Microscopic imaging of biological samples using coherent soft x-rays from free-electron laser and synchrotron sources

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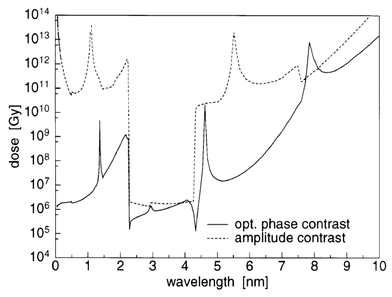
MOTIVATION

Soft X-rays

Biological samples show high contrast at energies between the K-absorption edges of carbon and oxygen ("water window"). The contrast achievable is similar in both amplitude and phase, while radiation damage is minimized for a given desired spatial resolution [1].



Dose applied if 30 nm protein features in 10 µm vitreous ice are detected.
Schneider, Ultramicroscopy 75 (1998) 85-104

Ptychography

Recently developed ptychographic algorithms for phase retrieval [2-4] attract growing interest in the field of nanoscale X-ray imaging of biological objects [5]. Their fast convergence, the possibility to image extended specimens, and a relatively simple experimental implementation overcome many limitations of other X-ray imaging methods.
We believe ptychography will be a key tool in future investigations of extended biological objects with lensless microscopy.

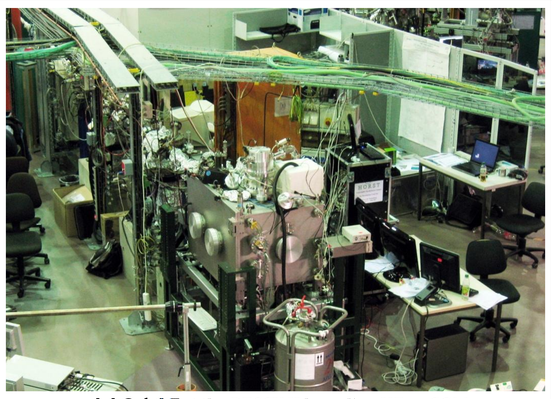
EXPERIMENTS

HORST

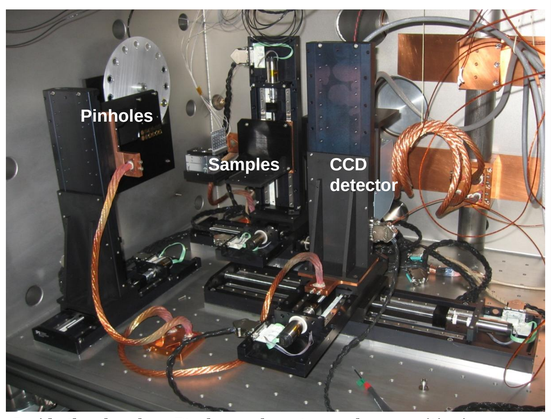
The UHV scattering chamber HORST (HOlographische RöntgenSTreuapparatur) is a versatile apparatus that has already been used for:

- Single pulse CXDI at the free-electron laser FLASH in Hamburg [5]
- Holographic imaging at the synchrotron BESSY II in Berlin [6-8] and at FLASH [5, 9, 10]
- Soft X-ray ptychography of biological objects at BESSY II [11]

Motorized stages with long travel ranges for detector, samples and pinholes in combination with high precision piezo-electric actuators provide a remarkable flexibility for different imaging methods. Within minutes, experimental setups can be fundamentally changed and different samples can be investigated without having to ventilate the chamber.



HORST at the BESSY II beamline UE52-SGM



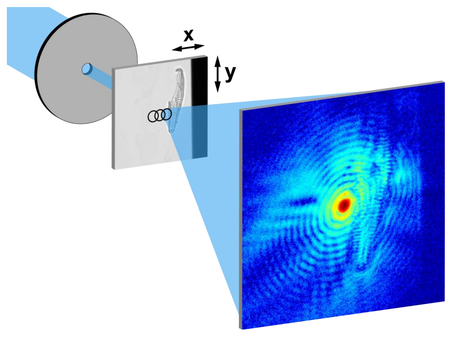
Inside the chamber: Ptychography setup on large positioning stages (150 mm travel range)

PTYCHOGRAPHIC ALGORITHM

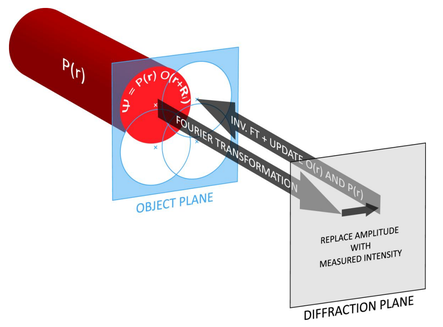
By laterally moving the specimen and illuminating overlapping regions, high redundancy in the collected diffraction data is achieved.
The specimen (object function $O(\mathbf{r})$) will introduce changes in phase and amplitude to the penetrating beam (probe function $P(\mathbf{r})$). At every sample position R_i:

- Calculate $\Psi_i = P(\mathbf{r})\ O(\mathbf{r}+\mathbf{R_i})$ (overlap constraint)
- Fourier transform Ψ_i to the diffraction plane
- Replace $|\Psi_i|^2$ with the measured intensity (Fourier constraint)
- Transform back to real space for an improved guess of Ψ_i

In the original version of the algorithm, an update function is used to improve $O(\mathbf{r})$ before moving to the next sample position [2]. It still relies on *a priori* knowledge about the illumination function. Thibault et al. recently demonstrated a method to retrieve both the object and the probe function with each iteration [4]. Thereby, the illumination profile at the object plane can be extracted.



