

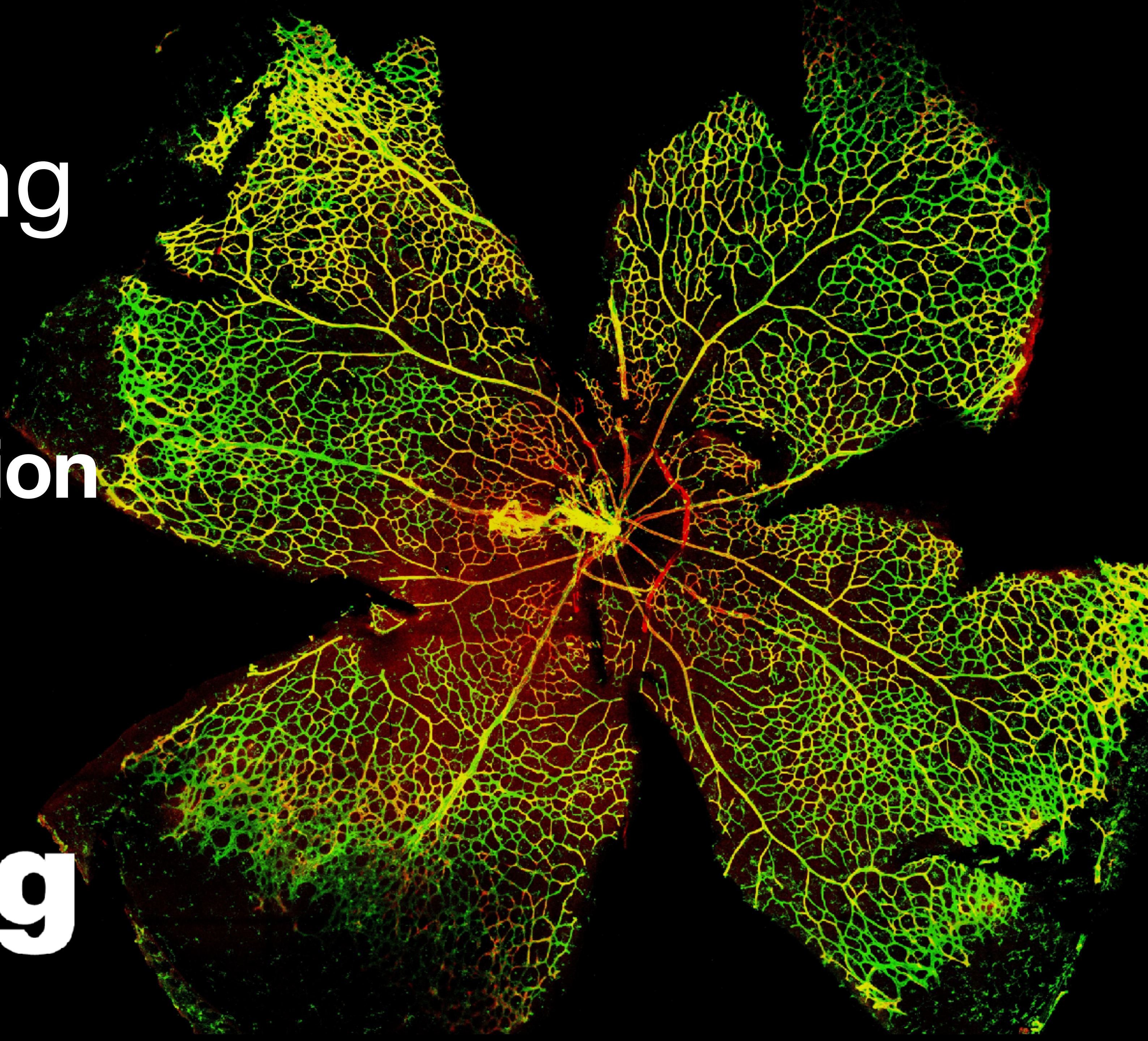


cellular imaging

Wellcome Centre for Human Genetics

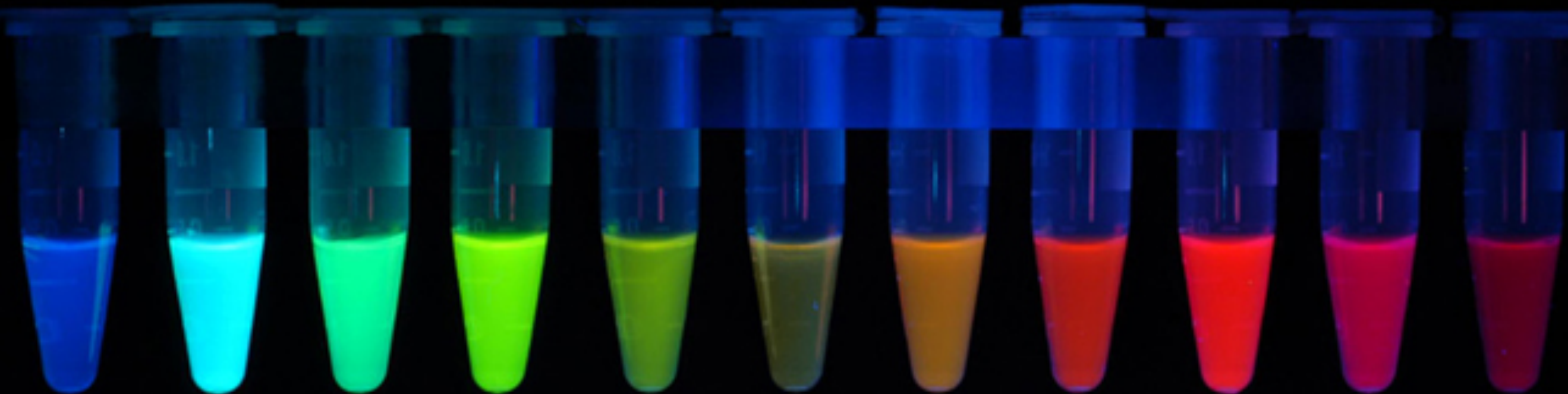
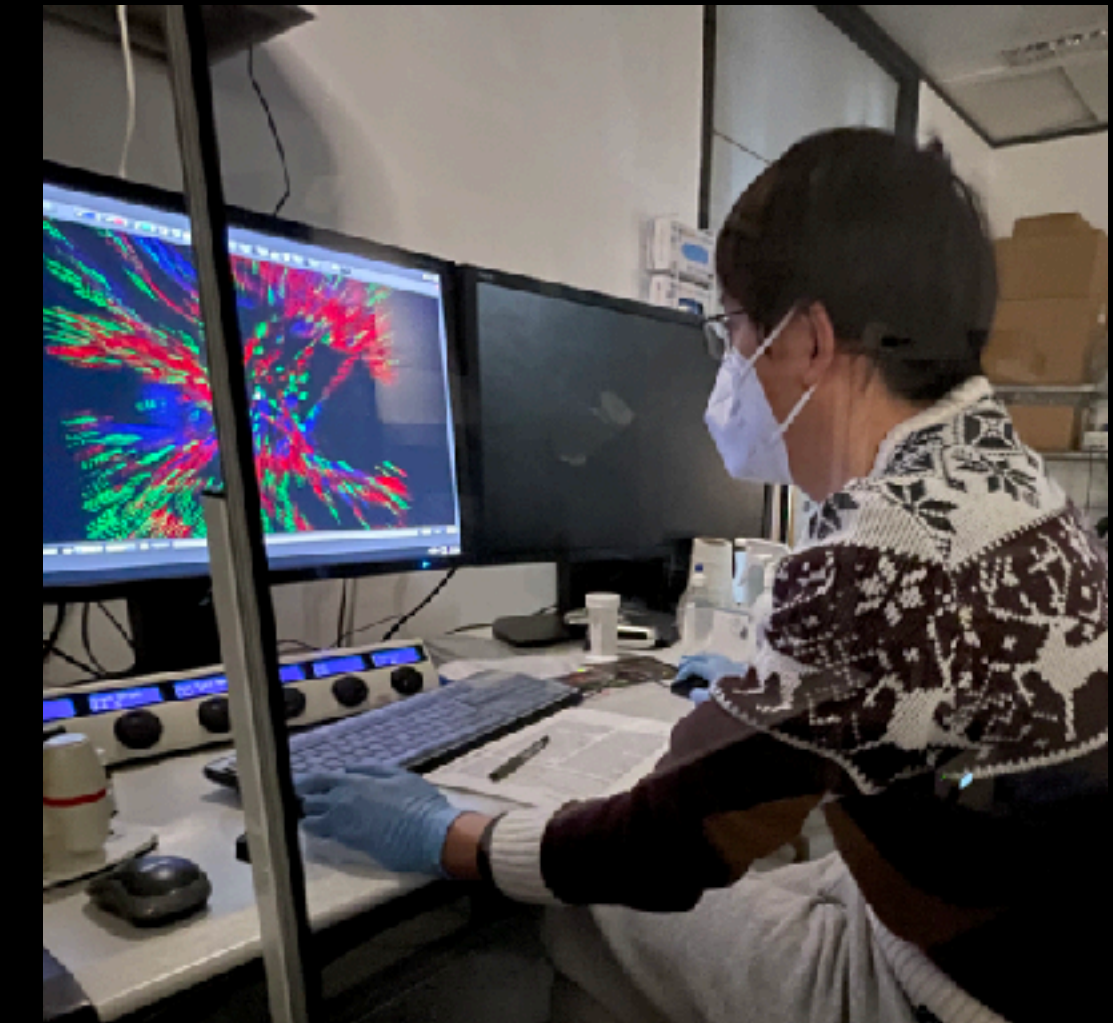
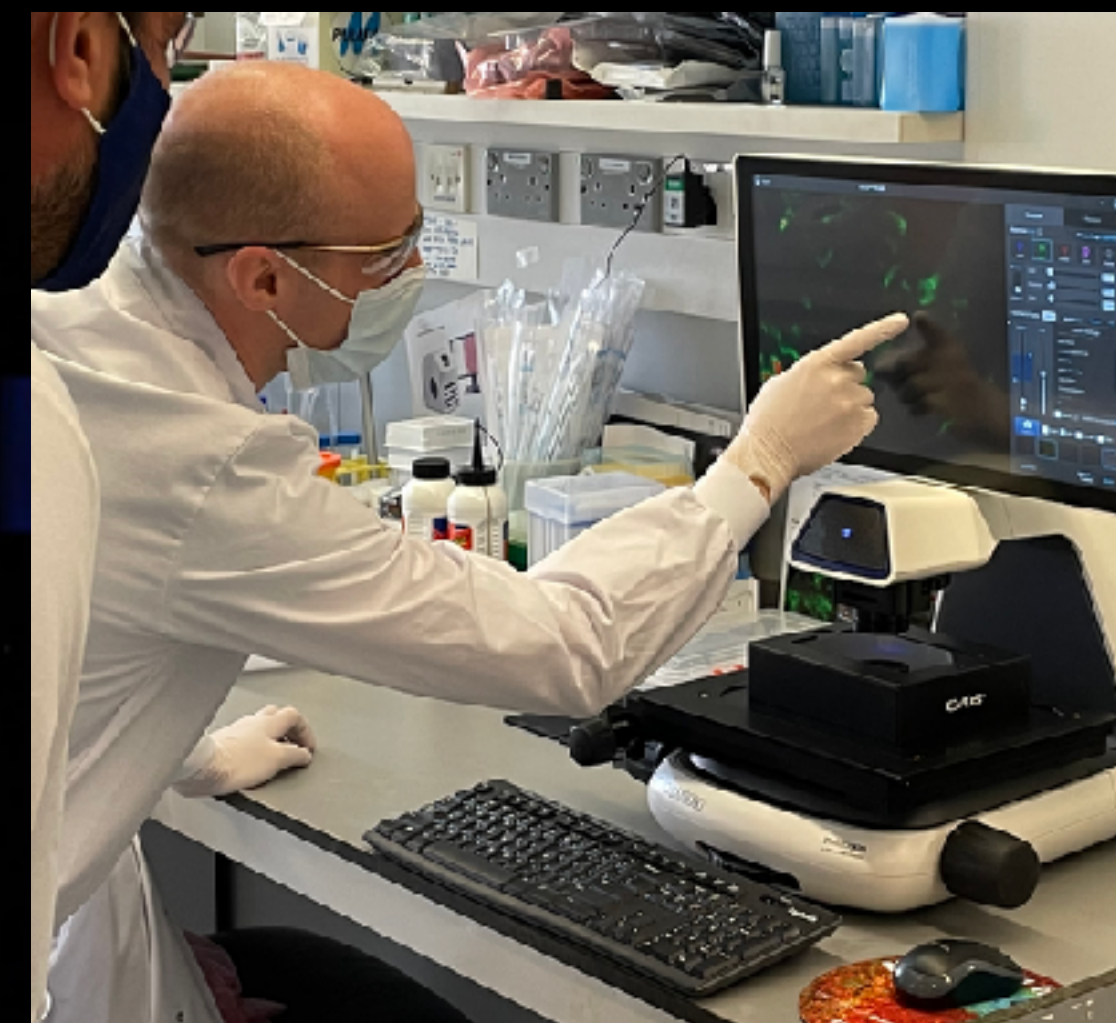
Facility Introduction

