EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

ESRF	**Experiment title:** Pushing the limits of grating-based phase-contrast imaging for biomedical and paleontological applications	**Experiment number**: MI-983
Beamline: ID19	**Date of experiment**: from: 26/11-2009 to: 30/11-2009	**Date of report**:
Shifts: 15	**Local contact(s)**: Timm Weitkamp, Irene Zanette	*Received at ESRF:*

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1. Introduction & Motivation

Phase-sensitive x-ray imaging, which uses the phase shift rather than the absorption as the imaging signal has the potential of substantially increased contrast in samples that offer only very little contrast in absorption, e.g. biological tissue samples. Various phase-sensitive x-ray imaging methods were developed in the past years. They can be classified into interferometric methods, techniques using an analyzer, and free-space propagation methods. Some of the methods not only generate a form of phase contrast, but also provide quantitative phase information, which can be used for advanced image analysis. The extension to computed tomography (CT) is one such interesting possibility providing three-dimensional information about the specimen.

One of the more recently developed methods is an approach based on a grating interferometer. This approach has been pioneered by us at ESRF starting with first experiments at BM05 [Weitkamp SPIE 2004, Weitkamp APL 2005]), in which we investigated the x-ray optical properties gratings and mirrors. We continued the development toward phase-contrast radiography and first phase-contrast tomography experiments at ESRF in 2005 (Experiment MI-764, Refs. [Weitkamp OE 2005, David ME 2007, David SE 2008]), using moderate x-ray energies of 17.5 keV. During the last to years, we then turned specifically toward biomedical applications and pushed the technique to higher energies (up to 25 keV), larger fields-of-view (up to 18 x 18 mm^2), and higher density sensitivity in the reconstructions of soft tissues (Experiments MI-825 & MD-328 Refs. [Pfeiffer PMB 2007, Pfeiffer SPIE 2008, Mueller SPIE 2008, Weitkamp ER 2008, Pfeiffer PRL 2008, Baruchel JSR 2008]).

Based on the above mentioned previous individual experiments, we filed this long-term proposal with the **overall goal** to establish a joined and coordinated effort to further push the current limitations of a recently **grating-based phase-contrast imaging approach** to open up new application fields, specifically for biomedical and paleontological applications.

As stated in the application proposal, the **main four technical development goals** of the whole experiment series in the three year duration of the LTP are:

(T1) **Increasing the density sensitivity** of grating-based phase-contrast tomography from currently ~ 1 mg/cm^3 to 0.2 mg/cm^3.

(T2) **Increasing the spatial resolution** of grating-based phase-contrast imaging from currently ~ 4 micron to below 1 micron.

(T3) **Increasing the energy range** of grating-based phase-contrast tomography from currently 25 keV to 90 keV.

(T4) **Development of a standardized and user-friendly** software package for grating-based phase-contrast tomography.

These technical goals will be coupled to the following **scientific goals** :

(S1) The increased density sensitivity of < 0.2 mg/cm^3 will be used for establishing the potential of grating-based phase-contrast tomography for the **visualization of pathologies associated with neurodegenerative diseases.**

(S2) The increased special resolution of < 1 micron will be used for establishing the potential of grating-based phase-contrast tomography for the **visualization of brain micro-vessels in small-animal brains.**

(S3) The increased energy range of up to 90 keV will be used for establishing the potential of grating-based phase-contrast tomography in imaging of fossils, with specific aim on the **visualization of fossils such as inclusions in opaque amber and hominoid teeth at in the 1-40 microns resolution range.**

2. Experiments and Results so far

Within the long-term proposal we requested a total of six experiments each with 15 shifts distributed over 3 years. Up until now, one such experiment has been performed (15 shifts at the end of November 2009). During this beamtime, the following scientific goals (as also outlined in the full proposal) could be achieved:

2.1. Test and characterization of novel grating structures for higher x-ray energies beyond 25 keV (work package T.3.1 & T.3.2 of the original proposal)

In order to move grating-based phase-contrast imaging to higher energies, a series of novel grating structures where fabricated by the Karlsruhe Micro- and Nanotechnology Facility (J. Mohr et al) and at the Paul Scherrer Institute (C. David et al) within the framework of a scientific collaboration with the main proposer. New fabrication techniques, based essentially on LIGA technology at the ANKA synchrotron, allowed the fabrication of very high aspect ratio structures (70 micron high structures at 2.4 micron period, see also Fig. 1). These structures allowed us – for the first time – to test grating-based phase-contrast imaging at energies beyond the previously accessible energy range, which was limited to ~25 keV (due to the limited height of the previously used grating structures).

