Confocal nl
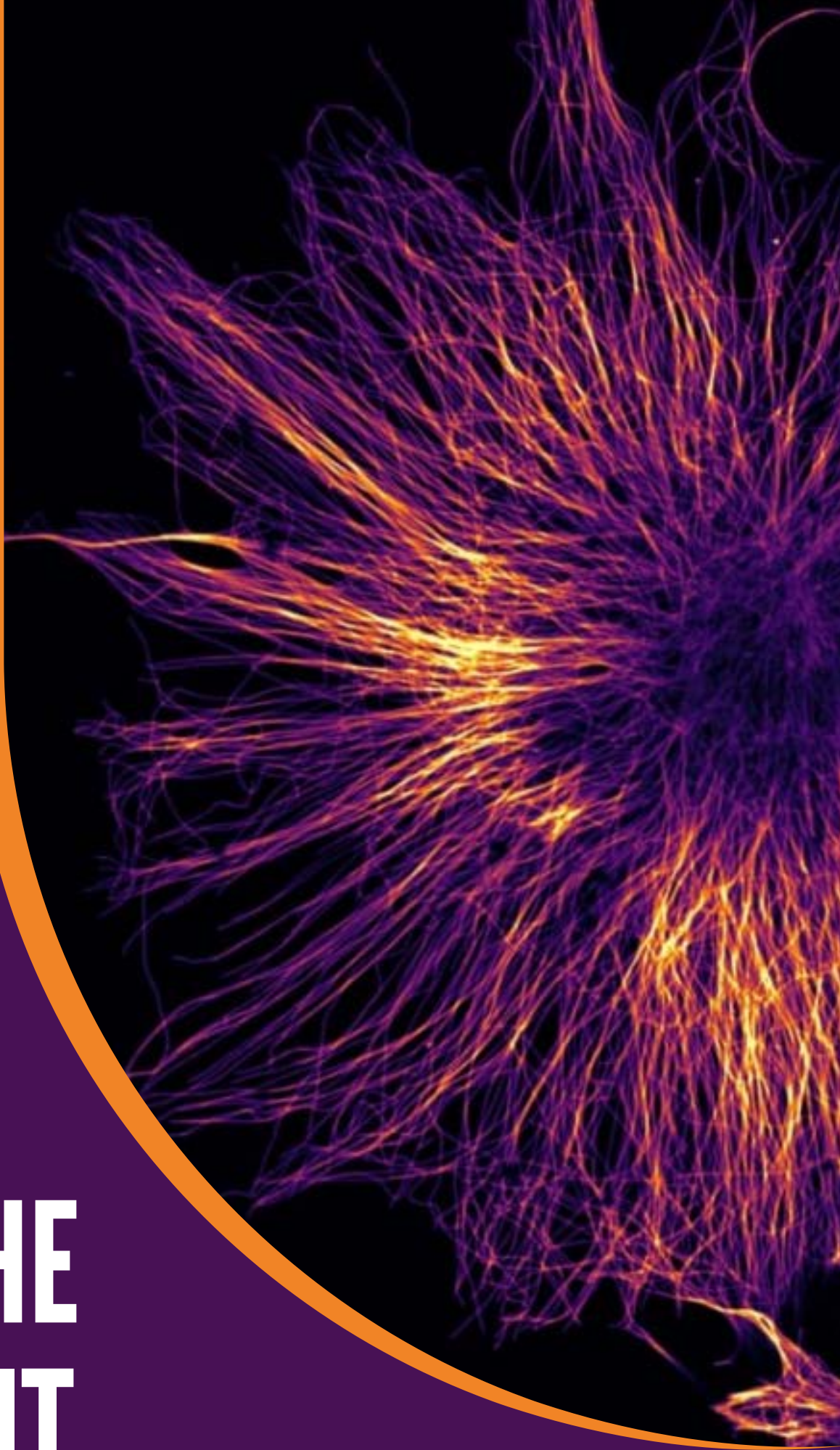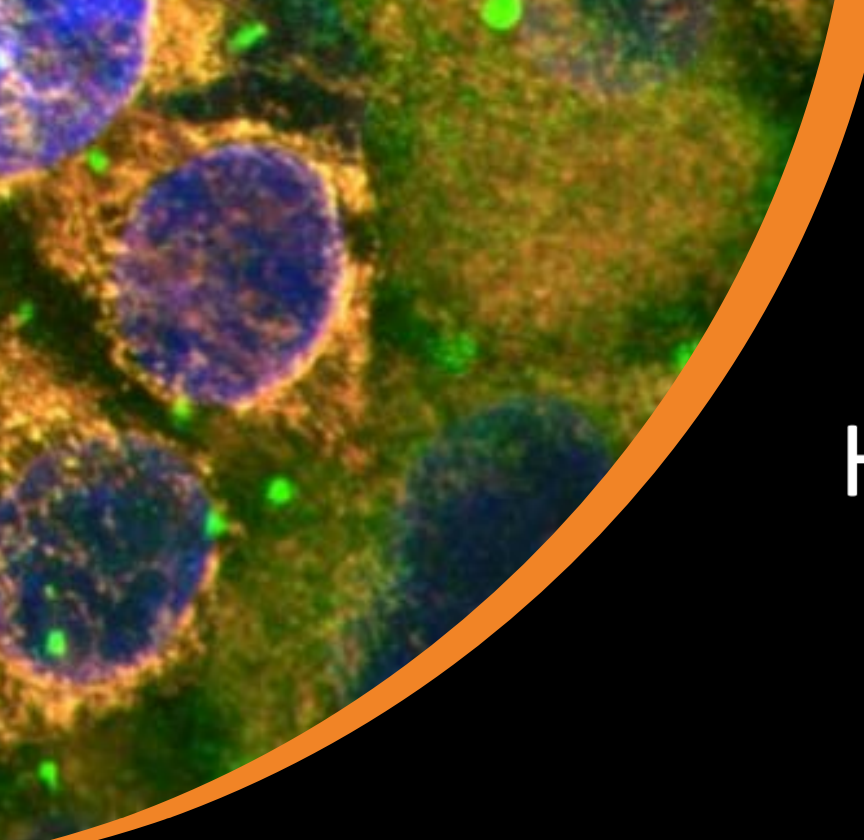