Smaller, denser, better illuminators for computational microscopy

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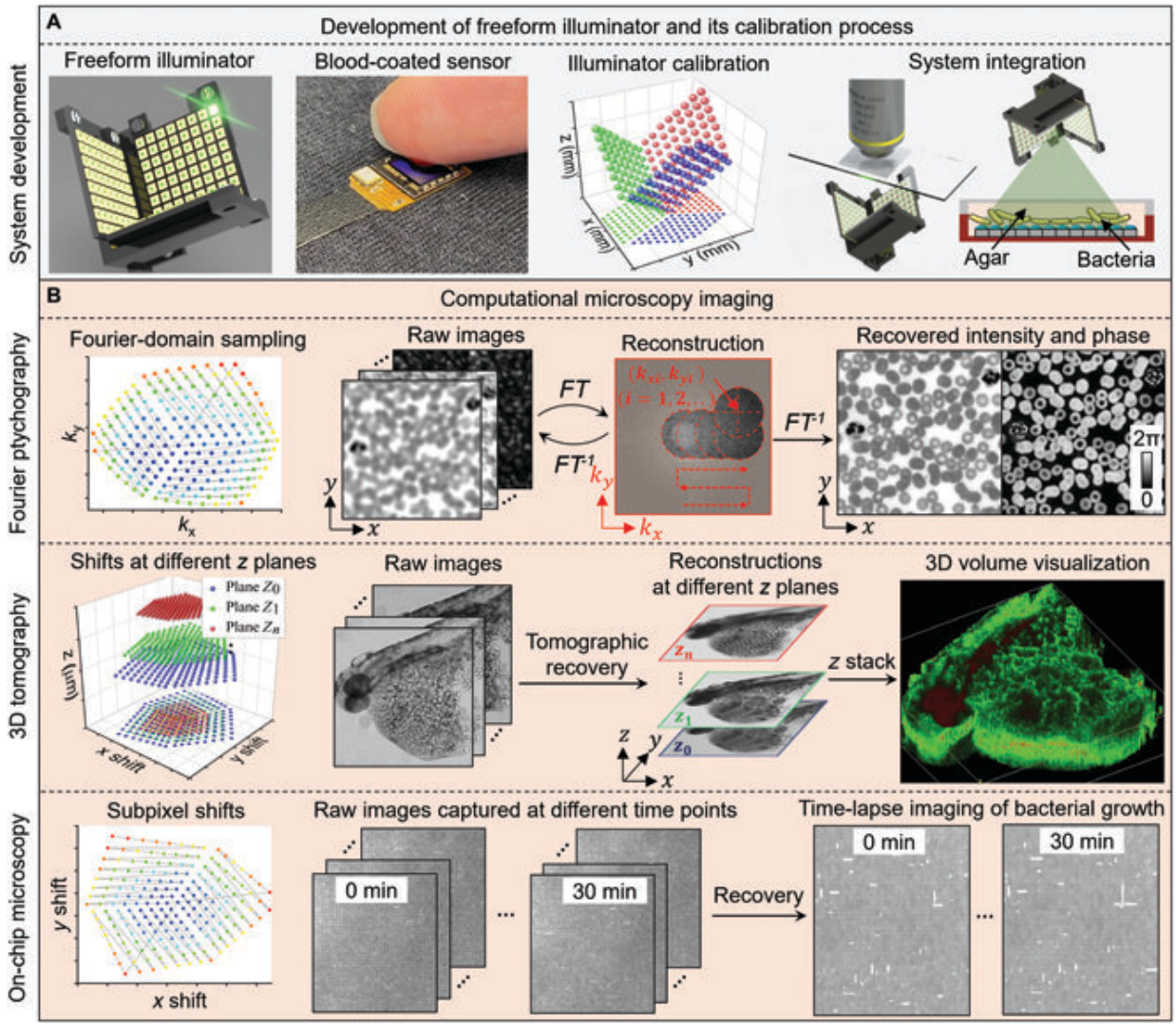

(A) The development of an illuminator and its calibration process. A blood-coated image sensor was used to determine the 3D light source positions. (B) The illuminator was used for Fourier ptychographic microscopy (first row), 3D

tomographic imaging (middle row) and on-chip microscopy (bottom row). Credit: Pengming Song et al.

Seeking to expand the possibilities offered by programmable illumination, a group of researchers at the University of Connecticut developed a strategy for constructing and calibrating freeform illuminators offering greater flexibility for computational microscopy. Their calibration method uses a blood-coated sensor for reconstruction of light source positions.

They demonstrated the use of calibrated freeform illuminators for Fourier ptychographic microscopy, 3D tomographic imaging and on-chip microscopy and used a calibrated freeform illuminator in an experiment to track bacterial growth.

The group's research was published Feb. 20 in *Intelligent Computing*.

New possibilities for experimental setup using freeform illumination allow not only more flexibility but also greater efficiency: "With this platform, we can start to transit Petri-dish-based experiments from the traditional labor-intensive process to an automated and streamlined process," the research paper states.

Programmable light sources simplify the work of illuminating samples in different kinds of microscopy contexts, but conventional programmable arrays consist of a flat, fixed grid of individual lights. An array consisting of lights that can be placed anywhere in three dimensions offers experimenters the opportunity to reduce the size of the illuminator, to place the lights closer to the sample, and to increase the density of the lights and adjust the angle of illumination according to their specific requirements.

Freeform illumination can be further enhanced in the future with better calibration methods, denser lighting arrangements, lights with a greater diversity of wavelengths and adjusted position estimates.

The authors designed and built four different freeform illuminators: a tilted flat surface, a triangular pyramid, a dome and a Mobius band. The pyramid illuminator had several advantages. It was angled towards the sample, it had the highest number of lights at the point farthest from the sample and it could be placed very close to the sample to deliver light efficiently.

The authors calibrated the illuminators using a blood-coated sensor. Coating the sensor with a thin but dense layer of particles enabled the authors to calculate the position of each lighting element using ray tracing. The advantage of using human blood from a finger prick is that no sophisticated or expensive tools are needed. However, other substances with similar properties can be used in its place. The authors verified their calibration method, finding that the recovered positions matched the actual positions with only small deviations.

The authors demonstrated the use of their freeform illuminators in several experiments. All four illuminators were used successfully for Fourier ptychographic microscopy, a computational microscopy technique that combines different images produced with lighting at varying angles.

The pyramid illuminator was used to capture separate images lit with red, green and blue light which were combined to make a full-color image. It was also used for 3D tomography, a technique that builds a 3D image by stacking cross-section images of a 3D sample.

The pyramid illuminator was used for on-chip microscopy, a setup where the sample sits directly on the sensor, just like the blood smear in

the calibration step. Combining the images produced with separate lights in the freeform illuminator results in a final image with higher resolution than any of the raw images alone, which is why this technique is called super-resolution microscopy. One experiment produced a still image of blood cells, and another produced a time-lapse series of the growth of a colony of E. Coli bacteria on a block of agar.

Guoan Zheng conceived the idea and designed the experiments. Pengming Song and Tianbo Wang conducted the experiments. Their co-authors on this paper are Shaowei Jiang, Chengfei Guo, Ruihai Wang, Liming Yang and You Zhou.

More information: Pengming Song et al, Freeform Illuminator for Computational Microscopy, *Intelligent Computing* (2023). DOI: 10.34133/icomputing.0015

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