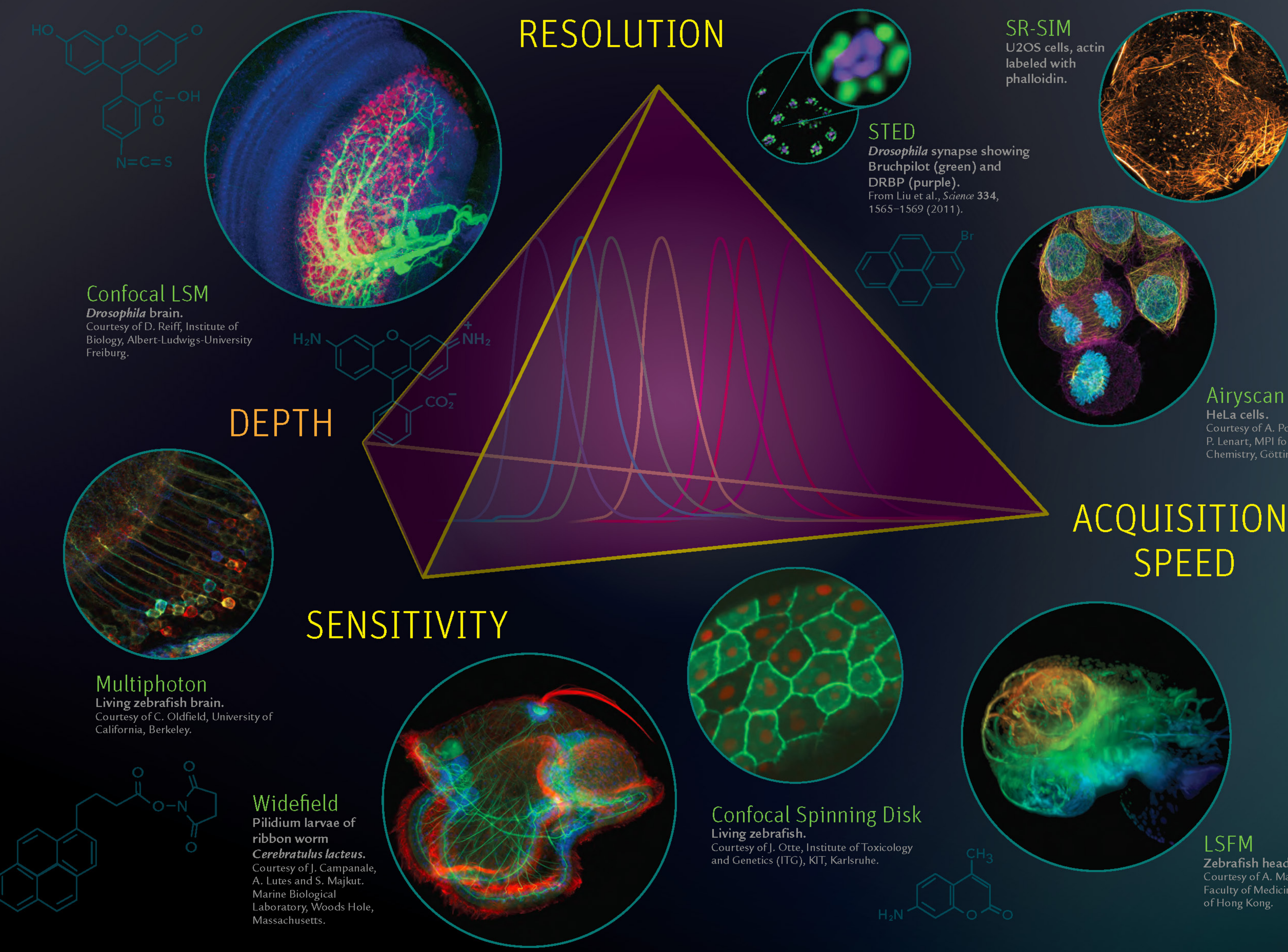
Taming the Imaging Triangle

Widefield[1] · SR-SIM[2] · LSFM · Confocal Spinning Disk · Confocal LSM · Airyscan[3] · Multiphoton · STED

Sensitivity · Resolution · Speed · Depth penetration · Sample size · Spectral parallelization · Flexibility[4]

1. No deconvolution or Apotome; 2. With Lattice SIM; 3. With Multiplex mode; 4. Objective, immersion medium, sample carrier.



