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Lensless Fluorescent On-Chip Microscopy using a Fiber-Optic Taper

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***Abstract*— We demonstrate a lensfree on-chip fluorescent microscopy platform that can image fluorescently labeled cells over ~60 mm^2 field-of-view with <4 μm spatial resolution. In this lensfree imaging system, micro-objects of interest are directly located on a *tapered* fiber-optic faceplate which has > 5-fold higher density of fiber-optic waveguides in its top facet compared to the bottom facet. For excitation, an incoherent light source (e.g., a simple light emitting diode - LED) is used to pump fluorescent objects through a glass hemi-sphere interface. Upon interacting with the entire sample volume, the excitation light is rejected by total internal reflection process occurring at the bottom of the sample substrate. Fluorescent emission from the objects is then collected by the smaller facet of the tapered faceplate and is delivered to a detector-array with an image magnification of ~2.4X. A compressive sampling based decoding algorithm is used for sparse signal recovery, which further increases the space-bandwidth-product of our lensfree on-chip fluorescent imager. We validated the performance of this lensfree imaging platform using fluorescent micro-particles as well as labeled water-borne parasites (e.g., *Giardia Muris* cysts). Such a compact and wide-field fluorescent microscopy platform could be valuable for cytometry and rare cell imaging applications as well as for micro array research.**

I. Introduction

Over the last decade, lens-based optical microscopes have been extensively used for imaging of biological specimens and associated micro-fluidic platforms. With recent advances in fluorescent labeling technologies and imaging components, various discoveries have been made in biological sciences through the use of lens-based microscopes. However, typical imaging field-of-view of such a lens-based optical microscope is not yet compatible with the scale of the micro-fluidic devices that are especially used for high-throughput screening applications. In addition to this, their relative complexity also makes conventional lens-based microscopes rather bulky and expensive, limiting their use to relatively advanced laboratory settings.

To provide an alternative solution to this need, we have recently introduced various lensless microscopy techniques [1-7] that compensate for the lack of optical components in the digital domain. Along the same lines, here we demonstrate a lensless on-chip microscopy platform that can image fluorescent objects over ~60 mm^2 field-of-view with <4 μm spatial resolution without the use of any lenses, mechanical scanning or thin-film interference filters. In this on-chip microscopy system, fluorescent objects are placed on a tapered fiber-optic faceplate that has >5-fold higher density of fiber-optic waveguides in its top facet compared to the bottom one. For fluorescence excitation, an incoherent source such as a simple light emitting diode (LED) is used to pump fluorescent objects through a glass hemi-sphere interface (see Fig. 1). Upon interacting with the entire sample volume, the excitation light is rejected by total internal reflection process occurring at the bottom of the sample substrate. With the removal of the weakly scattered excitation light using an inexpensive absorption filter (Fig. 1), only the fluorescent emission from the sample can be collected by the smaller facet of the tapered faceplate to be delivered to a detector array (e.g., a CCD or CMOS chip). A

