Opportunities for phase-based computed tomography in the laboratory

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***Abstract*—It has been demonstrated in many instances that phase-based computed tomography (CT) can provide superior contrast-to-noise ratio for weakly attenuating samples than attenuation-based CT. In order to exploit this benefit on a wider scale, phase-based tomography implementations must be compatible with standard x-ray equipment. The edge illumination method, which is based on aperturing a beam and measuring spatial displacements caused by refraction, is an attractive choice for such use due to its low requirements on spatial and temporal coherence. This document provides a brief introduction to the working principle of the edge illumination method and reviews recent advances that lead to increased robustness, faster acquisitions and lower dose delivery. Moreover, it reports on a recent study in which the edge illumination method was applied to samples from the field of tissue engineering, yielding synchrotron-like image quality with exclusively commercially available, laboratory-based x-ray equipment.**

***Index Terms*—computed tomography, phase contrast imaging, x-ray imaging**

I. Introduction

COMPUTED tomography (CT) has come a long way since its invention by Hounsfield and Cormack in the sixties. Regardless of whether it is used for clinical decision making, pre-clinical biomedical research, materials science or homeland security, CT is nowadays an integral part to various aspects of our society. The strength of this three-dimensional imaging modality lies in the wide range of spatial resolution regimes that can be accessed (on the macro-, micro- and even the nano-scale) and the fact that it can be implemented with a variety of x-ray sources (from compact hospital x-ray tubes to large, highly specialized synchrotron facilities) and detectors (from CCD cameras to cutting-edge energy resolving photon counters). Despite this versatility, CT suffers from one limiting issue; since image contrast is based solely on x-ray attenuation (dominated by photoelectric absorption or Compton scattering, depending on the energy used), materials with low atomic number suffer from a low contrast-to-noise ratio (CNR), leading to poor feature detectability. Although higher photon statistics can in principle improve the CNR, this option is often ruled out in practice by tight constraints on dose delivery and acquisition time.

On the other hand, attenuation is not the only physical effect that x-rays undergo when they travel through matter; phase shifts occur at the same time, since the wave velocity is different for different media. A sample's ability to attenuate and shift the phase of an x-ray beam is commonly described by the complex refractive index: $n(k) = 1 - \delta(k) + i\beta(k)$; δ and β drive phase shift and attenuation, respectively, and k is the wave number. For weakly attenuating materials and within the diagnostic energy range, δ can be up to three orders of magnitude larger than β, implying that CNR can be largely improved if phase effects are exploited [1].

Several methods have been developed to use x-ray phase shifts for imaging [2]. When a beam has a sufficient degree of spatial coherence, interference fringes develop as it propagates after exiting the sample, converting phase shifts into measureable intensity variations. This so-called propagation-based phase contrast imaging is however restricted to synchrotrons or micro-focal x-ray sources, due to its stringent coherence requirements. Another way of measuring phase shifts is by exploiting the Talbot effect, which creates an interference pattern (a self-image) at some specific distances downstream of a diffraction grating. Grating interferometry-based phase contrast imaging methods seek to measure this interference pattern as well as any disturbances to it caused by additional refraction introduced by the sample. The Talbot effect intrinsically requires coherence; however,

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implementations with low-coherence x-ray sources are possible by splitting the beam into an array of mutually incoherence but self-coherent sub-sources. Other phase contrast imaging methods also measure x-ray refraction, i.e. the macroscopic manifestation of the phase shift. These methods make use of an analyzer positioned in the beam path behind the sample, which modulates the beam intensity depending on the refraction angle. The analyzer can be a crystal (exploiting the laws of Bragg diffraction) or a single aperture/an array of apertures (exploiting spatial beam displacements resulting from refraction). While demanding less spatial coherence, crystal-based methods still require a temporally coherent beam due to the crystal's narrow-band energy acceptance. In turn, aperture-based methods tolerate low spatial *and* temporal coherence; thus, they can be implemented with conventional x-ray tubes, making them attractive for use in laboratory environments.

