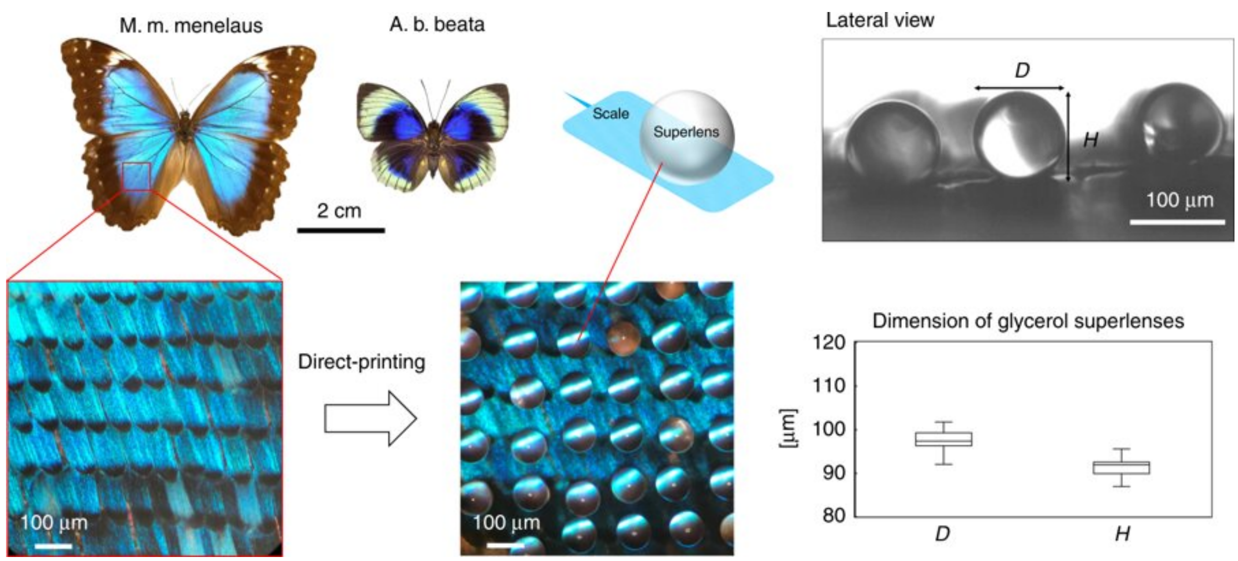
PHYS.ORG

Biophotonics: In situ printing liquid superlenses to image butterfly wings and nanobiostructures