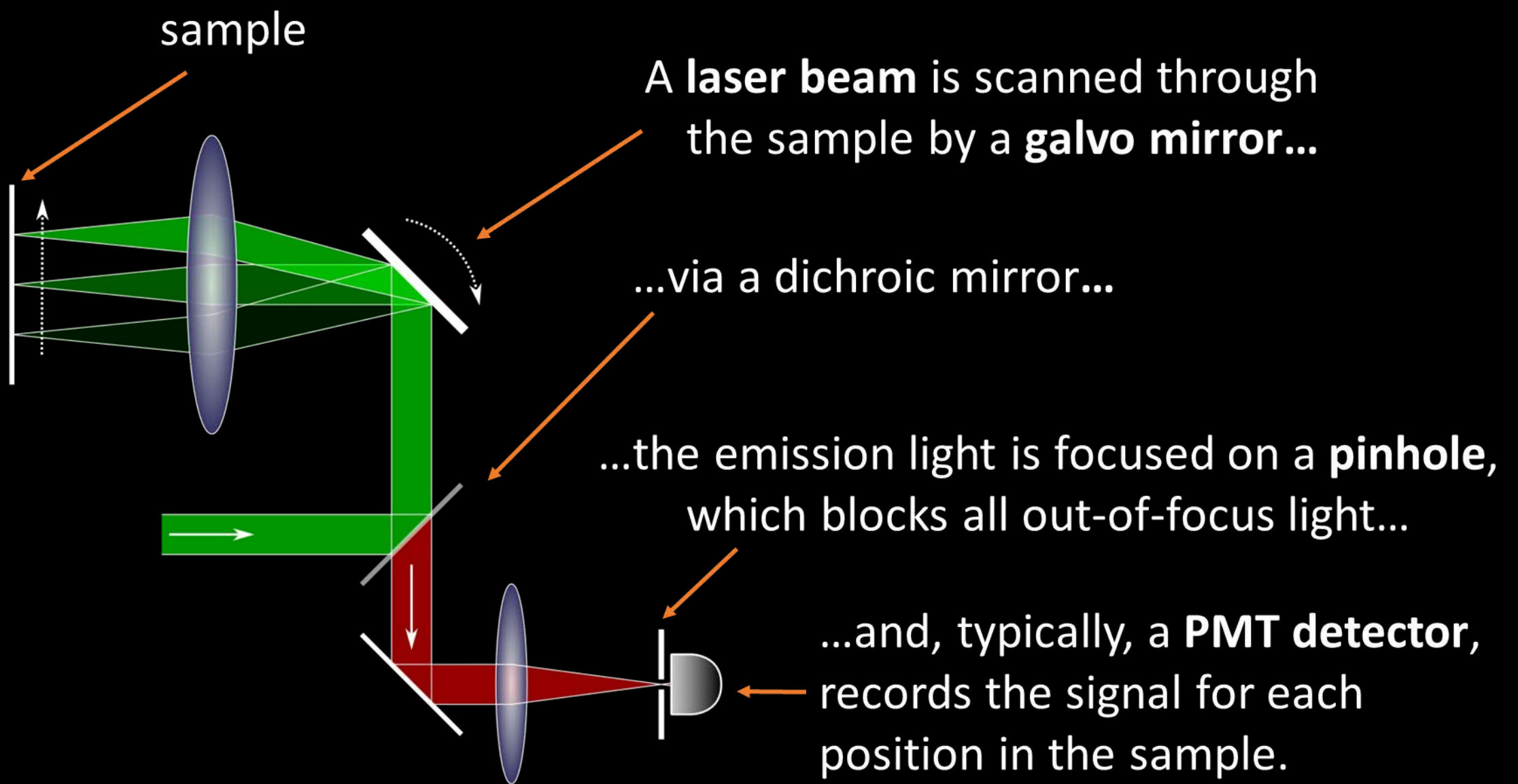
Quick Knowledge PART 1

