



HAL
open science

3D optical microscopy for quantifying T lymphocyte activation

Md Rasedujjaman, Guillaume Maire, Hugues Giovannini, Patrick C. Chaumet, Kamal Belkebir, Anne Sentenac, Philippe Robert

► **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-02181388>

Submitted on 12 Jul 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

3D optical microscopy for quantifying T lymphocyte activation

Md RASEDUJJAMAN^{1*}, Guillaume MAIRE¹, Hugues GIOVANNINI¹, Patrick C. CHAUMET¹

Kamal BELKEBIR¹, Anne SENTENAC¹, Philippe ROBERT²

1. Aix-Marseille Université, Institut Fresnel, 13013 Marseille, France

2. Aix-Marseille Université, Adhesion & Inflammation Lab, 13005 Marseille, France

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

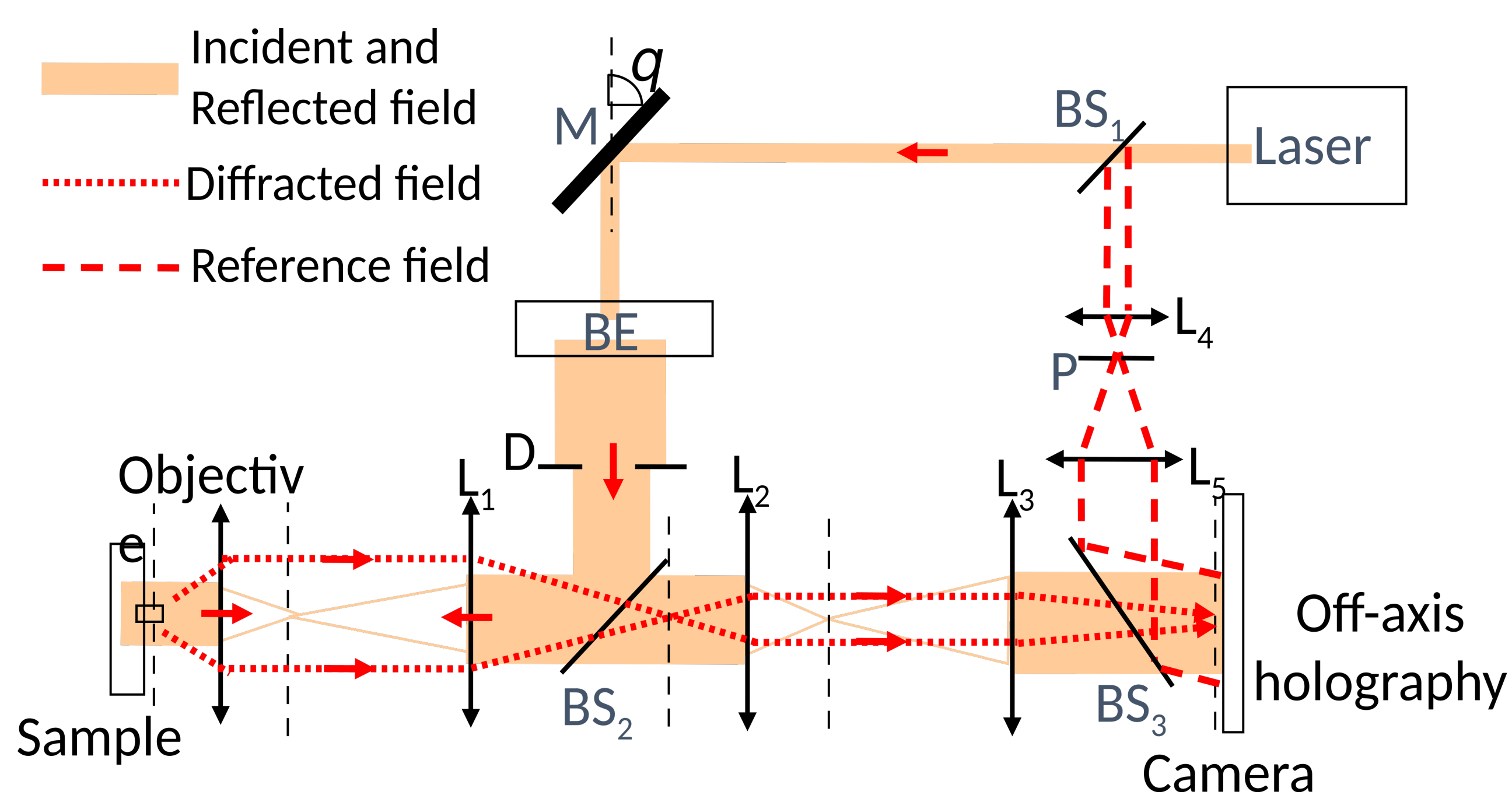


Fig. 1. Block diagram of the TDM in reflection configuration : M, rotating mirror; BE, beam expander; D, diaphragm; L1, tube lens; L2, ..., 5 lenses; BS1, ..., 3, beam splitters.

