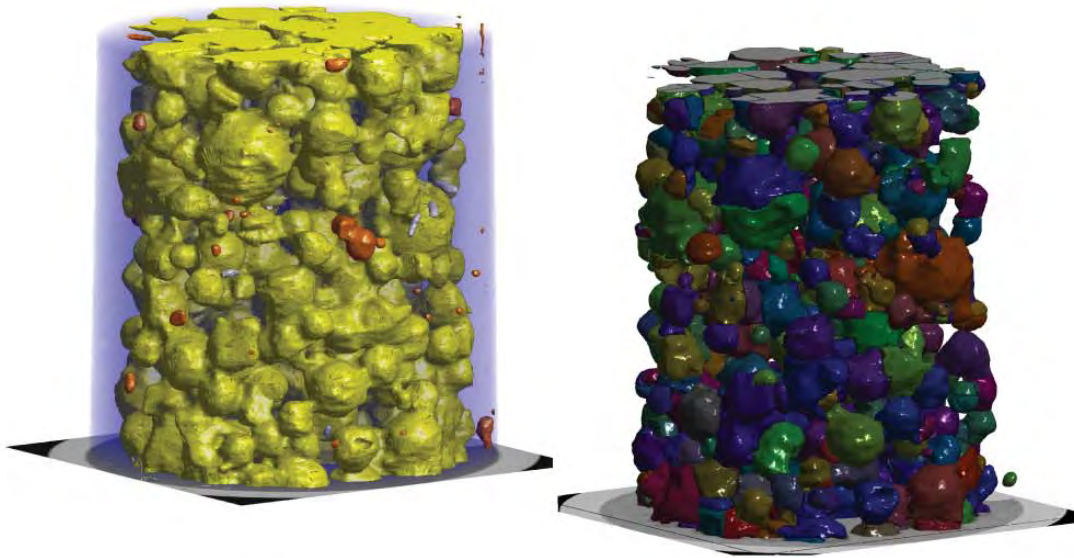




Applications of novel X-ray imaging modalities in food science

PhD Thesis

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Applications of novel X-ray imaging modalities in food science

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Abstract

In recent years, the interest for non-destructive imaging of the internal structures in food products has increased. First of all, the food industry shows an increased interest for automated quality inspection of food products. Secondly, food microstructure has become more important within food science for understanding and designing food products. In both of these aspects, X-ray imaging methods such as radiography and computed tomography provide a non-destructive solution.

However, since the conventional attenuation-based modality suffers from poor contrast in soft matter materials, modalities with improved contrast are needed. Two possible candidates in this regard are the novel X-ray phase-contrast and X-ray dark-field imaging modalities. The contrast in phase-contrast imaging is based on differences in electron density which is especially useful for soft matter materials whereas dark-field imaging produces a contrast based on differences in microstructure.

In order to increase the use of X-ray imaging within food science, possible applications of X-ray phase-contrast and X-ray dark-field imaging should be studied. To reach these applications, improvements are needed on several aspects of the imaging process. From the initial step of *taking* the image, the information in the image needs to be *translated* through image analysis before data analysis can be applied to *treat* the image quantitatively and answer the questions at hand.

In this work, a number of studies were carried out to investigate possible applications of novel X-ray imaging modalities within food science. The first two studies mainly concern the image acquisition process of *taking* the image. Using dark-field radiography, raw, frozen and defrosted fruit were distinguished, and structural changes in barley seeds during germination were monitored. Furthermore, the process of *translating* the image in image analysis was addressed. For improved handling of multimodal image data, a multivariate segmentation scheme of multimodal X-ray tomography data was implemented. Finally, quantitative data analysis was applied for *treating* the images. Quantitative studies were conducted on the microstructure of a dairy-like food emulsion as well as the structural changes in meat due to heat treatment.

In conclusion, the studies indicated a strong potential of using novel X-ray imaging modalities within food science.

Dansk resumé

I de senere år er interessen steget over for ikke-destruktive metoder til billeddannelse af de indre strukturer i fødevarer. Først og fremmest skyldes dette en øget interesse i fødevareindustrien over for automatiseret kvalitetsinspektion af fødevareprodukter. For det andet er mikrostrukturen i fødevarer blevet vigtigere inden for fødevarevidenskab for at opnå en dybere forståelse af fødevareprodukter og designe nye. I begge af disse aspekter præsenterer røntgenbilledmetoder såsom radiografi og tomografi en ikke-destruktiv løsning.

Siden den konventionelle dæmpningsbaserede røntgenbilledmodalitet lider under en række begrænsninger pga en ringe billedkontrast i de lettere organiske materialer. Inden for fødevarer kræves derfor modaliteter med forbedret kontrast. To mulige kandidater er de nye fasekontrast og dark-field røntgenbilledmodaliteter. Kontrasten i fasekontrast er baseret på forskelle i elektrontætheder, hvilket er særligt anvendeligt ved organiske materialer, mens dark-field danner en kontrast baseret på forskelle i mikrostruktur.

For at øge udbredelsen af røntgenbilledmetoder inden for fødevarevidenskab bør mulige anvendelser af fasekontrast og dark-field røntgenbilleder således studeres. For at nå frem til disse anvendelser kræves forbedringer inden for flere aspekter af billedannelsen. Fra det første skridt i at *optage* billedet (en: “*taking the image*”) skal informationen *oversættes* igennem billedbehandling (en: “*translating the image*”) før databehandling kan anvendes til at *omdanne* billedet til kvantitativ information (en: “*treating the image*”), så de ønskede spørgsmål kan besvares.

I dette ph.d. arbejde blev en række studier udført for at undersøge mulige anvendelser af ny røntgenbilledmodaliteter indenfor fødevarevidenskab. De første to studier omhandler hovedsageligt billedannelsesprocessen i at *optage* billedet. Ved at bruge dark-field radiografi kunne rå, frossen og optøet frugt skelnes fra hinanden, og strukturelle ændringer i spirende bygkerner kunne monitoreres. Derudover blev processen i at *oversætte* billedet under billedbehandling adresseret. For at forbedre håndteringen af multimodal billeddata blev en multivariat segmentering implementeret for multimodale røntgentomogrammer. Endelig blev kvantitativ databehandling anvendt for at *omdanne* billederne. Her blev dels mikrostrukturen af en fødevareemulsion og dels de strukturelle ændringer i kød grundet varmebehandling studeret kvantitativt.

Slutteligt konkluderedes det, at studierne viste et stort potentiale for at anvende nye røntgenbilledmodaliteter inden for fødevarevidenskab.

Papers on which this thesis is based

Paper I

M. S. Nielsen, L. B. Christensen & R. Feidenhans'l,
Frozen and defrosted fruit revealed with X-ray dark-field radiography.
Food Control. (2014) **39**, 222-226

Contribution: Conducted the design of the study, carried out measurements and data analysis, and wrote the initial draft of the paper.

Paper II

M. S. Nielsen, K. B. Damkjær & R. Feidenhans'l,
Quantitative in-situ monitoring of germinating barley seeds using X-ray dark-field radiography.
Submitted to Journal of Food Engineering. (2015)

Contribution: Participated in designing the study, carried out X-ray imaging measurements and data analysis, and wrote the initial draft of the paper with input from KBD.

Paper III

H. Einarsdóttir, **M. S. Nielsen**, R. Miklos, R. Lametsch, R. Feidenhans'l, R. Larsen & B. K. Ersbøll,
Analysis of micro-structure in raw and heat treated meat emulsions from multi-modal X-ray microtomography.
Innovative Food Science and Emerging Technologies. (2014) **24**, 88-96

Contribution: Participated in designing the study, carried out X-ray imaging measurements and initial data processing, and contributed in writing the paper. The main data analysis and development of the multivariate segmentation scheme was performed by HE.

Paper IV

M. S. Nielsen, M. B. Munk, A. Diaz, E. B. L. Pedersen, M. Holler, S. Bruns, J. Risbo, K. Mortensen & R. Feidenhans'l,
Ptychographic X-ray computed tomography of extended lipid networks in food emulsions.
Submitted to Food Structure. (2015)

Contribution: Participated in the design of the study, carried out X-ray imaging measurements, initial data processing and the bulk of the data analysis, and wrote the draft of the paper.

Paper V

R. Miklos, **M. S. Nielsen**, H. Einarsdóttir, R. Feidenhans'l & R. Lametsch,
Novel X-ray phase-contrast tomography method for quantitative studies of heat induced structural changes in meat.
Meat Science. (2015) **100**, 217-221

Contribution: Participated in designing the study, carried out X-ray imaging measurements, initial data processing and the calibration for quantitative voxel values, and contributed in writing the paper. The bulk main data analysis was performed by HE, and the draft and conclusions were made by RM.

Other papers not included in the thesis

Paper VI

T. Stankevič, S. Mickevičius, **M. S. Nielsen**, O. Kryliouk, R. Ciechonski, G. Vescovi, Z. Bi, A. Mikkelsen, L. Samuelson, C. Gundlach & R. Feidenhans'l,
Measurement of strain in InGaN/GaN nanowires and nanopillars.
Journal of Applied Crystallography. (2015) **2**, 344-349

Contribution: Participated in X-ray measurements and initial data processing, and contributed in editing the manuscript.

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Chapter 1

Introduction

The work in this thesis has been carried out within the field of X-ray imaging. More specific, the main focus has been applications of novel X-ray imaging modalities within food science. In this work, the aim was to evaluate the potential for novel X-ray imaging modalities in the food industry and food science. This potential includes both new types of automated quality inspection systems as well as 3D microstructural investigations.

In order to apply novel and non-standard imaging modalities, developments in a number of areas have become important. These relate to the necessary steps in an imaging experiment. From the initial process of *taking* the image, the information in the image needs to be *translated* through image analysis before data analysis can be applied to *treat* the image quantitatively and answer the questions at hand. These steps, *taking*, *translating* and *treating* the image, will be the common theme throughout the thesis.

The importance of the last two steps has grown in the last 10 years. With a decrease in image acquisition times due to high-brilliance X-ray sources, a drastic increase in data have followed. Whereas a PhD student in X-ray imaging used to spend most of the PhD doing measurements, today a few days at an imaging beamline can produce enough data for a full PhD project. Conversely, image processing, visualization and quantitative data analysis now take up the majority of the PhD work. The aim of the thesis is to present the steps in X-ray imaging to the reader and stress their importance.

The reader is assumed to have a basic knowledge of X-ray physics, image analysis and optics, though the most important theoretical background is included. The thesis is written as a synopsis, thus primarily working as an introduction to and presentation of the attached papers.

This first chapter will start by providing a general motivation for the need for

new X-ray imaging methods in food science in section 1.1. Then in section 1.2, a brief introduction to a number of refraction- and scattering-based X-ray imaging methods is given. Finally in section 1.3, an outline of remainder of the thesis will be given.

1.1 General motivation: Why X-ray imaging of food?

Since its discovery in 1895 ([Röntgen, 1895](#)), conventional X-ray imaging has found widespread use. Publicly, though, it is probably best known for its medical applications. As a non-invasive imaging method with high penetration depth, it is widely used in diagnostics at hospitals. This includes 2D radiographic images and since the 1970s also 3D computed tomographic images ([Hounsfield, 1973](#)). A main appeal is that diagnostic analysis can be performed on the X-ray image directly without doing a lengthy data analysis beforehand. After the image has been taken, the medical examiner translates and treats the image qualitatively.

Thus, to apply X-ray imaging within food science may not seem obvious. In recent years, however, two trends have heightened the interest for X-ray imaging within the food industry and food science research.

First of all, the food industry shows an increased interest for automated quality inspection of food products due to public concerns ([Haff and Toyofuku, 2008](#)) as well as retailer demands ([Jha, 2010](#)). This has led to a shift from manual to automated on-line inspection systems such as machine vision, metal detectors and X-ray inspection. One of the main applications of X-ray inspection is for detection of foreign bodies in food products ([Graves et al., 1998](#)). Hard materials such as bone fragments, glass, metal and stones are readily detected while soft materials such as paper, wood, hair, plastics and insects are more difficult ([Chen et al., 2013](#); [Graves et al., 1998](#); [Schatzki et al., 1996](#)). In addition using respectively dual- or low-energy X-ray systems, quality parameters such as fat content in meat ([Damez and Clerjon, 2013](#)) or defects from e.g. insect infestation in grain may be assessed ([Haff and Toyofuku, 2008](#); [Kotwaliwale et al., 2011](#)). Since on-line inspection gives a high throughput, computer-based decision systems using image analysis and quantitative data processing are needed to translate and treat the images ([Kotwaliwale et al., 2011](#)).

Secondly, food microstructure has become more important within food science for understanding and designing food products ([Aguilera, 2005](#); [Heertje, 2014](#)). Fat crystal networks in fats such as cocoa butter, milk fat and palm oil have been found to exhibit complex fractal microstructures ([Narine and Marangoni, 1999a,b](#)). In fruits, the pore space microstructure is important for gas flow as well

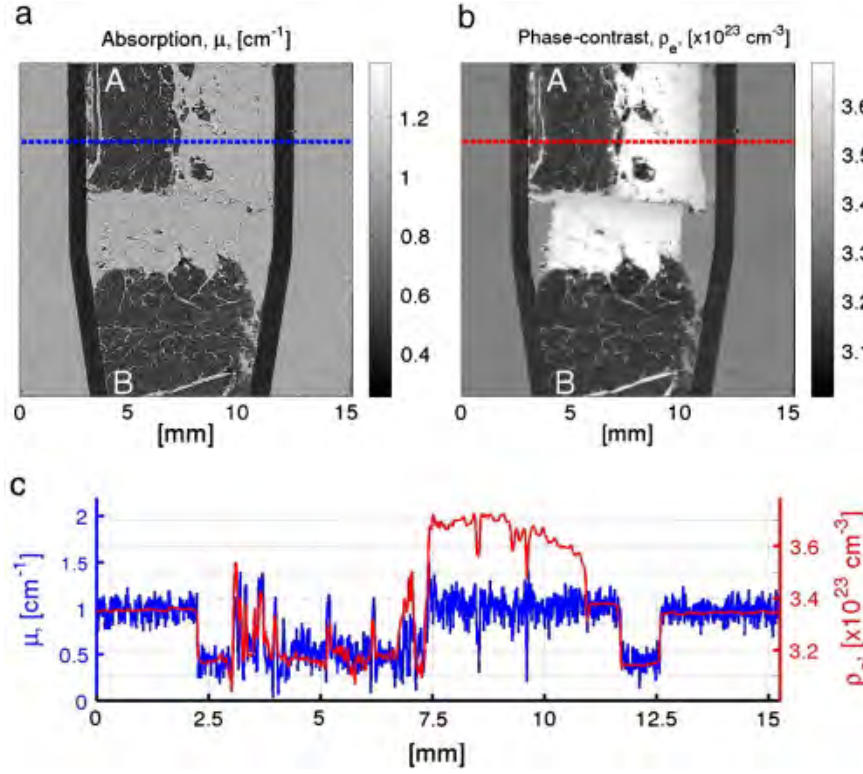


Figure 1.1: Frontal slice from a (a) conventional CT and (b) phase-contrast CT tomogram. (c) Plot of the attenuation-contrast and phase-contrast through the lines marked in (a) and (b). *Reproduced with permission from Jensen et al. (2011).*

as texture (Mendoza et al., 2007; Schotsmans et al., 2004). And both the rheology and function of food emulsion systems depend on the microstructure (Heertje, 2014). Techniques often employed to study food microstructure include various types of light and electron microscopy such as confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) (Aguilera et al., 2000; El-Bakry and Sheehan, 2014). In addition, X-ray computed microtomography (μ CT) has proven useful in studying the microstructure of a range of food products (Frisullo et al., 2010; Lim and Barigou, 2004; Mendoza et al., 2007).

Even though conventional X-ray imaging has proven useful in a number of studies of foods, both radiographic and tomographic applications face limitations due to the poor contrast in soft matter materials (Haff and Toyofuku, 2008; Kotwaliwale et al., 2011). Thus, methods with improved contrast are needed.

Two such novel imaging methods could be X-ray phase-contrast and X-ray dark-field imaging. Compared to conventional μ CT, X-ray phase-contrast has demonstrated superior contrast in porcine tissue (Jensen et al., 2011) and enhanced the detection of pore walls in fruits (Verboven et al., 2008). While the conventional X-ray image modality uses absorption or more generally attenuation

as contrast mechanism, X-ray phase-contrast relies on the refraction of X-rays to form the image contrast. This provides higher contrast in soft matter materials as can be seen from figure 1.1 when comparing (a) the conventional CT to (b) phase-contrast CT.

The X-ray dark-field signal is formed by scattering of X-rays from microstructures. Within food science, X-ray and neutron-based scattering techniques have long been used to study food structures. A recent example is the spin-echo small-angle neutron scattering (SESANS) which has been used to e.g. study microstructures in dairy products ([Tromp and Bouwman, 2007](#)). Unlike normal scattering techniques, the structural sensitivity in dark-field imaging provides an image contrast which can spatially distinguish different microstructural compositions. Thus, heterogenous and multiphase samples can readily be inspected. Within food science this has recently been applied for foreign body detection in food products ([Kottler et al., 2010](#); [Nielsen et al., 2013](#)).

In order for these novel X-ray imaging modalities to develop further, not only advances in the image acquisition are needed. In addition, development of methods for image analysis and quantitative data analysis are needed in order to translate and treat these new types of images.

In this thesis, developments in novel X-ray imaging modalities within food science will be introduced. Applications for novel contrast mechanisms, image analysis and quantitative data analysis are presented. Some of the imaging techniques have potential for industrial use while others find their main use within scientific research.

1.2 Review of phase-contrast and dark-field imaging techniques

Several techniques exist for X-ray phase-contrast and dark-field imaging. In the following we will give a short review of a number of the important refraction- and scattering-based methods. The techniques covered may be grouped into three categories (1) propagation-based imaging, (2) grating-based imaging and (3) coherent diffraction imaging.

Although they employ somewhat different approaches, all techniques require some degree of beam coherence. The use of a coherent illumination can cause a variation in the recorded intensity due to sample-induced phase-effects. This principle is illustrated in figure 1.2. At increasing sample-to-detector distance, phase-effects from the disk-like objects in the simulated phantom become stronger. In the Fresnel region beginning at a distance of 10 mm, edge enhancement effects are

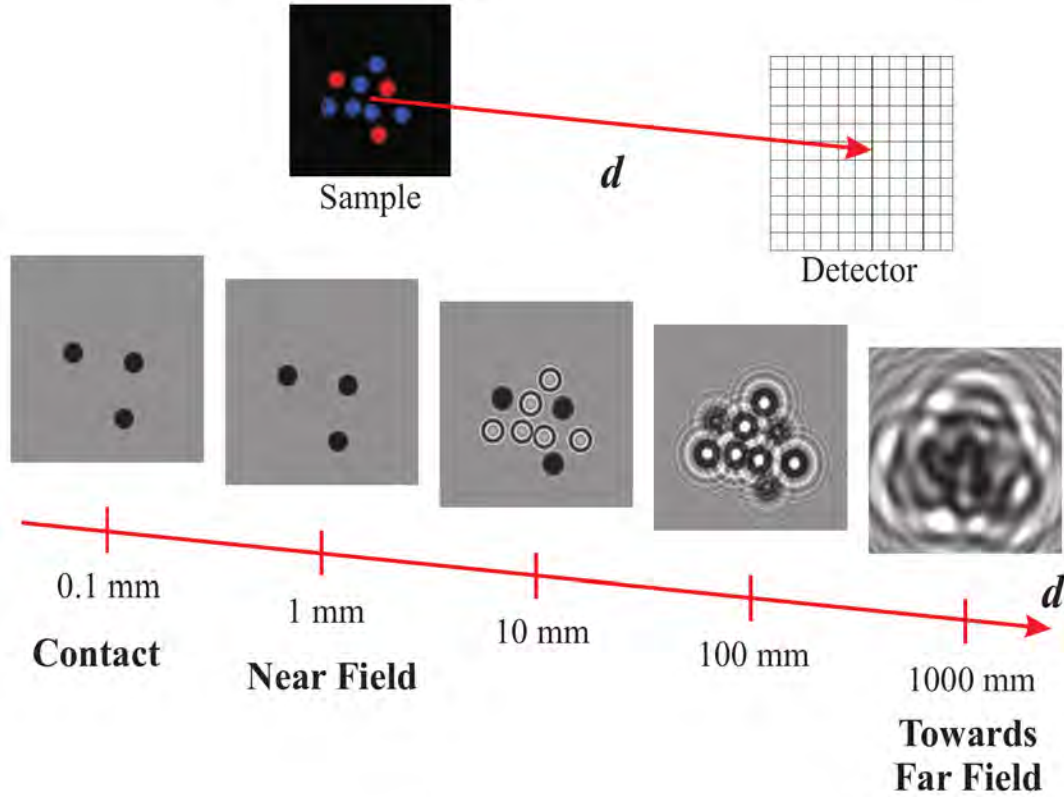


Figure 1.2: *Top:* Radiographs of small disk-like objects (red: zero transmission, blue: no absorption, phase shift π) with a diameter of $5\ \mu\text{m}$ were simulated using wave propagation. A wavelength of $1\ \text{\AA}$ and a detector with pixel size of $100\ \text{nm}$ placed at the noted distances (0.1 , 1 , 10 , 100 , and $1000\ \text{mm}$) were assumed. *Bottom:* Illustration of phase-effects from coherent X-ray illumination at increasing sample-to-detector distance. The transition takes place from pure absorption contrast (in the contact regime) via edgeenhancing in-line phase contrast (near field) to stronger phase contrast (Fresnel region) and towards the far-field regime (although the final Fraunhofer image is not given). *Reproduced from Als-Nielsen and McMorrow (2011).*

seen which reveal the presence of pure phase-objects (in blue). At larger distances beyond $1000\ \text{mm}$, the Fraunhofer region is approached where the phase-effects are seen as speckles in the diffraction patterns. The phase-contrast imaging techniques described in the following operate in either the Fresnel or Fraunhofer regime.

In only a few pages, a comprehensive review of each method cannot be achieved. Rather a short presentation of each will be provided as well as a few notes on the benefits and limitations. As investigations and further developments of the different methods are still being conducted, the advantages and drawbacks may change accordingly.

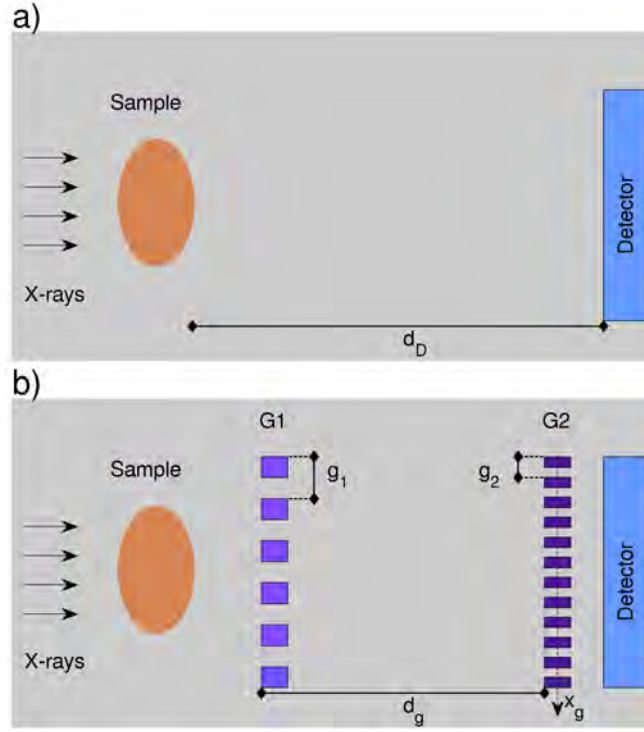


Figure 1.3: Schematic overview of two X-ray imaging methods using a transverse coherent X-ray beam. **a)** In Propagation Based Imaging (PBI), the detector records exposures at a number of sample-to-detector distances d_D . **b)** In Grating Based Imaging (XGI), a phase difference is induced by the phase-grating G1 which results in an intensity variation at the analyzer grating G2. A number of exposures are recorded with G2 moved in steps to analyze the interference pattern.

1.2.1 Propagation-based imaging

In propagation-based imaging (PBI), refraction effects due to Fresnel diffraction are detected. No optical elements are needed. By increasing the distance between the sample and detector d_D , phase-differences induced by the sample result in intensity variations in the outgoing beam. A schematic of a PBI set-up is shown in figure 1.3 (a). The method measures the Laplacian of the phase (Cloetens et al., 1997). The technique was first demonstrated in the 1990s using synchrotron sources (Cloetens et al., 1996; Snigirev et al., 1995), and was later implemented with a laboratory-based X-ray source (Wilkins et al., 1996). While the method allows the use of polychromatic X-ray sources, a high degree of spatial coherence is necessary (Zhou and Brahme, 2008). For laboratory setups, microfocus sources or similar are needed.

PBI allows for absorption and phase-contrast imaging for both radiography and tomography. If several different sample-to-detector distances are used, quantitative determination of both absorption and phase can be achieved (Cloetens

et al., 1999). However, since this approach increases both the time and dose received by the sample, measurements at a single sample-to-detector distance are preferable. Thus, some attention has been devoted to algorithms which perform phase-retrieval from measurements at a single distance (Burvall et al., 2011). Although only exact under certain conditions, e.g. single homogeneous known sample material, the methods are often used as an approximative phase-retrieval. An algorithm by Paganin et al. (Paganin et al., 2002) has found use at several synchrotron facilities in Europe and is applied regularly for tomography measurements.

Dark-field imaging is not obtained with this method, and scattering is generally neglected within this framework. A clear advantage of the method is the lack of optical elements. Among the limitations are the requirement of high resolution to resolve the interference caused by the Fresnel diffraction. In addition, a high spatial coherence is needed which limits the available flux at a laboratory setup. The development of new microfocus X-ray sources such as the liquid metal jet source (Larsson et al., 2013) may help to overcome the last limitation.

1.2.2 X-ray grating interferometry

X-ray grating interferometry (XGI) also works in the Fresnel region. The technique relies on an interferometer using periodic gratings. A schematic of a setup for XGI is shown in figure 1.3 (b). One grating G1 is used to produce an interference pattern consisting of periodic fringes transverse to the beam direction. The change in position and amplitude of the periodic fringes can be probed using a second grating G2. From this the attenuation, refraction and (ultra) small-angle scattering can be determined. The method was first demonstrated in the beginning of the 2000s using synchrotron sources (David et al., 2002; Momose, 2003; Weitkamp et al., 2005), and later it was adapted to laboratory-based setups (Pfeiffer et al., 2006). With tomography the method allows for reconstruction of the full complex refractive index (Nielsen et al., 2012; Pfeiffer et al., 2007; Weitkamp et al., 2006), and it is also possible to perform dark-field tomography (Bech et al., 2010). The method can be applied using polychromatic sources. However, since it relies on interference a certain degree of spatial coherence is needed. In a laboratory setup the latter can be achieved by using a third grating that acts as an array of line sources.

XGI has the powerful advantage that absorption, phase-contrast and dark-field images are obtained at the same time (Bech et al., 2010; Pfeiffer et al., 2008) allowing for direct comparison on voxel basis. Furthermore the method can be implemented at lab-based sources and still retain a large flux. The field of view is limited only by the area of the gratings used and should be scalable to tens of centimeters. In cone beam geometries, bent gratings have been proposed

to assure perpendicular incident angles on the grating lines for large areas (Revol et al., 2011). The energy range is only limited by the height of the grating lines. Experiments at 82 keV have been conducted (Willner et al., 2013).

A great deal of work has been performed to move the technique towards commercial scanners (Koehler et al., 2015; Tapfer et al., 2012; Yaroshenko et al., 2013), and recently the first commercial μ CT scanner was made available (Bruker microCT, 2015).

1.2.3 Coherent Diffractive Imaging

In the Fraunhofer regime, phase-effects from a coherent X-ray beam result in a speckle pattern in the diffracted beam (Veen and Pfeiffer, 2004). Using coherent diffractive imaging (CDI) methods, phase-contrast images can be retrieved from a single speckled diffraction pattern through an iterative process (Miao et al., 1998). First constraints are imposed on an input object function in real space. Then after Fourier transformation, the measured diffraction intensities are used to update the magnitude in reciprocal space before backprojecting to real space. To ensure phase determination, the diffraction pattern must be oversampled above the Bragg sampling frequency (Miao et al., 1998; Veen and Pfeiffer, 2004). The approach has been used on both crystalline and non-crystalline materials (Miao et al., 1999; Robinson et al., 2001).

A recent scanning variant of CDI is X-ray ptychography (Rodenburg et al., 2007; Thibault et al., 2008). A Schematic of the technique is shown in figure 1.4 reproduced from (Dierolf et al., 2010). A pinhole creates a localized coherent illumination on part of the sample as seen in panel a). The illumination is moved in a pattern across the specimen as in panel b) and diffraction patterns are recorded from partly overlapping illuminations. Through iterative methods, the complex transmission function may be retrieved from the diffraction patterns (Rodenburg and Faulkner, 2004; Thibault et al., 2009). The necessary oversampling is ensured by requiring sufficient overlap of the illuminations.

As opposed to other CDI methods, ptychography does not rely on assumptions of homogeneity, negligible absorption, or small phase advance (Dierolf et al., 2010). In addition the iterative phase-retrieval methods are faster (Rodenburg et al., 2007).

The method has recently demonstrated a spatial resolution in 3D of 16 nm (Holler et al., 2014) and thus bridges the gap between electron microscopy and μ CT. Furthermore, in ptychographic X-ray tomography quantitative electron density values are reconstructed (Diaz et al., 2012) which allows for sensitive studies of electron density variations in soft-tissue samples (Esmaeili et al., 2013).

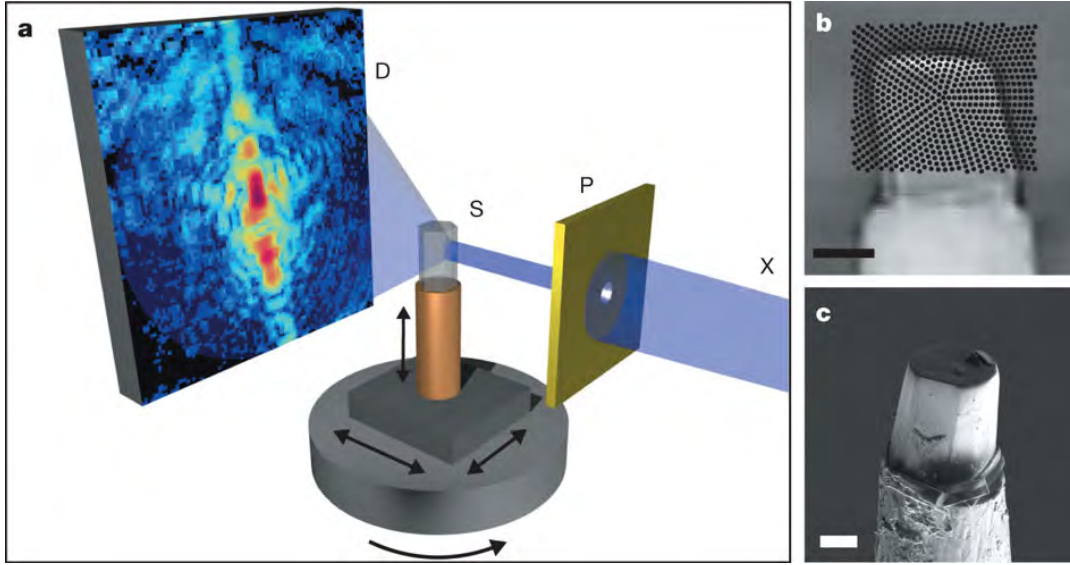


Figure 1.4: **a)** Schematic of experimental set-up. The X-ray beam (X) impinges on the pinhole (P), which creates a localized illumination on the sample (S). Diffraction patterns are recorded with a detector D. **b)** Projection image of the specimen. The black scan points are placed on concentric circles starting from the centre. **c)**, Scanning electron micrograph of the specimen. Scale bars, 10 μm . Reproduced from (Dierolf et al., 2010).

The need for high coherence, high beam flux and small tailored illumination, makes CDI techniques in general and X-ray ptychography in particular unsuited as a lab-based technique. Due to a relative small field-of-view of some tens of μm , the preparation of sufficiently small samples requires some work. With the advance of diffraction-limited fourth generation synchrotrons, X-ray ptychography is foreseen to improve in both smaller acquisition times and higher quality images (Thibault et al., 2014).

1.3 Outline of thesis

The remainder of the thesis is outlined as follows.

In Chapter 2 we will provide the theoretical background for contrast mechanisms and X-ray imaging. The chapter provides the background for the experiments and methods presented in the thesis and Papers I-V. First, three basic radiographic contrast mechanisms are presented: attenuation-contrast, phase-contrast and dark-field contrast. Furthermore, the phase-contrast imaging techniques of X-ray grating interferometry and X-ray ptychography are introduced.

The focus of Chapter 3 is how novel contrast modalities may be utilized in

taking X-ray images. The chapter discusses the complementarity of attenuation- and dark-field contrast and the ongoing development of the theoretical framework. In addition it serves as an introduction to [Paper I](#) and [Paper II](#). These papers demonstrate two applications of X-ray dark-field radiography and highlights the difficulties in a quantitative interpretation of the dark-field signal. In [Paper I](#), X-ray dark-field radiography is used to detect whether fruits and berries are in a raw, frozen or defrosted state. [Paper II](#) applies X-ray dark-field radiography to study the breakdown of starch granules in barley seeds during germination.

Chapter 4 provides a background in image analysis and works as an introduction to [Paper III](#). Multimodal imaging requires further development of image segmentation and classification methods in order to *translate* the raw images of several modalities. In [Paper III](#), multivariate image analysis on multimodal image data is shown to give improved feature detection. Three image modalities are combined in multimodal segmentation and quantitative analysis of meat emulsion samples.

Chapter 5 discusses how data analysis can extract quantitative measures from images. The focus is to *treat* images as quantitative data rather than qualitative depictions of the sample. The chapter serves as an introduction to [Paper IV](#) and [Paper V](#). [Paper IV](#) investigates the structure of an extended lipid network in a dairy-like food emulsion using ptychographic X-ray computed tomography. In [Paper V](#) heat induced changes to the structure and composition of a piece of meat are studied quantitatively.

Finally in Chapter 6, an overall conclusion and outlook of the thesis is given.

Chapters 3-5 are based on five papers in total. The papers are referred to by roman numerals as Paper I-V and are included in their full length in Appendix A. Paper I, III and V have already been published, while [Paper II](#) and [Paper IV](#) have been submitted for publication.

Chapter 2

X-ray image modalities

In this chapter, a short background to X-ray imaging is given. First, the physical interactions that form the main contrast mechanisms in X-ray imaging are presented. Secondly, an introduction is given for two novel X-ray imaging techniques: X-ray grating interferometry and ptychography. For both techniques reconstruction algorithms are presented for producing the associated radiographs, i.e. transmission (attenuation), differential phase-contrast and dark-field for grating interferometry and attenuation and phase-contrast in the case of ptychography.

The chapter is not intended as a thorough review, and the reader is assumed to have some familiarity with the concepts beforehand. For more information, we refer the readers to [Als-Nielsen and McMorrow \(2011\)](#); [Nugent \(2010\)](#); [Veen and Pfeiffer \(2004\)](#).

2.1 Contrast mechanisms

In this thesis, three types of image modalities are utilized: Transmission, phase-contrast and dark-field. They in turn each rely on a physical interaction. In a transmission image, the contrast is formed by the attenuation of the X-ray beam. The attenuation is e.g. caused by absorption of the X-rays in the material. For the phase-contrast modality, the image contrast depends on the (coherent) refraction of X-rays. Finally, the dark-field modality is based on scattering effects. In the following, an introduction to these interactions is given.

2.1.1 Attenuation and refraction

We will begin by regarding attenuation and refraction. A combined way to describe these is through the complex refractive index for X-rays

$$n = 1 - \delta + i\beta, \quad (2.1)$$

where the dimensionless δ and β account for the refraction and attenuation, respectively. Both are small quantities ($< 10^{-5}$) and are related to the attenuation length μ and the electron density ρ_e (Als-Nielsen and McMorrow, 2011)

$$\delta = \frac{2\pi\rho_e r_0}{k^2}, \quad \beta = \frac{\mu}{2k}, \quad (2.2)$$

where the X-ray wave number $k = 2\pi/\lambda$ is given in units of inverse length by the wavelength λ , and $r_0 = 2.82 \times 10^{-15}$ m is the Thomson scattering length.

While several absorption and scattering processes may attenuate an X-ray beam, photo-electric absorption is the dominating contribution for energies below 100 keV. As the measurements in this thesis have been done for X-ray energies in the range 5-40 keV, we will only consider the contribution of photo-electric absorption to attenuation.

For energies above the k-edge, the attenuation length for photo-electric absorption and the electron density can be expressed in terms of the mass density ρ_a , the atomic number Z , and the photon energy E (Als-Nielsen and McMorrow, 2011)

$$\mu \propto \rho_a \frac{Z^4}{E^3} = \frac{N_A}{M} \rho_m \frac{Z^4}{E^3}, \quad \rho_e = Z \rho_a = \frac{Z N_A}{M} \rho_m, \quad (2.3)$$

where ρ_a is expressed in terms of the atomic mass number, M , mass density ρ_m , and Avogadro's number N_A . For non-homogeneous materials ρ_e and μ will vary with the position in the material. Thus in general, the complex refractive index $n(x, y, z)$ depends on the coordinate position.

The effect of the refractive index can be illustrated by comparing two monochromatic plane waves traveling in the $\mathbf{z}$ direction. As shown in figure 2.1, the first wave travels in vacuum with refractive index 1 while the second travels through a homogeneous material with complex refractive index n . The wave in vacuum can be described as $E(z, t) = E_0 e^{i(kz - \omega t)}$.

For the X-ray wave entering the material, the wavenumber changes from k to

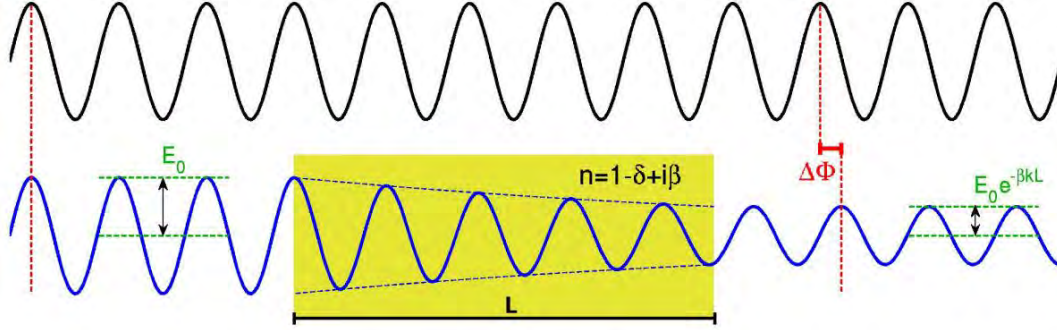


Figure 2.1: Index of refraction: Comparison of a plane wave traveling in vacuum (black) and in a material with refractive index n (blue). In the material the amplitude of the wave is reduced with a factor of $e^{-\beta k L}$ due to absorption, and the phase changes by $\Delta\Phi$ due to refraction. (Reproduced from Nielsen (2012)).

nk , and the wave inside the material can be described as:

$$\begin{aligned}
 E'(z, t) &= E_0 e^{i(nkz - \omega t)} \\
 &= E_0 \underbrace{e^{-\beta k z}}_T \underbrace{e^{-i\delta k z}}_{\Delta\Phi} e^{i(kz - \omega t)}
 \end{aligned} \tag{2.4}$$

Thus, after traversing a length L in the material, the wave amplitude is attenuated by a factor of $e^{-\beta k L}$, and the phase of the wave is shifted by $\Delta\Phi = -\delta k L$. In general, the phase shift can be written as

$$\Delta\Phi = k \int \delta(x, y, z) dz, \tag{2.5}$$

The attenuation can be measured by a reduction in the detected intensity. This will reduce the X-ray transmission T when compared to vacuum

$$T = \frac{I}{I_0} = \frac{|E'|^2}{|E|^2} = e^{-\int 2\beta(x, y, z) k dz} = e^{-\int \mu(x, y, z) dz}, \tag{2.6}$$

Since detectors only measure the intensity, the X-ray phase is not recorded directly. However, the induced phase-shift can cause a refraction of the wavefront which affects the spatial intensity pattern. This can be illustrated by considering the refracted beam with phase $\Phi(\mathbf{r}) = \mathbf{k}' \cdot \mathbf{r}$ which travels in the direction $\mathbf{n}' = \mathbf{k}'/k' = (\lambda/2\pi)\nabla\Phi(\mathbf{r})$. Thus the angle of refraction in the (x, y) plane perpendicular to the incident beam direction can be expressed as

$$\alpha_x = \frac{1}{k} \frac{\partial\Phi(x, y)}{\partial x} = \frac{\partial}{\partial x} \int \delta(x, y, z) dz, \tag{2.7}$$

$$\alpha_y = \frac{1}{k} \frac{\partial\Phi(x, y)}{\partial y} = \frac{\partial}{\partial y} \int \delta(x, y, z) dz, \tag{2.8}$$

Thus, measuring the angle of refraction is a measure of the gradient of the phase.

The full complex refractive index describes two physical interactions which constitute complementary contrast mechanisms for X-ray imaging. However, they are linked together by the Kramers-Kronig relations ([Als-Nielsen and McMorro, 2011](#)).

2.1.2 Scattering

When an X-ray photon strikes an atom, every electron becomes the source of a scattered wave ([Glatter and Kratky, 1982](#)). For X-ray energies above the k-edge of the atom and far below the Compton energy ($E_c = 511\text{keV}$), the dominating process will be elastic Thomson scattering. For this case, all secondary waves have the same intensity given by ([Als-Nielsen and McMorro, 2011](#); [Glatter and Kratky, 1982](#))

$$I(\theta) = r_0^2 \frac{I_0}{R^2} \frac{1 + \cos^2(2\theta)}{2} \quad (2.9)$$

where r_0 is the Thomson scattering length, I_0 the intensity of the incoming beam, R the distance from the object to the detector, and 2θ the scattering angle.

When dealing with scattering within X-ray imaging, only forward scattering at small 2θ angles is considered. In this small-angle approximation, the polarization factor $(1 + \cos^2(2\theta))/2$ is close to one, and the angular dependence of I will be negligible. This means that the intensity of the scattered wave from each single electron is equal. Differences in scattered intensity are due to interference between scattered waves.

In the case of ordered lattice structures, scattering angles of constructive interference follow the well-known Bragg's law

$$q = \frac{4\pi}{\lambda} \sin(\theta) \quad (2.10)$$

which relates the scattering angle 2θ and wavelength λ to the scattering vector $\mathbf{q}$. The constructive interference occurs if and only if $\mathbf{q}$ coincides with a reciprocal lattice vector of the sample structure ([Als-Nielsen and McMorro, 2011](#)). For small angle scattering, Bragg scattering occurs for rather large lattice distances in the $nm - \mu m$ range which limits the relevance to samples with corresponding lattice distances. Within food science fat crystals and fibers are examples of such samples.

In general, the scattered intensity $I^s(\mathbf{q})$ from the volume V can be calculated from the distribution of the scattering lengths density $\rho(\mathbf{r})$ by ([Lindner and Zemb,](#)

2002)

$$\begin{aligned} I^s(\mathbf{q}) &= \int_V \int_V \rho(\mathbf{r}) \rho(\mathbf{r}') e^{-i\mathbf{q} \cdot (\mathbf{r} - \mathbf{r}')} d\mathbf{r} d\mathbf{r}' \\ &= V \int_V \gamma(\mathbf{r}) e^{-i\mathbf{q} \cdot \mathbf{r}} d\mathbf{r} \end{aligned} \quad (2.11)$$

where the real space correlation function $\gamma(\mathbf{r}) = \frac{1}{V} \int_V \rho(\mathbf{r}') \rho(\mathbf{r} + \mathbf{r}') d\mathbf{r}'$ has been introduced. For X-rays the Thomson scattering length r_0 is equal for all electrons, and $\rho(\mathbf{r}) = r_0 \rho_e(\mathbf{r})$ is often replaced by the electron density $\rho_e(\mathbf{r})$.

This general formulation can be used in imaging methods where the full scattering curve is measured. However, in imaging methods where this is not the case such as grating-based imaging other approaches are needed.

An inspiration might be gained from small-angle scattering where approximations can be used to obtain a rough understanding of the scattering curve. These often consider particles embedded in some material that forms a solvent or matrix. For dilute systems of particles at very low $\mathbf{q}$ and thereby small θ , the Guinier approximation can be used. In the Guinier approximation for spherical particles, the scattered intensity per volume relative to the solvent is (Lindner and Zemb, 2002)

$$I_m^s(\mathbf{q}) = \eta V_p \Delta \rho^2 e^{-(qR_G)^2/3} \quad (2.12)$$

where η is the volume fraction of particles, V_p the particle volume, $\Delta \rho = \rho_p - \rho_s$ the difference in scattering length distribution between particle and solvent and R_G the rotation of gyration of the particle given by

$$R_G = \frac{1}{2} \frac{\int_{V_p} \gamma(\mathbf{r}) r^2 d\mathbf{r}}{\int_{V_p} \gamma(\mathbf{r}) d\mathbf{r}} \quad (2.13)$$

The Guinier approximation states that the scattered intensity will have an almost Gaussian-like distribution in small $\mathbf{q}$.

In absence of the full $\mathbf{q}$ -dependence, an approach in imaging is to approximate the scattered intensity by a Gaussian distribution $A(\theta)$ in scattering angle 2θ (Bech et al., 2010)

$$A(\theta) = \frac{1}{\sqrt{2\pi}\sigma^2} \exp\left(-\frac{(2\theta)^2}{2\sigma^2}\right) \quad (2.14)$$

where σ is the width of the Gaussian scattering distribution. The form of this distribution describes isotropic scattering only. In the case of anisotropic scattering from e.g. fibrous structures, the scattering distribution will be directional dependent, and a 2D Gaussian distribution should be adopted instead (Jensen

et al., 2010). It should be noted that this formulation is rather simple. For a more detailed description, a more advanced approach should be adopted such as a tensor model for the 3D scattering length distribution (Malecki et al., 2014). However, for illustrative purposes we will use the simple scattering distribution formulation here.

The effect of scattering from the sample is to spread out the profile. This can be described by an convolution of the beam intensity I_0 with the scattering distribution $A(\theta)$.

$$I^s = I_0 \otimes A(\theta) \quad (2.15)$$

In full-field imaging, small-angle scattering from places in the sample will cause a divergence in the local beam profile. Normally, this will not cause any effect on the recorded image as long as the translation resulting from the scattering is much smaller than the pixel size. For coherent imaging, however, even scattering by small angles can reduce the transverse coherence, and a local decoherence of the beam can occur due to scattering events. This effect is what is utilized in the X-ray dark-field imaging modality of X-ray grating interferometry.

2.2 Grating-based interferometry

X-ray grating interferometry is a phase-contrast imaging technique that operates in the Fresnel regime. The technique relies on full-field imaging where the field-of-view (FoV) is given by the detector size, and the spatial resolution is restricted by the pixel-size.

An X-ray grating interferometer consists of two or three linear phase- or absorption gratings. In the case of two gratings, the interferometer is called a Talbot interferometer while the three-grating interferometer is called a Talbot-Lau interferometer ([Weitkamp et al., 2006](#)). For simplicity, we will simply use the term (X-ray) grating interferometer for both cases.

Here, we will present a short introduction to X-ray grating interferometry and discuss some method requirements. The introduction follows along the lines of [Nielsen \(2012\)](#) and [Jensen \(2010\)](#).

2.2.1 The Talbot effect

In grating-based interferometry, the phase-contrast effects are detected by using an interferometer comprised of several periodic gratings. In figure 2.2, a schematic of an interferometer with two gratings is shown. The phase grating G1 is a beam splitting phase grating that induces a fixed phase-shift in part of the incoming beam. G1 is normally designed with a 0.5 duty-cycle, with a typical period of $g_1 = 4 \mu\text{m}$, and with phase-shifts of π or $\pi/2$ at the design energy. In order to record the phase-contrast effects, the analyzer grating G2 with absorbing lines is placed in front of the detector at a distance d after G1. The requirements on this distance will be discussed in the following.

As shown in figure 2.2, the simulation ([Als-Nielsen and Bech, 2009](#)) of a coherent monochromatic X-ray beam incident on G1 results in an interference pattern downstream. Bright (dark) shades depict points in space where constructive (destructive) interference has increased (decreased) the intensity of the incident beam shown in gray. For creating the pattern in MATLAB, the Fresnel propagator ([Bech, 2009](#)) was used¹. After a certain distance, the interference pattern in figure 2.2 is seen to repeat itself. This effect of periodic wavefront propagation is named the *Talbot effect* after Henry Fox Talbot who discovered it in 1836 ([Talbot, 1836](#)).

¹The Fresnel propagator in reciprocal space $\hat{P}_d(k_x, k_y)$ for propagating a distance d in the z -direction is given as $\hat{P}_d(k_x, k_y) = e^{ikd} e^{-kd(k_x^2 + k_y^2)/2k}$ where k is the wavenumber of the beam ([Bech, 2009](#)). The propagation of a wavefunction $\Psi_0(x, y)$ a distance d can then be evaluated as $\Psi_d(x, y) = \mathcal{F}^{-1} \left(\mathcal{F}[\Psi_0(x, y)] \hat{P}_d(k_x, k_y) \right)$ where $\mathcal{F}$ denotes the Fourier transform.

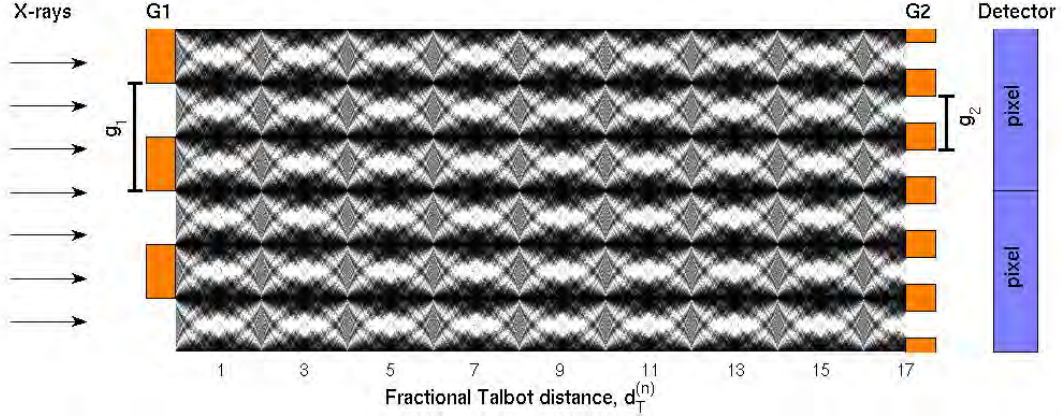


Figure 2.2: Interference pattern generated by an ideal phase-grating with a phase-shift of π and a 0.5 duty cycle. White is maximum intensity and black is no intensity. Gray is the intensity of the incoming coherent monochromatic parallel beam. Gratings and detector are shown. (*Reproduced from Nielsen (2012)*).

Thus, the induced phase-modulation by G1 results in an intensity pattern. This is further illustrated for different propagation distances in figure 2.3. The largest intensity differences for the 0.5 duty cycle G1 grating with a phase-shift of π are found at the odd fractional Talbot distances $d_T^{(n)}$ given as:

$$d_T^{(n)} = n \frac{g_1^2}{8\lambda}, \quad (2.16)$$

For n odd, the intensity pattern is box-shaped as indicated for the 5th fractional Talbot distance in figure 2.3 (green curve) (Pfeiffer et al., 2005; Weitkamp et al., 2006). The period of the pattern is seen to be half the period of G1. For a wavelength $\lambda = 0.5 \text{ \AA}$ and grating period $g_1 = 4 \text{ }\mu\text{m}$ the distance is $d_T^{(5)} = 20 \text{ cm}$.