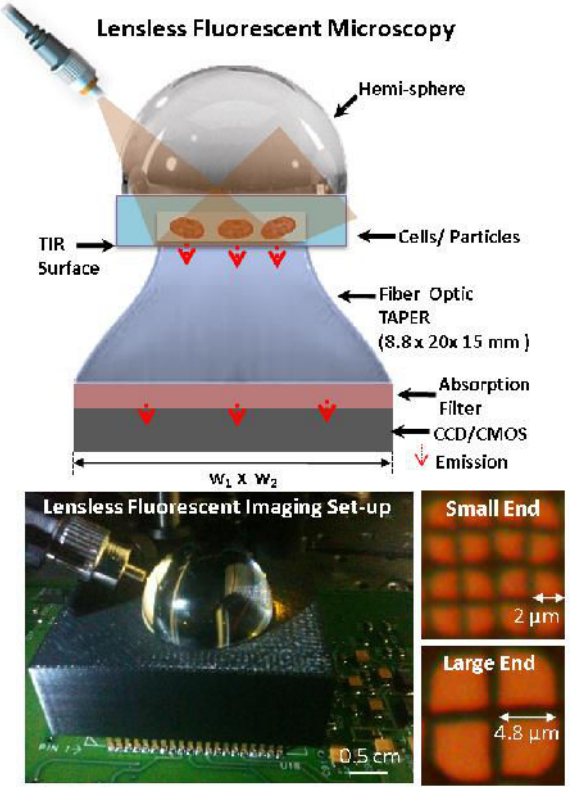

Fig. 1. (Top) The schematic of the lensless fluorescent microscopy platform is shown. (Bottom) The picture of the set-up and the microscopic images of the tapered faceplate surfaces are demonstrated.

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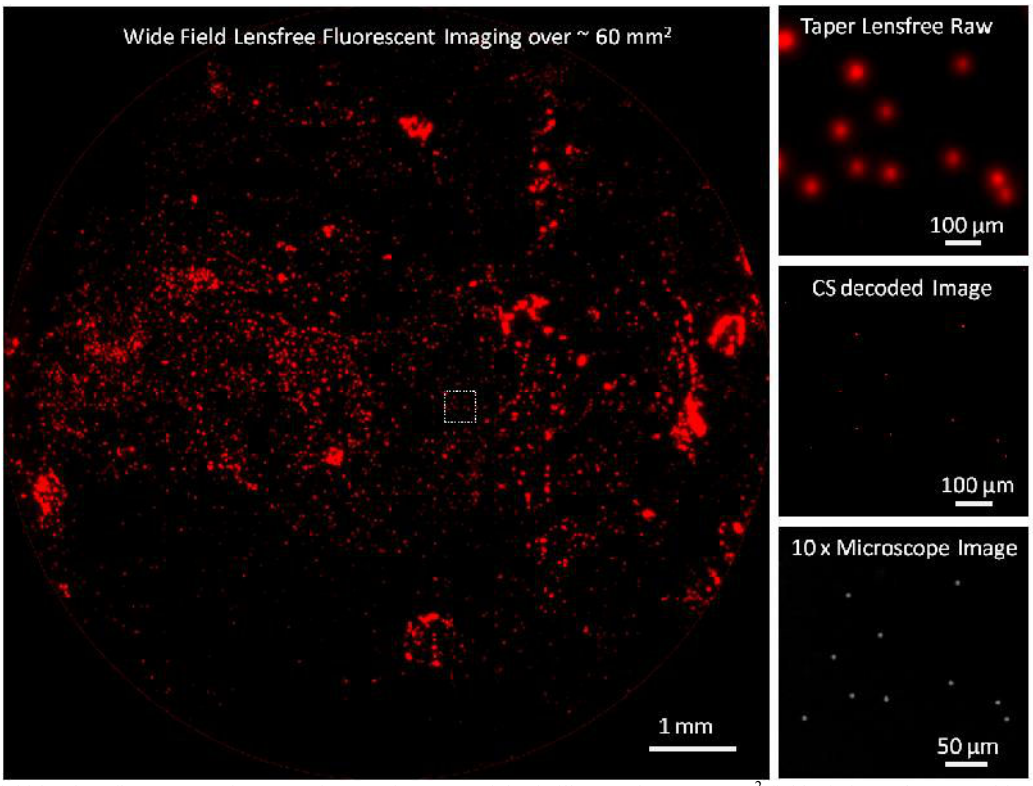

Fig. 2. Wide-field lensless fluorescent microscopy image of 4 µm particles is illustrated over 60 mm^2 field-of-view. The zoomed images on the right column also show the raw lensless image, decoded lensless image and the 10x microscope image comparison, which agree well with each other.

compressive sampling based decoding algorithm is then used to process the recorded lensless images to achieve <4 µm spatial resolution on a chip. We have validated the performance of this platform using fluorescent micro-particles as well as labeled *Giardia Muris* cysts. As a result of this compressive decoding process, the space-bandwidth-product of the on-chip imager can be digitally increased to enhance the throughput of our imaging platform.