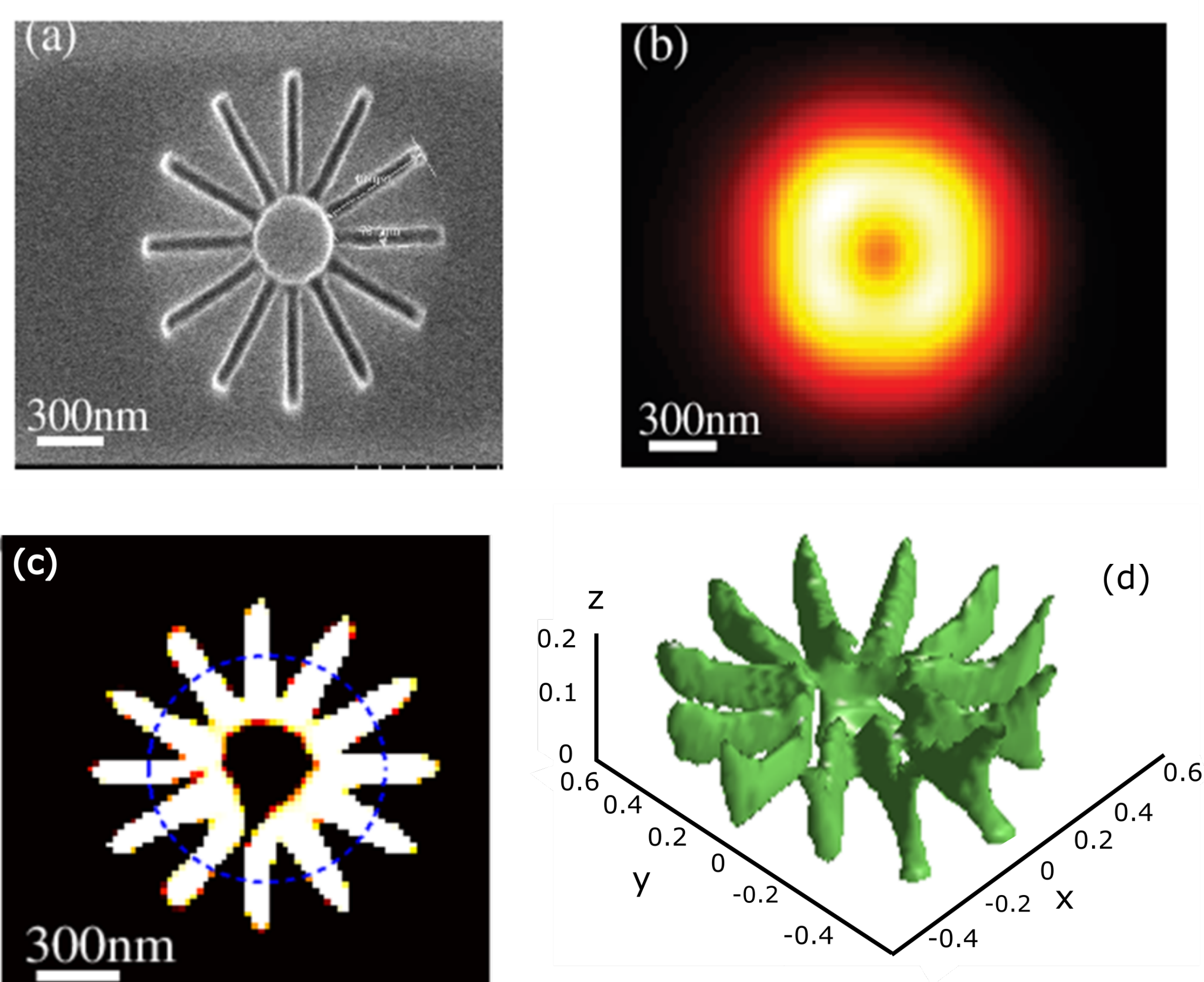
Theory of off-axis holography

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