In Paper I and Paper II the 5th fractional Talbot distance was used while the 3rd fractional Talbot distance was used in Paper III and Paper V. Additional details can be found in section 2.4.

As seen in figure 2.3 (purple and orange), a small change in position from the 5th fractional Talbot distance results in large intensity changes. These continues until the 4th fractional Talbot distance (blue) where a flat intensity curve is reached. Thus, the grating interferometer must be calibrated precisely to achieve an optimal interference pattern.

As a quantitative measure for the intensity amplitude of the interference pattern, the visibility V is introduced

$$V \equiv \frac{I_{max} - I_{min}}{I_{max} + I_{min}} \quad (2.17)$$

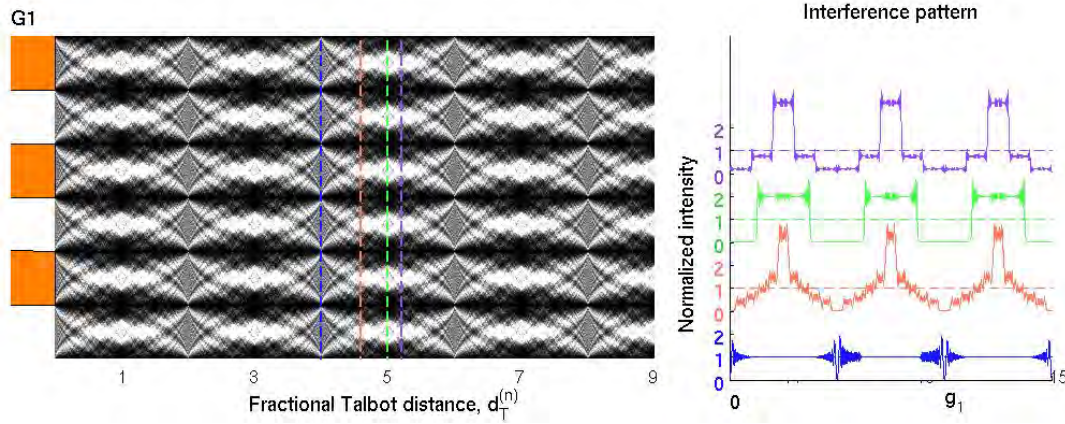


Figure 2.3: *Left:* Interference pattern generated by the same grating as in figure 2.3 with lines indicating 6, 6.5, 7 and 7.2 fractional Talbot distances. *Right:* Normalized intensity patterns at the indicated propagation distances. (Reproduced from Nielsen (2012)).

The visibility is a relative measure with values between zero and one. Often, V is expressed as a percentage. The minimum $V = 0$ corresponds to $I_{max} = I_{min}$ as seen for the 4th fractional Talbot distance, and the maximum $V = 1$ when $I_{min} = 0$ as seen for the 5th fractional Talbot distance.

2.2.2 Measurements using a grating interferometer

When a sample is placed in the grating interferometer, it will change the interference pattern through attenuation, refraction and scattering effects. The principle of grating-based imaging is to record the induced changes and from these retrieve images of the sample. In order to do this, a way to determine the interference pattern is needed. As the period of the interference pattern is typically $2\ \mu\text{m}$, a pixel size of around $100\ \text{nm}$ would be needed to resolve the pattern directly. Since this would be impractical for imaging samples in the order of $10\ \text{mm}$, a second analyzer grating G_2 is used instead. This has a 0.5 duty cycle and absorbing gold lines with a period g_2 that matches the interference pattern. By inserting G_2 at an uneven fractional Talbot distance, half of the interference pattern is blocked. When G_2 is moved in the x-direction perpendicular to the grating lines, different parts of the interference pattern will be detected.

In figure 2.4, this stepping procedure is illustrated for simulated detector read-outs of a cherry tomato at different positions of G_2 . At first, in panel a) where the grating is aligned with the interference pattern at $x_g = 0$, the maximum intensity is recorded by the detector. When the grating in panel c) is moved half a period at $x_g = g_2/2$, most of the intensity is blocked, and only refracted and scattered X-rays are recorded. At the position $x_g = g_2/4$ in panel b), an intermediate

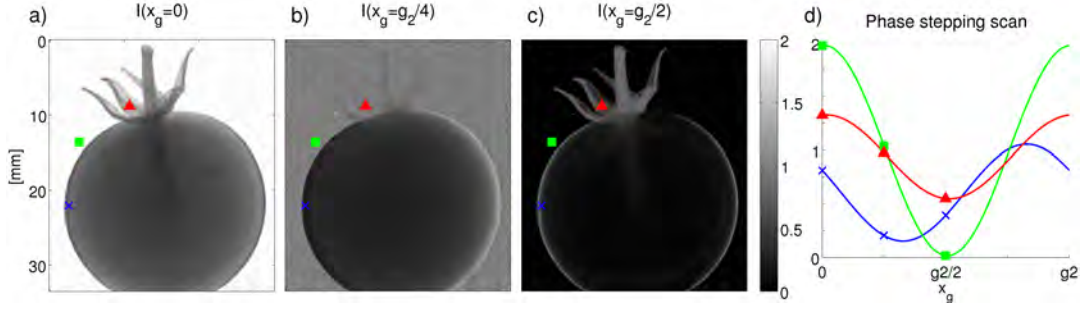


Figure 2.4: The stepping procedure a)-c) Illustration of idealized detector read-outs of a cherry tomato for the grating positioned at $x_g = [0; g_2/4; g_2/2]$ respectively. d) Plot of the intensity in three pixels as a function of the grating position. The three pixels are indicated in panels a)-c).

intensity level is recorded. Thus, by obtaining a series of intensities as function of x_g , the interference pattern can be analyzed. In panels a)-c), three pixels are indicated with a $\times$, a $\blacksquare$ and a $\blacktriangle$. These will be referred to in the following.

Ideally, the recorded intensity variations from the stepping procedure will be a triangular- shaped top-hat function (Als-Nielsen and McMorrow, 2011; Bech, 2009). However, in non-ideal setups several imperfections such as grating imperfections and partial coherence effects will cause a smearing of the detected intensity pattern (Weber et al., 2011). For real life setups, the recorded intensities can be shown to follow a sinusoidal curve (Bech et al., 2010). Thus, in practice the acquired data can be modeled by a first order Fourier expansion (Bech et al., 2010; Pfeiffer et al., 2008).

$$I(j,k,x_g) = \sum_{m=0}^{\infty} a_m(j,k) \cos \left[\frac{2\pi m}{g_2} x_g - \phi_n(j,k) \right] \\ \simeq a_0(j,k) + a_1(j,k) \cos \left[\frac{2\pi}{g_2} x_g - \phi_1(j,k) \right], \quad (2.18)$$

where (j,k) refers to the pixel numbers, a_m are the amplitude coefficients, ϕ_m the corresponding phase coefficients, and g_2 the period of G2. The coefficients a_0 , a_1 and ϕ_1 can be calculated from a sinusoidal fit using e.g. a *Fast Fourier Transform* (FFT) algorithm. An alternative approach is to apply an analytical linear least squares model of the data and calculate the parameters from this, as in Weber et al. (2011).

An example of the intensity variations with x_g in a pixel is shown in figure 2.5. The black curve illustrates the recordings from an empty (reference) pixel where a_0 describes the average intensity, a_1 the oscillation amplitude and ϕ_1 the position of the interference pattern. When a sample is present, X-ray interactions alter the amplitude, position, and average intensity of the recorded signal as illustrated in the purple curve. In figure 2.5 and the following, the subscripts s and r denote the

sample and reference measurement, respectively. By comparing the two curves from a reference and sample measurement, the effect of the sample itself can be extracted.

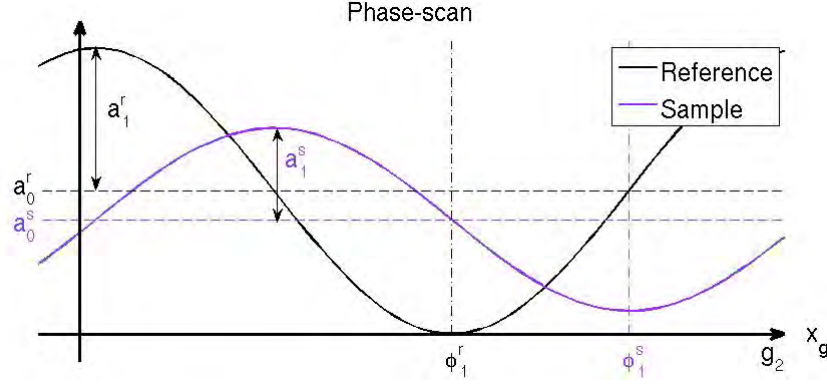


Figure 2.5: Signals: Comparing reference (black) and sample (purple) measurement three signals can be computed. The transmission can be calculated from the ratio of the mean values, a_0^s/a_0^r , the change in phase $\phi_1^s - \phi_1^r$ gives the differential phase-contrast and the ratio of the relative amplitude, $(a_1^s/a_0^s)/(a_1^r/a_0^r)$, gives the dark-field signal.

The transmission T can be seen from (2.6) to be the reduction in mean intensity due to attenuation. Since a_0 is proportional to the mean intensity, T can be calculated as:

$$T(x, y) = e^{-\int \mu(x, y, z) dz} = \frac{a_0^s}{a_0^r} \quad (2.19)$$

In figure 2.4, the effect of attenuation can be seen in the pixel indicated with a $\times$ in the tomato. Here the average curve intensity is reduced to 0.65 in comparison to average of 1 in the background pixel indicated with a $\blacksquare$ as seen in panel d).

The sample-induced shift in the interference pattern $\phi_1^s - \phi_1^r$ corresponds to an angular deviation in the x -direction of

$$\alpha_x = \frac{g_2}{2\pi d} (\phi_1^s - \phi_1^r), \quad (2.20)$$

where d is the G1-G2 distance, and the scaling factor $g_2/(2\pi d)$ is introduced since the value $\phi_1^s - \phi_1^r = 2\pi$ corresponds to a lateral shift of g_2 after a distance d . From (2.7) we thus find that $\phi_1^s - \phi_1^r$ is proportional to the partial derivative of the phase (Pfeiffer et al., 2006)

$$\frac{\partial \Phi(x, y)}{\partial x} = k \frac{\partial}{\partial x} \int \delta(x, y, z) dz = k \alpha_x(x, y) = k \frac{g_2}{2\pi d} (\phi_1^s - \phi_1^r), \quad (2.21)$$

As a result $\phi_1^s - \phi_1^r$ is a measure for the x -component of the phase gradient and forms the differential phase-contrast (dpc) radiographs of the grating interferometer. Subsequently the phase can be reconstructed by integration (Pfeiffer et al.,

2006). In figure 2.4, the phase-shift difference is illustrated by comparing the $\times$ pixel in the tomato with the background pixel $\blacksquare$. While the background ($\blacksquare$) has maximum at $x_g = 0$, the ($\times$) pixel has maximum between $x_g = \frac{3}{4}g_2$ and $x_g = g_2$.

The visibility V introduced in section 2.2.1 is related to the oscillation amplitude a_1 as the relative amplitude $V = a_1/a_0$. In presence of a sample with microstructures, the visibility will be reduced by small-angle scattering in the stepping direction. As in section 2.1.2, isotropic scattering by the sample can be modeled by a Gaussian scattering distribution $A(\theta)$. At G2 a distance z from the sample, this angular spread results in a horizontal translation x which for small angles can be related to θ by $2\theta = x/z$. As described in equation (2.15) the beam intensity I^s is found by a convolution of the reference beam intensity I^r with $A(x)$ (Bech et al., 2010)

$$\begin{aligned} I^s(x) &= \left\{ a_0^r + a_1^r \cos\left(\frac{2\pi}{g_2}x - \phi_1\right) \right\} \otimes A(x) \\ &= a_0^r + a_1^r \exp\left(-\frac{2\pi^2}{g_2^2}\sigma^2 z^2\right) \cos\left(\frac{2\pi}{g_2}x - \phi_1\right), \end{aligned} \quad (2.22)$$

where (2.18) has been used for the reference intensity, and z is the distance from the sample to G2. As seen, the amplitude a_1^s/a_0^s is reduced by the factor $V^s = \exp\left(-\frac{2\pi^2}{g_2^2}\sigma^2 z^2\right)$ due to the scattering distribution $A(x)$. A measure for the (relative) visibility can be found by the ratio of sample and reference amplitude.

$$V(x, y) = \frac{V^s}{V^r} = \frac{a_1^s/a_0^s}{a_1^r/a_0^r}, \quad (2.23)$$

It turns out that the visibility reduction due to isotropic scattering depends on the sample thickness in much the same way as beam attenuation from e.g. absorption (Bech et al., 2010, 2012). Thus, a dark-field attenuation coefficient μ_{DF} has been introduced (Lynch et al., 2011; Strobl, 2014).

$$V(x, y) = e^{-\int \mu_{DF}(x, y, z) dz} \quad (2.24)$$

We will return to the dark-field attenuation approach in chapter 3 when discussing quantitative values for the dark-field signal.

In figure 2.4, a reduction in visibility can be seen in the pixel indicated with a $\blacktriangle$ on the stem of the tomato compared to the background pixel $\blacksquare$. As seen in panel d), the background curve amplitude ($\blacksquare$) of $a_1 = 1$ is reduced to $a_1 = 0.35$ in the stem ($\blacktriangle$) due to scattering from microstructures. Compared to the mean curve values a_0 , this gives a drop in visibility from $V = 1/1 = 100\%$ to $V = 0.35/.95 = 37\%$. As the images produced using the visibility reduction are formed by the scattered X-rays, they are called dark-field images. The dark-field signal will be discussed in more detail in chapter 3.

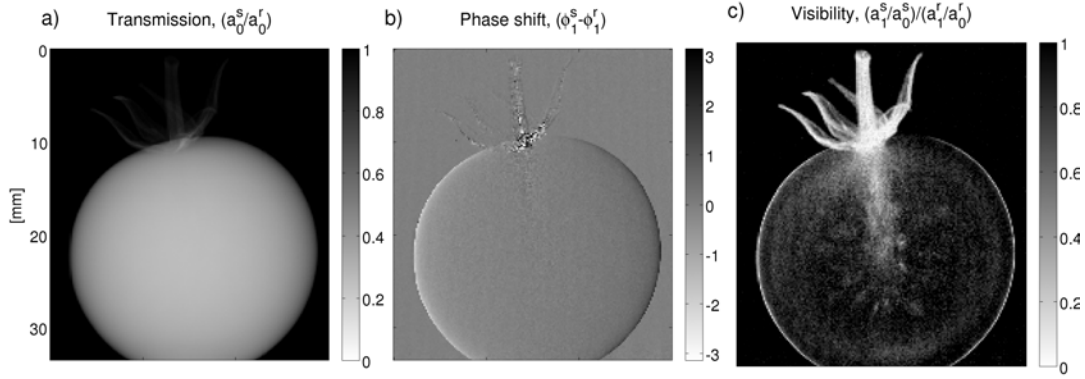


Figure 2.6: Three types of images are recorded with the grating interferometer: **(a)** The transmission signal which is the conventional X-ray absorption-contrast image, **(b)** the differential phase-contrast image, the measured phase-shift which is sensitive to electron density variations and **(c)** the dark-field image which is the reduction in visibility and shows scattering structures of the sample.

The three different grating interferometer images are presented in figure 2.6 using a cherry tomato as an example. In panel (a) the conventional transmission image can be seen with bright shades indicating low transmission, i.e. high absorption. The image in panel (b) depicts the differential phase. The bright (dark) shade on the left (right) parts of the tomato corresponds to an increase (decrease) in phase. The dark-field image in panel (c) measures the loss of visibility due to scattering or unresolved edges. Scattering is seen to take place from both the stem and seeds inside the tomato.

2.2.3 Method considerations

For practical implementations of a grating interferometer setup, a number of requirements must be considered. In the following three such considerations are discussed: Beam divergence, transverse coherence and monochromaticity.

Beam divergence

When using a diverging beam, the gratings must be designed to take the magnification into account. Both the Talbot distances and the period of the pattern will change. If L_1 is taken as the distance from the source to G1, and G2 is placed a distance L_2 from G1 (corresponding to a fractional Talbot distance d_T), the magnification M_g is

$$M_g = \frac{L_1 + L_2}{L_1} = \frac{L_1 + d_T}{L_1} \quad (2.25)$$

This magnification leads to the magnified Talbot distance d_T^* and magnified period of G2 g_2^* (Engelhardt et al., 2007).

$$d_T^* = M_g d_T = \frac{L_1 + d_T}{L_1} d_T \quad (2.26)$$

$$g_2^* = M_g g_2 = \frac{L_1 + d_T}{L_1} g_2 \quad (2.27)$$

Thus, for a setup with a large beam divergence a specific source-to-G1 distance L and inter-grating distance d apply for a set of gratings. Thus new gratings are required if a change in Talbot distance is needed. This is the case for commercial and lab-based setups. At synchrotrons, the beam is almost parallel, and the inter-grating distance can be varied.

Transverse coherence

As the grating interferometer relies on the Talbot effect, the incoming beam must have a large enough transverse coherence length for the pattern revival to occur. However, since the gratings are only periodic in one direction, coherence is only required in this direction.

One common definition of the transverse coherence length is (Weitkamp et al., 2006)

$$\xi_t = \frac{\lambda L_1}{s}, \quad (2.28)$$

where L_1 is the distance from the source to G1, λ is the wavelength and s is the source size. It can be shown (David et al., 2002; Weitkamp et al., 2006) that the minimum coherence length required for the n th fractional Talbot distance (with a G1 with a phase-shift of π) is

$$\xi_t > n \frac{g_1}{2}, \quad (2.29)$$

By inserting this into (2.28) we can express the lower bound of the coherence length as a limit on the source size of

$$s < \frac{2\lambda L_1}{n g_1} = \frac{L_1 g_2}{2 d_T}, \quad (2.30)$$

where in the second step equation (2.16) for the expression for the n th fractional Talbot distance has been used, and $g_1 = 2g_2$ (when assuming no magnification) has been inserted.

At a synchrotron with $L = 100$ m. $g_2 = 2$ μm and $d = 4.5$ cm for the first Talbot length, this gives a limit $s < 2$ mm. This should be compared to a lab-based setup with $L = 1.5$ m where the limit becomes $s < 33$ μm . Since a typical source size is 1mm for a lab-source, the source size for lab-based setups must be reduced to use the grating interferometer.

One way is to use a micro-focus source with a sufficiently small source size (Engelhardt et al., 2007). However, the flux of such a setup is rather limited. Another approach is to introduce another absorption grating G0 where the individual grating lines adds constructively to the intensity (Pfeiffer et al., 2006). The period g_0 of this source grating should be (Weitkamp et al., 2006)

$$g_0 = g_2 \frac{L_1}{d_T}, \quad (2.31)$$

As the width of the grating lines have to fulfill equation (2.30), the duty cycle should be less than 0.5.

Monochromaticity

A monochromatic beam is characterized by a narrow spectral bandwidth of the beam. This is important for the longitudinal coherence length ξ_l which for a Lorentzian spectrum is given by (Veen and Pfeiffer, 2004)

$$\xi_l \simeq \frac{2}{\pi} \frac{\lambda^2}{\Delta\lambda} \quad (2.32)$$

where $\lambda/\Delta\lambda$ is the fractional bandwidth of the beam.

The grating interferometer depends on the spectrum of the X-ray beam in two ways: The fractional Talbot distances $d_T^{(n)}$ from equation (2.16) and the grating height h for ensuring the phase-shift $\Delta\Phi = \delta kh$ of G1. For a synchrotron source using a monochromator, the X-ray beam is approximately monochromatic, and no further assumptions are necessary.

In contrast, when using a lab-based or commercial source, the obtainable visibility will be affected. For a polychromatic spectrum, a range of wavelengths will not match the design fractional Talbot distance. As seen in figure 2.3, an unmatched position in the Talbot carpet will divert from the square form as for the purple and orange curve. In addition, an unmatched phase-shift will also lead to a distortion of the interference pattern. Both effects tend to reduce the overall visibility. Despite these considerations, the grating interferometer setup has no limitations on source bandwidth in general (Engelhardt et al., 2008). Thus, the grating interferometer can also be installed at a lab-based source albeit at lower visibility.

2.3 X-ray ptychography

X-ray Ptychography is a type of coherent diffractive imaging (CDI) which allows for phase-contrast imaging at the nanoscale. As all CDI techniques, ptychography operates in the Fraunhofer regime and relies on phase-retrieval from coherent diffraction patterns. Opposite to the full-field imaging in grating-based imaging, ptychography is a scanning technique. The coherent X-ray illumination is shaped into a small probe, and a set of diffraction patterns are obtained by scanning across the sample in a partially overlapping pattern. As a scanning technique, the FoV is determined by the scanning area, and as a CDI technique the spatial resolution κ_s is restricted by the maximum momentum transfer $q_{\max} = 2\pi/\kappa_s$ (Veen and Pfeiffer, 2004).

In the following, the principle of ptychography is introduced. A discussion on method considerations is included as well. For more on ptychography see also Pedersen (2015) which has been a source of inspiration.

2.3.1 Principle of ptychography

The setup used in ptychography is illustrated in figure 2.7. A narrow coherent X-ray beam is incident upon a sample with complex refractive index $n(\mathbf{r})$. Prior to reaching the sample, the beam can be described by the probe function $P(\mathbf{r})$. When traveling through the sample, the X-rays will interact with the object via the refractive index as described in section 2.1.1. We will denote this interaction by the object function $O(\mathbf{r}) = \exp(i \int (n(\mathbf{r}) - 1) \mathbf{k} \cdot d\mathbf{r}) = \exp(ik \int -\delta(\mathbf{r}) + i\beta(\mathbf{r}) dz)$ where the integral is taken over the sample thickness in the beam direction $\hat{\mathbf{z}}$.

As the beam leaves the object, $P(\mathbf{r})$ has changed to the exit field $E(\mathbf{r})$. In ptychography, the exit field is simply described as the product of the probe and object functions (Rodenburg and Faulkner, 2004):

$$P(\mathbf{r})O(\mathbf{r}) = E(\mathbf{r}) \quad (2.33)$$

Then, the exit field $E(\mathbf{r})$ propagates towards the detector where it forms the diffraction space wave function $D(\mathbf{k})$. Here $\mathbf{k}$ is the reciprocal space position. For a fully coherent beam, the two are related by a Fraunhofer Fourier propagator (Nugent, 2010) and $D(\mathbf{k})$ is simply the Fourier transform of $E(\mathbf{r})$

$$D(\mathbf{k}) = \mathcal{F}[E(\mathbf{r})] = \mathcal{F}[P(\mathbf{r})O(\mathbf{r})] \quad (2.34)$$

At the detector, the intensity of the diffraction pattern $I(\mathbf{r}) = |D(\mathbf{k})|^2 = |\mathcal{F}[P(\mathbf{r})O(\mathbf{r})]|^2$ is measured. The task in ptychography is then to reconstruct

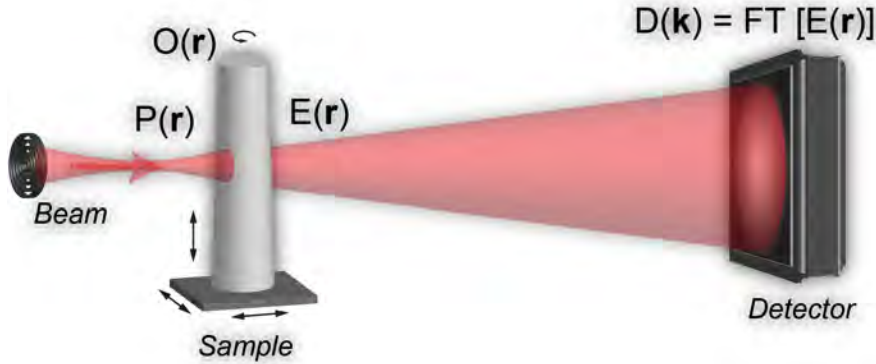


Figure 2.7: Ptychography setup. A coherent X-ray beam described by the probe function $P(\mathbf{r})$ is incident on a sample with object function $O(\mathbf{r})$. When traveling through the sample, $P(\mathbf{r})$ is transformed to the exit field $E(\mathbf{r})$. The exit field propagates towards the detector where it forms the diffraction pattern $D(\mathbf{k}) = \mathcal{F}[E(\mathbf{r})]$. (Adapted from [Pedersen \(2015\)](#).)

the object function $O(\mathbf{r})$ from the measured diffraction patterns. For this, a phase-retrieval method is needed.

2.3.2 Phase-retrieval

Phase-retrieval methods in CDI are typically iterative methods that shifts between real and reciprocal space while applying one or more constraints. For X-ray ptychography, two constraints are used; an overlap and an intensity constraint ([Maiden and Rodenburg, 2009](#); [Rodenburg and Faulkner, 2004](#); [Thibault et al., 2009](#)). The two constraints are illustrated in figure 2.8 and summarized below.

- i) In ptychography a set of multiple overlapping measurements of the sample area is applied. This introduces an object constraint since the same part of object is in view several times with the same probe. Thus the overlap constraint stems from an overdetermined object. The overlapping scanning pattern is illustrated in figure 2.8 a).
- ii) The measured intensity forms a constraint on the modulus of the diffraction wave function $I(\mathbf{k}, \mathbf{r}_j) = |D(\mathbf{k}, \mathbf{r}_j)|^2$.

Several methods for phase-retrieval in ptychography exist. In the following we will give a short introduction to the ptychographic iterative engine (PIE) algorithm. PIE formed the first ptychographic phase-retrieval algorithm. Furthermore, some additional developments are discussed.

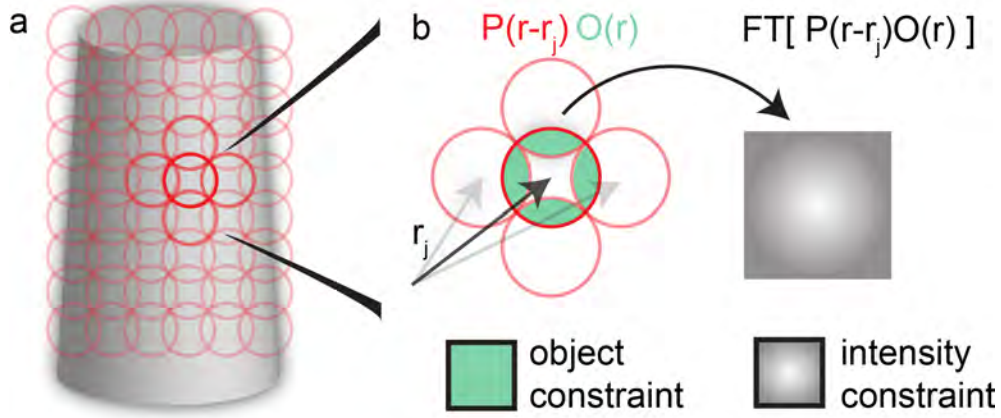


Figure 2.8: Ptychographic scanning and constraints. a) The object is scanned with a series of overlapping exposures. b) Overlapping exposures can be described by moving the probe function $P(\mathbf{r}-\mathbf{r}_j)$ to a set of positions $\mathbf{r}_j$. The overlap supplies an object constraint. In the far field, the intensity $I(\mathbf{k}, \mathbf{r}_j)$ is measured which provides the intensity constraint $I(\mathbf{k}, \mathbf{r}_j) = |\mathcal{F}[P(\mathbf{r}-\mathbf{r}_j)O(\mathbf{r})]|^2$. (Adapted from Pedersen (2015).)

Ptychographic iterative engine

In the PIE algorithm developed by Rodenburg and Faulkner (2004), an iterative approach is used where constraints are applied in turn for each probe position. By continuously cycling through all positions one-by-one, the (complex) object function $O(\mathbf{r})$ index is slowly reconstructed. For this, the probe function P is assumed to be known and constant. From the real and imaginary parts of the reconstructed object function, the transmission and phase-contrast radiographs can be retrieved.

Starting from an initial guess g for the object function $O_{g,0}(\mathbf{r})$, the algorithm iteratively improves on the solution. In practice the initial guess can be a 2D matrix of random complex numbers representing the real and imaginary part of the refractive index of the object. Denoting the object function for the n th iteration $O_{g,n}$ we will consider the iterative procedure from step n to $n+1$.

1. By multiplying the current guessed object function by the known probe function at position $\mathbf{r}_j$, the guessed exit wave function for position $\mathbf{r}_j$ is produced.

$$E_{g,n}(\mathbf{r}, \mathbf{r}_j) = P(\mathbf{r}-\mathbf{r}_j)O_{g,n}(\mathbf{r}) \quad (2.35)$$

2. Calculate the corresponding guessed detector field by a Fourier transform to reciprocal space:

$$D_{g,n}(\mathbf{k}, \mathbf{r}_j) = \mathcal{F}[E_{g,n}(\mathbf{r}, \mathbf{r}_j)] = |D_{g,n}(\mathbf{k}, \mathbf{r}_j)|e^{i\theta_{g,n}(\mathbf{k}, \mathbf{r}_j)} \quad (2.36)$$

where $\mathbf{k}$ is reciprocal space position, and in the last step $D_{g,n}$ is written as an amplitude and (guessed) phase $\theta_{g,n}$

3. Use the measured intensities $I(\mathbf{k}, \mathbf{r}_j)$ to correct the detector field amplitude:

$$D_{c,n}(\mathbf{k}, \mathbf{r}_j) = \left| \sqrt{I(\mathbf{k}, \mathbf{r}_j)} \right| e^{i\theta_{g,n}(\mathbf{k}, \mathbf{r}_j)} \quad (2.37)$$

4. Inverse Fourier transform back to real space to obtain a new (and improved) guess for the exit wave function

$$E_{c,n}(\mathbf{r}, \mathbf{r}_j) = \mathcal{F}^{-1}[D_{c,n}(\mathbf{k}, \mathbf{r}_j)] \quad (2.38)$$

5. Update the object function for step $n+1$ using the guessed object function and guessed and corrected exit functions:

$$O_{g,n+1}(\mathbf{r}) = O_{g,n}(\mathbf{r}) + \frac{|P(\mathbf{r} - \mathbf{r}_j)|}{|P_{\max}(\mathbf{r} - \mathbf{r}_j)|} \times \frac{P^*(\mathbf{r} - \mathbf{r}_j)}{|P(\mathbf{r} - \mathbf{r}_j)|^2 + \alpha_P} \times \beta_P \{E_{c,n}(\mathbf{r}, \mathbf{r}_j) - E_{g,n}(\mathbf{r}, \mathbf{r}_j)\} \quad (2.39)$$

where P^* is the complex conjugate of the probe, and $|P_{\max}(\mathbf{r} - \mathbf{r}_j)|$ is the maximum value of the probe amplitude, and the parameters α_P and β_P are chosen appropriately.

6. Move to a position $\mathbf{r}_{j+1}$ where the probe overlaps with the previous position. Repeat step 1-6 until convergence.

As mentioned, measurement constraints are essential for a successful phase-retrieval. In the PIE algorithm, the intensity constraint is applied in step 3 to correct the detector field amplitude, and the overlap constraint is ensured in step 1 and 6 by moving to an overlapping probe position.

The crucial step of the PIE algorithm is the update of the object function in step 5. The last term of equation (2.39) updates regions at the current probe position where the corrected and guessed exit waves differ. The amount of update feedback is weighted by the parameter β_P and by the relative probe strength $|P(\mathbf{r} - \mathbf{r}_j)|/|P_{\max}(\mathbf{r} - \mathbf{r}_j)|$. The parameter α_P is included to avoid division by zero if $|P_{\max}| = 0$. Typically α_P is a small number i.e. $\alpha_P = 10^{-4}$, and β_P is in the range $0.5 - 1$ (Rodenburg and Faulkner, 2004).

The convergence is monitored through the sum squared error (SSE) which is the deviation of the guessed detector field amplitudes from the measured intensities

$$SSE = \frac{\{|D(\mathbf{k}, \mathbf{r}_j)|^2 - |D_{g,n}(\mathbf{k}, \mathbf{r}_j)|^2\}^2}{N} \quad (2.40)$$

where N is the number of pixels of the reconstructed FoV.

One of the strengths of the PIE algorithm is its apparent simplicity. In each step a single update is carried out at a single probe position. However, the requirement of a known probe limits the applicability of the method.

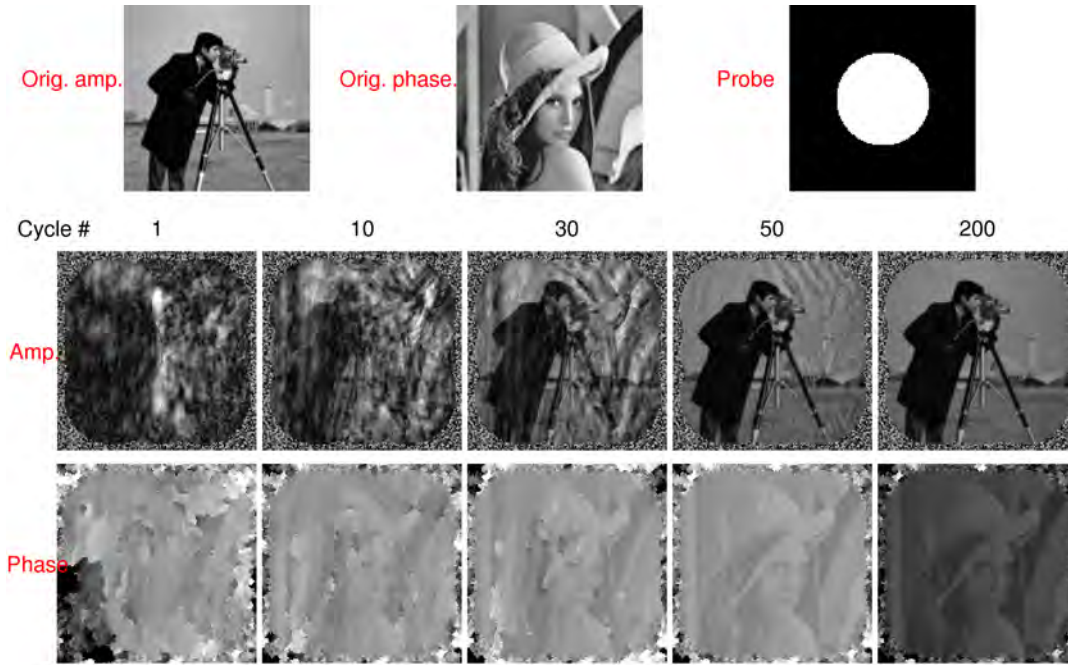


Figure 2.9: PIE algorithm. The top row displays the amplitude and phase of the object function as well as the probe function used. In the two other rows, the reconstructed amplitude (middle row) and phase (bottom row) are shown after 1, 10, 30, 50 and 200 cycles of the probe positions in the PIE algorithm.

An example of the PIE algorithm is illustrated in figure 2.9 using an implementation in NumPy by [Thibault \(2012\)](#). Two images are used as the amplitude and phase of the object function as shown in the top row. In the bottom two rows, the reconstructed amplitude (middle row) and phase (bottom row) of the PIE algorithm are shown after a number of cycles of probe positions. A circular probe was used as seen in the top right part of the image.

Further developments

An improvement on the original PIE is the extended ptychographic iterative engine (ePIE) ([Maiden and Rodenburg, 2009](#)). By introducing a second update step for the probe function, the probe can be reconstructed as part of the algorithm. Thus, the requirement of a known probe is removed. In addition rather than moving to a neighboring probe position in each step, the algorithm uses a random sequence of positions for the probe.

Another way to improve on the PIE algorithm would be to update all probe positions at the same time. This is the idea of the ptychographic difference map (PDM) approach by [Thibault et al. \(2008, 2009\)](#). In this approach, the exit wave fields are collected into a single state vector. In the high-dimension Euclidean space occupied by this vector, the intensity and overlap constraints are applied as distance-minimizing projections ([Thibault et al., 2009](#)). This results in a more compact formulation, and as with ePIE the probe can be reconstructed as part of the algorithm. An issue with PDM is that a periodic scanning pattern can resolve in a set of non-unique solutions. More irregular scanning schemes such as the Fermat spiral ([Huang et al., 2014](#)) can help to overcome this issue.

More recently, X-ray ptychography has been extended to tomographic studies ([Dierolf et al., 2010](#)). This presents the additional challenge of ensuring alignment of angular projections on the nano-scale. To overcome this, a method for post-measurement refinement has been proposed ([Guizar-Sicairos et al., 2011](#)).

2.3.3 Method considerations

X-ray ptychography is a demanding technique in terms of setup requirements. Thus, the technique has only been implemented at synchrotron facilities. We here briefly touch upon a number of requirements: Sample thickness, transverse coherence, monochromaticity and probe position.

Sample thickness

Since the simple factorization in equation (2.33) assumes an (optically) thin object ([Rodenburg and Faulkner, 2004](#)), ptychography sets a condition on the sample size. While most 2D projection studies consider thin film samples where this is easily fulfilled, thicker objects are imaged in tomography studies. In the supporting material of their paper, [Thibault et al. \(2008\)](#) investigate the validity of equation (2.33) for extended objects. They find that the object thickness must be kept within $Z = \kappa_s a_P / \lambda$ where κ_s is the spatial resolution and a_P is the extent of the probe at the object.

For typical experimental conditions, [Thibault et al. \(2008\)](#) found a maximum object thickness Z of 50 μm . The typical size of objects imaged in ptychography are a few tens of μm which falls within the allowed range. In addition, note that improvements in resolution will tend to increase the maximum allowed thickness.

In order to extend ptychography to even thicker samples, the multislice ePIE (3PIE) algorithm ([Maiden et al., 2012](#)) has recently been proposed. Using this approach, a reconstruction of two layers placed with a 105 μm gap between

them was demonstrated experimentally (Suzuki et al., 2014). Thus, new methods within X-ray ptychography may help to overcome the restrictions on sample thickness

Transverse coherence

A transverse coherent beam is a requirement for X-ray ptychography. As with CDI techniques in general, full transverse coherence ensures coherent diffraction patterns (Nugent, 2010) which allows for phase-retrieval (Veen and Pfeiffer, 2004). In lack of a fully coherent beam, partial coherence can lead to artifacts in final ptychographic reconstructions (Rodenburg et al., 2007). A way to deal with partial coherence effects is to expand the illumination into several modes by spectral decomposition (Thibault and Menzel, 2013)

To ensure transverse coherence, the beam must be shaped which can reduce the flux by several magnitudes at e.g. third generation synchrotron sources (Thibault and Menzel, 2013). In this regard, the advent of fourth generation synchrotron facilities poses a great potential for ptychography. Since these diffraction limited sources provide a major improvement in coherence, an increase in both quantity and quality in ptychographic measurements can be expected (Thibault et al., 2014)

Monochromaticity

X-ray ptychography in line with other CDI techniques requires a monochromatic beam. In case of a broad energy band, the diffraction patterns will be smeared, and the information necessary for phase-retrieval will be lost.

To resolve an object of size a_o with a spatial resolution κ_s , following restriction applies for the bandwidth when using CDI techniques (Veen and Pfeiffer, 2004)

$$\frac{\lambda}{\Delta\lambda} < \frac{\kappa_s}{a_o} \quad (2.41)$$

For an object of size $a_o = 10 \text{ } \mu\text{m}$ and a spatial resolution of $\kappa_s = 10 \text{ nm}$, the bandwidth should be kept below $\lambda/\Delta\lambda = 1 \times 10^{-3}$ using a crystal monochromator.

Probe position

In the ptychographic reconstruction algorithms such as PIE, the probe position is assumed known for each diffraction pattern. Uncertainties in probe position caused by e.g. vibrations and mechanical instabilities will induce errors in the

final reconstruction (Faulkner and Rodenburg, 2005). Thus, an accurate determination of the probe position is crucial for improvements in spatial resolution especially for tomographic studies (Holler et al., 2012).

One approach for improving the precision in probe position with respect to the beam-defining optics is to use laser interferometry (Holler et al., 2012). By using a laser interferometric feedback for probe position, a 3D spatial resolution of record 16 nm was recently demonstrated (Holler et al., 2014).

2.4 Setup parameters

Above the methods of grating interferometry and ptychography were introduced. In the experiments presented in this thesis, these two methods were applied using a number of different experimental setups. Both lab-based setups as well as beam-line instruments at third generation synchrotrons were used. For an overview, key experimental parameters for the setups of papers I-V are listed in table 2.1.

A key parameter to note is the spatial resolution. As shown in table 2.1, the spatial resolution in the experiments spanned three orders of magnitude. The X-ray imaging techniques used range from the nano-scale with 300 nm in Paper IV to the macro-scale with 0.5 mm in Paper I and Paper II. Novel X-ray imaging techniques can be applied to a broad range of investigations from microstructure to macro-scale objects.

	HCØ lab		TOMCAT		cSAXS
Paper	I	II	III	V	IV
Energy [keV]	28	28	25	25	6.2
Monochromator	No	No	Yes	Yes	Yes
Prop. dist. [mm]	120	200	124	124	7225
FoV (h,v) [mm ²]	30 × 56	27 × 47	12.7 × 3.8	12.7 × 3.8	0.04 × 0.03
Resolution [µm]	500	500	22.5	22.5	0.3
	Gratings				
Talbot order	5th	5th	3rd	3rd	NA
Talbot dist [cm]	20	20	12.4	12.4	NA
g_1 [µm]	3.5	3.5	3.98	3.98	NA
g_2 [µm]	2.0	2.0	2.0	2.0	NA

Table 2.1: List of experimental parameters for X-ray experiments in papers I-V. Grating interferometry is used in papers I, II, III and V while paper IV utilizes X-ray ptychography. The resolution was estimated from the resulting tomograms.

Chapter 3

Taking the image - the X-ray dark-field modality

In an imaging experiment, one of the first steps involves acquiring i.e. *taking* the image. For this, a physical contrast mechanism is needed to form the image features. In chapter 2, three such mechanisms were introduced; attenuation (absorption), refraction and scattering. The degree of interaction between the X-ray beam and an object will determine the gray level pixel values of the final image. Thus, the discernible features in an image are determined through the contrast i.e. the difference in gray level values. In this chapter, the focus will be contrast formation when *taking* the image. The novel X-ray dark-field image modality based on scattering contrast will be used as an example. In addition, the chapter functions as an introduction to [Paper I](#) ([Nielsen et al., 2014](#)) and [Paper II](#) ([Nielsen et al., under review 2015a](#)).

When a new X-ray image modality is introduced, a great effort lies in understanding and interpreting the image contrast and how it relates to an observed material. In X-ray dark-field imaging, the image contrast is formed by the loss of coherence of the X-ray beam due to internal microstructures and interfaces of the material ([Pfeiffer et al., 2008](#)). This loss of coherence can be due to X-ray scattering from sub-resolution microstructures in the material ([Lynch et al., 2011](#); [Yashiro et al., 2010](#)) or due to strong refraction from unresolvable edges ([Yashiro and Momose, 2015](#)). The former case has been ascribed to (ultra) small-angle X-ray scattering and is caused by micron-sized structures in the material. Although advances have been made in recent years, the theoretical framework for dark-field imaging is still undergoing development. At this point, a quantitative description of the signal can only be made in a few cases.

One of the appealing qualities of X-ray dark-field imaging is the complementarity to conventional X-ray imaging. While conventional X-ray attenuation only

depends on the elemental composition of a material, the scattering of dark-field imaging also depends on the microstructure. This complementarity has been utilized for studies within several fields. For medical applications, combined X-ray attenuation and dark-field imaging have been applied for e.g. mammography (Michel et al., 2013; Stampanoni et al., 2011), non-invasive differentiation of kidney stones (Scherer et al., 2015) and investigations of pulmonary emphysema in lungs (Meinel et al., 2013; Schleede et al., 2012; Yaroshenko et al., 2013). In food science the complementary image modalities have been applied for detection of foreign bodies (Kottler et al., 2010; Nielsen et al., 2013).

In this chapter, the complementary contrast modality of dark-field imaging will be illustrated. Furthermore, possible applications of X-ray dark-field radiography are presented.

3.1 Dark-field radiography for foreign body detection

In order to highlight the complementarity of the X-ray dark-field and attenuation modalities, an example of a possible application is given in the case of foreign body (FB) detection where we will follow the work¹ of Nielsen et al. (2013).

In modern food production automatic online screening for FBs using e.g. conventional X-ray scanners are an important part of quality assurance. However, even though conventional X-ray attenuation is able to detect heavy FBs such as glass, stones and metals, many soft material and organic FBs are difficult if not impossible to detect. To overcome this challenge, X-ray dark-field imaging could be employed for detection of soft material FBs with internal microstructures.

Figure 3.1 displays (a) a transmission and (b) a dark-field radiograph of a piece of minced beef meat with three FBs inserted. The FBs are from left to right: A piece of glass, four layers of paper and a ladybug. Whereas the glass (blue square) in panel a) has a stronger attenuation signal compared to the minced meat (yellow squares), there is no visible difference between the minced meat and the paper (green square) or ladybug (red squares). In the dark-field radiograph in panel b), both the paper and ladybug give a stronger scattering signal than the minced meat. In contrast, only the edges of the glass can be seen in panel b).

This can be explained by the structure of the three FBs. In contrast to the glass, both the paper and exoskeleton of the ladybug contain microstructures which give rise to the X-ray scattering detected in the dark-field signal. This signal creates a contrast to the minced meat where the X-ray scattering is less

¹This work was done as part of the MSc thesis of Mikkel Schou Nielsen.

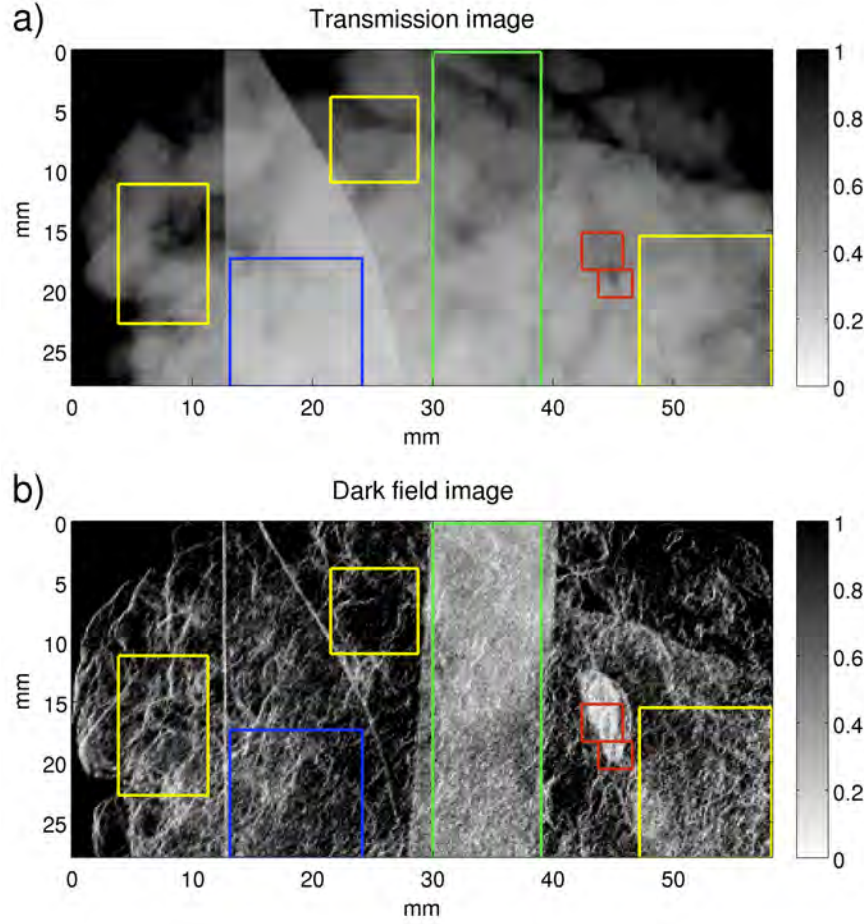


Figure 3.1: X-ray images of minced beef meat with three FBs; glass (left), 4 layers of paper (middle) and a ladybug (right). a) Transmission radiograph b) Dark-field radiograph.

pronounced. On the other hand, the glass contains Si ($Z=14$) which has a stronger attenuation than the organic materials in the minced meat, paper and ladybug. In this way, the attenuation and dark-field modalities display complementary contrast properties.

To quantify the contrast between FB and food matrix in the transmission and dark-field modalities, an image contrast measure is introduced in the form of a contrast-to-noise ratio (CNR). We define the CNR between two region-of-interests (ROI) A and B as (Song et al., 2004)

$$\text{CNR} = \frac{|\mu_A - \mu_B|}{\sqrt{w_A \sigma_A^2 + w_B \sigma_B^2}} \quad (3.1)$$

where μ_j denotes the mean and σ_j^2 the variance of the pixel values in the j th

ROI. Since the area of the two ROIs may differ, σ_j^2 is weighted with the factor w_j which is the ratio of the number of pixels in the j th ROI relative to the total number of pixels in both ROIs. From this definition, the CNR values are positive numbers with a lower bound of zero in the case of no difference in μ_A and μ_B . The CNR values have no upper bound and may tend towards infinite in the hypothetical case of zero variance and non-zero difference of μ_A and μ_B .

We illustrate the CNR measure by calculating CNR values between the FBs and minced meat in figure 3.1 using the regions indicated by colored squares. As seen from table 3.1, these calculated values confirm the qualitative observations made above. Whereas the transmission CNR is high for the glass and low for the paper and ladybug, the dark-field CNR is low for glass and high for paper and ladybug.

Material	Transmission CNR	Dark-field CNR	w_{FB}
Glass	2.3	0.0	0.30
4 sheets of paper	0.1	1.8	0.48
Ladybug	0.5	1.9	0.06

Table 3.1: Transmission and dark-field CNR values for foreign bodies in minced meat.

In summary, complementary contrast mechanisms may be combined to improve image detection systems. Even though our observations here have been rather simple, they illustrate how the physical contrast mechanism determines what kind of objects will be observed. Thus, the combination of X-ray dark-field and conventional transmission could be a way to increase the sensitivity of FB detection using X-ray scanning systems. Rather than improving the detection of existing materials, a range of additional materials could be detected by including a scattering-sensitive X-ray modality.

3.2 Structural sensitivity of the dark-field signal

As has been stated previously and illustrated qualitatively in the previous section, the dark-field modality is sensitive towards scattering from microstructures. Still, a quantitative relation between the size of structures and the dark-field signal is missing. This structural sensitivity is relevant when optimizing the design of an imaging setup. However, for this a theoretical framework is needed.

So far, the theoretical investigations have mostly considered scattering from disordered microstructures - e.g. diluted microspheres in a matrix material (Lynch et al., 2011; Yashiro et al., 2010). For ordered structures, quantitative

theoretical studies are still missing. Since fibrous structures such as bone, wood and reinforced carbon fibers have been proposed as possible applications for X-ray dark-field imaging, these could strongly benefit from a deeper theoretical understanding. Furthermore, in general an extension of the framework to polychromatic sources remains to be done. This would be required for practical applications using commercial scanners.

