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13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT This DURIP proposal purchased a lens-based scanning nanoscopy system to significantly improve the speed of progress in our existing ARO project, which designs field-portable and cost-effective lensfree imagers to detect nano-particles and viruses across a very large field-of-view. While this wide-field imaging capability is very exciting, especially for high-throughput nanoscopy needs in field-settings, it also creates significant bottlenecks for cross-validation and calibration of our results because existing lens-based nanoscopy tools, without automated scanning, have extremely limited FOVs. This necessitates several hundred images to be captured using a lens based

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Final Report - Summary

This DURIP was used to purchase a lens-based scanning optical nanoscopy system to supplement and significantly improve the speed of progress in our existing Army Research Office (ARO) project (W911NF-13-1-0419 – PI: Dr. Ozcan), which is titled “Ultra-wide field imaging of single nano-particles, viruses and bacteria using field-portable computational on-chip microscopy and self-assembled liquid nano-lenses”. In this currently existing ARO project, we utilize stable and biocompatible thin wetting films to self-assemble aspheric liquid nano-lenses around individual nano-particles or other nano-objects of interests, including viruses, to be able to detect ultra-weak signatures of these sub-wavelength objects using a portable on-chip microscope. This approach, when combined with digital holography and lensfree imaging, enables us to image and detect sub-50 nm particles (i.e., smaller than $\lambda/10$ – one tenth of the wavelength of the illumination light) using field-portable and cost-effective imaging designs, across a very large field-of-view (FOV) of $>20\text{-}30\text{ mm}^2$, i.e., about three orders-of-magnitude larger than other nano-imaging techniques. While this wide-field computational imaging capability of our technique is very exciting, especially in a cost-effective and portable design (making it suitable for use in field settings), it also creates a significant bottleneck for comparison and validation of our results because existing lens-based nanoscopy tools, without automated scanning features, have extremely limited FOVs – typically less than 0.05 mm^2 per image. This necessitates several hundred different optical images to be captured using a nanoscope to validate one of our self-assembly enabled lensfree reconstructed images.

This DURIP proposal solved this bottleneck in comparison, cross-validation and training of our reconstructed lensfree images at the nano-scale by purchasing a lens-based scanning nanoscope that can rapidly screen large sample areas for detection and quantification of nanoscopic objects, e.g., sub-100 nm particles and viruses across large FOVs ($> 20\text{-}30\text{ mm}^2$). The purchase of this scanning nanoscopy platform significantly assists us in large scale cross-validation efforts for our self-assembly based lensfree imaging of organic and inorganic nanoparticles, mono-disperse samples, complex poly-disperse mixtures, cultured viruses and sub-500 nm bacteria and will help us build gold standard image libraries to compare and extract the lensfree image signatures for these nano-scale objects of interest. Without an automated scanning nano-scale imaging interface, existing benchtop optical microscopes that we have in our lab cannot be efficiently used to test and validate our new lensfree nanoscopy techniques because of their limited resolution, detection sensitivity, scanning speed and precision.

While we have a dedicated optical microscope in our lab that we frequently use for micro-scale imaging of specimen, besides being heavily used and overbooked, this existing optical microscope is optimized for micro-scale imaging and cannot be used for high-throughput and high-speed detection of nano-scale objects due to its limited detection sensitivity, resolution and precision. Therefore, none of the existing optical microscopy tools that we have in our lab can truly address the demanding needs of our ARO project for capturing gold standard nanoscopic images of sub-wavelength specimen across very large FOVs that are imaged using our self-assembly enabled lensfree on-chip microscopes.

In addition to these, this newly purchased automated scanning optical nanoscope is also being used as part of Dr. Ozcan’s graduate and undergraduate courses on BioPhotonics, where the students (in

groups) will have some hands-on experience and demonstrations toward high-throughput nanoscopy. The same instrument will also be used as part of our outreach activities while hosting e.g., high-school students in the form of science demonstrations.