James Bancroft, PhD

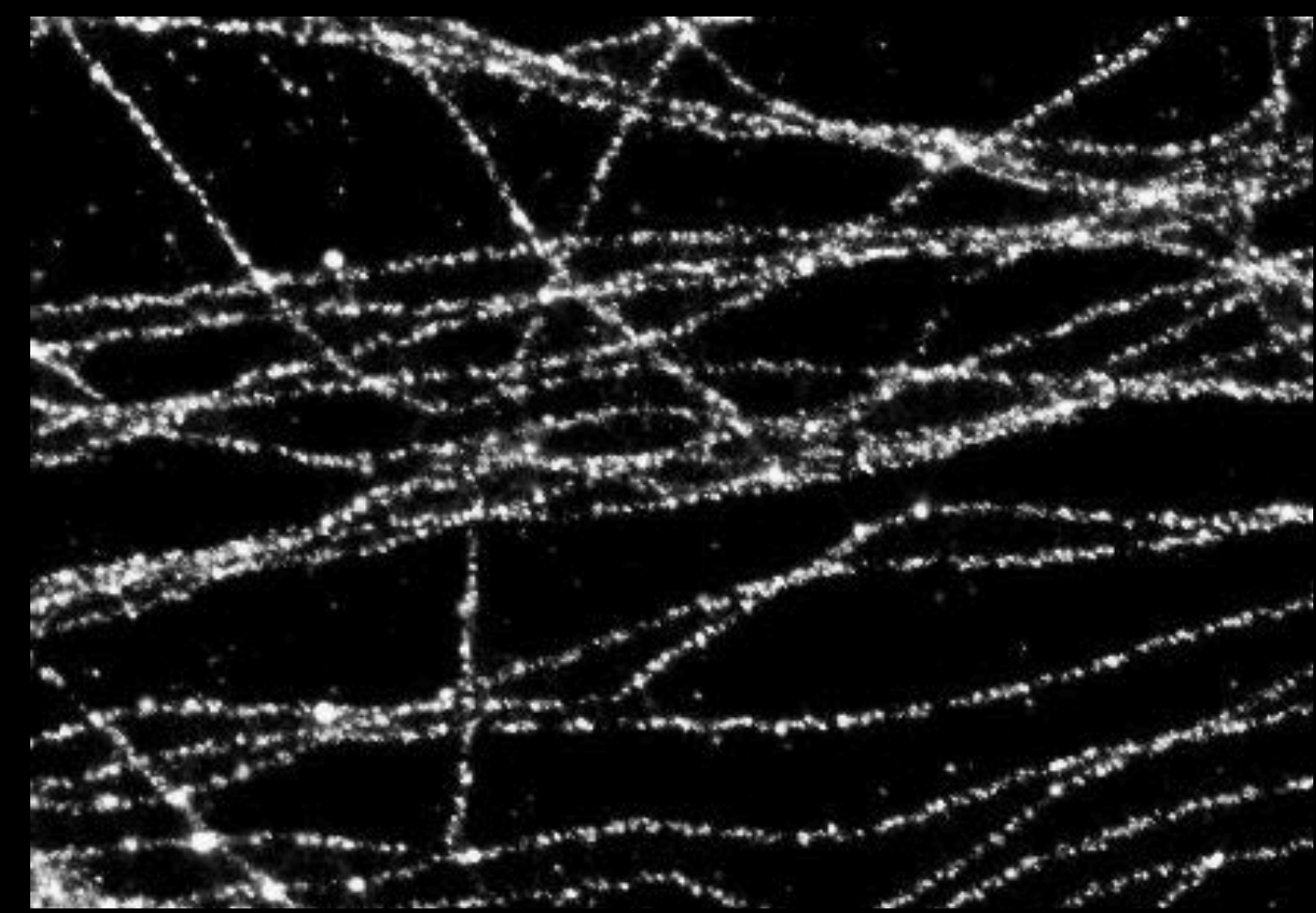
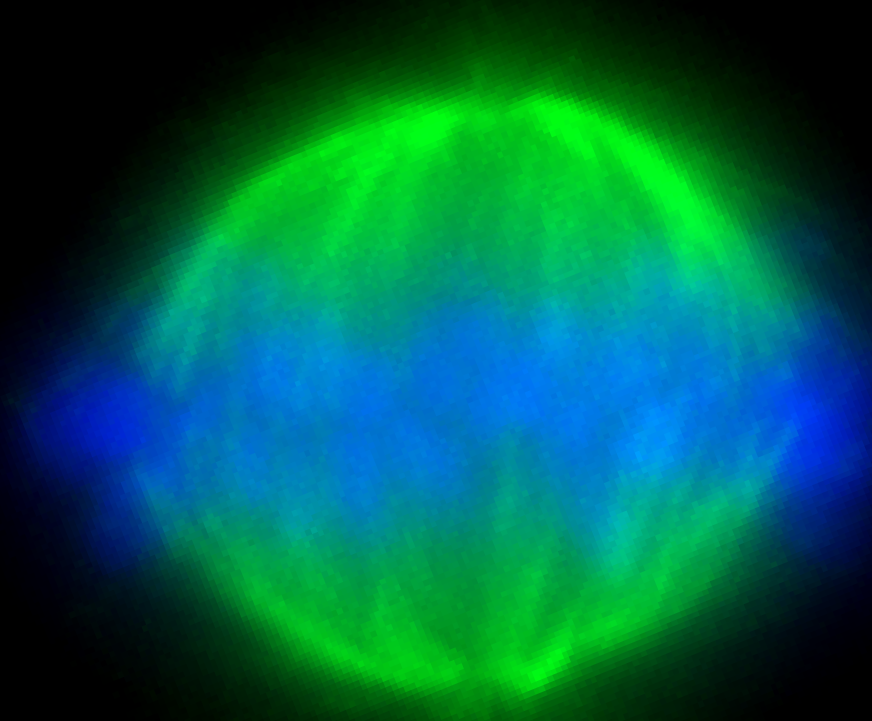
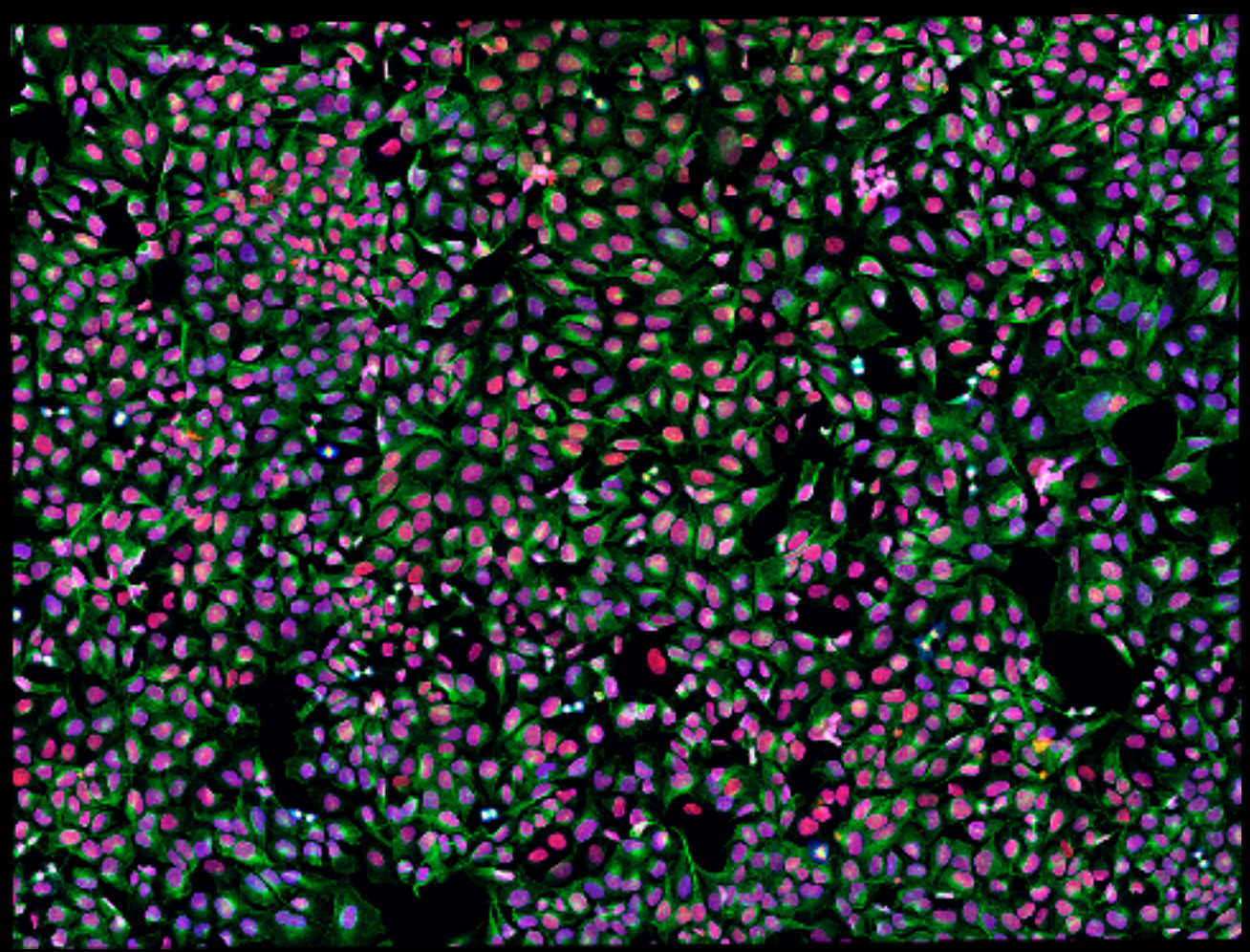


