Through a Sensor, Holographically

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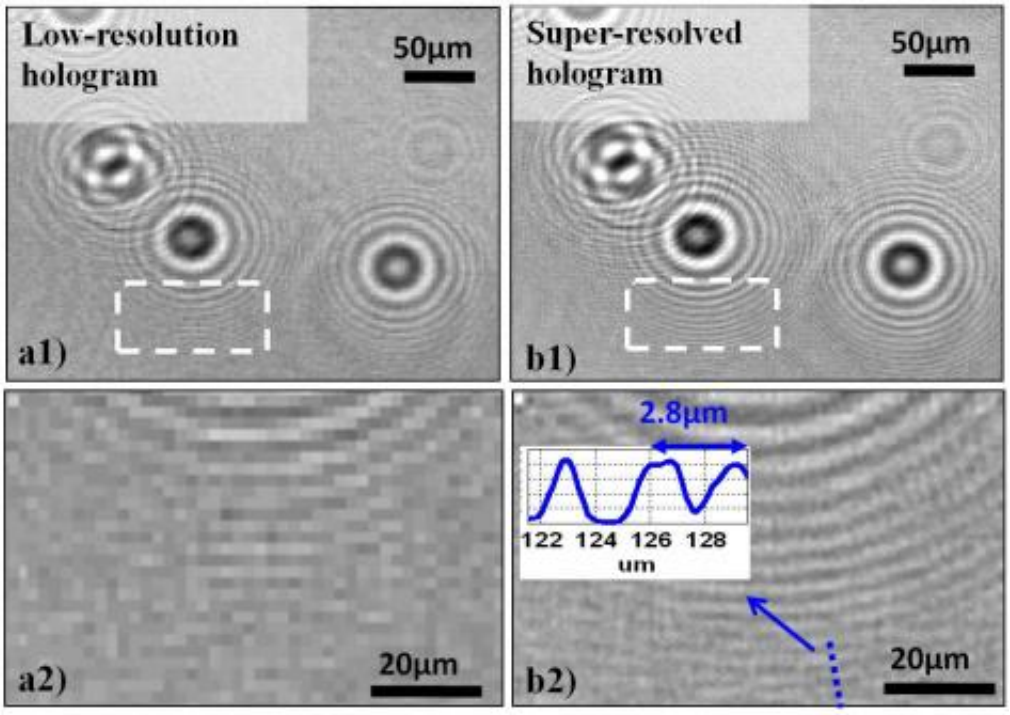

(A1) A single, low-resolution (LR) hologram obtained by vertical illumination of 10µm beads in a chamber. (A2) Zoomed region from the LR hologram, showing the aliasing effect due to undersampling of high-frequency interference fringes. (B1) A digitally synthesized pixel superresolved (SR) hologram using multiple subpixel shifted LR holograms. (B2) Zoomed region from the SR hologram, showing the digitally recovered high-frequency fringes. The inset shows the profile along the dashed line on the SR hologram, where an interference fringe with 2.8µm period is recovered with high SNR, which is normally undersampled by the detector with a physical pixel size of 2.2µm. (c) *PNAS*, doi:10.1073/pnas.1015638108

(PhysOrg.com) -- The power and resolution of lens-based optical microscopes have improved by orders of magnitude since their invention around 1595. Nevertheless, relying on a high-magnification lens for image clarity has limitations that become more relevant as larger and larger sample volumes need to be viewed. Moreover, achieving these advances in lens-based optical microscopy has increased their size and complexity. At the same time, microfluidic systems are becoming increasingly sophisticated while decreasing in size, creating a need for miniaturized optical microscopy that can be integrated onto a lab-on-a-chip to allow simultaneous analysis and imaging of small biological samples.

Enter Prof. Aydogan Ozcan, associate professor of electrical engineering at UCLA's Henry Samueli School of Engineering and Applied Science. Ozcan and his team – notably lead researchers Serhan Isikman and Dr. Waheb Bishara – have created a lens-free chip and image processing algorithm that utilizes optical sensors, holography and digital tomography combination to render high-resolution, high-contrast images while avoiding the limitations of standard lens-based optical microscopy. “The sensor,” Ozcan notes, “is an inexpensive five megapixel CMOS chip, 5MP with a 2.2 micrometer pixel size. It’s almost the same sensor that we have at the back of a Blackberry or iPhone, except that it’s monochrome rather than RGB.”