This document focusses on the edge illumination method [3,4], a specific aperture-based phase contrast imaging method that was developed initially at the Elettra synchrotron (Trieste, Italy), and more recently in the radiation physics laboratories of University College London (UCL). Following a brief introduction to the working principle of the method, its potential for use in standard laboratory environments is discussed through recent advances on robustness, scan speed and dose delivery. Finally, imaging examples of soft tissue specimens from the field of tissue engineering are presented.

II. The edge illumination method

The edge illumination method converts x-ray refraction into image contrast. This is achieved by illuminating only the edges of a row of pixels with a narrow (typically < 20 μm) laminar beam (collimated by a slit-shaped aperture): a refraction of the beam towards/away from the pixels' active areas causes an increase/decrease in the measured intensity. In order to obtain a two-dimensional image, the sample has to be scanned through the beam. This implementation stems from early developments at the Elettra synchrotron where the flux is sufficiently high to perform fast acquisitions despite the need for sample scanning. When x-ray flux is limited (e.g. with conventional x-ray sources), the edge illumination method is typically implemented in full-field mode [Fig. 1]. In this setup, a mask, i.e. an array of slit-shaped apertures in front of the sample ("pre-sample mask") splits the beam into an array of physically separated beamlets, and a second mask in front of an area detector ("detector mask") creates insensitive areas ("edges") between the pixels. By positioning the pre-sample mask such that each individual beamlet falls partially on a pixel and partially on an absorbing detector mask septum, the edge illumination principle is replicated over the entire field of view (FOV), eliminating the need for sample scanning.

A prototype of such a setup has been built at UCL, based exclusively on commercially available x-ray equipment. The setup features a Rigaku MicroMax 007 HF x-ray tube with rotating molybdenum target (focal spot ≈ 70 μm), a Hamamatsu C9732DK flat panel detector with CMOS read-out (pixel size = 50 μm x 50 μm) and two sets of masks fabricated by electroplating gold strips onto a graphite substrate (Creatv MicroTech Inc., Potomac, MD, USA). The aperture widths of the pre-sample and detector masks are 23 μm and 29 μm, respectively, and their periods are 79 μm and 98 μm.

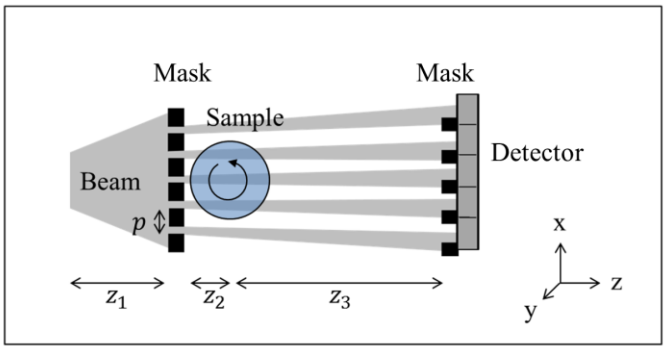

Figure 1. Schematic showing the full-field implementation of the edge illumination method (as seen from top). The distances in the prototype scanner at UCL are z_1 =1.6 m, z_2 = 0.05 m and z_3 = 0.35 m.

Intensity variations (contrast) in a single radiograph are typically due to a combination of x-ray attenuation and refraction. The acquisition of a second image frame, after the pre-sample mask is re-positioned such that the beamlets fall onto the opposite sides of the detector mask apertures ("opposite illumination configuration"), and the subsequent processing of these two images according to a dedicated procedure ("phase retrieval") enables the separation of the attenuation and refraction channels [5,6]. If the sample contains scattering structures, each radiograph contains an additional (the so-called "dark field") channel. In that case, the acquisition of a third image frame, with the pre-sample and detector mask apertures fully aligned, is required to separate the attenuation, refraction and dark-field information [7]. In order to acquire a CT dataset, the sample must be rotated and two (or three, for scattering samples) projection images must be acquired at each CT angle. For each transverse sample slice (y = *const.*) this yields sinograms of the following form [8]:

$$S_\beta(x,z;\theta) = -2k\int \beta(x',z')\,dz \quad \text{(attenuation)} \quad (1)$$

$$S_\delta(x,z;\theta) = {}^{\partial}/_{\partial x}\int \delta(x',z')dz \quad \text{(refraction)} \quad (2)$$

