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3D optical microscopy for quantifying T lymphocyte activation

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Abstract

The tomographic diffractive microscope (TDM) can be implemented in either transmission configuration or reflection configuration. TDM in reflection configuration has higher Fourier spatial frequency data along the optical-axis of the microscope in comparison to the transmission configuration and also reflective samples can be imaged. We have recently exploited the specific features of such a configuration. This optical tomographic microscope coupled to sophisticated inversion schemes could be a good candidate for detecting the immunological synapse of T lymphocyte activation. Presently, no technique permits to perform a fast detection of T lymphocyte activation at an early stage which is very promising in medical diagnosis applications. In doing so we have first considered polystyrene bead (comparable to the size of T-cell) in water medium and detected the interface. This same experiment could be used for detecting the immunological synapse.

Keywords: Reflection tomography, T lymphocyte, Immunological synapse

Experimental setup : reflection diffraction tomography

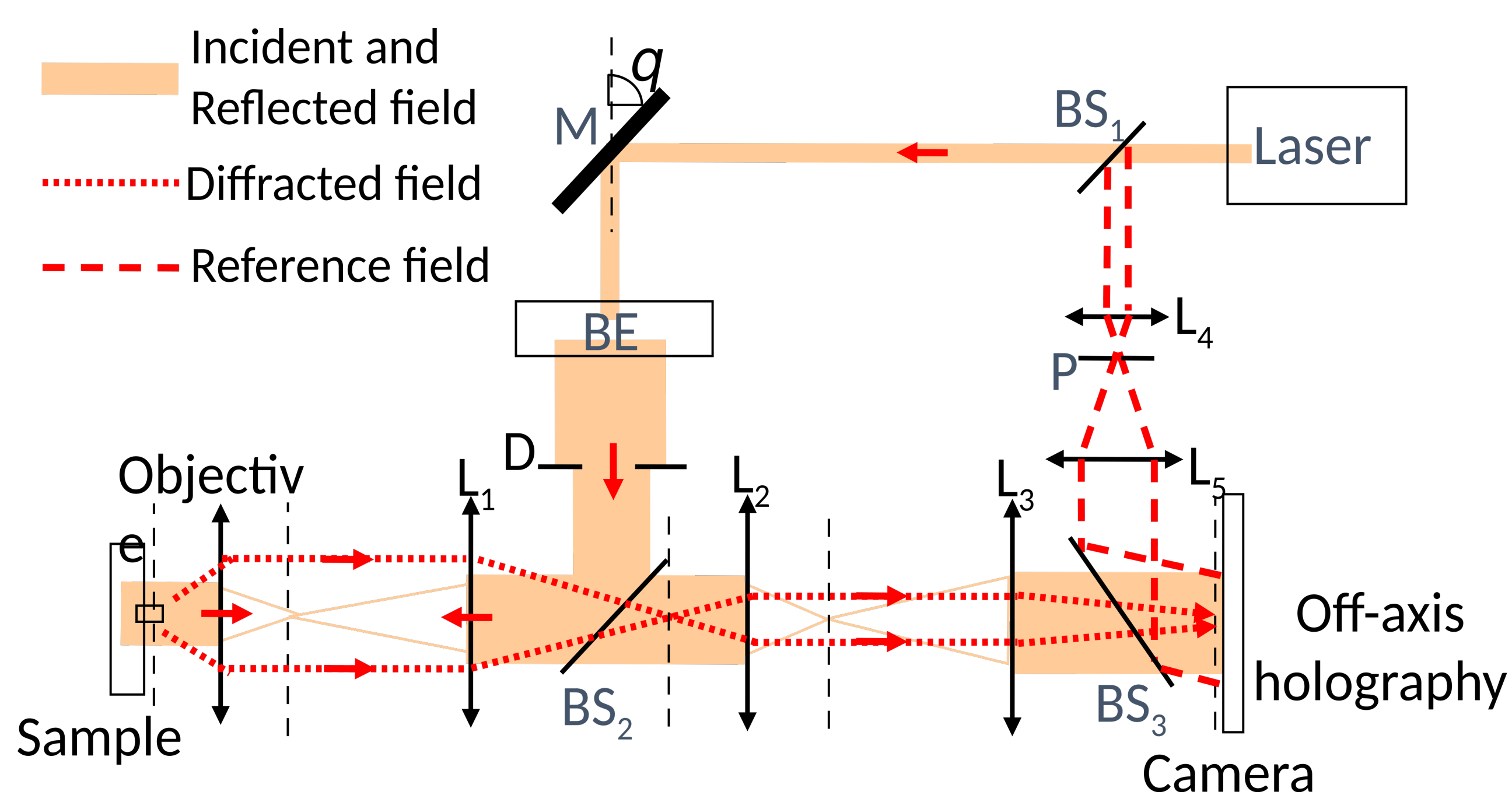


Fig. 1. Block diagram of the TDM in reflection configuration : M, rotating mirror; BE, beam expander; D, diaphragm; L1, tube lens; L2, ..., 5 lenses; BS1, ..., 3, beam splitters.