Such a compact and wide-field fluorescent microscopy platform, with a rapid digital decoder behind it, could be very valuable for cytometry, rare cell imaging as well as for micro array research needs.

II. RESULTS AND DISCUSSION

For our on-chip lensless fluorescent microscopy set-up, a CCD sensor-array, a thin absorption filter, a tapered fiber-optic faceplate and a glass hemi-sphere are assembled together as shown in Figure 1. An incoherent fiber coupled source is also used for fluorescent excitation in the same geometry. Once assembled, we used this compact lensless microscopy platform to image 4 and 2 µm diameter fluorescent micro-beads as well as *Giardia Muris* cysts.

In Figure 2, a lensless raw image of 4 µm fluorescent micro-objects is illustrated over a field-of-view of ~60 mm^2. This image looks blurry due to its lensless operation (see e.g., the top right image in Fig. 2); however, a compressive sampling (CS) based decoding algorithm can be used to digitally improve the spatial resolution.[5-7] For comparison purposes, a 10x objective-lens microscope image for a zoomed FOV of the same objects is also included in Figure 2, which agrees well with our CS decoded lensless image shown in the same figure.

We quantified the spatial resolution of our platform by resolving closely-packed fluorescent bead-pairs. This is illustrated in Figure 3, where lensless raw images of 2 µm fluorescent bead-pairs are decoded using CS and compared against their corresponding 40x conventional lens-based microscope images. These results verify a spatial resolution of <4 µm, which could be valuable for microscopic analysis of cells and model organisms. We should emphasize that the pixel size at the CCD chip of this lensfree on-chip imager is 9 µm, such that a resolution of <4 µm is quite significant in this case.

Next, lensfree imaging of labeled *Giardia Muris* cysts is demonstrated using the same compact microscopy interface (see Figure 4). The CS decoded lensless images agree well with their corresponding microscope comparison images shown in the same figure. These presented results, when combined with large FOV micro-fluidic chips, could be useful for rapid screening of water borne parasites in field settings.

Finally, we would like to discuss that the tapered fiber-optic faceplate in our lensless on-chip microscopy platform (Fig. 1) serves several purposes: (1) The lensfree point-spread-function (PSF) of our on-chip imaging system can be engineered using a tapered faceplate geometry such that a better spatial resolution can be achieved; (2) A magnification (~2.4X) is introduced to the lensless imaging system through