$$S_{\sigma^2}(x,z;\theta) = \int \sigma_\Phi^2(x',z')dz \quad \text{(dark-field)} \quad (3)$$

where σ_Φ^2 describes the scattering properties of the sample, $(x',z') = (x\cos\theta - z\sin\theta, x\sin\theta + z\cos\theta)$ are the rotating coordinates of the sample and θ is the rotation angle. These sinograms enable the reconstruction of tomograms of $k\beta$ (attenuation), δ (phase), σ_Φ^2 (dark-field) via standard reconstruction methods, e.g. the filtered back projection (FBP) formula. The derivative in the refraction sinogram imposes the use of an additional integration step or the use of a dedicated filter function in the FBP.

III. Robustness, speed and dose

Since the edge illumination method is compatible with conventional x-ray tubes, it offers potential for a widespread use outside specialized synchrotron radiation facilities. However, the implementation of an imaging setup in non-synchrotron environments such as academic research laboratories or hospitals imposes several stringent requirements: a) the experimental setup should be easy to align and robust towards environmental vibrations, b) acquisitions should be fast, and c) the delivered radiation dose should be low (although the definition of "low" is obviously application dependent). Recently, strategies were developed for the edge illumination method in order to increase its compatibility with these criteria:

a) While the alignment of the pre-sample and detector masks is relatively straight forward and is currently carried out via a semi-automatic procedure [9], manufacturing-related mask imperfections prevent the alignment from being perfect, and therefore a certain, mask-dependent accuracy limit exists which cannot be overcome. Hence, local variations of the illumination level (the fraction of each beamlet falling into the detector mask aperture and therefore onto the pixel active area) across the field-of-view cannot be avoided. However, by applying a "local phase retrieval" procedure [10], this misalignment can be completely accounted for and its negative effect on the retrieved absorption, refraction and dark-field channels eliminated. In fact, the "local" method has been shown to tolerate mask imperfections of up to a few tens of micrometres, which is way above current manufacturing standards.

b) Due to the limited flux of conventional x-ray tubes and the fact that, until now, the separation of attenuation and refraction contrast required two input images, edge illumination CT has suffered from relatively lengthy scan times. To tackle this problem, an alternative phase retrieval method ("reverse projection"), which was first published by Zhu et al. (2010) for grating interferometry [11], has been further developed to make it applicable to EI datasets [12]. Reverse projection retrieval relies on the observation that two images acquired with a rotation offset of 180 degrees between them provide the same information as two acquired in the opposite illumination configurations as described above. This retrieval simplifies the experimental procedure and enables a more efficient (and thus faster) acquisition. In fact, it allows a continuous rotation of the sample while previously this had to be interrupted to reposition the pre-sample mask from one to the opposite illumination configuration at each rotation angle. Keeping the pre-sample mask in a fixed position throughout scans also improves robustness, as it eliminates any potential misalignment caused by repeated motor movements.

c) As a possibility for dose reduction, we have developed a new phase retrieval algorithm that does no longer require two images as input [13]. Instead, it relies only on a single image, and yields the projected thickness of a sample (in a similar manner to the widely used method developed by Paganin *et al.* for propagation-based phase contrast imaging [14]). This retrieval method reduces the number of image frames needed for a CT acquisition/reconstruction and, therefore, the dose and the acquisition time by a factor of 2. Since the method relies on the assumption of a single material sample, it cannot be considered strictly quantitative. However, so far high image quality results were obtained for all biological soft tissue samples that have been investigated.

IV. Application in tissue engineering

Tissue engineering, a sub-discipline of regenerative medicine, aims at the development of replacement organs by combining appropriate scaffolds and cells. An important question is how to produce scaffolds enabling cell adhesion, proliferation and differentiation. There is strong evidence that scaffold microstructure, biomechanical properties and extracellular matrix composition play a crucial role in this. Typically, microstructure and matrix composition are analyzed using histology and electron microscopy. These imaging techniques, however, require destructive sample preparation; hence they are not suitable for a volumetric analysis, longitudinal studies or *in vivo* translation. In a 2013 review paper on imaging modalities used for tissue engineering applications, x-ray phase contrast imaging was identified as a potential method to overcome the current lack of non-destructive, three-dimensional imaging technology that provides detailed scaffold information [15].

