

## Development of Laboratory Grating-based X-ray Phase Contrast Microtomography for Improved Pathology.

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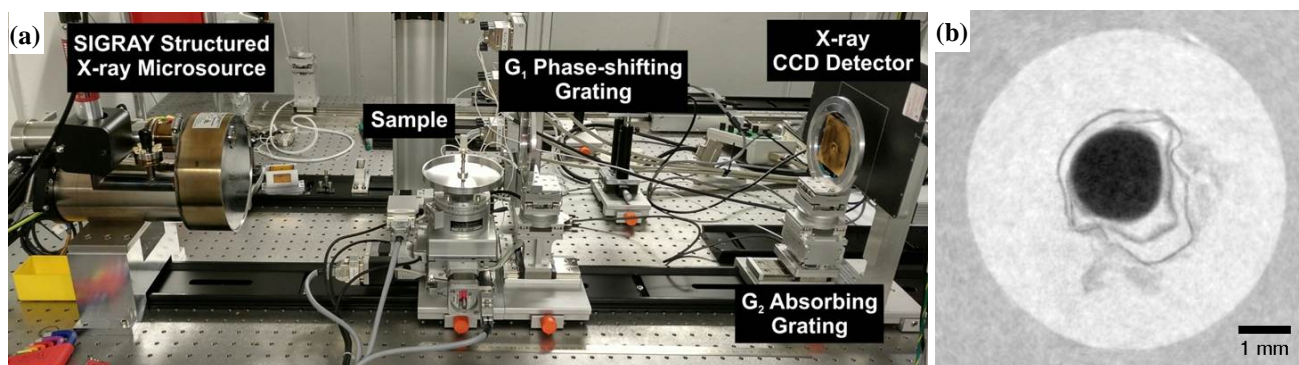
Histopathological examination of tissue specimens is a central diagnostic technique in clinical medicine. The examination relies on the patient's tissue preparation including chemical fixation, very thin sectioning, staining, and subsequent optical microscopy inspection. In this work, we aim to investigate and promote the use of X-ray phase contrast microtomography [1, 2, 3] in histopathology. Taking advantage of the higher sensitivity of X-ray phase contrast is especially well-suited for biological soft tissues, for which standard X-ray absorption does not typically yield enough signal to noise contrast. Furthermore, the use of X-ray phase contrast could possibly reduce the requirement of chemical staining in some conditions, thus enabling an examination of a tissue that is closer to its natural state and X-ray microtomography does not damage the tissue as the sectioning is virtually performed a posteriori on the three-dimensional reconstructed image of the sample.

We are developing a new laboratory X-ray grating interferometry system using an X-ray microsource, in-house fabricated X-ray gratings and a high resolution scintillator-based X-ray detector. The setup is expected to have a field of view up to 2 cm and a high spatial resolution ( $<10\ \mu\text{m}$ ) while using an effective X-ray energy of 20 keV and an approximate geometric magnification of a factor of 2. Our most relevant advances are focused in 1) the use of a structured X-ray source developed by SIGRAY, Inc. [4], that removes the requirement of using a  $G_0$  grating; 2) the use of high Talbot orders using small grating periods of  $1\ \mu\text{m}$  [5], to greatly increase the phase sensitivity of the interferometer; and 3) the investigation of different sample preparation techniques to optimize the quality and quantitiveness of the phase contrast tomographic reconstructions. Fig. 1(a) shows the current laboratory  $G_0$ -less X-ray phase contrast microtomography setup at the Paul Scherrer Institut using structured X-ray microsource (SIGRAY, Inc.) and fig. 1(b) shows a slice from a phase contrast tomographic reconstruction of the eye of mouse obtained with such a  $G_0$ -less system and with an approximate spatial resolution of  $20\ \mu\text{m}$ . The data was acquired combining the structured X-ray anode with a set of gratings  $G_1$  and  $G_2$  with periods of  $3\ \mu\text{m}$ . As reported in ref. [5], we have also developed a new microfabrication technique combining deep reactive ion etching of silicon with atomic layer deposition to fabricate gratings with a period of  $1\ \mu\text{m}$  and a groove depth of  $30\ \mu\text{m}$ . Such gratings can be used to build a system using a high Talbot order interferometer, which enables a much higher phase sensitivity while keeping the total length of the setup short. Finally, fig. 2 and tab. 1 show the quantitative phase tomographic reconstruction of human breast cancer biopsy in formalin demonstrating that biological specimens can be imaged with minimal sample

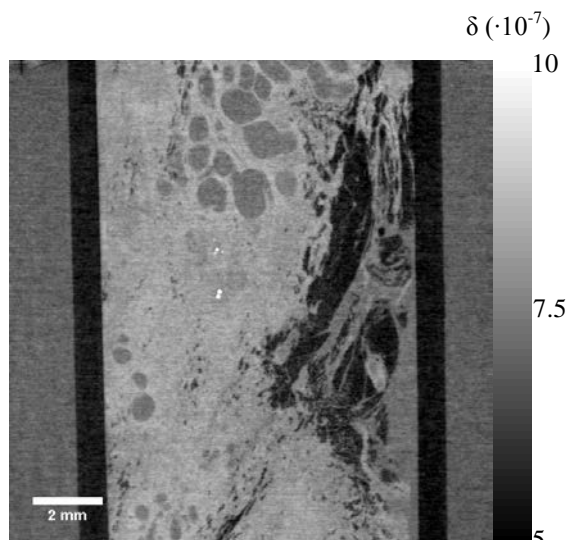
preparation. In summary, the proposed X-ray phase contrast system has the potential to become a new valuable clinical tool for non-destructive histopathological examination [6].

References:

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**Figure 1.** (a)  $G_0$ -less X-ray grating interferometer setup at the Paul Scherrer Institut using a structured X-ray source from SIGRAY, Inc. (b) Reconstructed slice of a mouse eye embedded in a paraffin block by X-ray phase contrast tomography.



**Figure 2.** Quantitative (refraction index coefficient) reconstructed slice of a breast tissue biopsy in liquid formalin by X-ray phase contrast tomography.

	Measured $\delta$ coefficient	Calculated $\delta$ coefficient
Water	$0.72 \pm 0.01$	0.712
Eppendorf tube	$0.60 \pm 0.01$	~ 0.658
Liquid formalin	$0.74 \pm 0.01$	0.68
1) Subcutaneous fat	$0.61 \pm 0.02$	-
2) Breast tissue	$0.83 \pm 0.02$	0.68
3) Fat	$0.77 \pm 0.01$	0.69
4) Calcification	$1.25 \pm 0.06$	1.90

**Table 1.** Comparison between the measured and the calculated refraction index coefficient,  $\delta$ , for the breast tissue biopsy.