



Building a Microscopic Microscope

by Changhuei Yang and Demetri Psaltis

Do you see floaters drifting in your eyes when you look up into a clear blue sky? The floater phenomenon is the inspiration for our recent invention, *the optofluidic microscope*. The optofluidic microscope is a high-resolution, chip-size microscope that, remarkably, operates without lenses. It is already the world's smallest microscope, and we aim to make it the world's cheapest. The future use of the optofluidics microscope by bioscientists, clinicians, and doctors may mark a new era in discovery and healthcare.

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icroscopes are ubiquitous in biology and clinical analysis laboratories. They are the workhorses of bioscience research and our surrogate eyes into the wondrous world of microbes and cells. Yet,

since the development of the microscope beginning about 1590 by two Dutch spectacle makers, Hans and Zaccharias Janssen, then by Galileo and Antonie van Leeuwenhoek, the basic microscope design has undergone very few fundamental changes over the intervening centuries. In almost all microscopes, you can expect to find precise and expensive optical lenses and plenty of space for the light to travel and reshape itself—the long distance between the objective lens and the eyepiece lens isn't there for aesthetic reasons! way to create small and precise optical lenses on chips easily. Second, the space requirements of conventional microscopy conflict with the size constraints of chip-based devices.

These difficulties aside, we can ponder "what if?" situations for miniature microscopes. An on-chip microscope implementation method can dramatically change the way we use microscopes. The application range of an on-chip microscope is wide and will be discussed below—at this junction, we would like to point out that a typical bioscience laboratory contains less than ten microscopes (size and cost are both factors in this). We invite the reader to consider the enhanced efficiency if the number of microscopes per laboratory is to increase by a factor of ten or a hundred.

Rethinking the Microscope

The antiquated nature of the conventional microscope design stands out even more in the context of the ongoing lab-on-a-chip research and development, in which laboratory instruments are being systematically miniaturized into chipsize devices. While a wide range of bioanalysis methods have been successfully miniaturized and implemented in a labon-a-chip format, there has not been a commercially viable approach to miniaturizing microscopy until recently. The difficulty is twofold. First, there isn't a cheap and efficient To implement a cost-effective and commercially viable on-chip microscope, it was necessary to break with tradition, abandon the old microscope design, and rethink the whole imaging problem from the ground up. Fortunately, optical technology has come a long way since the 16th century, and we now have access to a broader range of devices than existed even 50 years ago!

To motivate this redesign process, let us enumerate the functions that the microscope must perform. There are three



primary functions. First, a microscope should be able to replicate an image of the object onto a person's retina-or at least provide an image to an observer. Nowadays, electronic detector grids have been substituted in place of the retina in many optical devices. Nevertheless, the vast majority of microscopes still operate by relaying and replicating an object's image onto a sensor grid of some sort.



Figure 1. (a) The basic OFM scheme. The device is uniformly illuminated from the top. The sample passes over the hole array and blocks the light transmissions through the holes. (b) A prototype next to a quarter; the actual device is about the size of Washington's nose on the quarter. (c) The physical dimensions of the prototype. Holes a and b are both placed in the channel's center. By measuring the time difference between when the target first passes over each hole, we can determine the speed of the target by dividing the holes' separation distance with the time difference.

Second, a microscope should be able to magnify the image so that objects are adequately resolved. Twenty-twenty vision roughly corresponds to a pixel size of about 5 μ m on the retina. To resolve sub-micron features, magnification is invariably required. Third, a microscope should be able to select a specific plane in the object for imaging—this is called optical sectioning.

Drawing Inspiration From Floaters

What exactly are floaters? And why do we see them? For those of you who are unacquainted with the term 'floaters,' chances are you simply do not know them by name. Floaters are the spindly objects that float in your field of view and can be most clearly seen when you look up at a clear blue sky. Those highly resolved images are caused by debris in your vitreous humor that has gotten very close to your retinal layer. Under uniform illumination (such as that of a clear sky), they project clear shadows onto your retinal layer. Remarkably, the lens in the eye plays no part in this imaging process. To verify this, the next time you see floaters, try focusing and defocusing your sight. The floaters should remain equally sharp.