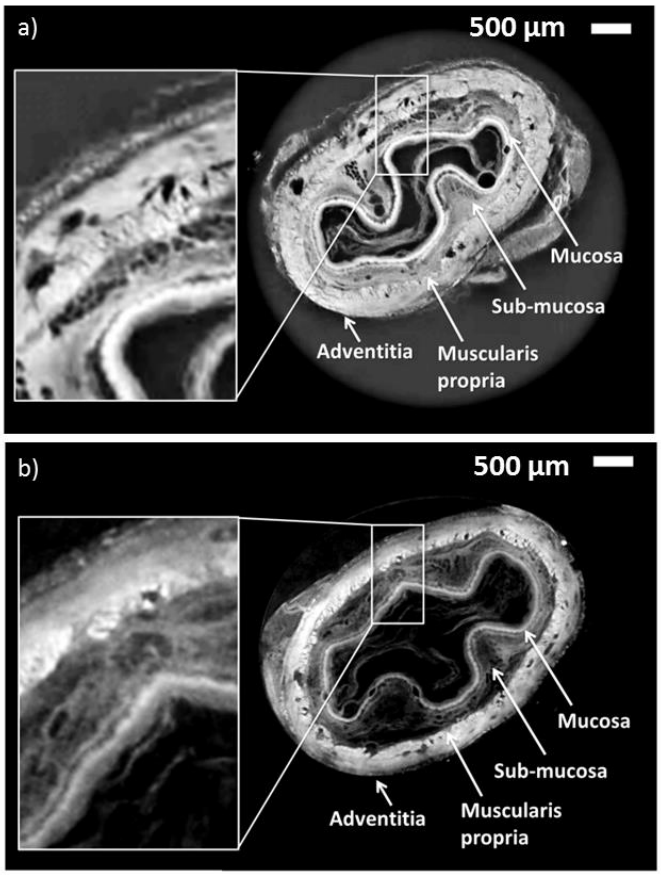

Figure 2. Phase tomograms of a decellularized rabbit esophagus acquired with a) propagation-based phase contrast CT at the ESRF (ID17) and b) the edge illumination method in the

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To demonstrate this potential, a range of tissue engineering scaffolds obtained via decellularization of small rodent organs was scanned recently [16], first with propagation-based phase contrast CT at beamline ID17 of the European Synchrotron Radiation Facility (ESRF), and then with the laboratory-based edge illumination setup at UCL. Phase tomograms were reconstructed, showing high image quality for both the propagation-based and the edge illumination data. As an example, Figure 2 shows tomograms of a rabbit esophagus scaffold; in both images, contrast is sufficiently high to identify all native anatomical layers of the esophagus (mucosa, sub-mucosa, muscularis propria, adventitia, as indicated by arrows in the figure), and to assess their structural integrity. The latter is important to judge the performance of the decellularization method used. Most importantly, the fact that an image quality comparable to that of synchrotron-based phase contrast CT was obtained in a standard laboratory using exclusively conventional x-ray equipment indicates that not only does phase contrast CT have the capability to replace histology and SEM for this range of applications, but also that imaging could be performed inside tissue engineering research laboratories, enabling a high-throughput and wide uptake.

V. Summary

Despite many advantages and widespread exploitation, CT imaging is still limited by poor CNR when applied to weakly attenuating samples like biomedical soft tissue. Phase contrast imaging methods can overcome this problem, since the phase exploited by these modalities for contrast generation can be much larger than attenuation effects. The edge illumination method's sensitivity to x-ray refraction is realized through the use of apertures in the beam path. Low demands on spatial and temporal coherence make the method attractive for use outside specialized synchrotron facilities such as research laboratories and hospitals. In order to ensure the method's compatibility with "real life" applications encountered in such environments, strategies for improved robustness, increased acquisition speed and dose reduction have been developed. These advances, together with the recent achievement of synchrotron-like image quality with laboratory equipment for tissue engineering samples, suggest that the edge illumination method provides opportunities for a widespread exploitation of phase-based tomography.