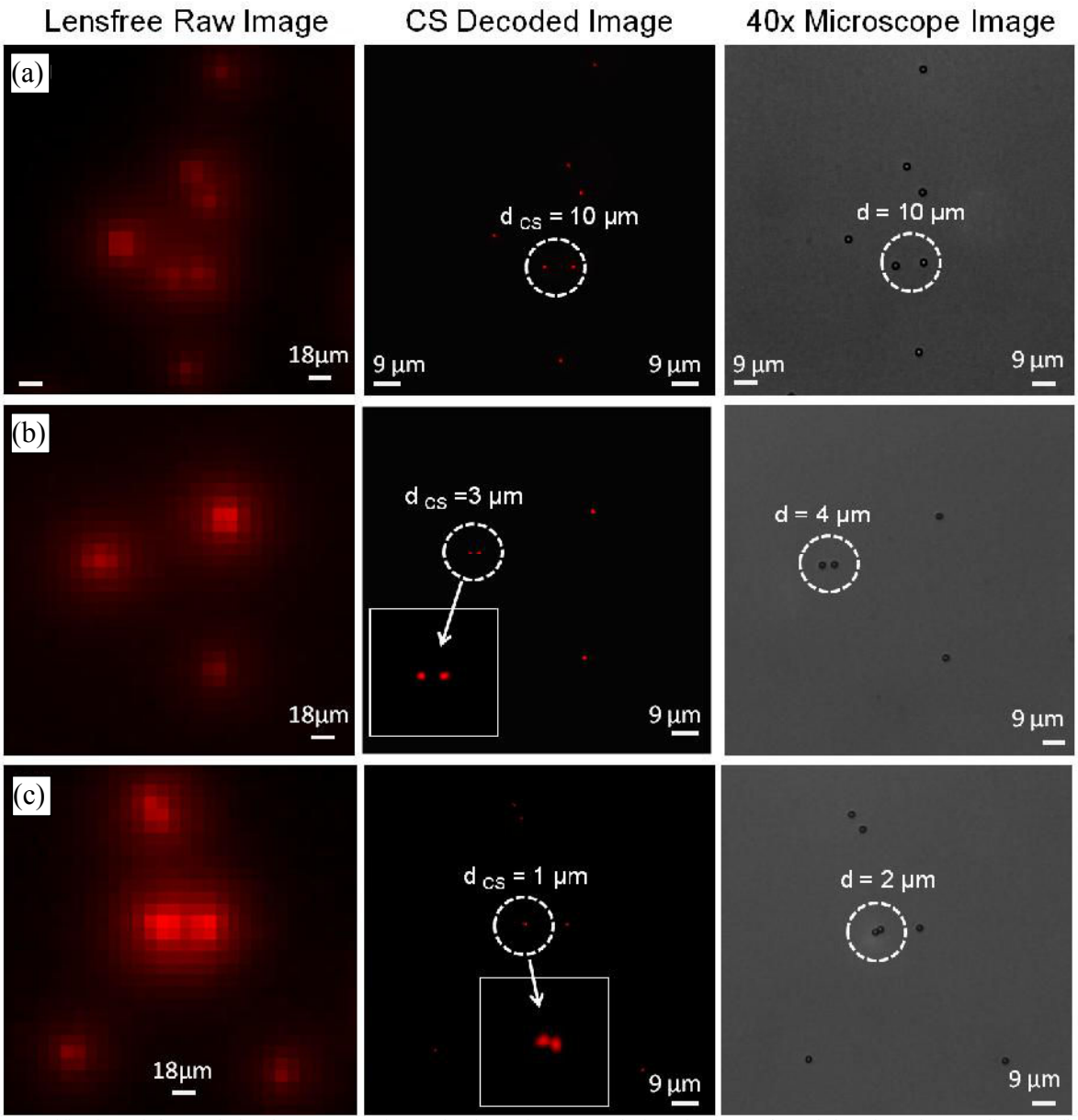

Fig. 3. Quantification of the resolution improvement in our lensless microscopy platform is shown through imaging of 2 µm diameter fluorescent particles. The middle column shows our CS decoded lensfree images and the right column provides 40x microscope image comparisons, which verify <4 µm resolution after decoding.

collection of the fluorescent signal with a denser fiber-optic grid (i.e., ~2 µm period at the top facet of the taper, compared to ~4.8 µm period at its bottom facet); and (3) Thermal isolation of the samples from the CCD/CMOS chip can be achieved, which could be valuable to implement active cooling of the sensor array without affecting the samples. Although not implemented in this current work, active cooling of the sensor-array could further improve the signal-to-noise ratio and contrast of lensless images, potentially providing a better spatial resolution for our CS decoded images.

III. MATERIALS AND METHODS

Our on-chip microscopy platform is assembled on an 11MPixel CCD chip (KODAK, KAI-11002). A 40 µm thick absorption filter (a detailed fabrication recipe is described in [6],[7]) is placed on this CCD chip using a vacuum pen and then a tapered fiber-optic faceplate (Edmund Optics, NT55-134) is positioned on top of the filter, where the small facet is facing the objects to be imaged. A 3D printed plastic housing is used to cover the optical components, except the active imaging area (see Fig. 1). The excitation is delivered through a fiber coupled incoherent source. Together with the assembly of the glass hemi-sphere onto the micro-fluidic chip, the samples are placed on the imaging area of the tapered faceplate (see Fig. 1). Lensfree fluorescent images are then acquired using a custom-developed LabView interface.

