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► **To cite this version:**

Md Rasedujjaman, Guillaume Maire, Hugues Giovannini, Patrick C. Chaumet, Kamal Belkebir, et al.. 3D optical microscopy for quantifying T lymphocyte activation. Journées Des Doctorants de l'Institut Fresnel, Jun 2019, Marseille, France. hal-02181388

HAL Id: hal-02181388

<https://hal.science/hal-02181388v1>

Submitted on 12 Jul 2019

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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

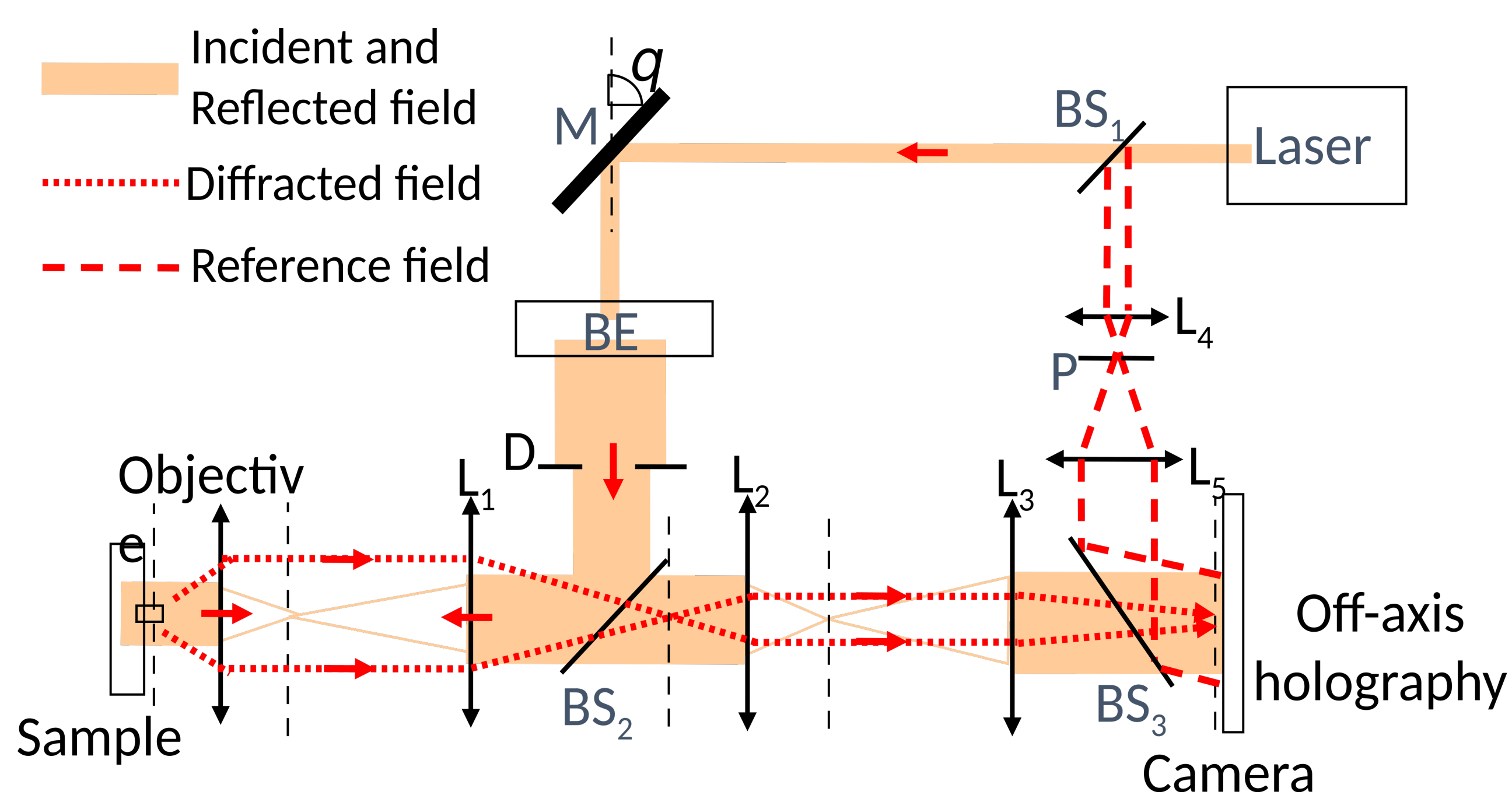


Fig. 1. Block diagram of the TDM in reflection configuration : M, rotating mirror; BE, beam expander; D, diaphragm; L1, tube lens; L2, ..., 5 lenses; BS1, ..., 3, beam splitters.

