

Chapter 2

Lensfree On-Chip Fluorescence Microscopy for High-Throughput Imaging of Bio-Chips

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Abstract On-chip fluorescence microscopy is an emerging platform that enables high-throughput screening of bio-chips over a wide field-of-view without the use of any lenses, thin-film filters or mechanical scanners. In this review, we summarize the recent advances in lensfree fluorescence microscopy and also discuss some of its unique capabilities toward high-throughput screening applications, including rare-cell imaging, on-chip cytometry as well as micro-array research.

Introduction

Optical Microscopy has become an indispensable tool for many scientific disciplines especially in biomedical sciences. Although rapid advancements in modern microscopy techniques allow us to visualize microscale structures and processes in

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unprecedented details, they are still relatively bulky and low-throughput, necessitating a tedious mechanical scanning to image e.g., large-area microfluidic devices and biochips [1]. To provide an alternative imaging toolset for this high-throughput screening challenge of bio-chips, we have recently introduced an on-chip fluorescence imaging platform that can rapidly monitor fluorescently labeled cells or small animal models over an ultra-wide field-of-view (FOV) of e.g., $>9\text{ cm}^2$ without the use of any lenses, thin-film filters or mechanical scanners [2–6]. This emerging lensfree fluorescence microscopy platform, achieving $<4\text{ }\mu\text{m}$ spatial resolution, provides at least an order of magnitude larger FOV compared to a conventional $10\times$ objective lens and lends itself to a compact architecture that can easily be integrated with microfluidic chips for massively parallel screening of fluorescently labeled cells or small animals. This high-throughput lensfree fluorescence microscopy platform, combined with the state-of-the-art bio-chips, could pave the way toward rapid on-chip diagnostic systems for biomedical applications, including rare-cell research, high-throughput cytometry as well as micro-array analysis.

Materials and Methods

Our on-chip lensfree fluorescent imaging modality utilizes an excitation interface (e.g., a prism, a hemisphere or a planar waveguide) to pump the objects of interest located within a bio-chip, where the excitation light is mostly rejected through total internal reflection (TIR) occurring at the bottom facet of the sample holder (Fig. 2.1[a1, b1, c1]). In addition to TIR rejection, an inexpensive absorption filter is also used to remove the weakly scattered excitation light that does not obey the TIR process. Upon removal of the excitation, only the fluorescent emission from the objects is collected using e.g., free-space, fiber-optic-faceplate (FOF) or fiber-optic-taper (FOT) based optics, and is then delivered to a large-format sensor-array (e.g., CMOS or CCD) that has an active area of e.g., $>9\text{ cm}^2$, which is also equivalent to the sample FOV. Finally, by using an image reconstruction method (e.g., deconvolution or compressive decoding), the detected lensfree fluorescent images are rapidly processed to yield higher-resolution microscopic images of the specimen across a wide FOV. Typical reconstructed images of this lensfree fluorescence microscopy platform are demonstrated in Fig. 2.1[a3, b3, c3], where $4\text{--}10\text{ }\mu\text{m}$ diameter micro-beads are imaged using the lensfree on-chip imager.

Components of the Lensfree Fluorescence Imaging Platform

In this sub-section, we will discuss some of the key components of the lensfree on-chip fluorescence microscopy platform.

Excitation/Illumination Design: Fluorescently labeled specimen located within micro-fluidic devices can be probed with various illumination configurations: the