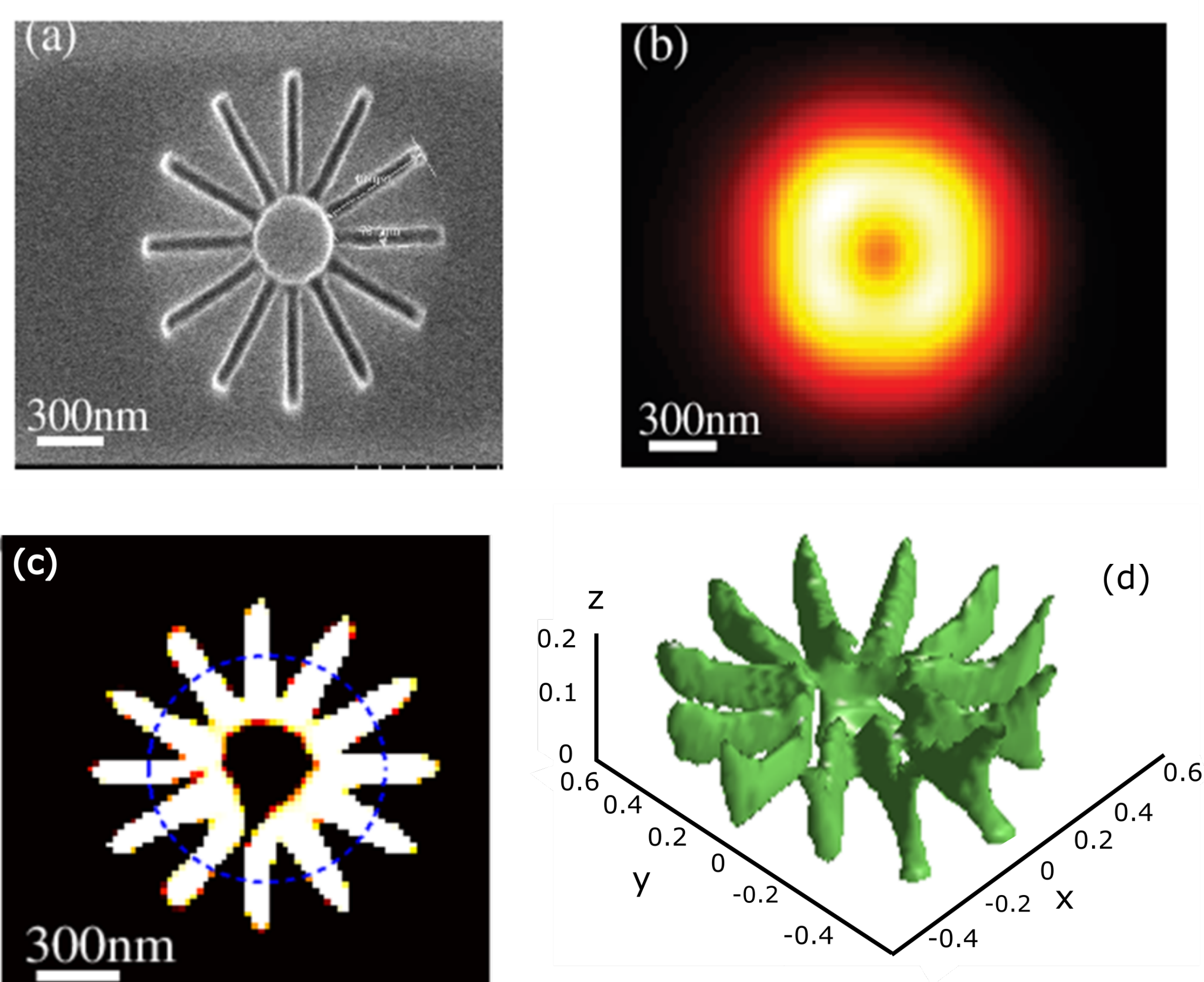
Theory of off-axis holography

The signal as reflected by the sample E_s and the reference wave E_{ref} interfere and the camera record the hologram.

$$I_{intf}(r) = |E_s(r)|^2 + |E_{ref}(r)|^2 + E_s E_{ref}^* + E_s^* E_{ref}$$

The signal E_s (both phase and intensity) is then separated using 2D Fourier transform from the hologram in k-space.