January 31 2019, by Thamarasee Jeewandara

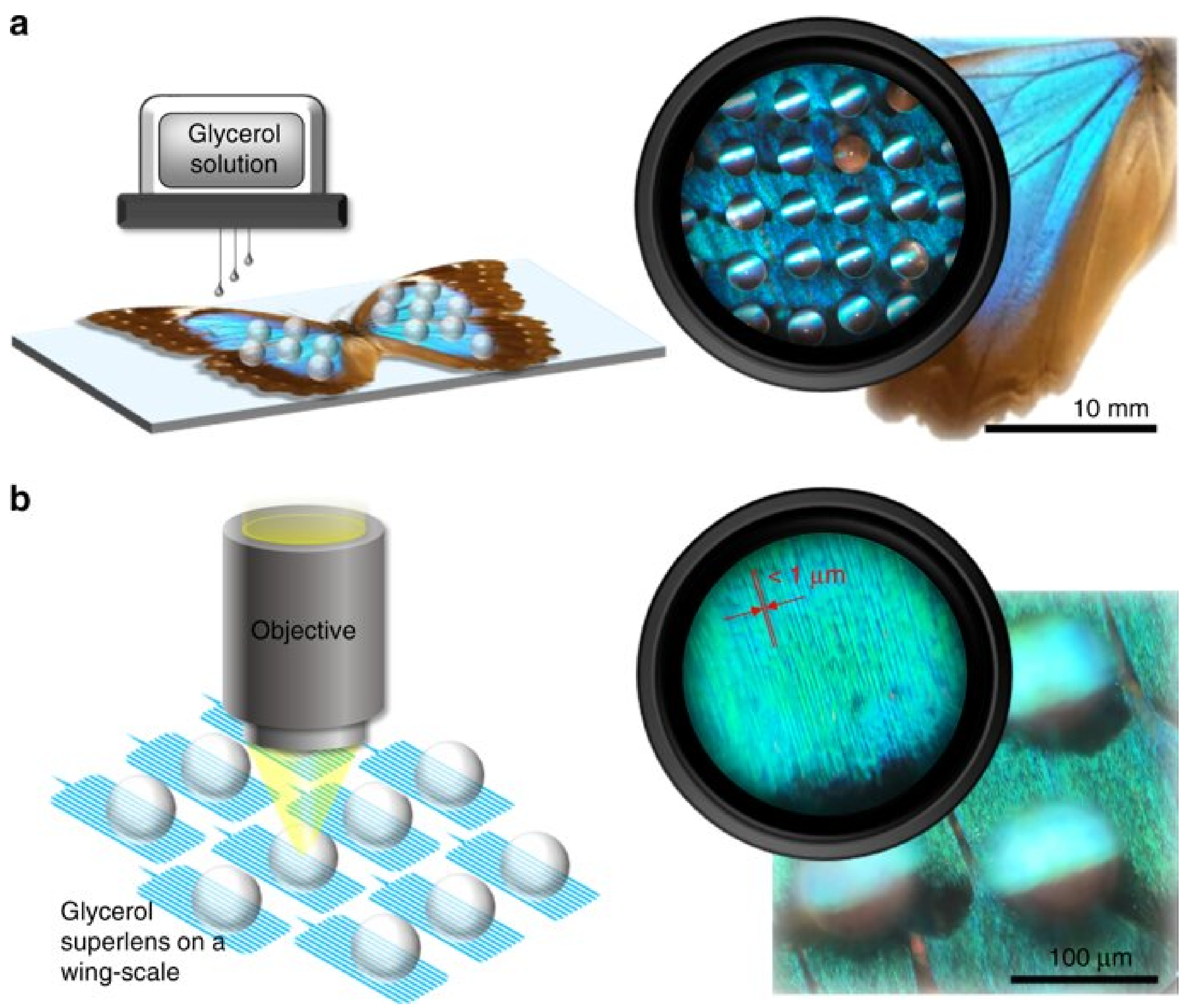


Schematic of the subdiffraction-limited imaging of a butterfly sample using in situ printed glycerol superlenses. The Morpho menelaus menelaus (M. m. menelaus) and Agrias beatifica beata (A. b. beata) samples were placed flat on a clean glass slide for printing. The microscopic images show the scale arrangement of the ventral wing of M. m. menelaus (bottom left) and the superlens array printed on the wing scales (middle). The superlenses exhibited a sphere-like geometry on the wing scales. The lateral image (upper right) was acquired using the inverted microscope (Nikon, Ti-E). The dimension statistics include data from 13 measured lenses based on their lateral images. Credit: Microsystems & Nanoengineering, doi: https://doi.org/10.1038/s41378-018-0040-3

Nanostructures and natural patterns have long fascinated researchers in bioinspired materials engineering. Biological samples can be imaged and observed at the nanoscale using sophisticated analytical tools in materials science, including scanning electron microscopy (SEM) and transmission electron microscopy (TEM). While imaging methods contribute to the understanding of structures by revealing material properties for biomimetic materials synthesis, they have often done so with the loss of photonic properties inherent to the materials.

In a new method, materials scientists Boliang Jia and colleagues at the departments of mechanical engineering and robotics presented a printable biocompatible superlens placed directly on objects of interest to observe subdiffraction-limited features (resolution beyond the diffraction limit). They then viewed the natural features using an optical microscope to demonstrate nanoscale imaging of butterfly wings in color. The study allowed super-resolution imaging and a larger field of view (FOV) compared to the previous dielectric microsphere-based optical systems of super-resolution microscopy.

