14. ABSTRACT
In this proposal timeline (1 Year total) we developed a new lensless on-chip imaging platform, termed LUCAS, to enable both diagnostic and sensing capabilities within a cost-effective hand-held unit that is specifically suitable for the battlefield settings. We established the proof of concepts of this imaging modality to achieve reliable and repeatable quantified whole blood analysis and compared our results against commercially available blood analyzers. Furthermore, we investigated the fundamental limits on the size of the detectable cell/bacteria with this

15. SUBJECT TERMS
lensfree imaging, blood analysis on a chip
Report Title

Lensless Imaging for Battlefield On-Chip Blood Diagnostics

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List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)


Number of Papers published in peer-reviewed journals: 14.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

None

Number of Papers published in non-peer-reviewed journals: 0.00

(c) Presentations
1. A. Ozcan, “Lab-on-a-Cellphone as an Emerging Telemedicine Platform,” University of California, Global Health Day, UCI Campus 30 November 2010, Irvine CA

2. A. Ozcan, “Lensfree Imaging for Microscopy and Diagnostics,” First Look LA Meeting, USC Campus 16 November 2010, Los Angeles CA

3. A. Ozcan, “Photonics based Telemedicine Technologies toward Smart Global Health Systems,” UC Irvine Biomedical Engineering Department Seminar Series, 12 November 2010, Irvine CA

4. A. Ozcan, “Photonics based Telemedicine Technologies toward Smart Global Health Systems,” mHealth Summit, 9 November 2010, Washington DC

5. A. Ozcan, “Photonics based Telemedicine Technologies toward Smart Global Health Systems,” Los Angeles IDEA Project, 7 November 2010, Los Angeles


8. A. Ozcan, “Photonics based Telemedicine Technologies toward Smart Global Health Systems,” Body Computing Conference, University of Southern California, 23 September 2010, Los Angeles, CA

9. A. Ozcan, “Photonics based Telemedicine Technologies toward Smart Global Health Systems,” Google, 10 September 2010, Mountain View, CA

10. A. Ozcan, “Photonics based Telemedicine Technologies toward Smart Global Health Systems,” Cisco, 2 August 2010, Milpitas, CA


15. A. Ozcan, “Incoherent Lensfree Cell Holography for Global Health Applications” 7th International Conference on Optics-Photonics Design and Fabrication, (April 19-21 2010) Yokohoma, Japan


17. A. Ozcan, “Photonics based Telemedicine Technologies toward Smart Global Health Systems” Stanford University Electrical Engineering Department, 12 April 2010, Stanford CA


19. A. Ozcan, “Photonics based Telemedicine Technologies toward Smart Global Health Systems” UC Berkeley EECS Department, 12 March 2010, Berkeley CA


Number of Presentations: 27.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

None

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):


33. A. Ozcan, “Incoherent Lensfree Cell Holography for Global Health Applications” 7th International Conference on Optics-Photonics Design and Fabrication, (April 19-21 2010) Yokohoma, Japan (Invited Talk)


Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 40

(d) Manuscripts

None

Number of Manuscripts: 0.00

Patents Submitted

None

Patents Awarded

None

Awards

• LAUNCH Health Innovation Award, presented by NASA, USAID, Department of State, and NIKE, 2010
• Bill & Melinda Gates Foundation, Grand Challenges Explorations Award, 2010
• National Geographic Emerging Explorer Award, 2010
• Popular Mechanics Breakthrough Award, 2010
• Netexplorateur Award, Netexplorateur Observatory and Forum, France, 2010
• PopTech Science and Public Leaders Fellowship, 2010
• University of Southern California - Body Computing Slam Prize, 2010
• NIH Director’s New Innovator Award, 2009
• Vodafone Americas Foundation - Wireless Innovation Award, 2009
• MIT’s Technology Review Magazine, TR 35 Award, 2009
• IEEE Photonics Society Young Investigator Award, 2009

Graduate Students

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Names of Post Doctorates
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**Student Metrics**

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: ...... 2.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields: ...... 2.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields: ...... 1.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale): ...... 1.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering: ...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense ...... 1.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: ...... 0.00

**Names of Personnel receiving masters degrees**

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**Names of personnel receiving PHDs**

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**Names of other research staff**
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FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)
Final Report for 56556-MS-DRP (PI: Aydogan Ozcan, UCLA)

(1) BAA Number: DARPA-BAA-09-31

(2) Technical Area: Photonics (DSO: LtCol John Lowell, Ph.D.)