What we offer

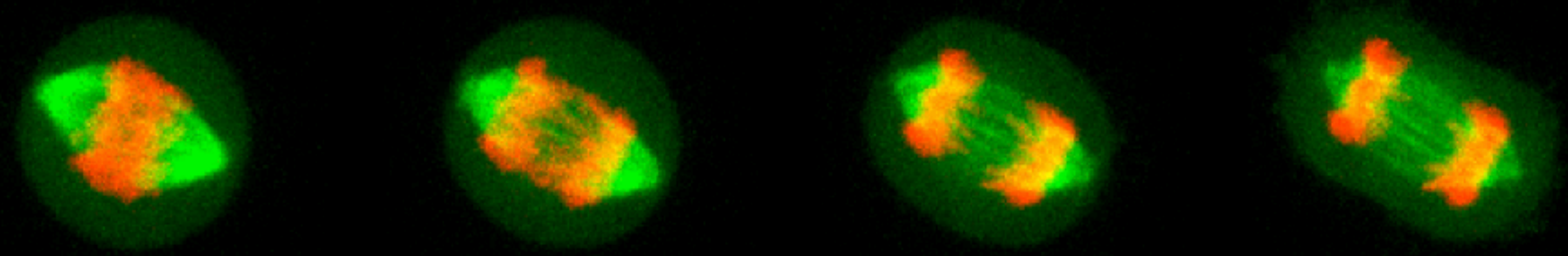
- Training and ongoing support
- Consultation - project planning, protocols, trial reagents
- Collaborative projects
- Analysis
- Seminars and workshops
- Drive purchase and implementation of new equipment