HOW TO BREAK THE DIFFRACTION LIMIT DURING LIVE CELL IMAGING?

Here's standard laser scanning confocal microscopy (LSCM).

sample

A **laser beam** is scanned through the sample by a **galvo mirror...**

...via a dichroic mirror**...**

...the emission light is focused on a **pinhole**, which blocks all out-of-focus light...

...and, typically, a **PMT detector**, records the signal for each position in the sample.

From the detector signal at each position, an image of the sample is calculated.
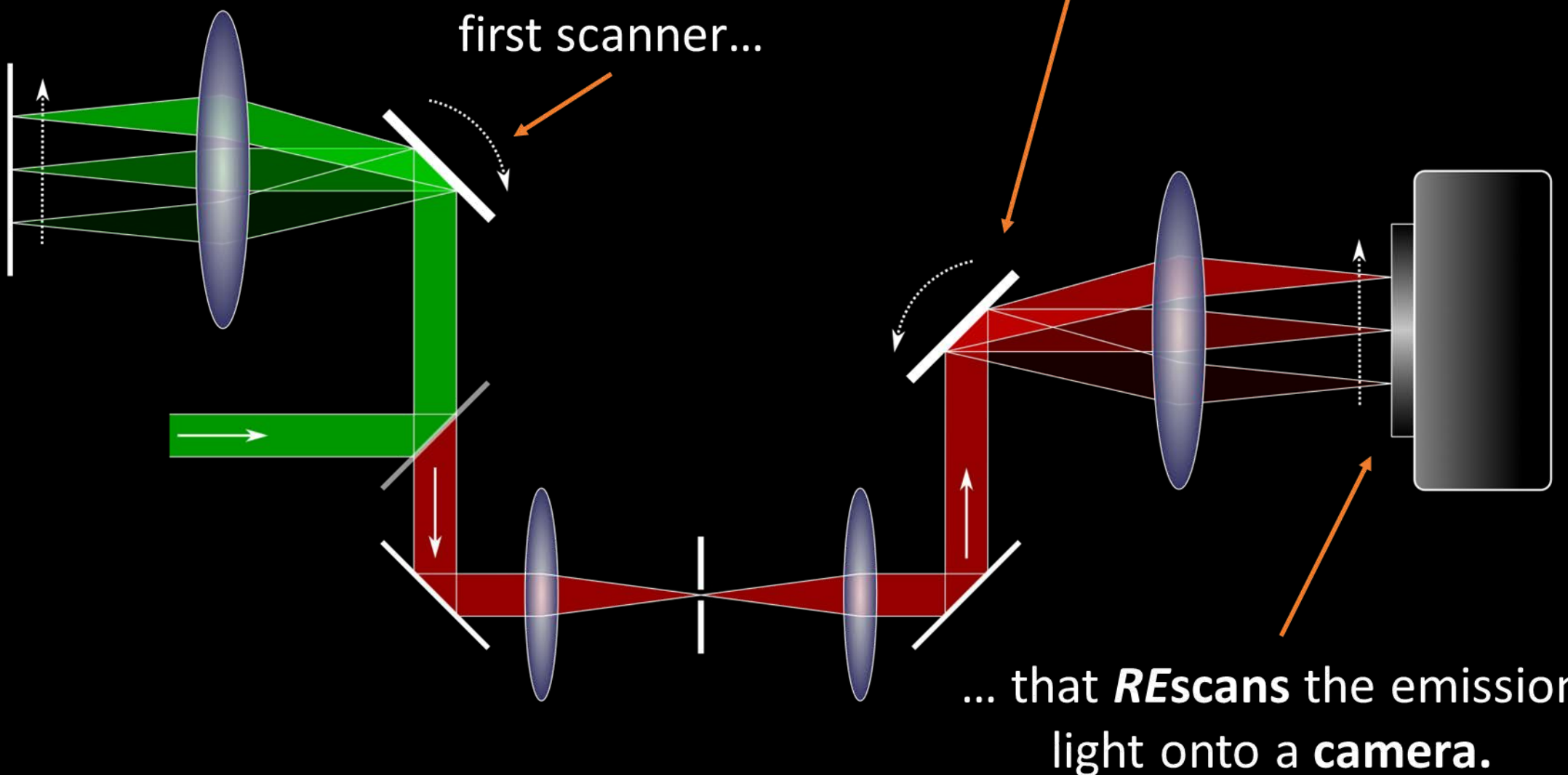
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In *REscan* microscopy,
we *REthink* confocal technology.

Instead of a single detector,
we use a **second scanner**...

... **synchronized** with the
first scanner...

