XUV Ptychographic imaging of mouse hippocampal neurons with 50nm resolution using the Artemis HHG source

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Introduction

The availability of coherent sources of XUV and soft X-ray radiation from sources based on high harmonic generation (HHG) means that imaging in these spectral regions can be performed using lens-less techniques such as ptychography[1]. This allows for much increased resolution, as no imaging optics are required, and for measurement of the full complex transmission of the sample. In this paper, we describe coherent imaging of mouse hippocampal neurons grown on silicon nitride substrates at a wavelength of 29 nm with diffraction-limited resolution of ~100 nm. Transmission imaging was performed with an illumination-forming pinhole close to the sample, and also with an illumination-forming aperture at a large distance (~1m) from the sample demagnified by EUV reflective optics. This allows much greater flexibility in designing measurement geometries without the need for a pinhole in close proximity to the sample.

Ptychography

Traditional imaging works by illuminating an object with light, and then collecting the light transmitted or scattered by the object using an objective lens, which forms a real image behind the lens. In contrast, coherent imaging works by illuminating an object with coherent light, and then collecting the scattered light pattern from the object directly onto an array detector placed in the position of the objective lens. The scattered light pattern has intensity information but no associated phase information once detected, so a reverse transform back to an image is not possible. In order to create an image, the phase of the scattered light on each pixel of the array detector is determined by an iterative algorithm, which makes use of the existence of constraints in object plane and detector plane in order to make the determination of the unknown phase of each pixel possible.

Many algorithmic techniques exist to reconstruct the phase, but one of the most successful is ptychography. In a ptychography measurement, the illuminating light, or 'probe', is scanned over a large object, and scattered light collected from each probe position. The probe positions are designed so that the illuminated areas overlap significantly, and this spatial overlap in object space, along with a constant illumination probe, provides the second constraint, the first being the knowledge of the intensity of the scattered light at the detector.

Experimental details

The ptychographic imaging algorithms work most effectively when the illumination probe at the sample has a spatial profile which contains structure. Gaussian illumination, for example, makes phase reconstruction very difficult. The classical way to provide illumination with structure is to illuminate through a pinhole placed close to the sample, so that the sample is in the near field of the pinhole and is illuminated with a highly-structured Fresnel diffraction pattern. For the wavelengths in this experiment, this means the pinhole is typically ~100 µm upstream from the sample. These experiments used an FIB-cut pinhole of 10 µm diameter.

The XUV light at a wavelength of around 30 nm was generated on the imaging beamline of the Artemis HHG source, using argon gas as the generation medium. Pulses centred at 800 nm, with energies in the range 1-2mJ, and duration 40fs were focused into a 3mm gas cell for phase-matched generation. Two 200nm thick Al filters were used to separate laser from XUV, which was focused onto the pinhole using a curved multilayer XUV mirror. The sample and pinhole could be moved independently of the XUV focus using combinations of stepper and piezo stages.

Scattered radiation was collected using an in-vacuum XUV CCD camera, which was positioned very close to the sample to increase the numerical aperture (NA) of collection. In a ptychography experiment, the resolution is determined by the NA of the detector and signal to noise ratio, just as it is for traditional microscopy.

Samples for these experiments were mouse hippocampal neurons, grown at the Institute for Life Sciences at the University of Southampton by J. Bailey, K. Deinhardt, and J. Chad[2]. To achieve this, hippocampal neurons from E17 mouse embryos were cultured on poly-D-lysine-coated 50nm SiN substrates before fixation at different growth times.

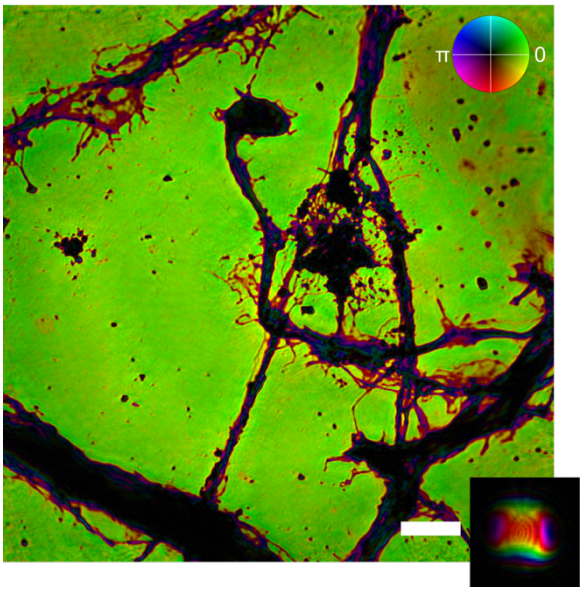

Figure 1 - Transmission image at 29 nm of 7DIV neurons grown on SiN substrate. Scale bar is 10 µm.

Figure 1 shows the complex transmission function of a sample of neurons grown for 7 days *in vitro* (7DIV) on a 50 nm SiN substrate. Colour in the figure shows the relative phase of the object's transmission function, and the circle in the top right corner shows the phase scale. Amplitude of the transmission function maps to intensity in the figure. This figure was

reconstructed from 1792 collected scatter patterns. Reconstruction was performed using a modified ePIE method[3], in particular using orthogonal probe relaxation[4] to account for variations in the HHG output of the source, and taking account of the spectrum of the source, which extended over several harmonics of the laser. The peak wavelength was 29 nm. The image's pixel size was 105 nm, corresponding to the diffraction-limited resolution governed by the size and position of the detector. The probe illumination function calculated during the reconstruction is shown in the lower right corner of figure 1, at the scale as the image, with colour representing phase as in the main image. This probe function can be numerically back-propagated to the position of the pinhole, giving a sharp-edged image of the 10 µm pinhole with a saddle-shaped phase profile, as expected for radiation from an off-axis focused spherical mirror.