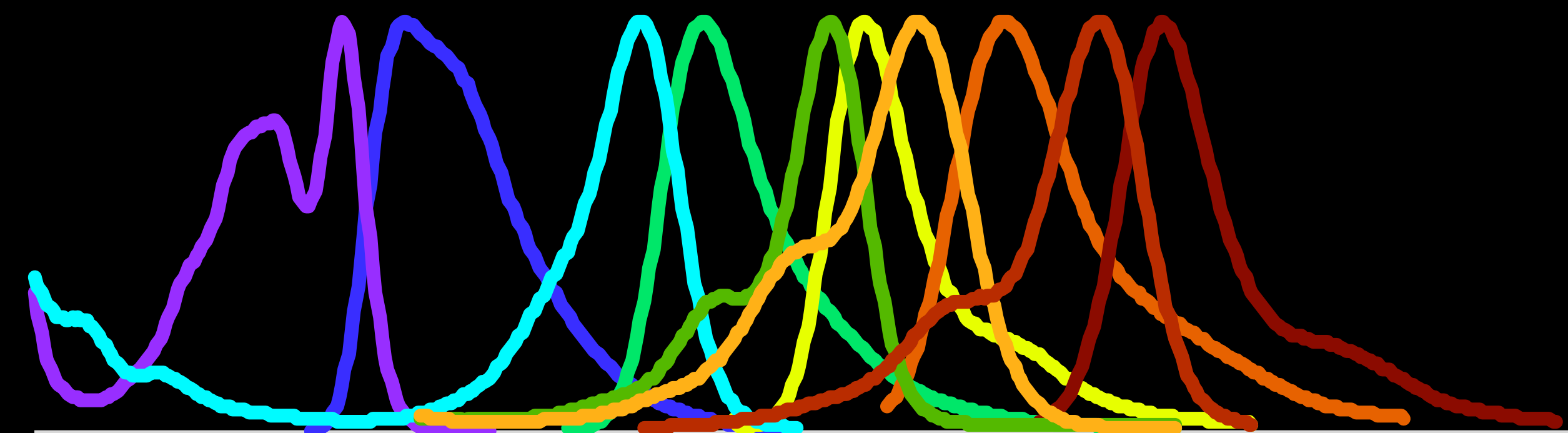
The right kind of resolution



Spatial resolution



Temporal



300nm 400nm 500nm 600nm 700nm 800nm

Spectral



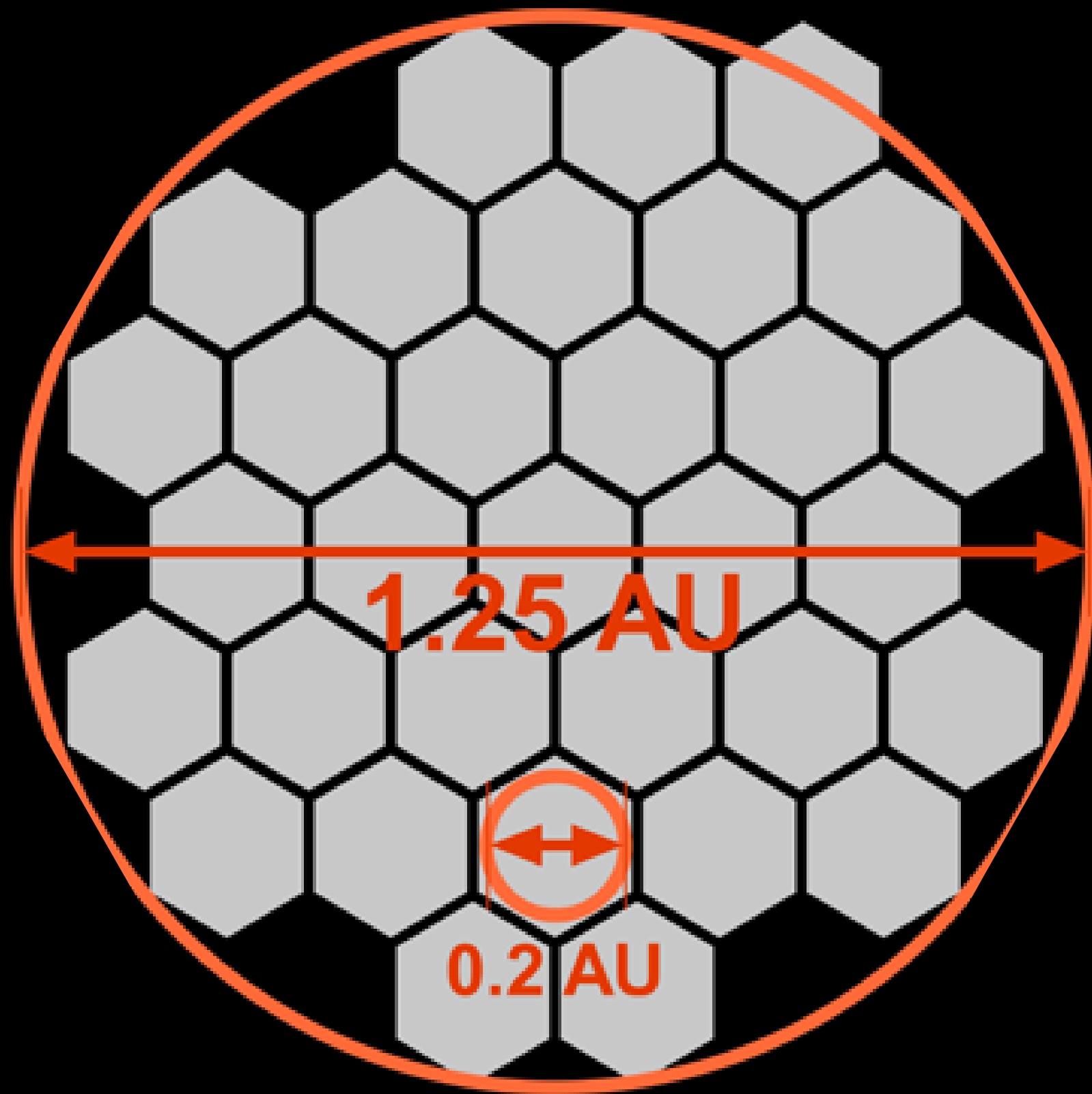
Technology to deliver resolution



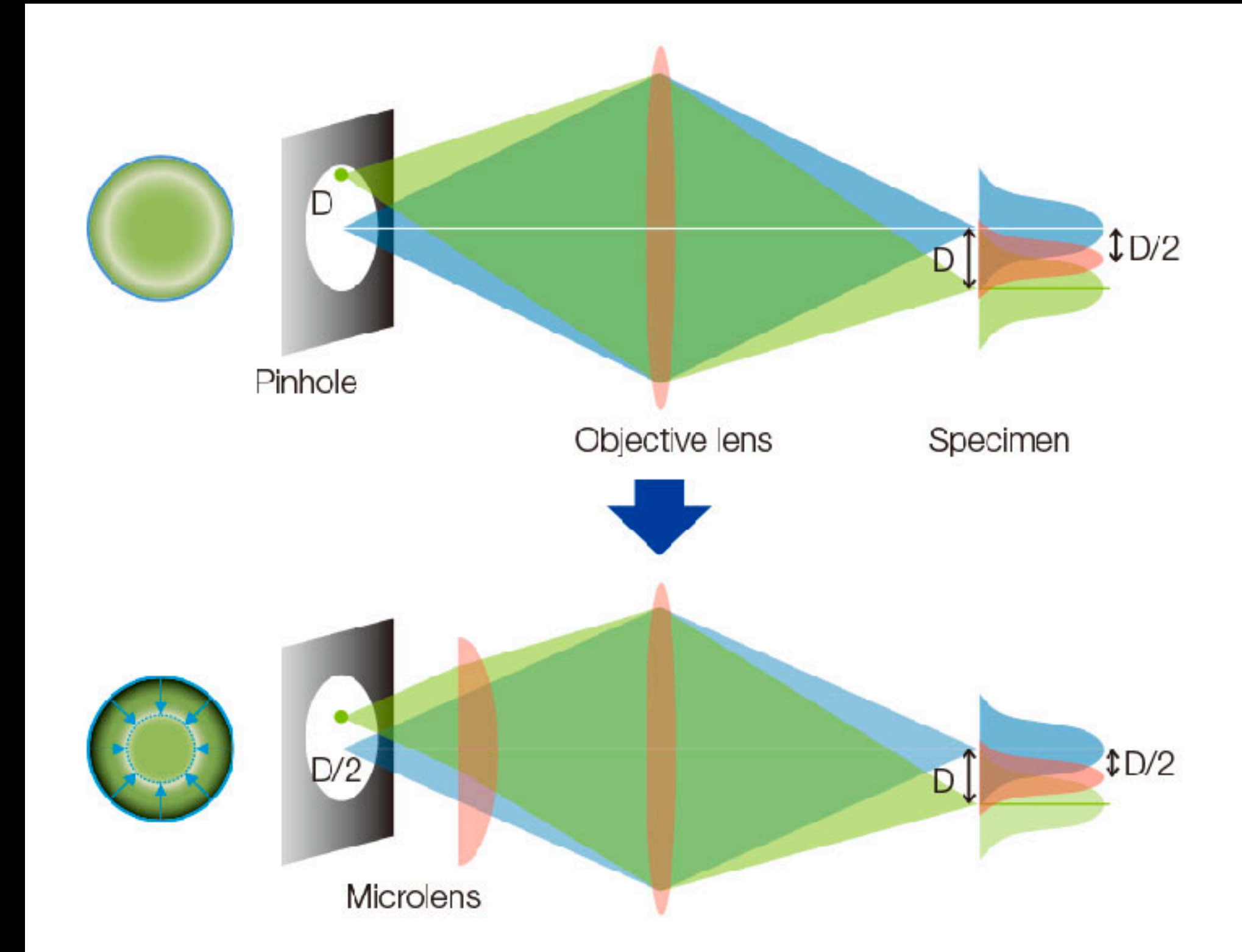
5 confocal, 2 advanced widefield, 1 STORM/SMLM/TIRF, 1 laser capture micro-dissection