Experimental setup. A pinhole defines a coherent beam, illuminating overlapping regions of a movable specimen. A CCD detector records the diffraction pattern in the far field.

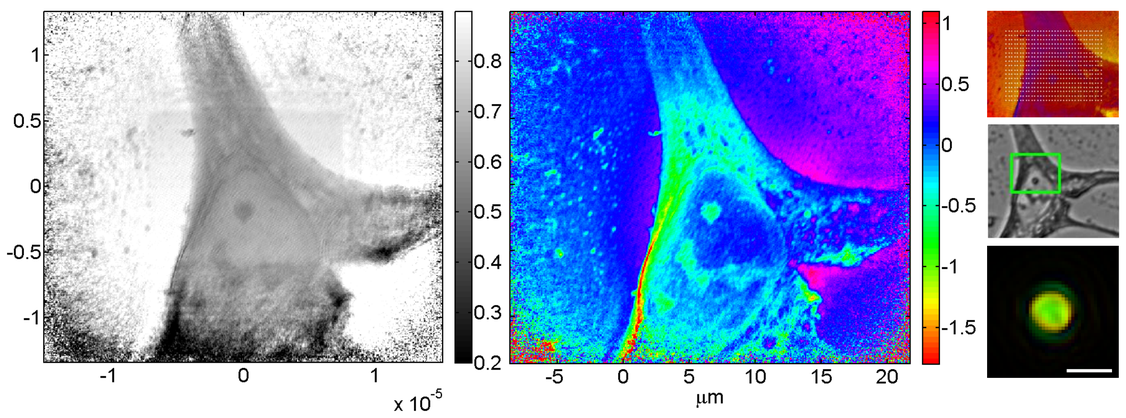


Schematic of the algorithm and its constraint set, shown for one iteration at one sample position R_i.

RESULTS

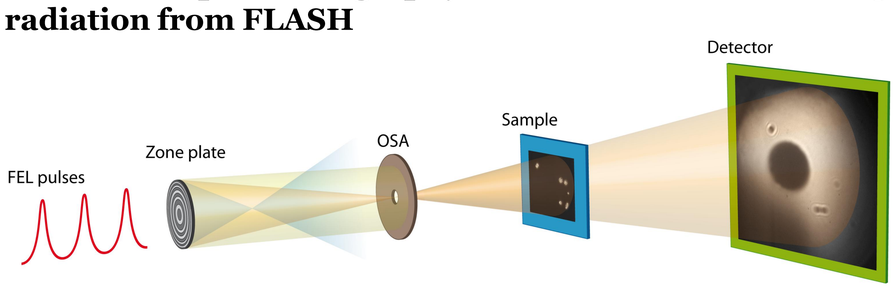
Ptychography

- A human stem cell cultivated on a 100 nm silicon nitride membrane was freeze-dried and diffraction images were recorded at 34 x 17 positions at a photon energy of 517 eV (~ 2.40 nm) at the synchrotron source BESSY II.
- The reconstruction reveals absorption as well as phase contrast. The phase shift for soft x-rays is remarkably high, compared to hard x-rays.
- Ptychography proves as a reliable method for high-resolution imaging. Especially for weakly scattering biological samples the phase contrast enhances the information provided by absorption contrast.

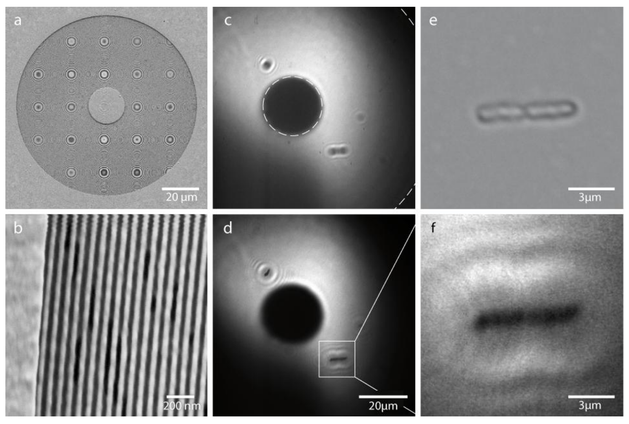


Absorption contrast (left) and phase contrast (middle) reconstructions of a stem cell (brightness encodes amplitude, color encodes phase in rad). Right column: Combined phase and absorption contrast image showing the 34x17 scan positions (top), optical micrograph of the sample with highlighted ROI (middle) and the wavefront of the illumination function backpropagated into the plane of the pinhole aperture (bottom, scalebar is 1 µm).