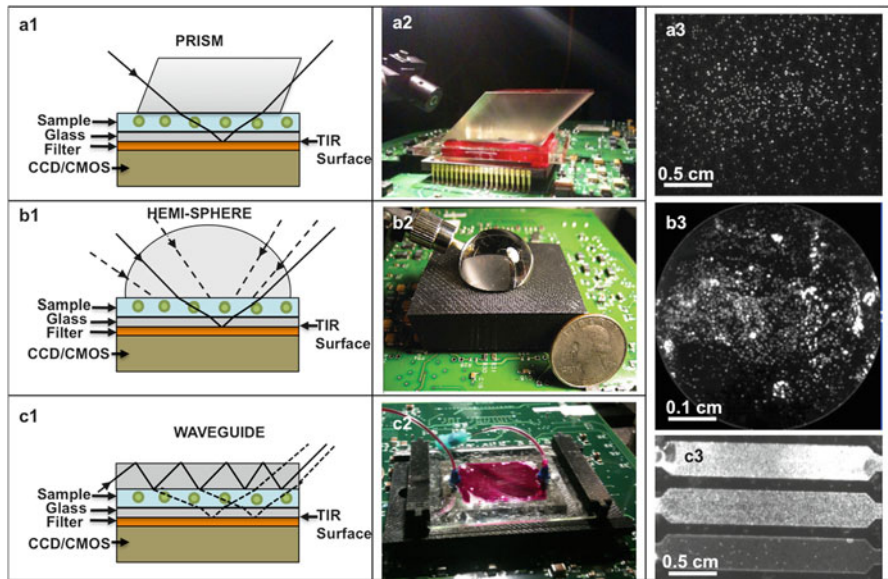


Fig. 2.1 The schematics (a1, b1, c1), corresponding experimental set-ups (a2, b2, c2) and typical wide-field lensfree fluorescence images (a3, b3, c3) of various excitation methods are shown

fluorescence excitation can be achieved through e.g., a prism (e.g., rhomboid, dove prisms – Fig. 2.1[a1, a2]), a hemi-sphere (Fig. 2.1[b1, b2]) or a waveguide (Fig. 2.1[c1, c2]), where incoherent sources such as simple light emitting diodes (LEDs) can be used to provide uniform illumination over a wide FOV.

Light Collection and Sampling: In this on-chip imaging platform, once the specimen is excited through one of the illumination methods presented above, the fluorescence emission is collected and is then delivered to an optoelectronic sensor-array. As for the collection of the fluorescence signal, three different configurations can be utilized, incorporating free-space, an FOF or alternatively an FOT (see Fig. 2.2).

Although free-space collection enables monitoring of bio-chips over a wide FOV, since the fluorescent emission is not directional and rapidly diverges, the detected raw lensfree images become rather broad at the sensor plane. Therefore, to better control the spatial spreading of fluorescent signal in our platform, we can employ a planar optical component, i.e. an FOF, which is located between the object and the sensor planes [3, 4]. A typical FOF (Fig. 2.2[b1, b2]) is composed of a 2D array of fiber-optic cables that carry two-dimensional optical intensity information from one plane to another. Its main function in lensfree imaging is to engineer the point-spread function (PSF) (Fig. 2.2[b3]) of the on-chip imager, improving the signal-to-noise ratio (SNR) and the spatial resolution of the microscopy platform. As an alternative to a regular FOF, an FOT (Fig. 2.2[c1, c2]) can also be used, which has a larger density of fiber-optic cables on its top facet compared to the bottom one [5]. FOT not only provides a better PSF (Fig. 2.2[c3]), but also achieves

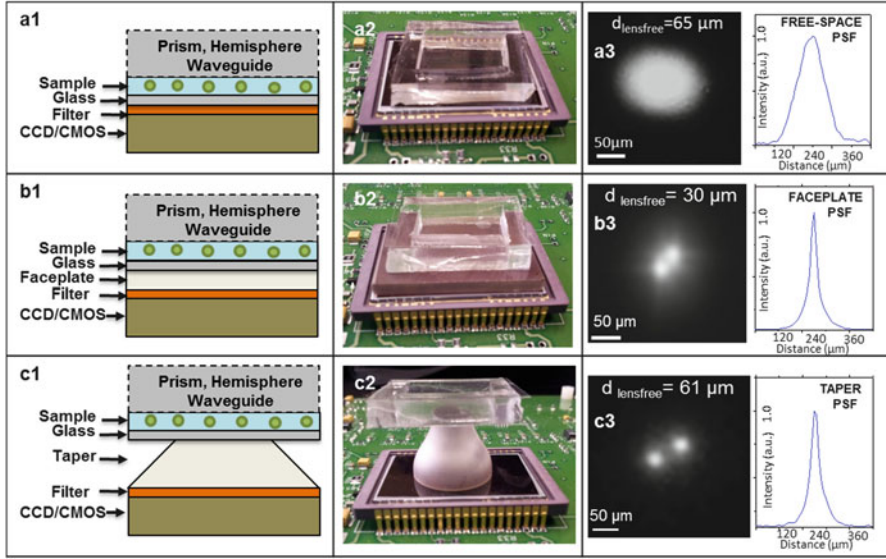


Fig. 2.2 The schematics (a1, b1, c1), corresponding experimental set-ups (a2, b2, c2), lensfree images and PSF analysis (a3, b3, c3) of the various light collection methods are shown

magnification in our platform (e.g., 2–3 \times), which further helps us to increase spatial resolution, despite the reduced FOV due to the taper geometry. Typical lensfree images of micro-particles and the PSFs of various configurations are demonstrated in Fig. 2.2[a3, b3, c3].