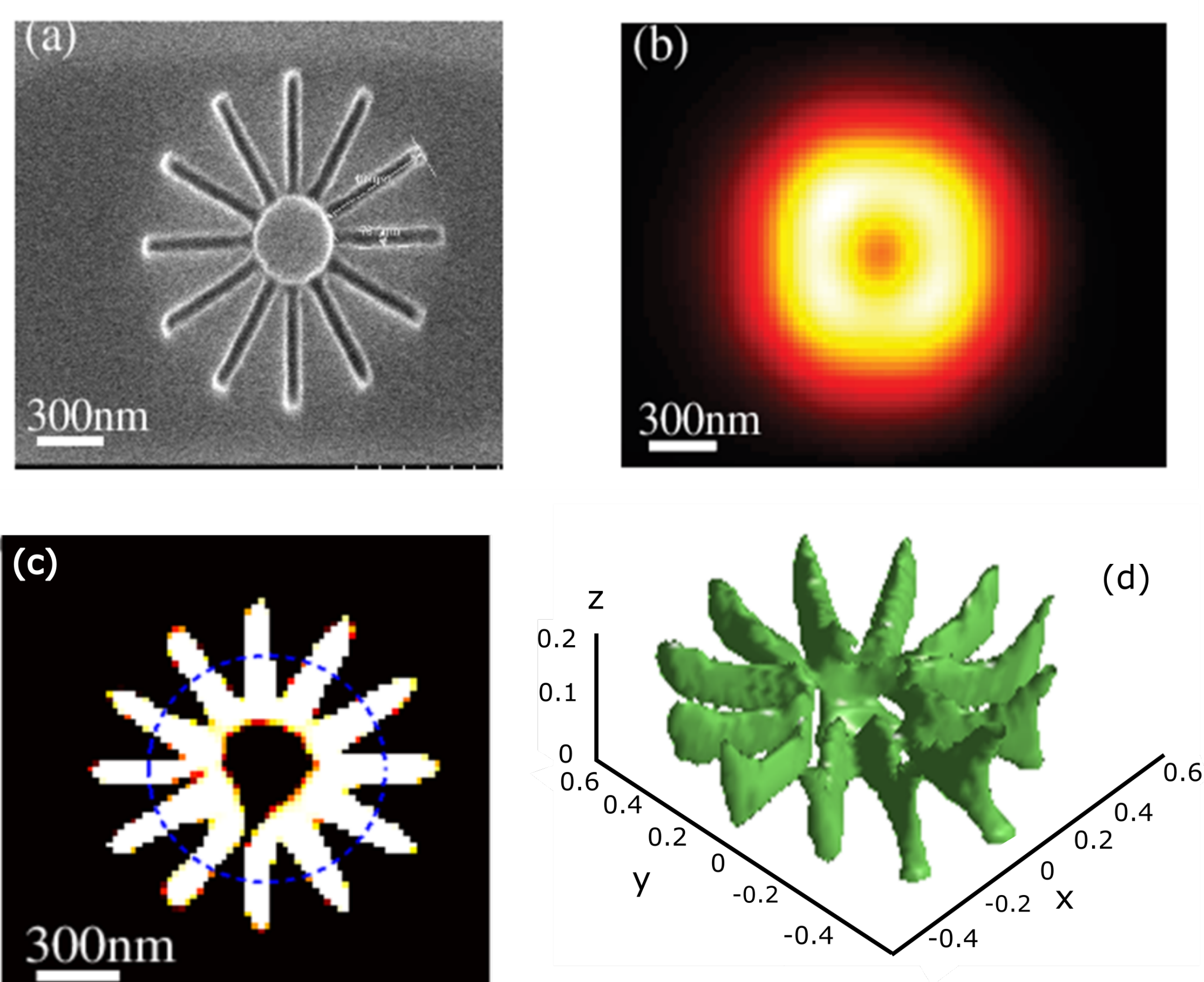
Theory of off-axis holography

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

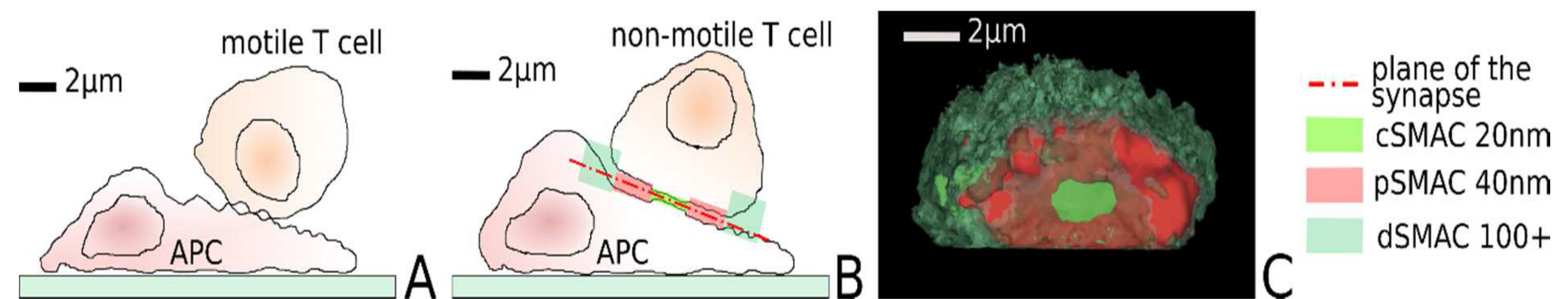


Fig. 3. (A) Motile T cell on Antigen presenting cell (APC), (B) Formation of synapse, (C) fluorescence confocal image of the synapse.