The floater phenomenon points the way to an imaging method that does not require the use of lenses or any other optical elements. Specifically, we can perform imaging by simply placing the object of interest directly onto a sensor grid, such as a CCD or CMOS sensor (this is the chip that is the heart and engine of your digital camera). By illuminating the object uniformly, a transmission image of the object can be directly recorded by the sensor grid. Recently, this strategy has been implemented as an imaging method by researchers at Stanford University and NASA Ames Research Center. One must excuse the microscopists of the 16th century for not inventing this sooner—the only sensor grid available to them was embedded in the human eye, and there is no practical way of introducing objects of interest directly onto the retinal layer.

This direct-imaging approach has several advantages. First, the lack of optical elements in this arrangement implies that there is no aberra-

tion of the optical elements to worry about. Second, this is an intrinsically space-conserving method and as such is highly attractive as a chip-based microscopy method. However, this imaging method is non-magnifying and its resolution is fundamentally limited by the pixel size of the sensor grid. In other words, we can resolve two points on the object as long as they map onto two different pixels on the sensor grid: the denser the grid, the higher the resolution of this imaging system.

Unfortunately, this last characteristic is a disadvantage in practice. Currently the smallest available pixel size in a commercial CCD or CMOS sensor is about 5 μ m, so this direct-imaging approach cannot be expected to perform better than a conventional microscope, which has a resolution range of about 1 μ m to 0.2 μ m. While we hope for the day when commercial CCD or CMOS sensor pixels will shrink in size by an order of magnitude, this day is unlikely to come soon since the creation of such sensors is hindered by very significant fabrication challenges. Until this day comes, is it possible to get around the problem of pixel size?

The Optofluidic Microscope Method Explained

The nascent field of optofluidics—the fusion of microfluidics and optical technologies—offers us a way around this problem. A new microscopy method, termed Optofluidic Microscopy (OFM), which we recently developed at Caltech, enables the imaging of fluid-immersible objects with microscope-level resolution (and as we shall see, super resolution as well).

An OFM device may be fabricated as follows. First, a layer of metal is coated onto a linear sensor array to block out

light. A line of holes is then punched into the metal layer. Finally, a microfluidic channel is added on top of the entire chip.

In operation, the device is uniformly illuminated from the top and the target object is flowed across the array of holes via the microfluidic channel (see Figure 1). The time-varying light transmission through each hole constitutes a transmission image line trace across the object. By stacking the lines traces from all the holes together, we are able to



Figure 2. (a) Non-OFM technique. A transmission image of the object can be obtained by simply stacking up the transmission time traces collected as the object passes over the holes. To achieve high-resolution imaging, we need to space the holes closely across the channel. This particular arrangement gives poor image resolution as we cannot space the holes closer than a sensor-pixel width without mapping multiple holes onto a single sensor pixel. (b) OFM technique. The line of holes along the channel is skewed. This way, we can space the holes as closely across the channel as we want (in latitude), while at the same time ensuring that there is a unique 1-to-1 mapping of holes to sensor pixels. As the object will pass over each hole at different times, we need to correct for the time delays in the transmission traces prior to image construction. This is easily done by unskewing the traces based on the flow velocity of the sample (as measured with holes a and b).

construct a transmission image of the object.

The exact arrangement of the array of holes with respect to the underlying pixel grid is the key novel aspect of the OFM. Rather than placing them in a line exactly parallel to the flow direction, (for instance, in the absolute center conventional microscope. The similar quality verifies that the OFM method is capable of delivering high-resolution images. Our prototype contains 600-nm wide holes that are spaced at 5-µm intervals. We note that as a demonstration unit, the metal layer of this device is not directly bonded

onto the linear array sensor. Instead, the transmissions

through the holes are projected onto a linear CCD array by

relay optics. Recently, we successfully implemented an on-

Dual Advantage: Compact &

of each pixel), they are placed only on the centerline of each pixel on the vertical axis. In the horizontal direction, they are *slightly* offset from each other—so a diagonal line with

respect to the flow is created by the holes. (a) Put another way, the longitude of the holes stays constant, but the latitude (b) changes ever so slightly from one hole to the next. Assuming the specimen is rigid,