Projected pinhole imaging

For many experimental imaging geometries, having to place a pinhole very close to the sample surface is very restrictive and can make experiments too difficult. In principle, a highly-structured probe can be imaged onto the sample if high NA illumination optics are available, for example in visible light ptychography. In the XUV, the absence of good imaging optics means that any image of the probe is not accurate – however, as ptychography reconstructs the probe illumination, accurate probe imaging is not important, but retention of structure in the probe is important.

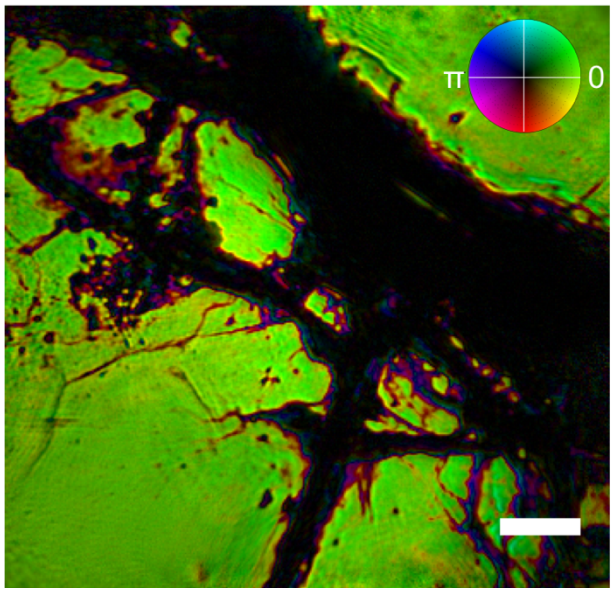

Figure 2 - reconstructed image of 7DIV neurons using a projected pinhole. Colour represents phase in the same manner as figure 1. Scale bar is 5 µm.

To demonstrate the principle, instead of a 10 µm pinhole placed 100 µm from the sample, an iris with diameter of 1.75 mm was placed about 50 cm before the multilayer mirror that focuses the XUV onto the sample. XUV coming through the pinhole creates an unfocused image of the iris on the sample with dimension of ~5 µm. The image in is a ptychographic image of another area of the same 7DIV neuron sample collected by scanning the projected probe over the sample. The quality of the image is slightly lower than the image in figure 1, but recognizable structure of the biological sample on the scale of the 100 nm pixel size is visible.

The probe beam reconstructed in parallel with the object in figure 2 is shown in **Figure 3**, with displayed colour mapping to probe field phase, and displayed intensity to probe amplitude. The illumination wavefront is smoothly-varying, as expected for a beam close to focus, but there is still sufficient phase structure for ptychography to be possible. The overall size of the probe is ~ 5 µm, as shown by the scale bar in the figure. The lack of probe structure makes reconstruction more difficult, but it is still possible, with good results. In future, more structured illumination can be achieved with the use of higher NA illumination optics.

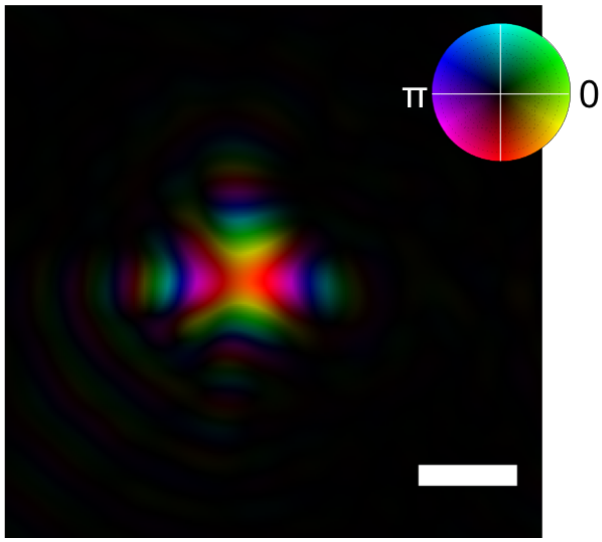

Figure 3 – Reconstructed probe illumination function at sample for projected probe image in figure 2. The phase is indicated by the colour, as in figure 1. Scale bar is 5 µm.

The probe illumination function shown in figure 3 was numerically propagated back to the plane 50cm before the multilayer XUV focusing mirror. Again, a sharp-edged probe function is recovered at the position of the pinhole, indicating that the reconstructed probe at the sample is accurate.

Conclusions

XUV ptychographic imaging in transmission mode of mouse neurons has been demonstrated with the Artemis XUV source. Resolution of 100 nm has been obtained, limited by data collection time. As well as imaging using a pinhole close to the object, the use of a pinhole situated near the HHG source and imaged onto the object has been demonstrated. Imaging using this 'projected pinhole' technique is experimentally convenient for many sample geometries, including reflection-based measurements.

Acknowledgements

This work builds on the investment and research from the EPSRC Basic Technology grant GR/R87307/01. PhD studentships were funded by EPSRC, and the EU FP7 Erasmus Mundus Joint Doctorate Programme EXTATIC under framework partnership agreement FPA-2012-0033. The K5200 graphics card used for this research was donated by the NVIDIA Corporation.

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