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3D optical computational microscopy in reflection configuration

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Introduction

The noninvasive diffraction 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 noninvasive microscope coupled to sophisticated inverse schemes could be a good candidate for detecting the immunological synapse of T lymphocyte activation. Previously, 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-cells) in water medium and detected the interface. This same experiment could be used for detecting immunological synapse.

Keywords: Reflection microscopy, T lymphocyte, Synapse

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).

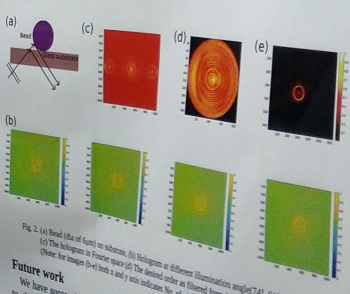


Fig. 2. (a) Schematic of the experimental setup. (b) Holograms at different illumination angles (74°, 61°, 23°, 67°). (c) The filtered order in filtered form. (d) The beam interface. (e) The reconstructed interface of the sample.

Experimental setup

The noninvasive microscope is presented in Fig. 1. The light source is a low cost laser diode (405 nm) with a beam diameter of 1 mm. The laser beam is collimated and focused by a lens (f = 100 mm) onto a sample. The sample is a polystyrene bead (100 nm) in water medium. The reflected light is collected by a lens (f = 100 mm) and focused onto a camera. The camera is a high-speed camera (1000 fps) with a resolution of 1024 x 1024 pixels. The camera is connected to a computer (Intel Core i7, 16 GB RAM) which controls the camera and the data acquisition. The data are stored in a hard drive (1 TB) and processed by MATLAB. The results are displayed on a monitor (24 inch) and printed on a printer (A4).

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 therefore a 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 synapse [2] with the same experimental setup and data acquisition method as used for the bead.

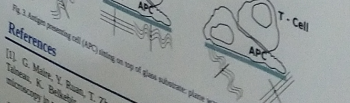


Fig. 3. Schematic of the experimental setup for T-cell imaging. The setup includes a light source, a beam splitter, a sample, and a camera.

References

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