In the following ordered structures will be omitted, and only the dark-field attenuation coefficient $\mu_{DF}(x, y, z)$ from disordered microstructures will be considered. In accordance with [Yashiro et al. \(2010\)](#), [Strobl \(2014\)](#) in a general description divides μ_{DF} into a macroscopic scattering cross section Σ and a (real-space) correlation function $\mathcal{G}$

$$\mu_{DF}(x, y, z) = \Sigma(x, y, z) (1 - G(x, y, z, d_{GI})) , \quad (3.2)$$

where $\mathcal{G}$ depends on the geometry of the sample particles and the auto-correlation distance d_{GI} of the grating interferometer. The beam propagates in the z -direction. The latter parameter d_{GI} gives a measure for the size sensitivity of the interferometer which is related to the geometry through:

$$d_{GI} = \frac{L_s \lambda}{g_2} \quad (3.3)$$

where λ is the wavelength, g_2 the grating period of G2, and L_s is the distance z from the sample to G2 when the sample is placed between G1 and G2. In the case where the sample is placed between G0 and G1, L_s is given by $L'_s = L_2 \times (L_1 + L_2 - L_s) / L_1$ ([Strobl, 2014](#)) where L_1 is the distance between G0 and G1 and L_2 is the distance between G1 and G2. In a parallel beam geometry without a source grating G0, $\lim_{L_1 \rightarrow \infty} L'_s = L_2$ which corresponds to the fractional Talbot distance d_T of the grating interferometer.

In order to be applicable, the general formulation of equation (3.2) must be made more explicit. According to [Yashiro et al. \(2010\)](#), the scattering cross section Σ for disordered particles is proportional to λ^2 , the electron density difference $|\Delta\rho_e|^2$ and the particle volume fraction η . In addition, they propose an approximation for the autocorrelation function

$$G(x, y, z, d_{GI}) \simeq \exp \left[-(d_{GI}/\xi(x, y, z))^{2H} \right] , \quad (3.4)$$

where ξ and H are parameters to be empirically determined in each case by measuring μ_{DF} for different values of d_{GI} . Although this approximation gives some insight into the microstructure of the sample, the applicability is limited since an empirical determination must be made for each sample.

For a sample of diluted monodisperse spheres with radius r , an analytical expression for Σ and $\mathcal{G}$ has been found ([Lynch et al., 2011](#); [Strobl, 2014](#); [Yashiro](#)

et al., 2010). Using $\zeta = d_{GI}/(2r)$, this is given as² (Lynch et al., 2011)

$$\Sigma = \frac{3\pi^2}{2} \lambda^2 \eta |\Delta\rho_e|^2 r \quad (3.5)$$

$$\mathcal{G}(\zeta) = \begin{cases} \sqrt{1-\zeta^2} \left(1 + \frac{\zeta^2}{2}\right) + \zeta^2 \left(1 - \frac{\zeta^2}{4}\right) \ln \left(\frac{1-\sqrt{1-\zeta^2}}{1+\sqrt{1-\zeta^2}} \right) & \text{for } d_{GI} < 2r \\ 0 & \text{for } d_{GI} \geq 2r \end{cases} \quad (3.6)$$

This expression has been verified both experimentally using monodisperse silica microspheres in water (Lynch et al., 2011) and in simulations (Malecki et al., 2012). In addition, a dark-field contrast agent using microbubbles has been investigated using the above formalism (Velroyen et al., 2013).

The general size-dependent shape of μ_{DF} from equation (3.5) is shown in figure 3.2. The values of μ_{DF} has been normalized by the maximum value, and plotted versus the particle diameter $2r$ in units of d_{GI} on a log-scale. The curve displays a single peak, and trails off towards zero for both small and large particle sizes.

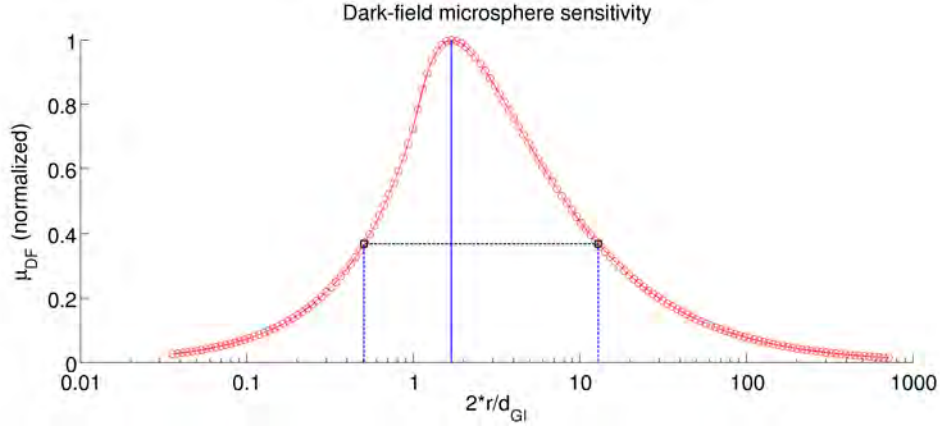


Figure 3.2: Illustration of size-dependence of the dark-field signal. Curve on semi-log plot of normalized $\mu_{DF} = \Sigma(1 - \mathcal{G})$ using the expressions from (3.5) is shown in red. The values of μ_{DF} are normalized by the maximum value, and the particle diameter $2r$ in units of d_{GI} . Positions of maximum value as well as $1/e$ of maximum are indicated in blue lines.

The position for the maximum value of the dark-field signal is found to be at $2r \simeq 1.8 d_{GI}$. At $2r \simeq 0.5 d_{GI}$ and $13 d_{GI}$, the value of μ_{DF} is reduced to $1/e$ of the maximum value. Thus, as a measure for the size-sensitivity of the dark-field signal, this size-range will be used. For a sample containing particles with

²In the literature, the logarithmic factor is given in two ways. Here the formulation by (Lynch et al., 2011) is used where the result from (Strobl, 2014; Yashiro et al., 2010) is $\ln \left(\zeta / (1 + \sqrt{1 - \zeta^2}) \right)$.

a broad size-distribution, the particles with diameter between $0.5 d_{GI}$ and $13 d_{GI}$ will contribute the most. However, a sample only containing particles outside this range might still give a measurable dark-field signal. Therefore it is not possible to uniquely determine the sizes from the dark-field signal. The curve indicates that even rather large particles result in a dark-field signal.

In order to illustrate the dependence on wavelength and setup geometry, values for μ_{DF} for a specific sample and setup is shown in figure 3.3. The values for μ_{DF} are again plotted versus the sphere diameter on a log-scale. The sample is assumed to be a 10 vol% solution of starch granules in water placed between G1 and G2 with a grating period $g_2 = 2 \mu\text{m}$.

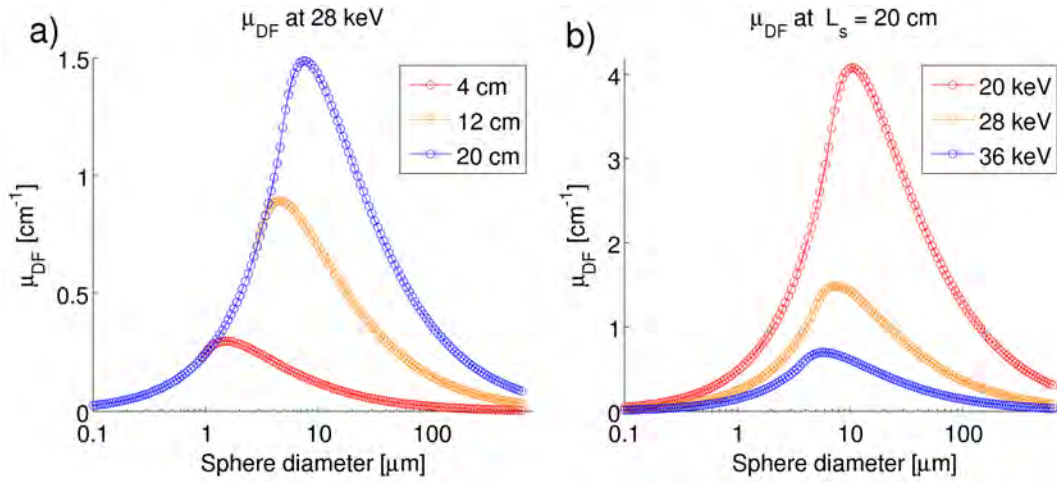


Figure 3.3: Illustration of dark-field dependence on photon energy and L_s distance. The plots show μ_{DF} values for a 10 vol% solution of starch granules of various sizes in water. a) μ_{DF} values at 28 keV photon energy for $L_s \in \{4 \text{ cm}; 12 \text{ cm}; 20 \text{ cm}\}$. b) μ_{DF} values for $L_s = 20 \text{ cm}$ and a photon energy of 20 keV, 28 keV or 36 keV.

In figure 3.3 a), the dependence on the sample-to-detector distance L_s is illustrated for three different distances at constant photon energy at 28 keV. As can be seen, the left side of the curve is the same for all distances. When the distance is increased, the maximum position is shifted to the right. At 4 cm (20 cm) distance, the dark-field signal is sensitive for approximately $0.5 - 12 \mu\text{m}$ ($2 - 60 \mu\text{m}$) diameter spheres. In effect L_s functions as a cut-off level for the sensitivity. For a focus on a factor K smaller sizes, the distance should be decreased by the same factor.

Furthermore, at larger distances the maximum value for μ_{DF} is seen to increase linearly with L_s . Thus, making the dark-field signal sensitive towards larger structures results in a larger attenuation and therefore a smaller penetration depth. In turn, increasing size sensitivity will restrict the measurements to thin samples.

The wavelength or energy dependence is illustrated in figure 3.3 b) at constant distance $L_s = 20$ cm. Here the μ_{DF} values are seen to decrease for all particle sizes when the photon energy is increased. Thus a decrease in wavelength allows for measuring thicker samples. In addition, the peak position is seen to shift towards smaller sphere diameters for increasing energy, i.e. decreasing wavelength.

Since Σ depends on λ^2 as seen from (3.5), a change in wavelength (and thus photon energy) results in a larger change in μ_{DF} than a change in L_s . As d_{GI} depends linearly on both λ and L_s , a relative change in either will cause the same change to $\mathcal{G}$. Thus, by finding the right combination of λ and L_s , a grating interferometer setup can be optimized for a specific particle diameter $2r$ and sample thickness.

3.3 Introduction to Paper I and II

In the following two sections we summarize the most important points of [Paper I](#) (Nielsen et al., 2014) and [Paper II](#) (Nielsen et al., under review 2015a). Important figures are included. For the full details the reader is referred to Appendix A where the papers are attached in full length.

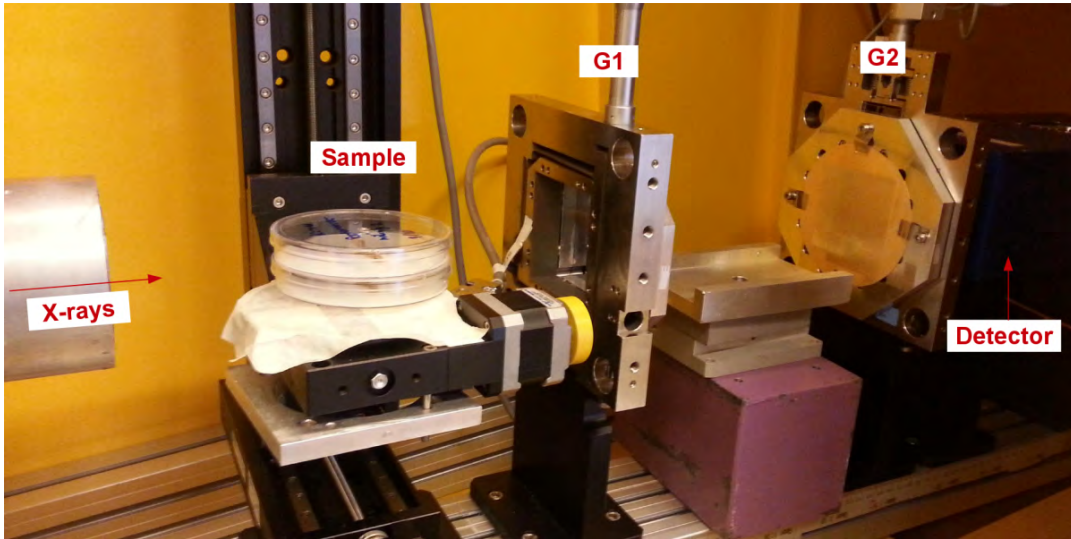


Figure 3.4: The grating interferometer setup at the Niels Bohr Institute. The X-ray beam is incident on the barley seed sample in front of the phase-grating G1. The analyzer grating G2 selects the part of the wavefront which is recorded by the detector. The source grating G0 is placed upstreams of the flight tube near the source (not shown).

Figure 3.4 shows the grating interferometer setup at the Niels Bohr Institute which was used for the experiments in [Paper I](#) and [Paper II](#). From the left through

the flight tube, the X-ray beam is incident on the barley seeds sample studied in [Paper II](#) which is placed in front of the phase-grating G1. Upstreams of the flighttube the source grating G0 (not shown) ensures sufficient transverse coherence of the beam. The analyzer-grating G2 selects which part of the wavefront is recorded in the detector. A nanoconverter motor is used to step G2 in order to scan the intensity pattern. In [Paper I](#), the only modification was that the sample was placed between G1 and G2.

Both in [Paper I](#) and [Paper II](#), the dark-field modality was used to investigate sample microstructures. Thus, the sensitivity range of the dark-field signal is a relevant parameter. However, since the studies were conducted using a rotating anode source at 40 kV acceleration voltage, a broad X-ray spectrum was used and the assumption of a monochromatic beam is not fulfilled. In lack of in-depth studies of the effect of polychromatic sources on μ_{DF} , we propose an approximation by using the grating design energy of 28 keV as an effective energy for the calculations in section 3.2. Using this and the grating period $g_2 = 2 \text{ } \mu\text{m}$, we find a sensitivity range of $1.2 - 36 \text{ } \mu\text{m}$ for $L'_s = 12 \text{ cm}$ in [Paper I](#) and $2 - 52 \text{ } \mu\text{m}$ for $L'_s = 18 \text{ cm}$ in [Paper II](#).

The setup parameters can be found in table 2.1 on page 33, and the acquisition details are listed in the papers in A.

3.3.1 Introduction to Paper I

The title of [Paper I](#) is 'Frozen and defrosted fruit revealed with X-ray dark-field radiography' ([Nielsen et al., 2014](#)). The paper investigates whether X-ray dark-field radiography may be applied to distinguish between the fresh, frozen and defrosted state of fruit. In this proof-of-concept study, two different types of berries and a piece of mandarin were studied.

During growth of fresh fruit and berries, temperatures below freezing point can cause freeze injuries in the crop. The resulting structural changes are unwanted as they influence functionality and may result in a spoiled crop. In e.g. California, sudden drops in temperature during winter is a cause of freeze injuries in the citrus industry. Since California Department of Food and Agriculture regulations do not permit oranges to be sold if more than 15% of fruit in a lot have freeze damage ([usd, 2013](#)), automatic detection of microstructural changes due to freeze injuries could be of industrial relevance. Furthermore, since many crops are frozen post-harvesting, techniques to monitor the microstructural effects of the applied freezing could also be of interest.

Previously, various detection methods have been proposed including magnetic resonance imaging (MRI) ([Gamble, 1994](#); [Hernández-Sánchez et al., 2004](#); [Kim](#)

et al., 2008) as well as ultra-violet (UV) fluorescence and machine vision for surface inspection (Slaughter et al., 2008). However, these methods have fundamental challenges with respect to industrial requirements for high speed inspection capacity and revelation of internal features. In this paper, we propose to apply X-ray dark-field radiography as a possible method to detect microstructural changes due to freezing or defrosting.

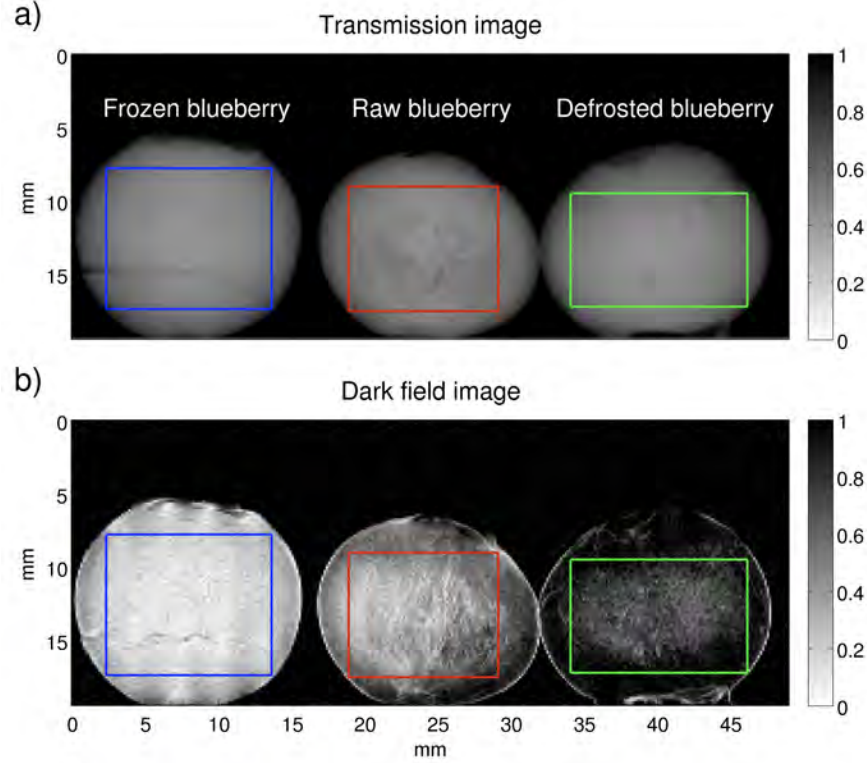


Figure 3.5: Blue berries: X-ray images of blue berries in different states; frozen (left), raw (middle) and defrosted (right). a) Transmission radiograph b) Dark-field radiograph.

Figure 3.5 shows an example of the results for the blueberries. From left to right, a frozen (blue square), raw (green square) and defrosted (red square) blueberry are shown in both transmission, panel a), and dark-field, panel b), radiography. As seen from the transmission radiograph in panel a), there is no difference in attenuation of X-rays in blueberries whether they are frozen, raw or defrosted. However, in comparison a large difference in scattering signal is observed in the dark-field radiograph in panel b). A higher signal is seen for the frozen than for the raw blueberry whereas a reduced signal is seen for the defrosted compared to the raw blueberry. Thus, freezing causes an increase in the scattering from the microstructures in the blueberry which may arise due to formation of ice crystals. Furthermore, the decrease in signal for the defrosted berry may be interpreted as a disruption of the blueberry microstructure following

Sample	Transmission CNR	Dark-field CNR	w_F
Blueberry, frozen	0.2	2.3	0.55
Blueberry, defrosted	0.1	2.9	0.52

Table 3.2: Transmission and dark-field CNR values of frozen and defrosted samples compared to raw sample.

the freeze-thaw treatment.

These observations are confirmed by CNR values comparing the frozen or defrosted berry to the raw as seen in table 3.2. Almost no contrast is found for the transmission CNR with values close to 0, while the dark-field CNR is above 2 for both frozen and defrosted. It is interesting to note that both the higher dark-field signal of the frozen berry as well as the reduced signal for the defrosted berry result in a high dark-field CNR.

The results highlight the complementarity between the absorption and dark-field modality and the potential for using dark-field radiography in food science. The observed contrast due to freezing indicate a potential to apply dark-field radiography to monitor the changes while the product is being frozen. This could also involve studying different types of freezing as only rapid freezing was used in this paper. Since the measurements in the study were performed using a lab-based X-ray grating interferometer setup, these results demonstrate a potential for using X-ray dark-field radiography in industrial applications.

3.3.2 Introduction to Paper II

The title of [Paper II](#) is 'Quantitative in-situ monitoring of germinating barley seeds using X-ray dark-field radiography' ([Nielsen et al., under review 2015a](#)). In this pilot study, X-ray dark-field radiography was used to monitor microstructural changes relating to water uptake and degradation of starch granules in barley seeds. A time-resolved study of twenty germinating seeds over 43-55 h was performed.

In the production of malt, water uptake in barley seeds is followed by enzymatic degradation of the barley endosperm during germination. The degradation process, known as modification, causes structural changes in the barley endosperm as cell walls, starch granules and the surrounding protein matrix are partially hydrolyzed by enzymes. A sketch of a section through a barley seed can be seen in figure 3.6. The degradation initiates in the scutellum end of the seed. Ideally, the front then advances in parallel to the scutellar face ([Briggs, 2002](#); [Briggs and Macdonald, 1983](#); [Gianinetti, 2009](#)). This has also been confirmed by theoretical enzyme kinetic models which also predict that the front will move at a constant speed ([Fowkes and O'Brien, 2010](#); [O'Brien and Fowkes, 2005](#)). However, experimental investigations are needed to verify these results ([Fowkes and O'Brien, 2010](#)).

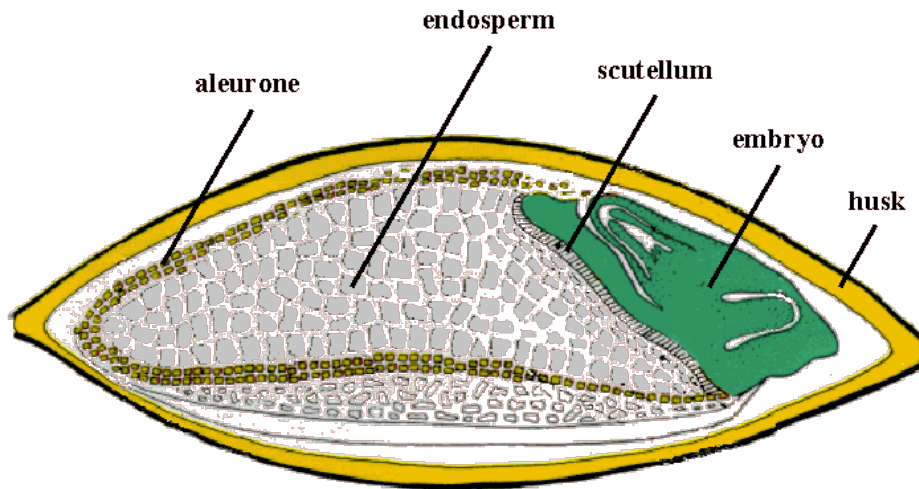


Figure 3.6: An illustration of a cut through a barley seed. The endosperm contains starch granules which are degraded during germination. The endosperm is separated from the embryo by the scutellum. (*Reproduced from [Forskningscenter \(1999\)](#).*)

Although modification patterns have been investigated using microscopy methods ([Aastrup et al., 1981](#); [Brennan et al., 1997](#); [Briggs and Macdonald, 1983](#); [Ferrari et al., 2010](#)), these involve destructive sample preparation as the barley seeds need to be halved through e.g. use of sand paper. Accordingly, in-situ monitoring in a single seed cannot be done using microscopy. Instead a non-destructive

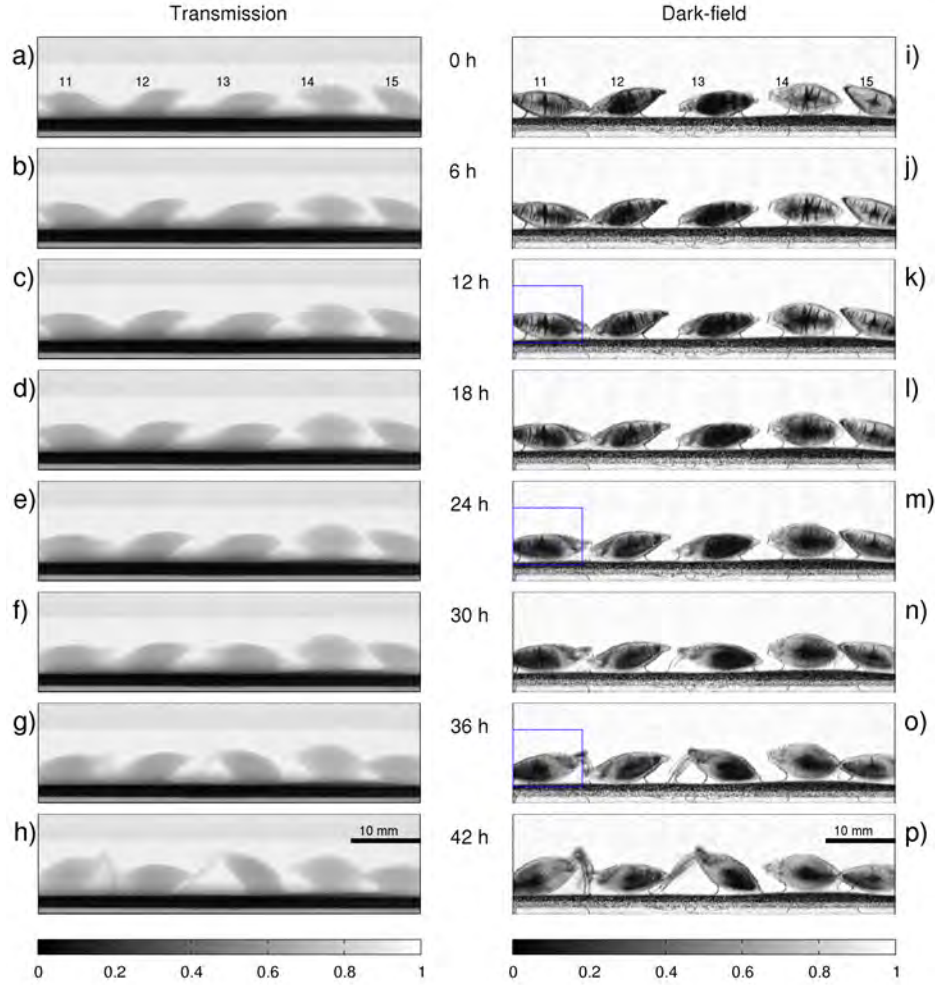


Figure 3.7: Transmission and dark-field projections of five barley seeds (numbered 11 to 15) in 6 h intervals from $t=0$ h to $t=42$ h. The blue squares in panels k), m) and o) indicate the ROI used in figure 3.8.

imaging method is needed. In [Paper II](#), we applied non-destructive X-ray dark-field radiography to monitor barley germination of 2×10 seeds during a 43 & 55 h period, respectively. Since this modality is sensitive towards microstructures, degradation of barley microstructures due to endosperm modification was detectable.

The seeds numbered from 1-20 were placed on wetted paper and were measured with radiography using grating interferometry. Figure 3.7 displays radiographs of seeds 11 to 15 using transmission in panels a)-h) and dark-field in panels i)-p). Radiographs in intervals of 6 h were selected starting at $t=0$ h and ending at $t=42$ h.

While the barley seeds appear homogeneous over time with transmission, two

internal changes can be observed in the dark-field images.

- Vertical dark lines are present within all of the seeds at $t=0$ h. Over time these lines are seen to fade until at $t=30$ h where all have disappeared.
- Dark areas in the central part of the barley seeds are seen. In some seeds such as 11 to 13 they are present at $t=0$ h, and in others such as 14 and 15 they seem to become more pronounced until $t=18$ h. As time passes, these areas shrink in all seeds.

Similar to the vertical lines in the dark-field radiographs, stress cracks in several types of seeds have been observed using conventional radiography (Demyanchuk et al., 2013). Stress cracks can occur during a drying process, and barley seeds for malt production are dried prior to germination. As unresolvable edges (Yashiro and Momose, 2015) and cracks (Lauridsen et al., 2015) previously have been shown to give a dark-field signal, stress cracks seem to be a likely explanation.

Regarding the dark areas, the reduction in dark-field signal begins near the scutellum part of the barley seed and spread through the endosperm. This pattern and movement resemble the modification pattern as observed from microscopy studies. To explain this behavior, we note that the dark-field signal in the measurements were sensitive towards microspheres with diameters in the range of $2 - 60 \mu\text{m}$ as discussed in section 3.2. As barley starch granules have diameters within the same range between $0.5 - 48 \mu\text{m}$ (Ao and Jane, 2007; Briggs, 1978; Lindeboom et al., 2004; MacGregor, 1979; Stoddard, 1999), we suggest that the starch granules produced the signal. Thus, the degradation of starch granules during modification would cause a reduction in signal as observed. Hence, we propose that the X-ray dark-field signal can be used to monitor the modification process. An extra advantage using X-ray dark-field imaging is that single seeds can be monitored in-situ.

To illustrate this, a close-up of seed 11 at 12 h, 24 h, 36 h and 48 h can be seen in figure 3.8 panels a)-d). Here the front of the degradation has been identified (shown in magenta) using edge analysis. In section 4.2.3 this analysis will be described further. In panel e), a plot of the relative position of the front along the major axis (shown in red) with time is shown. After an initial time, from 28 h to 55 h the position is seen to change approximately linearly in time. Thus the degradation front moves with constant speed in this time range. The initiation time and speed of the front was extracted using a first-order polynomial linear least-squares regression (shown in blue).

The same analysis was applied to rest of the seeds, and quantitative values of front speed and initiation times were successfully obtained from sixteen out of twenty seeds. For the four remaining seeds, the edge detection algorithm failed. The initiation times and front speeds are shown in table 3.3. The times vary from 0 h to 32 h and the speeds from 0.05 mm/h to 0.14 mm/h . Here 0 h means

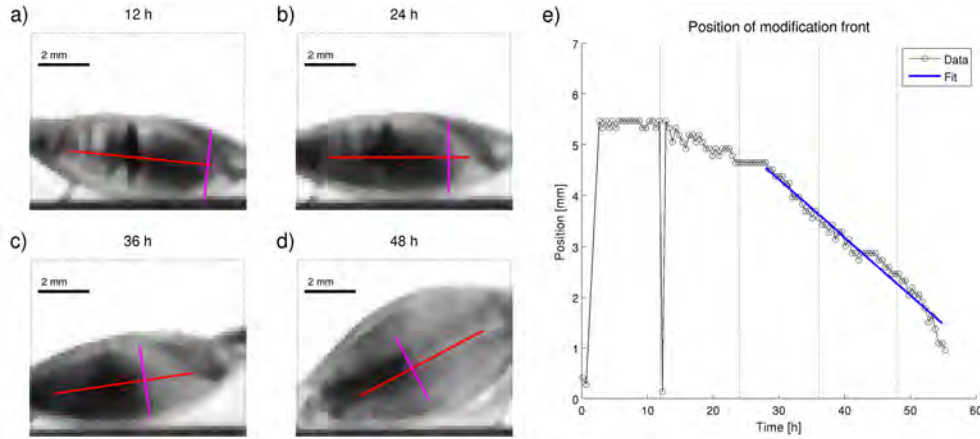


Figure 3.8: Image analysis. a)-d) Dark-field radiographs of a barley seed (number 11) at $t=12, 24, 36, 48$ h. Using image analysis, the major axis and dark-field front were identified as indicated in red and magenta, respectively. e) The position of the dark-field front with time. The fit obtained using a linear least-squares regression is indicated in blue.

within the first 30 min. Also included are the R^2 values of the least-squares fits. The result of obtaining constant speeds for the barley degradation front is in line with theoretical results (Fowkes and O'Brien, 2010; O'Brien and Fowkes, 2005).

The paper then discusses similarities and differences compared to conventional microscopy methods. Although, the shape of the areas obtained with both methods are similar, micrographs measure the reflection from a single section whereas dark-field radiography measures the cumulative signal from the full seed volume. Furthermore the paper considers the dose imparted on the seeds and discusses the effect on germination.

In conclusion, X-ray dark-field radiography was successfully applied to monitor the modification process in germinating barley. As this was performed in-situ, individual barley seeds could be followed, and a constant speed of the degradation front was obtained. Again, the study illustrates the complementarity between transmission and dark-field radiography.

Seed no	Initiation time [h]	Front speed [mm/h]	R ² (fit)
1	28	0.11	0.95
2	17	0.06	0.97
3	28	0.14	0.94
5	7	0.10	0.97
6	11	0.07	0.97
7	11	0.10	0.91
8	24	0.11	0.97
9	15	0.06	0.93
10	28	0.06	0.95
11	28	0.11	0.97
12	18	0.08	0.92
15	27	0.07	0.95
16	32	0.05	0.79
17	28	0.12	0.98
19	0	0.06	0.94
20	15	0.06	0.91
Mean	20 ± 9	0.09 ± 0.03	

Table 3.3: Barley seed front speeds and initiation times as found through image analysis. R² values for the linear least squares regressions are indicated. Mean values with an interval of a single standard deviation are listed as well.

3.4 Outlook

We have in this chapter and in Papers I-II introduced the complementary capabilities of the X-ray dark-field contrast, and demonstrated possible food science applications of X-ray dark-field radiography.

In both papers, the dark-field contrast revealed structural changes within the food product. In [Paper I](#) freezing of fruits and berries and in [Paper II](#) degradation of starch granules in germinating barley altered the microstructure. Thus both papers have demonstrated a potential for using the dark-field modality to assess and monitor the microstructural state of a food product. Accordingly, dark-field could be applied to investigate other microstructural changes in food products.

The contrast sensitivity towards freezing in [Paper I](#) could be applied to monitor the dark-field signal while food products are being frozen. An interesting future study could be to investigate whether different types of freezing result in different dark-field signals. How freezing affects the microstructure of a food product is important for food storage. A potential use could also be to monitor frozen food products during storage.

Dark-field radiography of germinating barley could be employed in [Paper II](#) for quality assurance when making malt. Current techniques use e.g. Calcoflour to stain sanded barley grains followed by inspection by microscopy which is time-consuming. X-ray dark-field radiography could potentially provide an instantaneous assessment of the degree of modification of the seeds. However, future studies are needed to validate the measurements from X-ray dark-field radiography.

In order to link the structural changes observed with the dark-field modality to the water content in barley seeds during modification, further studies are needed. Neutron radiography using a neutron grating interferometer (nGI) could be a way to monitor both the water uptake and structural changes in barley seeds. Towards neutrons, water is a strongly attenuating material. Thus, the neutron transmission modality via nGI might be used to monitor the water uptake while the structural changes are monitored using neutron dark-field radiography.

The studies presented in both papers were performed using a lab setup with a polychromatic source. Thus the results are comparable to what should be expected from a commercial grating interferometer scanning system. Recently, the first such commercial grating-based μ CT scanner was made available by Bruker microCT ([Bruker microCT, 2015](#)), and the first user results have recently been obtained ([Senck et al., 2015](#)). In this light, grating based X-ray phase-contrast and dark-field may see increasing use in the coming years.

However, as the Bruker microCT system is a compact desktop setup, its

applicability is limited to research and development purposes. An implementation at production lines is not straightforward. For widespread industrial use of X-ray dark-field imaging, the grating interferometer must be implemented in a conveyor-based X-ray scanner. If successfully accomplished, a conveyor-based X-ray grating interferometer scanner could readily replace existing systems. Since the conventional attenuation-based X-ray image is also reconstructed in the grating interferometer, no existing image information is lost while two complementary contrast modalities are gained. As this chapter has demonstrated, complementary image modalities can provide new modes of image contrast and open up for novel applications.

Efforts toward designing and realizing a conveyor-based setup have been made using a Moiré fringe scanning approach. Here a Moiré fringe pattern is created by either detuning the distance L_2 between G1 and G2 (Kottler et al., 2007b) or tilting the angle of G2 relative to G1 (Arboleda et al., 2014). Then in place of the conventional phase-stepping scan where a grating is stepped, the sample is moved in steps through the Moiré fringe pattern. A phase-scanning curve can then be constructed using pixels at different positions along the Moiré fringe period, and the image reconstruction is performed as usual. Recently, this approach has been realized in a proof-of-concept study using a grating interferometer implemented in a Phillips mammography scanner (Koehler et al., 2015). If expanded to continuous movement of the sample rather than step-wise, this approach could be implemented in a conveyor-based scanning system. Thus the prospect of industrial use of grating-based X-ray imaging does seem promising.

Chapter 4

Translating the image using image analysis

Having acquired an image, the next step is to understand its content. Here a method is needed to *translate* the image features into information. For qualitative image inspection, the human eye is a very well-adapted instrument. Thus for qualitative feature detection and analysis, *taking* the image followed by manual inspection is enough. However, for automated image inspection, a computer-based image analysis is needed for *translating* the image.

Since image analysis methods are used for all images regardless of the modality, a range of methods exist from fields such as optical imaging, electron microscopy and X-ray imaging. Thus, when dealing with image analysis of an X-ray image, a host of knowledge can be drawn upon.

However, an interesting aspect is when dealing with more than a single type of image. In chapter 3 we illustrated how a novel imaging modality can provide a complementary image contrast. If several of these imaging modalities are combined, the resulting multimodal image will contain additional information over the individual images. This can be used within image analysis to perform e.g. improved segmentation (Nielsen et al., 2012). However, new methods for image analysis are needed to utilize the full potential of this kind multimodal data.

The focus of this chapter will be image analysis methods. In addition, the chapter will serve as an introduction to Paper III (Einarsdóttir et al., 2014). When *translating* the initial projections into image information, several image analysis methods are applied along the way. In order to acquire a 3D image from a set of 2D projections, a tomographic reconstruction method is needed. In order to gain structural information from the resulting tomographic slices, the different components must be segmented. Prior to this segmentation, filtering operations are often applied for e.g. denoising or enhancement of features such as edges.

In the following, an introduction to some of these methods will be given. These can be used for both multimodal images or images of a single modality.

4.1 Tomographic reconstruction

In computed tomography (CT) a material property $f(x,y,z)$ of a sample is reconstructed in 3D from a number of projections at sufficiently many angles. Examples of material properties are the attenuation length $\mu(x,y,z)$ and electron density $\rho_e(x,y,z)$.

The challenge in tomography is to relate the recorded signal at each angle to the 3D sample geometry. The signal is recorded in the laboratory coordinate system (x', y', z') which will be rotated by the angle θ in the (x', y') plane relative to the sample coordinates (x, y, z) as shown in figure 4.1 (c). This corresponds to a rotation around the z axis wherefore $z' = z$. As shown, a line $p_\theta(x')$ at height z' of the 2D projection $p_\theta(x', z')$ records the integrated sample property $f(x,y,z)$ in the y' -direction.

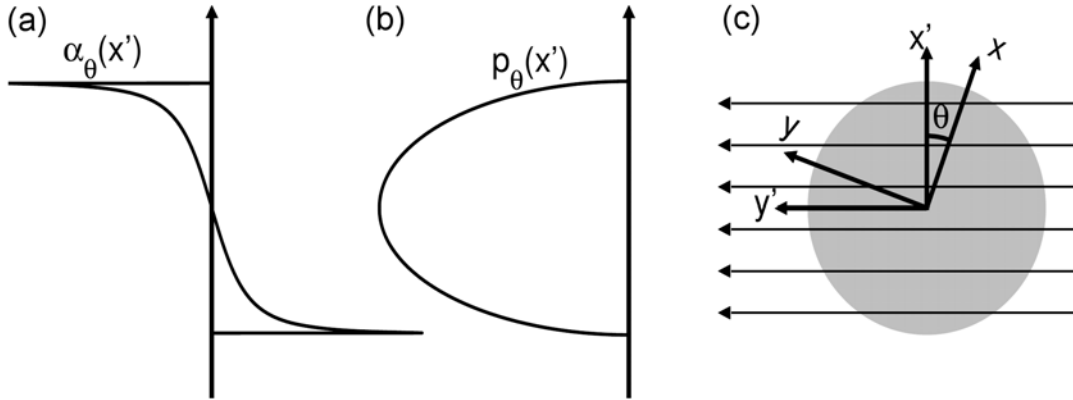


Figure 4.1: Projection geometry: Relating the recorded signal to the sample geometry. (c) The beam (from the right) is transmitted through a horizontal section of the sample and the projected signal is recorded. The sample coordinate system (x, y, z) is rotated by the angle θ with respect to the detector system (x', y', z') . (a) The projection p_θ . (b) The refraction angle α_θ . (*Reproduced with permission from Jensen (2010).*)

For a parallel beam geometry, the relation between the 2D projection $p_\theta(x', z')$ and the sample coordinate system can be done rather straightforward by use of the Radon transform (Kak and Slaney, 2001). Since the beam is parallel we can reduce the problem to a set of horizontal lines through the object. Then, the line projection $p_\theta(x')$ at height z' can be calculated as

$$p_\theta(x') = \int f(x', y') dy' = \int \int f(x, y) \delta(x \cos \theta + y \sin \theta - x') dy dx \quad (4.1)$$

where δ is the Dirac-delta function, and the detector coordinates are related to the sample coordinates as

$$x' = x \cos \theta + y \sin \theta, \quad y' = -x \sin \theta + y \cos \theta, \quad z' = z \quad (4.2)$$

Although equation (4.1) might seem cumbersome, the relation in Fourier space is more direct. In effect, it turns out that the Fourier transform of $p_\theta(x')$ corresponds to a line in Fourier space $\tilde{f}(v, w)$. This is called the Fourier slice theorem (Kak and Slaney, 2001).

An illustration of a number of line projections at different angular orientations from $0^\circ - 180^\circ$ can be seen in figure 4.2 a). Due to the rotation of the sample, the features of the object can be seen to follow sinusoidal-like curves. For this reason, the representation in panel a) is called a sinogram.

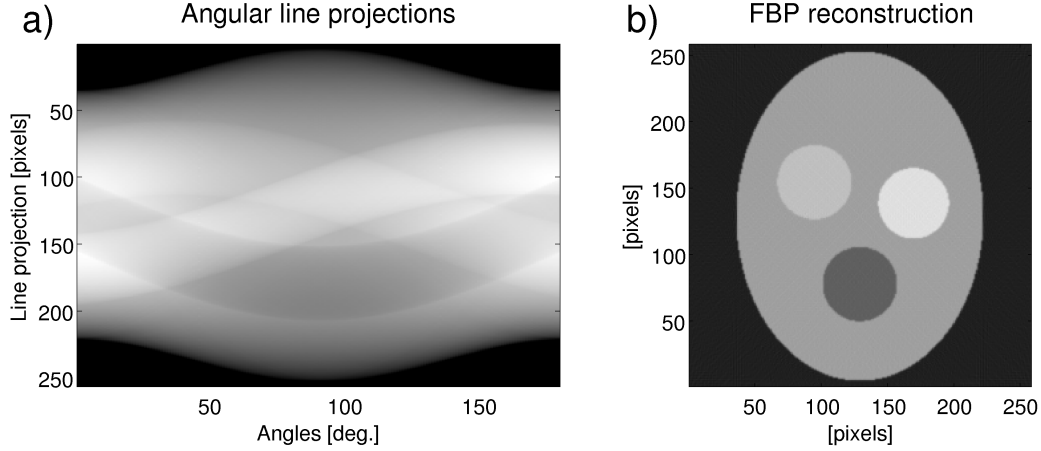


Figure 4.2: Line projections and reconstruction: a) Constructed line projections of a test object at different angular orientations from $0^\circ - 180^\circ$. b) Tomographic reconstruction of the object using the filtered back-projection (FBP) algorithm.

Having established the relation between the projection and sample in a parallel-beam geometry, a reconstruction method can be formulated. A well-known method is the filtered back-projection (FBP) algorithm as described in Kak and Slaney (2001).

$$f(x, y) = \int_0^\theta \mathcal{F}^{-1}[\tilde{p}_\theta(v') \tilde{H}(v')] d\theta. \quad (4.3)$$

where $\tilde{p}_\theta = \mathcal{F}[p_\theta]$ and $\tilde{H}$ is a filter function. A common filter for the reconstruction is the Ram-Lak filter $\tilde{k}$ shown in figure 4.3 (a) which is given by

$$\tilde{k}(v') = |v'|. \quad (4.4)$$

The Ram-Lak filter is the 'natural' filter which functions as a weighting factor in Fourier space. This weighting compensates the unequal sampling in Fourier

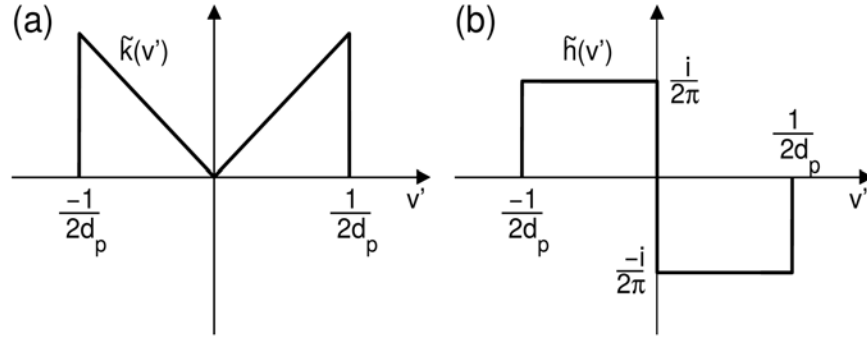


Figure 4.3: Filter functions for FBP (a) The Ram-Lak filter. (b) The modified complex Ram-Lak filter in FBP of differential phase-contrast. (*Reproduced with permission from Jensen (2010).*)

space at high frequencies. An result of applying the FBP algorithm to a set of angular line projections can be seen in figure 4.2 b).

The FBP algorithm has several assumptions on sample geometry and the X-ray interaction.

- First, a parallel and monochromatic beam is assumed. Although fulfilled at synchrotron facilities, at laboratory setups this is most often not the case.
- Furthermore, the interaction between the X-ray beam and the material is assumed not to depend on the projection angle. In general this will be fulfilled by scalar material properties such as the absorption of a monochromatic x-ray beam. However, vectorial or tensor quantities such as the local scattering vector of the material are not guarantied a successful reconstruction.
- Finally, the sample is assumed to remain constant during measurements. Thus, sample deformation or movement will distort the final reconstruction.

The FBP algorithm is applied for tomographic reconstruction of the measurements in [Paper III](#), [Paper IV](#) and [Paper V](#).

4.1.1 Attenuation tomography

In attenuation tomography, the material property is the attenuation coefficient μ in units of inverse length e.g. cm^{-1} . Since X-ray attenuation at energies 5-40 keV is dominated by photoelectric absorption, the modality is also sometimes described as absorption tomography. As μ is a scalar quantity, FBP can be directly applied.

Recalling the results from equation (2.19), the attenuation projection p_θ can

be derived from the transmission radiograph as:

$$\begin{aligned}
 p_\theta(x') &= -\log(T(x')), \\
 &= -\log\left(\frac{a_0^s}{a_0^r}\right), \\
 &= \int \mu(x', y') dy'
 \end{aligned} \tag{4.5}$$

Thus, using FBP from equation (4.3) on the projections in equation (4.5), the local attenuation voxel-values in the 3D volume are reconstructed.

4.1.2 Phase-contrast tomography

Most phase-contrast imaging techniques do not reconstruct the phase directly. In grating interferometry, the differential phase-contrast radiographs are reconstructed through measuring the angular change in the stepping direction of the gratings. As we recall from equation (2.21) this is given as

$$\begin{aligned}
 \alpha_{x'}(x') &= \frac{g_2}{2\pi d} (\phi_1^s - \phi_1^r), \\
 &= \int \frac{\partial}{\partial x'} \delta(x', y') dy'.
 \end{aligned} \tag{4.6}$$

Thus, the projection $\alpha_{x'}$ measures the x-component of the gradient of the refractive index δ . If the FBP is applied directly to equation (4.6), only the derivative will be reconstructed and not δ .

An alternative way is to utilize a property of the Fourier transform. Taking a Fourier transform of a derivative of a function is the same as taking the Fourier transform of the original function and multiplying by $2\pi i v'$. Thus, integration can be performed in Fourier space by division with the same factor. In effect, integration of $\partial_{x'} \delta$ can be implemented in the FBP by adjusting to an appropriate complex filter function (Pfeiffer et al., 2007)

$$\tilde{h}(v') = |v'| \frac{1}{2\pi i v'}. \tag{4.7}$$

The imaginary part of $\tilde{h}$ is shown in figure 4.3 (b).

Although this approach only uses part of the full gradient $\nabla \delta$, it is commonly very successful in the tomographic reconstruction of δ . Nonetheless, some improvements on the reconstruction have been reported in a tomographic study where the full gradient is measured (Rutishauser et al., 2011). By tilting the gratings by 45° with respect to the tomographic axis, both the horizontal and vertical differential phase-contrast projections could be extracted, and the full gradient

$\nabla\delta$ could be reconstructed. From the reconstructed gradient, the refractive index δ can be found as described in (Kottler et al., 2007a).

When performing quantitative tomography, the δ values are often converted into electron densities ρ_e as described in equation (2.2).

4.1.3 Dark-field tomography

The FBP algorithm has been applied for tomographic reconstruction of the dark-field signal in case of isotropic scattering (Bech et al., 2010). Similar to attenuation, the dark-field projection can be derived from the dark-field radiograph as:

$$\begin{aligned} p_\theta(x') &= -\log(V(x')), \\ &= -\log\left(\frac{a_1^s/a_0^s}{a_1^r/a_0^r}\right), \\ &= \int \mu_{DF}(x', y') dy' \end{aligned} \tag{4.8}$$

However, since scattering in general is angular-dependent, the conditions for FBP are not guaranteed to be fulfilled. Thus, except for the case of isotropic scattering, tomographic reconstruction of the dark-field signal cannot be performed using the standard FBP.

A recent approach for tomographic reconstruction of anisotropic scattering applied an algebraic reconstruction technique (Malecki et al., 2014). By modeling the dark-field signal as a (discrete) tensor quantity, the scattering vector in each voxel could be reconstructed by use of the simultaneous algebraic reconstruction technique (SART). However, in the present thesis work the SART method has not been considered.

4.2 Filtering

In image analysis, filtering is commonly applied for both image correction and in image analysis. An acquired image might e.g. suffer from noise or blurred features. To correct for these effects, filters can be used for noise reduction and image sharpening. (Gonzalez and Woods, 2008). In addition, feature detection in image analysis may be performed through filtering such as edge detection.

A common type of filters are linear filters. Filtering using linear filters rely on the convolution of an image $\mathcal{I}$ by a filter kernel H . The resulting image $\mathcal{I}'$

can be written as

$$\mathcal{I}'(\mathbf{x}) = (\mathcal{I} * H)(\mathbf{x}) \equiv \int \mathcal{I}(\mathbf{x} - \boldsymbol{\tau}) H(\mathbf{x}) d\boldsymbol{\tau} \quad (4.9)$$

where '*' indicates convolution. The convolution operation possess several algebraic properties such as commutativity, associativity and distributivity. In addition, (partial) differentiation of a convolution has the following useful property

$$\frac{\partial}{\partial x_i} (\mathcal{I} * H) = \frac{\partial \mathcal{I}}{\partial x_i} * H = \mathcal{I} * \frac{\partial H}{\partial x_i} \quad (4.10)$$

Thus, rather than taking the derivative of an image directly and then performing convolution, the convolution can be performed with the derivative of the filter kernel.

Another important relation regards the Fourier transform of a convolution and is called the Convolution theorem.

$$\mathcal{F}(\mathcal{I} * H) = \mathcal{F}(\mathcal{I})\mathcal{F}(H) \quad (4.11)$$

Thus by transformation to Fourier space, the convolution operation becomes a simple product. This property is used for practical implementations of convolution since fast FFT algorithms instead of direct integration can be used. The convolution of an image with a filter kernel can thus be computed by taking the inverse Fourier transform of the Fourier space product of the two.

$$\mathcal{I} * H = \mathcal{F}^{-1}[\mathcal{F}(\mathcal{I})\mathcal{F}(H)] \quad (4.12)$$

To illustrate the design and application of filter kernels, we will in the following begin by a small example.

4.2.1 Example: Using filters in image analysis

As an example of how to construct filters for image analysis, we will estimate the local (weighted) mean and gradients of an image through filters. Consider a neighborhood of the image pixel $\mathcal{I}(x,y)$ as follows:

$\mathcal{I}(-1,1)$	$\mathcal{I}(0,1)$	$\mathcal{I}(1,1)$
$\mathcal{I}(-1,0)$	$\mathcal{I}(0,0)$	$\mathcal{I}(1,0)$
$\mathcal{I}(-1,-1)$	$\mathcal{I}(0,-1)$	$\mathcal{I}(1,-1)$

For computational ease, the neighborhood is rewritten as a 9×1 column vector as $\mathcal{I} = [\mathcal{I}(-1, -1) \ \mathcal{I}(-1, 0) \ \mathcal{I}(-1, 1) \ (\dots) \ \mathcal{I}(1, 0) \ \mathcal{I}(1, 1)]^T = [I_1 \ (\dots) \ I_9]^T$.