As for sampling of the fluorescent signal, once the emitted photons are transmitted through one of the collection methods described above, a sensor-array is used to digitize the fluorescence signal. For lensfree fluorescent imaging, CCD sensors can in general provide better sensitivity and larger FOV, while CMOS sensors can be employed for relatively cheaper and lighter weight designs (e.g., for field use).

Bio-Chip Design: To handle fluorescently labeled specimen, various bio-chip designs can be used, including glass-tape-glass based devices, PDMS (Polydimethylsiloxane)-channel-glass devices, or wide-area glass capillary arrays. One can select any of these device designs and then combine it with e.g., surface-chemistry protocols to achieve highly specific and sensitive on-chip lensfree fluorescence microscopy and/or biosensing that could potentially be useful for e.g., rapid detection of pathogens, sub-population of cells as well as molecular assays.

Reconstruction Methods

Lensfree fluorescence raw images look blurry due to diffraction, and therefore, to partially undo the effect of diffraction and create higher resolution microscopic

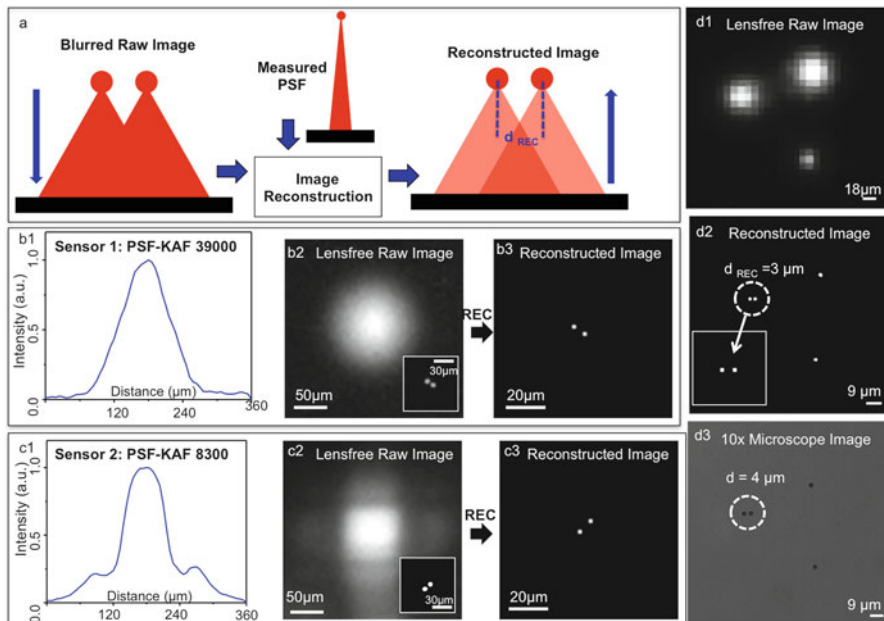


Fig. 2.3 The image reconstruction process (a) and PSFs of the different sensors (b1–3, c1–3) are shown. The resolving power of the imaging platform is quantified (d1–3)

images, these raw images are processed using image reconstruction methods, employing e.g., a *Lucy-Richardson deconvolution method* [2, 7, 8] or a *compressive sampling based decoding algorithm* [3, 9, 10]. Starting with an initial measurement of the incoherent PSF of the on-chip system, lensfree images are reconstructed within a few minutes (e.g., ~ 10 min for 9 cm^2 FOV using a standard PC – Fig. 2.3[a]). To quantify the spatial resolution, closely packed fluorescent bead pairs are reconstructed, verifying $< 4 \mu\text{m}$ spatial resolution based on an FOT collection platform (Fig. 2.3[d1, d2, d3]) [5]. Furthermore, to demonstrate the sensor independent performance of this platform, the PSFs of the two different CCD sensor-arrays are measured, showing a noticeable variance in their 2D patterns; however the reconstruction of closely packed fluorescent beads can still be achieved as illustrated in Fig. 2.3[b1–3, c1–3].

