Multi-perspective Fluorescence Talbot Microscopy

Yangyang Sun and Shuo Pang*
College of Optics and Photonics (CREOL), University of Central Florida. 4304 Scorpius St. Orlando, FL 32816, USA
Email: pang@creol.ucf.edu

Abstract: We demonstrate a long imaging depth, multi-perspective fluorescence scanning microscopy based on Talbot effect generated from a microlens array. An object with two layers that are 155μm apart was reconstructed from different perspectives.
OCIS codes: (110.1758) Computational imaging; (180.2520) Fluorescence microscopy

1. Introduction

The space-bandwidth limit of the optical systems imposes a practical bottleneck in high-throughput microscopy applications: the conventional microscope objectives with apertures greater than a few millimeters are incapable of maintaining the diffraction-limited resolution for a large field-of-view (FOV). Recently developed Talbot microscopy achieved the large FOV imaging by stitching each regional image together, and thus can scale up the imaging area [1]. However, for large FOV applications, such as digital pathology, maintaining the whole slide within the focal region is a challenging task. The depth information can be retrieved by multi-perspectives from the sample, as demonstrated in light field microscopy with the tradeoff of the lateral resolution [2]. In this paper, we demonstrate a setup using an aperture array mask and a microlens array (MLA) to achieve the large FOV multi-perspective Talbot microscopy with long imaging depth. The aperture mask changes the wavefront of each periodic structure to adjust the illumination perspective. The effective numerical aperture (NA) of each lenslet is limited to 0.08 to achieve an imaging depth of ~155μm. Since the Talbot microscope is an illumination based system, the reduction of the aperture does not affect the fluorescence collection efficiency.

2. Experiment setup and reconstruction

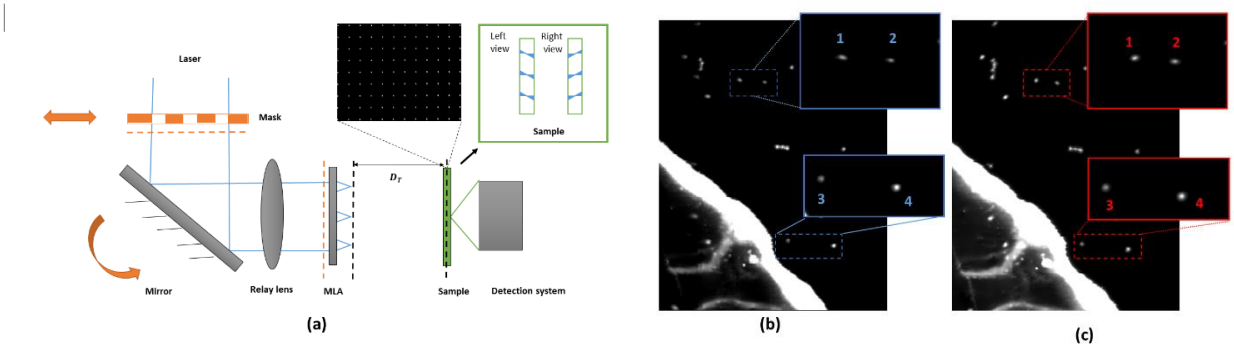

Fig. 1. (a) Schematic of the setup. The MLA is placed at the conjugate plane of the aperture mask. Insert: the Talbot spot grid on sample plane, acquired by CCD (manta G-145B, AVT) (b-c) Reconstructed image of the sample from the left perspective (b) and the right perspective (c). Fluorescent beads with 15 μm in diameter are placed on both sides of a cover slip (155μm) as a two-layer sample. Beads 1 and 2 are on the different sides of the cover slip, while Beads 3 and 4 are on the same side of the cover slip.