To estimate mean and gradients, we approximate the small neighborhood of $\mathcal{I}$ as a plane g centered on the neighborhood

$$g(x, y) = a + b\mathbf{x} + c\mathbf{y}. \quad (4.13)$$

where a is an approximation of the local mean, and b and c are approximations of $\frac{\partial \mathcal{I}}{\partial x}$ and $\frac{\partial \mathcal{I}}{\partial y}$, respectively. The image $\mathcal{I}$ can be estimated by g as

$$\mathcal{I} = g + \epsilon = X\beta + \epsilon, \quad (4.14)$$

where $\beta = [a, b, c]^T$ is a 3×1 column vector, X is a 9×3 coordinate matrix, and ϵ is a column vector containing the error.

In addition, a 9×9 diagonal weighting matrix W is introduced to emphasize near pixels over remote. A Gaussian kernel will be used for the weighting of the neighborhood pixels

1	2	1
2	4	2
1	2	1

Where the Gaussian kernel form the diagonal of W

$$W = \begin{bmatrix} 1 & & & & & & & & \\ & 2 & & & & & & & \\ & & 1 & & & & & & 0 \\ & & & 2 & & & & & \\ & & & & 4 & & & & \\ & & & & & 2 & & & \\ & & 0 & & & & 1 & & \\ & & & & & & & 2 & \\ & & & & & & & & 1 \end{bmatrix}. \quad (4.15)$$

From these considerations, the optimal filters can be found through weighted linear regression by minimizing

$$\|W^{1/2}(\mathcal{I} - X\beta)\|^2 = \epsilon^2, \quad (4.16)$$

with respect to β . By setting the differential equal to zero, the resulting normal equations become

$$X^T W \mathcal{I} = [X^T W X] \beta. \quad (4.17)$$

And solving for β we get

$$\beta = \begin{bmatrix} a \\ b \\ c \end{bmatrix} = \begin{bmatrix} \frac{1}{16}(I_1 + 2I_2 + I_3 + 2I_4 + 4I_5 + 2I_6 + I_7 + 2I_8 + I_9) \\ \frac{1}{8}(I_3 + 2I_6 + I_9 - I_1 - 2I_4 - I_7) \\ \frac{1}{8}(I_7 + 2I_8 + I_9 - (I_1 + 2I_2 + I_3)) \end{bmatrix}, \quad (4.18)$$

The coefficients appearing in front of the I_j s in the parameters a , b and c above form the filter kernels H_a , H_b and H_c

$$H_a = \frac{1}{16} \begin{bmatrix} 1 & 2 & 1 \\ 2 & 4 & 2 \\ 1 & 2 & 1 \end{bmatrix}, \quad H_b = \frac{1}{8} \begin{bmatrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{bmatrix}, \quad H_c = \frac{1}{8} \begin{bmatrix} -1 & -2 & -1 \\ 0 & 0 & 0 \\ 1 & 2 & 1 \end{bmatrix}, \quad (4.19)$$

Here H_a is seen to be a (normalized) Gaussian kernel, and H_b and H_c are the (normalized) Sobel kernels which describe the (weighted) horizontal and vertical derivatives.

The result of applying the filters H_a , H_b and H_c to an image can be seen in figure 4.4. By filtering with the Gaussian filter kernel, the high frequency features of the original in panel a) are seen to be (slightly) suppressed in the resulting image of panel b). A smoothing or blurring of the image has occurred. Filtering using the Sobel filter kernels results in the images $S_x = \mathcal{I} * H_b$ and $S_y = \mathcal{I} * H_c$ which shows an enhancement of the edges in the image. This enhancement occurs for edges along the horizontal or vertical direction as seen in panel c) and d), respectively.

In the following, the use of filtering will be examined further in the cases of noise reduction and edge detection.

4.2.2 Denoising

To some degree, noise will be present in all X-ray images. An illustration of a constructed noisy image can be seen in figure 4.5 b) which can be compared to the original noise free image in panel a). While improving counting statistics during measurements may help to reduce noise, noisy images will often be a challenge in further image analysis. Thus, denoising methods are an invaluable tool, and several methods exist.

A simple approach for dealing with noise is to perform a local (weighted) average. This can be performed by applying a linear filter such as the Gaussian

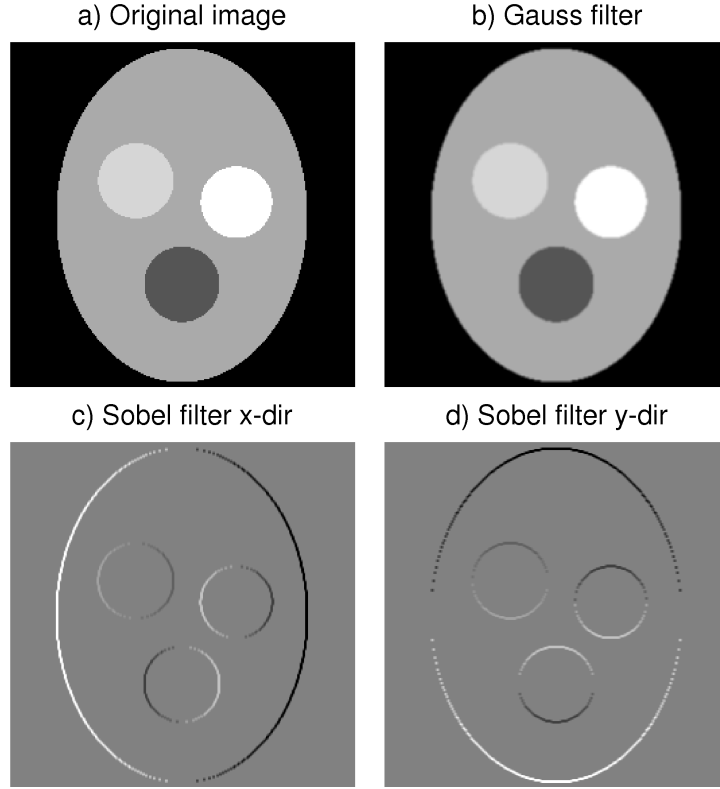


Figure 4.4: Example of filtering an image with different filter kernels. a) Original image. b) The image filtered by a Gaussian kernel. c)+d) The image filtered by a Sobel kernel in horizontal and vertical directions, respectively.

filter. The Gaussian filter acts as a low-pass filter and will suppress the high-frequency components of the noise. However, in the same manner high-frequency features in the image will also be suppressed as discussed in figure 4.4 b) leading to a loss of spatial resolution. A kernel can be created as in section 4.2.1 by approximating a Gaussian distribution given as

$$G(\mathbf{x}; h) = \frac{1}{\sqrt{2\pi} h^2} \exp\left(-\frac{x^2}{2h^2}\right) \quad (4.20)$$

on a support with $(0,0)$ in the center pixel. Here the parameter h controls the width of the Gaussian distribution. For $h \rightarrow 0$ the filter kernel only gives weight to the center pixel while for $h \rightarrow \infty$ it approaches a box filter with equal weight to each pixel in the support. The effect of using a $[7 \times 7]$ Gaussian filter with $h = 1$ for denoising can be seen in figure 4.5 d).

The median filter is one of the common non-linear filters used for denoising. As with the kernel for linear filters, the median filter also considers a support neighborhood around the center pixel. Instead of convolution with the kernel, the median filter works by replacing a pixel value in the noisy image with the

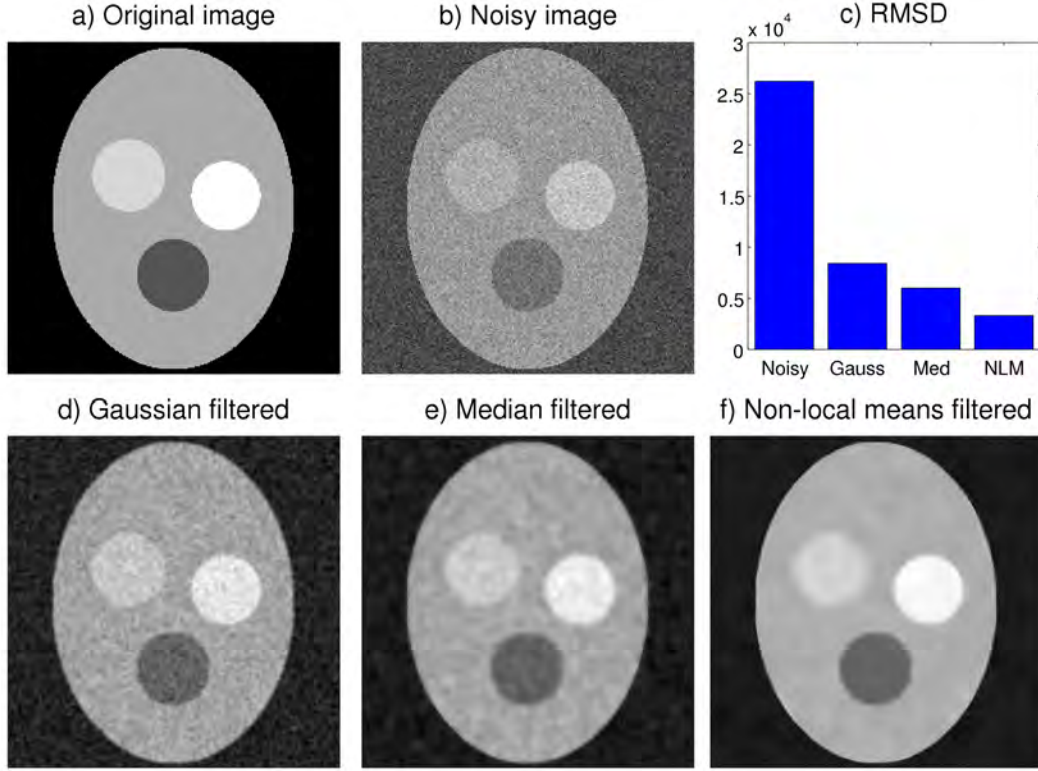


Figure 4.5: Illustration of denoising using different methods. a) Original noise free image. b) Noisy version of original image with white noise added. c) Root-mean square deviation (RMSD) of panels b) and d)-f) compared to original image. d) Denoising using filtering with a $[7 \times 7]$ Gaussian kernel. e) Denoising using median filtering with a $[7 \times 7]$ kernel. f) Non-local means denoising using $[15 \times 15]$ neighborhood windows.

median value of the neighborhood pixels. An illustration of applying a median filter for denoising can be seen in figure 4.5 e).

In contrast to the two simple filters considered above, a more sophisticated approach is the Non-local means (NLM) algorithm introduced by [Buades et al. \(2005\)](#). Here the denoised value at $\mathbf{x}$ in the image $\mathcal{I}$ is a mean of the values of all points whose Gaussian-weighted neighborhood looks like the neighborhood of $\mathbf{x}$. A similarity measure between point $\mathbf{x}$ and $\mathbf{y}$ is introduced as

$$(G_a * |\mathcal{I}(\mathbf{x} + \cdot) - \mathcal{I}(\mathbf{y} + \cdot)|^2)(0) = \int G_a(\boldsymbol{\tau}) |\mathcal{I}(\mathbf{x} + \boldsymbol{\tau}) - \mathcal{I}(\mathbf{y} + \boldsymbol{\tau})|^2 d\boldsymbol{\tau} \quad (4.21)$$

where the squared differences are convoluted with a Gaussian of width parameter a to define a neighborhood window. A small squared difference in a neighborhood around $\mathbf{y}$ compared to around $\mathbf{x}$ means a high degree of similarity. In the final denoising, this is used as input for the weighted average in the NLM

algorithm as

$$NL[\mathcal{I}](\mathbf{x}) = \frac{1}{C(\mathbf{x})} \int_{\Omega} \exp \left(-\frac{(G_a * |\mathcal{I}(\mathbf{x} + \cdot) - \mathcal{I}(\mathbf{y} + \cdot)|^2)(0)}{h^2} \right) \mathcal{I}(\mathbf{y}) d\mathbf{y}, \quad (4.22)$$

where $C(\mathbf{x}) = \int_{\Omega} \exp \left(-\frac{(G_a * |\mathcal{I}(\mathbf{x} + \cdot) - \mathcal{I}(\mathbf{y} + \cdot)|^2)(0)}{h^2} \right) d\mathbf{y}$ is a normalizing constant, and h is a filtering parameter. The weighting in NLM is performed in the region Ω around $\mathbf{x}$. While this can be over the full image, normally this is reduced to a smaller region. An illustration of the effect of the NLM algorithm for denoising is shown in figure 4.5 f).

As seen from figure 4.5, there are differences in the effect of the denoising methods. In order to compare the three filters used, the root mean squared deviation (RMSD) is computed and displayed in panel c). It is seen that the NLM has the lowest RMSD and also visually seems to give the best denoising.

In the work in this thesis, several of the above methods have been used. In [Paper II](#) and [Paper IV](#), the median filter has been used in relation to image analysis while an iterative variant of the NLM algorithm has been used in [Paper IV](#) for denoising.

4.2.3 Edge detection

In order to find the location of the boundaries between different regions in an image, edge detection can be used. The basic idea is to look at the image gradient since this will give a strong signal at edges as for the Sobel filter kernels in figure 4.4 panels c) and d). In Sobel edge detection, this is expanded by calculating the Sobel gradient magnitude as $|S| = \sqrt{S_x^2 + S_y^2}$. As seen in figure 4.6 b), the gradient magnitude gives a signal for the edges between regions. The strength of the signal is seen to be strongest for edges between regions of large difference in pixel value.

In the final step of Sobel edge detection, a threshold is used to choose which edges to include in the edge detection. As seen in figure 4.6 panels c) and d), the edges of the upper left circle is not included at a 0.45 threshold while detected at a 0.2 threshold.

While the edges in the original test image of figure 4.6 a) appear sharp, some image regions may be separated by a more diffuse boundary as seen from the circles in figure 4.7 a). For diffuse boundaries, the signal from the Sobel filter is less as seen in figure 4.7 b) where the Sobel derivative at an angle $\alpha = \pi/3$ is shown. This was calculated as $S_{\alpha} = \cos(\alpha)S_x + \sin(\alpha)S_y$.

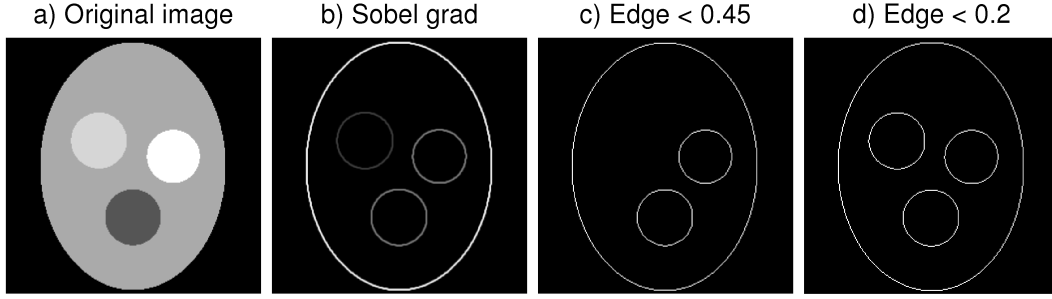


Figure 4.6: Illustration of Sobel edge detection. a) Original image. b) The Sobel gradient magnitude of the original image. c)-d) Edge detection at a 0.45 and 0.2 threshold.

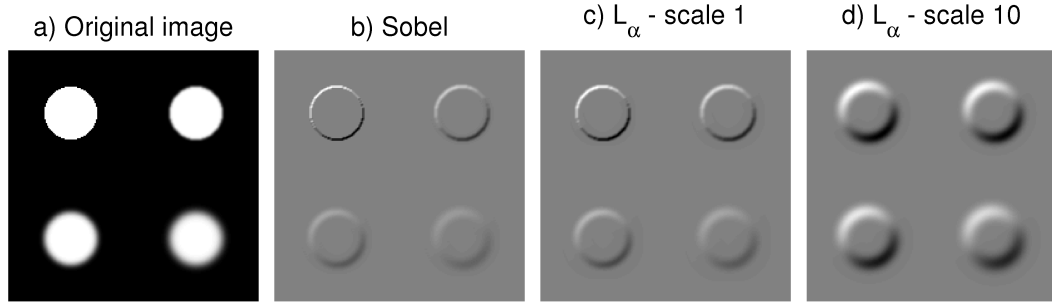


Figure 4.7: Illustration of response at angle $\pi/3$ of sharp and blurred features using Sobel or scale-space derivative. a) Original image. b) Image with Sobel filter applied. c)-d) Scale-space derivative L_α at scale 1 and 10.

An approach to handle features at different length scales such as sharp and diffuse edges is the scale-space representation ([Lindeberg, 2007](#)). The scale-space at scale t is the image $\mathcal{I}$ convolved with the Gaussian kernel G of width $t = h^2$ as in equation (4.20)

$$L(\mathbf{x}; t) = \mathcal{I}(\mathbf{x}) * G(\mathbf{x}; t) \quad (4.23)$$

One of the advantages with the scale-space representation is when dealing with derivatives of images. This can be seen from the scale-space representation of the n 'th order (partial) derivative of an image

$$L_{x_i^n}(\mathbf{x}; t) = \left(\frac{\partial}{\partial x_i^n} \mathcal{I}(\mathbf{x}) \right) * G(\mathbf{x}; t) = \mathcal{I}(\mathbf{x}) * \left(\frac{\partial}{\partial x_i^n} G(\mathbf{x}; t) \right) \quad (4.24)$$

where equation (4.10) has been used. Thus, rather than taking the derivative of the image directly, scale-space derivatives can be performed by convolution with the derivative of a Gaussian instead.

In addition the scale-parameter t allows an enhancement of features at selected length scales. This can be seen in figure 4.7 panels c) and d) from the scale-space

derivative $L_\alpha = \cos(\alpha)L_x + \sin(\alpha)L_y$ at angle $\alpha = \pi/3$ at two different length scales. At scale $t = 1$ in panel c), L_α is similar to S_α with a reduced edge signal from the diffuse circles. However, at scale $t = 10$ in panel d) a relative stronger signal is observed from the diffuse edges.

The angular scale-space derivative L_α was used in [Paper II](#) to locate the boundary between two regions in germinating barley seeds. In the dark-field radiographs a diffuse edge separated a region of strong dark-field signal from a region with low. As described in [Paper II](#), the boundary between these two regions defined the front of enzymatic degradation of starch granules in the barley seed.

4.3 Segmentation

Image segmentation is loosely speaking the act of partitioning an image into a number of mutually exclusive regions ([Li, 2009](#)). The partitioning constitutes a labeling problem which is based on one or more specified criteria. The segmented regions can e.g. be used to identify the presence or absence of objects or components in an image. In automated quality inspection of food products, segmentation is used for detection of foreign bodies ([Haff and Toyofuku, 2008](#)). Furthermore, segmentation is often used for identifying the different components in X-ray tomographic slices. A more mathematical stringent definition of image segmentation can be found in textbooks such as [Gonzalez and Woods \(2008\)](#).

The goal in the segmentation process is to define the most effective partition. Since the application of an imaging study varies from case to case, the type of segmentation will likewise differ. A number of different approaches to segmentation exist e.g. based on algorithms for edge detection, thresholding, region growing or morphology ([Gonzalez and Woods, 2008](#)). In the following, segmentation based on thresholding and Markov random fields (MRF) will be discussed.

4.3.1 Thresholding

In threshold segmentation an image is partitioned into regions based on pixel intensity values. Each region is assigned a discrete label l_i from the set of labels $\mathcal{L}$. For example when the possible regions are n objects or components, $\mathcal{L} = \{1, \dots, n\}$.

A simple case of labeling is the binary case where a pixel is either labeled 1 or 0. For studies of porous media, this formulation is used to separate the matrix material from the air of the pore space. In threshold segmentation, this is done by selecting a threshold value τ that separates the intensity value distribution of

the material from the air. Then for each pixel $p(i,j)$ a check is performed whether the value is above or below the threshold $p(i,j) > \tau$. The pixels of the segmented image $q(i,j)$ are then given by:

$$q(i,j) = \begin{cases} 1 & \text{if } p(i,j) > \tau \\ 0 & \text{if } p(i,j) \leq \tau \end{cases} \quad (4.25)$$

The number of regions can be increased by defining more than one threshold.

So far the thresholding method described has assumed images with only one intensity value for each pixel. Nonetheless, the scheme can be extended to cover multivariate images such as color images or multimodal X-ray images. Multivariate thresholding can be viewed as a distance computation, where for each pixel $\mathbf{p}(i,j)$ the distance in pixel value D from a specific pixel value point $\mathbf{a} = a_1, \dots, a_n$ is calculated. Here, the bold-face denotes a n -dimensional vector.

Different measures can be used for the distance calculation. A notable one is the well-known n -dimensional *Euclidean distance*. With this selected method a way to segment the multimodal input image is:

$$q(i,j) = \begin{cases} 1 & \text{if } D(\mathbf{p}, \mathbf{a}) \leq R_\tau \\ 0 & \text{otherwise} \end{cases}, \quad (4.26)$$

where the segmented output image q takes a single value. In this way of calculating the distance, the equation $D(\mathbf{p}, \mathbf{a}) = R_\tau$ describes a hypersphere (in n -dimensions), and the thresholding can be seen as selecting all pixels with a set of values within this hypersphere. Again the number of regions can be increased by defining more than one threshold.

Multivariate threshold segmentation was applied in [Paper II](#) as part of image analysis.

4.3.2 Markov Random Field

Using a Markov random field (MRF) framework for image segmentation makes it possible to include information on neighboring pixels. This can be helpful when dealing with e.g. image noise. The MRF approach uses a probabilistic model to describe the image and the labeling. Since X-ray imaging fundamentally relies on counting photons with, ascribing a random variable to the pixel has some appeal. Using this framework, the goal of the segmentation is to find the optimal labeling by e.g. maximizing the a posteriori probability. The following introduction to MRF follows the work by [Li \(2009\)](#).

The idea is that the image represents a finite number of different labels. For a porous media there are two labels, i.e. material and air. However in the process of *taking* the image random errors such as noise have been introduced. In MRF this is modeled by ascribing a random variable F_i to each pixel i from the set of pixel sites $\mathcal{S}$. The family of random variables $\mathbf{F} = \{F_1, \dots, F_m\}$ forms the random field in MRF.

In a realization of $\mathbf{F}$, each F_i takes one of the label values f_i from the set of labels $\mathcal{L}$. This event has an associated conditional probability $P(f_i|f_j, j \in \mathcal{S} \setminus \{i\})$ that in principle depends on all other pixels in the image. However, the Markov part of MRF assumes that only the neighboring pixels influence this probability.

$$P(f_i|f_j, j \in \mathcal{S} \setminus \{i\}) = P(f_i|f_j, j \in \mathcal{N}_i) = P(f_i|f_{\mathcal{N}_i}). \quad (4.27)$$

where $\mathcal{N}_i$ is the neighborhood of the i th pixel. A common neighborhood is the nearest neighbors which form the 4-neighborhood in 2D (left, right, top and bottom) and 6-neighborhood in 3D (left, right, top, bottom, front and back). The 6-neighborhood in 3D has been used within this thesis work.

Several models can be used to describe the probability of a given label value. In case of a binary labeling problem of $\mathcal{L} = \{-1, 1\}$, the Ising model can be used

$$P(f_i|f_{\mathcal{N}_i}) = \frac{1}{Z} \exp \left(\alpha_i f_i + \sum_{j \in \mathcal{N}_i} \beta_{ij} f_i f_j \right) \quad (4.28)$$

where Z is a normalization factor, α_i is the a priori probability for the label f_i , and β_{ij} is the interaction cost for the neighboring i th and j th site. By adjusting β_{ij} , neighboring sites can be set to favor similar labels. Thus, β_{ij} functions as a spatial smoothing parameter which can be used to produce homogeneous regions in the segmentation.

In the next step, the measured pixel values x_i are included by considering the conditioned labeling probability $P((f_i|f_{\mathcal{N}_i})|x_i) = P(f_i|f_{\mathcal{N}_i}, x_i)$. This can be calculated using Bayes theorem.

$$\begin{aligned} P(f_i|f_{\mathcal{N}_i}, x_i) &= \frac{P(x_i|(f_i|f_{\mathcal{N}_i})) P(f_i|f_{\mathcal{N}_i})}{P(x_i)} \\ &= \frac{P(x_i|f_i) P(f_i|f_{\mathcal{N}_i})}{\sum_{f_i \in \mathcal{L}} P(x_i|f_i) P(f_i|f_{\mathcal{N}_i})} \end{aligned} \quad (4.29)$$

where $P(x_i|f_i)$ is the expected data distribution of label f_i . Often a Gaussian distribution is assumed which for a single modality is given as

$$P(x_i|f_i) = \frac{1}{\sqrt{2\pi\sigma_{f_i}^2}} \exp \left(-\frac{(x_i - \mu_{f_i})^2}{2\sigma_{f_i}^2} \right) \quad (4.30)$$

where the mean μ_{f_i} and variance $\sigma_{f_i}^2$ can be estimated from the measured intensity values. In multimodal imaging, this should be replaced by a multivariate Gaussian distribution.

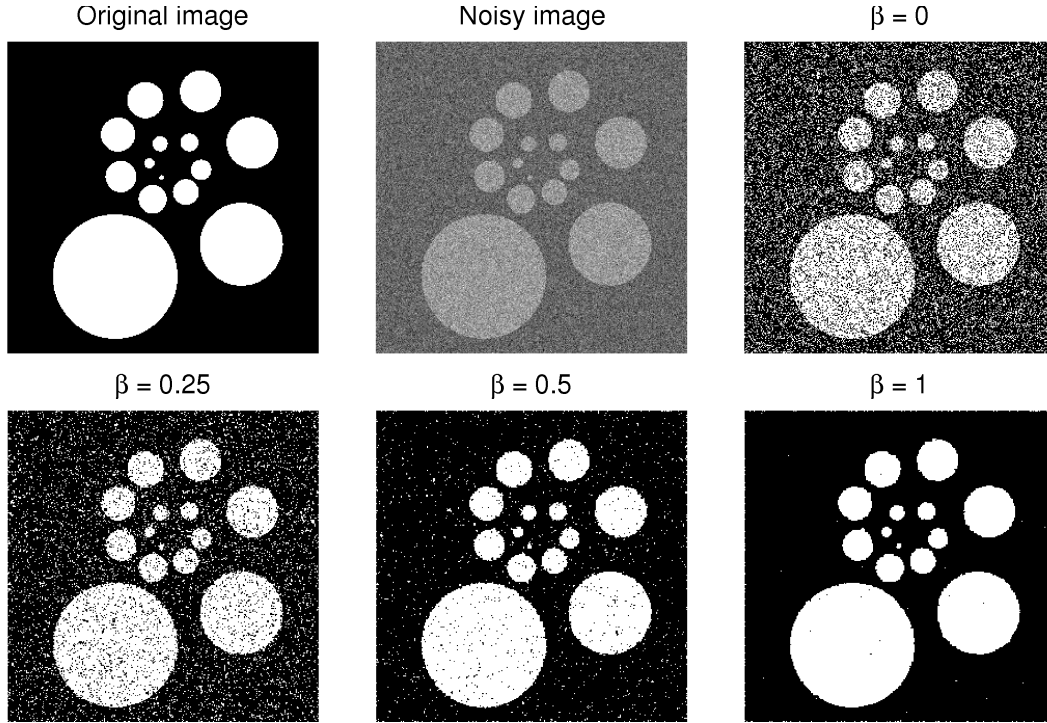


Figure 4.8: Illustration of MRF segmentation on the noisy image shown in top middle frame. When $\beta = 0$ as in the top right frame, no information on neighbors is used, and the segmentation is similar to threshold segmentation. In the bottom row, the effect of increasing the β value can be seen as increased homogeneity. For comparison, the original image is displayed in the top left frame.

An illustration of MRF segmentation is shown in figure 4.8. Here the Ising model has been used for segmentation of the noisy image shown in the top middle frame. The effect of increasing the β value is shown in the top right frame and the bottom row. With $\beta = 0$ the segmentation resembles that of threshold segmentation. It is seen that many pixels have received a wrong classification due to the image noise. In contrast, with $\beta = 1$ the segmented image is much closer to the original image.

For practical implementations, the MRF labeling can be performed iteratively by cycling all sites until a steady state has been found. However, this can both be time consuming and systematic tracing errors can be introduced. Another approach is to use graph cuts to find the optimal binary labeling scheme which has been described by [Boykov et al. \(2001\)](#) and [Boykov and Kolmogorov \(2004\)](#). By use of alpha extension, the graph cuts framework was expanded to cover multiple labels as well.

Two different implementations of MRF segmentation were used in [Paper III](#) and [Paper IV](#). In [Paper III](#), a multimodal segmentation was developed within the MRF framework while [Paper IV](#) relied on an implementation by [Pedersen et al. \(2015\)](#) for phase-contrast tomography data. Both implementations applied graph cuts with alpha extension for finding the optimal labeling.

4.4 Grating interferometer experiments at a synchrotron source

The measurements performed in [Paper III](#) and [Paper V](#) were carried out at the TOMCAT beamline of the Swiss Light Source (SLS) synchrotron in Switzerland. The grating interferometer setup is shown in figure 4.9. The setup consists of a sample stage, the grating interferometer with a G1 and G2 grating and a detector system (not shown).

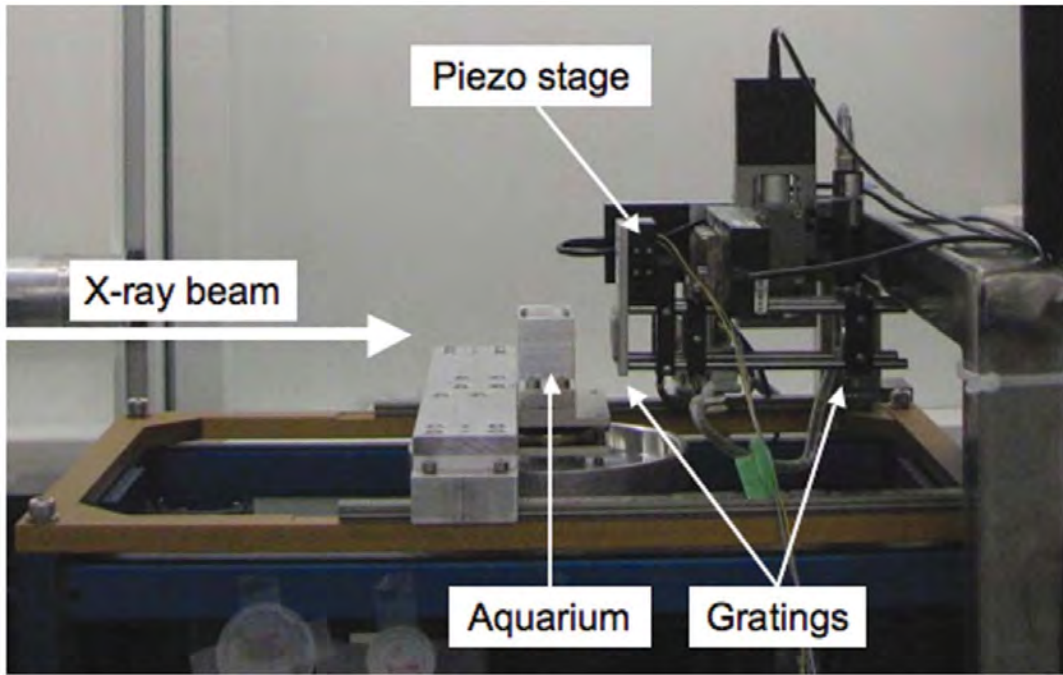


Figure 4.9: The grating interferometer setup at the TOMCAT beamline at SLS. The X-ray beam enters from the left and reaches the sample which is submerged in water in an aquarium. The sample stage allows for sample movement and includes a rotation stage for tomography. The grating interferometer is placed downstream of the sample stage before reaching the detector (not shown). (*Reproduced from McDonald et al. (2009)*)

At the sample stage, the sample is placed in a water aquarium to prevent phase-wrapping artifacts. Due to the periodicity of the gratings, a transverse shift from a refraction effect larger than the grating period g_2 will be 'wrapped' to a value between $0 - g_2$. By using water as a reference, large phase-shifts are suppressed which allows for a higher sensitivity. A water aquarium was used in both [Paper III](#) and [Paper V](#). The sample can be translated sideways out of the beam for collection of reference images. A downside of placing the sample in water is that the tomographic reconstructed values are relative to water and not on absolute scale.

The sample is placed in front of the phase-grating G1 of the grating interferometer. The setup parameters can be found in table 2.1 on page 33, and the acquisition details are listed in the papers of full length in Appendix A.

4.4.1 Introduction to Paper III

[Paper III](#) is entitled 'Analysis of micro-structure in raw and heat treated meat emulsions from multimodal X-ray microtomography' ([Einarsdóttir et al., 2014](#)). In this paper, the structure of a meat emulsion system was analyzed using grating-based X-ray CT. The analysis was made possible by the development of a multimodal segmentation algorithm using the three modalities of the grating interferometer; attenuation, phase-contrast and dark-field.

Meat emulsion systems are used for e.g. sausages of the frankfurter type. They consist of a mixture of proteins, fat particles, water, salt and often carbohydrates. In the preparation of the raw meat batter, the fat is chopped into tiny particles which are thoroughly mixed within the comminuted lean meat. During cooking, water will be expelled from the protein phase due to muscle fiber contraction. The challenge for meat emulsion preparation is to minimize/prevent fat and moisture separation from the product.

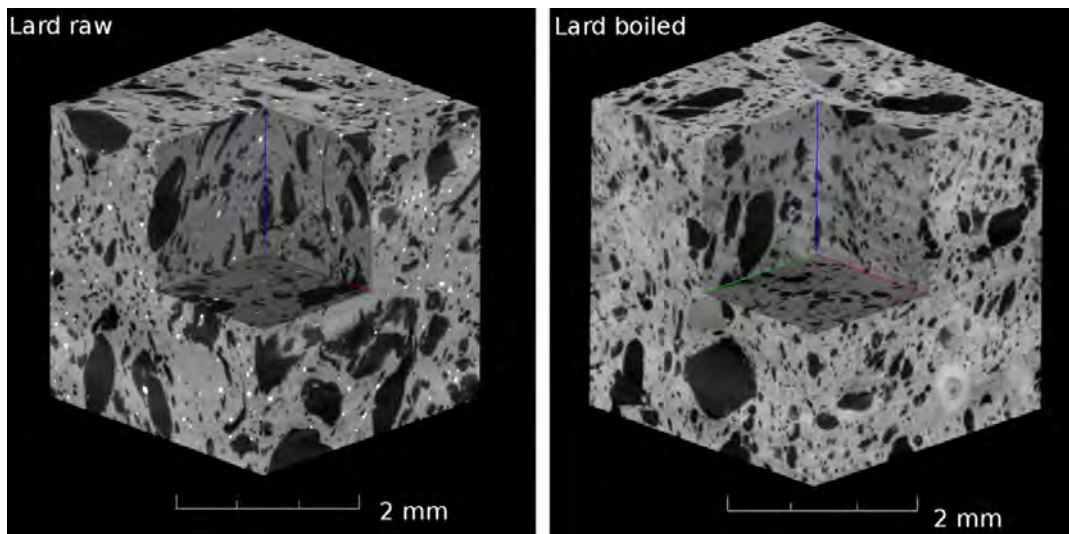


Figure 4.10: 3D representation of a meat emulsion using phase-contrast CT. The fat phase is seen in black, the protein phase in gray shades and salt particles in white. *Left:* Raw emulsion. *Right:* Cooked emulsion.

A 3D representation of a phase-contrast tomogram of a raw meat emulsion batter is shown in the left panel of figure 4.10. The lard fat particles shown in black are dispersed in the protein phase seen in gray shades. Undissolved salt grains are seen in white. In the right panel of figure 4.10, the meat emulsion is

shown after heat treatment. The salt grains have been dissolved, and pockets of expressible fluids have formed in the protein phase.

A potential for improved segmentation using multivariate analysis in grating-based imaging has previously been demonstrated for a piece of pork fat and a piece of beef muscle tissue (Nielsen et al., 2012). By combining conventional CT with phase-contrast CT into a multichannel image, manual bivariate threshold segmentation could be performed. In Paper III, an automated multivariate-segmentation algorithm is implemented which makes several improvements. Not only are all three modalities from the X-ray grating interferometer included, in addition advanced statistical methods are employed in both the description and segmentation of the data.

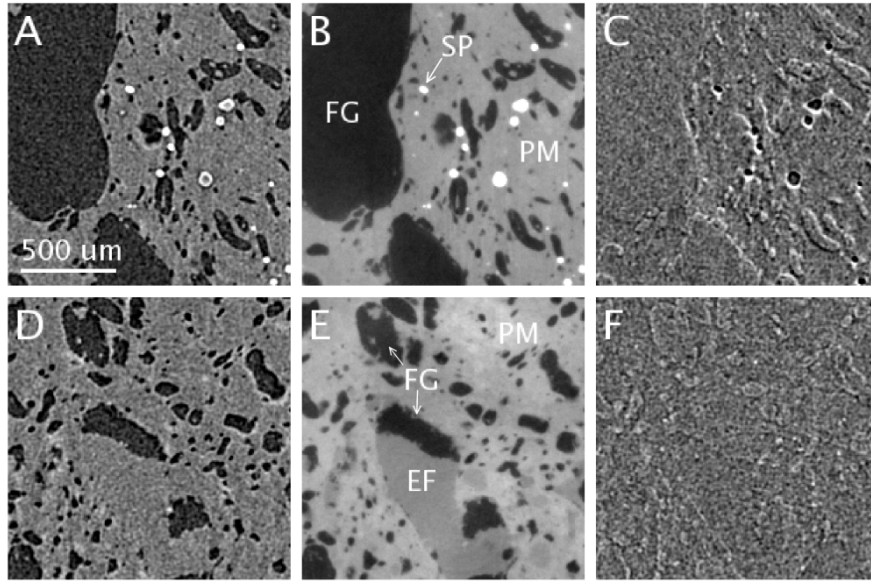


Figure 4.11: Partial transverse slices from the raw (top row) and cooked (bottom row) meat emulsion sample. In the three columns from left to right are presented the attenuation, phase-contrast and dark-field modalities. Labels are given in panels B and E: FG — fat globules; EF — expressible fluid; PM — mixture of protein, starch and moisture; SP — salt particle.

Tomographic slices are shown in figure 4.11 for all three modalities of the raw (top row) and cooked (bottom row) meat emulsion sample. From left to right, the attenuation, phase-contrast and dark-field modalities are presented. The complementarity of the signals are clearly seen. In the paper, the gray values of the three modalities were modeled as a mixture of multivariate Gaussian distributions using an expectation-maximization (EM) algorithm (Friedman et al., 2001). The number of known constituents were used as an input in the fitting to obtain the multivariate normal distributions. In figure 4.12 an example is shown where the distributions for the meat emulsion systems are represented by their covariance matrices.

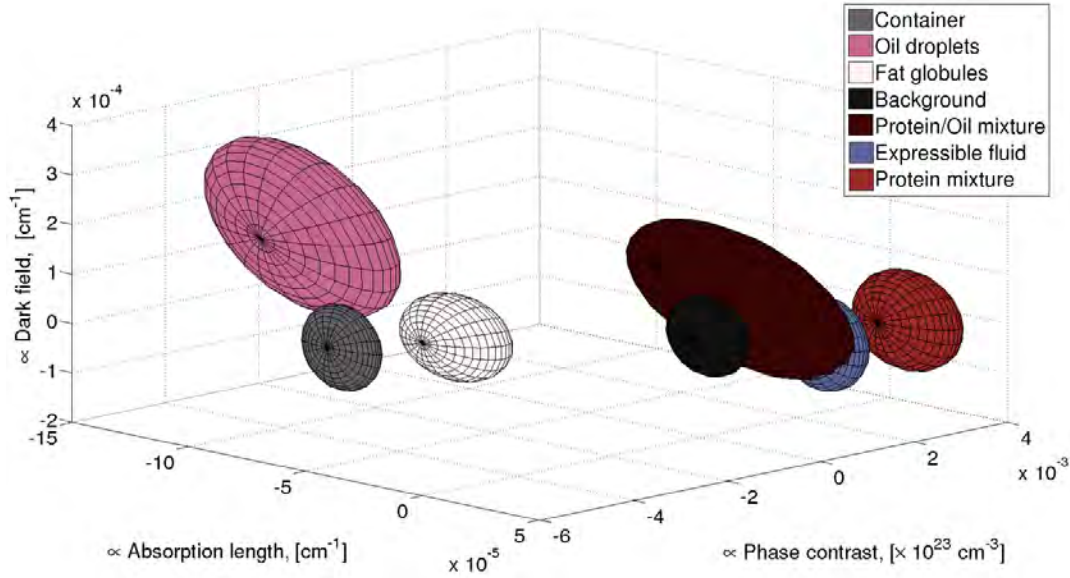


Figure 4.12: The result from the EM algorithm represented by the covariance matrices of the distributions. Note that the values are not to absolute scale, and zero point values are that of water.

In order to describe the spatial information, the data was modeled by a Markov random field (MRF) in 3D using six neighboring sites (voxels to the left, right, top, bottom, front and back). To find the optimal segmentation solution, the multi-labeling problem was solved using graph cuts with alpha expansions as described in [Boykov et al. \(2001\)](#). In figure 4.13, slices from the segmentation of one of the meat emulsions before (panel A) and after (panel B) heat treatment are shown. The multi-component segmentation depicts the protein phase in red and the lard in white. The salt particles present prior to heat treatment are shown in green, and the expressible fluid due to the heat treatment is represented in blue.

The paper also discusses the quality of the segmentation. Two experts established a ground truth through manual annotation. By comparing the segmentation classification to the ground truth, the overall accuracy of the segmentation was estimated to be approximately 97%.

From the segmented tomograms, the paper then goes on to calculate quantitative parameters for the meat emulsions. To characterize the fat particles, schemes for correct labeling are discussed, and a custom algorithm is implemented.

The paper highlights the need for new image analysis methods when novel multimodal image techniques are introduced. Multivariate approaches for image segmentation make use of all of the available information from all modalities.

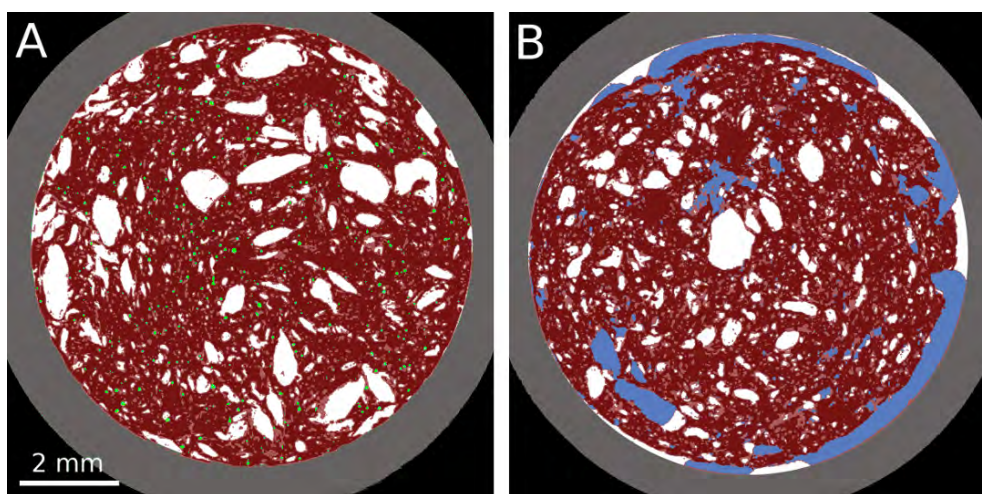


Figure 4.13: A slice from two of the segmented volumes. The protein phase is shown in red, salt-soluble protein in light red, the expressible fluid in blue, the salt particles in green and the lard in white. Panel A Raw meat emulsion sample, panel B cooked meat emulsion sample.

4.5 Outlook

We have in this chapter discussed a number of image analysis methods. In addition, possible applications were illustrated in this chapter and in [Paper III](#).

When novel and multimodal techniques are introduced, developments in image analysis are required to deal with the new possibilities. We saw for example how the Hilbert filter had been introduced to handle tomographic reconstruction of differential phase signals. Furthermore for multimodal images, additional information may be available which multivariate segmentation methods can utilize.

In this regard, [Paper III](#) was the first paper to demonstrate a multivariate segmentation method for all three modalities of the grating interferometer. By combining absorption, phase-contrast and dark-field CT in a multivariate Gaussian model, the full available image information was included in the segmentation process. This illustrates the benefit of combining complementary image modalities.

In addition, improvements in image segmentation may give a more robust feature detection in the images.

Effective techniques for tomographic reconstructions, denoising, segmentation and feature detection are necessary in order to extract information from images. This *translation* of the image is a prerequisite for quantitative analysis of the sample structures as well as for automated scanning systems. Improvement of the available image information can expand the applicability of a technique. For example with improved denoising methods, image information could be preserved at reduced exposure times. In turn, reduced scan times increase the temporal resolution of a technique. Furthermore by developing algebraic iterative reconstruction algorithms, improved reconstruction of differential phase-contrast can be achieved in parallel ([Fu et al., 2013](#)) and cone beam ([Fu et al., 2015](#)) geometries.

Chapter 5

Treating the image - Quantitative information

Often the goal with an imaging study is not reached by just obtaining the images. Rather, the processed and analyzed images form the starting point for acquiring quantitative information. In the previous chapters we discussed how to reach this starting point by *taking* and *translating* the image. In the final step of *treating* the image we can obtain quantitative physical information about the sample.

These quantitative measures may either be derived from spatial structures in the images or from quantitative voxel values which describe a material property such as the electron density.

Examples of spatial structural measures are object volumes, areas, thicknesses and shapes. These can often be related to mechanical properties of the sample. For e.g. porous media, the connectivity of the pore network and the wall thickness of the matrix are important for mechanical strength. In food science, this has been used to characterize porous products such as bread crumb (Falcone et al., 2005; Lassoued et al., 2007) and extruded cereal products (Chevallier et al., 2014). Furthermore, as X-ray CT is a non-destructive technique, tomographic time-series of pore evolution can also be obtained. This has been done e.g. in the case of bubble formation during bread-dough proofing and baking (Babin et al., 2006; Turbin-Orger et al., 2012).

The type of voxel value reconstructed in CT depends on the image modality used as discussed in 4.1. In conventional CT, the attenuation value can be obtained whereas the electron density is reconstructed in phase-contrast CT. The obtained values can be used to identify components or quantify deviations from expected values. As an example, a sub-voxel porosity in a material will give a reconstructed value smaller than the bulk value. The relative deviation can be used as a measure for the local sub-voxel porosity. In food science, an approach

using quantitative voxel values have not been reported for conventional CT. With grating-based phase-contrast CT, electron density values have been extracted and used to characterize porcine rind and fat ([Jensen et al., 2011](#)).

In some studies the quantitative measures are specific to the problem at hand. In [Paper II](#), edge detection was used to find the location of the degradation front in germinating barley seeds. By following the change in position with time, the movement of the degradation front could be quantified. In [Paper III](#), a sphericity measure was introduced to characterize the shape of the identified fat particles.

In the following, a few common measures and the algorithmic approaches to find them will be presented. In addition, the chapter will serve as an introduction to [Paper IV](#) ([Nielsen et al., under review 2015b](#)) and [Paper V](#) ([Miklos et al., 2015](#)).

5.1 Structural measures

As mentioned, quantitative structural measures deals with spatial shapes and sizes in the tomogram. Typically, the segmented tomogram is the outset for quantitative structural measures. At this point the gray levels of the tomogram has been reduced to a few integer values corresponding to the number of segmented phases. For computational ease, a further reduction to a set of binary images can be achieved by extracting each segmented phase individually.

Some of the more common structural measures are percent object volumes and size distributions of particles or objects.

5.1.1 Percent object volume

The percent object volume (POV) is a simple measure to quantify the sample composition from a segmented tomogram. The POV value for the j th component is calculated as the component volume V_j relative to the total sample volume V .

$$\text{POV}_j = \frac{V_j}{V}, \quad (5.1)$$

POV values can be calculated for each component or phase identified through image segmentation. For porous materials, the POV value for the pore phase is equal to the sample porosity. If the volumetric composition of the sample is known beforehand, the POV value can be used to validate the measured tomogram.

The POV measure was used in [Paper III](#), [Paper IV](#) and [Paper V](#).

5.1.2 Particle size distribution

In many imaging studies CT scans are employed to visualize internal particles or objects such as pores or inclusions. A simple way to characterize the particles or objects is to calculate their size distribution. An approach to do this is to identify each particle with a unique label. Then a size distribution can simply be calculated from the volume of each particle. In addition, other geometric parameters can be calculated once the labelmap is obtained. As an example, the sphericity of fat particles in [Paper IV](#) was calculated from the obtained labelmap.

In the following, two different approaches to create a labelmap are presented.

Flood fill algorithm

If the particles are spatially separated, then the labeling can be done by finding the connected components in a binary image through e.g. a flood fill algorithm. The flood fill is illustrated in figure 5.1.

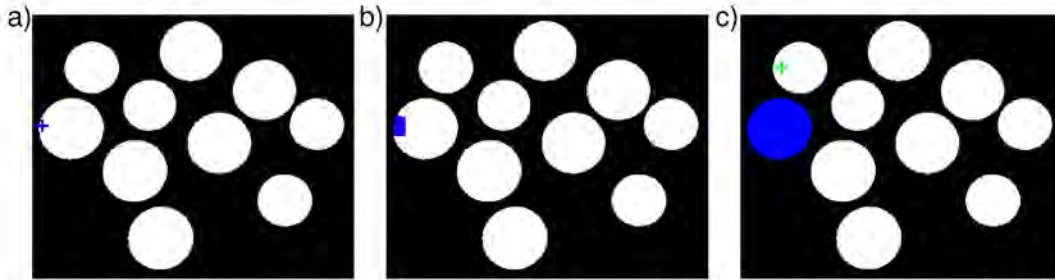


Figure 5.1: Illustration of the flood fill algorithm. a) The algorithm is initiated in one of the white pixels, and the color of the pixel is changed. b) In turn the neighboring pixels are probed, and the color of white pixels are changed while black pixels remain untouched. c) When no white neighboring pixels are left, a new seed point is found in another separate object, and the algorithm is initiated again using a new color.

The algorithm is initiated in a white pixel in one of the circular particles as seen in panel a) which is given a new label color. Then, the flood flows into the neighboring pixels which are given a new color if they are white as seen in panel b). When no white neighboring pixels are left, the algorithm starts in a new particle with another color as seen in panel c).

An example of the final result of finding connected components can be seen in figure 5.2 b). The ten circles have been colored in different colors, and in panel e) a histogram of their equivalent diameters (in blue) have been computed. The circles are found to have roughly two different diameters of 48 and 58 pixels.

These sizes can be compared to the (blue) scalebar in panel a) of a length of 50 pixels.