Spatial Resolution - User Friendly Super-Resolution

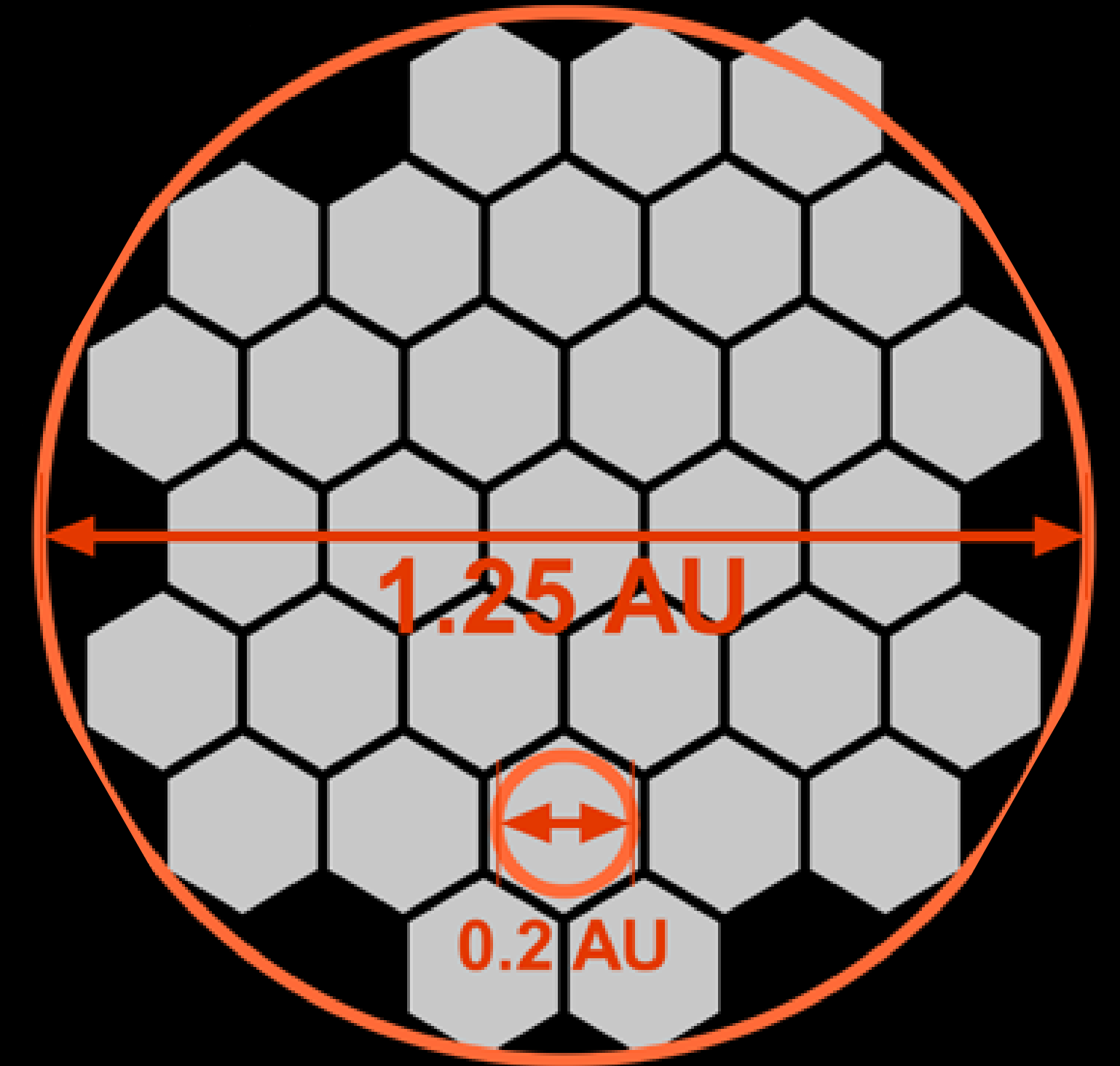
Airyscan



SoRa

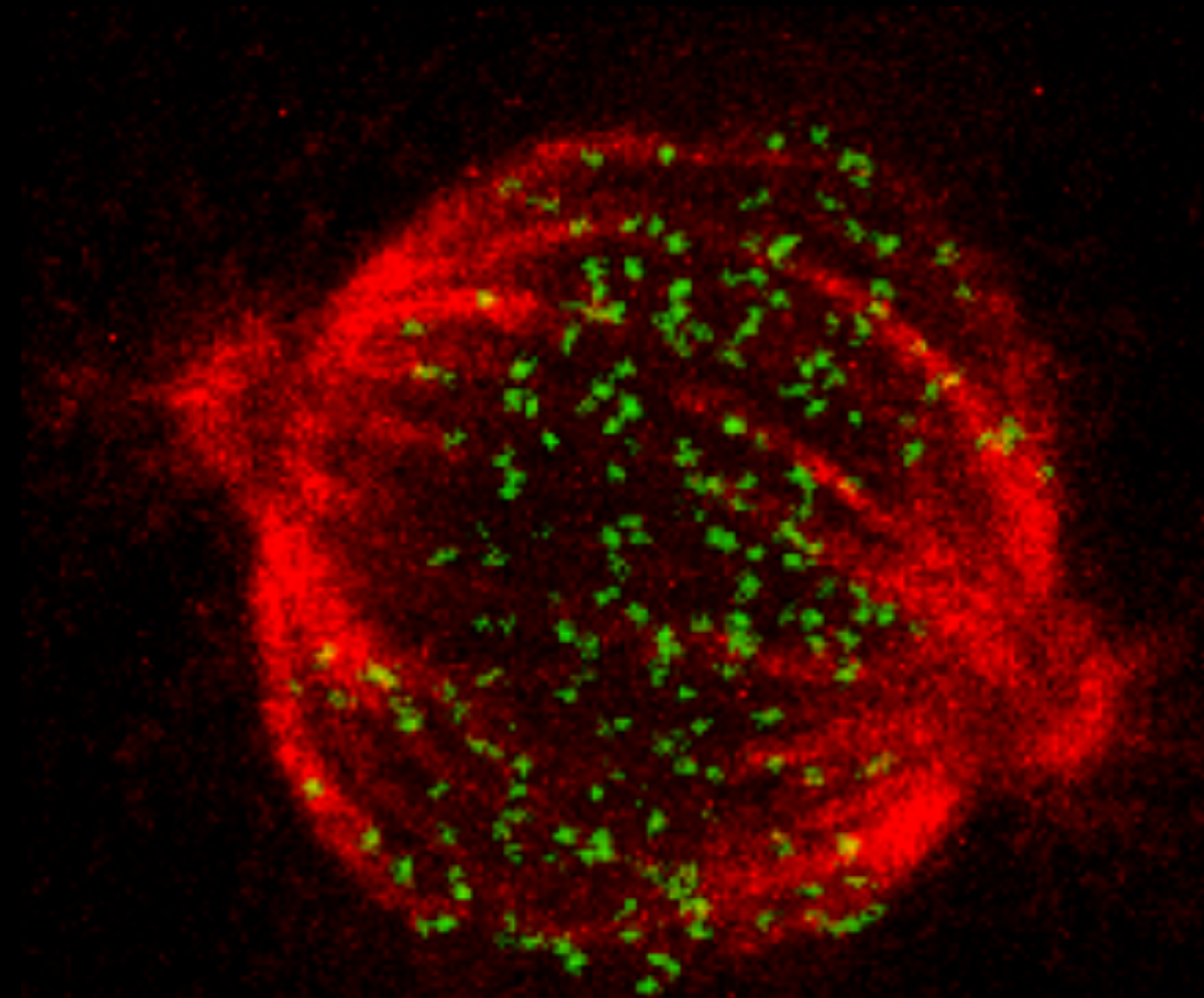


Airyscan2 - Point-scanning confocal super-resolution

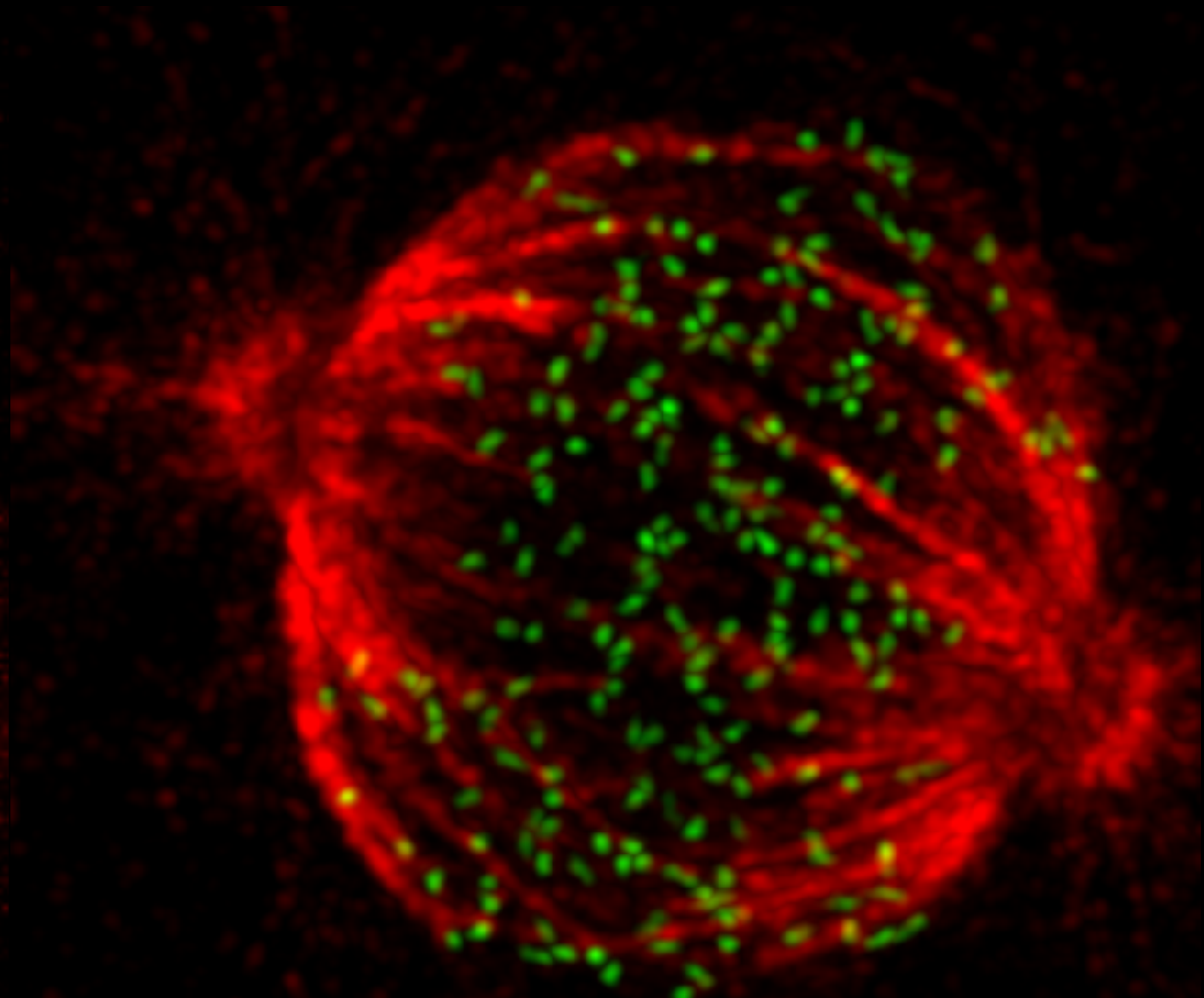


- Airyscan detector v2 - 4-8x increase in sensitivity
- 2x increase in resolution ($\sim 120\text{nm}$ x/y)
- Multiplexing increases acquisition speed (up to 18fps single channel)
- Variable sliding dichroic mirrors allow efficient spectral separation

Traditional Confocal vs Airyscan

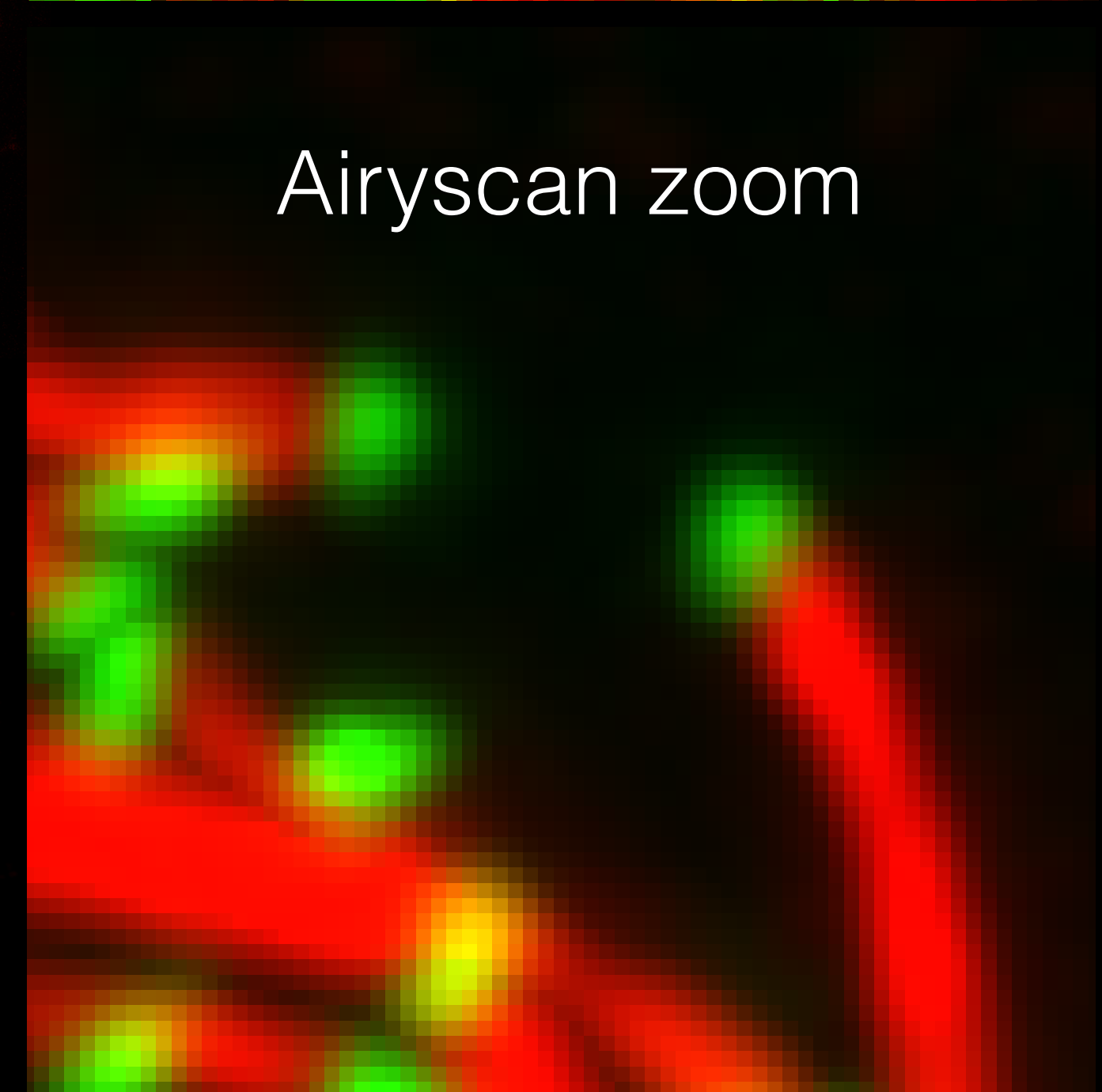
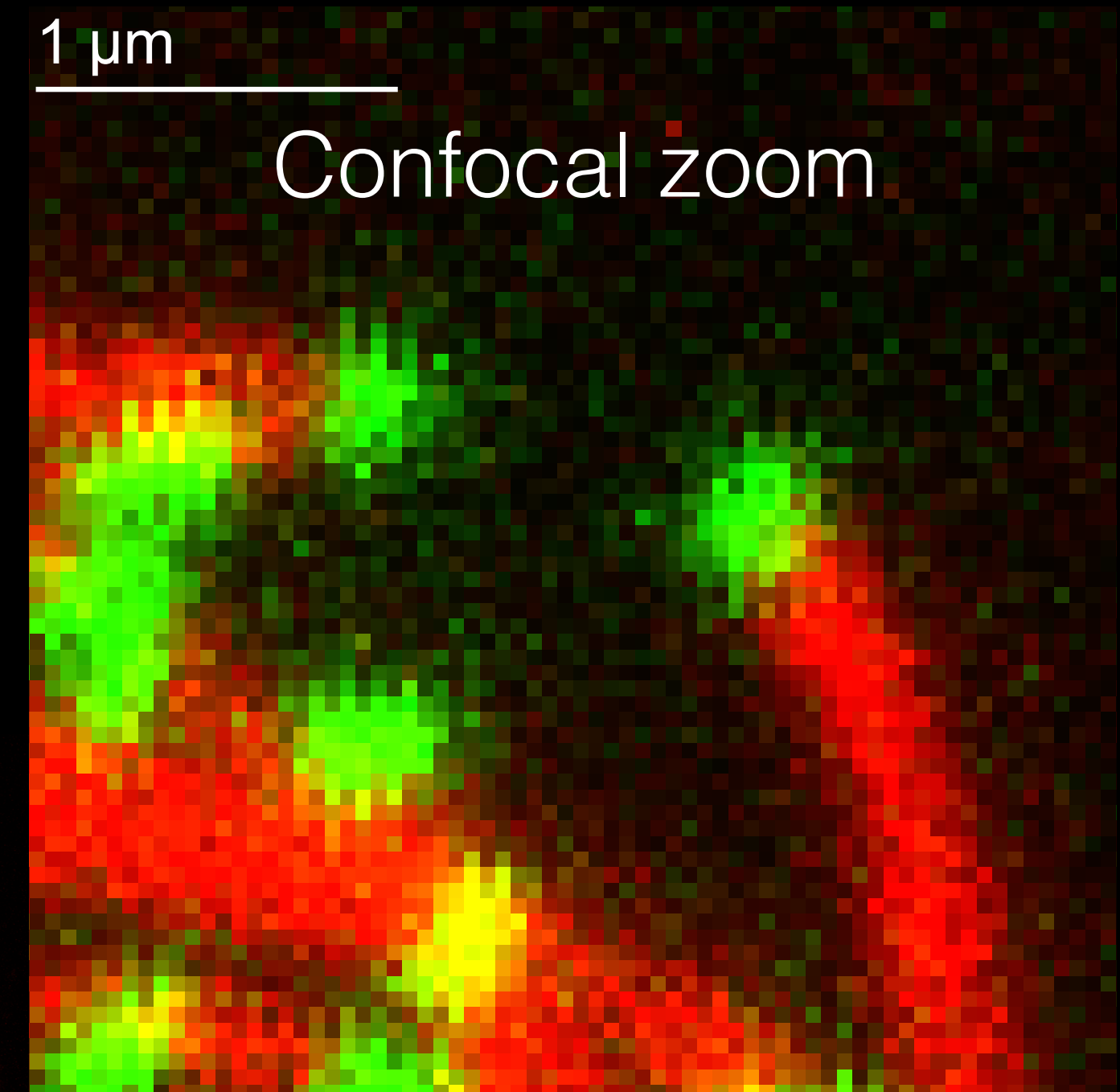
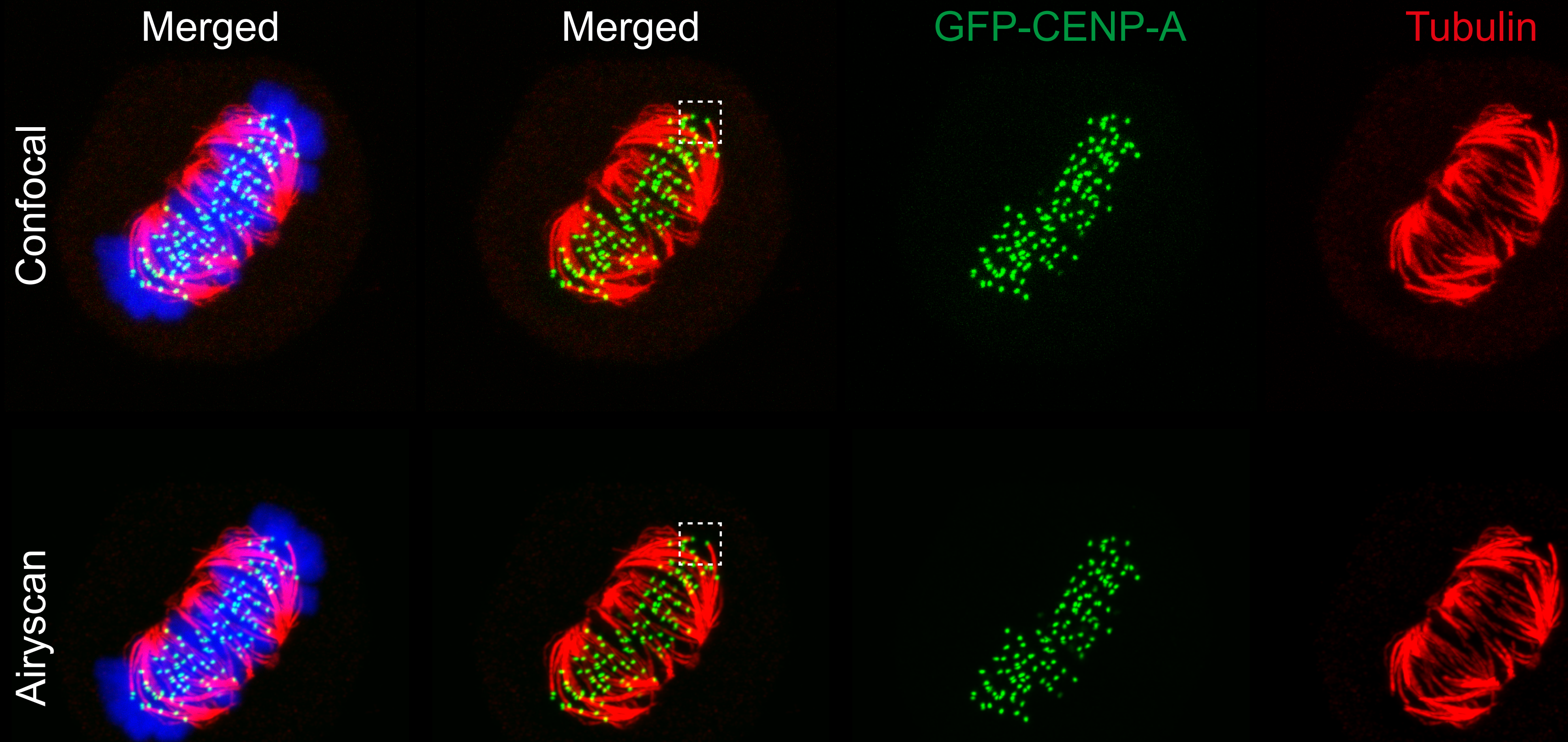