Improvement of resolution brought by diffraction tomography



Ref : T. Zhang et al., Optica, 3, 2016

Fig. 2. (a) SEM image of a resin star, (b) Standard dark field image, (c) Tomographic reconstruction (d) Axial resolution (improved)

Immunological synapse

Ref : K. Murphy et al., Immuno Biology, 9th edition

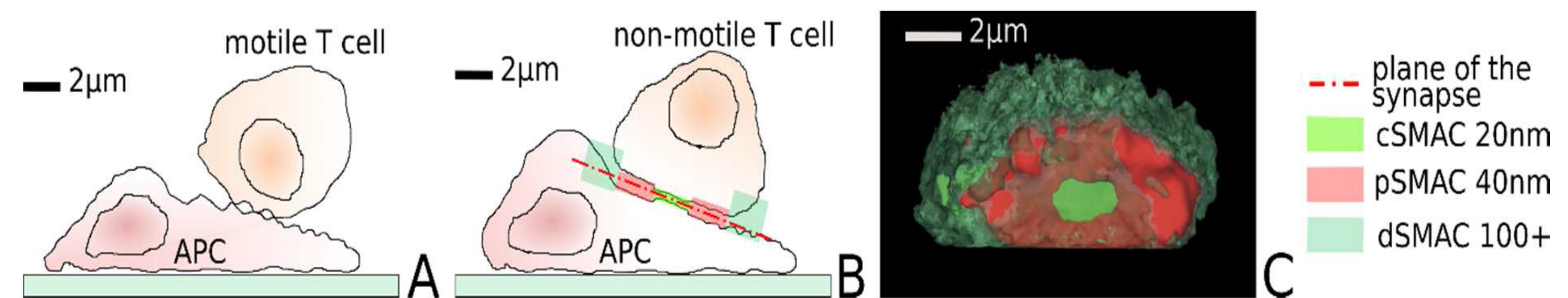


Fig. 3. (A) Motile T cell on Antigen presenting cell (APC), (B) Formation of synapse, (C) fluorescence confocal image of the synapse.

