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Quantitative Evaluation of Hard X-ray Damage to Biological Samples using EUV Ptychography

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Abstract.

Coherent diffractive imaging (CDI) has become a standard method on a variety of synchrotron beam lines. The high brilliance short wavelength radiation from these sources can be used to reconstruct attenuation and relative phase of a sample with nanometre resolution via CDI methods. However, the interaction between the sample and high energy ionising radiation can cause degradation to sample structure. We demonstrate, using a laboratory based high harmonic generation (HHG) based extreme ultraviolet (EUV) source, imaging a sample of hippocampal neurons using the ptychography method. The significant increase in contrast of the sample in the EUV light allows identification of damage induced from exposure to 7.3 keV photons, without causing any damage to the sample itself.

1. Introduction

The study of hippocampal neurons is of great interest to the scientific community to understand mechanisms that can lead to neuro-degenerative disease. To aid further understanding of biological processes, imaging at high spatial resolution and resolving internal structures beneath the sample surface is required.

Coherent diffractive imaging (CDI) is a rapidly evolving field capable of producing diffraction limited resolution of a sample's amplitude and phase, without the need for any imaging optics. A scanning variant of CDI known as ptychography is a proven robust imaging method to solve the phase retrieval problem. Ptychography records scatter patterns at multiple partially overlapping regions of the sample illuminated by spatially confined structured illumination [1]. The overlap provides redundancy in the collected dataset. The ptychography algorithms assume that there is identical sample transmission in the overlapping regions in combination with precise knowledge of relative sample shift and measured oversampled diffracted intensities to reconstruct both the complex exit wave field of the sample, and the complex probe illumination function.

Laboratory scale EUV sources such as plasma based sources and HHG sources have demonstrated the required coherence to successfully image via the ptychography technique [2,3]. Whilst the synchrotrons provide a much broader range of imaging wavelengths that spread from



IR to hard X-rays, the laboratory EUV sources are readily available, inexpensive, and in the case of HHG sources the coherent photon flux is approaching / comparable to synchrotron sources as shown in [4] where the flux was 1×10^{12} photons/sec .

The drawback of all short wavelength sources is that the sample is illuminated by ionising radiation that causes both primary and secondary damage effects in the sample. This radiation induced damage can significantly alter the sample structure, and in particular unfrozen biological samples are particularly sensitive to radiation damage. Below, we investigate the effect of radiation damage caused from an illumination of 7.3 keV synchrotron source using a 42 eV HHG laboratory source via the ptychography method.

2. Experimental method

The sample under investigation was a culture of mouse hippocampal neurons prepared on a 50 nm Si_3N_4 membrane coated in a thin layer of poly-D-lysine. The neurons extracted from the hippocampus of an E17 mouse embryo were cultured for 14 days before fixation. The cells were fixed using 4% paraformaldehyde fixing solution and 20% sucrose in phosphate buffered saline solution. Finally, they were fixed in 100% methanol for 20 minutes before air drying.

The sample was imaged at the I13 beam line at the Diamond Light Source. Photons generated from the undulator are monochromated using using a Si (1,1,1) crystal. 7.3 keV photons were focused down using a Fresnel zone plate of focal length 34 cm. The DC frequency is blocked using a central stop, and higher diffraction orders are removed using an order sorting aperture. The sample is placed after the focus, where the hard X-ray spot size was chosen to be $\approx 2 \mu\text{m}$ in diameter. The scatter pattern from the sample was measured a distance of 7.1 cm from the sample using a Merlin MEDIPix photon counting detector. The ptychography method was used to image the sample using a step size of $0.5 \mu\text{m}$ to ensure the ptychographic overlap constraint was fulfilled. The sample was positioned using piezoelectric actuators and the exposure times were set at 10 s. The photon flux on the sample was $1.4 \cdot 10^8$ photons/s.