... that *REscans* the emission
light onto a **camera.**

*The signal automatically ends up at the right place on the
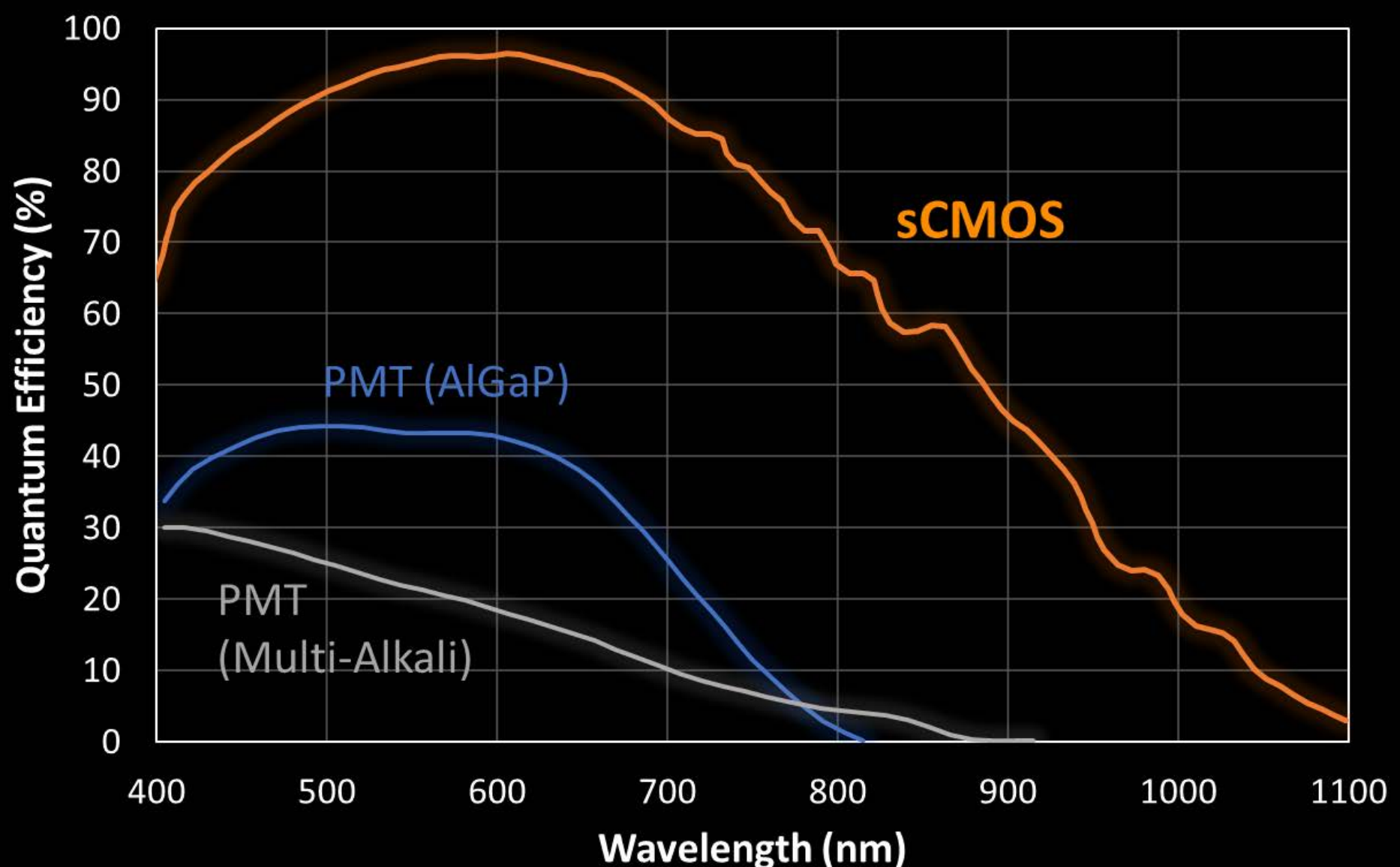camera, and the image is reconstructed all by itself.*

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REscan confocal has extra sensitive detection.

REscan uses sCMOS **camera** as detector.

Compared to PMT detectors, sCMOS have a **higher quantum efficiency** in a **wider wavelength range...**

...and, most importantly, they have **pixels.**
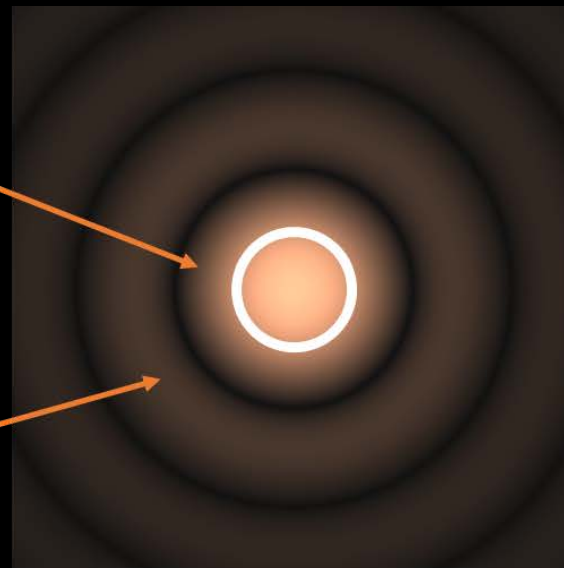
Here is why pixels are cool...

*RE*scan collects more light without losing **resolution**.