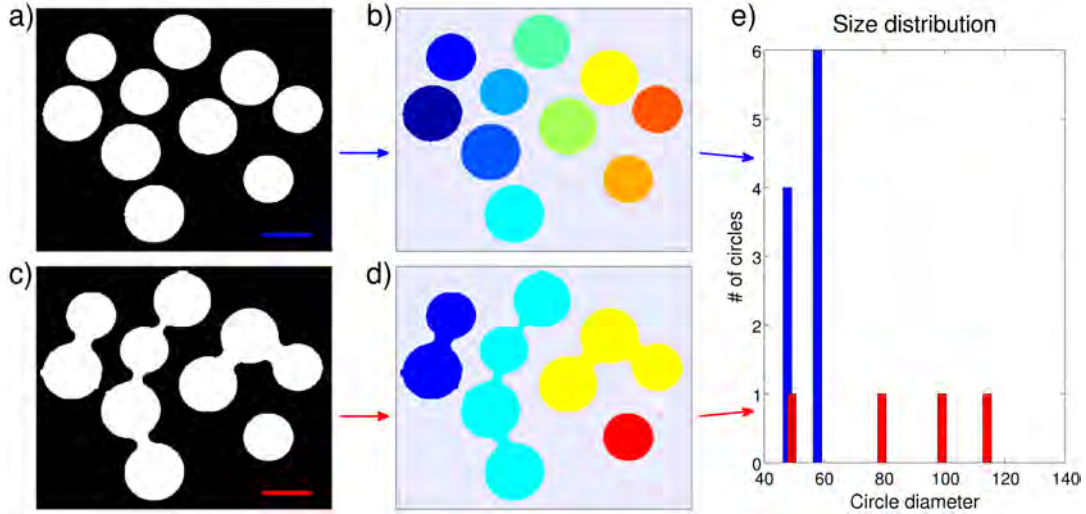


Figure 5.2: Connected components of a) separated white circles and c) touching white circles found by the flood fill algorithm. b) The labelmap for a). d) The labelmap for c). e) The equivalent diameters of the labeled components in b) and d).

While this approach works for spatially separated objects, it fails when objects are touching or connected by a thin segment as seen in figure 5.2 c). As seen in panel d), some of the objects receive the same color, and the size distribution changes as seen in red in panel e). In this case, another approach is needed such as the watershed algorithm.

Watershed

In the watershed approach, watershed lines are created to separate spatial structures in an image ([Beucher, 1982](#)). The idea is to interpret the image as a topographic measure with valleys and ridges. This can be done by calculating the gradient of a gray scale image or the distance map in a binary image. The latter computes the distance from the white pixels (ones) to the black pixels (zeros).

Using the topographic image, the watershed algorithm works by flooding the valleys (also called catchment basins) in steps starting from the lowest point (largest distance). When the water from two catchment basins meet, a dam is constructed to separate the two. When the water reaches the top of the basins the algorithm terminates, and watershed lines are constructed at the boundaries of the water and on the dams. A labelmap can then be extracted from the separated regions. For a more thorough introduction see [Kak and Slaney \(2001\)](#).

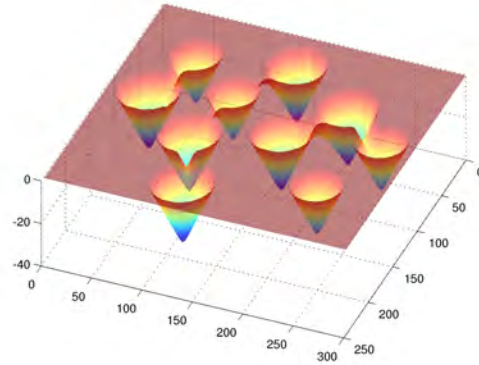


Figure 5.3: The connected white circles of figure 5.2 c) illustrated as a topographic representation. The depth in the surface corresponds to the distance to the black regions. The touching circles are connected by shallow channels.

An illustration of a topographic map can be seen in figure 5.3. The distance map of the white circles of figure 5.2 c) is shown as a 3D representation. The depth in the surface map corresponds to the distance from that pixel to the black pixels of the figure. The touching circles are seen to be connected by shallow channels. The idea is that the watershed algorithm will construct watershed lines in the shallow channels in order to separate the touching circles.

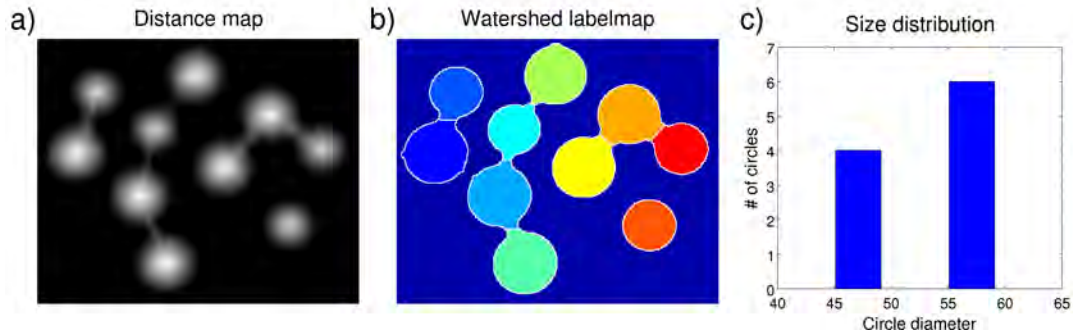


Figure 5.4: Illustration of applying the watershed algorithm. a) Distance map of figure 5.2 c). Bright colors correspond to a large distance. b) Resulting labelmap from the watershed algorithm. Watershed lines are shown in gray. c) Histogram of equivalent diameters.

The result of applying the watershed algorithm is summarized in figure 5.4. In panel a), the distance map is shown in color coding. Bright colors correspond to a large distance. The resulting labelmap from applying the watershed algorithm is seen in panel b). The watershed lines have separated the touching circles, and all circles have been given different colors. As seen from the histogram of the equivalent diameters in panel c), two different sizes of circles have been found which matches that of the separated circles.

5.2 Quantitative voxel values

When discussing quantitative voxel values, it is important to distinguish between relative and absolute values. When the gray level values of an image are used in e.g. edge detection and segmentation as discussed in chapter 4, it is the relative difference which is important. However, in order to link the voxels of a tomogram to the physical quantity they represent, values on an absolute scale are needed.

Having absolute voxel values transforms the image from a structural representation to a measurement of a material property. The material properties obtained in X-ray tomographic reconstruction were discussed in section 4.1. By comparing to calculated values, it becomes possible to validate whether the sample composition is as expected or not. To some extent, absolute tomographic values can also be used to identify materials in a sample of unknown composition. In [Paper V](#), absolute values were applied to validate the composition and identify the presence of microcrystalline cellulose.

For a sample that changes over time, absolute values can be used to track the diffusion of materials in the sample. This was used in [Paper V](#) to quantify the changes in electron density of a piece of beef due to cooking.

Two of the ways to ensure absolute values are either to perform a post-reconstruction calibration or to ensure it directly in the reconstruction.

- Calibration of a tomogram can be performed by including references in the sample. From tabulated values of the reference materials, a linear transformation can be applied to the measured tomogram to achieve absolute values. This approach was used in [Paper V](#).
- Absolute quantitative reconstructions require precise knowledge of the instrumental setup. Above all, the change in photon counts due to the sample must be measured precisely by e.g. including open beam images in the analysis. This is not always kept in absolute values in commercial scanners. Furthermore since the complex index of refraction is wavelength dependent, the X-ray spectrum of the source must be known. At synchrotron sources with monochromatic beams this is normally well-known. For X-ray sources used in labs and commercial setups, the broad X-ray spectrum is harder to account for. X-ray ptychography as used in [Paper IV](#) is an example of a quantitative technique where absolute values are directly in the reconstruction.

5.3 Introduction to Paper IV and V

Paper IV (Nielsen et al., under review 2015b) and Paper V (Miklos et al., 2015) both investigate the microstructure and electron densities of food products quantitatively using X-ray phase-contrast tomography. In the following, some main results and figures will be presented. For the full details we refer to appendix A where the papers in full length are attached.

A key difference between the two papers is the imaging method used. Whereas X-ray grating interferometry is applied in Paper V, the method of Ptychographic X-ray computed tomography is used in Paper IV. The reason for the choice of method lies in the relevant length scale. While Paper IV studies a food emulsion system consisting of structures on the sub-micron scale, Paper V investigates a piece of muscle tissue of a size of a cm. In the former, a nano-CT method is needed to resolve the individual microstructures. In the latter, a larger FoV is needed to study the different components in the muscle tissue.

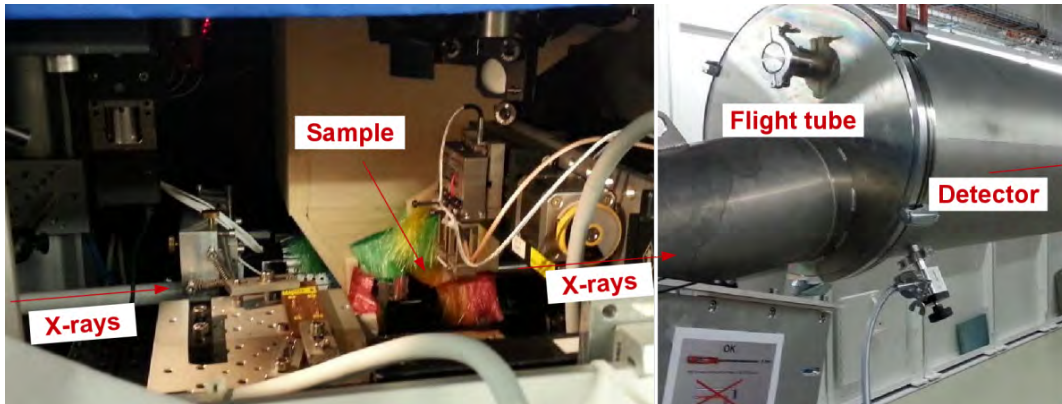


Figure 5.5: Photographs of the ptychography setup at the cSAXS beamline at SLS. The X-ray beam enters from the left and is focused by a Fresnel zone plate (not shown) before reaching the sample station in the middle. In order to reduce vibrations and instabilities, the sample area is enclosed. The sample is mounted by a robot which also allows for a high degree of automation in measurements. On the right, the X-ray beam exits into a evacuated flight tube before reaching the detector (not shown).

The setup for Paper V was the same as used in Paper III which has been described in section 4.4.

The setup for Paper IV is shown in figure 5.5. The X-ray beam enters from the left and is focused by a Fresnel zone plate (not shown) before reaching the sample station in the middle. On the right, the X-ray beam exits into a evacuated flight tube before reaching the detector (not shown). Rotation of the sample station allows for tomography measurements. The sample area is enclosed in order to reduce vibrations and instabilities.

The setup parameters can be found in table 2.1 on page 33, and the acquisition details are listed in the full length of the papers in A.

5.3.1 Introduction to Paper IV

The title of [Paper IV](#) is 'Ptychographic X-ray computed tomography of extended lipid networks in food emulsions' ([Nielsen et al., under review 2015b](#)). The paper presents the first application of ptychographic X-ray computed tomography (PXCT) for a food science sample. In the study, a quantitative study of a dairy-like oil-in-water (O/W) emulsion is performed using microstructural and electron density information. The main question is whether the lipid phase forms an extended lipid network or not.

Typically, extended lipid networks are not found in O/W emulsions. Formation of fat crystal networks are limited to within the individual lipid droplet. However, through a process known as partial coalescence droplets can be linked together following collision events. In extreme cases of partial coalescence, this can lead to formation of a space-filling lipid network even in an O/W emulsion. Recent studies indicated that this was the case for an almost semi-solid palm-kernel oil based O/W emulsion ([Munk et al., 2014](#); [Munk and Andersen, 2015](#); [Munk et al., 2013](#)). However, a 3D imaging technique with soft-material contrast and sub-micron resolution would be needed to confirm the existence of a partial-coalesced extended lipid network.

In [Paper IV](#), we demonstrate PXCT to be a suitable method for 3D nano-imaging of food emulsions. The high spatial resolution and soft-material contrast allows for resolving even finer details. A total of four tomographic measurements of four emulsion samples were conducted at room temperature and atmospheric pressure. The samples were made in the same way as in the previous studies and consisted of a 25 w% lipid and a 75 w% water phase.

A 3D representation of the segmentation of one of the resulting tomograms is shown in figure 5.6. The applied MRF segmentation was able to identify the lipid and water phases as intended. Depicted in panel a) in yellow and orange is the connected and unconnected lipid, respectively. Almost all of the water phase was connected as indicated in blue in panel a). Nonetheless a few small pockets of water were encapsulated by the lipid as shown in green in panel b). Furthermore, an additional high electron density component was found which was identified to be microcrystalline cellulose from the added stabilizer mixture. A zoom-in on three cellulose particles depicted in gray is shown in panel c).

As seen from panel a) of figure 5.6 almost all of the lipid phase was connected in a single space-filling network. This was in agreement with the hypothesis of

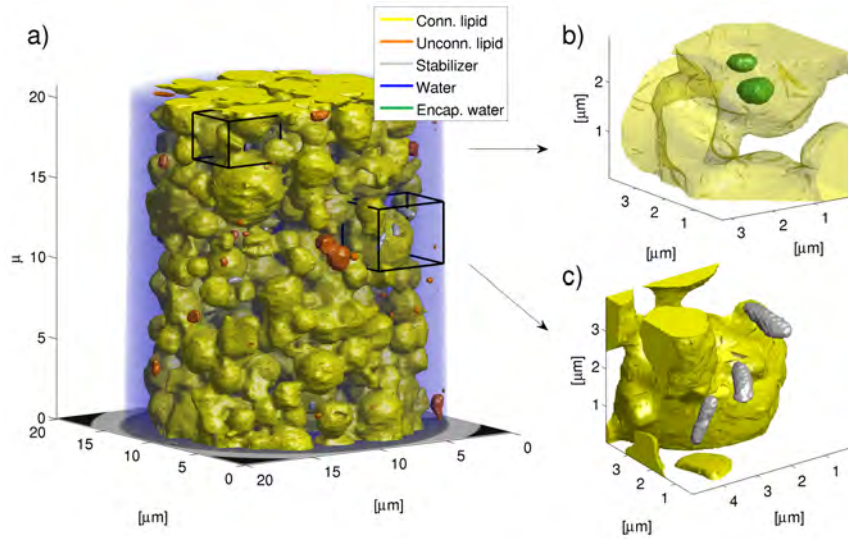


Figure 5.6: 3D representation of the sample emulsion. a) The full emulsion with the water phase shown in blue, the stabilizer in phase in gray, the connected lipid phase in yellow and isolated lipid regions in orange. b) A close-up of the sub-volume in the top box of a) depicting an isolated water pocket inside the connected lipid phase. c) A close-up of the stabilizer phase in the sub-volume of the bottom box of panel a).

partial coalescence of the lipid phase. To test this hypothesis further, a watershed algorithm was used to identify localized volumes of the lipid phase. A 3D representation of the resulting labelmap is depicted in panel a) of figure 5.7. The identified lipid domains appear approximately spherical in shape. A quantitative comparison of the size distribution of the lipid domains from all four tomograms are seen in panel b) of figure 5.7. The mean value of each distribution is listed in table 5.1. The found distributions and mean values from the tomograms compares well to particle size measurements of lipid globules using dynamical light scattering. The mean globule diameter found in a previous study (Munk et al., 2013) is also listed in table 5.1. These results are an additional indication that the lipid network has formed through partial coalescence.

To validate the measurements, additional quantitative parameters were found as shown in table 5.1 and compared to calculated reference values. The measured POV values are seen to vary between the four tomograms. Since values both larger and smaller than the calculated reference values are observed, this could reflect local variations in the bulk sample on the micrometer scale. The measured mean electron densities are also seen to be in fair agreement with the calculated reference values. The values for lipid and water are within 4%, while the deviation for the cellulose could be explained by partial voxel effects due to the small POV.

Thus, in Paper IV the use of PXCT allowed for a successful visualization and

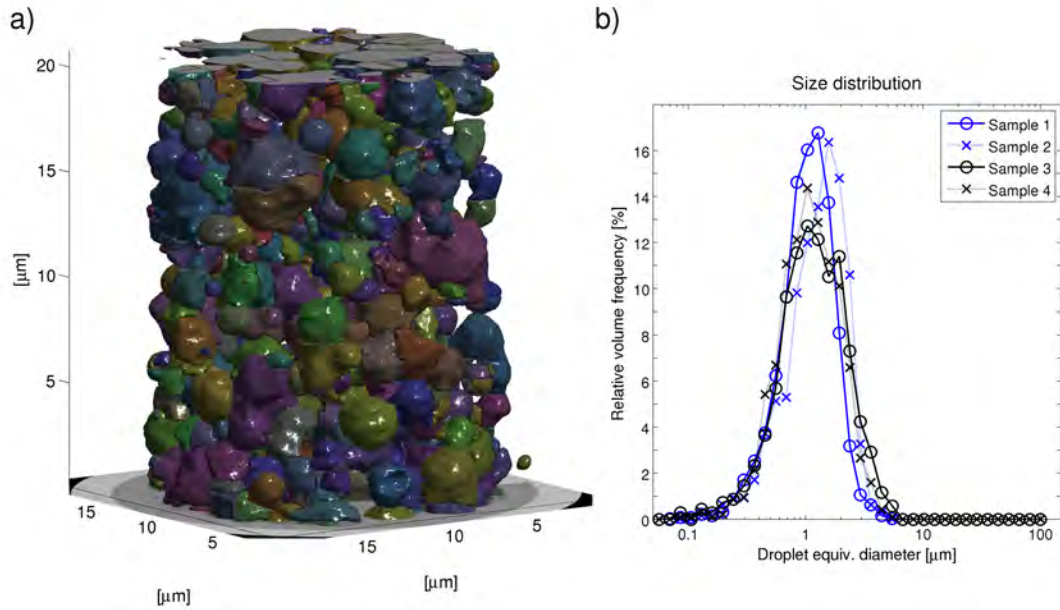


Figure 5.7: a) A 3D representation of the resulting labelmap after applying the watershed algorithm to the connected lipid phase from figure 5.6 panel a). The coloring distinguishes individual domains. b) The size distributions of the equivalent diameters for all four samples.

quantitative investigation of the 3D extended lipid network in a food emulsion. This demonstrated the potential for using PXCT in food science. Being a fairly recent imaging method, the possible applications of PXCT are currently being investigated. The quantitative nature of PXCT makes the technique appealing for food science cases albeit the limited availability is a disadvantage. However, as X-ray ptychography has been found especially promising at fourth-generation synchrotrons ([Thibault et al., 2014](#)), the technique could gain a wider use in Scandinavia with the opening of MAXIV in Lund.

Percent object volume (POV)					
Data type	Ref [vol%]	Sample 1 [vol%]	Sample 2 [vol%]	Sample 3 [vol%]	Sample 4 [vol%]
Lipid phase	28.4	31.2	38.1	37.7	26.5
Water phase	71.4	68.7	61.9	62.2	73.5
Cellulose	0.2	0.05	0.01	0.03	0.02
Electron densities, ρ_e					
	[e/Å ³]	[e/Å ³]	[e/Å ³]	[e/Å ³]	[e/Å ³]
Lipid phase	0.308	0.314	0.323	0.321	0.318
Water phase	0.348	0.362	0.363	0.360	0.360
Cellulose	0.477	0.397	0.382	0.391	0.374
Micropipette	0.666	0.668	0.671	0.673	0.668
Bulk	0.336	0.347	0.348	0.345	0.349
Mean equivalent diameter, d					
	[μm]	[μm]	[μm]	[μm]	[μm]
Lipid phase	0.98	1.15	1.41	1.40	1.25

Table 5.1: Percent object volume (POV), mean electron densities and equivalent lipid diameter from the four tomograms compared to reference values. The reference values for POV and electron density were calculated from the ingredients used in the sample preparation. The reference value for mean equivalent diameter was taken from [Munk et al. \(2013\)](#). The bulk emulsion electron density was calculated from the ρ_e and POV values of the emulsion phases in the table. Electron density values for the micropipette are included as well.

5.3.2 Introduction to Paper V

The title of [Paper V](#) is 'Novel X-ray phase-contrast tomography method for quantitative studies of heat induced structural changes in meat' ([Miklos et al., 2015](#)). In the paper, X-ray phase-contrast CT using a grating interferometer is applied as a quantitative method for studying heat-induced structural changes in a piece of raw beef. The phase-contrast modality was needed since inadequate contrast was provided by conventional attenuation-based CT as illustrated by figure 5.8. While the conventional slices of the top row show very few features, several are visible in the phase-contrast slices in the bottom row. Since a non-destructive method was applied, the same sample could be measured before and after heat treatment, and a direct comparison was possible. Through image segmentation and analysis, quantitative measures for the structural changes could be derived.

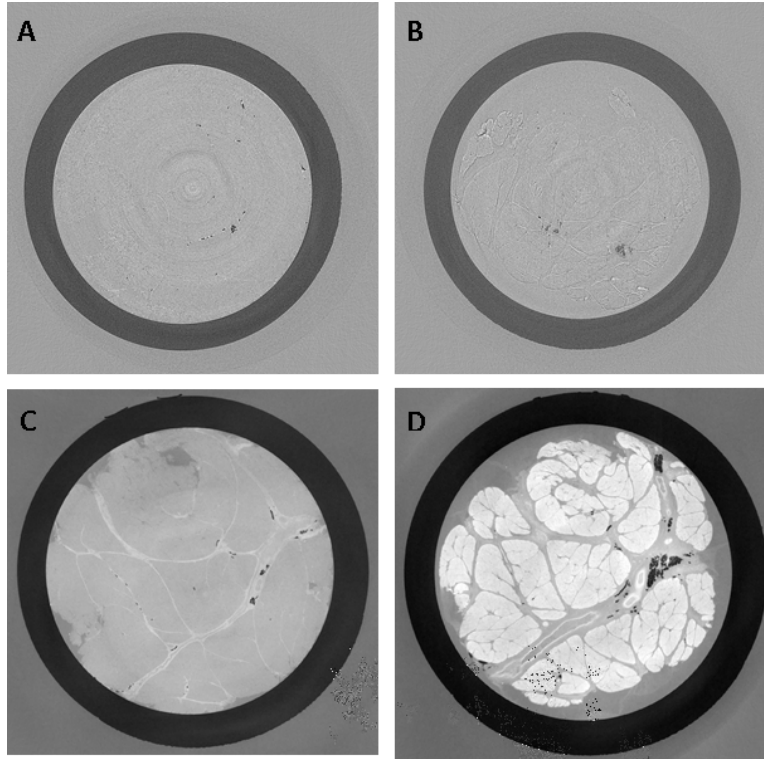


Figure 5.8: Tomogram slices in different modalities of the beef sample before and after heat treatment. A) Attenuation slice of raw meat. B) Attenuation slice of boiled meat. C) Phase-contrast slice of raw meat. D) Phase-contrast slice of boiled meat.

Cooking of meat results in extensive structural changes in both connective tissue and muscle fibers which are important for the eating quality of meat ([Christensen et al., 2011](#)). Denaturation of the different meat proteins causes conformational changes such as transversal and longitudinal shrinkage of the muscle fibers and the shrinkage and solubilization of the connective tissue ([Tornberg,](#)

2005). The heat induced structural changes of meat are conventionally studied by microscopy or indirect quantitative methods such as differential scanning calorimetry (DSC), turbidity measurements or protein solubility. However, as these methods are destructive in nature, it is not possible to monitor changes in a single sample. Although a non-destructive technique such as X-ray CT could be used, the soft-tissue contrast in attenuation-based CT is inadequate to distinguish the changes. In order to do this, the high sensitivity of X-ray phase-contrast CT is needed.

Measurements were performed on the sample before and after heat treatment. Two tomographic slices through the sample are shown in figure 5.8 for both the attenuation (panels A-B) and phase-contrast (panels C-D) modality. The raw beef is shown in A and C and the cooked in panels B and D. By comparison, the phase-contrast modality is seen to reveal more details of the beef sample. While only intramuscular fat can be distinguished in the attenuation slices, both muscle fibers, connective tissue, fat and the water phase surrounding the sample are distinguishable using phase-contrast. The superior contrast is highlighted even further when comparing the raw and cooked sample. Using the phase-contrast modality, huge changes in the muscle fiber, connective tissue and water phases are apparent as seen from panels C and D of figure 5.8.

In the paper, the segmentation method described in [Paper III \(Einarsdóttir et al., 2014\)](#) is applied on the tomograms. A view of the segmented horizontal slices are shown in figure 5.9 for the (A) raw and (B) cooked beef. The corresponding electron density histograms are shown in panels C and D for the raw and cooked beef, respectively.

A comparison of panels A and B of figure 5.9 indicates a shrinkage of the muscle fiber volume and an increase of the connective tissue and water phase after heat treatment. This observation is confirmed quantitatively by the percent object volume (POV) values shown in table 5.2. The muscle fiber POV of the full tomogram volume has shrunk from around 86% to 55%. Correspondingly, POV values for the water phase and connective tissue have increased from around 5% and 8% to approximately 20% and 25%, respectively. The volumetric changes may be explained by water being expelled from the muscle tissue and taken up by the connective tissue.

The explanation is supported by the electron density values in figure 5.9 panels C and D. After heat treatment, the muscle tissue contains less water relative to protein. As muscle fiber protein has a higher electron density than water, the value increases from roughly $3.52 \times 10^{23} \text{ cm}^{-3}$ to $3.64 \times 10^{23} \text{ cm}^{-3}$. In contrast, the uptake of water by the connective tissue lowers the electron density from around $3.58 \times 10^{23} \text{ cm}^{-3}$ to $3.52 \times 10^{23} \text{ cm}^{-3}$.

Furthermore the paper discusses the finer electron density variations of the

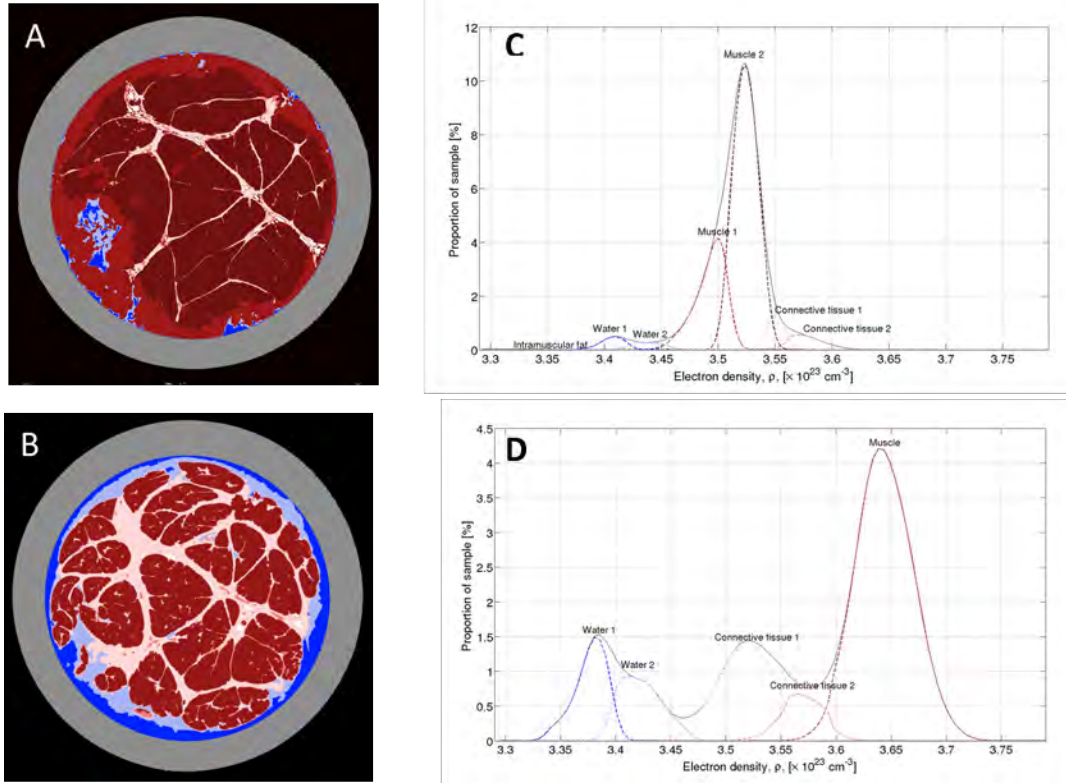


Figure 5.9: A-B: Slices of the segmented tomogram of the sample (A) before and (B) after heat treatment. The muscle fiber is shown in tones of red, the connective tissue in white, water in blue, lipid in black and the sample container in gray. C-D: The electron density distributions of the different sample components before and after heat treatment, respectively.

different components. For both muscle fibers, water phase and connective tissue more than a single phase can be identified. By measuring the heat induced changes on the same sample, this paper indicates future possibilities for quantitative dynamical studies using X-ray phase-contrast CT. A central limitation for these studies is the measurement time per tomogram. For X-ray grating interferometry at synchrotron facilities, acquisition times of approximately 1 h are needed. Thus, either the changes need to take place at much longer time scales than 1 h. Otherwise the processes must be controllable such that they are halted during measurements and restarted in-between.

Component	Raw	Cooked
Water	4.87	19.54
Muscle fibers	86.18	54.96
Connective tissue	8.41	24.89
Intramuscular fat	0.54	0.61

Table 5.2: Percent object volumes (POV) of the soft tissue components of the raw and cooked beef.

5.4 Outlook

We have in this chapter and in [Paper IV](#) and [Paper V](#) demonstrated two quantitative studies of food products using X-ray phase-contrast CT.

Although both of these papers describe results obtained with a synchrotron X-ray source, the quantitative methodologies could be applied to studies using laboratory or commercial setups with the accompanying limitations. In both papers, a combination of structural and material quantitative parameters were used to analyze the food product.

[Paper IV](#) was the first paper to apply ptychographic X-ray CT within food science. While soft tissue samples are often frozen for PXCT studies, the food product was kept in its native state at room temperature during measurements. Thus, this demonstrates that food products can be imaged non-destructively even at the submicron scale using PXCT without prior sample fixation measures. The obtained spatial resolution of around 300 nm brings PXCT within the range of conventional methods such as confocal laser scanning microscopy (CLSM). Although only accessible at synchrotrons, the technique could prove essential for high-resolution 3D imaging of food samples where CLSM fails.

The paper also highlights how quantitative structural parameters can be extracted from the segmented tomograms and compared to other types of measurements. In a next step, finite element modelling on the lipid structures could be used to obtain mechanical quantitative parameters. This could then be compared to direct measurements on bulk emulsions. Within food lipid networks, previous studies of the structure of fat crystal networks using 2D microscopy images have linked the fractal dimension of the network to the elastic modulus ([Narine and Marangoni, 1999a,b](#)). X-ray phase-contrast CT could be a way to expand this approach to 3D.

[Paper V](#) demonstrated quantitative measures of both structural and electron density changes in cooking of meat. The paper provided snapshots of the same meat sample before and after heat treatment. The changes in the sample could

thus be followed due to the non-destructive nature of X-ray phase-contrast CT. Using methods such as CLSM or histology which both require staining of the sample, different cuts would have to be compared. The paper indicates a potential for using X-ray phase-contrast CT quantitatively to follow changes in a sample. The next step would be to obtain a fully time-resolved study of the structural and electron density changes during cooking.

Already in recent years, time-resolved (or 4D) X-ray phase-contrast CT studies of living organisms have been performed. Using grating interferometry, the breathing of a worm could be followed in real-time ([Momose et al., 2011](#)), and by using propagation-based imaging, impressive in-vivo studies of a fly during flight have been conducted ([Mokso et al., 2015](#); [Walker et al., 2014](#)). In the latter case, quantitative structural parameters were extracted from the tomograms making it possible to study the mechanics of the fly motor.

The key limitation for time-resolved studies lies in the tomographic acquisition time. When using standard tomographic reconstruction approaches, the sample is assumed to be static for all angular projections. If the time-scale of sample changes are much slower than the acquisition time, then this is approximately valid. Otherwise if the changes are periodic in time, projection-based gating can be used to fulfill the reconstruction requirements. The latter was the approach used for the fly.

In many food products, the relevant changes are non-periodical. However, in contrast to in-vivo studies, the changes would often be induced by an external source which can be designed to match the acquisition time. This was the case in a recent time-resolved study of bubble flow in a foam ([Raufaste et al., 2015](#)). Here the flow velocity could be controlled in order to ensure successful tomographic acquisition. Similar experiments with food science relevance could be envisioned in coming years which could provide a novel rheological and mechanical understanding of food microstructure.

Due to improvements in both brilliance of synchrotron X-ray sources and detector acquisition rates, time-resolved CT measurements will become increasingly feasible during the next few years. This will also mean a dramatic increase in data size per measurement as the data becomes four-dimensional. The computational requirements thus become even more critical for a successful imaging experiment. Development of fast algorithms, standardized data processing pipe-lines and use of parallelized work-flows on computer clusters will be needed in order to keep up with the data production at synchrotron beam lines.

Chapter 6

Conclusion

A number of new applications of novel X-ray imaging modalities within food science have been presented in this thesis. This includes the introduction to and presentation of five original papers (Papers I-V). Copies of the original articles are attached in Appendix A. The thesis work has emphasized that an X-ray imaging study consists of several steps; from the initial image acquisition to the final quantitative result. These steps have been presented as *taking*, *translating* and *treating* the image.

When *taking* the image, knowledge of the physical interactions is important for image reconstruction and understanding contrast formation. The introduction to X-ray grating interferometry and X-ray ptychography in chapter 2 highlighted this point. For reconstruction of the sample properties in both techniques, a theoretical framework was presented. In grating interferometry, the sample influence on the interference pattern led to three complementary signals: transmission, differential phase-contrast and dark-field. From the coherent diffraction patterns in ptychography, the transmission and phase could be reconstructed using the Fraunhofer Fourier propagator.

The availability of complementary modalities expands the versatility of X-ray imaging when *taking* the image. This was demonstrated by the complementary transmission and dark-field radiographs of raw, frozen and defrosted fruits and berries. While no difference was observed in the conventional transmission radiograph, the novel dark-field modality could distinguish frozen from raw or defrosted. However, to achieve an optimal contrast the X-ray physics of the contrast mechanism must be understood as discussed in chapter 3. In case of the dark-field modality, the structural sensitivity of the setup determines the level of contrast. Using this knowledge, the dark-field signal from germinating barley seeds could be related to stress cracks and starch granules.

Through image analysis, the qualitative information of an image can be *trans-*

lated, and used to further data analysis. When novel X-ray imaging techniques are introduced, developments in image analysis methods may be needed as discussed in chapter 4. As an example, a multivariate segmentation approach was developed and applied to multimodal X-ray images from grating interferometry. By combining information from all modalities, the feature identification of a multicomponent food emulsion system could be improved.

Finally, quantitative results in an imaging study can be obtained by *treating* the image. As discussed in chapter 5, quantitative data analysis can rely on spatial structures or quantitative voxel values. As an example, a structural quantitative result was demonstrated for the 3D microstructure of a lipid network in a food emulsion system. Here the lipid network could be quantitatively characterized with a 300 nm spatial resolution. The connected lipid network could be determined, and the size distribution of the lipid domains was obtained. As an example of quantitative voxel values, the electron density changes in a piece of meat due to heat treatment were observed.

As described, a number of X-ray imaging studies within food science have been presented in this thesis. The goal of these was to determine the potential of novel X-ray imaging techniques within food science. Through the studies two advantages of X-ray imaging were apparent.

First, the non-destructive nature of X-ray imaging allows for in-situ time-dependent studies. This was indicated in [Paper III](#) and [Paper V](#) by monitoring the electron density of a meat emulsion system and a piece of meat before and after heat treatment. In addition, the in-situ potential of novel X-ray imaging techniques was illustrated in [Paper II](#) where the structural changes in germinating barley was monitored. As the latter study was performed using a laboratory setup, this indicates a potential for use in industrial settings. Industrial relevant applications could both be envisioned for quality test inspection in laboratories or in automated on-line inspection through development of a conveyor-based scanning system.

Secondly, the combination of complementary image modalities with 3D tomography studies demonstrates a potential for microstructural investigations of food systems. Especially the sensitivity in the phase-contrast modality towards small mass density differences has shown promise. In [Paper V](#) the phase-contrast modality demonstrated superior contrast over the conventional attenuation, and in [Paper IV](#) phase-contrast gave the first 3D visualization of a food system using X-ray ptychography. The two studies also demonstrated the range of structures accessible by X-ray imaging techniques. While computed tomography (CT) using grating interferometry at synchrotrons operates with resolutions of tens of μm , ptychographic X-ray CT (PCXT) successfully demonstrated a resolution of 300 nm.

In conclusion, the various applications presented in this thesis have indicated a strong potential of using novel X-ray imaging modalities in food science.

6.1 Comparison of X-ray phase-contrast imaging techniques

When preparing an imaging experiment, one of the first decisions is the choice of imaging technique. As mentioned in the introduction, several X-ray phase-contrast imaging techniques are available. Three of these are X-ray grating interferometry (XGI), propagation-based imaging (PBI) and PXCT of which the first and latter were applied in the thesis work.

These three techniques have different advantages and disadvantages. Both with regards to spatial resolution which is on very different scales, the quantitativeness of the the image voxel values and the applicability for commercial setups. A summary of the capabilities for the three methods is presented in table 6.1. In the table, the techniques are rated by 'X' (capable), '(X)' (partial capable) and '-' (not capable).

Technique	XGI	PBI	PXCT
Nanoscale res [$< 1 \mu\text{m}$]	-	-	X
Micronscale res [$1 \mu\text{m}$ - $100 \mu\text{m}$]	(X)	X	-
Quant. voxel values	(X)	(X)	X
Macroscale FoV [$> 1 \text{ cm}$]	X	X	-
High temp. res. [$< 1 \text{ s}$]	(X)	X	-
White beam	X	(X)	-
Coherent source	(X)	X	X
Synchrotron CT setup	X	X	X
Commercial CT setup	X	(X)	-
Conveyor belt potential	X	-	-

Table 6.1: Comparison of the three techniques X-ray grating interferometry (XGI), propagation-based imaging (PBI) and ptychographic X-ray CT (PXCT). The techniques are rated by X (capable), (X) (partial capable) and - (not capable).

As seen from the table, PXCT is the only of the three techniques that offers a spatial resolution on the nanoscale, and reconstructs quantitative electron densities on an absolute scale. On the other hand, PXCT requires a monochromatic beam and high transverse coherence which can only be found at synchrotron sources. Furthermore the technique is limited to a small FoV and has rather

long acquisition times on the order of hours. Thus, PXCT should be selected for (static) structural investigations of samples where a resolution on the nanoscale and quantitative electron densities are required. Given that a synchrotron is needed, the study should concern fundamental research questions of high scientific importance.

The strength of PBI is the very high temporal resolution which allows for studying dynamical processes in the sample. As mentioned in the outlook of chapter 5, PBI has been used for in-vivo studies of a fly during flight ([Mokso et al., 2015](#); [Walker et al., 2014](#)). Using a gating approach, an effective temporal resolution of 1.5–3 kHz was achieved. At synchrotrons, this high temporal resolution can be combined with a tunable spatial resolution in the order of $1\text{ }\mu\text{m} - 50\text{ }\mu\text{m}$. Thus, processes at different time- and length-scales can be investigated. However, in general quantitative voxel values cannot be obtained within this framework, and the phase-contrast reconstruction is only valid for samples of two materials. Thus, PBI should be selected for dynamical studies of samples where the absolute quantitative voxel values are not needed. The simple instrumental setup needed for PBI allows for a varied sample environment, and studies with a varying temperature, pressure or atmosphere are a promising venue for novel experiments.

XGI is the one of the three techniques with the largest potential for commercial applications. It is also the only technique which provides the dark-field contrast. As mentioned in the outlook of chapter 3, a dedicated XGI commercial scanner has recently become available. Compared to a synchrotron, acquisition times are longer, and the transverse coherence is lower with a commercial scanner. However, a local setup allows for other types of measurements than what can be done within a limited synchrotron beamtime. In addition, a commercial setup also makes X-ray phase-contrast imaging available for product development or quality assurance applications within the industry. Since XGI is also available at synchrotrons, lab-based studies can be conducted prior to or in relation to beam-times. Synchrotron setups using XGI are comparable to PBI in spatial resolution although most setups have a vastly reduced temporal resolution. However, XGI allows for a better reconstruction of quantitative voxel values compared to PBI and in addition provides the dark-field contrast. Thus, XGI should be selected for practical applications of X-ray phase-contrast imaging and for all dark-field imaging. In addition, studies of a longer duration can better be performed using a XGI at a local setup. At the synchrotron, XGI should be selected for providing quantitative voxel values when a larger FoV is needed than with PXCT.

6.2 Outlook

Since this thesis has been centered around *taking*, *translating* and *treating* the image, the three steps received independent chapters in the thesis. As a result, an outlook of contrast formation, image analysis and quantitative results in X-ray imaging studies have already been given. In the present chapter, some of the broader perspectives will be addressed.

Overall, the studies presented in this thesis were novel within their field. They represent proof-of-principle studies that need further validation in order to be of wider use. Thus, one way to proceed would be to conduct further studies to validate the proposed applications against conventional methods. This could be a study to compare modification patterns in germinating barley using light microscopy techniques versus the dark-field radiography approach proposed in [Paper II](#). Validating the measured electron density changes in heat treated food systems to water loss and texture measurements could be an additional example. Finally, it could be a possibility to compare 3D confocal laser scanning microscopy to PXCT of food emulsions or another food system.

Another venue to consider is commercial X-ray phase-contrast scanners. Within grating-interferometry, commercial scanners are either being developed ([Koehler et al., 2015](#)) or already available for sale ([Bruker microCT, 2015](#)). These scanners present a perspective for widespread use of grating interferometry within the next few years. In this regard, the demonstration of a wide range of possible applications increase the scientific or industrial case for using novel X-ray imaging modalities. The applications presented in this thesis illustrate several interesting cases within food science. In addition, the novelty of the approach within food science suggests that many other applications may still be possible.

Besides grating interferometry, other phase-contrast modalities may become commercial available in the future. The recent development of a new liquid metal jet X-ray source ([Larsson et al., 2013](#)) with increased beam brilliance could pave the way for propagation-based imaging or other techniques. At the Technical University of Denmark (DTU), a liquid metal jet source is being implemented in a nanoCT setup ([DTU Industry Portal, 2015](#)) with Zernike phase-contrast and a spatial resolution down to 50 nm.

The quantitative results discussed in chapter 5 indicate how X-ray imaging can be used to measure material properties. Within materials science, quantitative X-ray CT has become a commonly available tool within materials labs ([Maire and Withers, 2013](#)). Here, the segmented tomogram forms the starting point for further modeling rather than the final result. By calculating meshes from the segmented structure in a tomogram, finite element (FE) modeling can be performed using the volumetric data as input ([Youssef et al., 2005](#)). Through

the use of FE, micromechanical properties such as stress-strain relationships, fluid permeability and thermal transport can be modeled ([Maire and Withers, 2013](#); [Müter et al., 2015](#); [Youssef et al., 2005](#)). In addition to artificial material foams, the approach of using FE on tomography data has also been applied to a biomaterial i.e. the shell of a heart urchin ([Müter et al., 2015](#)). Within food science, the displacement field of a bread crumb has been modeled using X-ray tomography data from a compression load study ([Moussawi et al., 2014](#)).

The combination of X-ray tomography data and FE modeling can become a powerful tool in studying material properties of food materials. When the approach is developed and validated, the relation between microstructure and macro properties of food designs can be investigated. This could give fundamental knowledge on e.g. texture formation and hardness in food products.

Altogether, X-ray imaging is a promising technique within food science and food materials. Furthermore, the novel X-ray imaging modalities described in this thesis are especially appealing due to their sensitivity towards microstructural and electron-density differences present in food materials. The outlook of this PhD work points to an increasing interest for and more widespread use of X-ray imaging techniques within food science in the foreseeable future.

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Appendix A

Publications

The five papers, papers I - V, are attached here.

Publication I

Food Control

M. S. Nielsen, L. B. Christensen, R. Feidenhans'l.

Frozen and defrosted fruit revealed with X-ray dark-field radiography.

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Short communication

Frozen and defrosted fruit revealed with X-ray dark-field radiography

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ABSTRACT

A novel non-destructive method for distinguishing frozen and defrosted fruit and berries using X-ray dark-field radiography is proposed. In this proof-of-principle study we are able to discern between the raw and frozen state of two kinds of berries and a piece of mandarin as well as between the raw and defrosted state of one of the berries. Contrast-to-noise (CNR) values of around 2.5 are obtained with X-ray dark-field radiography whereas almost no contrast is found with conventional X-ray radiography with CNR values around 0.2.

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1. Introduction

Freeze injuries in fruits and berries are unwanted as the structural changes induced by freezing can influence the functionality of the crop. Therefore, great interest has been put in methods for detecting microstructural changes due to freeze injuries. Previous studies have employed a range of methods such as magnetic resonance imaging (MRI) for detection of the reduction in water proton density due to freeze injuries in blueberries, oranges and mandarines (Gamble, 1994; Hernández-Sánchez et al., 2004; Kim et al., 2008) as well as ultra-violet (UV) fluorescence and machine vision for detection of surface changes due to freeze injuries in oranges (Slaugther et al., 2007). However, these methods have fundamental challenges with respect to industrial requirements for high speed inspection capacity and revelation of internal features.

As a novel non-destructive method for detecting the frozen, raw or defrosted state of a piece of fruit, we propose to employ X-ray dark-field radiography which has recently been introduced (Pfeiffer et al., 2008) and to compare this approach to conventional transmission X-ray radiography. Conventional X-ray radiography has previously been applied for inspection of internal quality parameters of agricultural produce (Kotwaliwale, Weckler, Brusewitz, & Kranzler, 2007; Narvankar, Singh, Jayas, & White, 2009) but has to the authors' knowledge not been successfully used for detection of freeze injuries.

The advantage of dark-field radiography over the conventional transmission radiography is that it is sensitive to ultra small-angle X-ray scattering originating from the microstructure of the sample whereas transmission radiography measures the attenuation of the X-rays. This means that the dark-field signal is sensitive to structural differences on the micrometer scale while the contrast in the transmission signal originates from differences in mass density and/or atom number (Bech et al., 2010; Lynch et al., 2011; Yashiro, Terui, Kawabata, & Momose, 2010). This complementarity in the two image modalities has recently been employed for foreign body detection of paper, insects and textile in food products (Kottler et al., 2010; Nielsen, Lauridsen, Christensen, & Feidenhans'l, 2013).

One of the most promising techniques for dark-field radiography is grating-based interferometry (David, Nöhammer, Solak, & Ziegler, 2002; Momose, 2003; Weitkamp et al., 2005) which can be adapted to laboratory-based setups (Pfeiffer, Weitkamp, Bunk, & David, 2006). A great advantage using the X-ray grating interferometer is that it simultaneously records transmission and dark-field radiography, and can also be applied for CT.

In this study, we inspect berries and fruit using dark-field radiography with a lab-based X-ray grating interferometer.

2. Experimental method

The X-ray grating interferometer outlined in Fig. 1 has previously been described in detail (Pfeiffer et al., 2006; Weitkamp et al., 2005). It consists of an X-ray phase-grating G1 and an analyzer absorption grating G2. At laboratory setups a third grating, G0, is included to obtain satisfactory spatial coherence in the horizontal direction perpendicular to the grating lines.

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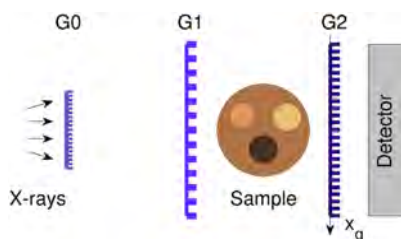


Fig. 1. X-ray grating-based setup with sample. The phase-grating, G1, and analyzer grating, G2, forms the interferometer. G0 acts as an array of line sources. G1 is the stepped grating.

An interference pattern is generated by G1 and creates a periodic intensity modulation at the position of G2. The latter is used to analyze the position, mean value and amplitude of the intensity modulation by moving one of the gratings in steps through the period of the pattern while recording an image at each step. In Fig. 1, G2 is the stepped grating.

The presence of a sample distorts the interference pattern and the distortion can be calculated by analyzing the intensity modulations with and without the sample present (Pfeiffer et al., 2009). From these calculations, the transmission and dark-field radiograms can be extracted. Due to the normalization using the reference scan without the sample present both radiograms will have pixel values in the range [0:1].

3. Materials and measurements

3.1. Grating interferometer setup

The experiments were carried out using an X-ray rotating anode tube with a grating interferometer setup at the Niels Bohr Institute at the University of Copenhagen. The Rigaku rotation anode tube had a copper target and was set at an acceleration voltage of 40 kV and a current of 150 mA. The effective source size was $1 \text{ mm} \times 1 \text{ mm}$.

The interferometer used a π phase-grating for G1 with a period of $3.5 \text{ }\mu\text{m}$, a G0 and G2 grating with periods of $14.1 \text{ }\mu\text{m}$ and $2.0 \text{ }\mu\text{m}$, respectively and was set up with a G0-to-G1 distance of 139 cm and a G1-to-G2 distance of 20 cm as described in Bech (2009). The gratings were optimized for 28 keV.

The images were recorded with a PILATUS 100k detector with 195×487 pixels and an effective pixel size at the sample of $155 \text{ }\mu\text{m} \times 155 \text{ }\mu\text{m}$.

3.2. Sample preparation

Three kinds of berries and fruits were used; blueberries, blackberries and a single piece of mandarin. In order to study freeze injuries, liquid nitrogen was applied as it allows for a short freezing time. The freezing of blackberries and blueberries was done by lowering the berries in liquid nitrogen such that the entire berry was covered and subsequently waiting around 150 s until the berry was completely frozen. One blueberry was frozen in advance of the others and allowed to defrost before being measured. By using quick freezing with liquid nitrogen, it can reasonable be assumed that the different berries experienced the same freezing regardless of size and shape and that the full volume of the berry had been frozen.

For the piece of mandarin, the tip of the mandarin was inserted into a few mL of liquid nitrogen allowing the freezing to spread such that the piece ended up being partly frozen.

3.3. Measurements

For both sample and reference scan 16 phase-steps were used, as described in Section 2, with an exposure time of 10 s for each exposure recorded with a total scan time of 3 min each for sample and reference scan. The measurements were carried out with air as a reference using the setup described in Section 3.1 and with the sample placed 8 cm downstream from G1 between the G1 and G2 gratings.

The settings were the same for the three measurements with similar environmental conditions of temperature and humidity of the air.

4. Image analysis

To compare the contrast between the frozen/defrosted and raw fruit in the transmission and dark-field signal, respectively, an image contrast measure is introduced in the form of a contrast-to-noise ratio (CNR) which is defined as (Song et al., 2004):

$$\text{CNR} = |\mu_F - \mu_R| / \left(w_F \sigma_F^2 + w_R \sigma_R^2 \right)^{1/2}$$

where μ denotes the mean value and σ^2 the variance of the frozen/defrosted (F) and raw fruit (R) in the transmission and dark-field images, respectively. The variances are weighted with the factor w which is the ratio of the number of the frozen/defrosted fruit (F) or raw fruit (R), relative to the total number of the two, i.e. $w_F = N_F / (N_F + N_R)$ where N is the number of pixels in the area, and $w_R = 1 - w_F$. If the two areas are equal, w will take the value one-half. A high CNR value means that the contrast between frozen/defrosted and the raw fruit is higher than the noise in the image whereas a low value means that there is no significant contrast.

5. Results

In Figs. 2–4, the images of the blueberries, blackberries and the mandarin, respectively, are shown with transmission in panel a) and dark-field radiograms in panel b) of each figure. The regions used for calculating CNR values between frozen/defrosted and raw fruit or berry are indicated with rectangles. It should be noted that the vertical fringe pattern observable in the dark-field images are an artifact originating from the interferometer.

A visual comparison of the images of the blueberries in panels a) and b) in Fig. 2 indicates that the dark-field image yields a much higher contrast difference between frozen, raw and defrosted berries than the transmission image. This reflects that although the X-ray attenuation measured by the transmission image only varies a little, the microstructure in the berries changes dramatically when being frozen or defrosted, and these variations in structure are recorded in the dark-field image.

The CNR values in Table 1 confirm that there is little difference in contrast between the frozen or defrosted blueberry and the raw blueberry in the transmission image ($\text{CNR} < 0.5$) while a significant CNR value ($\text{CNR} > 2.0$) is found in the dark-field image. Interestingly, the difference in contrast seems to be of the same order when comparing either the frozen or the defrosted to the raw berry.