Widefield

The entire field of view is simultaneously illuminated, and the emitted light is captured by a camera. This allows high-speed, sensitive image acquisition of, preferably, thin samples. Optical sectioning and increased resolution can be achieved by Apotome imaging or postacquisition deconvolution, capitalizing on the high number of available photons.

SR-SIM

Superresolution-Structured Illumination Microscopy also capitalizes on full field of view illumination and camera-based image acquisition. A moving pattern overlaid on the sample, captured at different positions, generates an optical section with increased resolution below the diffraction limit. This robust superresolution technique works best in low-scattering samples.

LSFM

Light Sheet Fluorescence Microscopy has a unique perpendicular arrangement of excitation and detection beam paths. Only the focal plane is excited, and the full field of view is acquired by a camera. Large optically sectioned volumes can be very sensitively acquired at high speed. Some systems allow the rotation of samples for multiview acquisition. Sample size and resolution depend on individual setups and objective lenses.

Confocal

Out-of-focus light is rejected by a confocal pinhole aperture, creating a high-quality optical section. Spinning-disk systems use multiple pinholes to achieve fast acquisition from thinner samples. The popular point-scanning systems stand out for their flexibility, increased depth penetration, and image quality even in scattering samples.

Airyscan

This unique confocal detection method overcomes the limitations of a closed pinhole for optical sectioning. Instead, 32 concentrically arranged detector elements in the conjugate image plane allow for increased sensitivity and resolution. At the same time, the main advantages of inherent optical sectioning and enhanced imaging depth are preserved. The additional spatial information collected by Airyscan allows high-speed acquisition with better signal-to-noise ratio.

Multiphoton

Another laser point-scanning approach: Here, it takes two or more photons to excite fluorophores, which only occurs at the focal spot, resulting in optical sectioning. The infrared excitation wavelengths allow for imaging at great depth, down to several millimeters into the tissue.

STED

Stimulated Emission Depletion microscopy is a laser point-scanning method in which one laser line excites the dye at the focal spot, while a second laser of longer wavelength suppresses emission in an outer donut. This combination currently achieves the highest possible imaging resolution. The required high-illumination intensities and acquisition times make it hard to apply this technique to dynamic processes in living samples.

FINDING THE BEST MICROSCOPE FOR YOUR LIVE SAMPLES

To understand biological processes, scientists study a variety of model systems ranging from single cells, tissue cultures, spheroids, and organs, to whole organisms. Each model system offers specific insights, helping scientists to address unique questions. Fluorescent labels can be genetically inserted to target subcellular structures. The labels and the excitation light, however, can affect or even harm the samples. It is often crucial for live-cell applications to use the smallest amounts of label and light to keep experimental conditions as close to nature as possible.

This necessity for gentleness contrasts with the need for spatial and temporal resolution in live-cell imaging. So, researchers must compromise. A common strategy to gain higher imaging speeds is to use larger pixels, which sacrifices achievable resolution; and an increase in resolution reduces imaging speed and often exposes the sample to more light. In this "Devil's Triangle" – ***speed***, ***resolution***, and ***sensitivity*** – improving one parameter causes the others to suffer. These compromises become even more important as sample volumes and time ranges of physiologically relevant studies keep increasing, which potentially exposes the sample to higher dosages of light. Consequently, a method's ***depth*** performance – its ability to image deep inside thicker samples – must also be considered.

In laboratories and imaging core facilities, scientists can often choose from several different fluorescence microscopy technologies, each providing a unique set of advantages and weaknesses. This poster aims to provide a quick guide to selecting the best method for answering your specific scientific question.

Head of an ant.
Courtesy of J. Michels, Institute of Zoology, Christian-Albrechts-Universität zu Kiel.

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