The schematic of setup is shown in Fig. 1 (a). A MLA, with a pitch size of 100 μm, is placed at the image plane of the aperture mask. We use the MLA to create an array of focal points. The sample is placed at one Talbot distance from the origin focal spot grid. This reformed spot grid provides us a longer working distance. Selecting the imaging perspective is performed by adjusting the image of aperture mask over the back plane of MLA. The aperture mask consists of circular aperture array with same period as that of the MLA. Each aperture has a diameter of 50μm. The aperture mask is driven by an actuator (LHA-HS, Newport) in the horizontal direction. The vertical perspective can be achieved by adding in an actuator in the vertical direction. The MLA is illuminated by a collimated laser beam with a wavelength of $\lambda = 489$ nm and an output power of 78mW. To construct an image, the mirror scans the grid on sample in two dimensions using a piezo stage (AGM 100L, Newport). Because of the long working distance (D_T = 40.19mm), the maximum scan angle of the mirror is only ±0.0726 °. While the aperture scanning over MLA with an NA of 0.17 can achieve ±9.78 °perspective view at focal distance so as one Talbot distance away. In this way we

can decouple the raster scanning process and perspectives scanning process. In our experiment, we move the mask 45 μm in horizontal direction to get two different perspectives in ±4.45 °.

We prepared the air dried micro beads (480/520, FS07F, Bangs Laboratories) on both surfaces of a cover slip as a two-layer sample. The sample are illuminated from two different perspectives by moving the aperture mask laterally. The reconstruction results are shown in Fig. 1 (b, c). The mean diameter of the micro beads is 15 μm and the thickness of cover slip is 155 μm. The bright band in the figure is quantum dot layer. The step size of the raster scan is 0.7 μm over an area of 1.2mm × 1.2mm. It is worth noting that this area can be further increased by replacing the objective lens in detection system with a high NA photography lens. This lens would not affect resolution as long as two adjacent spots in reformed grid could be distinguished.

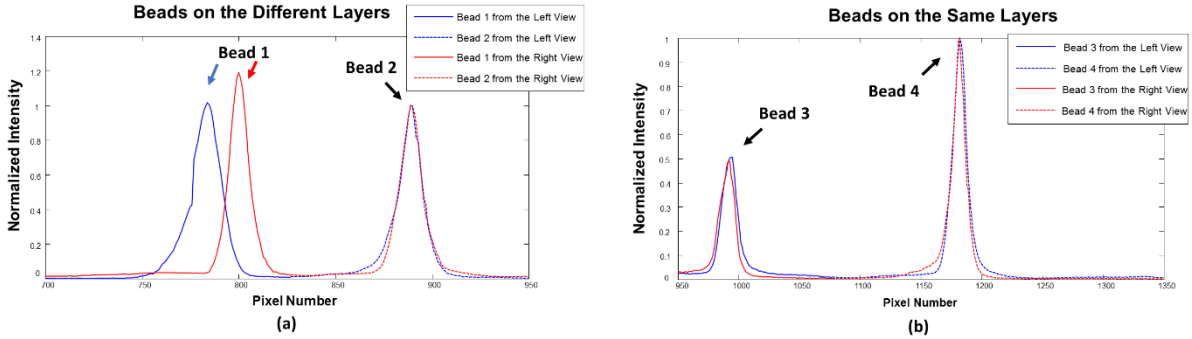

Fig. 2. (a) Normalized intensity profiles of Beads 1 and 2 on the different layers. (b) Normalized intensity profile of Beads 3 and 4 on the same layer. Blue and red line are from left and right perspectives, solid and dash line indicate profiles of different beads.

3. Results and conclusion

Under a conventional microscope (C80i, Nikon), we identified two beads on different layers (Bead 1 and 2) and two beads (Bead 3 and 4) on the same layers as reference, marked in the reconstruction results in Fig. 1. Normalized intensity profiles, indicating the horizontal positions of each bead, are shown in Fig. 2.As shown in Fig. 2, the horizontal distance of beads 3 and 4, those in the same layer, are unchanged in different perspectives. While the horizontal distance of beads 1 and 2, those in the different layers, varies in different perspectives. From the different perspectives, horizontal distance between the two beads changes 10.67 μm (16 pixels in reconstruction). The resolution of reconstruction is determined by the NA of lenslet of MLA, which can be calculated to be 1.44 μm. The depth of focus is 32.8 μm, take the aperture mask in account, the depth of focus is 131.2 μm. Within this depth of focus, the sample can be scanned like in tomography. In our proof-of-concept setup, we reconstruct perspective in ±4.45 °in one dimension, but it can be easily extended to larger angle in 2-dimension to perform a 3-dimension reconstruction. The angular resolution is determined by the aperture mask. The smaller apertures in the mask increases the angular resolution and reduces the lateral resolution, which is similar to the case in the light field camera [2]. The setup we proposed places the aperture mask and the MLA before the sample, so that the collection efficiency is only determined by the detection optics.

4. References

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