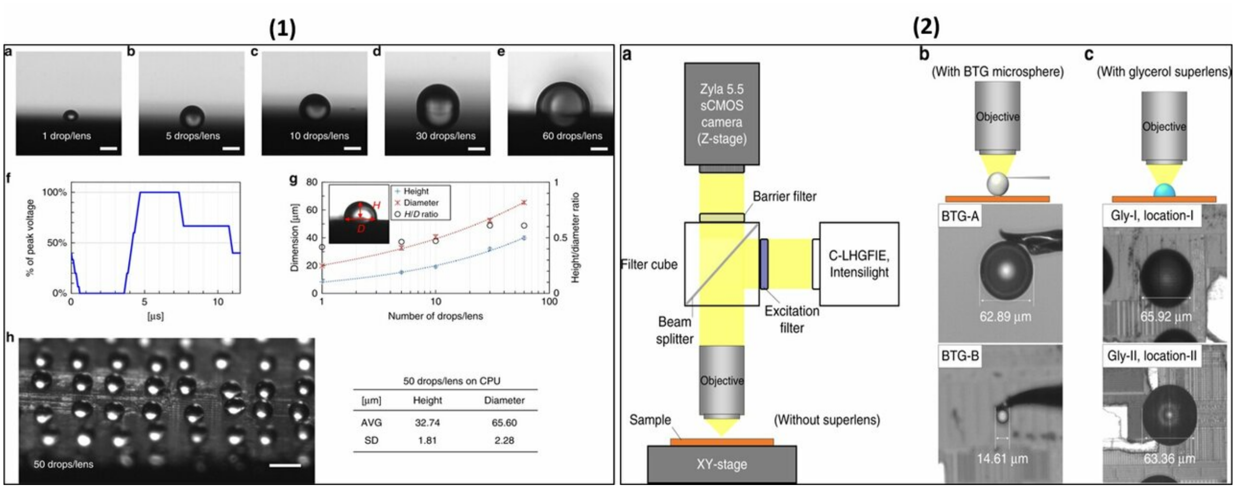
The new approach created a fast and flexible path to observe the direct colors of biological features at the nanoscale in the visible range. The results are now published in *Microsystems and Nanoengineering*, where the work allowed optical measurements at the subdiffraction-limited scale. A superlens is based on an optical material with a negative index of refraction (optical metamaterials) that could experimentally reverse nearly all known optical phenomena. Technically, a thin negative-index film can function as a 'superlens' to provide image detail with a resolution beyond the diffraction limit to which all positive-index lenses are subjected to.



In situ printing glycerol superlenses for nanoscale imaging of butterfly wings. a) Illustration of the printing process and a microscopic view of the formed superlens array on the wing scales. b) Conceptual image of the direct nanoscale observation of butterfly wing scales via superlenses, and the magnified image obtained through the superlens indicating a resolution of features with sizes less than 1 µm on the wing scale. Credit: Microsystems & Nanoengineering, doi: https://doi.org/10.1038/s41378-018-0040-3

In the study, Jia et al. devised a method to print glycerol (transparent liquid) on to butterfly wings and observe nanoscale wing structures hitherto unobserved via conventional optical microscopes. The work will pave the way for advanced liquid superlenses coupled with fast and flexible methods in optics. The results will assist nanostructural inspection via biophotonics in biological and non-biological samples.

The butterfly wings of Morpho cypris were first observed via high-resolution SEM in 1942, which led to the discovery of detailed structures below the diffraction limit using sophisticated tools. Since then, Morpho butterflies have been a subject of interest in bioinspired materials research due to their iridescent color and distinct photonic properties. For decades, the properties of light interference resulting from their brilliant nanostructures have attracted great interest in nanophotonics and biomimetic materials research. However, direct optical observations of the subdiffraction-limited structure of the wings at the nanoscale yet remain to be reported.