In **LSCM**, the pinhole lets **only the center of the spot** through (<1 Airy unit)
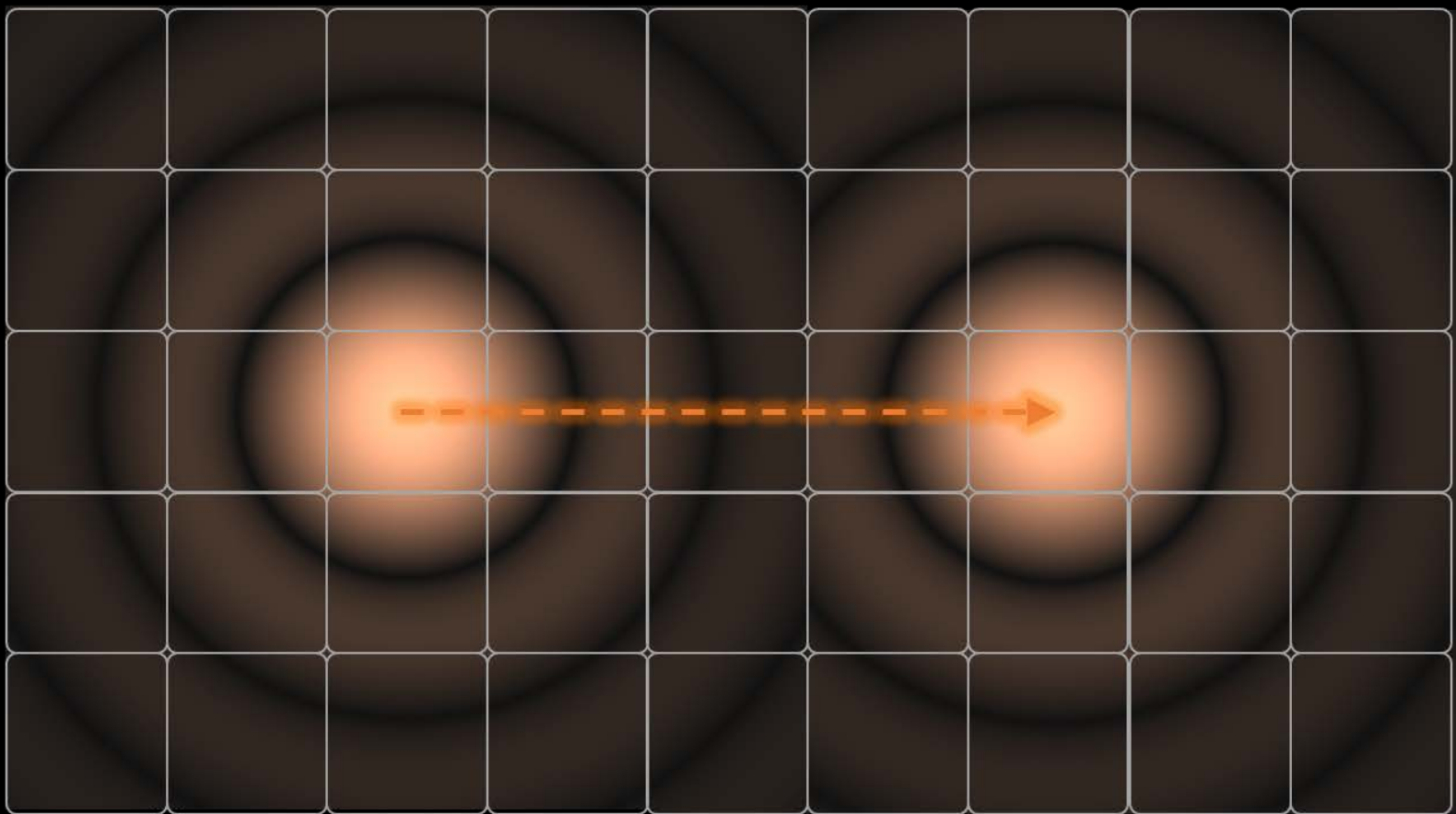
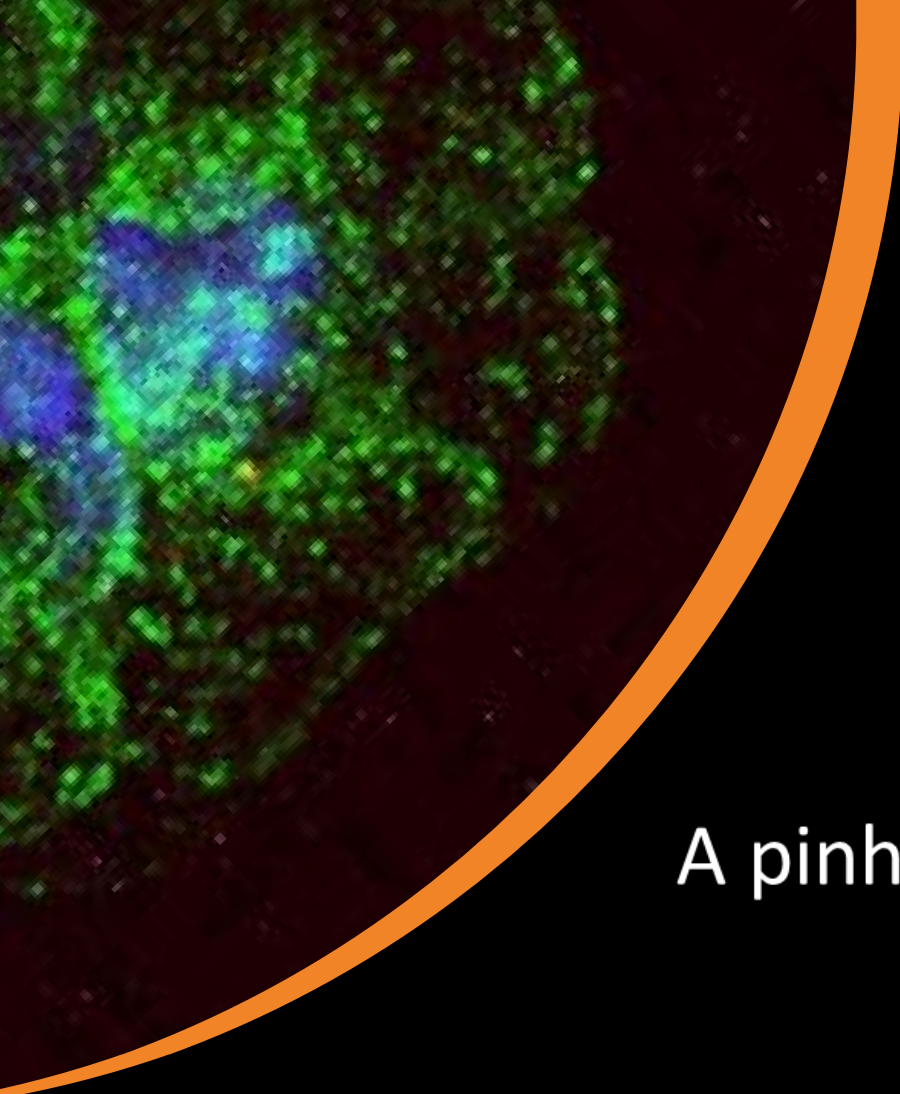Opening the pinhole more lets in light from different positions, and resolution is lost.