References

[1] A. Bravin, P. Coan and P. Suortti. (2013). X-ray phase-contrast imaging: from pre-clinical applications towards clinics. *Phys. Med. Biol.* 58, R1–R35. doi: 10.1088/0031-9155/58/1/R1

[2] S.W. Wilkins, Y.I. Nesterets, T.E. Gureyev, S.C. Mayo, A. Pogany *et al.* (2014). On the evolution and relative merits of hard x-ray phase-contrast imaging methods. *Philos. Trans. A Math. Phys. Eng. Sci.* 372, 20130021. doi: 10.1098/rsta.2013.0021

[3] A. Olivo, F. Arfelli, G. Cantatore, R. Longo, R.H. Menk *et al.* (2001), "An innovative digital imaging set-up allowing a low-dose approach to phase contrast applications in the medical field", *Med. Phys.* 28, 1610-9. Available: http://www.ncbi.nlm.nih.gov/pubmed/11548930

[4] A. Olivo and R. Speller. (2007) A coded-aperture technique allowing x-ray phase contrast imaging with conventional sources. *Appl. Phys. Lett.* 91, 074106. doi: 10.1063/1.2772193

[5] P.R.T. Munro, C.K. Hagen, M.B. Szafraniec and A. Olivo. (2013). A simplified approach to quantitative coded aperture x-ray phase imaging methods. *Opt. Express* 21, 11187-201. doi: 10.1364/OE.21.011187

[6] P.C. Diemoz, M. Endrizzi, C.E. Zapata, Z.D. Pesic, C. Rau *et al.* (2013). X-ray phase-contrast imaging with nanoradian angular resolution. *Phys. Rev. Lett.* 110, 138105. doi: 10.1103/PhysRevLett. 110.138105

[7] M. Endrizzi, P.C. Diemoz, T.P. Millard, J.L. Jones, R.D. Speller *et al.* (2014). Hard x-ray dark-field imaging with incoherent sample illumination. *Appl. Phys. Lett.* 104, 024106. doi: 10.1063/1.4861855

[8] F.A. Vittoria, M. Endrizzi, P.C. Diemoz, A. Zamir, U.H. Wagner *et al.* (2015). X-ray absorption, phase and dark-field tomography through a beam tracking approach. *Sci. Rep.* 5, 16318. doi: 10.1038/srep16318

[9] T.P. Millard, M. Endrizzi, K. Ignatyev, C.K. Hagen, P.R.T. Munro *et al.* (2013). Method for automatization of the alignment of a laboratory based x-ray phase contrast edge illumination system. *Rev. Sci. Instrum.* 84, 083702. doi: 10.1063/1.4816827

[10] M. Endrizzi, D. Basta and A. Olivo. Laboratory-based x-ray phase-contrast imaging with misaligned optical elements. *Appl. Phys. Lett.* 107, 124103. doi: 10.1063/1.4931778

[11] P. Zhu, K. Zhang, Z. Wang, Y. Liu, Z. Wu *et al.* (2010). Low-dose, simple, and fast grating-based x-ray phase-contrast imaging. *Proc. Natl. Acad. Sci USA* 107, 13576-81. doi: 10.1073/pnas.1003198107

[12] Hagen *et al.*, submitted

[13] P.C. Diemoz, F.A. Vittoria, C.K. Hagen, M. Endrizzi, P. Coan *et al.* (2015). Single-image phase retrieval using an edge illumination x-ray phase-contrast imaging setup. *J. Synchrotron Radiat.* 22, 1072-7. doi: 10.1107/S1600577515008978

[14] D. Paganin, S.C. Mayo, T.E. Gureyev, Miller P.R. and S.W. Wilkins. (2002). Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. *J. Microsc.* 206, 33-40. Available: http://www.ncbi.nlm.nih.gov/pubmed/12000561

[15] A.A. Appel, M.A. Anastasio, J.C. Larson and E.M. Brey. (2013). Imaging challenges in biomaterials and tissue engineering. *Biomaterials* 34, 6615-30. doi: 10.1016/j.biomaterials.2013.05.033

[16] C.K. Hagen, P. Maghsoudlou, G. Totonelli, P.C. Diemoz, M. Endrizzi *et al.* (2015). High contrast microstructural visualization of natural acellular matrices by means of phase-based x-ray tomography. *Sci. Rep.* 5, 18156. doi: 10.1038/srep18156