Confocal - Deconvolved

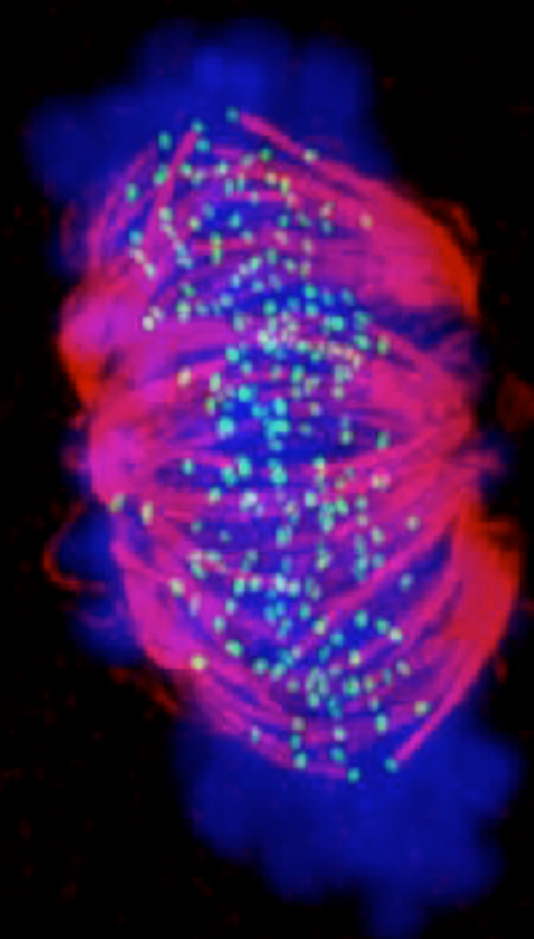
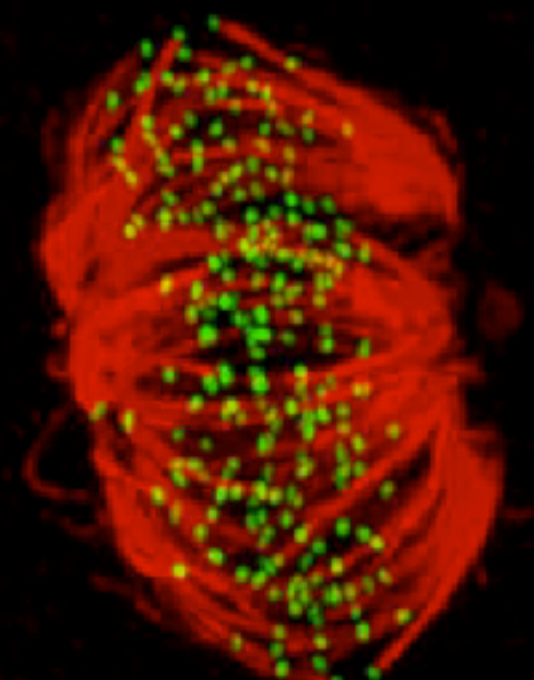
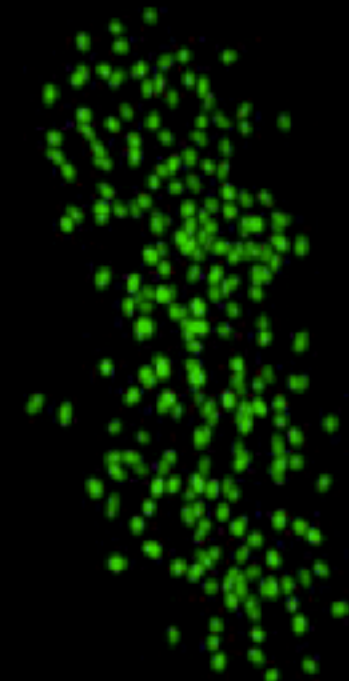
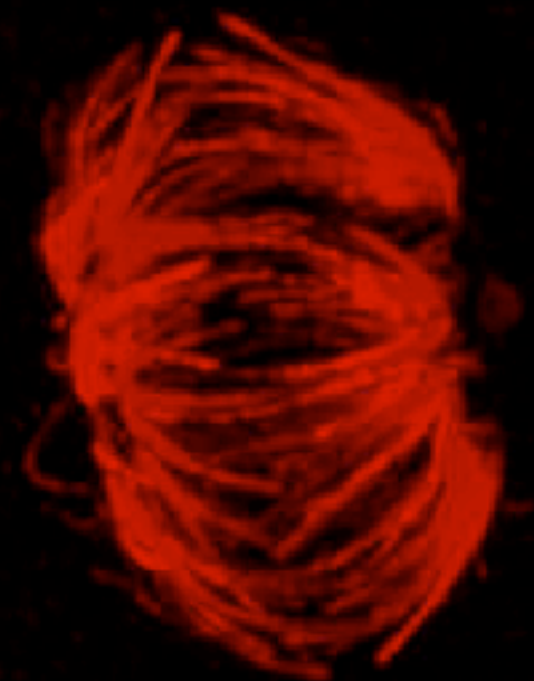