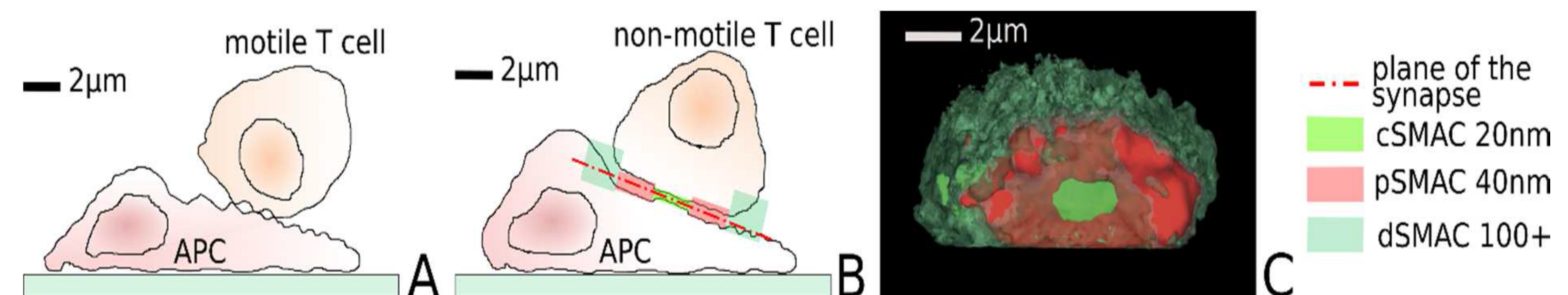
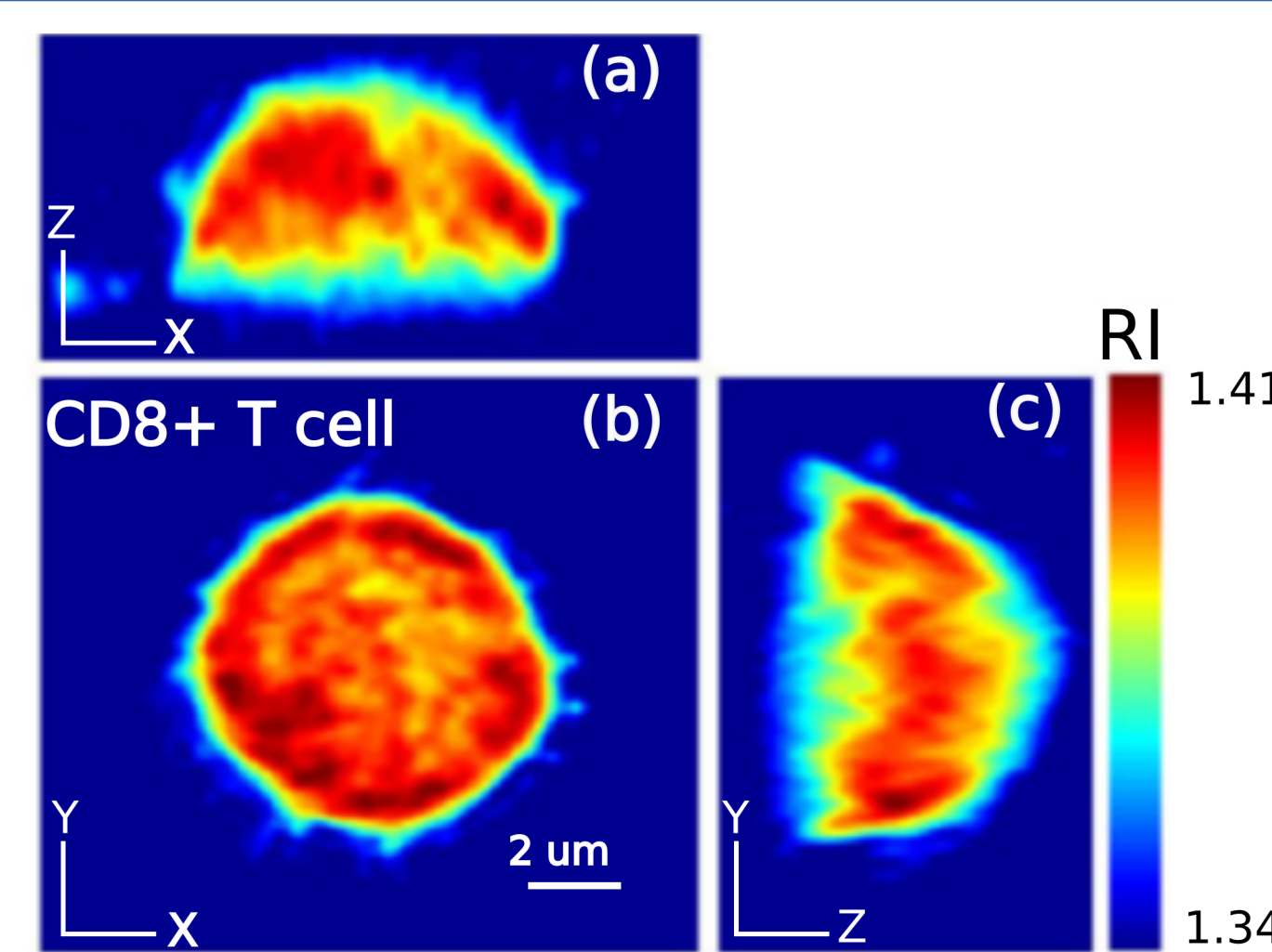