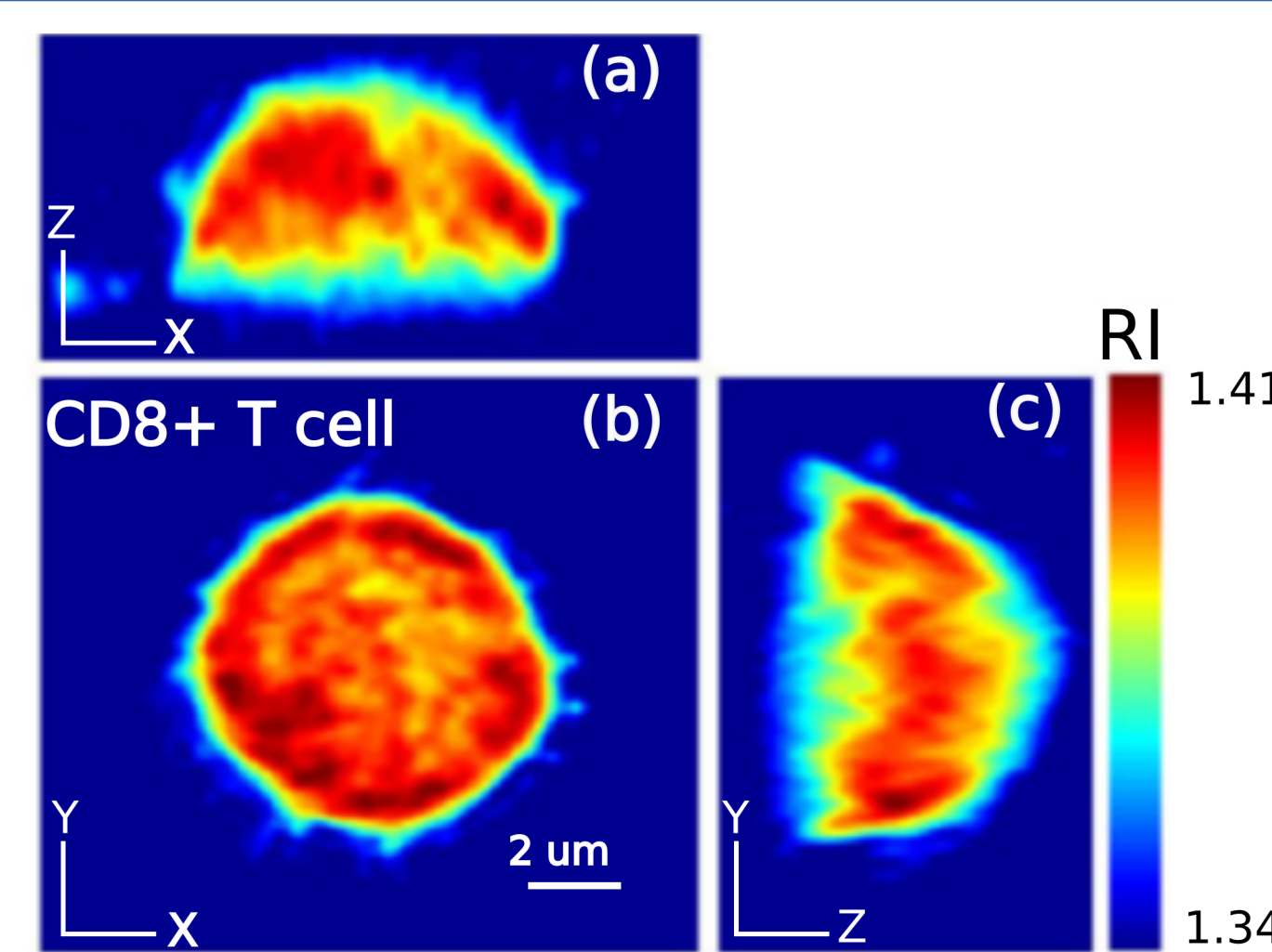


Fig. 4. 3-D Refractive Index (RI) tomograms of a T lymphocyte cell from mice peripheral blood

To reconstruct a 3-D RI tomograms, multiple 2-D holograms of a cell are measured at various angles of illuminations using an interferometric microscope in transmission.

Ref : J. Yoon et al., Sci. Rep. 7, 6654, 2017

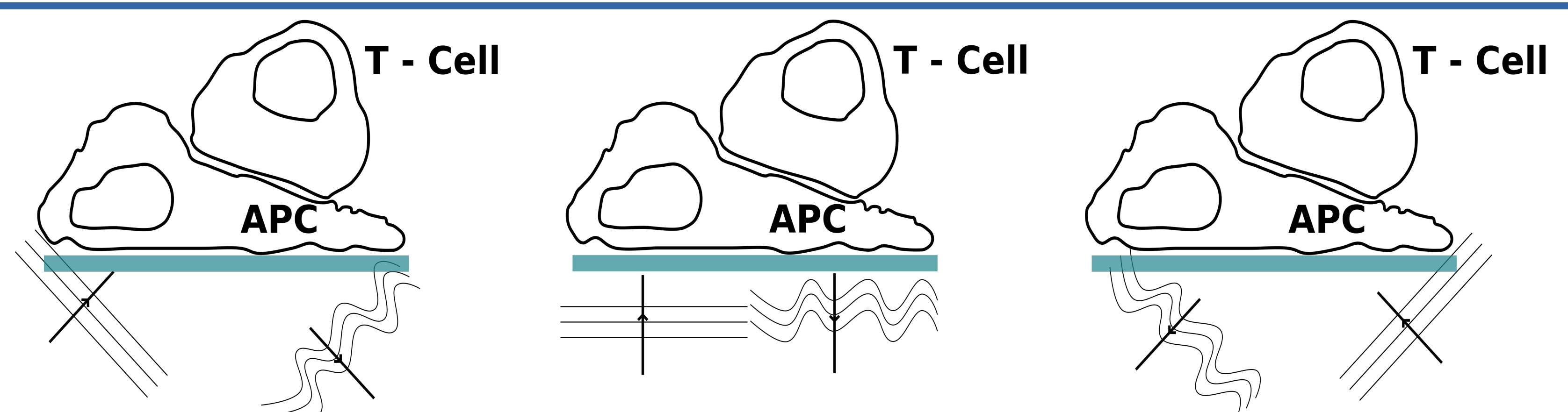


Fig. 5. Antigen presenting cell (APC) sitting on top of glass substrate: plane wave illumination at various angle