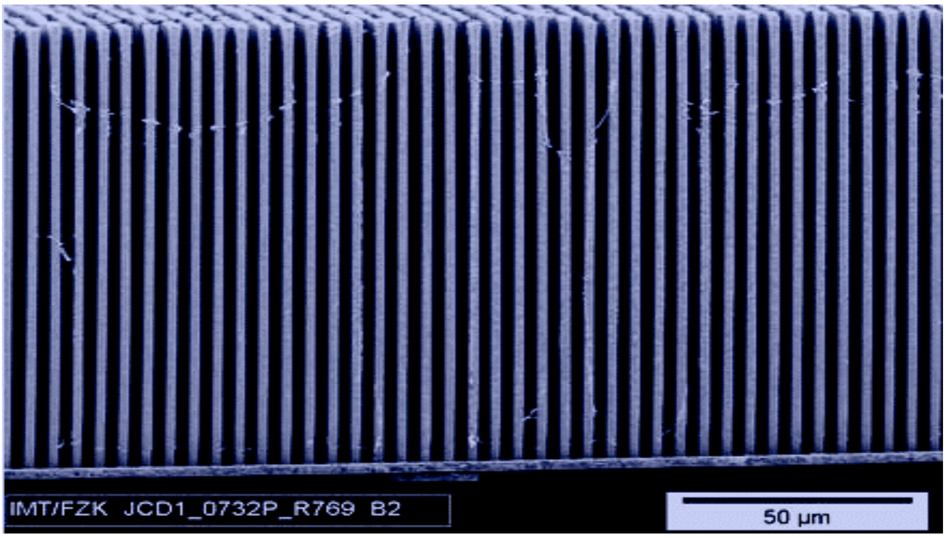


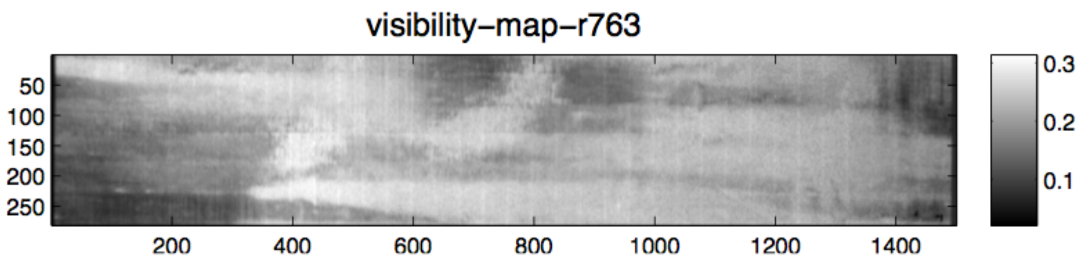
Figure 1: *SEM of a high-aspect ratio absorption grating structure manufactured using LIGA at the Microstructuring Facility of the Karlsruhe Institute for Technology by J. Mohr et al.*

These novel grating structures were intensively tested during this first beamtime, in a way that numerous visibility maps were measured, which can be used to characterize the grating quality and uniformity. The x-ray energy was 35.0 keV and 82 keV, and the distances between the absorption and analyzer gratings corresponded to the 1st, 3rd and 5th fractional Talbot order. Different combinations of phase gratings and absorption grating were tested.