('Airy Disk', the shape of the excitation spot.)

When the light is *RE*scanned on the camera, each part of the spot automatically ends up in the **correct pixel**.

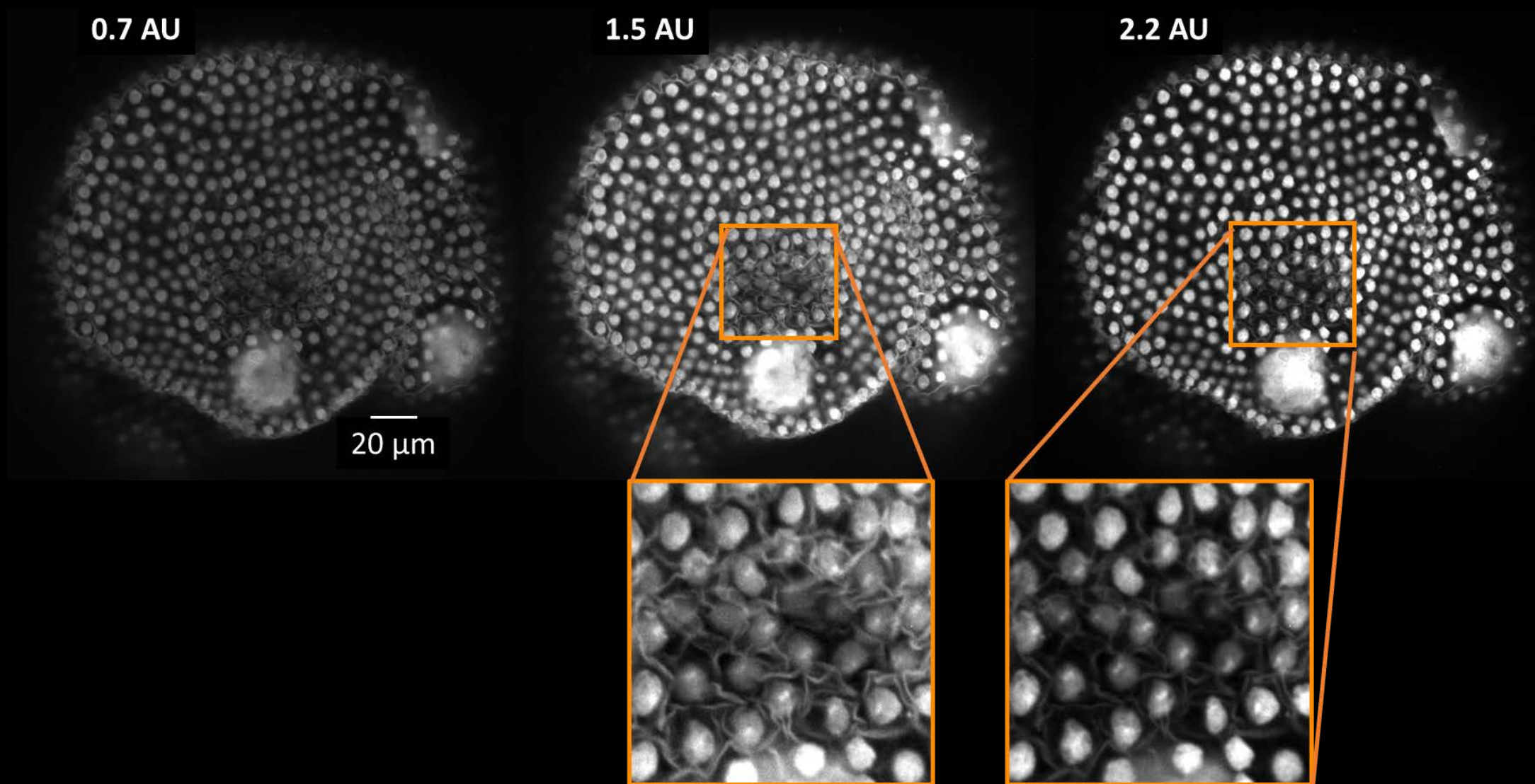And the pinhole stays **wide open** to collect **more light**

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The optimal **pinhole size** in *REscan* confocal microscopy?