(3) Lead Organization Submitting Proposal: University of California, Los Angeles

(4) Type of Business: "OTHER EDUCATIONAL"

(5) Other Team Members (if applicable) and Type of Business for Each: NONE

(6) Proposal Title: Lensless Imaging for Battlefield On-Chip Blood Diagnostics

(7) Technical Point of Contact to include: Prof. Ozcan, Aydogan, Engineering IV Building, Electrical Engineering, UCLA, Los Angeles, CA, 90095, Phone # 310 500 6568, Email: ozcan@ucla.edu;

(8) Administrative Point of Contact to include: Zhu, Julia, 11000 Kinross Avenue, Suite 102 Box 951406, Los Angeles, CA 90095-1406, Phone # (310) 794-0155, Email: JZhu@research.ucla.edu;

(9) Total Funds received from DARPA: 200,000 USD

(10) Duration (in months) of Proposed Work: 12 months
Statement of the Problem Studied and our Related Achievements

The needs and the requirements of medical diagnostics in resource limited settings such as the battlefield are significantly different than advanced medical laboratories. On the battlefield, medical resources, as well as trained personnel capable of running advanced diagnostic devices are difficult to find. Meanwhile, there is a growing need for cost-effective, compact, light-weight, and high performance diagnostic devices, equipped with advanced technologies, that can be used with minimally trained personnel to accurately analyze various bodily fluids such as blood, urine, saliva, sputum, sweat, etc. Analysis of these samples for diagnostic and sensing purposes requires a high-throughput platform that can specifically and accurately identify the characteristic signatures of target cells, bacteria or other bio-markers among thousands to millions of other micro- to nano-scale objects. To provide a solution to these problems, within this proposal timeline (1 Year total) we developed a new lensless on-chip imaging platform, termed LUCAS, to enable both diagnostic and sensing capabilities within a cost-effective hand-held unit that is specifically suitable for the battlefield settings. We established the proof of concepts of this imaging modality to achieve reliable and repeatable quantified whole blood analysis and compared our results against commercially available blood analyzers. Furthermore, we investigated the fundamental limits on the size of the detectable cell/bacteria with this platform. These accomplishments resulted in several refereed journal and conference articles. This research effort included one postdoctoral scholar as well as one graduate student working under the leadership of the PI.
Summary of our Achievements on Lensfree Holographic Imaging and Blood Analysis on a Chip:

Lenses for several decades have been helping detectors (analog or digital) to operate at the lowest possible space-bandwidth product that is determined by the desired FOV and the resolution of the image. However, the recent digital revolution, driven mostly by consumer electronics market, has already advanced the state of the art for digital imagers such that a 2D space-bandwidth product of >10-20 Million is readily available nowadays. This implies that today’s detector arrays are now much better suited to handle the information distortion caused by diffraction, which may then raise questions on the absolute necessity of the use of lenses in optical imaging. Moreover, today’s digital processors (such as GPUs) together with novel algorithms are also in much better shape to process, almost instantaneously, the acquired information at the detector end for taking the job of a physical lens. Therefore, we can conclude that the widespread use of lenses (or similar wavefront shaping elements) in optical imaging can now be replaced for several application needs by cost-effective, compact and much simpler optical architectures that compensate in the digital domain for the lack of complexity of optical components.

The fruits of this thinking have already appeared in the literature, where various lensfree on-chip imaging architectures were successfully demonstrated.[1-20] Among these approaches, lensfree digital holography deserves a special attention since with new computational algorithms and mathematical models,[21] it has the potential to make the most out of this digital revolution. For this end, as part of this funding, our group has recently led the way to a new incoherent holography platform termed LUCAS (Lensless Ultra-wide-field Cell monitoring Array platform based on Shadow imaging).[7-20]

LUCAS is fundamentally different from existing holographic approaches since it does not require a spatially or temporally coherent source. Instead, it works with an incoherent source (e.g., an LED) that is emanating from an unusually large aperture (e.g., 0.1mm). The key to the design of LUCAS is to realize that light waves pick up partial spatial coherence as they propagate, which implies that just over a few centimeters of free-space propagation they can effectively behave like a perfect coherent source for holographic imaging of micro-objects.[10] In its unique hologram recording geometry (see e.g., Fig. 1), LUCAS detects the holographic shadow signatures of the objects with unit magnification, which are then processed to enable digital recognition and microscopic imaging of the objects, achieving ~1-1.5 µm resolution over >20 fold larger FOV when compared to a conventional microscope objective-lens of similar resolution.[7,10]

There are several aspects of this LUCAS platform that makes it transformative for imaging and detection applications in especially field settings.