After inducing radiation damage in a localised region, the sample was imaged using 42 eV photons from a HHG source at the University of Southampton. The coherent EUV radiation is generated from a highly non-linear interaction between ultra-fast pulses and an inert gas. The driving laser beam was generated from a 2 mJ, 40 fs, 800 nm, 1 kHz repetition rate CPA Ti:sapphire laser focused into a argon filled gas cell. The fundamental wavelength was removed by a single 200 nm Aluminium foil and partial spectral filtering and focusing of the EUV radiation was obtained using a curved MoSi multilayer mirror. After filtering, the spectrum illuminating the sample consists of three harmonics centered around 29 nm (27-th harmonic) with a 12% bandwidth [5]. The sample is placed at the focus of the EUV radiation, where the spot size is measured to be $\approx 12 \mu\text{m}$. $80 \mu\text{m}$ upstream of the sample, a $10 \mu\text{m}$ aperture is placed to spatially confine the beam. The resulting flux incident upon the sample is $2 \cdot 10^8$ photons/s. Further detail on this experiment can be found in Ref. [3]. The scatter pattern of the sample was collected in the far field using an EUV sensitive cooled CCD, where the maximal collected NA was 0.26. The dynamic range of the CCD was numerically extended by 3 orders of magnitude, as described in Ref [3], from 10^4 to 10^7 counts, however despite this, the poor dynamic range of the CCD detectors is still a limiting factor, when imaging weakly scattering real biological samples.

The synchrotron datasets were reconstructed by the difference maps method and then refined by the ePIE algorithm [6]. However, for the case of the diffraction patterns collected using the HHG source, where both pointing stability and intensity stability are significantly poorer than the synchrotron source, the orthogonal probe relaxation ptychography (OPRP) method [7] was used for the reconstruction. This method relaxes the overlap constraint, and allows for beam fluctuation, reconstructing a different probe for each scanning position.

3. Experimental results

Fig. 1 shows the comparison of the reconstruction quality between the EUV ptychography reconstruction (Fig. 1(a)) and the X-ray ptychography (Fig. 1(b)). The detail of the EUV reconstruction is showing artefact-less reconstruction quality of the fine neural structures. On the other hand, any structure in the thick cell soma of the neurons are lost due to low penetration depth in biological material (≈ 50 nm). The X-rays easily penetrate through the thick cell bodies however, due to very low contrast, no details of the thinner neural connections can be seen.

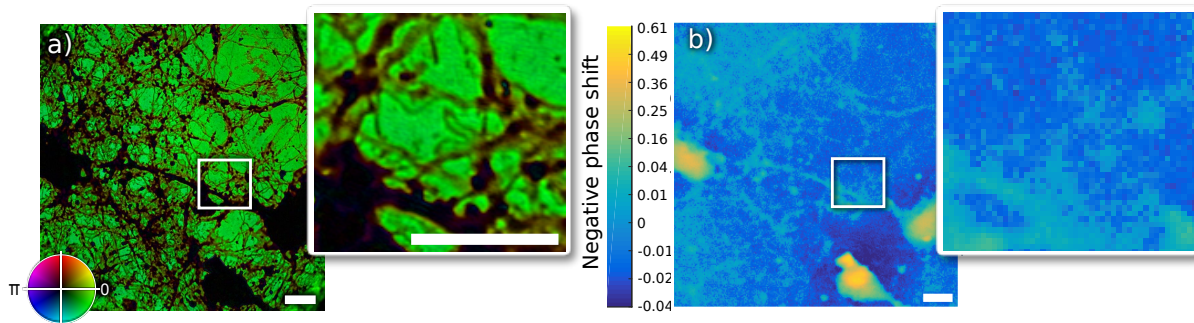


Figure 1. A reconstruction of neural structure in transitivity. (a) Complex colorscale reconstruction using 42 eV, (b) shows reconstructed phase from a ptychography scan at 7.3 keV where the sample dose was $1.4 \cdot 10^3$ Gy. Scalebar is 10 μ m.

It was assumed that because the inelastic interaction of the hard X-ray photons with the sample is very weak, the dose deposited in the sample would be below the radiation damage threshold. Using the formula derived by Howells et al [8] the sample dose D in Gray (J/kg) is calculated

$$D = \frac{N_0 h \nu}{L_a n S} , \quad (1)$$

where L_a is the attenuation length, $h \nu$ is the photon energy, N_0 is the number of incident photons per area S , and n is the average sample mass density.

