Sub-cellular structure of eukaryotic cells by holographic imaging

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Introduction: The aim of the proposed experiment was to image the structure of unsliced and unstained eukaryotic cells (Dictyostelium Discoideum) at the organelle and sub-organelle level. Coherent x-ray imaging offers a new route to image the ultra-structure of unsliced and unstained biological cells and tissues without invasive sample preparation at high resolution [1,2]. Hard x-rays can yield quantitative electron density and effective mass density maps due to the negligible absorption and the simple asymptotic behavior of the optical constants above the absorption edges of the biologically relevant elements. As a special variant of lensless coherent imaging, phase reconstruction in highly curved wavefronts has particular advantages (convergence, uniqueness). The setup for propagation imaging installed at the coherence beamline P10 [3] provides well controlled, coherent and highly curved illumination fields with lateral source dimensions down to 10 nm using the filtering properties of x-ray waveguides [4].

Figure. 1: (a) A 13.8 keV x-ray beam is focused by two KB-mirrors onto two crossed waveguides (WG), serving as a virtual quasi-point-source. A Dictyostelium Discoideum cell (C) is placed in the divergent beam at a distance $z_1$ behind the WG exit. A pixel detector (D) at a distance $z_2$ away from the sample records the magnified in-line hologram (Fresnel diffraction pattern). (b) Optical micrographs of the cells used in the experiment. (c,e) Holographic intensity diffracted from the cells shown in (b), placed at different distances $z_1$, normalized by the empty beam intensity distribution. (d,f) Corresponding quantitative phase reconstructions obtained with a modified HIO scheme, pixel sizes are 88 nm (d) and 62 nm (f). The colorbar is scaled in rad and also mg/cm$^2$. (g) Normalized diffracted intensity, obtained by placing the cell closer to the WG for higher magnification (M=1763). The corresponding region of the cell is marked in (c). A composition of 28 high-magnification holograms, obtained by scanning the cell through the field of view, results in a phase reconstruction of the whole cell with 31 nm pixel size. (h) One part of the high-resolution reconstruction corresponding to the region marked in (d). (i) Magnified image of the region marked in (d) illustrating the coarser sampling compared to (h).

Results: A 13.8 keV x-ray beam was focused by two KB-mirrors onto a system of two crossed x-ray waveguides (WG), yielding a virtual quasi-point-source with 10.7 nm × 11.4 nm (FWHM) lateral dimensions in the horizontal and vertical direction (Fig. 1) [3, 4]. The maximum integrated intensity in the focal spot of the KB-beam was $2.4 \cdot 10^{11}$ cps, as measured by a pixel detector (Pilatus). A maximum photon flux of $1.0 \cdot 10^8$ cps exiting the WG was measured. Dictyostelium
Discoideum cells (stem AX2) were starved and entered a state of high motility in which they build pseudopodia while doing chemotaxis [5]. The cells were rapidly injected into liquid ethane (Leica Gridplunger GP2), preventing the formation of ice crystals, and subsequently freeze-dried in high-vacuum below the glass-transition temperature. Two different cells were placed in the highly curved wavefronts at different distances $z_1$ behind the WG exit (Fig. 1). A pixel detector (Maxipix) with pixel size $\Delta_D = 55\mu m$ was placed at a distance $z_1 + z_2 = 5.29\text{m}$ behind the WG to record the magnified in-line hologram (Fresnel diffraction pattern). The geometric magnification $M = (z_1 + z_2)/z_1$ results in a demagnified (effective) detector pixel size $\Delta_D/M$ and the experiment can be described in a well-known equivalent parallel-beam geometry with an effective sample-detector distance $z_{\text{eff}} = z_1 z_2/(z_1 + z_2)$. Fig. 1 (c,e) shows the holographic intensity diffracted from the cells after normalisation by the empty WG beam intensity distribution, the total illumination time was 1.5 minutes (c) and 30 minutes (e), respectively. Phase reconstructions were obtained by application of a modified version of the classical Hybrid-Input-Output (HIO) scheme which circumvents the well-known twin-image problem [6]. Quantitative projected mass density maps could be extracted from the phase reconstruction (see colorbar scaling). The geometric parameters were $z_1 = 8.5 \text{ mm}$ and $z_1 = 6 \text{ mm}$, resulting in $M = 622$ and $M = 881$ with effective pixel sizes of $\Delta_D/M = 88 \text{ nm}$ and $\Delta_D/M = 62 \text{ nm}$ for the reconstructions shown in (d) and (f), respectively. The cells exhibit different global shapes, a characteristic that can be expected for motile cells. The reconstruction of the cell shown in (f) exhibits bulges which can be tentatively attributed to the formation of pseudopodia. Additionally, several sub-cellular features of the cells are visible. To exploit the “zoom” capabilities of the method and for higher resolution, the first cell was positioned closer to the waveguide ($z_1 = 3 \text{ mm}$) to increase the magnification ($M = 1763$). The sampling of the diffraction pattern shown in (g) is therefore much higher than the corresponding region recorded at $z_1 = 8.5 \text{ mm}$, shown in (c). A composition of 28 high-magnification holograms, obtained by scanning the cell through the field of view, resulted in a phase reconstruction of the whole cell with 31 nm pixel size. The azimuthal averaged power spectral density (not shown) indicates a half-period resolution of about 50 nm. One part of the high-resolution reconstruction, corresponding to the region marked in (d), is shown in (h). A magnified region of the reconstruction at $z_1 = 8.5 \text{ mm}$ marked in (d) is shown in (i), illustrating the coarser sampling compared to the high-resolution reconstruction (h).

Conclusion: Unsliced and unstained freeze-dried Discoideum cells were imaged successfully with quantitative projected mass density contrast. Shapes attributable to the formation of pseudopodia as well as sub-cellular structures could be visualized. Spatial resolution compared to our previous holography and ptychographic experiments with biological cells could be increased. Ongoing research - ideally backed up by a long term proposal - is directed towards: (i) tomographic reconstruction, (ii) imaging of frozen-hydrated samples (cryogenic environment), (iii) further increase of resolution by optimizations of waveguide fabrication, vibrational stability and geometrical parameters as well as (iv) a combination of imaging with nano-diffraction, which has already been successfully tested. To unambiguously indentify cellular components, a correlative microscopy approach will include staining by optical fluorescence markers.

Literatur

[1] D. Shapiro et al., PNAS 102, 15343 (2005); P. Cloetens et al., PNAS 103, 14626 (2006)