TUTORIAL

SCAPE 2.0 Microscopy

Speeding up light-sheet imaging of live biological specimens.

Light-sheet fluorescence microscopy offers advantages over point-scanning approaches like confocal or two-photon microscopy, allowing the rapid collection of large volumes of data with less photobleaching of fluorophores. But light-sheet approaches commonly involve awkward, multiple-objective setups at the sample that make it difficult to change imaging orientations, limit the kinds of samples that can be studied, and prevent the rapid data capture necessary to image "at the speed of life."

An alternative developed by OSA Fellow Elizabeth Hillman of Columbia University, USA, Swept Confocally Aligned Planar Excitation (SCAPE) microscopy, uses clever geometry and optics to get around these limitations, allowing imaging of live animals at blistering speeds. Hillman and colleague Citlali Perez Campos walked OPN through the latest iteration of the system, dubbed SCAPE 2.0.

SCAPE targets the imaging of biological specimens "at the speed of life."

1. Laser sources

The SCAPE 2.0 layout begins with one or more laser sources, which provide the excitation light for the fluorophores embedded in the sample. At present SCAPE 2.0 can accommodate up to five different sources, allowing excitation of multiple fluorophores in a single live sample.

2. Shaping the beam

The Gaussian beam from the lasers, bright in the middle and dim at the edges, next passes through a Powell lens—a "really cool type of lens," says Hillman—that affords a more flat-topped profile to the eventual light sheet. The beam then moves through cylindrical lenses and an adjustable slit (to modify the light sheet's numerical aperture).

3. From galvo mirror to sample

A dichroic mirror next shunts the excitation light to an off-the-shelf, single-axis galvanometer mirror. In a sense, this mirror is the heart of the SCAPE 2.0 system, as its scanning motion both allows the light sheet to be swept through the sample and de-scans the returning fluorescence signal, removing the need to independently refocus detection optics on the light sheet as it moves.

The excitation light, bouncing off of the galvo mirror, passes through a telescope that images the galvo mirror onto the back focal plane of the single, primary microscope objective at the sample.

4. Sweeping the sheet

At the objective, the beam penetrates the sample as an angled sheet, exciting the fluorophores in the sample to emit light, which is returned back through the objective. Illuminating with a sheet, Hillman notes, boosts efficiency relative to techniques like confocal and two-photon microscopy, since "you're giving the photons numerous chances to interact with the tissue as they're traveling along the sheet."

Meanwhile, as the galvo mirror scans, the light is swept in parallel oblique sheets through the sample, capturing a 3D volumetric image. The simple galvo mirror setup, according to Hillman, allows scanning to proceed "incredibly fast," imaging at a rate of 100 data volumes per second with no other moving parts.

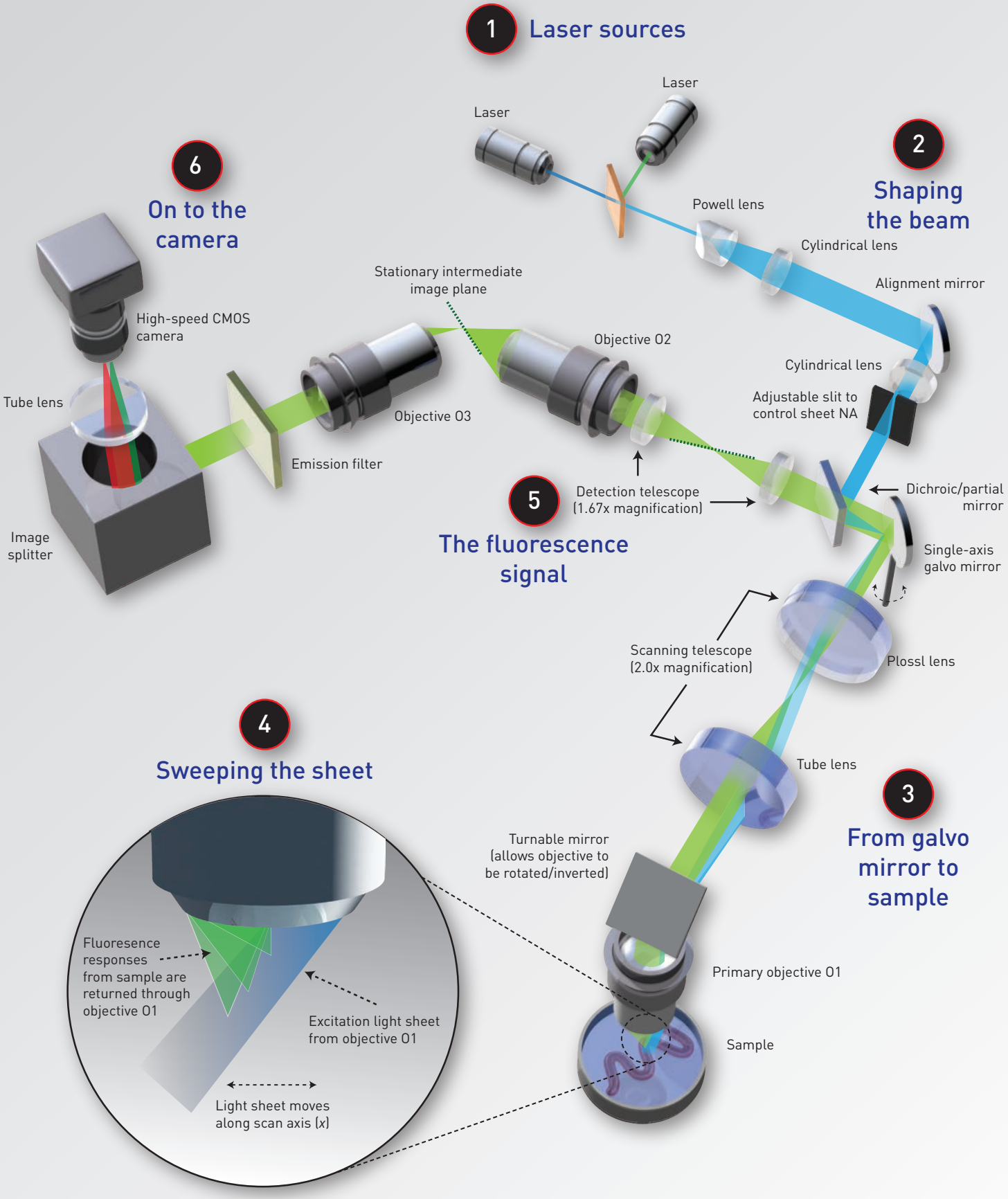
5. The fluorescence signal

The fluorescence light, returned through the objective, passes back through the telescope and reflects back off of the galvo mirror, to be sent through the dichroic to a detection telescope setup, which relays the light into the back of another objective lens.

This objective forms a focused, stationary image of the obliquely illuminated plane in the sample. A third objective lens, aligned to be focused on this oblique plane, effectively rotates the image so that its orientation is mapped to that of the camera focal plane.

6. On to the camera

Finally, an image splitter separates individual fluorescence color channels, and projects them onto different zones of a high-speed CMOS camera. The acquired oblique image planes can then be stacked to create striking 3D images of biology in action. Hillman's team has used SCAPE to image neuronal activity in crawling *C. elegans* worms, as well as the beating hearts of zebrafish and the mouse brain [see V. Voleti et al., Nat. Methods **16**, 1054 (2019)]. OPN



Source: E.M.C. Hillman; adapted from https://hillmanlab.zuckermaninstitute.columbia.edu / Art by Phil Saunders

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The SCAPE 2.0 system is described in detail in V. Voleti et al., Nat. Methods **16**, 1054 (2019).