A pinhole **between 1 and 2 Airy units** (AU) gives the best image.
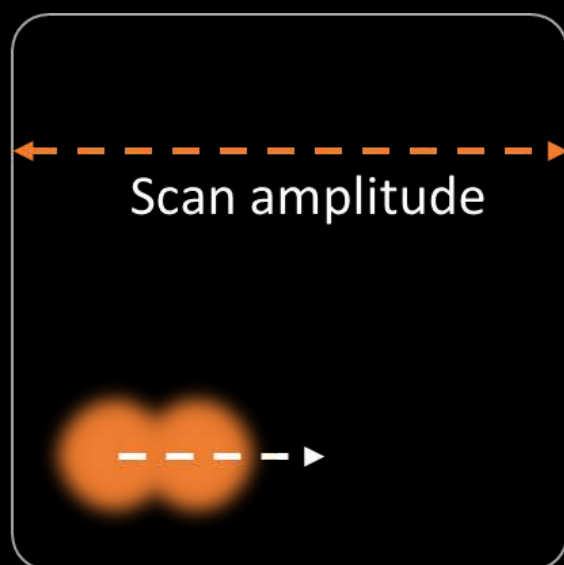
Smaller pinhole → sacrificed **signal**

Larger pinhole → lost **confocality**

0.7 AU

1.5 AU
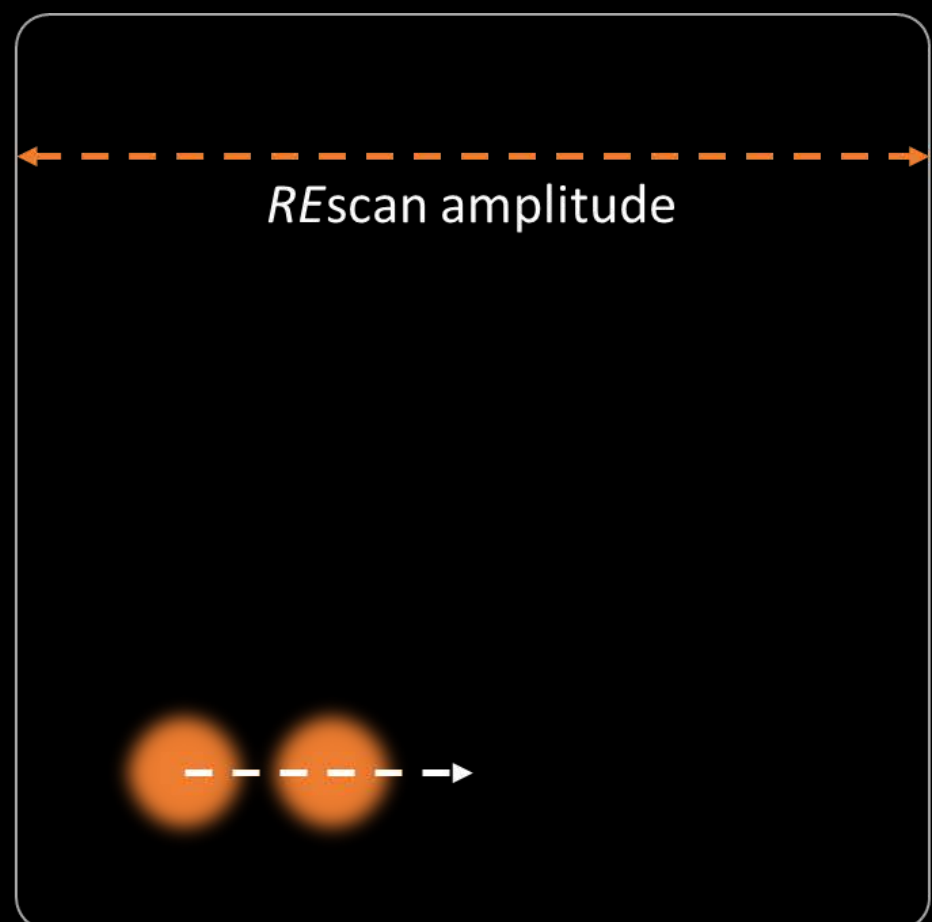
2.2 AU

20 µm

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With *RE*scan you can image in **Super Resolution**.

In *RE*scan, image **magnification** and **spot** size are **decoupled**.

We can **magnify** the image compared to the spot by giving the *RE*scanner a larger amplitude than the scanner.