One of the biggest challenges facing the team was reducing noise artifacts resulting from spatial and temporal coherence due to illuminating the sample with lasers – especially at oblique angles. This coherence-induced noise appears as speckling patterns that obscure images of the actual sample structure. The team addressed the issue by replacing laser illumination with *partially-coherent light* that emanates from a large aperture of ~0.05-0.1mm diameter with a bandwidth of 1-10 nm, finding that recording in-line holograms using partial coherence provided a gating function which allowed the device to filter

noise beyond a defined resolution level.

Using partially-coherent light provided a high signal-to-noise ratio (SNR) that dramatically improved clarity and legibility of fine structural details. Moreover, the team developed a sample illumination approach that rotates the partially-coherent light source around the sample, rather than requiring the sample platform to be rotated within the illumination field, which is rather inconvenient to achieve practically, especially for large sample volumes.

Ozcan comments that, of the many innovations in this lens-free optical tomographic microscope, three are key: *partially-coherent illumination with unit-magnification; pixel superresolution to achieve deeply subpixel lateral resolution;* and *dual-axis tomographic illumination*. In their setup, dual-axis illumination is achieved by rotating the light source using a motorized stage; alignment is not sensitive and robustness is maintained. At every illumination angle, a series of subpixel shifted holograms are recorded for implementing pixel superresolution, such that submicron lateral resolution can be achieved even under unit fringe-magnification. “The chip uses dual-axis illumination to mitigate our limited angles of illumination such that a decent axial resolution can be achieved. The spatial frequencies that are collected from each axis is merged together to fill in some gaps in the 3D Fourier spectra of our objects. Moreover,” he adds, “this is the first time that dual-axis illumination has been applied in optical computed tomography schemes.”

This pixel superresolution approach effectively increases the sensor pixel density without physically adding additional pixels or sacrificing the imaging field of view (FOV). This is accomplished by capturing different images resulting from motion of either the illumination source or the sample and subsequently merging these lens-free frames to synthesize a higher spatial resolution holographic image. In microfluidic applications, for example, the fluidic motion of objects flowing by the

sensor array can be used to generate high-resolution holograms.

Ozcan acknowledges that a fundamental challenge to transmission optical microscopy in general (whether lens-based or lens-free) is photon scattering when imaging thick tissue samples. “However,” he adds, “I expect that this limitation can be partially released if the scattering properties of tissue were to be reduced through some sample preparation steps – at least for certain class of objects. There is some very promising work in the literature around this major issue and researchers are working hard with various innovative schemes toward this end.”

Ozcan sees the primary applications of lens-free microscopy being in cell and developmental biology – especially in *microfluidic integration*. “Microfluidic integration would permit rather interesting lab-on-a-chip devices that could do *optofluidic microscopy and tomography* (also referred to as *holographic optofluidic microscopy*, or HOM) on the same chip. This way the compact and cost-effective platform of lab-on-a-chip devices could be coupled with high-resolution 3D micro-analysis tools on the same platform.”

In fact, Ozcan has done previous work in lens-free opto-fluidic microscopy, last year publishing a paper with Dr. Waheb Bishara and Dr. Hongying Zhu entitled Holographic Opto-Fluidic Microscopy (*Optics Express* 18:27499–27510, 20 December 2010, Vol. 18, No. 26). In that paper, the authors note that their HOM platform does not involve complicated fabrication processes or precise alignment, nor does it require a highly uniform flow of objects within microfluidic channels. Relatively recently the Ozcan group has also demonstrated, for the first time, optofluidic tomography, soon to be published in *Applied Physics Letters*.

Of great interest is that when asked if further miniaturization and integration – such as an on-chip partially-coherent light source – could

potentially enable *in vivo* applications, Ozcan did not rule out the possibility. “Over the last decade microfluidics has created a versatile platform that has significantly advanced the ways in which microscale organisms and objects are controlled, processed and investigated, by improving the cost, compactness and throughput aspects of analysis. Microfluidics has also expanded into optics to create reconfigurable and flexible optical devices such as reconfigurable lenses, lasers, waveguides, switches, and on-chip microscopes.”

More information:

-- The Ozcan Research Group at UCLA innovate.ee.ucla.edu/

-- Lens-free optical tomographic microscope with a large imaging volume on a chip, *PNAS* Published online before print April 19, 2011, doi: 10.1073/pnas.1015638108

-- Holographic Opto-Fluidic Microscopy, PubMed, doi: 10.1364/OE.18.027499

-- Optical microscope without lenses produces high-resolution 3-D images on a chip www.physorg.com/news/2011-04-o … gh-resolution-d.html