Furthermore, undergraduate researchers who work in Ozcan's lab are also given access to this scanning nanoscopic imaging system for their training and research projects. To set the background, every quarter Dr. Ozcan's lab has >40 undergraduate researchers (<http://org.ee.ucla.edu/undergraduate-research/>) who work in teams on bio- and nano-photonics related projects, and are mentored by senior graduate students as well as postdoctoral scholars of Ozcan lab. These undergraduate researchers routinely publish with us in refereed journal articles and conference proceedings, and they have so far co-authored more than 60 journal articles and more than 150 conference proceedings on their research projects in our lab. This massive volume of trainees and researchers in our lab also creates a bottleneck in terms of availability and scheduling of our existing optical imaging instruments, and that is why the newly acquired automated scanning nanoscopic imaging system is very timely and important for our lab to improve the productivity of our research projects.

In summary, the purchase of this scanning nanoscope with a very sensitive, high-speed and precise automated imaging interface is helping us better compare and validate our self-assembly based wide-field lensfree nano-imaging techniques, funded by ARO W911NF-13-1-0419, while also providing an important infrastructure for other DoD funded projects as well as education and training of undergraduate and graduate students at UCLA.

The details of the purchased imaging equipment

Our new microscope is based on the IX83P2F two deck epi-imaging platform from Olympus. The microscope base is fully customizable for various imaging needs. Our microscope utilizes its expansion slots by incorporating a motorized fluorescent filter turret, and an IX3-CAS magnification changer (1 \times , 1.5 \times , and 2 \times) into the body. The fluorescent turret is equipped with 8 filter sets to detect various fluorescent dyes spanning over the visible spectrum (Table 1)

Filter set	Excitation [nm]	Emission [nm]
DAPI-5060C	377	447
FITC-2024B	485	522
CY3-4040C	531	593
CY5-4040C	628	660
QD625-C	435	625
TXRED-4040	562	624
LF405-LP	405	Visible (Qdot)

Table 1: Fluorescent filter sets in the microscope system

The microscope houses two illumination units: a 100W halogen light bulb for transmission microscopy, and a 130W mercury lamp for fluorescence applications. The fluorescent illumination uses light guide

coupling, and houses the mercury bulb in a standalone unit yielding to an extended bulb lifetime of 2000 hours.

The microscope can be equipped with either a long working distance condenser with NA of 0.55 for taller specimen or it can also house the U-UCD8-2 universal condenser unit, with a maximum NA of 0.95 (dry), and 1.4 (oiled) for imaging thin specimen. Both condensers can be used for phase contrast imaging as well. The universal condenser unit can also be used for dark field observations.

The microscope is equipped with an autofocus/z-drift compensation system (IX3-ZDC2) utilizing an infrared laser to determine and keep the focus during imaging. The microscope sample stage is also fully motorized with a travel range of 114mm × 75mm, and an accuracy of 0.06 μm/mm. The stage is housing a universal sample holder for microscope slides and small petri dishes. For live cell imaging applications it can hold our INUF-IX3D-F1 incubation system, capable of maintaining a controlled temperature and air-gas mixture around a petri dish or a 96 wellplate.

The microscope is equipped with two cameras: a Qimaging Retiga 4000R cooled CCD for color brightfield and fluorescent readout, and a Hamamatsu Orca-Flash 4.0V2 low noise, monochrome scientific CMOS camera for low intensity fluorescent imaging applications.

We have two sets of the highest quality microscope objectives offered by Olympus, the UPLSAPO series featuring super apochromatic lenses, and a plan fluorite long working distance set for phase contrast imaging (Table 2).

Objective	Magnification	Numerical Aperture	Phase contrast
UPLSAPO4X	4×	0.16	N
UPLSAPO10X2	10×	0.4	N
UPLSAPO20X	20×	0.75	N
UPLSAPO40X2	40×	0.95	N
UPLSAPO100XO	100×	1.4	N
UPLSAPO100XOPH	100×	1.4	Y
UPLFLN4XPH	4×	0.13	Y
UPLFLN10X2PH	10×	0.3	Y
UPLFLN20XPH	20×	0.7	Y
LUCPLFLN40XPH	40×	0.6	Y

Table 2: Available objectives in the microscope

The microscope is controlled by the Metamorph software. It is capable for fully automated long term imaging and scanning of the specimen automatically. Multi-dimensional acquisition schemes can be set up, such as sequential imaging with multiple fluorescent illuminations, X-Y-Z-scanning, timelapse imaging, or any combination of these. The software also incorporates powerful image processing tools, i.e. for stitching the images, 3D deconvolution with objective PSF, etc.