Results

This on-chip fluorescence imaging platform, combining a compact experimental set-up and rapid image reconstruction algorithms, together with its wide field imaging capability could especially be useful for high-throughput screening applications. To demonstrate its proof-of-concept, we performed experiments with

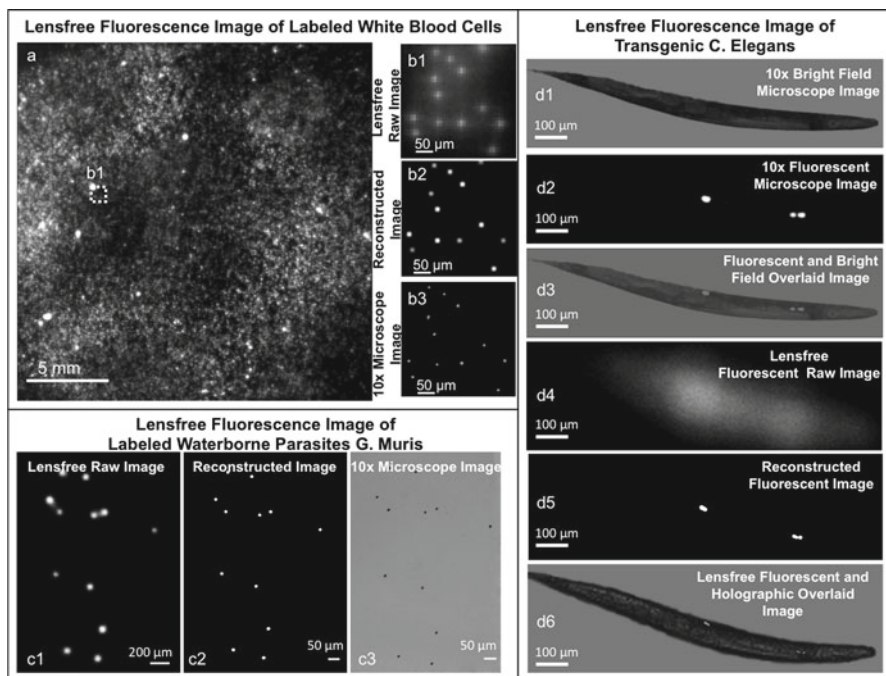


Fig. 2.4 Lensfree fluorescence images of white blood cells (**a**, **b1–2**), *G. muris* parasites (**c1–2**) and *C. elegans* (**d4–6**) are demonstrated, which agree well with 10× microscope comparisons

bodily fluids (e.g., whole blood samples with labeled white blood cells) [6], water-borne parasites (e.g., *Giardia muris*) [5] as well as genetically modified small model animals (e.g., transgenic *Caenorhabditis elegans*) [4].

The results of these experiments are presented in Fig. 2.4 which also includes comparisons against lens-based conventional microscope images of the same specimen. In Fig. 2.4[a] wide-field fluorescence image ($\sim 9 \text{ cm}^2$ FOV) of labeled white blood cells are shown. Figure 2.4[b2] illustrates the reconstruction results of digitally zoomed images of some white blood cells (Fig. 2.4[b1]), providing a decent agreement to a conventional fluorescent microscope image (Fig. 2.4[b3]). For water quality applications, in Fig. 2.4[c1] lensfree imaging of water-borne parasites is also presented, with the reconstructed results and microscope comparisons as shown in Fig. 2.4[c2, c3], respectively. Finally, we also present lensfree imaging of a *C. elegans* sample in Fig. 2.4[d4–6].

Conclusions

In this chapter, we reviewed an emerging wide-field lensfree fluorescent imaging modality, achieving $<4 \mu\text{m}$ spatial resolution over a large FOV of e.g., $>9 \text{ cm}^2$, that can rapidly monitor the state-of-art microfluidic chips toward high-throughput

screening applications, including on-chip cytometry, rare-cell analysis as well as microarray research. The presented imaging platform can also leverage other techniques to further increase its spatial resolution, incorporating e.g., higher-magnification FOTs to increase the resolving power of the imager, pixel super-resolution approaches [11] by shifting the fluorescent specimen to effectively create smaller size pixels, or through the use of nano-structured surfaces [12] to spatially modify the PSF of the on-chip imager. Such a lensfree fluorescence imaging platform, combined with better optical components and computational approaches could in general be useful for wide-field imaging of bio-chips.

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