Fluorescent micro-beads with 4 and 2 µm diameters

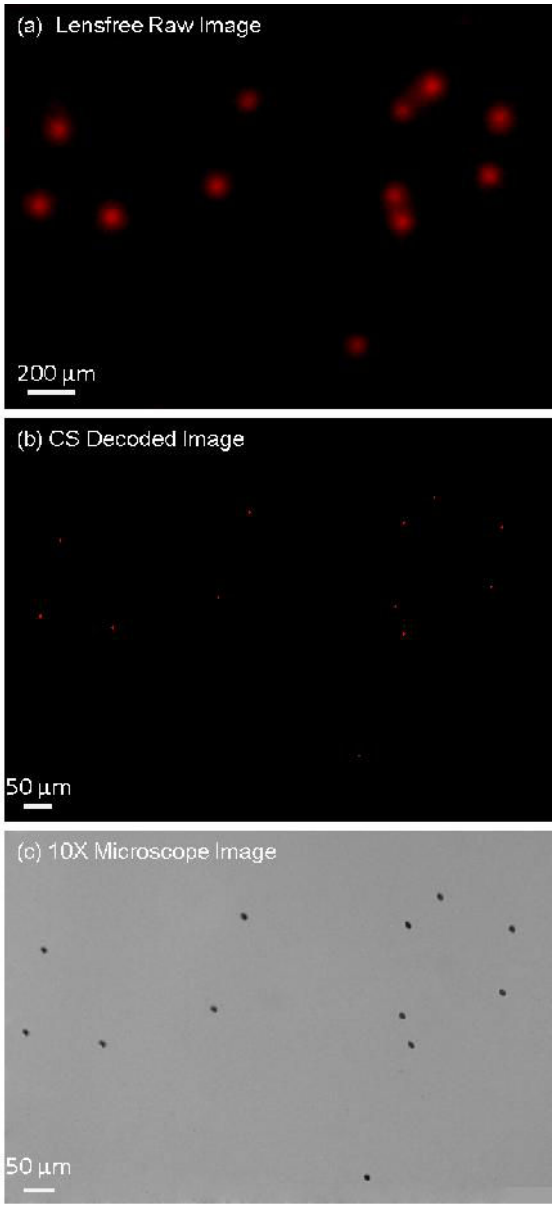

Fig. 4. Lensless on-chip microscopy of water-borne parasites (i.e., *Giardia Muris* cysts) is illustrated. The decoded image agrees well with the 10x microscope image comparison.

(Invitrogen, 580nm excitation & 605nm emission) are diluted 4000 times with DI water and transferred to micro-fluidic chips or sandwiched between glass slides using a pipette for lensless microscopy experiments.

Giardia Muris cysts (Waterborne Inc.) and 1mM SYTO®64 nucleic acid label (619nm emission) were mixed with 100 µL and 5µL of volume ratio, respectively. After incubation of ~30 min, the sample is centrifuged and resuspended in PBS to decrease the undesired background. The final solution is transferred to the sandwiched glass slides for lensless microscopy experiments.

A compressive sampling based decoding algorithm [8-10] is used to digitally increase the resolution in our on-chip microscopy system. First, the PSF of the lensless imaging system is measured using small and isolated fluorescent particles (e.g., 2 µm diameter). Then, for any arbitrary distribution of fluorescent objects, this *measured* PSF is used to calculate the *expected* lensless fluorescent image at the detector plane which is digitally sampled with a grid of pixels.[5] In our system, to process an acquired lensless raw image through compressive decoding, a cost-function is iteratively minimized (using truncated Newton-interior method [11]) which is constructed as l_1-regularized least square error between the *expected (i.e., calculated)* fluorescent image and *detected (i.e., measured)* lensless fluorescent raw image.[5] This processing takes typically ~40-50 iterations and ~0.5-2 minutes for smaller field-of-views as shown in e.g., Figures 2-3 using a 3.2 GHz Processor (Intel Core(TM)).

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