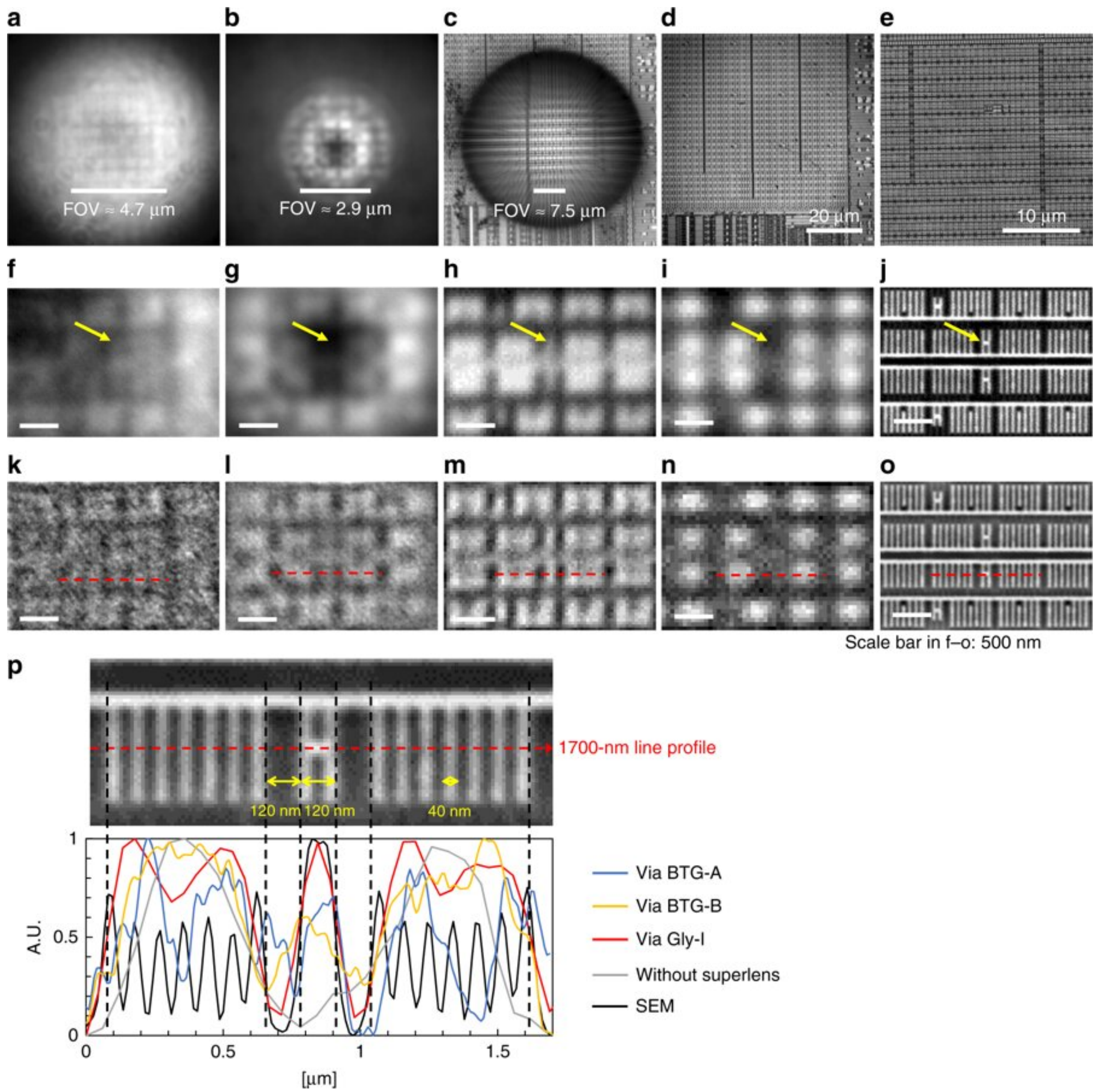


(1) Characterization of printed glycerol superlenses with different numbers of drops/lens. a–e) Lateral images of glycerol lenses with 1, 5, 10, 30, and 60 drops/lens on a clean silicon wafer. f) The jetting waveform used in the experiment. g) Plots of lens height (blue cross), diameter (orange star), and H/D ratio (black circle) with respect to the number of drops/lens. h) An on-chip

printed glycerol superlens array (50 vol%, 50 drops/lens) observed via a 4× (NA 0.10) objective at a 45° angle-of-view using a Nikon, Ti-E microscope (left). The table (right) shows the dimension statistics. Scale bar: a–e 20 µm, h 100 µm. (2) Configurations of the experimental setup a) Schematic of the imaging system based on the Nikon Ni-E platform without the use of a superlens. The major components include an Andor Zlya 5.5 sCOMS camera with a motorized focusing stage (Z), an Intensilight mercury-fiber illuminator (C-LHGFIE), a filter cube, an objective, and a motorized sample stage (XY). b) The configuration with a BTG microsphere (top) and the optical images of two BTG microspheres, BTG-A (middle) and BTG-B (bottom), mounted on a microprobe (5 µm tip diameter) with NOA63 (Norland) adhesive. c) The configuration with a printed glycerol superlens (top) and the optical images of two lenses printed at location-I (middle) and location-II (bottom) of the CPU samples. Credit: Microsystems & Nanoengineering, doi: https://doi.org/10.1038/s41378-018-0040-3

High-refractive-index microspheres in aqueous media have attracted great interest in recent years for observing liquid-immersed biological samples such as biological cells in vivo. Yet, the method is not favorable for samples with high-refractive index in dry conditions. In the present work, Jia et al. presented an in situ printed biocompatible glycerol superlens (SL) with higher resolution and larger FOV than barium titanate glass (BTG) microspheres under dry conditions. The scientists chose glycerol since it is a transparent liquid with a relatively high refractive index that is capable of printable droplet formation across a wide size range.