	Analyzer grating			Phase grating						
Fig.	**Name**	**Mat.**	**Period**	**Name**	**Mat.**	**Period**	**Energy**	**T.O.**	**Pixel size**	**Visibility**
1	IMT-062jk	Au	2.4 μm	PSI-3rd-A	Si	4.792 μm	35 keV	3rd	30 μm	<3%
2	IMT-R763	Au	2.4 μm	PSI-3rd-A	Si	4.792 μm	35 keV	3rd	30 μm	20%
3	IMT-063jk	Au	2.4 μm	PSI-3rd-A	Si	4.792 μm	35 keV	3rd	30 μm	24%
4	IMT-R841	Au	2.4 μm	PSI-3rd-A	Si	4.792 μm	35 keV	3rd	30 μm	21%
5	IMT-063jk	Au	2.4 μm	PSI-5th-A	Si	4.787 μm	35 keV	3rd	30 μm	27%
6	IMT-063jk	Au	2.4 μm	PSI-5th-New	Si	4.787 μm	35 keV	3rd	30 μm	18%

Table 1: *Mean visibility measured for different conditions during MI983 run 1, 26.11 - 01.12 2009 ESRF ID19*.

Table 1 and Figure 2 show examples of these results, which are now being used to optimize further the LIGA fabrication processes at the Karlsruhe Institute for Technology. A detailed analysis of these data and its comparison to SEM and light microscopy investigations is in progress, but not yet finished by now.



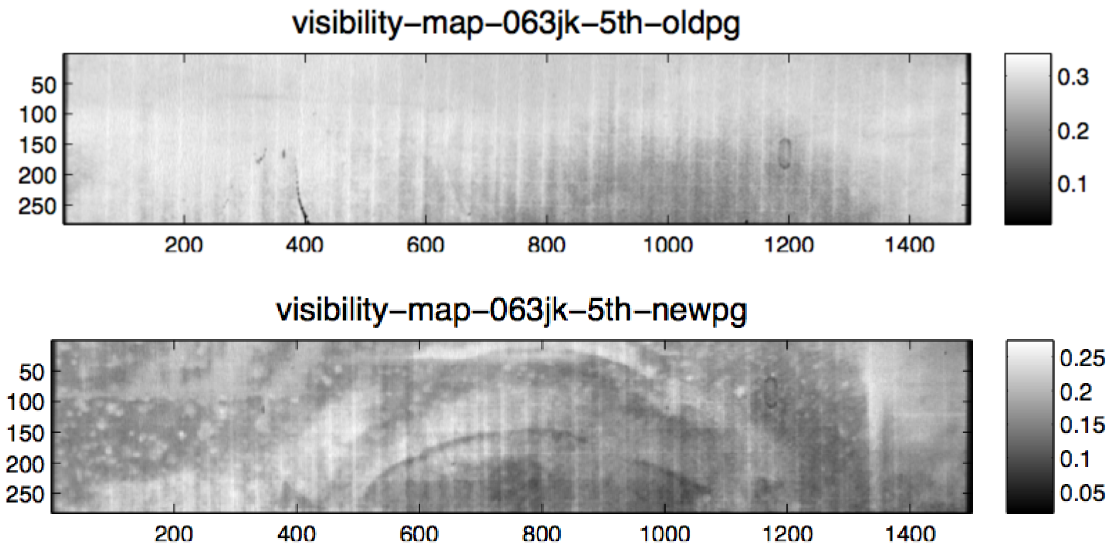


Figure 2: *Three exemplary visibility characterization maps (out of several ten) taken for the novel grating structures manufactured for 35 keV x-ray energy.*

2.2. Proof-of-principle biomedical application experiments with higher energy grating structures for phase-contrast imaging of small-animal models at 35 keV (work package T.3.1 of the original proposal)

Subsequent to the detailed characterization of the novel LIGA grating structures for 35 keV x-ray energy, several test experiments were performed. One such example is shown in Figure 3, which displays phase-contrast and absorption microCT slices obtained for a formalin-fixated mouse specimen. These results were obtained with 35 keV x-ray energy, 15 micron detector optics, and 900 projection angles. The inter-grating distance was set to 36 cm.

