Large-field-of-view, multi-perspective Talbot microscopy

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A novel low-cost scanning microscope enables robust, scalable imaging for microfluidic systems and photonic integrated circuits.

Common microscope objectives have fields of view (FOVs) of less than a few millimeters because of limits imposed by optical aberrations. To scale up the FOVs, additional lens elements and heroic design efforts are required to compensate for the aberrations, leading to reduced transmissions and higher system costs. Such FOV limitations have thus become a major bottleneck in microscopy for large-scale imaging applications (e.g., phenotype screening and semiconductor wafer inspection). In state-of-the-art commercialized systems, for instance, the samples are transported under a conventional microscope to increase the effective FOV, but this involves extended imaging times.

For our previous development of a compact microscopic imaging system,¹ we did not follow a single-aperture optical design approach. Instead, we used the self-imaging effect to project a grid of excitation light spots onto a sample. This self-imaging effect—also known as the Talbot effect—was first explained by Lord Rayleigh using diffraction theory. In conventional high-throughput scanning microscope setups, the sample is directly scanned by the focal spots of a microlens array. Our Talbot microscope, which uses Talbot images of the focal spots, however, has a longer working distance and a higher phase sensitivity. A slight gradient of the global incident wavefront on the microlens array can therefore shift the Talbot focal spots by a significant distance, without introducing much off-axis aberration. In addition, the self-imaging has a self-healing effect, which generates an improved uniformity among the focal spots. By scanning the grid of focal spots across the sample in our Talbot microscope setup, we can collect a sequence of local images and thus reconstruct a high-resolution image with a large FOV.¹ In contrast to conventional microscopes, the resolution and the FOV of our system are not coupled to each other. Using a prototype of this system, we have already demonstrated a resolution of 1.1 μm and an FOV that is 100 times greater than that of a 20× magnification, 0.4 numerical aperture (NA) conventional microscope objective.²

Major challenges in the development of large FOV microscopes are the sample alignment and calibrations (i.e., a small tilt of the sample can cause a substantial defocus in local images). Including a focus-adjustment mechanism in the z-direction, however, would compromise the compactness and the cost advantage of our Talbot microscope design. Alternatively, if

Figure 1. Large-field-of-view, multi-perspective microscope that is based on the Talbot effect. A microlens array (white) generates a grid of Talbot focal spots for parallel scanning. Local wavefront (red) engineering enables multi-perspective imaging.

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images from multiple perspectives of the sample are acquired, the defocus can be numerically compensated with post-processing (similar to plenoptics refocusing methods). In our latest development of the Talbot microscope, we change the wavefront locally within each period rather than changing the global wavefront gradient. This approach provides another degree of freedom in engineering the point spread function of each of the focal spots.

The schematics of our most recent system are illustrated in Figure 1. In our experimental setup we use a microlens array (SUSS MicroOptics) with a pitch of 100μm (each microlens has an NA of 0.17). We adjust the local wavefront by shifting a binary transmission aperture mask at the back focal plane of the microlens array. The Talbot image of the aperture mask is then optically relayed to the microlens array. Although smaller aperture diameters increase the focal depth and provide higher angular resolution, there is a trade-off with lateral resolution. By taking advantage of the phase sensitivity of the Talbot effect, our microscopy system decouples the focal spot array scanning from the point spread function engineering of individual focal spots.

We have also imaged a two-layer patterned photoresist (of micro cylindrical pillars) with our Talbot microscope. The two layers of SU-8 (the photoresist) are patterned on both sides of a microscope cover slip. The height of the pillars is 15μm and the thickness of the cover slip is 155μm. In addition, each cylinder has a diameter of 20μm and the period of the pattern is 40μm. Our resultant image is shown in Figure 2 (top left). We also shifted the perspective, from −5 to +5°, with a step size of 2° and the zoomed-in multi-perspective images are shown on the right of Figure 2. We can only retrieve depth information by scanning in the z-direction with a conventional microscope. Images of the sample, obtained with a 10× magnification microscope, are shown in the bottom left of Figure 2. Furthermore, we have demonstrated our imaging technique for a microfluidic fluorescent sample.

In summary, we have developed a large-FOV imaging system that is more robust than conventional microscopes. We attain this improvement through the use of a long focal depth and multi-perspective imaging. We achieve multi-perspective imaging by adjusting the local wavefront of the Talbot illumination. In our future work, we will make further developments by engineering the local point spread function. The method we have demonstrated is suitable for compact imaging systems that can be used in multi-layer integrated photonic and microfluidic systems.

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References