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3D optical computational microscopy in reflection configuration

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Introduction

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 [1]. 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, Synapse

Theory and Design

Theory

Tomographic diffraction microscopy is based on the same principle of the off-axis digital holography. Lets consider the incident field in space domain as follows:

$$E_{inc} = E_0 e^{i\varphi(r)}$$

But we do not observe the filed rather intensity is recorded and consequently phase information is lost.

$$I_{inc}(r) = \left|E_{inc}(r)\right|^2$$

When the $\mathbf{E_{inc}(r)}$ is incident on an object, the object scatters the incident field, this is called scattered field $\mathbf{E_d}$ **(r)**. Then the total field is given by $\mathbf{E(r)} = \mathbf{E_{inc}}$ **(r)** + $\mathbf{E_d}$ (r). If the total field **E(r)** is interfered with another field called reference field $\mathbf{E_{ref}}$ whose phase is known, the field detected $\mathbf{E_{intf}}$ (interference) will be as follows, $\mathbf{E_{intf}}$ **(r)** = **E(r)** + $\mathbf{E_{ref}}$ **(r)**. Then the resulting intensity from the above equation has the desired phase term φ(r) as shown below,

$$I_{intf}(r) = \left|E(r)\right|^2 + \left|E_{ref}(r)\right|^2 + 2\left|E(r)\right|.\left|E_{ref}(r)\right|.\cos\left[\Delta\varphi(r)\right]$$

where, Δφ(r) represents the phase difference. Now we can extract both the phase and intensity information.

Experimental setup

The tomographic microscope is presented in Fig. 1. The light source is a low coherence supercontinuum laser (NKT Photonics SuperK Extreme EXW-12), that can be filtered from 450 nm to 800 nm. The laser beam is linearly polarized and then divided into a reference beam and a beam directed towards the sample called object beam. The object beam and the reference beam are interfered off-axially. Due to the low coherence any parasitic reflection from the optical components in the object beam path does not interfere with the measurement. A galvanic mirror (M, Newport FSM-300) permits to control the deflection of the sample beam, while a beam expander and diaphragm D generate a wide collimated beam with near homogeneous power density. This beam illuminates the sample after transmission through the microscope objective (can be varied to have different NA) and the associated tube lens (L). The field scattered by the object is collected by the microscope objective (Nikon Apo TIRF oil 100×, NA =1.49) and imaged on a sCMOS camera with pixel size of 6.5µm(Andor Zyla) after passing through relay lenses L2 and L3 to obtain a global magnification of about 300.

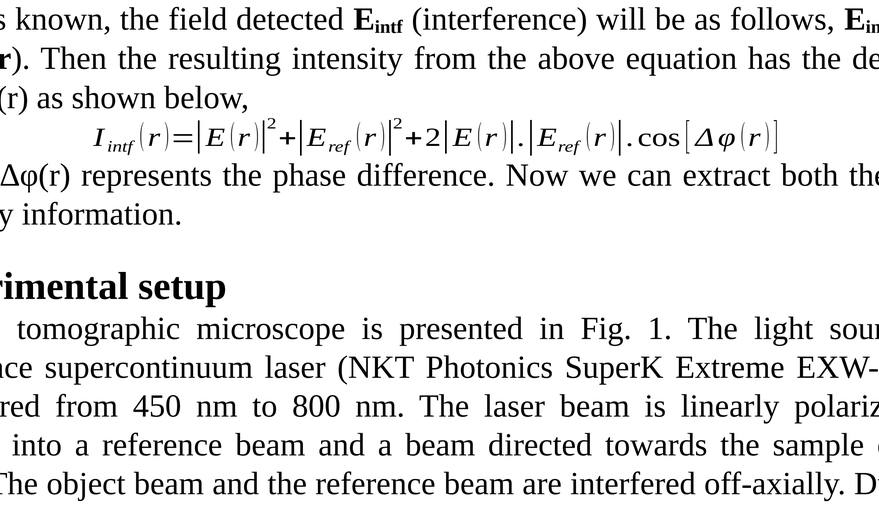

Fig. 1. Block diagram of the TDM in reflection configuration : M, rotating mirror; BE, beam expander; D, diaphragm; L1 ,tube lens; L2,...,5 lenses; BS1,...,3 , beamsplitters.

Data acquisition and Results

For data acquisition we use a custom made Linux based software (OpticsbenchUI). With this software the galvanic mirror is controlled for illuminating the sample at desired angle and the image sequences are recorded by the camera accordingly. Few of the recorded holograms are shown in Fig. 2(b). The images are recorded as HD5 format and then the necessary data are extracted by MATLAB. IFFT algorithm is then used to go to k-space from image space as shown in Fig 2(c). This allows us to filter the data. Once this process is done we go back again in image space by using FFT and combine all the images to reconstruct the interface of the sample as in Fig 2(e).