Fig. 3. (A) Motile T cell on Antigen presenting cell (APC), (B) Formation of synapse, (C) fluorescence confocal image of the synapse.



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

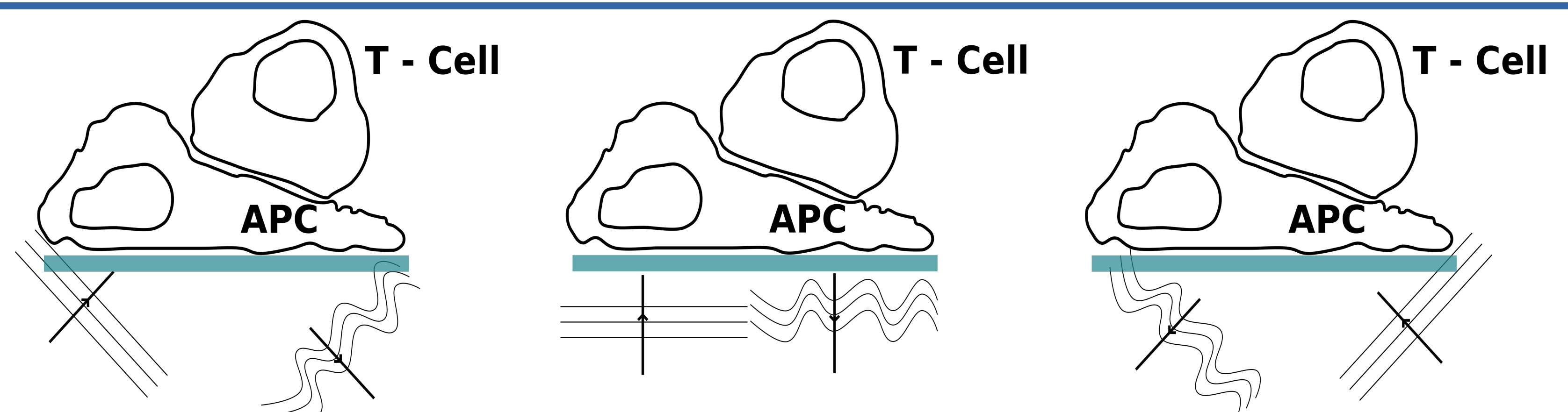


Fig. 5. Antigen presenting cell (APC) sitting on top of glass substrate: plane wave illumination at various angle