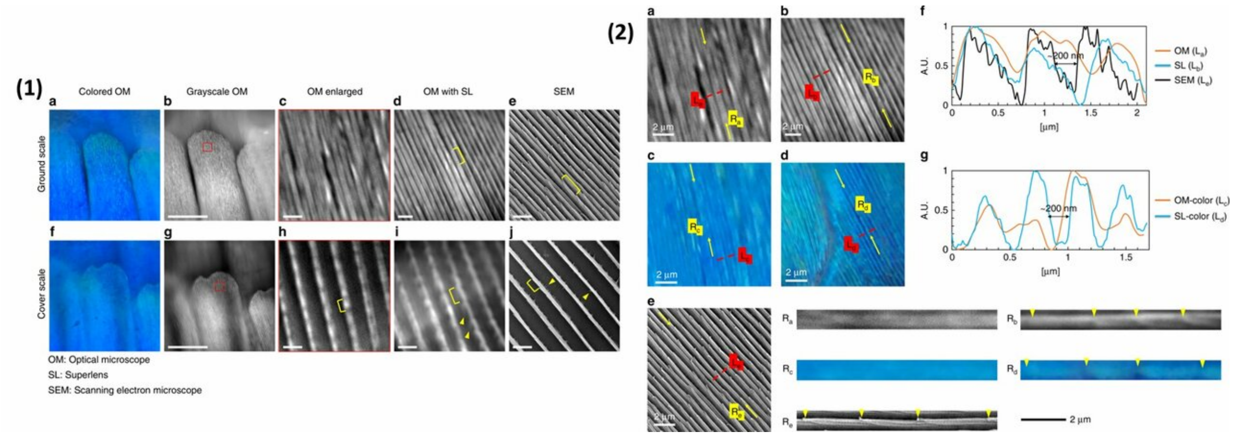
As an important feature, glycerol contains strong inter-molecular interactions and is therefore highly resistant to evaporation. Although microdroplets of water typically evaporate almost instantly, by comparison, glycerol printed as droplets with a volume of 50 percent could exist at least for a day on substrates without significant size changes. Jia et al. therefore directly printed glycerol superlenses on a Morpho butterfly wing using an ink-jet printing machine. Thereafter, they characterized the glycerol images using a central processing unit (CPU)-integrated circuit (IC). The scientists observed nanobiostructures ranging from 50 nm to 200 nm in scale. In the work, the scientists adjusted the viscosity of the glycerol solution via dilution tests with MiliQ water to select an optimal concentration of 50 volume percent (50 vol%) for printing.



Experimentally acquired images at location-I on the CPU sample. a–d) Optical images taken via BTG-A (a), BTG-B (b), Gly-I (c), and without a superlens (d). The objective used was 100× (NA 0.90). The estimated fields-of-view (FOVs) in a, b, and c are 4.7, 2.9, and 7.5 μm in diameter, respectively. e) The SEM image over the same area. f–j) Enlarged images over an approximate area of 3.9 μm × 2.7 μm from the center of a–e, respectively. The yellow arrows point to an "H"-like pattern approximately 120 nm in width. k–o) Bandpass-filtered images of f–j, respectively. The scale bar in f–o: 500 nm. p) Profiles of the red lines in k–o with normalized intensity. The 1700-nm line profiles are aligned with the features in the SEM image above. Credit: Microsystems & Nanoengineering, doi: https://doi.org/10.1038/s41378-018-0040-3

In optics, solid immersion lenses (SILs) can enhance optical resolution by increasing the effective numerical aperture (NA) of the imaging medium. The droplet lens is considered a liquid version of SILs with a flawless surface. The scientists first characterized the printed glycerol superlenses in the study using a different number of drops per lens on a clean silicon wafer before administration on butterfly wings. They selected the ideal number of drops per lens after a few trials; the resulting diameters of the glycerol lenses were comparable to BTG microspheres. Thereafter, they compared configurations of the experimental setup for the BTG microspheres and the glycerol superlens. The work showed that large BTG microspheres provided a large FOV, while a higher resolution was obtained with smaller BTG microspheres.