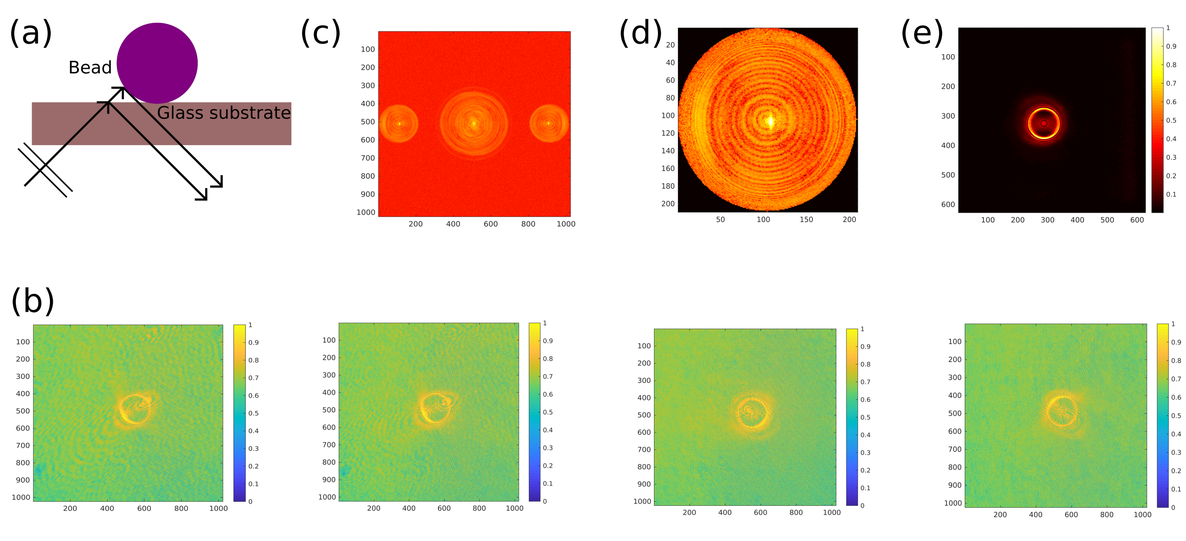

Fig. 2. (a) Bead (dia of 6µm) on substrate, (b) Hologram at different illumination angle(74^0, 61^0, 23^0, 67^0). (c) The hologram in Fourier space (d) The desired order as filtered form (c). (e) The bead interface (Note: for images (b-e) both x and y axis indicates No. of pixel)

Future work

We have successfully reconstructed a polystyrene bead with dimension comparable to that of T-cell. Of course the bead is well defined in geometry and therefor mathematical model for reconstruction is greatly simplified. For T lymphocyte as shown in Fig. 3, the shape is not regular and requires more advanced algorithm for detecting the synapses [2] with the same experimental setup and data acquisition method as used for the beads.

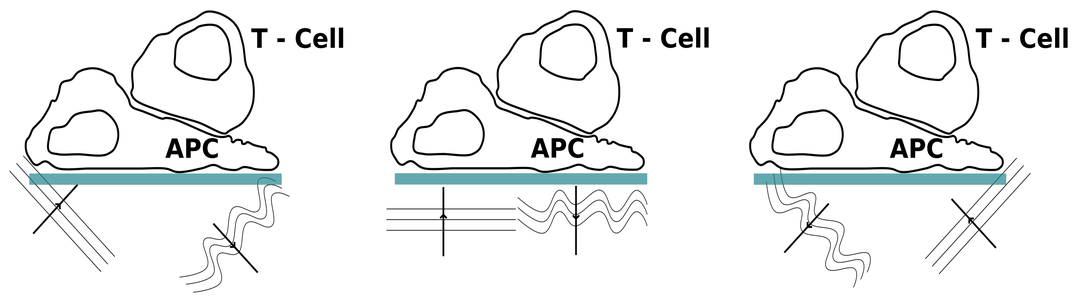

Fig. 3. Antigen presenting cell (APC) sitting on top of glass substrate: plane wave illumination at various angle

References

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Acknowledgment

I sincerely express my gratitude to the summer school on Intelligent signal processing for FrontIEr Research and Industry (INFIERI 2019), PETLAB, HUST for giving me the opportunity to participate this wonderful event. I also would like to acknowledge Marie Skłodowska-Curie Actions - European Commission, AMidex foundation and RPACA for funding this research project.