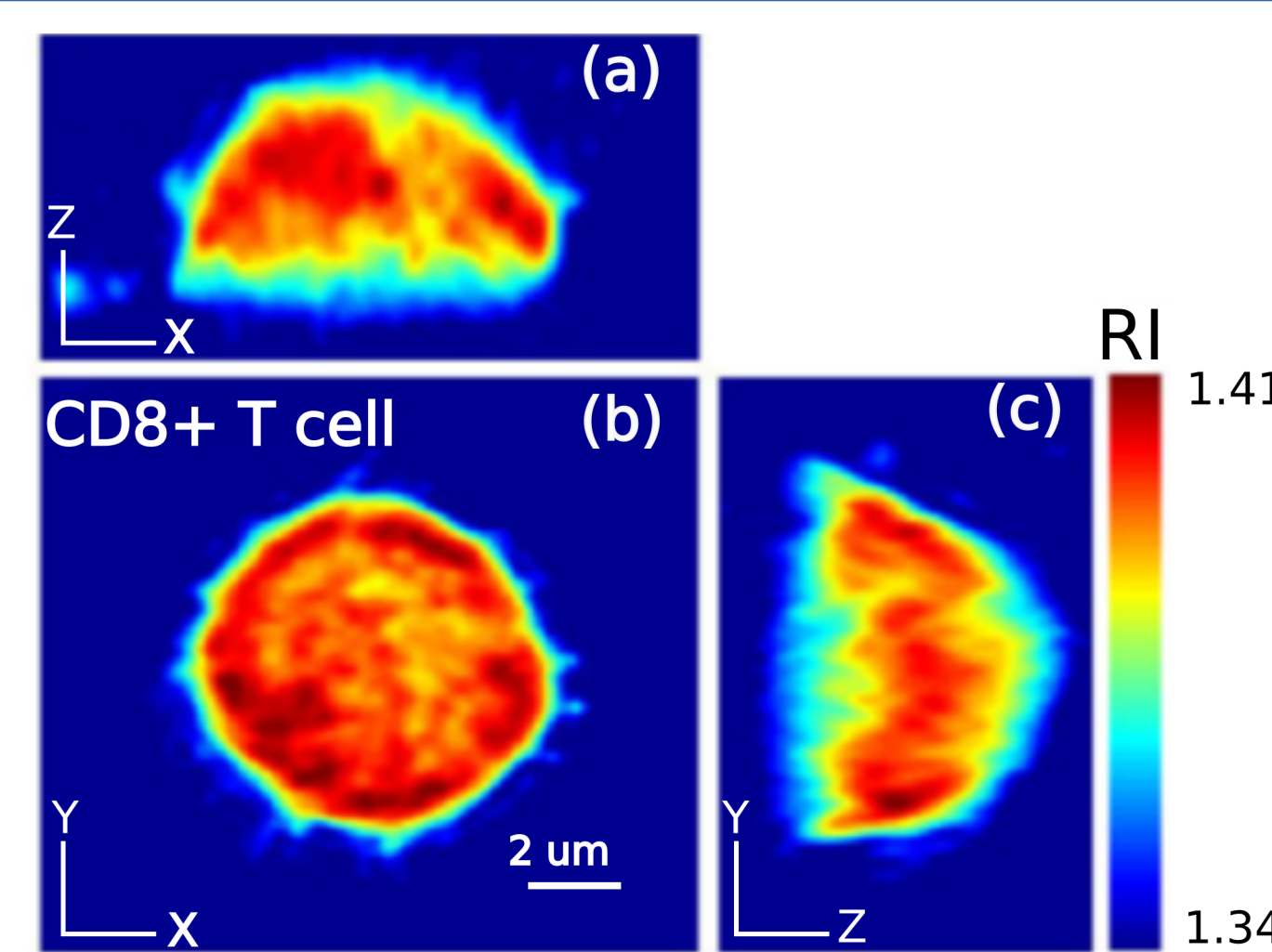


Fig. 4. 3-D Refractive Index (RI) tomograms of a T lymphocyte cell from mice peripheral blood

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