The sample received a spatially dependent dose distribution. The white dashed box in Fig. 2(a) shows the region where the sample received a dose of $3.6 \cdot 10^5$ Gy, whereas outside this region the sample received a dose of only $1.4 \cdot 10^3$ Gy. The 42 eV ptychographic reconstructions shown in Fig. 2(a) show the region of radiation induced sample damage that can be clearly identified. A detailed image of the transition region is shown in Fig. 2(2). The damage can be approximately quantified when looking at the exponential intensity decay constant, α , of each EUV diffraction pattern extracted from a fit of $y = x^\alpha$, and plotting this as a function of spatial position, shown in Fig. 2(c). The region of higher radiation does have a more negative value as a function of spatial frequency, which indicates a steep exponential reduction in the high frequency signal.

Due to the strong photon/sample interaction, the EUV dose given to the sample is approximated to be $4.4 \cdot 10^7$ Gy during 15 s exposure, which is 2 orders of magnitude more than the X-ray dose. Despite the high dose of EUV light that is capable of free radical generation [9], no radiation damage was observed. We think the dose given from the 42 eV illumination is not causing any changes to the sample that can be measured using our microscope. It is also possible that other damage mechanisms occurred in the X-ray reconstruction such as thermal damage. Both the heat damage and secondary radiation damage from diffusion of free radicals within the sample can be mitigated through cryo sample preparation as discussed in Ref. [8].

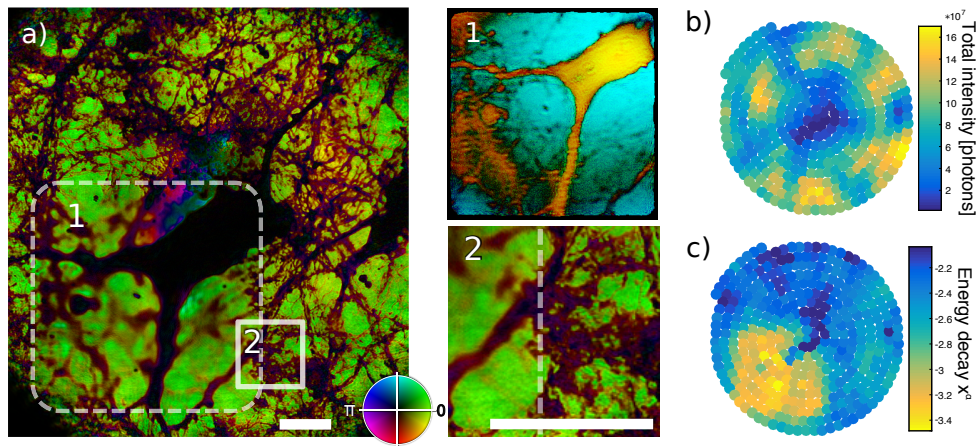


Figure 2. Radiation damage caused by X-ray ptychography at 7.3 keV. (a) EUV ptychography reconstruction after the X-ray ptychography scan. Subregion (1) was scanned by a $2\ \mu\text{m}$ X-ray beam with 10 s exposure. The X-ray ptychography reconstruction of this subregion is shown in a contrast enhanced colorscale. The transition region around the damaged region from EUV ptychography image is shown in (2). The image (b) shows a scanning transmission (STXM) image of the sample, plotted at each real space scanning position, while (c) shows the measured exponential decay of the scattering energy in the Fourier (detector) plane against the spatial frequency. Scale bar denotes $10\ \mu\text{m}$.

4. Conclusion

We have shown that the damage threshold for hippocampal neurons prepared without any measures to mitigate radiation damage is between $1.4 \cdot 10^3$ and $3.6 \cdot 10^5$ Gy when measured at the resolution of our EUV microscope (lateral resolution $=80\ \text{nm}$ axial resolution $10\ \text{nm}$). We have confirmed radiation damage using highly sensitive EUV radiation from a coherent laboratory based EUV source, using the OPRP algorithm to overcome the shortcomings of HHG EUV stability.

Acknowledgments

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