High-throughput, high-resolution X-ray phase contrast tomographic microscopy for visualisation of soft tissue

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Abstract. The use of conventional absorption based X-ray microtomography can become limited for samples showing only very weak absorption contrast. However, a wide range of samples studied in biology and materials science can produce significant phase shifts of the X-ray beam, and thus the use of the phase signal can provide substantially increased contrast and therefore new and otherwise inaccessible information. The application of two approaches for high-throughput, high-resolution X-ray phase contrast tomography, both available on the TOMCAT beamline of the SLS, is illustrated. Differential Phase Contrast (DPC) imaging uses a grating interferometer and a phase-stepping technique. It has been integrated into the beamline environment on TOMCAT in terms of the fast acquisition and reconstruction of data and the availability to scan samples within an aqueous environment. The second phase contrast approach is a modified transfer of intensity approach that can yield the 3D distribution of the phase (refractive index) of a weakly absorbing object from a single tomographic dataset. These methods are being used for the evaluation of cell integrity in 3D, with the specific aim of following and analyzing progressive cell degeneration to increase knowledge of the mechanistic events of neurodegenerative disorders such as Parkinson’s disease.

1. Introduction
Phase sensitive X-ray imaging methods can provide substantially increased contrast over conventional absorption based imaging, in particular for biological samples, and therefore exploiting the coherent nature of synchrotron radiation has evident advantages. Phase contrast tomography gives fast access to 3D structural information in near-native biological samples without need for time consuming tissue alteration/modification for contrast enhancement, such as casting and staining as currently required by most high-resolution imaging techniques. An added advantage is that phase signals are produced with much lower dose deposition, which can be very important when radiation damage becomes an issue. Various phase-sensitive X-ray imaging methods have been developed, including interferometric methods [1], propagation methods [2], and techniques using a crystal analyzer, such as diffraction enhanced imaging [3]. These methods differ significantly in terms of the required experimental setup.
In this paper the application of two complementary techniques for high-throughput, high-resolution X-ray phase contrast tomography is illustrated. A Modified Transport of Intensity approach [4] yields a good approximation of the 3D phase distribution of a weakly absorbing object from a single tomographic dataset without the need of additional hardware. It is particularly suited for small specimens when high resolution is required. The Differential Phase Contrast (DPC) method [5], based on grating interferometry, is characterized by a higher sensitivity and suited to larger samples. These methods are being used towards the evaluation of soft tissue and cell integrity in 3D, with the specific aim of following and analysing progressive cell degeneration to increase knowledge of the mechanistic events of neurodegenerative disorders such as Parkinson’s disease.

2. Cell Visualisation
The possibility to visualise the integrity of encapsulated cell populations will allow a more robust screening of encapsulated clones for clinical application. Unstained human fibroblast cells were encapsulated within a polymeric hollow fibre containing PVA foam. X-ray microtomography measurements of the capsule were carried out both in absorption and using a phase contrast approach based on Transport of Intensity, which can yield the 3D distribution of the phase (refractive index) of a weakly absorbing object from a single tomographic dataset [4]. Figure 1(a) compares reconstructed slices from datasets scanned using both methods.

![Figure 1](image)

(a) Showing slices through the polymer capsule as scanned using conventional absorption and a Transport of Intensity phase algorithm. The diameter of the capsule is 0.7 mm. Inset: section of foam with cells attached. (b) Labelling of cells based on a nearest neighbourhood method to give isolated objects in 3D.

Use of the Transport of Intensity phase algorithm has enabled easier segmentation of the cells from the PVA foam. Steps have been taken to make some quantitative analysis of the cells. Figure 1(b) illustrates the method taken to label the cells for a small section attached to the wall of the capsule. Clustering of cells has been observed.

3. Visualisation of the Substantia Nigra of a Rat Brain
Differential Phase Contrast (DPC) imaging uses a grating interferometer, consisting of a phase grating and an analyser absorption grating, and a phase stepping technique. Figure 2 shows the experimental setup installed at the TOMCAT beamline of the Swiss Light Source.

A focus has been placed on the fast acquisition and reconstruction of data. A full DPC scan over 9 phase steps and 721 projections can be performed in 20 minutes (energy 17.5 keV, 5th Talbot distance). The technique has been used to visualise the internal soft tissue features of a rat brain. Figure 3(a) shows an overview slice through the reconstructed 3D volume of the brain. Density differences can be observed at a spatial resolution of 10 microns.
Figure 2. Showing the setup of the interferometer with aquarium at the TOMCAT beamline (SLS). The brain was scanned within liquid paraffin.

Figure 3(b) shows a reconstructed slice of a local region of interest of the brain. The tissue structure is observed at a spatial resolution of 3.6 microns. The aim now is to focus around the region of the substantia nigra to follow the progressive degeneration of the dopaminergic neurons.

Figure 3. Showing (a) a reconstructed overview slice through the 3D tomographic volume of a rat brain, and (b) a slice of the local region of interest indicated.

4. Summary
Differential Phase Contrast microtomography with grating interferometry has enabled the visualisation of density differences in the soft tissue of a rat brain, with fast scan times. The Transport of Intensity phase contrast algorithm has facilitated the segmentation of encapsulated cells compared to conventional absorption contrast.

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