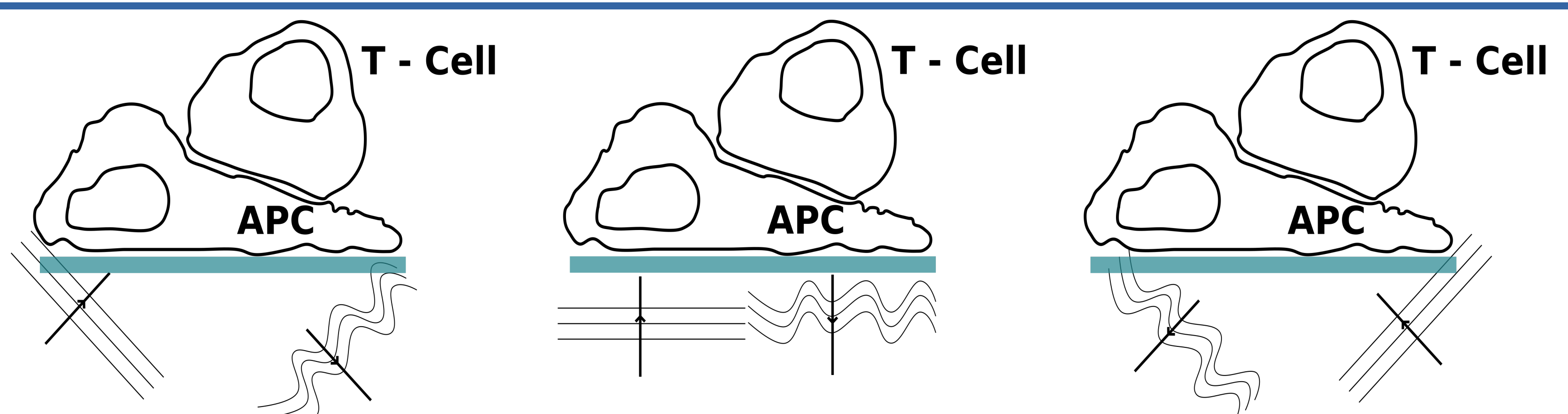


Fig. 5. Antigen presenting cell (APC) sitting on top of glass substrate: plane wave illumination at various angle