For the blackberries shown in Fig. 3, the transmission and dark-field images of the raw and frozen berries demonstrate the same behavior as above. While the transmission signal in panel a) is similar, the dark-field signal in panel b) varies significantly between the frozen and raw blackberry. This is also reflected in the CNR value in Table 1 which is comparable to the values for the blueberries.

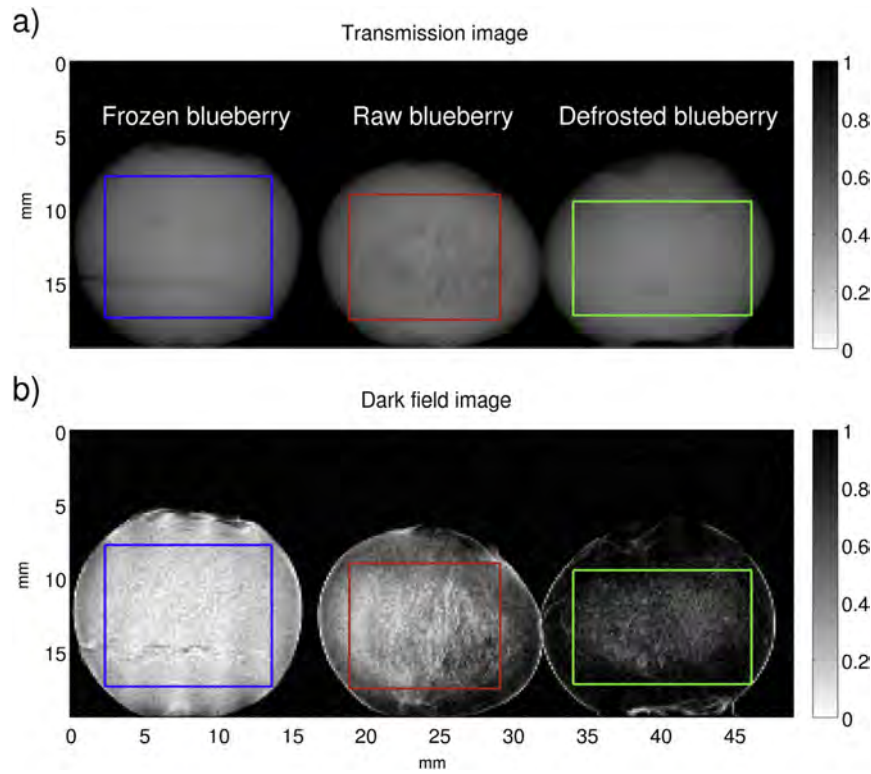


Fig. 2. X-ray images of blueberries in different states; frozen (left), raw (middle) and defrosted (right). The regions used for calculating the CNR values are indicated. a) Transmission radiogram b) Dark-field radiogram.

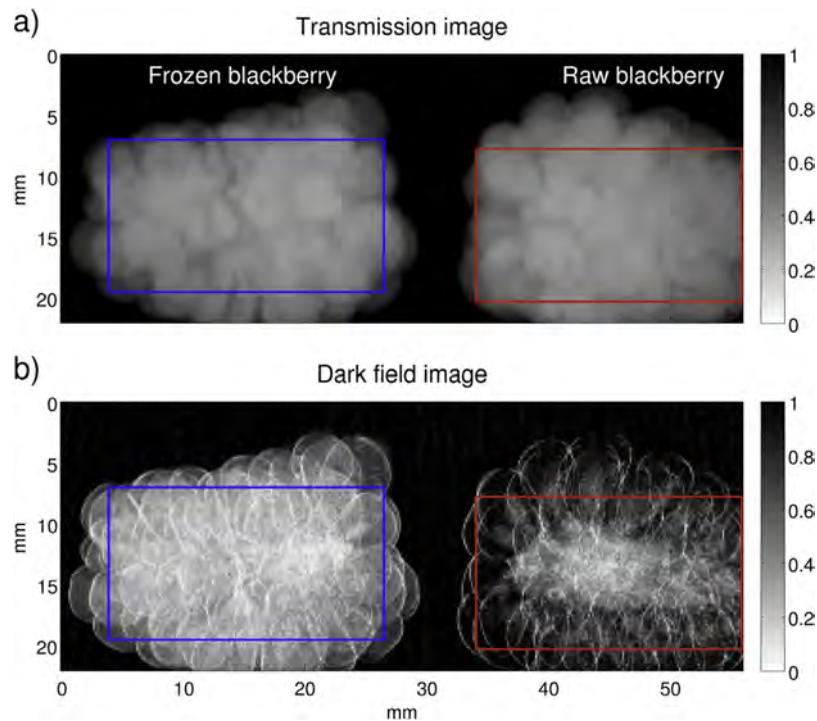


Fig. 3. X-ray images of blackberries in different states; frozen (left) and raw (right). The regions used for calculating the CNR values are indicated. a) Transmission radiogram b) Dark-field radiogram.

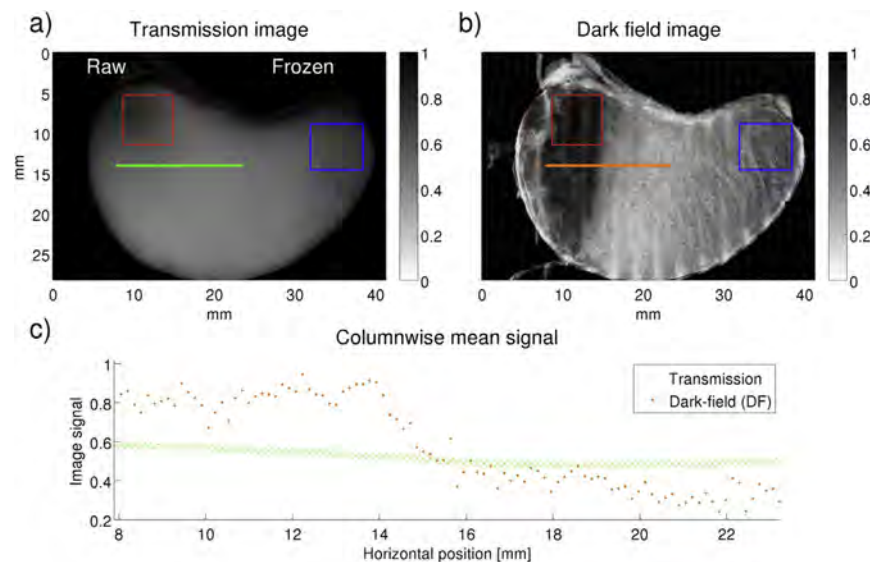


Fig. 4. X-ray images of a partly frozen mandarin with raw to the left and frozen to the right. The regions used for calculating the CNR values are indicated. a) Transmission radiogram b) Dark-field radiogram. c) Transmission and dark-field signal along the line indicated in panels a) and b).

In Fig. 4, images of a piece of partly frozen mandarin are shown. The right tip was inserted into liquid nitrogen and the freezing spread from there to the left. Again, little variation is visible in the transmission image in panel a) while the raw and frozen part is clearly discernible in the dark-field image in panel b). In Table 1, a CNR value between an unfrozen region in the top left and a frozen region in the top right of the mandarin has been calculated for both radiograms. The CNR values found are comparable to those for the blue and blackberries showing no contrast in the transmission but a considerable contrast in the dark-field radiogram.

The transition from the raw to the unfrozen region is shown in panel c) as the pixel values of the colored lines. While the transmission values (in green) change gradually and smooth across the phase transition zone of the mandarin, the dark-field image (in orange) has a high value in the left and a low in the right part with a transition region at 14–16 mm. The change in transmission can be attributed to a variation in thickness of the mandarin whereas the change in dark-field signal follows the freezing of the mandarin.

From the curves, it can also be noted that while the dark-field signal exhibits a higher contrast between raw and frozen, it is hampered by a higher noise level compared to the transmission signal.

6. Discussion and outlook

The results presented here, demonstrate that the X-ray dark-field radiography is sensitive to the raw, frozen or defrosted state of fruits which shows the complementarity of the contrast modality of X-ray dark-field to conventional X-ray radiography. Whereas almost no contrast is obtained with conventional X-ray radiography when comparing the raw state to either frozen or defrosted, CNR

values of around 2.5 are obtained with X-ray dark-field radiography.

However, while the dark-field signal is clearly sensitive to whether a piece of fruit is in a raw, frozen or defrosted state, it is still unknown what kind of change in microstructure is responsible for the improved image contrast. One possible explanation might be that it originates from ultra small-angle scattering from the cell walls in the fruit, and this scattering is enhanced if the cells are frozen – keeping the cell walls more rigid and ordered – while defrosting the fruit might break the cell walls. Further research experiments are needed to say anything conclusive.

A point not addressed by this study is the influence of the temporal development of the freezing transition on the dark-field signal. In this study only rapid freezing has been used, and it is left to future work to investigate whether the dark-field signal will change for a different phase transition dynamic.

The proof-of-principle experiments in this work indicate the potential for applying X-ray dark-field radiography in further studies of freeze injuries in fruits. However, an experiment on a larger scale of a single type of fruit with a focus on the defrosted state would be needed to fully evaluate the strength of the proposed method.

Further work also is needed before the technique can be implemented in an automatic detection system, e.g. as part of a conveyor belt line, but a proposal has already been made for a grating-based interferometry scanning system (Kottler, Pfeiffer, Bunk, Grüntzweig, & David, 2007) using a number of line detectors.

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Table 1
Contrast-to-noise ratios of frozen and defrosted samples compared to raw sample.

Sample	Transmission CNR	Dark-field CNR	w_F
Blueberry, frozen	0.2	2.3	0.55
Blueberry, defrosted	0.1	2.9	0.52
Blackberry, frozen	0.1	2.0	0.49
Mandarin, frozen	0.2	2.9	0.49

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Publication II

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Quantitative in-situ monitoring of germinating barley seeds using X-ray dark-field radiography.

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Quantitative in-situ monitoring of germinating barley seeds using X-ray dark-field radiography

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Abstract

During production of malt from barley seeds, cell walls and starch granules in the endosperm are degraded. Although this modification process is important for malt quality, the modification patterns of individual barley seeds have yet to be reported. The use of destructive microscopy methods have previously limited the investigations to ensemble averages.

X-ray dark-field radiography is a recent non-destructive imaging method which is sensitive to microstructural variations. In this study, the method was applied for quantitative in-situ monitoring of barley seeds. Microstructural changes relating to water uptake and modification were monitored over a 43-55 h period.

Sub-resolution stress cracks as well as a dark-field signal believed to originate from starch granules were detected. The evolution of the dark-field signal followed the known modification pattern in barley seeds.

Based on these findings, X-ray dark-field radiography could be a novel approach to monitor modification of germinating barley seeds.

Keywords: X-ray dark-field radiography, water uptake, barley, modification, quantitative monitoring, starch degradation.

1. Introduction

40 High quality malt from barley seeds is crucial in the production of high quality beer and malt whisky. In the production of malt, uptake of water during steeping is followed by enzymatic degradation of the barley endosperm during germination. The degradation process, known as modification, causes structural changes in the barley endosperm as cell walls, starch granules and their surrounding protein matrix are
45 partially hydrolysed by enzymes. The degradation initiates next to the scutellum and ideally advances in parallel to the scutellar face followed by endosperm degradation beneath the aleurone layer (Briggs and Macdonald, 1983; Briggs, 2002; Gianinetti, 2009).

50 The time needed for modification depends on the speed of the degradation front (Gianinetti, 2009) and can vary between different barley cultivars (Brennan et al., 1997). Theoretical enzyme kinetic models predict that the front will move parallel to the scutellar face at a constant speed (O'Brien and Fowkes, 2005; Fowkes and O'Brien, 2010). However, experimental investigations are needed to verify these
55 results (Fowkes and O'Brien, 2010).

Although the modification over time has been investigated extensively using microscopy methods (Aastrup et al, 1981; Briggs and Macdonald, 1983; Brennan et al., 1997; Ferrari et al., 2010) so far only ensemble averages have been obtained.
60 Since the barley seeds are sectioned and stained using e.g. calcofluor (Aastrup et al., 1980; Aastrup et al., 1981) before photographed, the applied methods are destructive in nature. Conversely, in order to follow the modification process in individual seeds, a non-destructive imaging method would be needed.

65 X-ray dark-field radiography is a novel, non-destructive imaging technique introduced by (Pfeiffer et al., 2008). It uses an X-ray grating interferometer (David et al., 2002; Momose, 2003; Weitkamp et al., 2005) which can be adapted to laboratory-based setups (Pfeiffer et al., 2006). The dark-field signal originates from ultra small-angle X-ray scattering of microstructures within the material and is thus sensitive to
70 structural differences on the micrometer scale (Bech et al., 2010; Lynch et al., 2011; Yashiro et al., 2010). In addition, sub-resolution edges may be detected (Yashiro and Momose, 2015; Lauridsen et al. 2015). Within food science, the structural sensitivity has been used for foreign body detection of paper and insects in food products (Nielsen et al., 2013). In addition, microstructural differences between fresh, frozen
75 and defrosted fruit and berries were recently distinguished (Nielsen et al., 2014).

Several structures on the micrometer scale are present in the barley seeds. The thickness of the barley cell walls in the aleurone layer and endosperm ranges from 0.5-4 μm (Fincher, 1975; Gram, 1982; Lazaridou et al., 2008; Palmer, 1998).
80 Endosperm starch granules typically range from 0.5-48 μm in diameter (Ao and Jane, 2007; Briggs, 1978; Lindeboom et al., 2004; MacGregor, 1979; Stoddard, 1999) depending on factors such as barley variety and growth conditions (Briggs, 1978). In

accordance, since the X-ray dark-field signal is sensitive to microstructural changes, degradation of barley microstructures resulting from endosperm modification could be detectable using X-ray dark-field radiography.

The aim of this study was to apply X-ray dark-field radiography to quantitatively investigate and monitor microstructural changes relating to water uptake and modification in barley seeds during a 48 hour germination period. The X-ray dark-field radiographs were compared to conventional X-ray transmission radiographs.

2. Materials and methods

2.1. X-ray grating interferometry

The X-ray grating interferometer has previously been described in detail (Pfeiffer et al., 2006; Weitkamp et al., 2005). As seen in figure 1, it consists of an X-ray phase-grating G1 and an analyzer absorption grating G2. At laboratory setups a third grating, G0, is included to obtain satisfactory spatial coherence in the horizontal direction perpendicular to the grating lines.

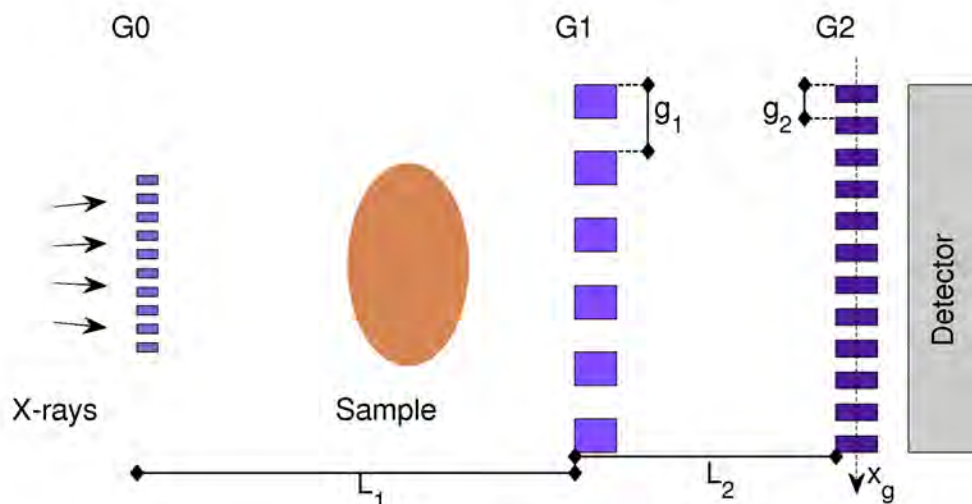


Figure 1: Sketch of the X-ray grating interferometer setup with distances indicated. The phase-grating G1 creates an intensity pattern which is analyzed by the analyzer grating G2. The spatial coherence is assured by the source grating G0.

An interference pattern is generated by G1 and creates a periodic intensity modulation at the position of G2. The latter is used to analyze the position, mean value and amplitude of the intensity modulation by moving one of the gratings in steps through the period of the pattern while recording an exposure at each step. The presence of a sample distorts the interference pattern and the distortion can be calculated by analyzing the intensity modulations with and without the sample present.

110 (Pfeiffer et al., 2009). From these calculations, the transmission and dark-field radiographs can be extracted.

Following (Strobl, 2014), the microstructure sizes that the dark-field contrast of a grating interferometer is sensitive to may be gauged by introducing the autocorrelation length, dGI, of the setup:

115

$$dGI = \lambda / g_2 * LS$$

where λ is the X-ray wavelength, g_2 is the grating period of G2, and LS relates to the position of the sample in the interferometer. When the sample is placed between G0 and G1, LS is given by $LS = (L1 - zS) / L1 * L2$ where L1 (L2) is the distance between G0 and G1 (G1 and G2) and zS is the distance of the sample upstreams to G1.

120 For spherical microparticles in a matrix, an analytical expression for the dark-field signal has been derived (Yashiro et al., 2010; Lynch et al., 2011; Strobl, 2014).
125 In this case, the microstructures that contribute to the dark-field signal are in the size range of $0.5 * dGI$ to $15 * dGI$.

So far, the wavelength-dependent dGI has only been defined for a monochromatic X-ray beam. As an approximation in the case of a white X-ray beam,
130 an effective energy could be introduced to calculate an effective dGI.

2.2. Barley samples

135 Barley samples of malting-quality (*Hordeum vulgare* L. cv. Odyssey) grown 2014 were obtained from Danish Malting Group A/S (DMG). The barley had a moisture content of 13.5%, a protein content (dry matter) of 9.7% and a grading of $1.9\% < 2.2$ mm and $95.9\% > 2.5$ mm (analyses done by DMG). The barley was stored in an airtight container at 5°C.

140 The germination energy was determined with 4 x 100 seeds using the BRF method (EBC method 3.6.2)(Analysis Comitee of the EBC, 1998).

2.3. Experimental X-ray grating interferometer setup

145 The experiments were done at the Niels Bohr Institute using an experimental X-ray rotating anode tube setup with a grating interferometer. The Rigaku rotation anode tube had a copper target and was set at an acceleration voltage of 40 kV and a filament current of 50-70 mA. The effective source size was 1 mm x 1 mm.

150 The interferometer used a π phase-grating for G1 with a period of 3.5 μ m, and G0 and G2 gratings with periods of 14.1 μ m and 2.0 μ m, respectively. The grating design energy was 28 keV. The interferometer was set up with a G0-to-G1 distance of

139 cm and a G1-to-G2 distance of 20 cm. The sample stage was positioned 15 cm upstreams of G1. Using the listed distances and a design energy of 28 keV, this gives
155 an effective dGI= 4 μm . The images were recorded with a PILATUS 100k detector with 195x487 pixels and an effective pixel size at the sample of 134 μm x 134 μm .

2.4. Sample preparation

To standardize the conditions for the barley germination a modified version of the
160 BRF method (EBC method 3.6.2) was used. Deviations were as follows: five seeds with the ventral side facing the wetted filter papers were placed in each Petri dish. Four Petri dishes were used in total, and the seeds were numbered from 1 to 20. The seeds were not removed during the experiment which lasted 43-55 hours.

2.5. X-ray radiography measurements

165 Two measurement time series, A and B, were performed. In both, a stack of two Petri dishes each containing 5 seeds were imaged using X-ray grating-based radiography. Seeds 1 to 10 were measured in time series A and 11 to 20 in B. The total duration was 43 h and 55 h, respectively.

170 At ten minute intervals a phase-stepping scan of the sample was conducted. In addition, five reference phase-stepping scans were conducted prior to each time-series. For both sample and reference scans of measurement series A (B) 16 phase-steps were used. The exposure time was 10 s (14 s) for each exposure recorded giving
175 a total scan time of 3 min (4 min) per phase-stepping scan. The measurements were carried out with air as background using the setup described in section 2.3. The temperature and humidity for the two measurements were similar with a temperature of 19 degrees C. From the acquired phase-stepping scans, X-ray transmission and dark-field radiographs were calculated.

2.5.1. Dose calculation

180 The dose d imparted on the barley seeds was estimated using the expression $d = N \cdot E \cdot \mu/\rho$ where μ/ρ is the mass attenuation coefficient, N is the number of incident photons per unit area and E is the photon energy (Howells et al., 2009). In place of the
185 polychromatic energy spectrum, E was approximated as an effective energy of 25 keV. Assuming the attenuation of barley seeds to be roughly the same as for water, this gave $\mu/\rho = 0.53 \text{ cm}^2/\text{g}$.

190 The number of photons $N = 1.3 \cdot 10^{10} \text{ photons/cm}^2$ was estimated as follows. First, the total number of photons detected in an empty area over the 16 phase-steps was estimated. From this, the number of incident photons on the 320 μm thick Si detector chip was calculated by dividing with the detection efficiency of 16% at 25 keV. Finally, N was found by multiplying by a factor of two since 50% of the
195 number of photons incident on the sample were assumed to be absorbed by the gratings.

Finally, this gave a dose per radiograph of $d = 28$ mGy and a total dose for the two time series of roughly 6 Gy and 9 Gy respectively.

2.6. Image analysis

In order to monitor the barley seeds quantitatively, image analysis was applied to the radiographs. First, noise reduction of the transmission and dark-field radiographs was performed using a median filter.

205 Secondly, a mask for each barley seed in a region-of-interest (ROI) was created using a bivariate threshold segmentation combined with a morphological opening. By convoluting the masked dark-field radiograph with a first-order derivative of a gaussian filter, the internal border of the scattering signal along the seed major axis was found. A variance of 4 pixels was used for the gaussian filter.

210 Thirdly, the position of the interface was recorded in intervals of 30 min using every third radiograph. The position was measured with respect to a line through the center-of-mass of the seed and along the major axis.

215 The area covered by the seed in the radiographs was calculated from the segmentation of the seed from the mask. An additional segmentation was made of the part of the seed with a high dark-field signal, and the area was extracted. Finally, the ratio of the two areas was calculated.

3. Results

220 3.1. Germination energy

Table 1 display the data for a four-time determination of the germination energy. The average germination energy was 98%. On average 20% and 83% of the barley seeds were chitted within 24 hours and 48 hours. Normally, the percentage after 24 hours is
225 higher. However, it varies between barley types.

Table 1. Four-time determination of germination energy (Analytica-EBC 3.6.3) for barley variety Odyssey harvested in 2014.

	Germination energy [%]			
	A	B	C	D
24 hours	15	20	21	24
48 hours	63	67	62	60
72 hours	19	12	15	13
Cumulated [%]	97	99	98	97

3.2. X-ray radiography of germinating barley seeds

230 Figure 2. panels a)-h) and i)-p) display a subset of transmission and dark-field
 radiographs, respectively, of germinating barley seeds 11 to 15. Radiographs in
 intervals of 6 h were selected starting at $t=0$ h and ending at $t=42$ h (for full time
 series see supplementary materials). On the gray-scale images, dark shades
 correspond to a high signal while bright shades correspond to a low signal.
 235

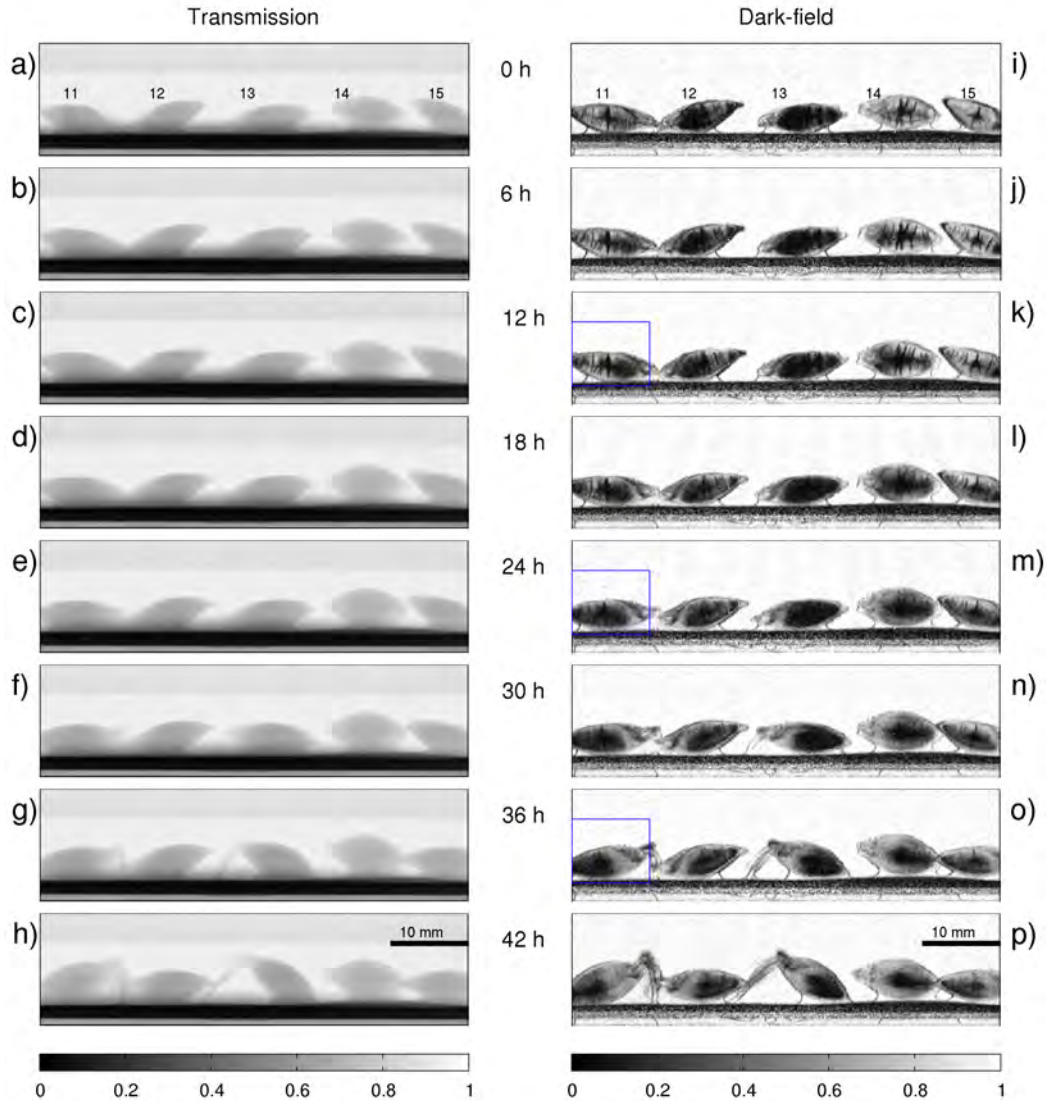


Figure 2: Transmission and dark-field projections of barley seeds 11 to 15 in 6 h intervals from $t=0$ h to $t=42$ h. The blue squares in panels k), m) and o) indicate the ROI used in figure 3.

In the transmission images in panels a)-h), the barley seeds appear homogeneous with no apparant internal changes over time. In the dark-field images in panels i)-p), however, changes over time of the internal seed structure can be observed. First, vertical dark lines are present within all of the seeds at $t=0$ h. Over time these lines are seen to fade until at $t=30$ h, all have disappeared.

Furthermore, overlaid on the vertical lines, dark areas in the central part of the barley seeds are seen. In some seeds such as 11 to 13 they are present at $t=0$ h, and in others such as 14 and 15 they seem to become more pronounced until $t=18$ h.

As time passes, these areas shrink in all seeds. This reduction in dark-field signal begins near the scutellum part of the barley seed, and is seen to spread through the endosperm region during the 42 h period. This pattern and movement resemble the modification pattern as observed from microscopy studies.

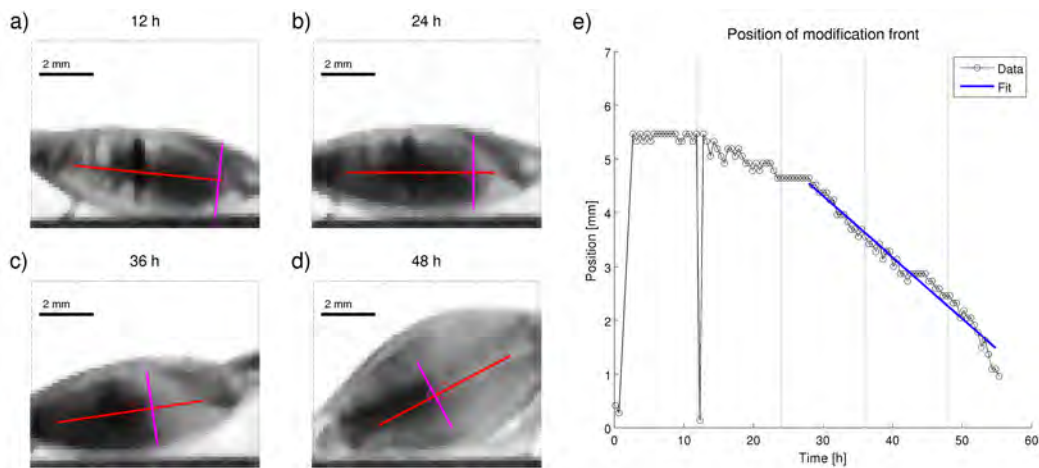


Figure 3: Image analysis of barley seeds. a)-d) Dark-field radiographs of barley seed 11 at $t=12, 24, 36, 48$ h. Through image analysis, the major axis and dark-field front were identified as indicated in red and magenta, respectively. e) The position of the dark-field front with time. A linear fit is indicated in blue.

A close-up of the time evolution of the dark-field signal for seed 11 can be seen in figure 3 panels a)-d) at 12 h, 24 h, 36 h and 48 h (for full time series see supplementary materials). The line along the seed major axis (shown in red) and the position of the signal front (shown in magenta) were found through image analysis. In panel e), a plot of the relative position of the front along the major axis with time is shown. In the first 12 hours the front is not moving. From 12 h to 28 h a small movement occurs whereafter the position changes approximately linearly in time. The sudden changes in position during the first few hours are artefacts of the edge detection algorithm. The initiation time and speed of the front was extracted using a first-order polynomial linear least-squares regression for 28 h to 55 h (shown in blue).

265 Similar patterns as for seed 11 were found in the other seeds. Specifically a
time period where the front moved at constant speed was observed. Using image
analysis, quantitative values of front speed and initiation times were successfully
obtained from sixteen out of twenty seeds. For the four remaining seeds, the edge
270 detection algorithm failed. The initiation times and front speeds are shown in table 2.
The times vary from 0 h to 33 h and the speeds from 0.05 mm/h to 0.14 mm/h. Here 0
h means within the first 30 min. Also included are the R^2 values of the least-squares
fits.

Table 2. Barley seed front speeds and initiation times as found through image analysis. R^2 values for the linear least squares regressions are indicated. No numbers were obtained for seed 4, 13, 14 and 18. Mean values with an interval of a single standard deviation are listed as well.

Seed no	Initiation time	Front Speed	R^2 (fit)
	[h]	[mm/h]	
1	28	0.11	0.95
2	17	0.06	0.97
3	28	0.14	0.94
5	7	0.10	0.97
6	11	0.07	0.97
7	11	0.10	0.91
8	24	0.11	0.97
9	15	0.06	0.93
10	28	0.06	0.95
11	28	0.11	0.97
12	18	0.08	0.92
15	27	0.07	0.95
16	33	0.05	0.79
17	28	0.12	0.98
19	0	0.06	0.94
20	17	0.06	0.91
Mean	20+/-9	0.09+/-0.03	

275 In figure 4, the fraction of the seeds covered by dark-field signal is
highlighted. On the 2D images, this will appear as an area. However, since the
radiographs are acquired from the transmitted beam, the full volume contributes to the
signal. In panels a)-c), dark-field radiographs of seed 1 to 20 are shown for $t=12$ h, 30
h and 42 h, respectively. The perimeter of the seed found by image analysis is
280 depicted in purple, and the perimeter of the dark-field signal is shown in green. From
the radiographs, it is again clear that the signal shrinks from $t=18$ h to $t=42$ h. The
signal is quantified as the ratio of the dark-field area to the seed area on the
radiograph. A histogram for the fractional areas are shown in panel d).

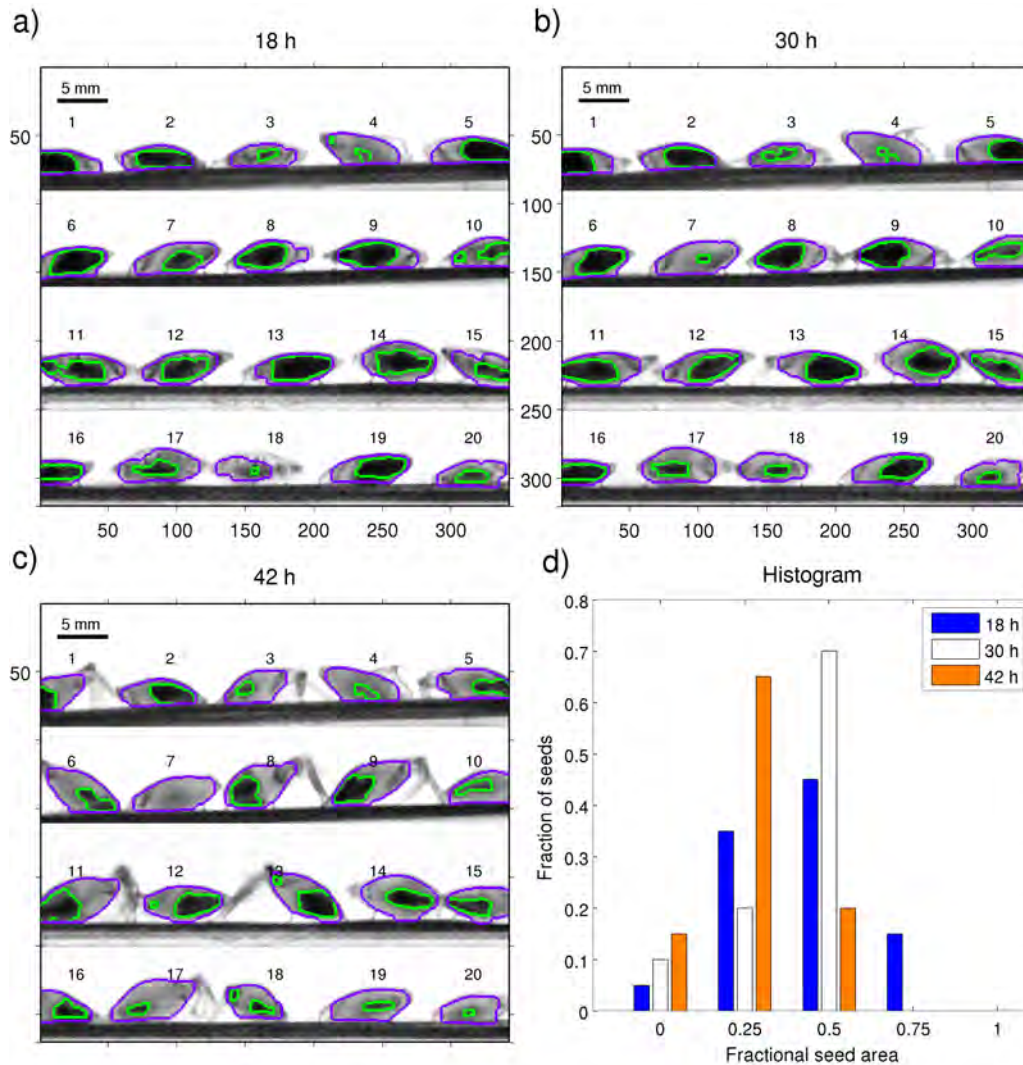


Figure 4: a)-c) Dark-field radiographs for barley seeds 1 to 20 at $t = 18, 30$ and 42 h. From image analysis, the area of the seeds and the dark-field regions were identified. The perimeters of these areas are indicated in purple and green, respectively. d) Histogram of the ratio of the dark-field areas to the full seed areas.

After the measurement, it was observed that all seeds had germinated except for seed number 2 and 19.

4. Discussion

290

Vertical lines as seen in the dark-field radiographs have previously been reported in studies of barley seeds using X-ray transmission radiographs (Demyanchuk et al., 2013). The lines originated from stress cracks in the endosperm. Such air-filled cracks smaller than the effective pixel size could result in a strong dark-field signal

295 due to the electron density difference between air and seed material (Yashiro and
Momose, 2015). Subsequently, filling of the stress cracks during water uptake would
lead to a smaller electron density difference, and the dark-field signal would diminish
as was observed. A recent study of water uptake in porous media found a similar
300 decrease in dark-field signal when the sub-resolution pores were filled with water
(Yang et al., 2014). According to (Fornal et al., 2000) stress cracks within the barley
endosperm do not seem to affect the ability to germinate .

The dark areas in the X-ray dark-field radiographs originate from X-ray
scattering from microstructures in the barley seeds. With an effective dGI of 4 μm ,
305 this means that the measurements were sensitive towards microspheres with diameters
in the range of 2-60 μm . As the starch granules are approximately spherical and fall
within this range, we infer that the observed dark-field signal originated from the
starch granules. Thus, the degradation of starch granules during modification would
cause a reduction in signal as observed. Hence, we propose that the X-ray dark-field
310 signal can be used to monitor the modification process. However, as the dark-field
radiographs project the scattered signal through the full seed volume, a direct
comparison to 2D sections from micrographs cannot be made.

Following the above argumentation, the quantitative parameters for front
315 speed and initiation times can be ascribed to the modification process. The constant
front speed can be interpreted as a constant velocity in the spread of starch
degradation as predicted from theoretical models (O'Brien and Fowkes, 2005;
Fowkes and O'Brien, 2010). The observed variation in the values between seeds
could be due to an uneven uptake of water from the wetted filter paper. Furthermore,
320 size, morphology and composition of the barley seed will have an influence on the
water uptake and therefore the modification pattern (Cozzolino et al., 2014;
MOLINA-CANO et al., 1995).

Since the total dose received by the barley seeds was several Gy, radiation
325 damage could be an issue. Indeed, this may have influenced that seed 2 and 19 failed
to germinate. However, as 90% of the seeds in the X-ray radiography measurements
did germinate, radiation damage was not a critical factor.

5. Conclusion and outlook

330 This pilot study demonstrates the first quantitative in-situ monitoring of germinating
barley seeds. Using X-ray dark-field radiography, microstructural changes within the
barley seeds during water uptake and modification could be successfully monitored
and assessed.

335 Initially, sub-resolution stress cracks were detected in the dark-field
radiographs through X-ray scattering from the air-filled pores. Water uptake in the
cracks could be followed through a diminished dark-field signal. The micrometersized
starch granules led to a X-ray scattering signal in the dark-field radiographs. During
modification, the degradation of starch granules led to a reduction in the observed

340 dark-field signal. By following the changes in the dark-field signal, the modification
proces could be quantified by the front speed, initiation time and fractional area of
undegraded starch. In accordance with theoretical predictions, a time range existed in
which the front speeds were observed to be constant.

345 However, further research is needed in order to validate and develop the
proposed method. More specifically, the dark-field radiographs should be compared
directly to conventional micrographs. Since the X-ray dark-field method is non-
destructive, the same barley seeds can be observed both in dark-field radiography and
in microscopy afterwards.

350 In future measurements, the dose imparted on the barley seeds could be
reduced in several ways. First of all, the measurement rate could be reduced since the
frequency of one radiograph per 10 min was much higher than the the changes taking
place. Furthermore, a detector with a higher detection efficiency than the present 16%
355 could be used which could reduce the exposure time per radiograph. Finally, as the
dark-field contrast does not rely on the attenuation by the sample, a higher X-ray
energy could be used which would reduce the absorption in the barley seeds. In this
way, the dose per radiograph could be reduced by at least a factor of 10 and for a full
time series by a factor of 50.

360 As the X-ray dark-field technique is not specific for barley, it could possibly
also be used for investigating and monitoring the germination pattern in other types of
seeds.

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Publication III

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Analysis of micro-structure in raw and heat treated meat emulsions from multi-modal X-ray microtomography.

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Analysis of micro-structure in raw and heat treated meat emulsions from multimodal X-ray microtomography

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ABSTRACT

This study presents a novel non-destructive X-ray technique for analyzing meat emulsions before and after heat treatment. The method is based on X-ray grating-interferometry where three complementary imaging modalities are obtained simultaneously measuring the absorption, refraction and scattering properties of the sample. Enhanced contrast capabilities of this X-ray technique makes studies on materials with similar attenuation properties possible. The emulsion samples were imaged both in a raw and cooked state. Additionally, different fat types were used in the emulsions in order to compare microstructural differences when either pork fat or sunflower oil was added. From the reconstructed tomograms the different constituents in the emulsions were segmented using a multivariate segmentation method. From this, a quantitative analysis was performed between the different samples, determining properties such as percent object volumes, porosity, average structure thickness and cooking loss. The grating-based X-ray technique and multivariate segmentation made the analysis of the microstructure possible which further gives insight to how both heat treatment, and the use of different lipid types, affect the final protein network quality.

Industrial relevance: Meat emulsions have previously been thoroughly studied, and the use of various fat substitutes and protein stabilizers has been investigated. The grating-based multimodal X-ray tomography method presented here is a feasible method to investigate the microstructural changes induced by heat treatment. It provides high-resolution three dimensional spatial information and in contrast to 2D imaging methods, quantitative parameters can be extracted by image analysis for the entire sample volume. Additionally, the non-destructive method allows for imaging the same sample before and after cooking.

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1. Introduction

The most important functional characteristics in comminuted meat products are the gel-forming abilities of the myofibrillar proteins. During comminution, salt-soluble myofibrillar proteins are extracted that, when heated, create a dense protein network referred to as gel (Tornberg, 2005). Some of the solubilized proteins will emulsify the added fat by forming an interfacial protein film around the fat globules, which are further stabilized by the protein gel (Barbut, 1995; Wu, Xiong, Chen, Tang, & Zhou, 2009). The fat globules act as fillers, reducing the porosity and increasing the stability of the gel. Differences in the physicochemical properties of saturated and unsaturated lipids, i.e. emulsification properties and physical state, will affect the distribution of fat and the influence on the gel stability and thereby the quality of the final product. Due to health aspects, substitution of animal fat with vegetable oil has generated interest in the meat processing industry

(Wood et al., 2004). The lower melting point of the vegetable oil facilitates an even distribution of small oil droplets in the meat batter leading to formation of a homogeneous gel structure. However, the higher mobility of the oil compared to the solid animal fat is a challenge. The coalescence of oil droplets may lead to channel formation in the protein network facilitating moisture transportation during heat treatment which can be observed as increased cooking losses (Barbut, 1995).

Studies on the quality of meat emulsions rely on a variety of measurements. These include determination of pH values, cooking loss, color composition, texture profiles, apparent viscosity, and emulsion stability (Choi et al., 2009, 2010; Gordon & Barbut, 1991; Shao, Zou, Xu, Wu, & Zhou, 2011). Current imaging techniques used to analyze the quality of meat emulsions have mainly focused on two dimensional measurements from either scanning- and transmission electron microscopy (Álvarez et al., 2012; Totosaús & Pérez-Chabela, 2009), light micrographs (Álvarez & Barbut, 2013; Youssef & Barbut, 2009, 2010), or confocal laser scanning microscopy (Sorapukdee, Kongtasorn, Benjakul, & Visessanguan, 2012). Due to the similar attenuation properties of the soft materials in meat emulsions, the use of X-ray microcomputed

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tomography (μ CT) has been limited. In Santos-Garcés et al. (2012) a feasibility study of X-ray μ CT for microstructure analysis of fermented sausages demonstrated that absorption tomography provided contrast between meat, fat and air holes. Although this μ CT analysis identified fat particles and air holes, the technique was not accurate enough to distinguish between pork lean and fat when these constituents were emulsified. Novel X-ray techniques based on grating-interferometry provide new imaging modalities that can be obtained simultaneously with absorption tomography (Bech et al., 2010). These modalities, phase contrast and dark-field imaging, measure the electron density and the diffusion length of the sample. Enhanced contrast capabilities of this X-ray technique makes studies on materials with similar attenuation properties, such as soft tissue, possible. Previous studies have demonstrated superior contrast with X-ray phase-contrast CT compared to conventional CT in a study of pork rind and fat (Jensen et al., 2011), and demonstrated the potential for improved segmentation when using multivariate analysis by combining conventional CT with phase-contrast CT for bivariate segmentation of a piece of pork back fat and a piece of beef muscle tissue (Nielsen et al., 2012).

In this paper, the novel X-ray technique is used to investigate the differences in microstructures of meat emulsions in three dimensions. Such analysis allows for determining structural parameters of the entire sample instead of inferring from partial information obtained by two dimensional imaging techniques. The information obtained from a three dimensional analysis is believed to further increase the understanding of emulsion microstructure. Additionally, the non-destructive technique offers the possibility to study the same sample in both raw and cooked condition. The samples used were raw and heat treated meat emulsions (10% protein, 25% fat, 60% moisture) prepared with either pork fat (lard) or sunflower oil. Absorption, phase contrast and dark-field tomograms were obtained at a synchrotron facility using a grating interferometer. From the reconstructed tomograms the different constituents in the emulsions were segmented using a multivariate segmentation method. A quantitative analysis was performed by measuring geometrical parameters in order to determine the microstructural differences of the emulsions when using lard or sunflower oil and also the effect heat treatment has on the emulsion quality.

2. Materials and methods

2.1. X-ray modalities

In Fig. 1 the three types of physical interactions — absorption, refraction and scattering — obtained from the absorption, phase-contrast and dark-field imaging modalities of grating-based interferometry are illustrated. The effect on an incoming Gaussian shaped beam profile (black) is depicted when elements with different physical properties are measured. The profiles shown in color represent what is recorded when a material is present. In green, the effect from an absorptive material is shown to attenuate the beam, while in blue, the effect of a refractive material is seen to cause a transverse shift in the position of the beam profile. Lastly, the small-angle scattering from a material with ordered micro-structures causes the beam profile, here shown in red, to broaden. By separating the attenuation, transverse shift and broadening of the beam, it is thus possible to measure three complementary imaging modalities. For further details on the X-ray modalities, the reader is referred to Bech, Jensen, et al. (2010) and Pfeiffer (2012).

2.2. Grating-based interferometry

One method to separate the three X-ray interactions is grating-based imaging (GBI), which relies on an X-ray interferometer consisting of periodic gratings for measurements. A schematic of a setup for GBI is shown in Fig. 2. Grating G1 produces a periodic intensity modulation, consisting of periodic fringes, transverse to the beam direction. The change in position, mean value and amplitude of the periodic fringes

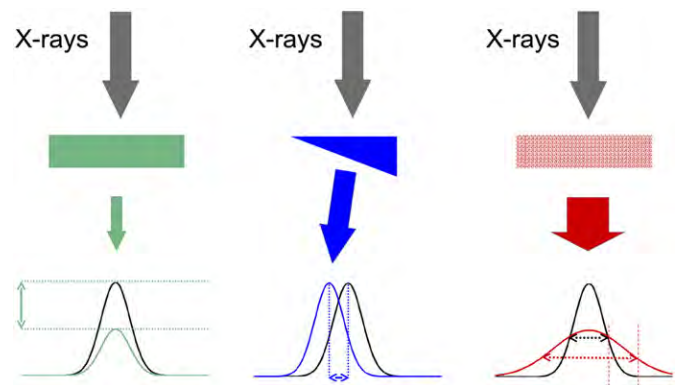


Fig. 1. The incoming X-ray beam changes when a sample is present. The effect on the beam from an absorptive material is shown in green, a refractive material in blue and a material with a homogeneous distribution of micro-structures in red.

can be probed using a second grating, G2, by physically moving one of the gratings in several steps, acquiring a projection image between each movement of the grating. From the same series of scans, both the absorption, refraction and small-angle scattering can be extracted giving an inherent pixel correspondence. Tomograms are then created for each modality using filtered back-projection. This results in absorption, phase-contrast and dark-field image volumes, measuring the attenuation length, electron density and the linear diffusion coefficient of the sample, respectively (Bech et al., 2010; Weitkamp, David, Kottler, Bunk, & Pfeiffer, 2006). GBI using synchrotron sources was first demonstrated in the beginning of the 2000s (David, Nohammer, Solak, & Ziegler, 2002; Momose, 2003; Weitkamp et al., 2005), and later adapted to laboratory-based setups (Pfeiffer, Weitkamp, Bunk, & David, 2006). The method can be applied using polychromatic sources but a certain degree of spatial coherence is needed. In a laboratory setup, spatial coherence can be achieved either by using a microfocus source or by using a third grating G0, which acts as an array of line sources for use with source sizes up to a square millimeter.

2.3. Meat emulsion samples

The meat emulsions used in this study were prepared in batches of 1 kg in a food processor (CombiMax 600, Braun, Germany). Thawed meat (480 g), potato starch (5 g), curing salt (NaCl with 0.6% nitrite) (17 g) and crushed ice (248 g) were comminuted at highest speed for 2 min. The temperature at this point was 1 °C in all batters. After addition of 250 g of either hand chopped cubes of lard or sunflower oil the batter was comminuted for 2 min. The temperature was measured (approx. 12 °C), and comminuting was continued for 1 min. End

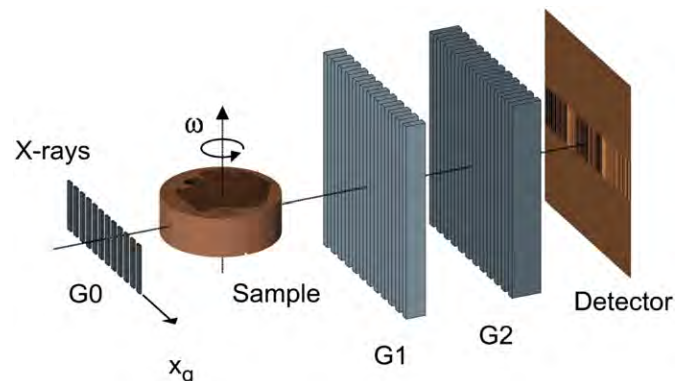


Fig. 2. A schematic of an X-ray tomography setup using a grating interferometer. Reprinted from Nielsen et al., 2012.

temperature was 14 °C. A portion of both the animal fat and sunflower oil batches was placed in sample containers. The samples were then centrifuged at 5000 g for 10 min, and had the lid closed under the surface of degassed PBS. The PBS-buffer was degassed to avoid bubble formation during scanning. The samples were imaged in this raw state prior to cooking. For the heating of the samples, a 200 mL glass of water was heated in a microwave oven up to boiling point. The sample in the container was then immediately placed in the water and left to stand for 10 min for the sunflower oil sample and 15 min for the pork fat sample. Both samples were then placed in a cold-water bath, 10 min for the sunflower oil sample and 15 min for the pork fat sample. The cooked samples were then imaged again. The increased times for the animal fat sample was to ensure that the sample was heated to a homogeneous temperature and likewise cooled to a stable cooling temperature. No difference due to heating and cooling times was observed in the data and therefore the shorter times for the sunflower oil sample are not believed to have affected the final result.

2.4. Tomography measurements

Absorption, phase-contrast and dark-field μ CT scans of both the raw and cooked meat emulsions were obtained at the TOMCAT beamline at the Swiss Light Source, Paul Scherrer Institut (PSI), Villigen, Switzerland. The setup is described in detail in McDonald et al. (2009). For this study the energy was set to 25 kV, and the third fractional Talbot distance (Weitkamp et al., 2006) was used. The full volumes obtained were $1720 \times 1720 \times 513$ voxels, with an effective voxel size at sample of $7.4 \mu\text{m} \times 7.4 \mu\text{m} \times 7.4 \mu\text{m}$. The total scan time was between 80 and 90 min per sample. Differences in scan time were due to fluctuations in motor movement times.