Scan amplitude

*RE*scan amplitude

In *RE*scan, all the details in the image are pulled further apart.
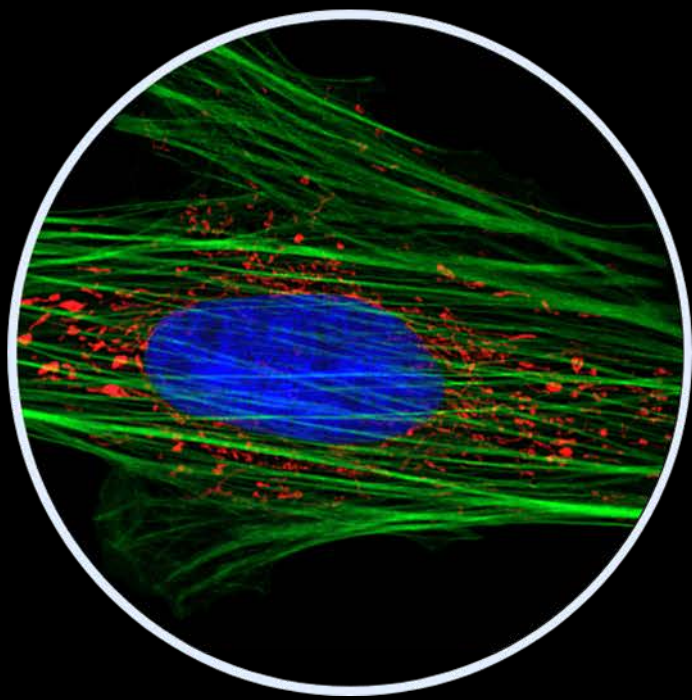
Twice as large *RE*scan improves resolution by **1.4x, breaking the diffraction limit.**

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Our solution: *REscan Confocal*

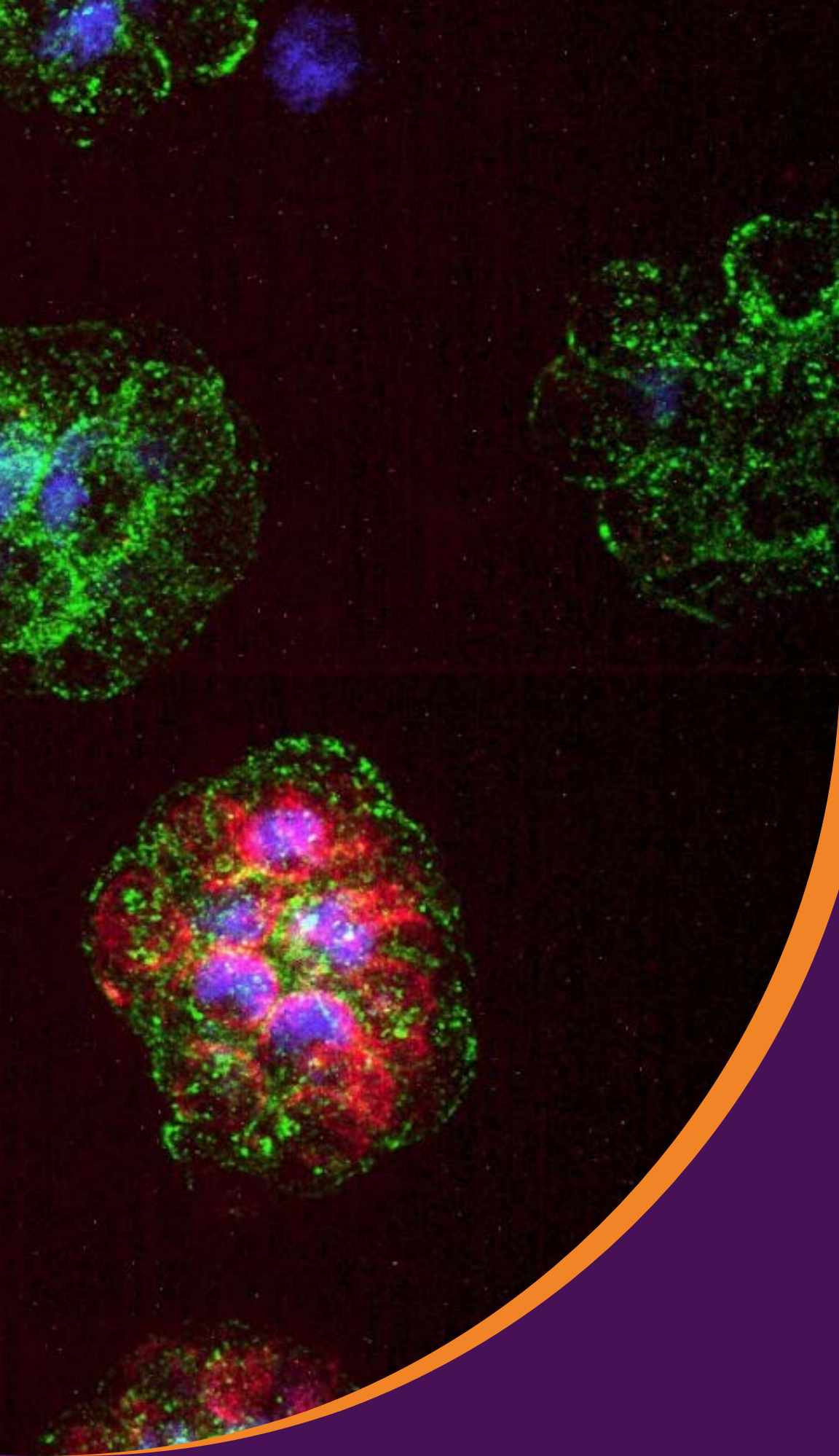Adding **camera** & **laser** makes a complete **confocal super resolution microscope.**

Adaptable on any microscope body.

Low laser power → **low phototoxicity** →

→ perfect for **live cell imaging**

www.confocalnl.com

Find out more at

www.confocalnl.com

With **REscan**, we not only get Super resolution, but also high speeds. Read more about that in PART 2.

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