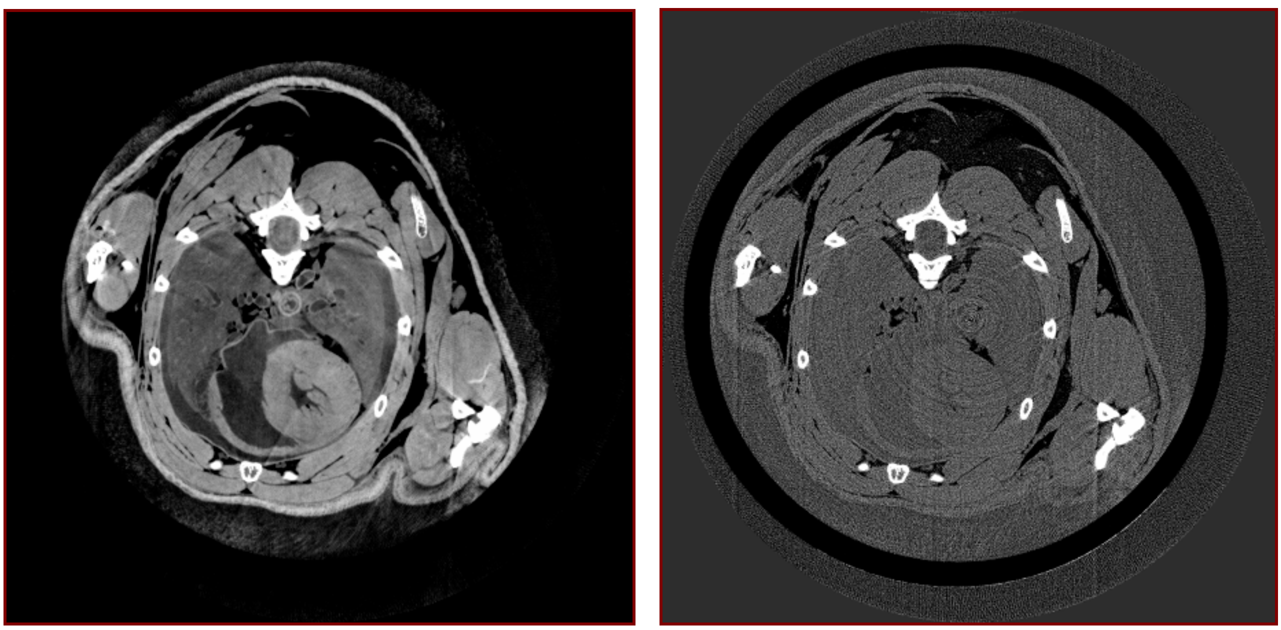


Figure 3: *Left: Grating-based phase-contrast microCT results for a formalin fixated mouse model aquired at 35 keV x-ray energy. Right: Corresponding absorption microCT result, obtained with the same amount of dose.*

The images clearly demonatrate that the novel grating structure fundtion well at these elevated x-ray energies. The results show, for example, that soft tissue details of the specimen are better visualized in the phase-contrast reconstruction (left panel) than in the conventional approach (right panel). In these particular slices, we can identify, e.g., the morphological structure of the heart muscle and several blood vessels in the liver of the mouse.

Presently, we are working on a more detailed evaluation of the data to precisely determine the dose that was used in the experiments and to analyse the signal-to-noise ratio gain in the different soft tissue materials present in the whole body of the animal. More experiments, in particular with small-animal tumor models are planned for the upcoming runs.

2.3. Proof-of-principle application experiments with higher energy grating structures for phase-contrast imaging of paleontological test samples at 82 keV (work package S.3.1 & S.3.2 of the original proposal)

Allthough not planned intitially for the very first run, we were already able to test grating structures for x-ray energies as high as 82 keV. Such high energies are necessary, e.g., for paleontological applications of measuring human fossilized teeth, as the transmission is too low at lower x-ray energies.