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

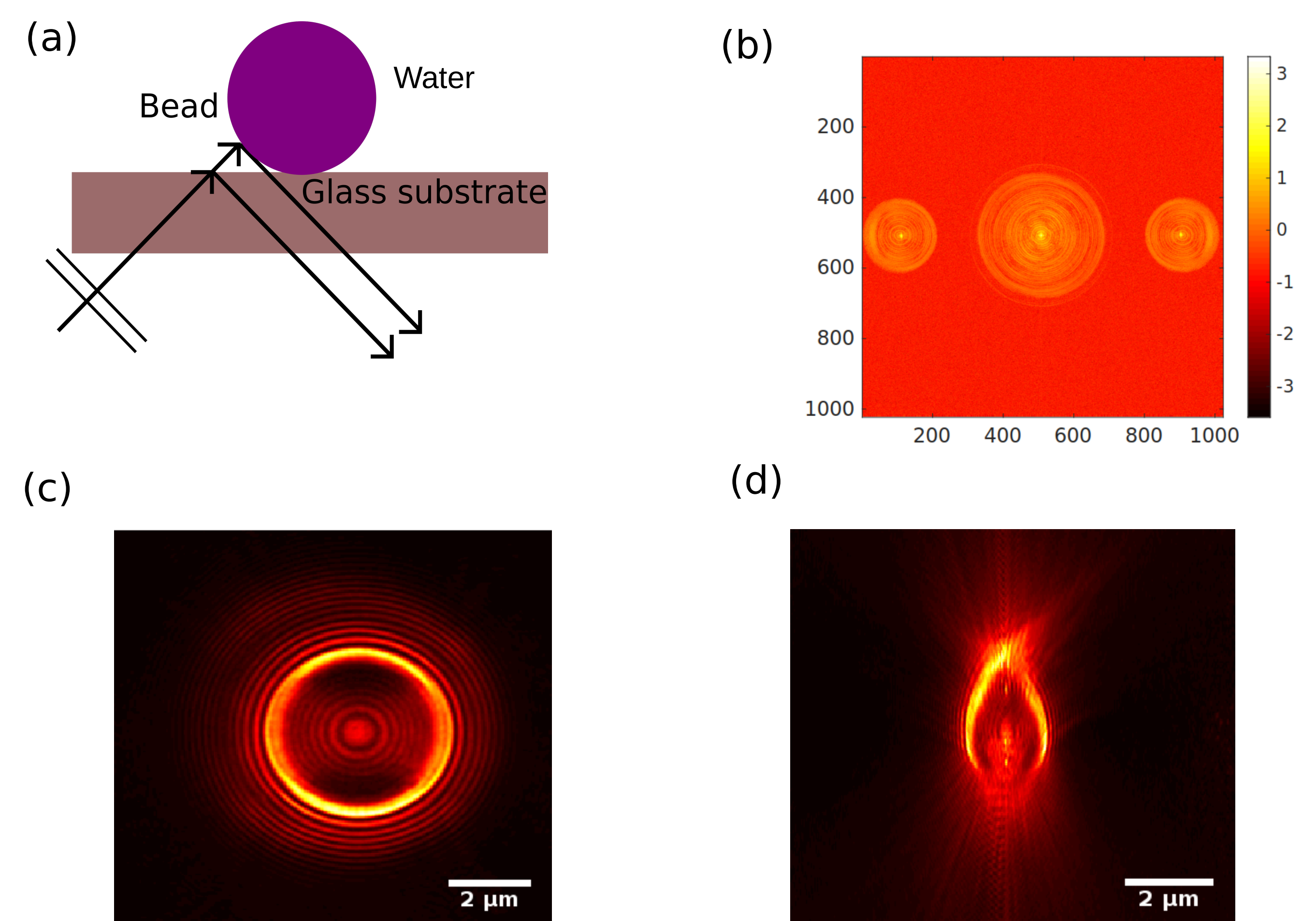


Fig. 6. (a) Polystyrene bead on glass, (b) Fourier transform of the hologram, (c) Dark field image of the bead (d) Axial cut of the bead

Acknowledgment