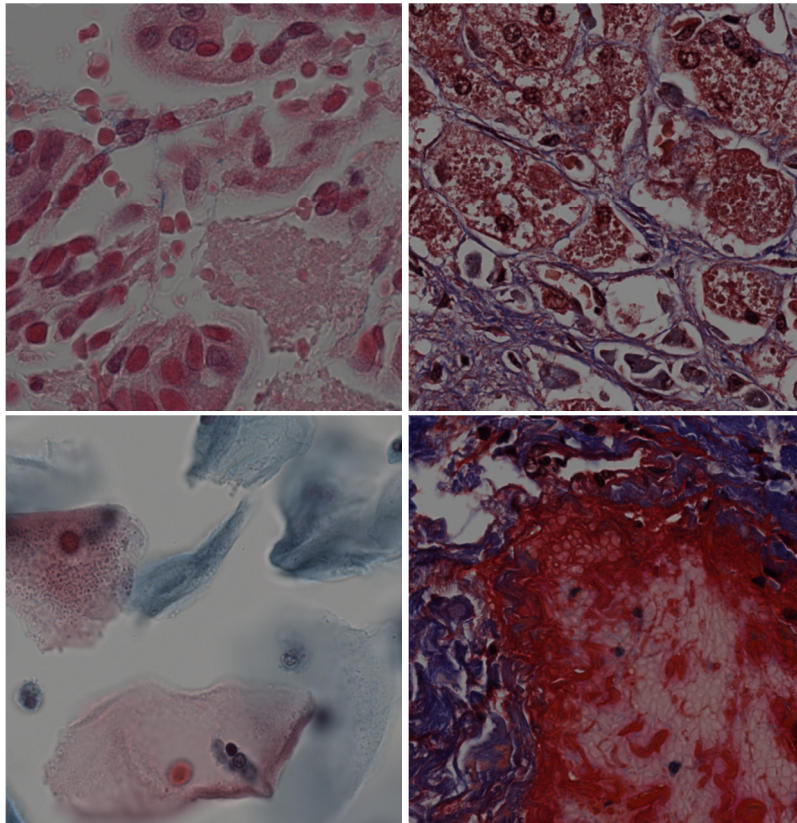


Figure 1: Bright field sample images of stained tissue samples

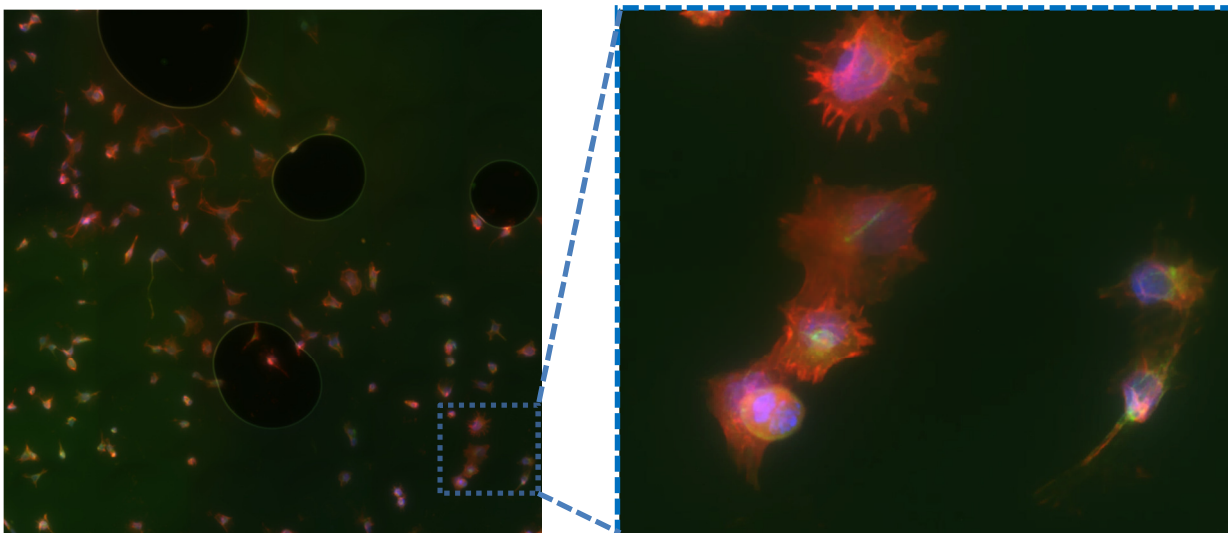


Figure 2: Triple stained merged fluorescent image captured by the microscope