Despite of the fact that the achieved visibility values were only on the order of 12-13%, and have to be improved in the upcoming experiments, some proof-of-principle results could be obtained. These are shown in Fig. 4, with the phase-contrast projection on the left, and the absorption projection on the right. We obviously observe also in this high energy regime that the phase-sensitive images reproduce much finer details in the images than in the absoprtion case (right panel).

The next steps include the demonstration of high-energy phase-contrast tomography and the further optimization of the visibility at such elevated energies.

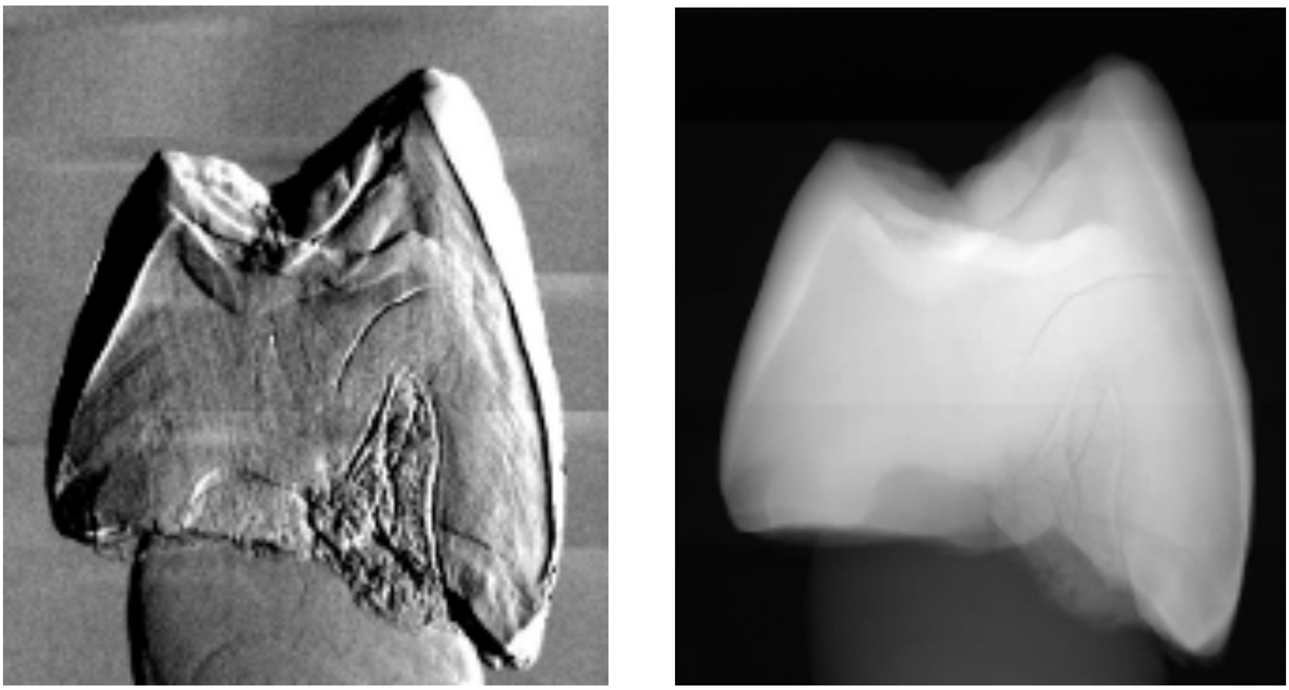


Figure 4: *Left: Grating-based phase-contrast micro-radiography results for a fossilized human tooth at 82 keV x-ray energy. Right: Corresponding absorption micro-radiography result, obtained with the same amount of dose.*

While many improvements can still be made, this result demonstrates the possibility of high energy phase-contrast imaging beyond 80 keV is indeed feasible using a grating interferometer.

3. Dissimination of the results

Parts of the results have been presented as poster contribution at the ICXOM20 conference with ESRF staff a co-authors (Irene Zanette, Timm Weitkamp, and Paul Tafforeau). Manuscripts for original publications of these first results are currently under preparation and should be submitted until mid 2010.