Lensfree on-chip holography facilitates novel microscopy applications

Aydogan Ozcan, Serhan Isikman, Onur Mudanyali, Derek Tseng, and Ikbal Sencan

Electrical Engineering Department, University of California at Los Angeles (UCLA), Los Angeles, CA

A new, lightweight, compact microscope is suitable for telemedicine applications in resource-poor settings.

The invention of the optical microscope has revolutionized our understanding of the microscopic world in biology, especially through shedding light on the structure and function of cells and micro-organisms. Over the last decade, the quest to understand the nanoscale world has demanded further advances in conventional-light microscopy, in particular to achieve spatial resolutions beyond the diffraction limit. As a result, today's advanced microscopes enable us to see structures that are much smaller than the wavelength of visible light. On the other hand, despite this recent renaissance in optical microscopy, the associated costs, complexity, and size of optical microscopes have increased proportionately, thus severely impeding their widespread use in resource-limited settings.

In parallel to these advances, recent progress in microfluidics has also enabled cost-effective, high-throughput handling and processing of cells and micro-organisms using miniaturized systems. Accordingly, device sizes and reagent volumes have been reduced significantly, along with the associated costs. Nevertheless, the relatively large size and limited field of view (FoV) of conventional optical microscopes still partially hinder their integration with such microfluidic platforms.

To provide a new imaging tool set, especially for telemedicine applications in resource-limited environments, we recently introduced an alternative microscopy platform,^{1, 2} which offers lensfree on-chip imaging with subcellular resolution over a wide FoV of $\sim 24\text{mm}^2$. This new imaging platform (see Figure 1) weighs $\sim 46\text{g}$ and has dimensions smaller than $4.2 \times 4.2 \times 5.8\text{cm}^3$. It provides tenfold larger FoVs compared to a 10X microscope objective lens with a similar numerical aperture ($\text{NA} \sim 0.2$).¹ Moreover, the lensfree design lends itself to a cost-effective assembly that is highly tolerant to misalignments (without the need for any light-coupling optics) enabling straightforward integration with microfluidic systems for rapid, massively parallel analysis of cells and micro-organisms on the same chip.

Our imaging platform is based on incoherent digital in-line holography and does not use any lenses, lasers, or other bulky optical components. Illumination is provided through an incoherent source such as a LED that is butt-coupled to a large pinhole of $\sim 50\text{--}100\mu\text{m}$ diameter, situated $\sim 2\text{--}10\text{cm}$ away from a CMOS or CCD sensor array. The sample, which is either sandwiched between cover glasses or contained within a microfluidic chamber, is placed directly on top of the sensor so that micro-objects are located $\sim 0.5\text{--}1\text{mm}$ away from the detector's active area. Because this geometry offers unit fringe magnification, the entire active area of the detector array becomes the object FoV, thus permitting significantly larger imaging throughput than a conventional microscope. The large sample-to-source distance creates partial spatial coherence on the sample plane, so that the scattered light from the objects can interfere with the unperturbed background light (the reference) to create holographic signatures of *individual* cells/micro-organisms on the sensor plane.^{1, 2} Digital

hologram reconstruction using iterative phase-retrieval algorithms allows rapid visualization of both phase and amplitude images of the objects on timescales of less than 1s. Importantly, the same compact, holographic microscope can also be converted into a Nomarski or differential-interference-contrast microscope using a cost-effective birefringent crystal.¹

We have tested the performance of our lensfree holographic on-chip microscope by imaging red and white whole-blood cells and platelets within a compact, lightweight, and cost-effective unit that is especially suitable for next-generation telemedicine systems¹ (see Figure 1). The spatial resolution in our holographic reconstructions is sufficient to reveal subcellular features of white blood cells to determine their types (e.g., granulocytes, monocytes, or lymphocytes), which is an important step forward for three-part differential imaging of white blood cells for whole-blood analysis.

Beyond telemedicine, our lensfree imaging modality within a benchtop platform may also impact numerous other applications where high-throughput microscopy is needed. For instance, we have tested our system for phenotypical characterization of *Caenorhabditis Elegans* (*C. elegans*) worms,² which are model organisms used extensively in drug discovery, genetics, and neurobiology. The wide FoV allows simultaneous monitoring of a large number of worms on the same chip with a resolution comparable to that of a 10X microscope objective, but over an order of magnitude larger imaging area: see Figures 2(a) and 2(b). Further, digital fusion of the reconstructed images obtained at three different wavelengths (450, 550, and 650nm) allows color imaging of the same *C. elegans* samples on the chip: see Figure 2(c).

Finally, we recently demonstrated integration of the same on-chip holographic imaging platform with lensfree fluorescent imaging (with a FoV of $>8\text{cm}^2$) for rapid screening of large-area microfluidic devices.³ This ultrahigh-throughput imaging platform, which can switch back and forth between lensless fluorescent and holographic on-chip operation, may be particularly important for rare cell-analysis applications, such as detection and quantification of circulating tumor cells in whole-blood samples. We are continuing development of on-chip holography applications.

Acknowledgments

A. Ozcan gratefully acknowledges support from the Office of Naval Research (through a 2009 Young Investigator Award) and the National Institutes of Health (NIH) Director's New Innovator Award (DP2OD006427 from the Office of the Director, NIH). The authors also acknowledge support from the Okawa Foundation, Vodafone Americas Foundation, the Defense Advanced Research Project Agency's Defense Sciences Office (grant 56556-MS-DRP), the National Science Foundation's Biophotonics, Advanced Imaging, and Sensing for Human Health Program (awards 0754880 and 0930501), the NIH (grant 1R21EB009222-01), and the Air Force Office of Scientific Research (project 08NE255).

Biography

Aydogan Ozcan received his PhD degree from Stanford University's Electrical Engineering Department in 2005. He joined UCLA in the summer of 2007 as an assistant professor, and currently heads the Bio-Photonics Laboratory.

References

1. Mudanyali O, Tseng D, Oh C, Isikman S, Sencan I, Bishara W, Oztoprak C, Seo S, Khademhosseini B, Ozcan A. Compact, light-weight and cost-effective microscope based on lensless incoherent holography for telemedicine applications. *Lab on a Chip*. 2010 In press, doi:10.1039/c000453g.

2. Isikman SO, Sencan I, Mudanyali O, Bishara W, Oztoprak C, Ozcan A. Color and monochrome lensless on-chip imaging of *Caenorhabditis elegans* over a wide field-of-view. *Lab on a Chip* 10. 2010:1109–1112. doi:10.1039/C001200A.
3. Coskun AF, Su T, Ozcan A. Wide field-of-view lens-free fluorescent imaging on a chip. *Lab on a Chip* 10. 2010:824–827. doi:10.1039/b926561a.

Figure 1.

(a) Lensfree holographic on-chip microscope weighing ~ 1.6 ounces. It uses a simple LED source (at 591nm) with an aperture of $\sim 50\text{--}100\mu\text{m}$ in front of the source. The source and the sensor are powered through a universal serial-bus connection from the side of the device. This lensfree holographic microscope operates with a unit fringe magnification to have the whole active area of the detector as its imaging field of view ($\sim 24\text{mm}^2$). Using a thin birefringent crystal that costs $\sim \$1\text{--}2$, the same assembly can also be easily converted into a differential interference contrast microscope.¹ (b) Lensfree on-chip holographic imaging results of whole-blood cells obtained with the unit in (a).¹ RBC: Red blood cell.

Figure 2. Color and monochrome imaging of *Caenorhabditis Elegans* over a wide field of view would allow high-throughput phenotypical characterization of worms for potential applications in drug discovery and neuroscience.