2.5. Image segmentation

Before quantitative parameters can be extracted it is necessary to segment the data volumes. This is done by classifying each voxel to a label l representing one of the elements present in the sample. These elements consist of the sample container and constituents including meat, fat, oil and salt. The set of possible labels for the classification task is given as $\mathbf{L} = (l_1, \dots, l_k)$. As the data obtained from GBI is multivariate, each voxel v_i can be represented by a vector of the three intensities $\mathbf{x}_i = (x_{i1}, x_{i2}, x_{i3})$, $i = 1, \dots, N$ where N is the number of voxels and (x_{i1}, x_{i2}, x_{i3}) represent the absorptive, refractive, and scatter intensities of the i -th voxel, respectively. In order to determine the likelihood of label l_j , $j = 1, \dots, k$, for voxel v_i and given $\mathbf{x}_i$, the data is modeled as a mixture of multivariate Gaussian distributions using an expectation-maximization (EM) algorithm (Hastie, Tibshirani, & Friedman, 2009). For each volume the number of known constituents is used as the number of Gaussians to fit, and the result obtained from the EM algorithm is multivariate normal distributions describing the constituents. From the distributions, the mean $\boldsymbol{\mu}_j = (\mu_{j1}, \mu_{j2}, \mu_{j3})$ and covariance matrix $\boldsymbol{\Sigma}_j$ for each constituent label l_j is known and the maximum likelihood label estimate for each voxel v_i can then be found for the label distribution that maximizes

$$P(\mathbf{x}_i | \boldsymbol{\mu}_j, \boldsymbol{\Sigma}_j) = \frac{1}{(2\pi)^{3/2} |\boldsymbol{\Sigma}_j|^{1/2}} \exp\left(-\frac{1}{2} (\mathbf{x}_i - \boldsymbol{\mu}_j)^T \boldsymbol{\Sigma}_j^{-1} (\mathbf{x}_i - \boldsymbol{\mu}_j)\right) \quad (1)$$

where the exponential term is the Mahalanobis distance providing a relative measure of the voxels distance to a given distribution.

In order to account for image noise and partial volume voxels the spatial context of the data is modeled by a Markov random field (MRF). Here, the data volume is considered as a random field defined on a set of sites, $\mathbf{S}$, where each site represents a voxel v_i . Each site has an associated stochastic variable, f_i , where $l \in \mathbf{S}$. The stochastic variables can take on a value within the set of labels $\mathbf{L}$, i.e. $f_i \in \mathbf{L}$. Subsequently

the neighborhood for each site i is defined as $\mathbf{N}_i$, which consists of six neighboring sites (voxels to the left, right, top, bottom, front and back). The probability of site i having label f_i is then given by

$$P(f_i | f_j, j \in \{\mathbf{S} \setminus i\}) = P(f_i | \mathbf{f}_{\mathbf{N}_i}) \quad (2)$$

where $\mathbf{S}$ is the set of sites and $\mathbf{f}_{\mathbf{N}_i} = f_j, j \in \mathbf{N}_i$. Given the possible set of labels $\mathbf{L}$ the smoothed labeling is found by minimizing the energy $E(f)$ of the labeling f

$$\arg \min_f \left(-\sum_{i \in \mathbf{S}} D(f_i) + \sum_{i,j \in \mathbf{N}_i} V(f_i, f_j) \right) \quad (3)$$

where $D(f_i)$ is the probability of label f to voxel site i given by Eq. (1), and $V(f_i, f_j)$ is the separation cost of f on the neighboring pixel sites i and j given by

$$V(f_i, f_j) = \begin{cases} -\beta_{ij} & \text{for } f_i = f_j \\ \beta_{ij} & \text{otherwise} \end{cases} \quad (4)$$

Here β is a parameter denoting the amount of desired homogeneity (smoothing). Hence the smoothness of the resulting segmentation can be steered by altering β . Fig. 3 illustrates the neighborhood relation of the MRF. To find the optimal segmentation solution the multi-labeling problem is solved using graph cuts with alpha expansions as described in Boykov, Veksler, and Zabih (2001).

2.6. Object labeling

In order to measure certain parameters for the segmented objects, such as mean volume and mean surface area, a labeling scheme is required. A normal connected components labeling algorithm (Sedgewick, 1998) will label multiple objects as a single object even if only one voxel connects them. Therefore a more sophisticated labeling scheme is required to separate connected objects. A custom region-growing labeling algorithm was developed for this purpose. The region-growing method relies on the distance map of a binary volume of the objects in question. The region is 'eroded' by eliminating all voxels that have a Euclidean distance smaller than a given threshold to the surrounding phase. The new binary volume is then labeled with conventional connected components labeling scheme. These labels are subsequently 'flooded' to previously eroded voxels starting with the largest distance under the threshold and iterating towards the edge between the object and surrounding phase.

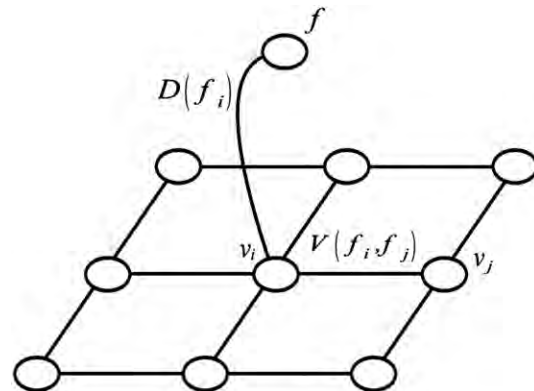


Fig. 3. The Markov random field illustrated for voxel v_i (only the left, right, top and bottom neighbors are shown). The cost of assigning label f to voxel site i is given by minimizing the energy of all possible labeling configurations.

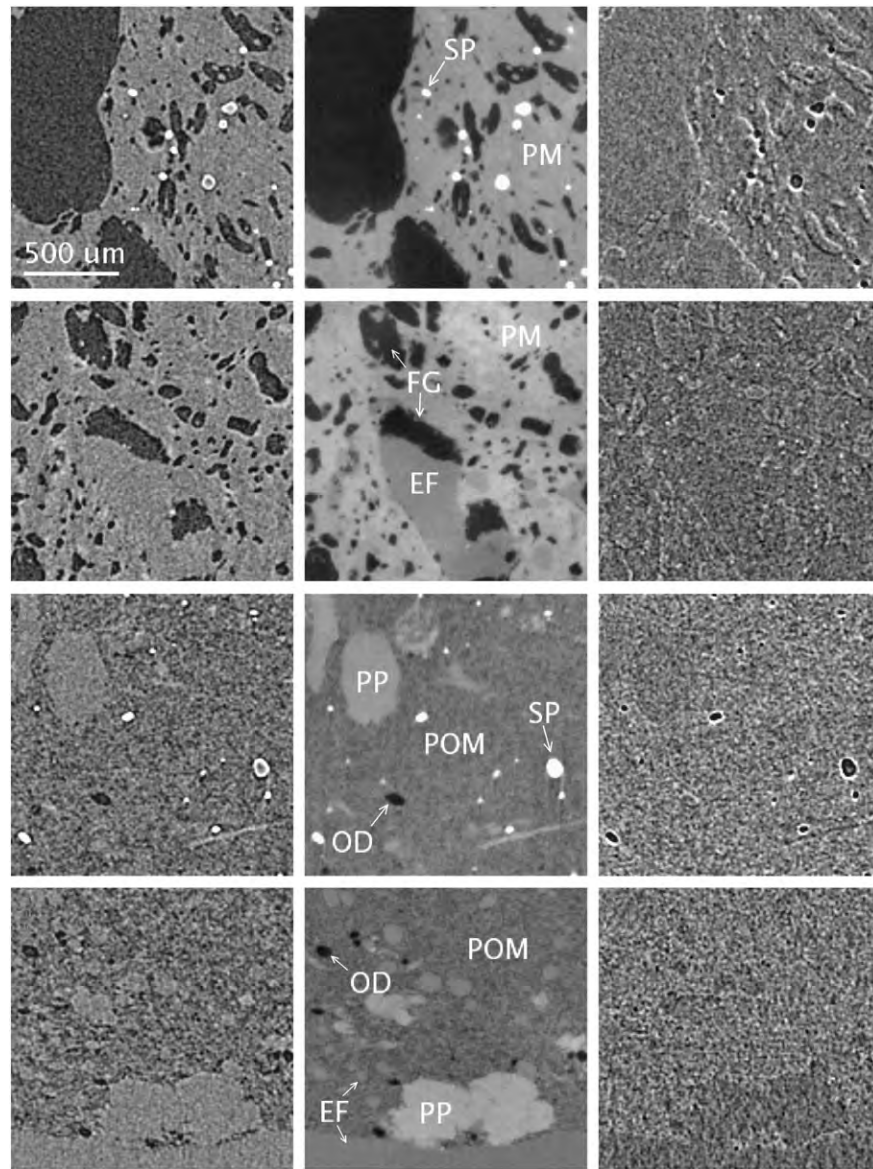


Fig. 4. Partial transverse slices from each modality for the emulsion samples where the top row shows the raw lard sample, row two the cooked lard sample, row three shows the raw sunflower oil sample and the bottom row shows the sunflower oil sample in cooked state. The left column shows the absorption modality, middle column shows the phase contrast modality and the right column shows the dark-field modality. Labels for the constituents are given in the phase contrast images: FG – fat globules; EF – expressible fluid; OD – oil droplet; POM – mixture of protein, oil, starch and moisture; PM – mixture of protein, starch and moisture; SP – salt particle. The images have been contrast enhanced for clarity.

The flooding of voxel labels is done at random to avoid directional artifacts.

2.7. Quantitative parameters

The following 3D geometric parameters for the constituents (fat, protein network, salt, expressible fluid (jelly) and oil droplets) were calculated using custom software written in Matlab: (i) the percent object volume (POV), percentage of volume for each constituent present in the sample volume; (ii) percent loss (PL), the percentage of volume for expressible fluid and fat segregated from the protein network; (iii) porosity (P), the fraction of the volume of pores (fat, salt, expressible fluid and oil droplets) within the protein network; (iv) scaled degree of anisotropy (DA), the degree of 3D asymmetry in the emulsion structure; (v) structure thickness (ST), the average of the local thickness of the protein network; (vi) mean volume (MV), a measure of the average volume of fat and expressible fluid; (vii) mean

sphericity (SP) of the expressible fluid and fat globules, which is found by

$$\psi = \frac{\pi^{\frac{1}{3}} (6V_p)^{\frac{2}{3}}}{A_p} \quad (5)$$

where V_p is the volume of the particle and A_p is the surface area¹.

3. Results and discussion

3.1. Tomography results

A partial transverse slice from each tomographic reconstruction of the emulsion samples obtained at TOMCAT is shown in Fig. 4. A

¹ The sphericity of a sphere is 1 and any particle which is not a sphere will have sphericity of less than 1.

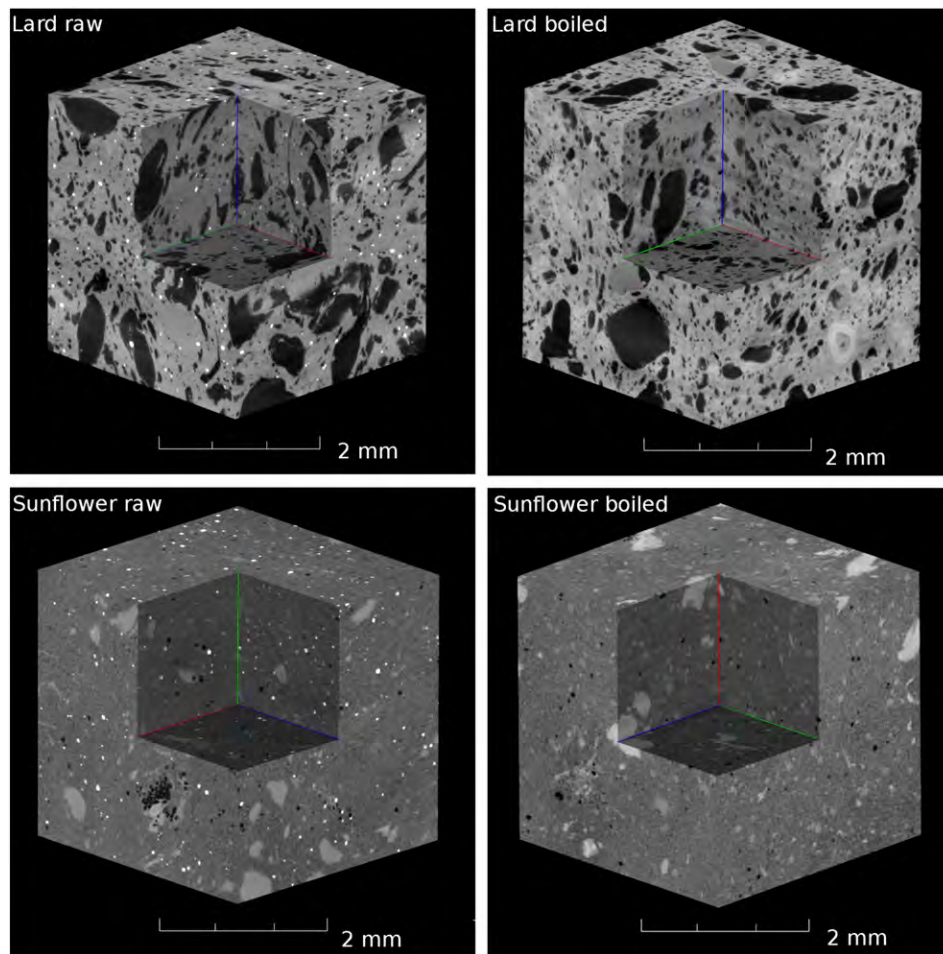


Fig. 5. Partial 3D visualization of the phase contrast sample volumes with VolView.

screenshot from a 3D visualization of the phase contrast modality using VolView can be seen in Fig. 5. Here, the pork fat is seen as darker globules in the lard emulsions and the slightly lighter regions in the emulsions with sunflower oil are pure protein that has not been mixed with the rest of the emulsion. In both the absorption and phase contrast slices the protein network has a darker intensity for the sunflower oil emulsions than the emulsions mixed with pork lard. This is due to the mixture of oil and meat, inseparable due to resolution limitations. It is apparent that the phase contrast modality results in the highest contrast

between the different constituents, and the expressible fluid is only distinguishable in the phase contrast modality. Although the dark-field modality seems mainly to consist of noise, further inspection shows that high contrasts can be seen at edges where different constituents meet. This is most notable at the salt–protein interface. The salt particles are completely dissolved after heat treatment. Additionally, expressible fluid has formed in both emulsion samples during cooking and as a result of this the protein mixture has obtained a slightly higher intensity in the phase contrast modality indicating an increased electron density.

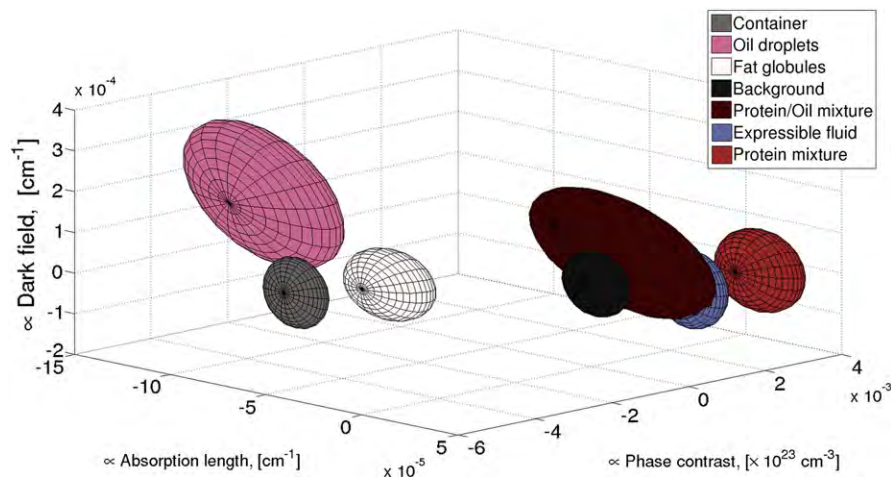


Fig. 6. The result from the EM algorithm represented by the covariance matrices of the distributions.

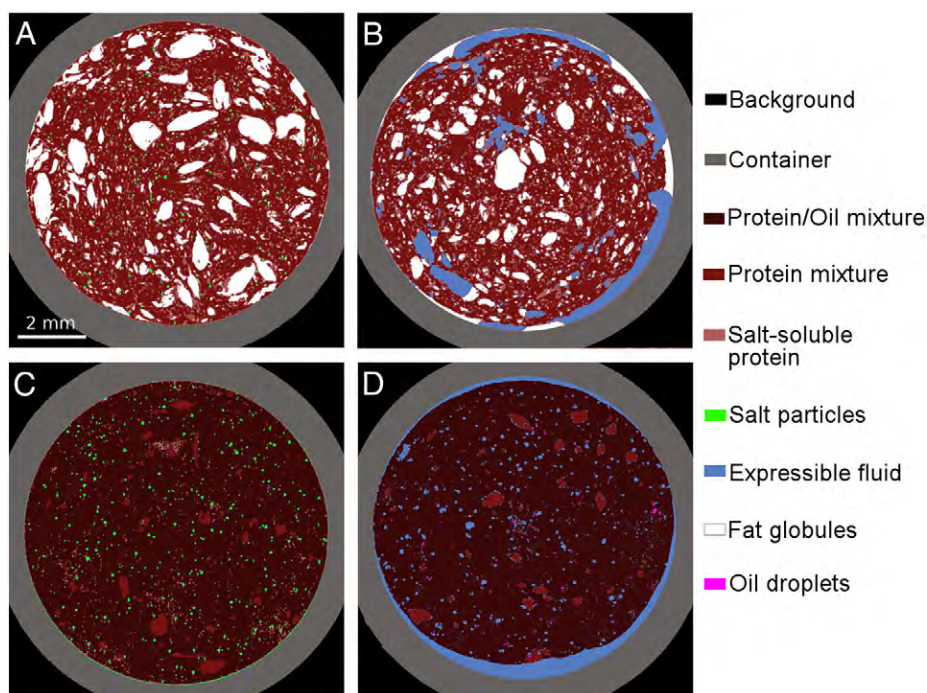


Fig. 7. A slice from each of the segmented volumes. A) Raw lard sample, B) boiled lard sample, C) raw sunflower oil sample and D) boiled sunflower oil sample.

It is apparent that the different modalities obtained provide complementary information, giving more detail of the sample imaged than previously possible with conventional X-ray absorption imaging.

3.2. Segmentation and object labeling

The mixture of Gaussians model obtained with the EM algorithm is illustrated in Fig. 6, where all constituents excluding salt are shown. The protein–oil mixture and oil phase have a higher variance in the dark field modality than the other constituents. This imaging modality can reveal information on features below the detector resolution (Pfeiffer et al., 2008). Thus oil droplets smaller than the resolution of the detector may be the cause for the high scattering in these two phases. The most distinct separation of the constituents is seen in the phase contrast modality. The absorption modality also contributes valuable information for the segmentation step. Thus, by combining the three modalities obtained in the X-ray grating interferometry tomography, additional information is available for the segmentation allowing for a better separation of the phases.

The segmentation results for each data volume can be seen in Fig. 7, where the color labeling scheme is explained to the right. These segmentation results were compared with annotations performed manually by two experts on a single slice from the phase contrast volumes. A confusion matrix is given in Table 1, giving the rate of correctly classified voxels. The main error of the segmentation constitutes of expressible fluid voxels incorrectly labeled as protein. However, the

expressible fluid is essentially protein that has solubilized, and therefore it can be difficult to determine precisely which voxels belong to the expressible fluid phase and which belong to protein. The bias of the annotation therefore varies with the user annotating the data. Additionally, most constituents interface with the protein network, and partial volume voxels containing both protein and another constituent are therefore the main cause for misclassification. Overall the segmentation accuracy of the multivariate contextual method is approximately 97%.

To highlight the importance of correct labeling of objects, before performing a further quantitative analysis, the results from the custom region growing method are compared with the standard connected components method in Fig. 8. Here the labeled objects are the fat globules in the raw lard sample. The standard method results in a large portion of the fat globules being labeled as a single object (represented in gray). The region growing method better separates single fat globules, giving a more realistic labeling result, and thereby also a more correct result from the quantitative analysis. The custom algorithm was calibrated to minimize the amount of ‘over’ segmentation. To validate the performance of the custom labeling algorithm, a ground truth was obtained by randomly selecting 20 objects labeled by the manual method and having two experts determine the number of globules it consisted of using 3D slicer visualization software. The results of the custom labeling method was then compared to this ground truth, resulting in a $(94.85 \pm 2.96) \%$ labeling accuracy compared to the $(8.42 \pm 1.36) \%$ of the manual labeling algorithm.

Table 1

Confusion matrix determined by the actual constituent labels and the predicted labels given in percentages (%). The ground truth labels are determined by two manual annotations, and the results are given as the mean $\pm$ the standard deviation from these annotations.

		Predicted class				
		Jelly	Oil	Fat	Salt	Protein
Actual class	Jelly	93.35 $\pm$ 3.04	–	0.2 $\pm$ 0.14	–	6.40 $\pm$ 2.90
	Oil	–	96.4 $\pm$ 1.9	–	–	3.6 $\pm$ 4.95
	Fat	0.07 $\pm$ 0.05	–	98.9 $\pm$ 1.13	–	1.05 $\pm$ 1.06
	Salt	–	–	–	99.05 $\pm$ 0.64	0.95 $\pm$ 0.64
	Protein	1.1 $\pm$ 0.42	0.2 $\pm$ 0.28	0.35 $\pm$ 0.50	0.1 $\pm$ 0.0	98.25 $\pm$ 0.35

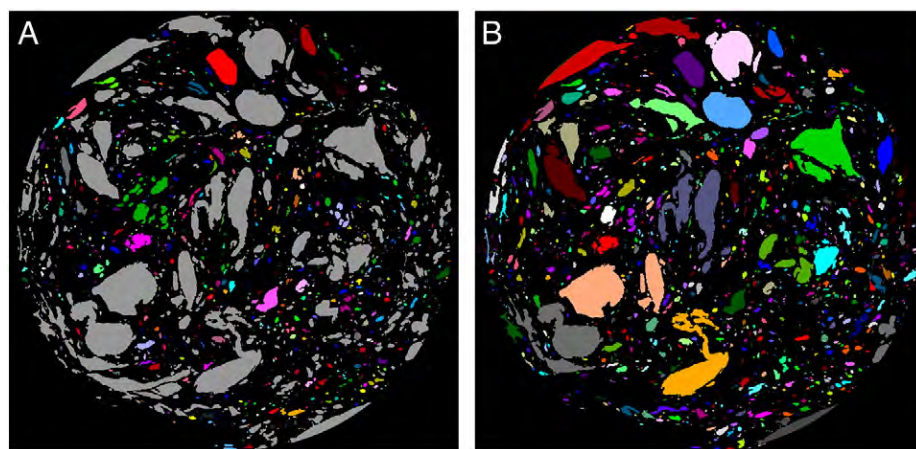


Fig. 8. Labeling results of fat globules from A) the normal connected components algorithm and B) the custom region growing algorithm applied to the segmented raw lard volume. Colors represent the separate objects labeled by the algorithms.

3.3. Quantitative analysis

Given the segmentations, a quantitative analysis of the emulsion microstructures was performed. Table 2 gives the percentage object volumes (POVs) for the constituents of the samples. The raw protein phase for the lard sample is found to be 73.9%, salt is 0.8% and fat 25.3%. When comparing the POV to the weighted ingredients (480 g meat, 5 g starch, 1.7 g salt, 248 g ice, 250 g fat) it is noted that the combined weight percentage of the meat, starch and ice amounts to 73.3% of the 1 kg batch. Since these ingredients are all segmented as the single protein mixture phase, the results fit well. The same goes for the sunflower oil sample. Here, the POV for the segmented protein and sunflower oil mixture was found at 98.3% and the POV for salt 1.7%, which is precisely the weighted percentage of these ingredients for the sunflower oil batch. Although weighted percentages and volume percentages are not the same measure, these results are reassuring. For the lard sample, the protein mixture volume decreases by 14.5% due to cooking loss after heat treatment. A smaller shrinkage is observed in the emulsion prepared with sunflower oil, and the protein network volume in this sample decreases by 11%. The cooking percent loss (PL) due to heat treatment is two-fold. First, the loss of expressible fluid is determined as the expressible fluid segregated from the emulsion. Secondly, the segregated fat located at the outer rim of the container is also considered as cooking loss. This combined cooking loss gives some insight into the moisture reserving capability of the emulsion. The expressible fluid cooking loss for the lard sample (7.4%) is slightly lower than for the sunflower oil sample (8.3%), corresponding to the findings of Barbut (1995). However, the POV of the entire expressible fluid

(including fluid trapped in the cooked emulsion) for the sunflower oil sample (12.7%) is lower than for the lard sample (15.6%). These results agree with previous findings (Choi et al., 2009; Vural, Javidipour, & Ozbas, 2004). The same results obtained for the lard sample have previously been shown in Miklos, Xu, and Lametsch (2011), where the water separation was found to be 15.2% of the total sample weight when lard was used. Additionally for the lard sample, 2.7% of the fat is segregated from the emulsion, contributing to the overall cooking loss of 10.1%.

Based on the segmented and labeled results, additional quantitative parameters were extracted, given in Table 3. The porosity of the lard sample is greater than for the sunflower oil sample due to the resolution limitations. The large fat globules are detectable, however the oil droplets are too small to be distinguished in the protein mixture. The porosity due to expressible fluid can however be detected and therefore the increase in porosity after heat treatment should preferably be considered. For the lard sample the porosity increases by 9.5% while only a 3.1% increase in porosity is observed for the sunflower oil sample. It is worth noting that the use of sunflower oil resulted in a larger number of expressible fluid populations. The mean volume of these populations is however smaller than for the lard sample. The scaled degree of anisotropy also reflects the homogeneity of the protein structure in the sunflower oil sample which has a greater 3D symmetry implying a more heterogeneous and stable protein network. The relative structure thickness of the protein network for both samples is found to decrease by approximately 21% due to heat treatment. Considering the pore structure of the samples, the expressible fluid populations within the cooked sunflower oil sample have a smaller average volume and higher sphericity, which contributes to the resulting homogeneity of the protein network. An interesting result is that the number of fat globules doubles after heat treatment, resulting in a decreased mean volume and surface area for the globules. During heating, within a temperature range of 43–70 °C, the fat within the protein encapsulated globules is in an expanding liquid form while the thin shell surrounding is in a semi-solid rigid state (Jones & Mandigo, 1982). The internal pressure in the globules can therefore cause ruptures to the shell at weak points, causing fat droplets to escape from the larger globules. These quantitative parameters illustrate how the use of sunflower oil results in a more stable and homogeneous protein network.

4. Conclusions

This paper has presented the use of a novel non-destructive X-ray technique to measure the microstructure of meat emulsions and the effect heat treatment and different lipid types have on the protein network. By utilizing the modalities obtained from the grating interferometer,

Table 2

Percent object volumes (POVs) for the constituents in the raw and cooked samples of meat emulsions containing either lard fat or sunflower oil. Cooking percent loss (PL) is given for the expressible fluid and fat segregated from the cooked emulsion.

Parameter ^a	Lard raw	Lard cooked	Sunflower oil raw	Sunflower oil cooked
POVs				
POV-meat matrix	73.9	59.4	98.1	87.1
POV-jelly	–	15.6	–	12.7
POV-fat	25.3	25.0	–	–
POV-salt	0.8	–	1.7	–
POV-oil droplets	–	–	0.2	0.2
Cooking loss				
PL-jelly	–	7.4 ^b	–	8.3 ^b
PL-fat	–	2.7 ^c	–	–

^a Percent object volumes: POV, percent object volume (%); PL, percent loss (%).

^b Percent loss of expressible fluid segregated from the cooked emulsion.

^c Percent loss of fat segregated from the cooked emulsion.

Table 3Three dimensional μ CT geometric parameters for the raw and cooked samples of meat emulsions containing either lard fat or sunflower oil.

Parameter ^a	Lard raw	Lard cooked	Sunflower oil raw	Sunflower oil cooked
<i>Meat matrix</i>				
P-meat matrix	26.1 ^b	35.6 ^b	1.9 ^b	5.0 ^b
DA-meat matrix	0.45 ^c	1 ^c	0.12 ^c	0.16 ^c
ST-meat matrix	80.16	62.97	152.25	119.80
<i>Pores^d</i>				
MV-fat	$3.2350 \cdot 10^5 \pm 1.7628 \cdot 10^7$	$1.7637 \cdot 10^5 \pm 1.6865 \cdot 10^7$	–	–
MV-jelly	–	$1.1297 \cdot 10^5 \pm 3.5930 \cdot 10^6$	–	$8.0778 \cdot 10^4 \pm 4.6515 \cdot 10^5$
MS-fat	$1.5344 \cdot 10^5 \pm 4.1755 \cdot 10^6$	$8.6519 \cdot 10^4 \pm 3.0862 \cdot 10^6$	–	–
MS-jelly	–	$7.6314 \cdot 10^4 \pm 1.2983 \cdot 10^6$	–	$8.0637 \cdot 10^4 \pm 2.6443 \cdot 10^5$
N-fat	173,995	341,994	–	–
N-jelly	–	49,072	–	63,750
SP-fat	0.7297 ± 0.1245	0.7848 ± 0.12	–	–
SP-jelly	–	0.6488 ± 0.1305	–	0.8055 ± 0.1388

^a Quantitative parameters: P, porosity (%); DA, degree of anisotropy (dimensionless); ST, structure thickness (μm); MV, mean volume (μm^3); MS, mean surface area (μm^2); N, number of objects (dimensionless); SP, sphericity (dimensionless).

^b Pores include fat globules, salt, oil droplets and expressible fluid within the protein network.

^c DA is scaled such that a perfectly isotropic cylinder has the value 0, and the most anisotropic structure (the cooked lard sample) has the value 1.

^d Results are given by the mean $\pm$ the standard deviation.

complementary contrasts were obtained, making it possible to distinguish expressible fluid from the protein network in the emulsions. Without the three modalities obtained from the grating-based technique, deriving microstructural parameters would not be feasible. The X-ray technique is presently the only one allowing for all three modalities and therefore shows great potential for imaging the microstructure of both meat emulsions and other food related products.

For the analysis of the data, a segmentation method based on Gaussian mixture models and MRF labeling with graph cuts was implemented. Quantitative parameters that represent the emulsion structure were then extracted. These parameters include the POVs of the different constituents and the porosity, degree of anisotropy and average structure thickness of the protein network. The results confirmed the difference in homogeneity of the protein network, which had already been inspected visually. As this study was limited to a single sample of each emulsion type due to limited beamtime no attempts were made to compare these findings with other quality parameters such as texture profile measurements, color composition, sensory panel evaluation, and apparent viscosity. Nevertheless, it has been shown that grating based X-ray imaging combined with multivariate contextual segmentation is a feasible method for the investigation of microstructural changes of meat emulsions due to heat treatment, and can serve as a valuable tool for further investigations.

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Publication IV

Submitted to Food Structure.

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Ptychographic X-ray computed tomography of extended lipid networks in food emulsions.

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Ptychographic X-ray computed tomography of extended colloidal networks in food emulsions

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Abstract

As a main structural level in colloidal food materials, extended colloidal networks are important for texture and rheology. By obtaining the 3D microstructure of the network, macroscopic mechanical properties of the material can be inferred. However, is hampered by the lack of suitable non-destructive 3D imaging techniques with submicron resolution.

We present results of quantitative ptychographic X-ray computed tomography applied to a palm kernel oil based oil-in-water emulsion. The measurements were carried out at ambient pressure and temperature. The 3D structure of the extended colloidal network of fat globules was obtained with a resolution of around 300 nm. Through image analysis of the network structure, the fat globule size distribution was computed and compared to previous findings. In further support, the reconstructed electron density values were within 4% of reference values.

Keywords: Food emulsion, Colloidal network, 3D microstructure, X-ray Ptychography, Computed tomography, X-ray phase-contrast imaging

1. Introduction

Extended colloidal networks constitute one of the main structural elements in multi-phase food materials such as butter, chocolate, cream cheese, whipped creams, ice cream, cheese and yogurt. Focus has been directed toward establishing the relationship between the structure of such networks on one side, and the macroscopic mechanical properties and sensorial textural properties on the other side [31, 17]. On a qualitative level, correlations have been established between microscopic obser-

15 vations of loose networks and low modulus or relationship between a more dense network and a higher modulus (see for example [23, 42, 5]). On a more quantitative level, a fractal description has been employed for inorganic colloidal networks [33], for protein systems [34, 41] and fat systems [25, 30, 26] leading to a quantitative relationship between structure and mechanical properties.

20 The experimental study of such networks has traditionally been based on light microscopy such as polarized light microscopy (PLS) and confocal laser scanning microscopy (CLSM). From the microscopy measurements, the network properties have for the most been analyzed using 2D slices. An extension to 3D imaging can be done by forming 3D image stacks from consecutive microgra-

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phy at different depths in the food product. In this way, Litwinenko used transmitted PLS and image deconvolution to study the fractal properties of a fat crystal network in two and three dimensions [22]. Similarly, CLSM can be applied as an 3D imaging method within food science. However, the obtained stacks are limited by the optical system and the laser penetration depth to some tens of microns from the top surface [10]. Furthermore, although a sub-micron spatial resolution is possible, scattering in the sample may limit the resolution along the vertical axis to the micron range [13]. In addition, the need of staining in CLSM may introduce artifacts and limit the in-situ applicability [10]. To our knowledge CLSM has not been used to image the 3D structure of colloidal networks in food materials.

In recent years, X-ray phase-contrast computed tomography (CT) has emerged at synchrotron facilities as a non-destructive 3D imaging modality, and has been successfully applied to study the microstructure of a range of food products [12, 40, 21, 27]. One of the most recent techniques for obtaining the X-ray phase-contrast modality is ptychographic X-ray computed tomography (PXCT) [8]. PXCT is a 3D nano-imaging technique that offers a spatial resolution in the 100 nm range. Unlike traditional X-ray microscopy, the spatial resolution is not dependent on objective lenses. Instead, spatial information is retrieved from the recorded diffraction of a coherent X-ray beam, and the spatial resolution is only limited by the angular spread of the scattered intensity. In addition, PXCT provides quantitative information by reconstructing the full 3D electron density distribution of the specimen [7, 38]. The technique is well-suited for in-situ measurements as it allows for sample environments at room temperature and ambient pressure. Previously, it has successfully been applied for an in-situ study of water uptake in a single silk fiber [11]. Altogether, PXCT is a promising candidate for 3D imaging of extended networks in food products.

As a model system for studying extended colloidal networks, a palm kernel oil (PKO) based oil-in-water emulsion is presented. These PKO emul-

sions are used as whippable creams for decorations of cakes where the fat globule network formation is important. Design of emulsions for whipping relies on tuning the propensity of partial coalescence of the oil droplets. Initially PKO emulsions are normally liquid, and first upon whipping the material is transformed to a foam of rather high viscosity and stability. However, too high propensity for partial coalescence can lead to product flaws such as solidification of the liquid emulsion to solid pastes during transport. As an example of a product flaw, a PKO emulsion exhibited pre-whipping solidification upon addition of two different combinations of lactic acid ester of monoglyceride (LACTEM) and unsaturated monoglyceride (GMU) [29, 28]. In these two systems, 2D CLSM micrographs of the lipid phase revealed large irregular aggregates and formation of extended networks of fat globules. In addition, increased hardness and viscoelastic modulus were observed. The added emulsifiers are believed to induce partial coalescence of the fat globules and transform the emulsion spontaneously from liquid to semi-solid [28]. However, due to strong multiple scattering of the laser light, the 3D structure of the network could not be determined using CLSM.

Thus, the exact extend and composition of the network of fat globules in 3D are still unknown. In addition, exactly how the water and lipid phases are located remain to be directly observed. In this study, a PKO emulsion with two combinations of LACTEM and GMU emulsifiers are measured with PXCT and compared to 2D CLSM micrographs. The 3D structure of both water and lipid phase as well as the quantitative electron density values are investigated.

2. The X-ray phase-contrast modality

The type of image contrast acquired in X-ray tomograms depends on the interreaction between the X-rays and matter. Both refraction and absorption of X-rays in matter are given by the full complex index of refraction [1]

$$n(\mathbf{r}) = 1 - \delta(\mathbf{r}) + i\beta(\mathbf{r}), \quad (1)$$

where the real part δ accounts for the refraction and the imaginary part β for the absorption. In X-ray phase-contrast techniques such as PXCT, the 3D distribution of $\delta(\mathbf{r})$ is reconstructed in the tomogram. Thus, the gray levels in the resulting images are due to the spatial variations of $\delta(\mathbf{r})$ in the material. These can be related to the electron density $\rho_e(\mathbf{r})$ as

$$\rho_e(\mathbf{r}) = \frac{2\pi\delta(\mathbf{r})}{r_0\lambda^2}, \quad (2)$$

where r_0 is the Thomson scattering length and λ the X-ray wavelength. For materials with known atomic composition, values of ρ_e obtained by PXCT can be compared to calculated values. For mixtures of materials of different weight-percentages w_j , the total electron density can be calculated as

$$\rho_e = N_A \rho_m \sum_j w_j \frac{Z_j}{M_j} \quad (3)$$

where N_A is Avogadro's constant, ρ_m is the mass density and Z_j and M_j are the number of electrons and the molar mass of the j th material, respectively.

3. Material and methods

3.1. Materials

Emulsifiers were of commercial food grade and all were provided by Palsgaard A/S (Juelsminde, Denmark): Lactic acid ester of monoglyceride (LACTEM) made from fully hydrogenated palm oil and rape-seed oil (C16:0 and C18:0 > 97% of fatty acids); unsaturated monoglycerides (GMU) made from sunflower oil (C18:1 > 81%). The stabilizer mixture (Palsgaard A/S, Juelsminde, Denmark) contained microcrystalline cellulose (MCC),

sodium carboxymethylcellulose (CMC) and disodium phosphate. Hydrogenated palm kernel oil (PKO) was obtained from AAK (Karlshamn, Sweden), sodium caseinate from DMV International (Veghel, The Netherlands) and sugar from Nordic Sugar (Nakskov, Denmark). Fatty acid composition of the PKO has previously been determined [28].

3.1.1. Emulsion blend preparation

Sodium caseinate (0.6 wt.%), stabilizer mixture (0.6 wt.%) and sugar (10 wt.%) were dispersed in water under continuous stirring and put aside for 4 h to hydrate proteins. Melted PKO (25 wt.%), either LACTEM (0.55 wt.%) alone or with GMU (0.15 wt.%) were mixed with the water phase, and the mixture was heated to 80 °C. A pre-emulsion was obtained by mixing with a high-shear blender (Ultra-Turrax, IKA, NC, USA) for approximately 20 s. Homogenization was subsequently carried out on a two-stage high-pressure valve homogenizer (PandaPlus 2000. GEA Niro Soavi, Parma, Italy) at 150/50 bar followed by cooling in a tubular heat exchanger to 30 °C. Immediately afterwards, small samples of the emulsions were prepared in micropipettes using a syringe. Subsequently, all emulsion samples were stored at 5 °C.

3.1.2. Micropipette preparation

The micropipettes were prepared from thin wall borosilicate capillaries with filament (Harvard Apparatus UK, Cambridge, UK) by using a micropipette puller (Sutter Instrument, CA, USA). The capillaries were pulled from the original 0.94 mm to an inner diameter at the tip in the range from 15 to 20 µm. Prior to measurements, the micropipettes were mounted on custom-made tips.

3.2. Confocal laser scanning microscopy

Micrographs of the lipid phase in the emulsion were obtained by an inverse confocal laser scanning microscope (Leica TCS SP5, Heidelberg, Germany). Total concentration of 1 ppm BOD-IPY 493/503 (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene) (Invitrogen, Carlsbad, USA) dissolved in dimethyl sulfoxide was

added to emulsions to stain lipids. The excitation wavelength was 488 nm and the emission bandwidth was 500–570 nm. A water immersion objective (HCX PL APO lambda blue 63.0 $\times$ 1.20 water UV) was used, and the image size was set to 1024 $\times$ 1024 pixels. A thin layer of stained emulsion was applied on a large standard cover glass and covered by another cover glass to avoid evaporation/drying. Around 20 micrographs were acquired of each emulsion preparation.

3.3. Ptychographic X-ray computed tomography

The PXCT measurements were conducted at the cSAXS beamline at the Swiss Light Source, Paul Scherrer Institut, Switzerland, using 6.2 keV X-rays and the setup described in [18]. A sketch of the setup is shown in figure 1

A coherently illuminated freznel zone-plate of diameter 170 μ m and 60 nm outer-most zone width was used to shape the illumination onto the sample. The sample was placed 1.8 mm downstreams of the focal point where the beam had a diameter of about 6 μ m. Because the initial illumination was causing clear radiation damage effects on the emulsion samples, the flux was reduced by a factor of 10 by opening the undulator gap by about 30 μ m from the maximum intensity. The final flux of about 1×10^8 photons/s did not cause any visible damage to the emulsions.

Ptychographic scans were performed over different fields of views (FoV) for each sample, as seen in table 1. The scanning pattern followed a Fermat spiral trajectory [20] with an average spacing between points of 2 μ m. A diffraction pattern was recorded at each point with an exposure time of 0.1 s with an Eiger 500k detector as in [9, 16] which was located 7225 mm downstream of the sample position behind a He-filled flight tube. Ptychographic scans were repeated at several angular positions of the specimen with respect to the X-ray beam, covering a 180° range. The total number of diffraction patterns and angular projections were different for each sample as detailed in table 1. The total measurement time varied between 3-10 hours.

Four tomograms were acquired in total. Two were of the emulsion containing both LACTEM

and GMU and two of the emulsion containing only LACTEM as indicated in table 1. The latter two tomograms were conducted on the same micropipette.

From the acquired data, first 2D ptychographic projections of the phase were reconstructed using a combination of the difference map algorithm [36] and a maximum likelihood optimization [15] used as a refinement step [37]. For ptychographic reconstructions In order to aim at a final 3D tomographic spatial resolution of 100 nm, an area on the detector of 400x400 pixels around the direct beam was selected such that the reconstructed ptychographic projections had a pixel size of 49 $\times$ 49 nm². Note that the pixel size and spatial resolution are not the same. Multiple pixels may be required to resolve features in the final images. The reconstructed phase projections for each tomogram were further processed and combined in a tomographic reconstruction using the filtered back projection as described in [14]. The tomographic slices were corrected for a constant bias off-set by forcing the mean value of the air outside the sample to be zero. The processing was done using a software implementation in MATLAB at the cSAXS beamline.

The spatial resolution of the final tomograms was evaluated by comparing their Fourier shell correlation (FSC) curves with the 1/2-bit threshold curve [39]. The sample stability turned out to be the limiting factor. Whereas the 3D resolution in a region with the micropipette was around 100 nm for all samples, the resolution in a region of the emulsions themselves were between 207-355 nm, as seen in table 1. This reduction can be ascribed to sample movement during measurements and could induce a blurring of the features in the final tomograms.

The dose d imparted on the specimen was estimated using the expression $d = N_0 \times E \times \mu/\rho_m$ where μ/ρ_m is the mass attenuation coefficient, N_0 is the number of incident photons per unit area, E is the photon energy, and ρ_m is the mass density [19]. Since the illumination, average step size and exposure time was kept constant, the dose was the same for all 2D ptychographic scans. Using $N_0 =$

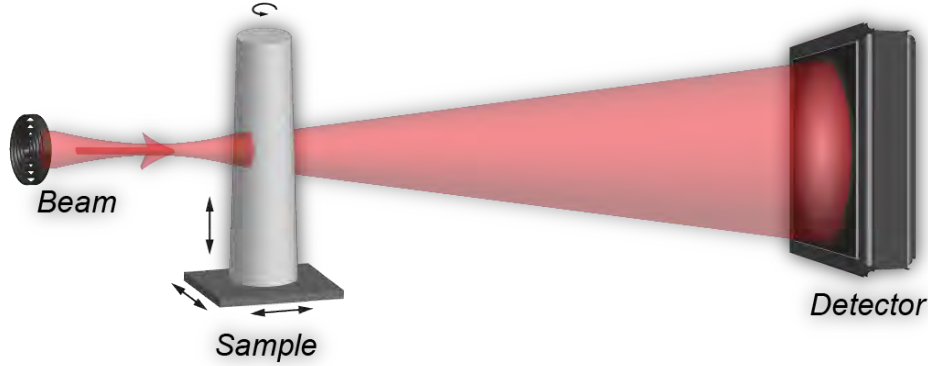


Figure 1: A sketch of the PXCT setup. The beam is shaped by a fresnel zone-plate before illuminating the sample. At the detector position, a diffraction pattern of the illumination is acquired. For the ptychographic scans, the sample may be translated in all directions. Rotation around the vertical axis is used for the tomography measurements.

Measurement details

	Sample 1	Sample 2	Sample 3	Sample 4
Emulsifier mix.	L+G	L+G	L	L
FoV [μm^2]	48x40	30x20	30x20	45x27
No of dif. pat.	481	151	170	311
Angular projs	200	250	300	400
Spatial res. [nm]	342	207	280	355
Total dose [MGy]	1.0	1.2	1.5	2.0

Table 1: Information on sample composition, settings for the PXCT measurements, and the spatial resolution in the reconstructed tomograms. L=LACTEM and G=GMU.

2.8×10^6 photons/ μm^2 and $\mu/\rho_m = 18 \text{ cm}^2/\text{g}$ for the emulsion at 6.2 keV [6], the dose was $d = 5.0 \times 10^3$ Gy per ptychographic scan. The total dose on each sample depended only on the number of angular projections, as shown in table 1.

3.4. Image analysis

To perform noise correction on the tomographic slices, a customized iterative 3D implementation of the non-local means algorithm [4] was applied. The level of noise and artifacts in the PXCT were assumed to be independent of the signal intensity. The denoising algorithm was implemented using Python.

An alpha-level Markov random field (MRF) segmentation as described in [32] was applied for segmentation of the tomograms into air, micropipette,

water, lipid and cellulose phases. First, the data was modeled as a mixture of distribution functions by assigning a probability distribution for each ascribed phase in the tomogram. Due to the sample movement during measurements, the water and lipid phases appeared blurred in the tomograms, and a model of two Gaussian distributions was used for these (see supporting material section Appendix A.2). After assigning probability distributions, the spatial information of the data was incorporated into the segmentation process by modeling the data as an isotropic MRF [24]. The MRF smoothing parameter was set to 0.5. To find the optimal segmentation solution the multi-labeling problem was solved using graph cuts with alpha expansions as described in [3]. Image analysis as well

as visualization of the tomograms was performed using own software implemented in MATLAB.

3.4.1. Quantitative parameters

From the segmented tomograms, the percent object volume (POV) values for the identified lipid, water and stabilizer phases were calculated. Reference POV values were obtained by calculation based on the emulsion blend preparation. The continuous phases and isolated regions of the phases were obtained by applying a connected components labeling algorithm. The volume fraction of the total lipid phase found in the largest connected network was calculated.

Assuming that the lipid phase in the emulsion had only partially coalesced, the individual fat globules would still be visible in the 3D network. To investigate this, the structure of the lipid phase was divided into smaller local domains using a watershed approach. From the segmented lipid phase, a distance map from the lipid to the surroundings was calculated before the watershed algorithm was applied. From the volume of each identified lipid domain, the equivalent diameter was extracted assuming a spherical shape. In addition, the mean diameter and the size distribution of these diameters were calculated.

A mean electron density was calculated for both the emulsion phases as well as the micropipette. The segmented phase was used as a mask on the acquired tomogram to identify the electron densities for each phase. Reference values were obtained from the known chemical composition and mass density of the components in the phases using equation (3) (see supporting material). The reference lipid phase value was calculated from the fatty-acid composition of the partly hydrogenated PKO which has been reported in [28]. For the micropipette, the atomic composition of the borosilicate material was supplied by the manufacturer [Apparatus].

4. Results

In figure 2, a tomographic slice from the PXCT measurement of sample 1 is displayed before and

after noise correction in panels a) and c), respectively. As the lipid phase has a lower mass density, and thereby lower electron density, than the water phase, it appears dark gray while the water phase appears light gray. Due to movement during the tomographic measurements, the lipid phase will be distributed over a larger volume. This causes a smearing in the reconstructed images which can be seen clearly in panel c) as brighter regions surrounding the lipid phase.

In panel b) a CLSM micrograph of the stained lipid phase is shown of an emulsion from the same batch as sample 1. When comparing the micrograph with the tomographic slice in panel c), some differences of the morphology of the lipid phase are seen. While the lipid phase in the micrograph forms large irregular aggregates, the tomographic slice shows individual fat globules or smaller aggregates.

Part of this discrepancy can be explained by the difference in vertical spatial resolution of the two methods. While the PXCT has an effective resolution of 200-300 nm with a slice thickness of 49 nm, the vertical resolution of the micrograph is in the micron range. Thus, more lipid phase will be visible in the micrograph than in a single tomographic slice. For a better comparison of the two modalities, the mean lipid phase from a stack of 41 tomographic slices with a total thickness of 2 μm is depicted in panel d). The segmented tomogram has been used as a mask to identify the lipid phase. As in the CLSM micrograph, the lipid phase in panel d) is seen to consist of large irregular aggregates with a similar morphology as in panel b).

A 3D representation of the segmented tomogram of sample 2 is illustrated in figure 3 (See supporting material section Appendix A.3 for sample 1, 3 and 4). Displayed in panel a) are the identified emulsion phases; lipid, water and stabilizer. No air pockets were found in the volume inside the micropipette in this nor any of the other tomograms. The largest connected part of the lipid phase is shown in yellow while the remaining isolated regions are shown in orange. As seen, the vast majority of the lipid phase was connected in

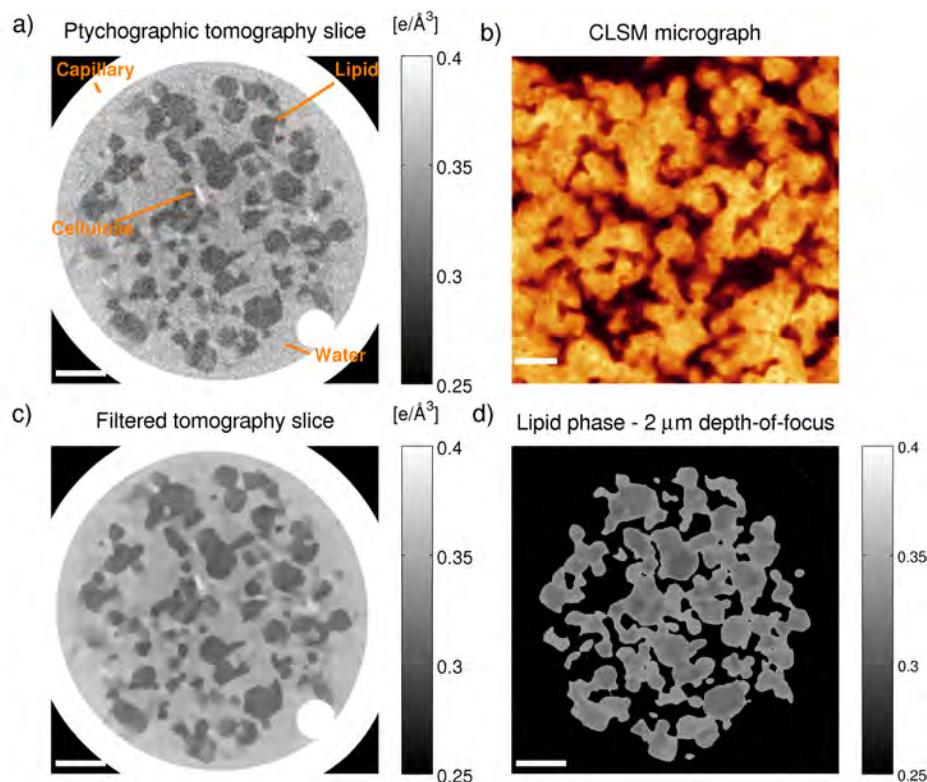


Figure 2: A ptychographic tomographic slice (panels a and c) of the sample 1 emulsion compared to a CLSM micrograph (panel b). The tomographic slice before a) and after c) noise correction depicts the electron density of the sample in $[e/\text{\AA}^3]$. In panel d) shows only the lipid phase of the tomographic slice where the depth-of-focus has been increased to 2 μm by averaging over 40 slices in the z-direction. The length of the scale bar corresponds to 10 μm .

a single network which was also observed for the other phases. This space-filling lipid network constituted more than 98% of the total lipid volume in all samples. The surrounding water phase is shown in blue in panel a) of figure 3. Even though more than 99% of the water volume was situated in this continuous phase, isolated water pockets inside the lipid phase were observed as illustrated in green color in the close-up in panel b). Besides the lipid and water phases, a third stabilizer phase was identified which is depicted in gray in panels a) and c). From the elongated shape and the location in the water phase, these regions were interpreted as microcrystalline cellulose from the stabilizer mixture.