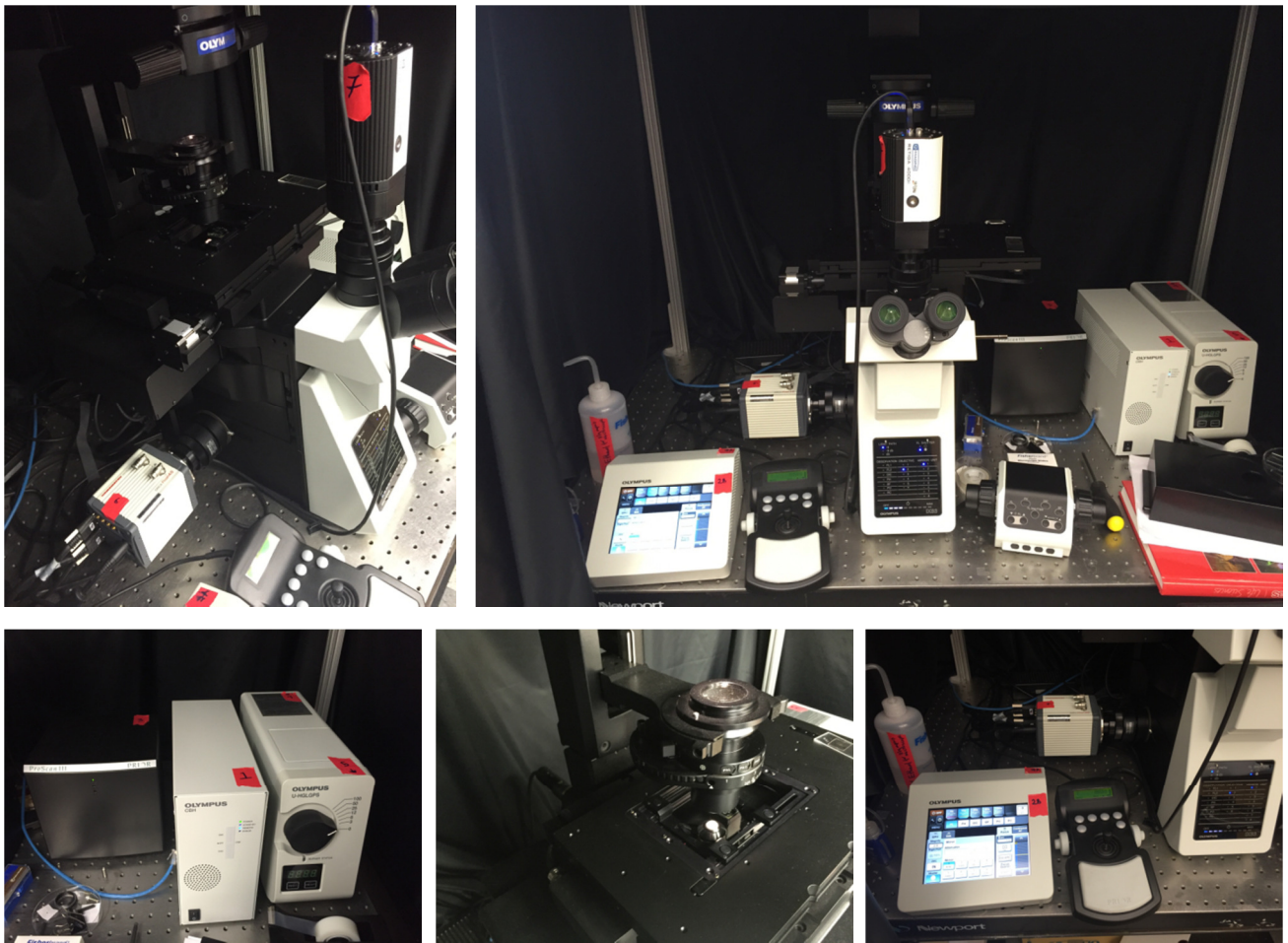


Figure 3: (TOP) Photos of the IX83 microscope system. (Bottom) Images showing the fluorescent illumination unit, the long working distance condenser unit, and the system controllers, respectively.