Airyscan

Traditional Confocal vs Airyscan



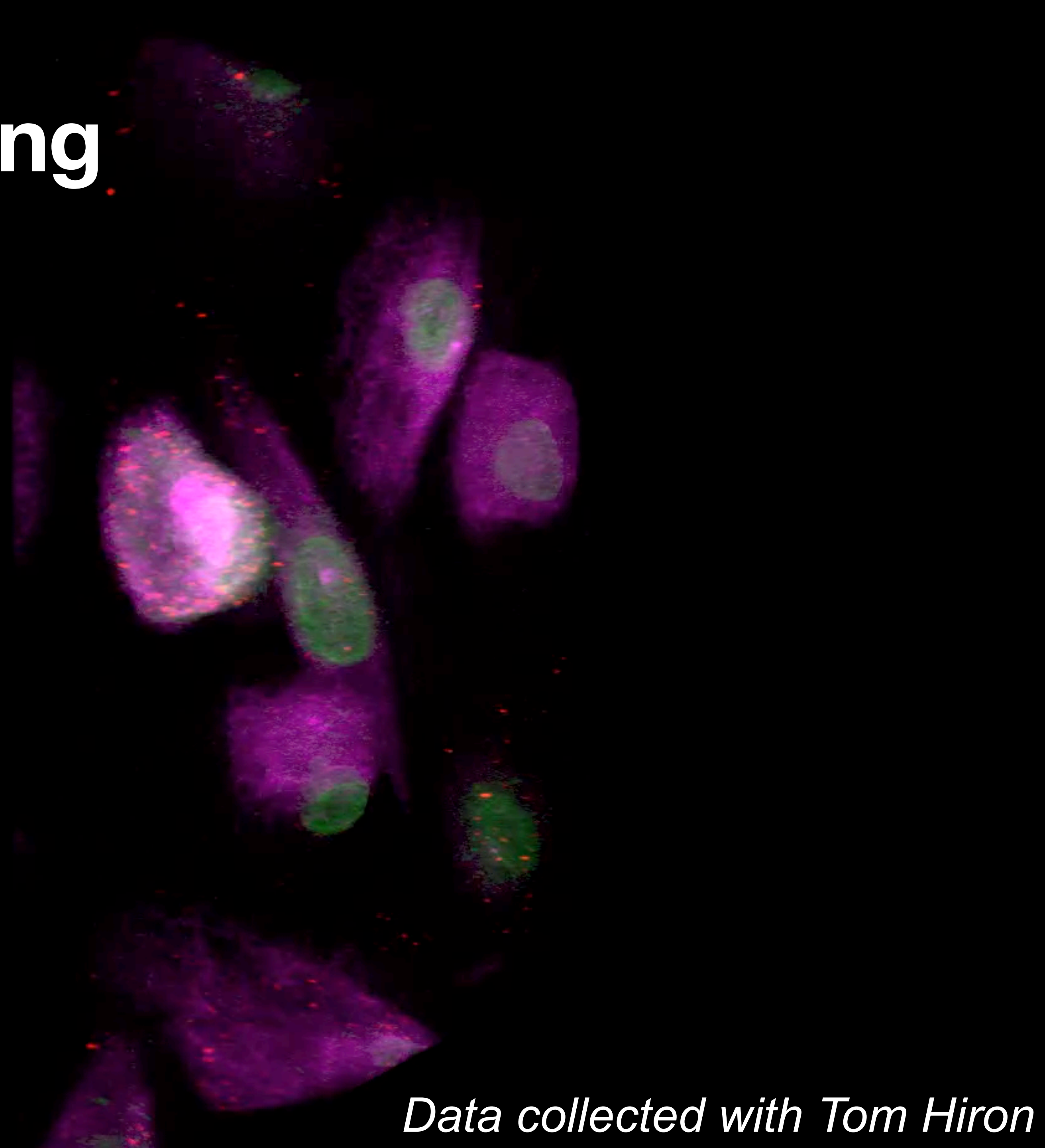
Airyscan 3D Reconstruction



Airyscan live cell imaging

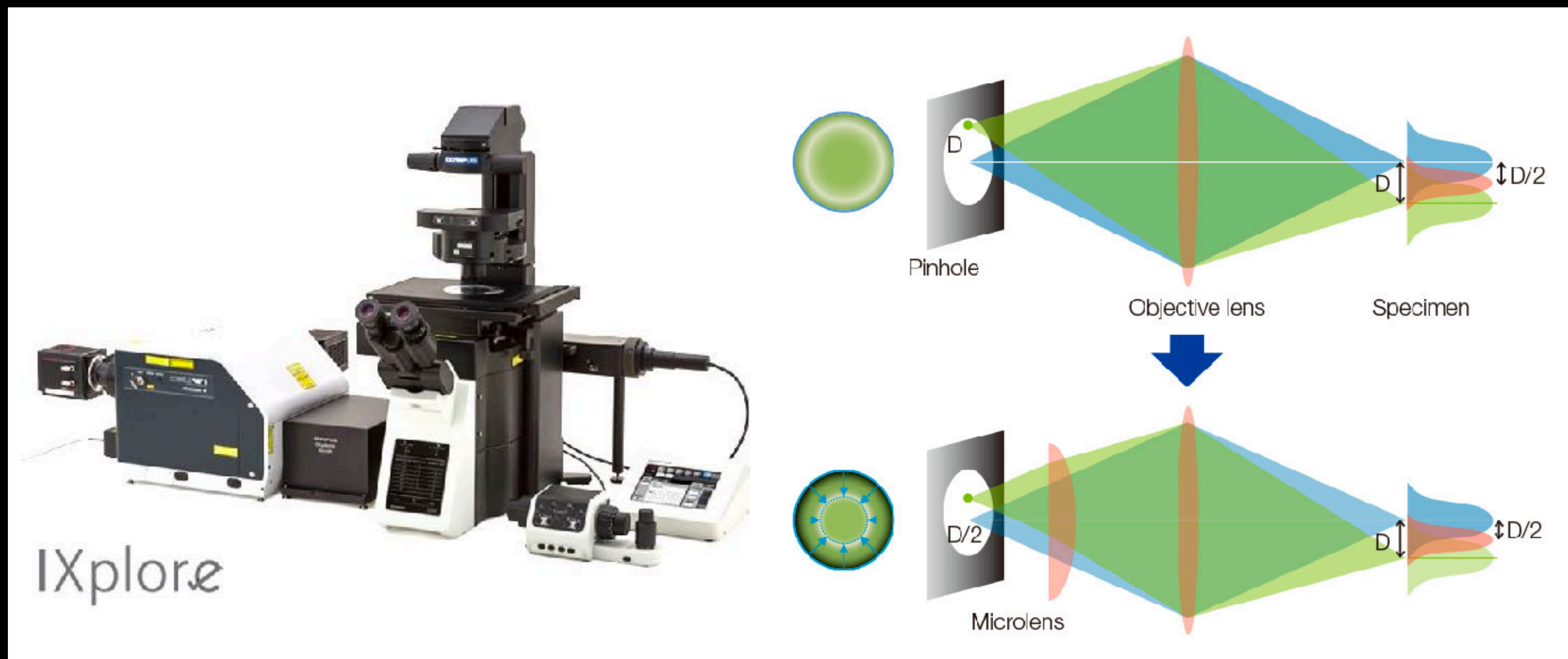
Super-resolution and sensitivity allowing cells to remain viable over long timelapse experiments

- 5 minute time lapse
- 20 hour duration
- 3 channels
- 50 z sections @ 0.19 μm
- 4 xy points



Data collected with Tom Hiron

SoRa - Super-resolution spinning disk



- Idealised pinhole and custom deconvolution achieving 120nm x/y resolution
- Very fast - up to 50fps
- Can also be run in standard SD confocal mode for viability and speed
- 2 camera options
- Standard sample preparation