![Fig. 1](image-url) (a) A LUCAS based lensless holographic microscope that weighs ~45 grams (<1.6 ounces) is shown. It utilizes a simple LED source (at 591 nm) with an aperture of ~50-100 µm in front of the source. The LED and the sensor are powered through USB connection from the side. This lensfree holographic microscope claims the entire active area of the sensor as its imaging field-of-view (FOV ~ 24 mm²), which constitutes >20 fold increase when compared to the FOV of a typical 10X objective-lens having a similar resolution. (b) Schematics of the holographic LUCAS microscope shown in (a). The target objects within the sample volume interact with the illumination light through scattering, absorption and refraction processes. This interaction then creates the holographic shadows of the objects on the digital sensor array, which contain their “fingerprints”, permitting digital recognition and microscopic image reconstruction within <1 sec. Drawing not to scale. Typical values: z₁~2-4cm, z₂<1-2mm, D~50-100 µm.
First, the light source in this holographic approach does not need to be a laser such that a spatially incoherent source like a simple LED can be used. This feature greatly simplifies the optical set-up, making it cost-effective and compact, as well as digitally cleaning the object holograms by eliminating the coherent speckle noise and substrate induced multiple-reflection effects (see Figs. 1-3).[7,10] Second, this on-chip holography approach is not hungry for spatial coherence and therefore does not require a small aperture size for illumination which improves the light throughput of the imaging system by orders-of-magnitude without causing an issue for hologram pattern analysis or digital image reconstruction. This large aperture size (e.g., 50-100 µm - Fig. 1) also makes it robust to mechanical misalignments or potential clogging problems permitting a long time of operation without imaging artifacts or the need for realignment. Third, because of its unit magnification (which is compensated entirely in the digital domain to achieve sub-pixel resolution), we can image a much larger field-of-view, typically by >20-100 fold than a conventional optical microscope (see e.g., Figs. 3-4). Fourth, apart from reconstructing microscopic images of objects through holographic processing, we can also detect a unique 2D holographic texture (i.e., a fingerprint) corresponding to each object, which provides an alternative source of digital information that complements the reconstructed object images. Through pattern/texture analysis of such holographic object signatures (both phase and amplitude) it is possible to recognize the type and the state of each object of interest without digital reconstruction [17,20], which is especially important for automated high-throughput detection/analysis applications.

This is an entirely new direction in lensfree holography which treats the amplitude and the phase of object holograms as fingerprints rather than data to be reconstructed. A major advantage of such an approach is that correlations calculated in the hologram domain are more sensitive than the image plane, especially for micro-scale
objects that are imaged with a unit-magnification lensfree system. This important feature of lensfree holographic imaging enabled us to achieve sub-pixel resolution of ~1.4µm with unit-magnification using a pixel size of ~2.2µm over an FOV of ~24mm²,[10,13] which would have been theoretically impossible for any lens-based system. Therefore, correlations and transformations occurring in the hologram domain exhibit several advantages toward high-throughput detection/analysis of target objects with significantly improved space-bandwidth products.

In addition to these, our group has also hit several other major milestones using the LUCAS platform. In particular, we achieved lensfree on-chip fluorescent imaging of labeled cells and particles over an ultra-wide FOV of >8 cm² [16,18] as well as differential interference contrast (DIC) imaging,[10,13] i.e., Nomarski phase contrast microscopy, within the same LUCAS platform (see Fig. 4). Furthermore, we succeeded in detection of various bacteria such as *E. Coli* or *Giardia lamblia*, [14] as well as imaging of blood cells including platelets, monocytes, granulocytes, lymphocytes, and red blood cells.[10,17] Beyond wide-field microscopy, the LUCAS platform also enabled automated high-throughput cytometry by counting red and white blood cells with an accuracy of <5%, each, as well as quantification of the hemoglobin content of whole blood within the same lensfree platform.[17]

Furthermore, we have also we combined lensless holographic imaging with antibody microarrays for rapid and multiparametric analysis of whole blood samples on the same chip.[11] For this purpose, monoclonal antibodies specific for leukocyte surface antigens (CD4 and CD8, both of which are especially important for immunity assessment) and cytokines were printed in an array so as to juxtapose cell capture and cytokine detection antibody spots. Lensfree holographic on-chip imaging was then used to rapidly enumerate CD4 and CD8 T-lymphocytes captured on antibody spots and to quantify the cytokine signal emanating from IL-2, TNF-α and IFN-γ spots on the same chip.[11]

And finally, we also demonstrated, for the first time, the use of nano-structured surfaces [8] for lensfree on-chip microscopy to further improve the resolution. In this nano-structured on-chip imaging modality, the object of interest is directly positioned onto a nano-structured thin

![Fig.4](image-url)
metallic film, where the emitted light from the object plane, after being modulated by the nanostructures, diffracts over a short distance to be sampled by a detector-array without the use of any lenses. The main function of the nano-structured surface is to encode the spatial resolution information into far-field diffraction patterns that are recorded in a lensfree configuration. This spatial encoding process is calibrated after the fabrication of the nano-structured surface, by scanning a tightly focused beam on the surface of the chip. For spatially incoherent imaging (e.g., for fluorescent objects on the chip) these calibration frames provide a basis which permits spatial expansion of any object distribution as a linear combination of these calibration images. Fortunately, calibration of a given structured chip has to be done only once, and any arbitrary incoherent object can be imaged thereafter using the same set of calibration images. Through a compressive sampling algorithm,[8,16] we decoded this embedded spatial information and demonstrated decomposition of a lensfree diffraction pattern into microscopic image of an incoherent object located on the chip with a sub-pixel spatial resolution of ~2µm. This imaging modality based on nano-structured substrates would especially be useful to create high-resolution lensfree fluorescent microscopes on a compact chip that could be used for specific imaging of labeled blood cells in resource poor settings.

References Cited