Towards Laser Driven Table-top Coherent Diffractive X-ray Microscopy of Cultured Hippocampal Neurons

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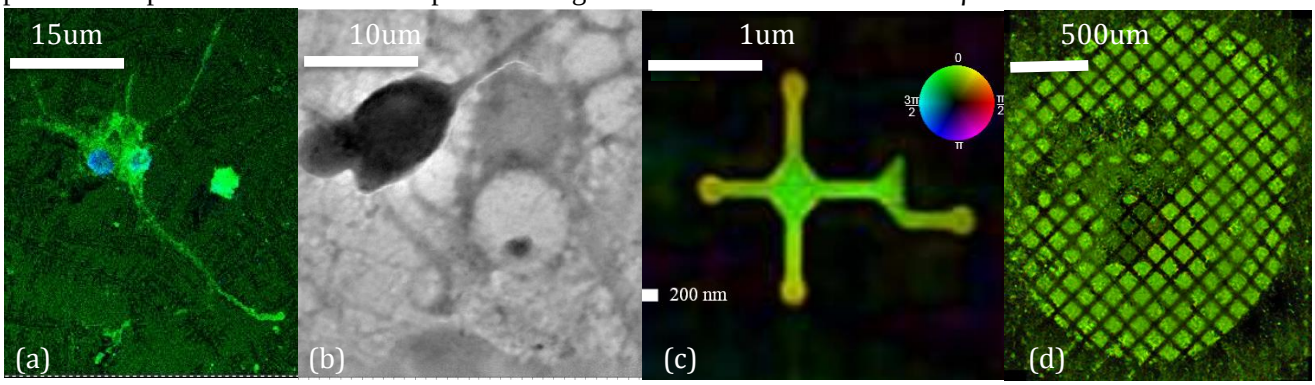
Neurodegenerative diseases such as Alzheimer's disease have a huge impact on the world population; over 44 million people worldwide and 850,000 in the UK were recorded as living with dementia in 2013. There are numerous theories attempting to explain the cause of Alzheimer's disease. Histology from the brains of people who had Alzheimer's disease shows neurofibilliary tangles and amyloid plaques. Their role in the mechanism of disease is not yet completely understood but we envisage that novel imaging techniques may aid understanding. We present initial data collected using confocal fluorescence microscopy and hard X-ray scanning diffractive microscopy (ptychography) on cultured neuron samples plus high resolution large field of view imaging of test samples from a soft X-ray lab based high harmonic generation (HHG) source.

Mouse hippocampal neurons were cultured on glass cover slides for confocal microscopy and 50 nm silicon nitride for X-ray (soft and hard) microscopy. The neuron cell structure is clearly visible with confocal imaging (Figure 1a); however in order to understand more about the mechanisms of the disease, the higher resolution available from X-ray imaging will be important. Contrast in X-ray imaging is available without staining, allowing imaging of the cells in a natural state, although in these examples the cells are not hydrated.

An unstained neuron sample was exposed to coherent hard X-rays (9keV) from synchrotron radiation (Diamond Light Source). Ptychography is a lensless imaging technique in which the sample is scanned at adjacent regions to the beam with intentional overlap that allows amplitude and phase information to be recovered. The sample was illuminated with highly curved beam and the data was collected in the nearfield diffraction mode in order to limit the dynamic range of weakly scattering biological samples. Computational algorithms are then used to retrieve both the phase and amplitude of the exit wave field of the sample from the measured diffraction intensities. At the photon energies used, attenuation of biological samples is negligible, therefor our images are presented in phase only (Figure(1b)). From the dataset of 900 diffraction patterns the phase of the neuron sample was imaged to 20 nm resolution over a 2 µm field of view



Figure(1). Figure 1a is confocal microscopy image showing a single neuron and Figure 1b shows the phase of the exit wave field from a ptychographic Xray image. A cell soma and apical dendrite appear resolved. Figure 1c shows a binary test sample image reconstruction using a high harmonic source (monochromatic at 27nm) reconstructed using Coherent Diffractive Imaging (CDI) Hybrid Input Output (HIO) algorithm to 60nm resolution. Figure 1d shows reconstruction of a test grid using near field ptychography from 27nm light generated from high harmonic source with a 2mm field of view

We aim to image both the phase and amplitude of the exit wave field from a neuron sample using a laser driven HHG source. Imaging using ptychography on our high harmonic set up has the potential to extract both amplitude and phase to sub 50nm resolution with little radiation damage, plus a large field of view. Soft X-rays also provide far more contrast than hard X-rays at 9keV. Looking further to the future, high harmonic sources have the potential to generate X-rays at 4 nm in the "water window", allowing ~10 nm resolution in hydrated samples, plus the ability to do time resolved imaging of biological samples, whilst remaining an accessible laboratory based imaging technique.