Figure 3. (a) A conventional microscope image of a nematode, C. elegans. (b) An OFM image of the same. Notice the tail end of his friend exiting the image at right.

each hole then captures information from the specimen at intervals that correspond to the *slight offset* of each hole from the previous (instead of intervals that correspond to

conventional microscopy, and yet it is capable of delivering high resolution. More importantly, it functions as a microscope without the use of bulky optical elements. OFM

By collecting images acquired over time of parts of the specimen separated by the latitudinal offset of the holes, and then reconstructing the image of the entire specimen, we get around the problem of pixel size. This is illustrated in Figure 2. Note that resolution may be increased by using smaller hole sizes and spacing them closer together in the horizontal direction.

Figure 3 shows OFM images of the C. elegans nematode acquired with our prototype in comparison with an image acquired with a comparable

chip version (see

Figure 4) and we

are in the process

of evaluating its performance.

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Figure 4. An on-chip implementation of the optofluidic microscope.

devices can be built onto chips with existing fabrication techniques and we estimate they can be mass manufactured at a very low cost (~ \$10's).

Why Is An On-chip Microscope Useful?

The dual advantages of compactness and low cost open up a wide range of possible applications. For example, the OFM can be used in white-blood-cell-counting cytometry devices as image-based cell-type discriminators. Clinicians can use such units as disposable, point-of-care microscopes and battlefield medics can easily carry these devices out into the field. Health workers in rural areas can use cheap, compact OFMs as part of their regular toolkit,

Low Cost

easily carrying them in their pockets from village to village for malaria diagnosis. Further, the OFM can change the way a bioscientist tackles imaging problems. Potentially tens or even hundreds of OFMs can be fabricated onto a single chip. Such a device can be used to parallelize the imaging of a large number of microorganisms and dramatically improve throughput. Taking a long view, the OFM can even form the imaging component of a bio-compatible device that may be implanted into a person to provide continual monitoring of objects in the blood stream. Such a device may be useful for screening circulating tumor cells and other abnormal objects to provide early warnings of developing diseases.

Looking Ahead

In the context of expanding the capabilities of the OFM, there are three directions that we will like to explore over the coming year.

PROGRESS REPORT

First, we would like to achieve super-resolution. A conventional microscope is limited in its resolution by the diffraction limit. Simply put, the projected image in a microscope is made up of propagative light rays from the object (in optical terms, these are far-field components) and is constrained in its resolution by the diffraction limit. In comparison, the OFM resolution is fundamentally tied to the size of the holes. As such, an OFM with small holes can, in principle, achieve resolution that is unattainable with conventional microscopes. We are in the process of demonstrating a super-resolution OFM.

Next, we are working on a fluorescence-capable OFM. Creating the equivalent of a fluorescence microscope with the OFM method is remarkably straightforward: we simply have to lay down a chromatic filter layer between the sensor and the metal layer. Another way to go about this is to start with a color sensitive sensor. Such a sensor has built-in filters.

Finally, we would like to demonstrate a phase-sensitive OFM. Creating a phase-imaging microscope system based on the OFM method is, again, remarkably simple. One approach will be to add a spacer medium between the sensor and the metal layer and punch hole pairs in place of individual holes. The hole pairs will function as miniature Young's double slits. By observing the interference pattern, we can determine the amplitude and phase of the transmission OFM image. In addition, this approach solves a problem associated with the OFM—the OFM does not provide optical sectioning capability; the plane of highest acuity is the plane immediately above the holes. With knowledge of the amplitude and phase distribution, we can actually compute the wavefront at any given plane. This will allow us to perform virtual focusing into the sample of interest.

Changhuei Yang is Assistant Professor of Electrical Engineering & Bioengineering and Demetri Psaltis is the Thomas G. Myers Professor of Electrical Engineering.

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For more information, visit these websites: http://www.optofluidics.caltech.edu http://www.biophot.caltech.edu http://optics.caltech.edu