When the scientists compared the images obtained with glycerol superlenses and those obtained using BTG, the results significantly improved in uniformity for images obtained using glycerol superlenses, alongside sharper nanoscale features. This implied that printed glycerol superlenses offered superior resolution capacity compared to BTG microspheres of equal and smaller sizes in air.



(1) Comparison of images of the M. m. menelaus ventral wing scales. Color images a and f were taken from the eyepiece using an iPhone 7 Plus camera. Grayscale images b–d and g–i were taken with an Andor Zyla5.5 sCMOS camera. Images e and j were taken by SEM; a–e are images of ground scales; f–j are images of cover scales; and c and h are the enlarged images of the red square areas in b and g, respectively. Yellow brackets indicate one of the lamellae tips on the ridges. All optical images were taken under a 100× (NA 0.90) objective. (2) Analysis with color images of sub-diffraction-limited structures. Ground scales of M. m. menelaus. a-d) were taken from the eyepiece using an iPhone 7 Plus camera without and via the glycerol superlens. Line profiles over the red dashed lines in a–e are shown in f and g. The ridges marked by yellow arrows were enlarged and are shown in the lower right. The inverted yellow rectangles mark the identified lamella tips along each enlarged section of the ridges. For ridges Ra and Rc imaged without the superlens, no lamella tips could be distinguished. The labels “La–e” correspond to line profiles, and labels “Ra–e” correspond to the enlarged ridges. Scale bar: 2 μm. OM optical microscopy, SL superlens, SEM scanning electron microscopy. Credit: Microsystems & Nanoengineering, doi: https://doi.org/10.1038/s41378-018-0040-3

In their work, Jia et al. observed two types of butterflies: Morpho Menelaus and Agrias beatifica beata. The scientists printed 60 glycerol drops (or lenses) on the butterfly samples to obtain spherical lenses approximating 95 µm in diameter. They observed the wing-scale features via an upright microscope system. The scientists were able to capture the ventral wing scales of the butterflies, where the Morpho species displayed two types of wing scales; ground and cover scales.

In comparison to SEM, glycerol superlenses were unable to resolve complete structures entirely, but they showed the existence of substructures between the ridges of butterfly wings. For example, Jia et al. showed that in situ glycerol superlenses could extend the limit for nanoscale structures in biological samples to approximate 200 nm in width. Additional experiments showed the ability to color-image subdiffraction-limited nanobiostructures using the superlenses.

The new method offers a cost-effective, fast and high-resolution imaging technique to visualize subdiffraction-limited nanobiostructures in situ. The work paves the way for water-immiscible liquids with high refractive indices to print liquid superlenses for water-immersion-based imaging applications. Biocompatible liquids such as silicone oil can be explored as superlenses underwater via low cost ink-jet printing next. Materials scientists continue to work toward engineering advanced liquid superlenses in nanobiophotonics. The scheme introduced by Jia et al. provides a fast and easy-to-implement strategy to observe nanobiostructures in biological and nonbiological samples.

More information: Boliang Jia et al. In situ printing of liquid superlenses for subdiffraction-limited color imaging of nanobiostructures in nature, *Microsystems & Nanoengineering* (2019). DOI: 10.1038/s41378-018-0040-3

Thomas F. Anderson et al. An Electron Microscope Study of Some

Structural Colors of Insects, *Journal of Applied Physics* (2005). DOI: 10.1063/1.1714827

Jingyun Huang et al. Controlled Replication of Butterfly Wings for Achieving Tunable Photonic Properties, *Nano Letters* (2006). DOI: 10.1021/nl061851t

Radwanul Hasan Siddique et al. Theoretical and experimental analysis of the structural pattern responsible for the iridescence of Morpho butterflies, *Optics Express* (2013). DOI: 10.1364/OE.21.014351

Pete Vukusic et al. Erratum: Photonic structures in biology, *Nature* (2003). DOI: 10.1038/nature01941