In-line zone plate holography in the water window with 3rd harmonic radiation from FLASH



Schematic drawing of the experimental setup. A zone plate (ZP) is creating the divergent light cone, which is required for digital X-ray holography. An order sorting aperture is filtering the direct beam as well as higher diffraction orders from the ZP.



Imaging the marine bacteria *Cobetia marina* in the water window at λ_3=2.67 nm using digital in-line holography. (a) SEM-image of used zone plate with clearly visible central beam stop. (b) Close-up of the zone plate's outermost zones. (c) X-ray hologram with *Cobetia marina* in the lower right corner. (d) Reconstruction of hologram (c). (e) Sample *Cobetia marina* under an optical microscope in bright field illumination (100x, NA=0.9). (f) Magnified ROI of the reconstructed image as indicated by rectangle in (d).

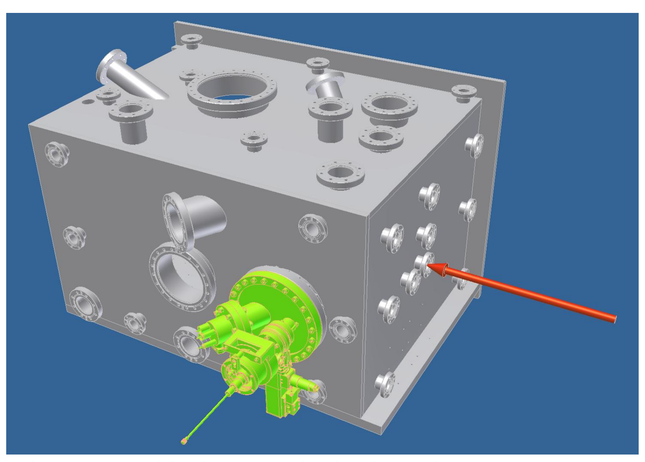
- Application of a zone plate instead of a pinhole led to an increase of flux seen by the sample by four orders of magnitude.
- This gain in efficiency allowed for using the 3rd harmonic at 2.67 nm and acquiring the first images of biological material in the water window at FLASH.
- Zone plate holography proves to be a direct imaging candidate for single pulse experiments as soon as FLASH reaches the water window with fundamental wavelengths.

OUTLOOK

Measurements in a cryogenic environment and in the liquid phase

1. Cryo transfer system

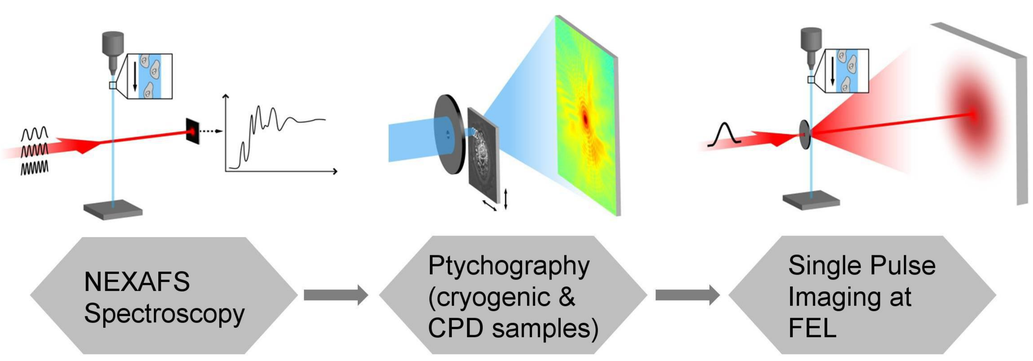
- Changing samples without the need of the time consuming venting of the whole vacuum chamber.
- The cryogenic sample stage eliminates the need to fixate and dry samples before the imaging process under vacuum conditions.



The green part of the the HORST chamber depicts the new cryogenic setup, which allows for an easy and fast transfer of vitrified samples in order to image them in vacuum conditions. The system is mounted on a CF200 flange at the back-side of the chamber. The same flange will be used to mount the liquid jet. The red arrow points in the direction of the X-ray beam.

2. Liquid jet

- The liquid jet allows for studying unstained living organisms in vacuum conditions.
- The liquid jet is part of an integrated workflow pipeline combining different imaging techniques and spectroscopy at different X-ray sources.
- This leads to a complete picture of a sample with knowledge about the elemental composition and distribution with high resolution structural information.



Sketch of the workflow pipeline where the combination of imaging techniques and spectroscopy at different X-ray sources deliver complementary information about a sample. First NEXAFS spectroscopy unveils the elemental composition of an unstained living biological organism. This information can then be used in a second step for resonant cryogenic ptychography leading to the spatial distribution of the detected elements. In a third step single pulse imaging at a FEL is applied in order to gain redundant information about the structure already available from ptychographic imaging but with the certainty of excluding artifacts caused by radiation damage.

LITERATURE

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ACKNOWLEDGEMENTS

The Heidelberg authors kindly acknowledge financial support from the BMBF projects 05KS7VH1 and 05K10VH4.