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

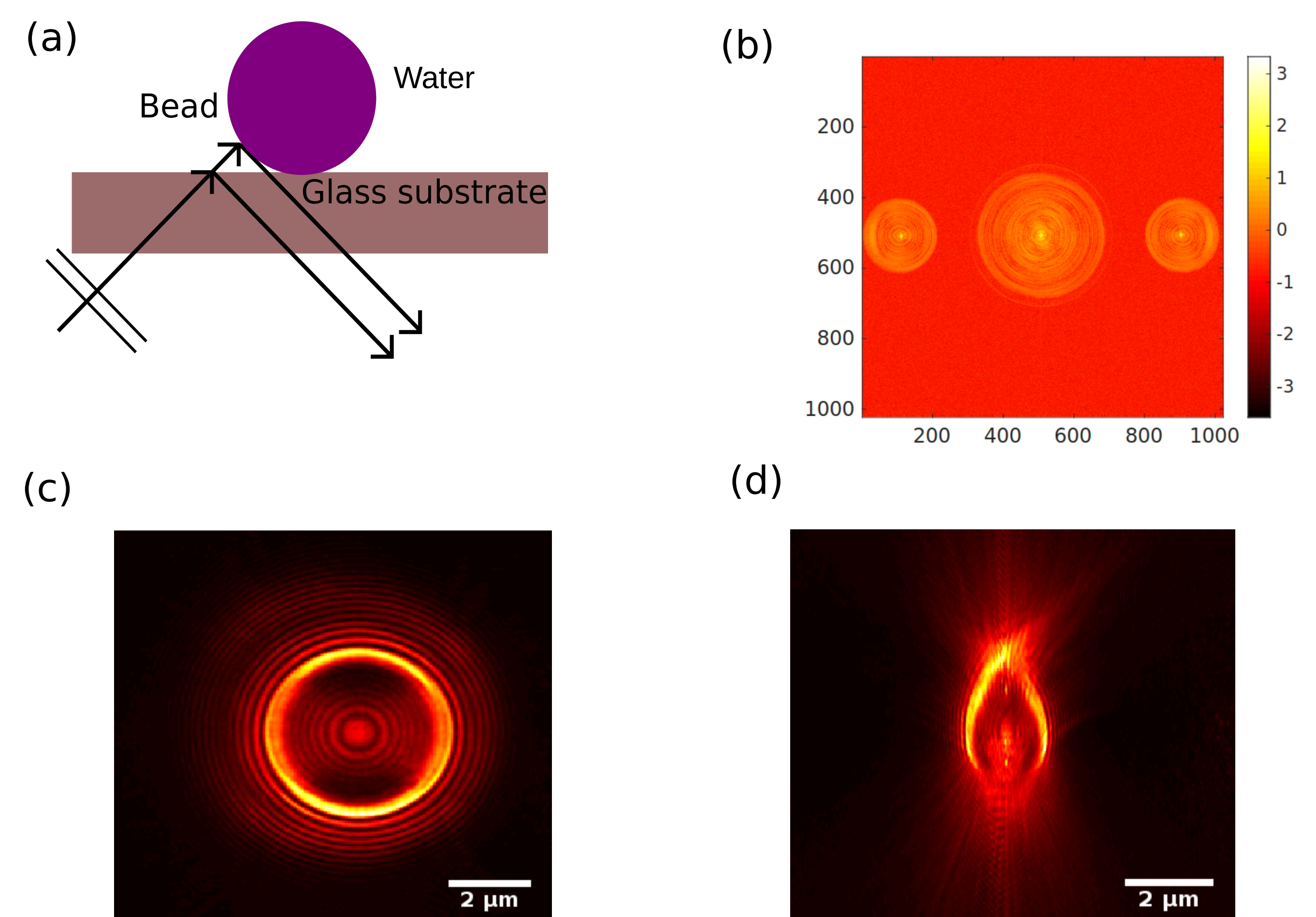


Fig. 6. (a) Polystyrene bead on glass, (b) Fourier transform of the hologram, (c) Dark field image of the bead (d) Axial cut of the bead

Acknowledgment