The morphology of the lipid network in figure 3 panel a) can be inspected more closely in figure 4. In panel a), the connected lipid phase is depicted

as a labelmap representation from the applied watershed algorithm where each lipid domain is represented in a different color. Visually, the lipid network seems to consist of spherical structures aggregated into a close packing. This is consistent with a partial coalescence of the fat globules. Although some labels are clearly non-spherical in shape, the algorithm has identified a large number of spherical lipid domains that resemble individual fat globules. The resulting size distributions of the equivalent diameters of all four samples are shown in panel b). It is seen that the samples give almost identical size distributions with the mode at around 1 μm . The mean values of the equivalent diameters are between 1.2 – 1.4 μm , as seen in table 2. These distributions closely resemble previously reported distributions of the fat globule sizes using

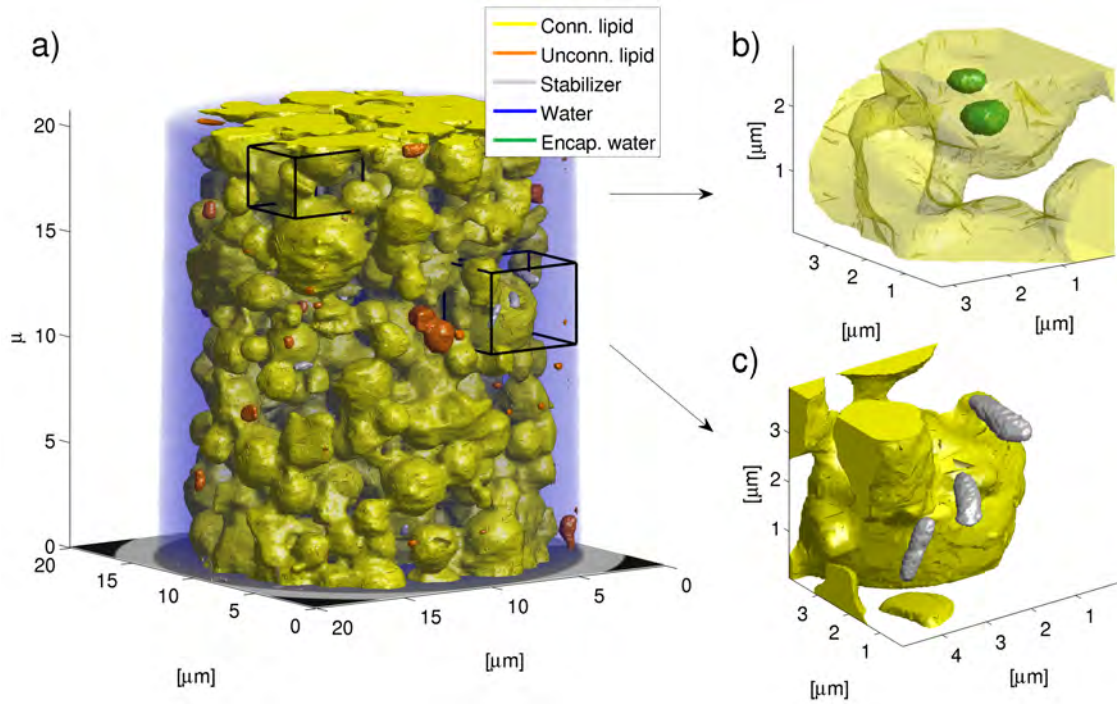


Figure 3: 3D representation of the sample 2 emulsion. a) The full emulsion with the water phase shown in blue, the stabilizer in phase in gray, the connected lipid phase in yellow and isolated lipid regions in orange. b) A close-up of the sub-volume in the top box of a) depicting an isolated water pocket inside the connected lipid phase. c) A close-up of the stabilizer phase in the sub-volume of the bottom box of panel a).

dynamic light scattering [29, 28]. Since these latter were measured on control PKO emulsions without added LACTEM or GMU, the present findings in panel b) support the hypothesis of partial coalescence.

Measured and reference POV values are shown in table 2. POV of the cellulose was lower than the expected in all tomograms. This might be due to the confined space in the micropipette which would not permit larger pieces of microcrystalline cellulose. The POV for lipid- and water phase varied between the tomograms with a larger lipid phase POV in sample 1-3 than expected and a smaller in sample 4. These variations demonstrate the challenge of obtaining a representative sampling with volumes on the micron scale. Although sampled within 100 μm on the same micropipette, large variations in the POV values of sample 3 and 4 were observed.

In addition to the spatial information, PXCT also provides information on the electron density distribution in the sample. In table 2, mean electron densities for the emulsion phases and the micropipette phase are shown for all measured samples as well as reference values. For all phases, the measured mean electron density values are quite similar across the samples. In addition, the precision of the PXCT measurements can be estimated by comparing the measured micropipette values with the reference values. For all samples, the obtained mean electron density for the micropipette phase was within 1% of the reference value as seen in table 2. A bit worse agreement was found when comparing measured and reference values of the emulsion phases in table 2. For the lipid and water phases, the measured mean electron densities were around 4% higher than the reference values. On the other hand, the cellulose from the stabilizer

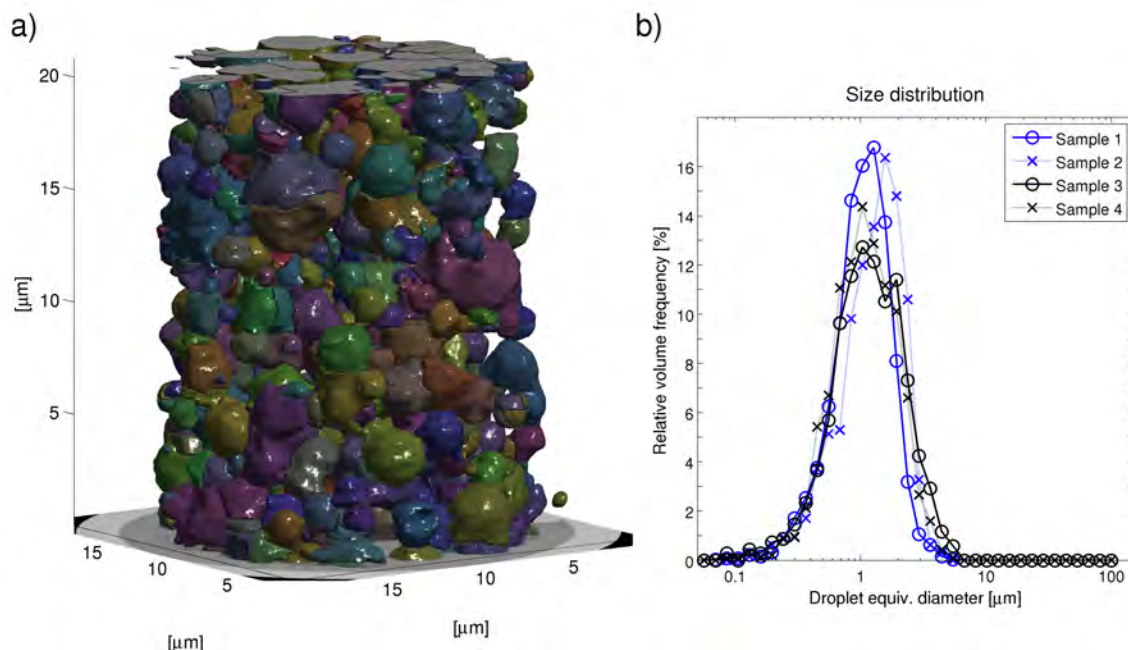


Figure 4: a) A 3D representation of the resulting labelmap after applying the watershed algorithm to the connected lipid phase from figure 3 panel a). The coloring distinguishes individual domains. b) The size distributions of the equivalent diameters for all four samples.

mixture displayed values 20% lower than the reference. For the cellulose, the size of the individual cellulose pieces was small compared to the spatial resolution which would result in a lower observed mean value.

5. Discussion

The main focus in the present study regards the extend, structure and composition of the lipid phase in the PKO emulsion. For all samples, the PXCT measurements revealed an extended network connecting almost all of the fat globules. In addition, the morphology of the network was consistent with a partial coalescence of the fat globules to an extreme degree. These findings were in accordance with previous studies [29, 28]. No clear structural differences between the two formulations with LACTEM and LACTEM+GMU were apparent. Previously, the two formulations have also been found to be similar in both microstruc-

ture and elastic modulus [29]. While a single- or two-phase system is often employed when studying extended networks in food systems, the sample PKO emulsion used here constitutes a full multi-phase food system. This indicates that PXCT can be used to investigate even complex food systems.

When considering the small sample volume in the PXCT measurements, a relevant question is how these findings relate to the bulk emulsion. As the micropipettes used for PXCT confined the PKO emulsions to a few tens of micrometers in diameter, the observed structure might be different than for bulk emulsion. This could be due to an applied shear stress during injection or a change in fat globule size distribution due to the micropipette width. However, while the amount and size of microcrystalline cellulose was reduced, the lipid morphology and distributions of domain diameters were in agreement with previous findings for bulk PKO emulsion. In addition, no air pockets larger than the spatial resolution were observed indicating an ab-

Percent object volume (POV)					
Data type	Ref	Sample 1	Sample 2	Sample 3	Sample 4
	[vol%]	[vol%]	[vol%]	[vol%]	[vol%]
Lipid phase	28.4	31.2	38.1	37.7	26.5
Water phase	71.4	68.7	61.9	62.2	73.5
Cellulose	0.2	0.05	0.01	0.03	0.02

Electron densities, ρ_e					
	[e/Å ³]	[e/Å ³]	[e/Å ³]	[e/Å ³]	[e/Å ³]
Lipid phase	0.308	0.314	0.323	0.321	0.318
Water phase	0.348	0.362	0.363	0.360	0.360
Cellulose	0.477	0.397	0.382	0.391	0.374
Micropipette	0.666	0.668	0.671	0.673	0.668
Bulk	0.336	0.347	0.348	0.345	0.349

Mean equivalent diameter, d					
	[μm]	[μm]	[μm]	[μm]	[μm]
Lipid phase	0.98	1.15	1.41	1.40	1.25

Table 2: Percent object volume (POV), mean electron densities and equivalent lipid diameter from the four tomograms compared to reference values. The bulk emulsion electron density was calculated from the ρ_e and POV values of the emulsion phases in the table. Electron density values for the micropipette are included as well.

sence of large shear stresses. Furthermore, a presence of sub-resolution air pockets would result in a reduction of the observed electron density. Thus, since the measured electron densities for the lipid and water phases were higher than the reference values, a large presence of sub-resolution air pockets is unlikely.

Since PXCT provides quantitative electron density values, these values may be used to identify the known phases in the sample and highlight unknown or unexpected. Hence, since the observed mean electron densities for the water and lipid phases were within 4% of the reference values, these phases could be easily recognized in the reconstructed tomograms. In addition, the quantitative electron densities allowed to distinguish the stabilizer phase even though the POV was below 1%.

When applying PXCT for food products, the sample stability becomes an issue. As described

above, radiation damage due to the initial high X-ray flux required a reduction in the beam flux. However, as this reduction prolonged the total scan time, movement of the sample during measurements became an issue for the spatial resolution. A way to avoid these challenges would be to measure the sample in a frozen state. Hence both stability and resistance towards radiation damage would be increased. However, this will not be an option for studies of the native state at room temperature as in the present study.

Compared to other X-ray phase-contrast imaging techniques, a main advantage of PXCT is the submicron spatial resolution offered. In comparison, X-ray grating interferometry is limited to the micron range by the period of the gratings used. Propagation-based techniques for X-ray phase-contrast imaging are restricted by the (effective) pixel size of the detection system, and are typically also in the micron-range resolution. An X-ray

phase-contrast technique which has achieved sub-
micron resolution for biological samples is full-
field tomographic microscopy with Zernike phase-
contrast [35]. Potentially, this could be an alterna-
tive way to resolve the structures of the PKO emul-
sion. However, quantitative reconstruction of the
electron density is challenging using this technique
compared to PXCT.

Compared with CLSM, PXCT offers a more
isotropic PSF which results in a uniform spatial
resolution in both horizontal and vertical direction.
Furthermore, PXCT is not limited to investigations
just below the surface of the sample. In addition,
PXCT measures the unaltered sample without stain-
ing as in CLSM, and hence provides the electron
densities of all phases present. Thus, besides the
structure of the lipid phase, also the water phase,
microcrystalline cellulose and the absence of air
pockets were observed in PXCT.

One of the limitations of PXCT is that the hori-
zontal FoV is restricted to a few tens of microm-
eters. Also, while e.g. CLSM can be performed
using tabletop lap equipment, PXCT is limited to
synchrotron facilities.

6. Conclusion

This study has shown the feasibility of PXCT
for food science applications. The obtained 3D mi-
crostructure of the PKO emulsion constitutes the
first reported direct measurement of an extended
network of fat globules in a food product. In the
PKO emulsion, the lipid network was structured
by aggregated lipid domains and almost the en-
tire lipid fraction was included in the extended net-
work. This demonstrated an extreme case of partial
coalescence of fat globules in a oil-in-water PKO
emulsion. The observed sizes and shapes of the
lipid domains were in agreement with CLSM mi-
crographs and previous measurements of fat globule
size distribution.

The electron density values measured by PXCT
were consistent across all measured samples. For
the micropipette, the measured mean values were
within 1% of the reference value. For the lipid
and water phases, the values were within 4% of

the expected value. Thus, as a quantitative method,
PXCT can be used to investigate the composition
of the sample. In the present study, this was used
to rule out the presence of sub-resolution air pock-
ets.

In further work, the 3D network structures ob-
tained from the PXCT measurements could be used
for fractal analysis as in e.g. [30, 26]. From such
analysis, a link between the microstructure and
macroscopic mechanical properties of the emul-
sion could be established.

Altogether, PXCT is a promising tool for spatial
and quantitative investigation of food products on
the sub-micron scale. This gives a novel quanti-
tative experimental approach for direct studies of
the 3D microstructure of food materials. In order
to achieve higher spatial resolution, considera-
tions regarding sample stability and radiation dam-
age must be taken into account.

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Appendix A. Supporting material

Appendix A.1. Sample composition

Information on the emulsion and capillary composition is shown in table A.3. The chemical compositions and mass densities ρ_m shown in the table were used to calculate electron density values for the lipid and water phases, the microcrystalline cellulose of the stabilizer phase and the capillary.

For calculating the dose on the sample, the mass attenuation coefficient μ/ρ_m for the full emulsion is needed. This was calculated from μ/ρ_m for the water and lipid phases shown in the table.

	Atomic composition	ρ_m [g/cm ³]	μ/ρ_m [cm ² /g]
Lipid ph.	3.4w% C ₈ H ₁₆ O ₂ + 3.2w% C ₁₀ H ₂₀ O ₂ + 45.7w% C ₁₂ H ₂₄ O ₂ + 16.1w% C ₁₄ H ₂₄ O ₂ + 9.0w% C ₁₆ H ₃₂ O ₂ + 19.0w% C ₁₈ H ₃₆ O ₂ + 3.1w% C ₁₈ H ₃₄ O ₂	0.92	10.3
Water ph.	86.4w% H ₂ O + 13.6w% C ₁₂ H ₂₂ O ₁₁	1.05	20.4
Cellulose	(C ₆ H ₁₀ O ₅) _n	1.5	
Capillary	80.9w% SiO ₂ + 12.9w% B ₂ O ₃ + 4.4w% Na ₂ O + 1.8w% Al ₂ O ₄	2.23	

Table A.3: Atomic composition and mass density ρ_m used for calculating reference electron density values. Mass attenuation coefficients μ/ρ_m at 6.2 keV used for dose calculation.

Appendix A.2. Segmentation of emulsion phases

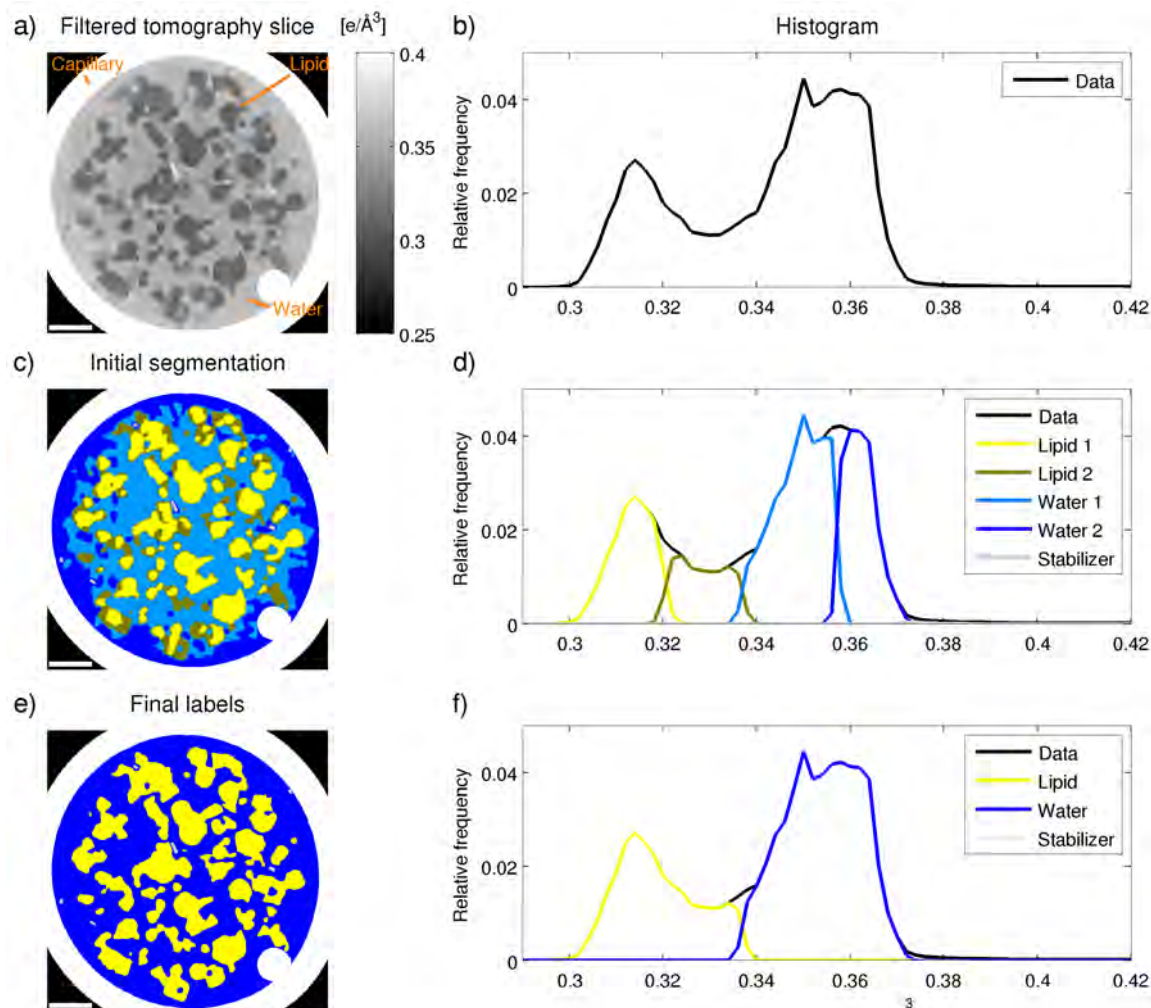


Figure A.5: Segmentation of the emulsion phases. a) Tomographic slice from sample 1 after noise correction. b) Histogram of voxel values of the slice. c) Initial segmentation of the emulsion with two lipid and two water labels. d) Histogram with electron density distribution of labels in the initial segmentation. e) Lipid and water labels have been combined in the final segmentation. f) Histogram with electron density distribution of labels in the final segmentation.

The segmentation of the emulsion phases is illustrated in figure A.5. As an example from sample 1, a tomography slice after noise correction is depicted in panel a). In panel b) the corresponding histogram of voxel electron density values is shown. Two broad peaks are evident in the electron density distribution in panel b) which correspond to the lipid and water phases of the emulsion. However, due to blurring of the lipid and water phases as seen in panel a), a broadening of the peaks have occurred, and they cannot be modeled as a single gaussian distribution.

Instead, each peak has been assumed to consist of two gaussian distributions which have been used as input for the initial segmentation algorithm. The result is depicted in panel c) with the corresponding

electron density distributions of the segmented phases shown in panel d). For the final labeling of phases, the two lipid and two water segmentations were combined as seen in panels e) and f).

Appendix A.3. Emulsion 3D representations

815 In this section, additional 3D representations of the segmented tomograms of sample 1, 3 and 4 are shown. Representations of the full sample volume are depicted in panel a) of figures A.6, A.7 and A.8. Displayed are the identified emulsion phases; lipid (yellow and orange), water (blue and green) and microcrystalline cellulose of the stabilizer phase (gray). No air pockets were found in the volume inside the micropipette.

820 The largest connected part of the lipid phase is shown in yellow while the remaining isolated regions are shown in orange. As seen, the vast majority of the lipid phase was connected in a single network which was also observed for the other phases. This space-filling lipid network constituted more than 98% of the total lipid volume in all samples as seen in table A.4.

825 Of the surrounding water phase, more than 99% of the water volume was situated in the continuous phase depicted in blue. However, small isolated water pockets inside the lipid phase were observed as illustrated in green color in the close-up in panel c) of figures A.6 and A.8 and panel b) of figure A.7. The percent object volumes of these pockets are listed in table A.4. Besides the lipid and water phases, a third stabilizer phase was identified which is depicted in gray in panels a) for all samples and panel b) in figures A.6 and A.8 and panel c) of figure A.7. From the elongated shape and the location in the water phase, these regions were interpreted as microcrystalline cellulose from the stabilizer mixture.

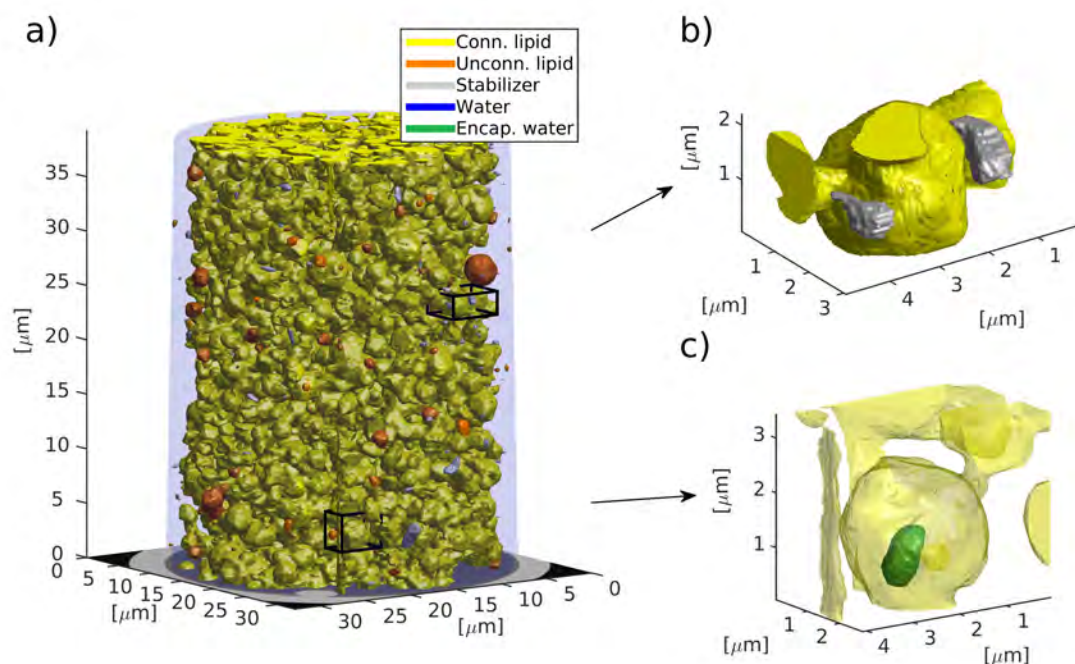


Figure A.6: 3D representations of the emulsion phases of sample 1. a) The full emulsion with the water phase shown in blue, the stabilizer in phase in gray, the connected lipid phase in yellow and isolated lipid regions in orange. b) A close-up of the stabilizer phase in the sub-volume of the bottom box of panel a). c) A close-up of the sub-volume in the top box of a) depicting an isolated water pocket inside the connected lipid phase.

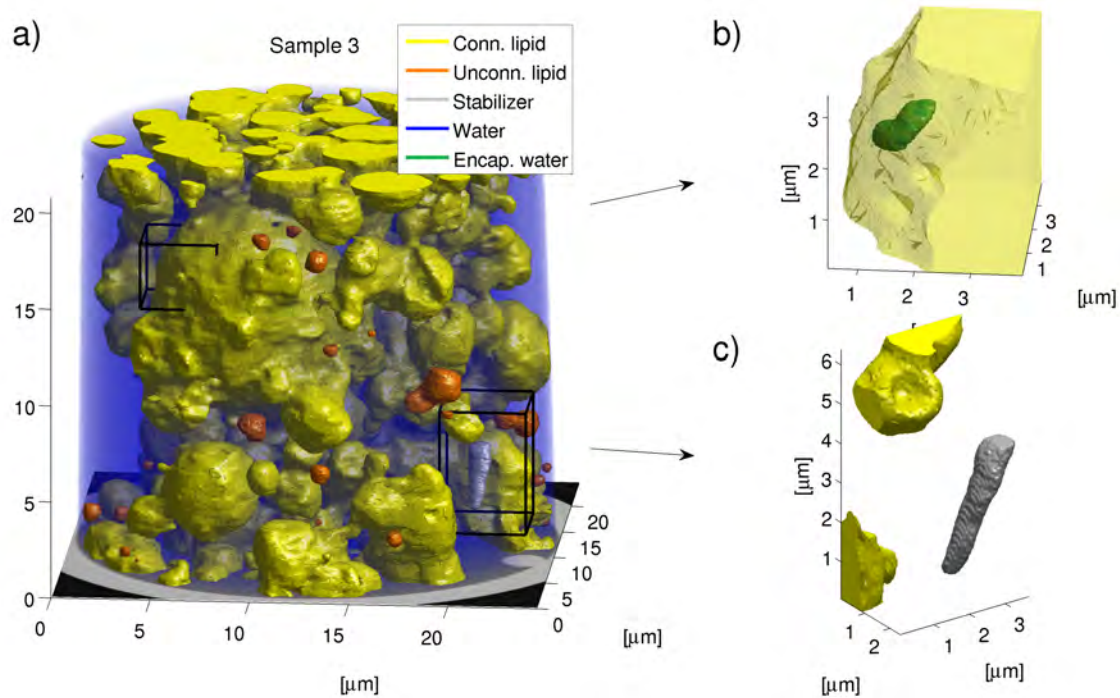


Figure A.7: 3D representations of the emulsion phases of sample 3. a) The full emulsion with the water phase shown in blue, the stabilizer in phase in gray, the connected lipid phase in yellow and isolated lipid regions in orange. b) A close-up of the sub-volume in the top box of a) depicting an isolated water pocket inside the connected lipid phase. c) A close-up of the stabilizer phase in the sub-volume of the bottom box of panel a).

Percent object volume (POV)				
Data type	Sample 1 [vol%]	Sample 2 [vol%]	Sample 3 [vol%]	Sample 4 [vol%]
Conn. Lipid phase	98.8	99.5	99.3	98.1
Isolated Water phase	0.02	0.12	0.08	0.0012

Table A.4: Percent object volume (POV) of the connected part of the lipid phase and of the isolated part of the water phase.

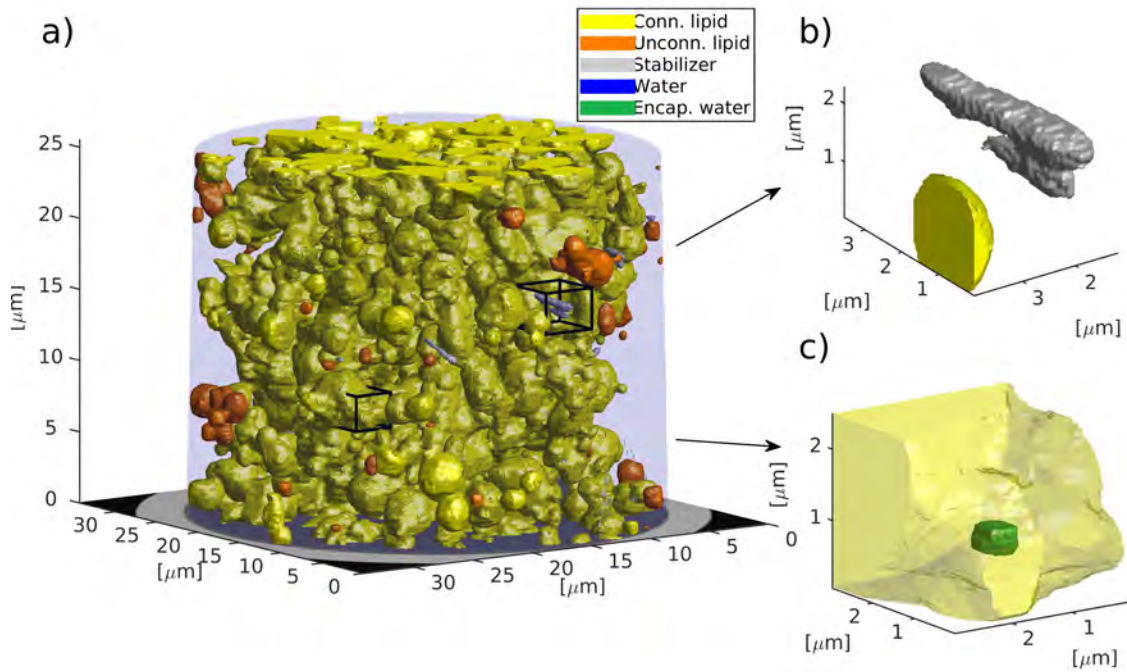


Figure A.8: 3D representations of the emulsion phases of sample 4. a) The full emulsion with the water phase shown in blue, the stabilizer in phase in gray, the connected lipid phase in yellow and isolated lipid regions in orange. b) A close-up of the stabilizer phase in the sub-volume of the bottom box of panel a). c) A close-up of the sub-volume in the top box of a) depicting an isolated water pocket inside the connected lipid phase.

Publication V

Meat Science

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*Novel X-ray phase-contrast tomography method for quantitative studies of heat
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Novel X-ray phase-contrast tomography method for quantitative studies of heat induced structural changes in meat



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ABSTRACT

The objective of this study was to evaluate the use of X-ray phase-contrast tomography combined with 3D image segmentation to investigate the heat induced structural changes in meat. The measurements were performed at the Swiss synchrotron radiation light source using a grating interferometric setup. The non-destructive method allowed the same sample to be measured before and after cooking. Heat denaturation resulted in a 36% decrease in the volume of the muscle fibers, while solubilization of the connective tissues increased the volume from 8.4% to 24.9%. The cooking loss was quantified and separated into a water phase and a gel phase formed by the sarcoplasmic proteins in the exudate. The results show that X-ray phase contrast tomography offers unique possibilities in studies both the meat structure and the different meat component such as water, fat, connective tissue and myofibrils in a qualitative and quantitative manner without prior sample preparation as isolation of single muscle components, calibration or histology.

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1. Introduction

Cooking of meat results in immediate and extensive structural changes of the meat proteins. These conformational changes are caused by the denaturation of the different meat proteins and include transversal and longitudinal shrinkage of the muscle fibers, aggregation and gel formation of the sarcoplasmic proteins and the shrinkage and solubilization of the connective tissue (Tornberg, 2005). The structural alterations are affected by heating time and temperature and are determinant of the eating quality of the meat (Christensen, Bertram, Aaslyng, & Christensen, 2011). The heat induced structural changes of meat are conventionally studied by microscopy or indirect quantitative methods as differential scanning calorimetry (DSC), turbidity measurements or protein solubility.

During the last decades X-ray computed tomography (CT) has generated interest as a valuable method in non-destructive three-dimensional imaging of microstructure of meat (Frisullo, Marino, Laverse, Albenzio, & Del Nobile, 2010; Furnols, Teran, & Gispert, 2009; Hollo, Szucs, Tozser, Hollo, & Repa, 2007) and meat products (Frisullo, Laverse, Marino, & Del Nobile, 2009; Santos-Garcés et al., 2013). CT has shown to be able to visualize the content and distribution of lean

meat, bones and fat, whereas detection of connective tissue is challenged by similarities in attenuation of connective tissue and muscle fibers. Recently, a novel grating based X-ray phase-contrast tomographic method with increased contrast has been demonstrated (Bech et al., 2010; Weitkamp et al., 2005). Where the image contrast formation in absorption tomography is based on differences in attenuation of the X-rays, the phase-contrast modality uses the refraction of X-rays caused by variation in electron densities for image generation, which results in improved contrast towards differences in mass density in soft tissues and serves to overcome challenges caused by similar attenuation properties of sample components. The superior contrast for soft tissues provided by X-ray phase-contrast CT compared to absorption CT has been demonstrated in a study of pork fat and rind (Jensen et al., 2011). With the use of phase-contrast even small density variations in the rind and variation in the fatty acid composition within the fat fraction were detected indicating the potential of the use of the phase-contrast modality in structure studies of meat.

The objective of this study was to evaluate the potential of the use of X-ray phase-contrast tomography to study the heat induced changes in the structure of meat. The non-destructive characteristics of the method allowed the same sample to be measured before and after heat treatment. The measurements were performed at a synchrotron facility with a grating interferometric setup. Advanced data segmentation allowed quantitative parameters as changes in volume of myofibrils and connective tissue, gel formation and cooking loss to be extracted from the data.

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2. Materials and methods

2.1. Sample preparation

One piece of raw beef *Semimembranosus* bought in the local supermarket was cut to fit into a 1.5 mL sample tube ($\varnothing = 10$ mm). The meat was placed with the fiber direction longitudinal to the tube. When placed in the tube, the lid was closed under the surface of degassed PBS buffer (0.01 M phosphate buffered saline, 0.138 M NaCl, 2.7 mM KCl, pH 7.4; Sigma-Aldrich) to avoid air bubble formation during the measurements. After measurements of the raw sample the exact same sample was heat treated by placing the sample tube in a glass of hot water (95 °C) for 30 min. After heat treatment the sample was cooled in tap water for 15 min and subsequently measured again.

2.2. X-ray tomography

Absorption and phase-contrast CT scans of the sample were obtained by use of a grating interferometer setup at the TOMCAT beam line, Swiss Light Source (SLS) at the Paul Scherrer Institute (PSI). The technique has been explained previously elsewhere (Bech et al., 2010), and the setup is described in detail in McDonald et al. (2009). Measurements were made at a photon energy of 25 keV and the third Talbot fractional distance was used (Weitkamp, David, Kottler, Bunk, & Pfeiffer, 2006) with a grating period of the phase-grating, called G1, of 3.9 μm and a period of 2 μm of the analyzer grating, called G2. The height of the grating lines was designed for photon energy of 25 keV. The sample was kept in a water bath during the measurements in order to reduce the difference in refractive index between the sample container and its surroundings. The scan time was around 90 min per tomogram, and three tomograms were conducted at three different heights in the sample. The volume of the sample in each tomogram was 0.25 mL giving a full volume of around 0.75 mL imaged per sample. Each volume consists of $1720 \times 1720 \times 513$ voxels with an effective voxel size of $7.4 \mu\text{m} \times 7.4 \mu\text{m} \times 7.4 \mu\text{m}$. Due to limited allocated time on the beamline only one sample was measured.

2.3. Data processing

The X-ray tomograms were reconstructed at the TOMCAT beam line using a local implementation of the filtered back-projection algorithm for absorption and phase-contrast tomography as described in Pfeiffer, Kottler, Bunk, and David (2007). The phase-contrast intensity values were calibrated to absolute electron density values through a linear regression using the sample container consisting of polyethylene and the surrounding water as references. The spatial resolution in the phase contrast images was estimated to be 23 μm by using the edge between container and surrounding water as a reference.

2.4. Data segmentation

A two-step segmentation algorithm was implemented as described in Grau, Downs, and Burgoyne (2006). The first step is to model the data as a mixture of Gaussians using an expectation–maximization (EM) algorithm (Dempster, Laird, & Rubin, 1977). This iterative process finds the maximum likelihood of parameters where the model depends on unobserved latent variables. The spatial information of the data is then incorporated into the segmentation process by modeling the data as a Markov random field (MRF) (Li, 2003). It models the a priori probability of neighborhood dependencies, and the field can either be isotropic or anisotropic. For the segmentation of connective tissue, the local information of the structure orientation and coherence is extracted to steer the smoothing (anisotropy) of the final segmentation. The MRF smoothing parameter β was set to 0.7.

3. Results and discussion

Absorption and phase contrast images of transverse cut of the raw and cooked beef sample are presented in Fig. 1. The grayscale images are reconstructed from a single slice of the data sets. The dark ring surrounding the samples is the sample tube. The absorption images (Fig. 1A+B) appear noisy and hardly any contrast between the muscle components is seen. In the absorption image of the raw meat (Fig. 1A) only the intra muscular fat can be separated from the muscle structure. In the cooked meat (Fig. 1B) the structure of the muscle fibers and connective tissue is faintly observed. In comparison, superior contrast is provided in the phase contrast images (Fig. 1C+D), where muscle fibers, connective tissue, intra muscular fat and the surrounding water phase appear clearly separated. In the phase contrast images intensity differences reflect differences in electron density of the structural components where the light intensity increases with increasing densities. In the raw meat (Fig. 1C) the muscle fiber part of the muscle is represented by the gray areas, whereas the connective tissue, mainly the perimysium, is the white areas. The small black spots are intramuscular fat. Towards the edge of the samples darker gray areas are seen representing a water phase. Due to changes in electron density of the muscle components as a consequence of heating an intensity shift is seen in the image of the cooked sample (Fig. 1, D) compared to the raw sample. In the cooked sample muscle fibers have contracted and thus increased in density, which is seen as the white areas: The connective tissue has decreased in density due to absorption of water and appears as light gray. The most remarkable changes in the structure are the shrinkage of both the total sample volume and the individual fiber bundles. In the cooked sample the fiber bundles appear clearly separated by the surrounding perimysium. The shrinkage of the myofibrils has forced water to be expelled, which can be observed as an increase in the

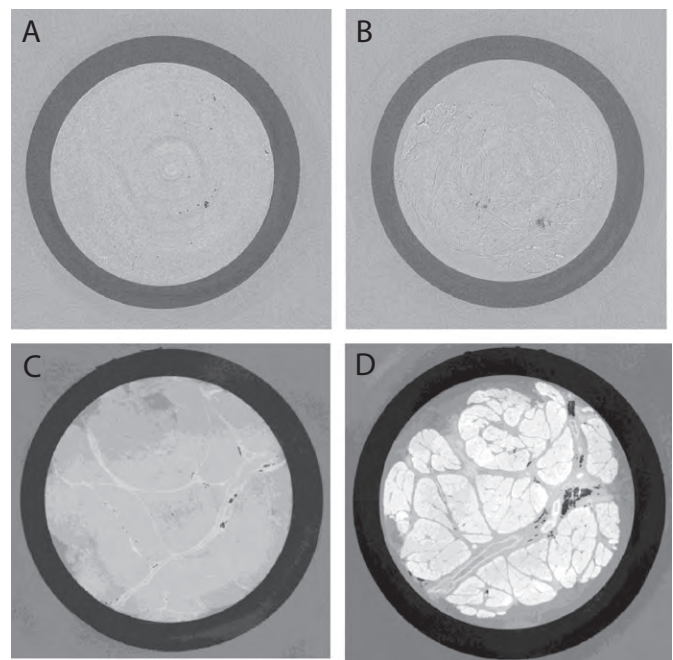


Fig. 1. Transverse sample cut reconstructed from the absorption (A+B) and the phase contrast (C+D) tomograms of raw (A+C) and cooked beef (B+D). In A+B the contrast is formed by differences in absorption of the X-rays by the sample components. Only fat (black) can be separated from the muscle (gray). In C+D the contrast is formed based on differences in refraction of the X-rays caused by differences in the electron density of the sample components. In C the components can be identified as: muscle (gray), fat (black), connective tissue (white), water (dark gray). In D the components can be identified as muscle (white), connective tissue (light gray), water (dark gray) and fat (black).

area of the water phase surrounding the meat. The area of the perimysium is increased indicating partly solubilization.

From the 3D data sets the observed difference in microstructure can be further explored by data segmentation of the full sample volumes. Due to the limited contrast provided from the absorption tomograms, the quantitative analysis were only performed on the phase contrast data sets. A 3D visualization of the segmented X-ray phase contrast tomograms of the raw and cooked beef is presented in Fig. 2A+B. An example of a segmented slice of the raw and cooked sample from the phase contrast tomograms is presented in Fig. 3A+B respectively, while the quantitative distribution of the structure components is presented in the corresponding histograms in Fig. 3C+D. From Fig. 3, A+C it is seen that the main components water (blue), muscle fibers (red) connective tissue (pink) and intra muscular fat (white) can be clearly separated. The use of the two-step data segmentation procedure further enabled segmentation of structural variations within these components, even though that no clear peak separation is observed in the histograms (Grau et al., 2006). In the raw sample (Fig. 3A) the muscle fibers from the inner and outer part of the sample can be separated into two populations based on differences in the electron densities. The outer part of the muscle fibers has a lower electron density compared to the inner part (Fig. 3C). This difference can be explained by an increase in the water binding capacity in the meat exposed to the surrounding buffer that had a relatively high pH (7.4) (Puolanne & Halonen, 2010). In the cooked meat shrinkage of the muscle fibers resulted in an increase in the electron density (Fig. 3D) compared to the raw sample (Fig. 3C). No difference between the inner and outer part is observed in the cooked sample as the shrinkage has forced the extra cellular water to be expelled as cooking loss. The connective tissue is segmented into two different populations. As seen in Fig. 3A+C this is mainly based on local variation in the electron densities of the perimysium in the junctions of the fiber bundles. The increase in the area of the connective tissue in the cooked sample compared to the raw sample indicating partly solubilization is further supported by the decrease in electron density of this component. This demonstrates that the meat has been undergoing a relatively severe heat treatment and reached a temperature above 80 °C and has started to gelatinize (Tornberg, 2005). In the water phase local variations in the protein content, ascribed to expelled sarcoplasmic proteins, result in two different populations that enable separation of the water phase based on the protein content. However, a strict division in exudate from the meat and the surrounding buffer is not possible. In Fig. 3 these water populations are designated as Water 1 and Water 2, where Water 2 has the highest protein content. In the raw sample the surrounding water phase is mainly constituted by buffer, but a small amount of exudate are detected in the regions at the surface of the meat. In the cooked sample the cooking loss results in an increase in the volume of the water phase, where the Water 2 population most likely will consist of a gel formed by expelled denatured sarcoplasmic proteins (Davey & Gilbert, 1974; Tornberg, 2005).

The volume changes of the main components are summarized in Table 1. In the table the volumes of the gel-like structure are included in the water phase. The observed heat induced changes in the muscle structure are similar to previously observations on the subject studied by other imaging methods as microscopy (Bendall & Restall, 1983; Tornberg, 2005) or MRI (Bouhrara et al., 2011). Compared to microscopy both MRI and X-ray tomography are non-destructive and three dimensional methods. Whereas, X-ray phase contrast tomography provides information about the electron density MRI, which is based on information about the proton density.

The superior contrast provided by phase contrast imaging compared to standard X-ray absorption imaging has previously been demonstrated by Jensen et al. (2011) in porcine fat and rind. Together with the present result for raw and cooked meat it is emphasized that the phase contrast modality is advantageous in the use of X-ray tomography for structure studies of meat as the small variation in the atomic composition between the structural components is insufficient to provide an absorption based contrast between main components as connective tissue and muscle fibers, while the variations in electron densities result in a high contrast even between structural deviations within the individual components, which demonstrates the potential of the method. Besides a better understanding of the interactions between the denaturation of the muscle fibers and the connective tissue, phase contrast X-ray tomography may be applied in studies of processing steps as salting, marinating, freezing or heating, that will result in changes in the electron density of the muscle fibers either caused by denaturation or affected water binding capacity. As indicated in the quantitative analysis of this study, increased water binding as a consequence of increased pH and salt affected the electron densities sufficiently to be detected.

In addition to monitoring of processing steps the non-destructive characteristics also contribute to the potential in relation to on-line selection of raw materials as fat, muscle and connective tissue can be non-invasively detected and quantified. Quality deteriorations as soft fat or PSE/DFD meat may be linked to variations in the electron density and used as marker in specifications for rejection or selection of raw materials for a specific production.

4. Conclusion

Phase contrast tomography offers unique possibilities to study structural changes of meat caused by cooking. The non-destructive characteristics of the method enabled studies to be made on the exact same sample before and after heat treatment. The high contrast in the data set made it possible to both visualize and quantify structural variation within the individual meat components. Even though the method might have an on-line potential, the relatively long measuring time currently limits the method to experimental purposes.

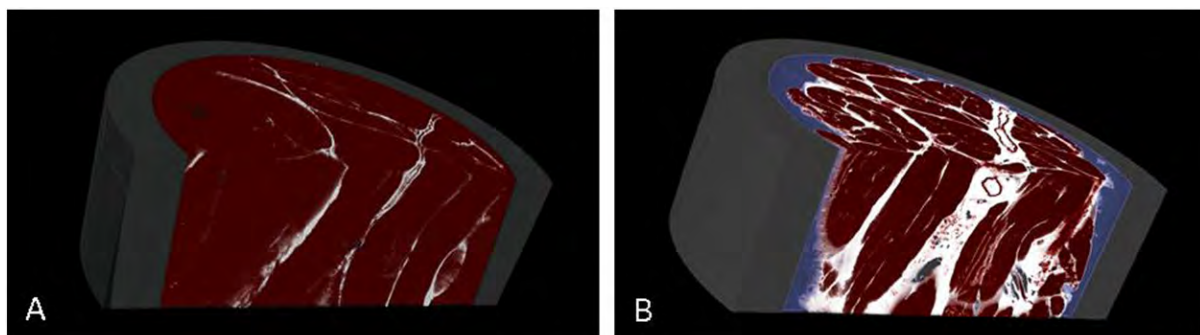


Fig. 2. 3D visualization of segmented X-ray phase contrast tomograms of raw (A) and cooked beef (B). Muscle tissue is shown in red, connective tissue in white, intramuscular fat in gray and the water phase is shown in blue.

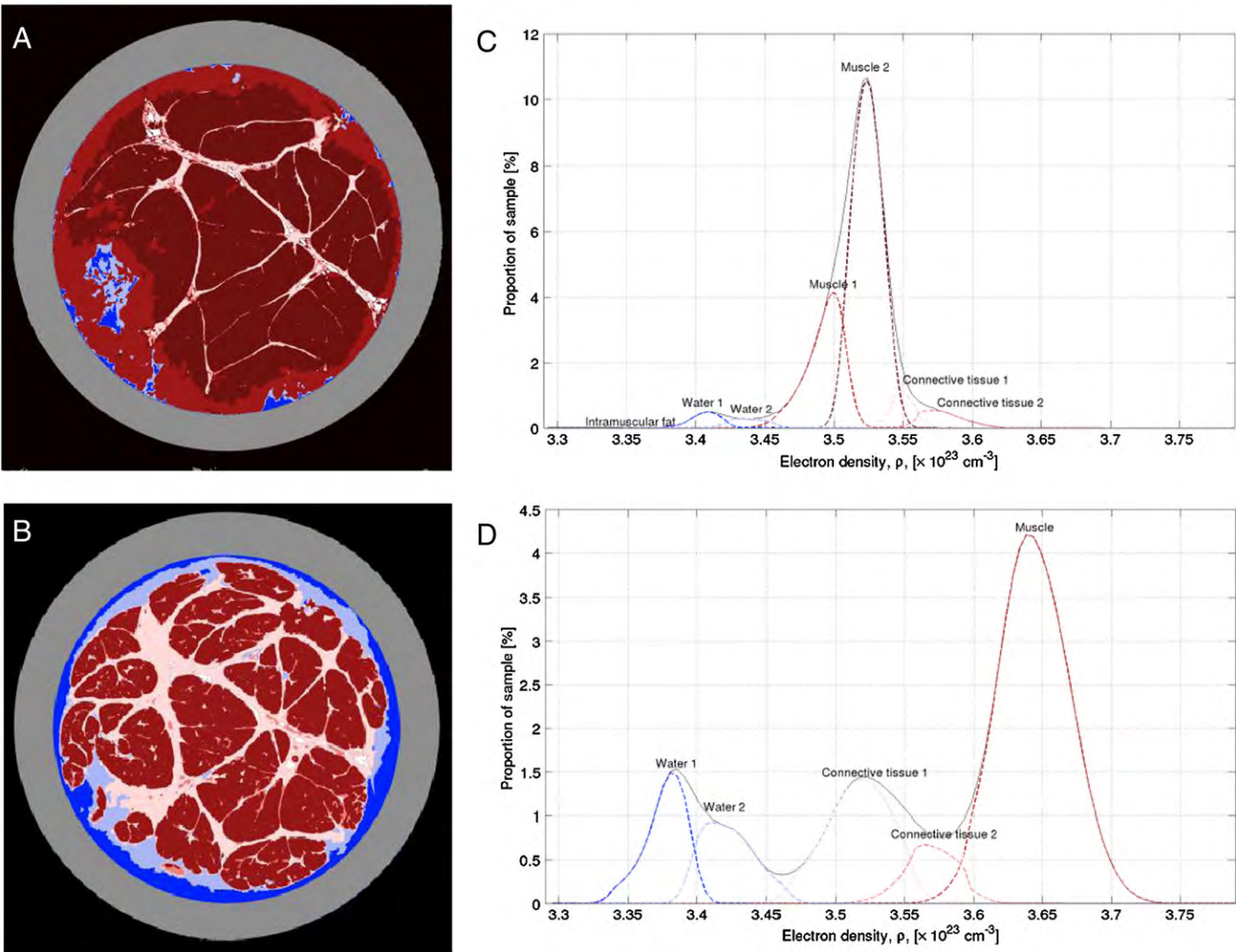


Fig. 3. Segmentation of beef sample in raw and cooked state. A+B: Examples of segmented slices from the datasets obtained from the raw and the cooked sample respectively. Muscle tissue is shown in red (1) or brown (2), connective tissue in light pink (1) or pink (2), water in blue (1) or light blue (2) and intramuscular fat in white. C+D: Histograms of the structure components generated from the full 3D data sets.

Table 1
Partial object volumes (POV) for the soft materials in the raw and cooked beef sample.

Component	Raw	Cooked
POV		
Water	4.87	19.54
Muscle fibers	86.18	54.96
Connective tissue	8.41	24.89
Intramuscular fat	0.54	0.61

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