High Throughput Microscopy With a Microlens Array

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Abstract: We demonstrate large field-of-view (30 mm²) scanning fluorescence microscopy with submicron resolution using a refractive microlens array. The large area of the array decreases image acquisition time over large fields-of-view by avoiding mechanical scanning.


1. Introduction

High throughput fluorescence microscopes are widely used in biological research to image large numbers of cells cultured in microwell plates.[1] The amount of optical information recorded throughout such experiments is very large – with image acquisition time presenting a severe bottleneck.[2] Because microscope objectives have limited fields-of-view (FOVs), the sample must be scanned large distances to build up a complete image, severely limiting the speed of such systems.

We present an approach to speed up fluorescence microscopy over a large surface area by using an array of microlenses to perform parallelized point scanning microscopy. Our approach allows us to read out the image sensor continuously over a large sample area, avoiding dead time resulting from long range motorized scanning and repetitive refocusing. We have reached pixel throughputs on par with current high throughput imaging systems, using a camera sensor region with only 352 x 352 pixels. Our experimental geometry also allows for the capture of distinct perspective views of a 3D scene, enabling a 3D appreciation of the sample without optical sectioning.

2. Experimental Setup

We use a microlens array to perform scanning microscopy without an objective lens (Fig 1a). A collimated laser beam (λ = 532 nm, P = 38 mW) passes through a dichroic beamsplitter and illuminates the microlenses, producing an array of focused spots on the fluorescent sample. The microlens array (Fig. 1b) is produced by reflow molding and covers 5.5 mm x 5.5 mm, consisting of a square grid of 10,000 elements (55 μm pitch). Each microlens has focal length 40 μm, diameter 36 μm and numerical aperture 0.41. The fluorescence emitted by the sample is collimated by the microlenses, passes through the beamsplitter and a long-pass filter (passes λ > 575 nm), and collected by a relay lens onto an image sensor. Confocal detection can be achieved for all microlenses simultaneously with an iris.
placed at the sample’s conjugate plane.[3] The relay lens is placed such that the image sensor records an image of microlens array (Fig. 1c). Each bright spot (Fig. 1c) corresponds to fluorescence collected by a particular microlens. An image of the sample is obtained by raster scanning the sample, while continually reading out the image sensor. For high throughput imaging (Figure 2a), we record a 352 x 352 pixel region of interest on a Phantom V7 camera, running at 404 frames per second (fps).

3. Fluorescence Imaging

The fluorescence micrographs in Figure 1d and 2a are constructed by stitching together the FOVs of individual microlens using a combination of custom written MATLAB code and Fiji image processing software.[4] Because our piezo stage travel is smaller than the microlens pitch, a 4x4 array of images is acquired for each microlens, with the stage repositioned for each image. This is a limitation of our piezo stage, easily overcome with a longer travel stage. The raw pixel throughput is 3.8 Mpx/sec. Using a 2 Mpx sensor, we expect that the throughput could be increased over 30-fold. Typical high throughput systems record 2 to 5 Mpx/s. The resolution of our imaging system is determined by the size of the focal spot created by the microlens and by the iris width. For images of microspheres, we have found that an iris twice the size of the illumination spot in the microlens imaging plane provides a good balance between signal to noise and reduced detection volume, giving a resolution of ~650 nm.

![Figure 2: a) Portion of the 30 mm² FOV captured with 9,500 microlenses out of a 10,000 element array. Sample consists of 2 µm fluorescent beads, scale bar is 2mm. Inset: Zoom-in of boxed region, scale bar is 25 µm. b) and c) Sample can be viewed from different perspectives by altering the angular distribution of collected rays. Green spot is the illumination point, red rays are emitted fluorescence. Red box on the CCD image (inset c)) corresponds to rays hitting the red boxed region of the microlens in c). d) Anaglyph of fluorescent beads constructed using rays impinging on two opposing sides on the microlens.

The raw data captured at the image sensor contains angular information about the emitted fluorescence. Rays emanating from the sample impinge on the microlens at a position determined by their angle with respect to the optical axis. This permits a stereoscopic image of the sample to be generated by combining two perspectives (Figure 2b), a technique known as lightfield microscopy [5]. Each pixel of the image of the microlens aperture on the image sensor (e.g. see red square of Figure 2c) represents a particular fluorescent emission direction. One can therefore produce an image of a sample, viewed from a chosen perspective, by building it from the appropriate pixels of the microlens images. In Figure 2d, we present a stereoscopic image of 2 µm beads, the result of overlaying two false-color overlaid images, each produced from different perspectives.

4. References