First measurements on a polystyrene bead. (diameter 6µm)

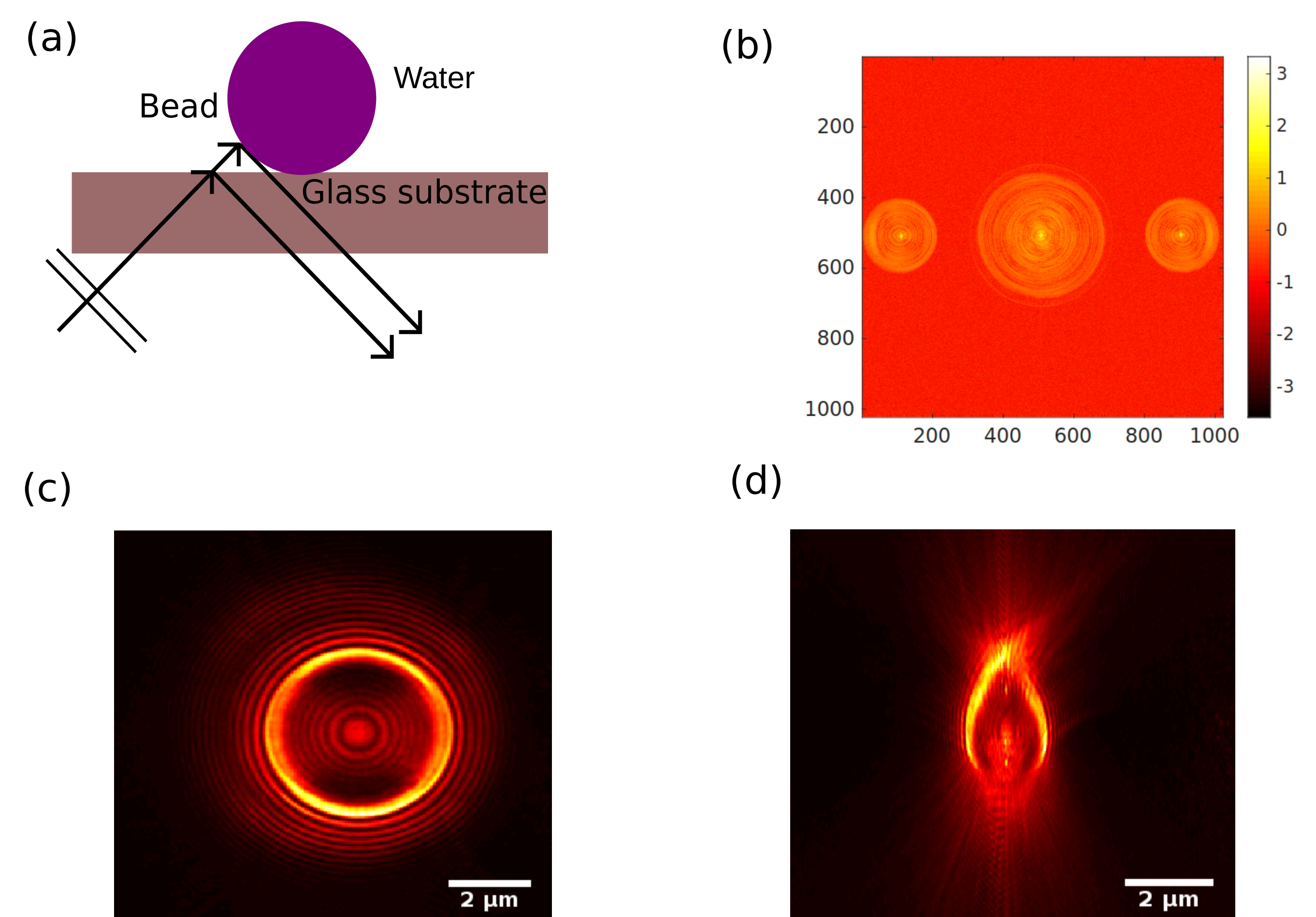


Fig. 6. (a) Polystyrene bead on glass, (b) Fourier transform of the hologram, (c) Dark field image of the bead (d) Axial cut of the bead

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