Temporal resolution

SoRa super-res

Live EB3 comets tracking microtubule tips

GFP-EB3

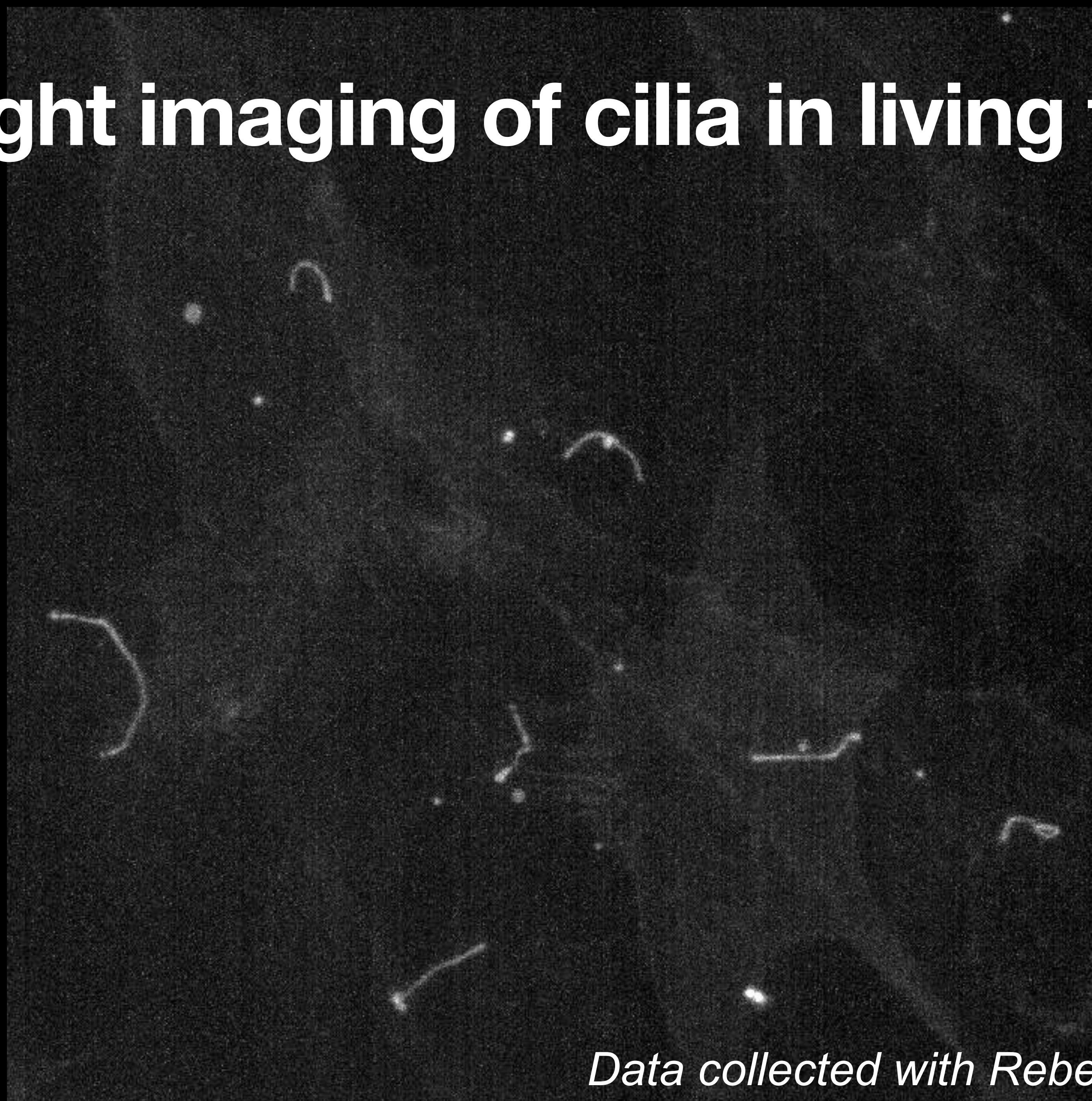
500 ms/frame

2 μ m



Fast low light imaging of cilia in living fibroblasts

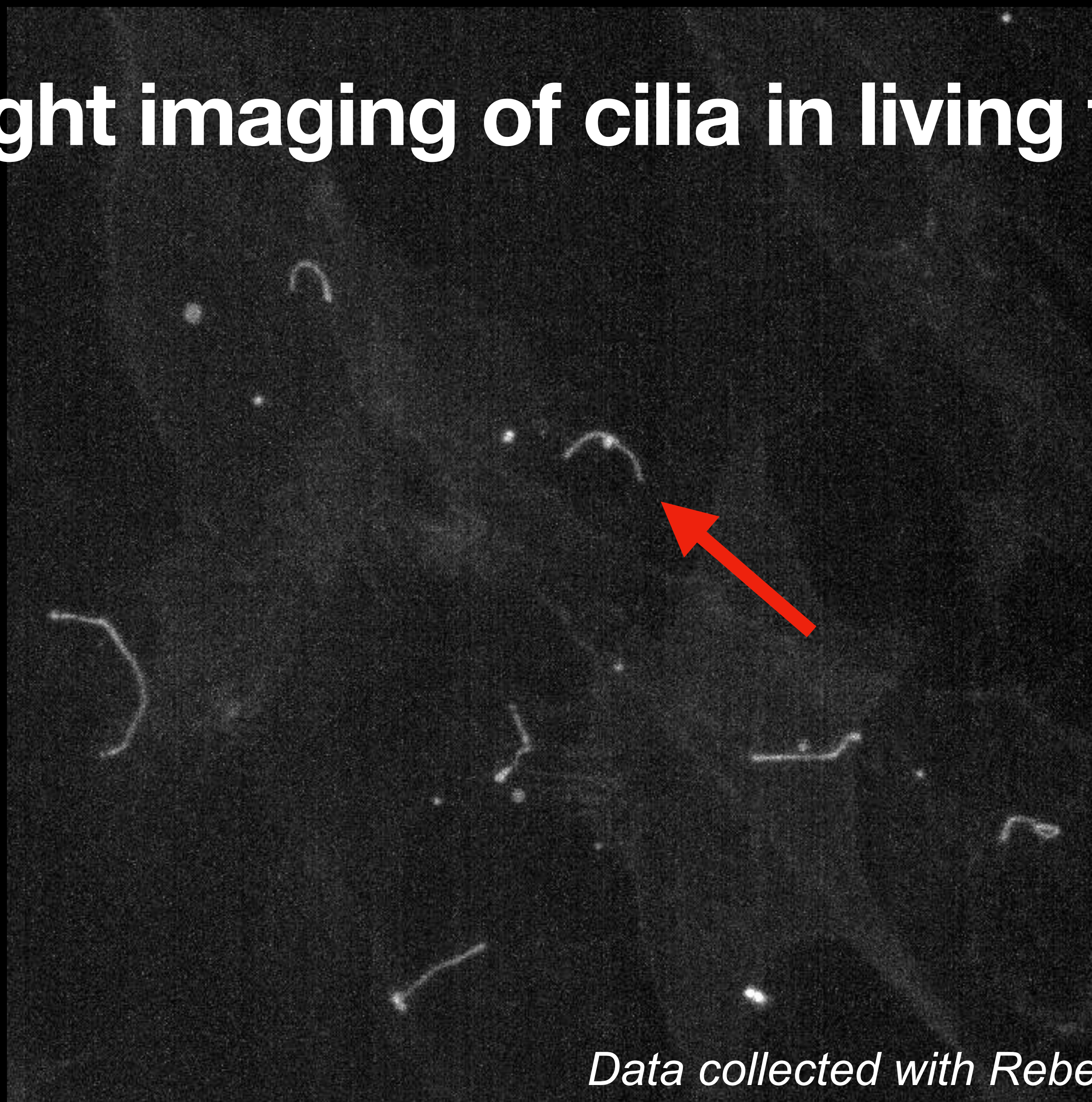
*16 fields collected
across 8 wells
every 3 minutes for
10 hours*



**SoRa
super-res**

Data collected with Rebekka Siebold-Schwab

Fast low light imaging of cilia in living fibroblasts



*16 fields collected
across 8 wells
every 3 minutes for
10 hours*

**SoRa
super-res**

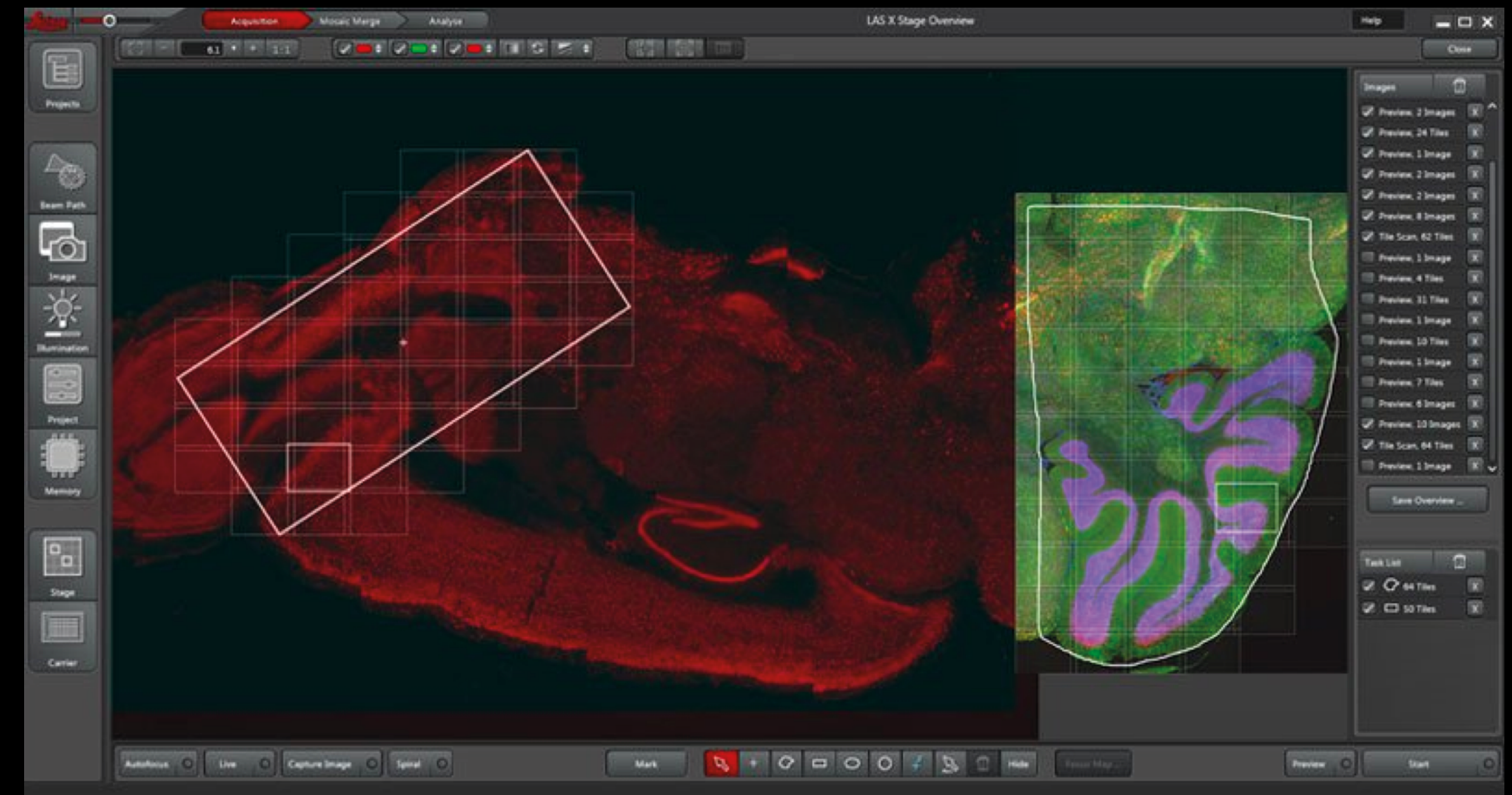
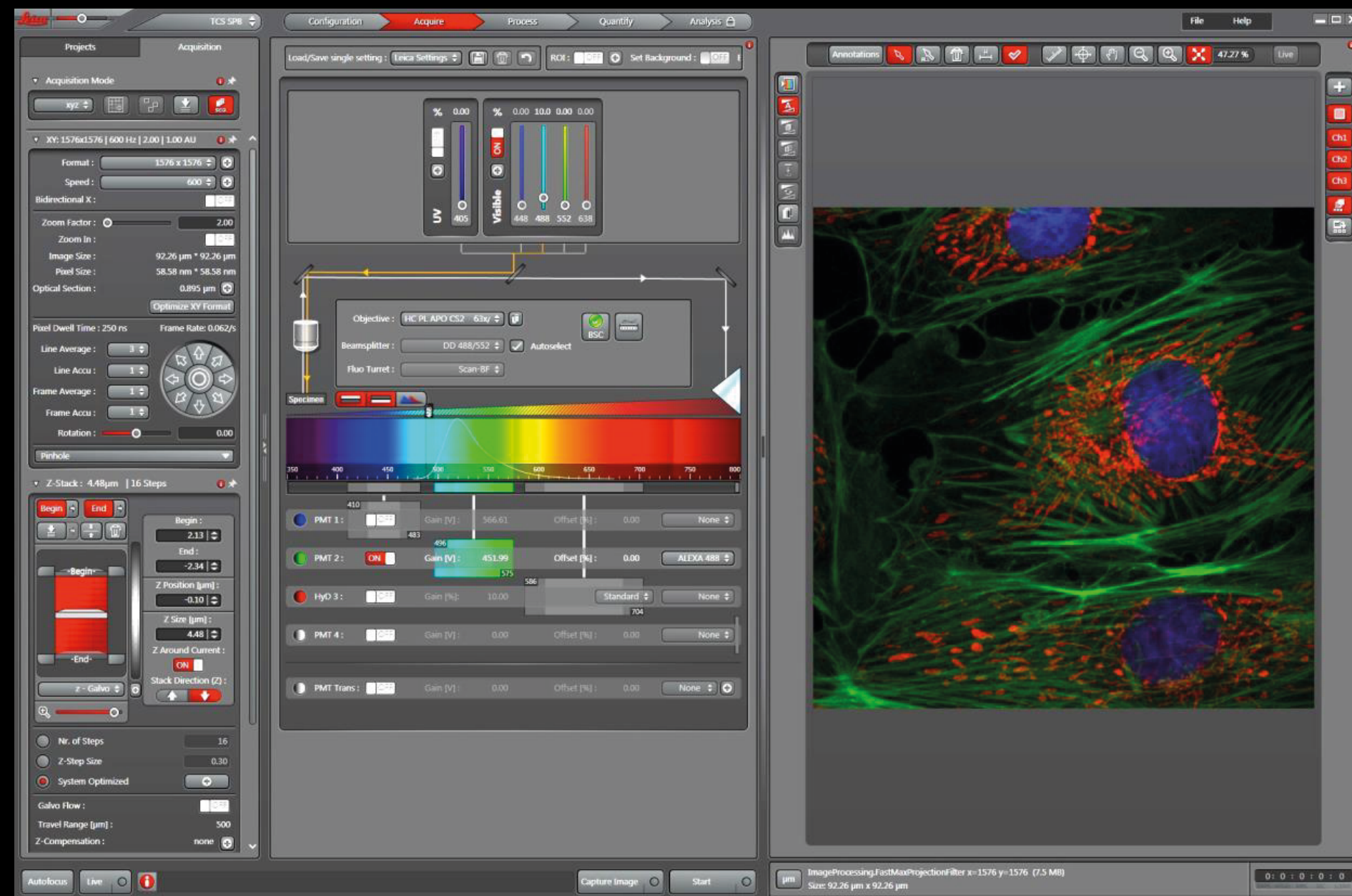
Data collected with Rebekka Siebold-Schwab

Point scanning confocal systems



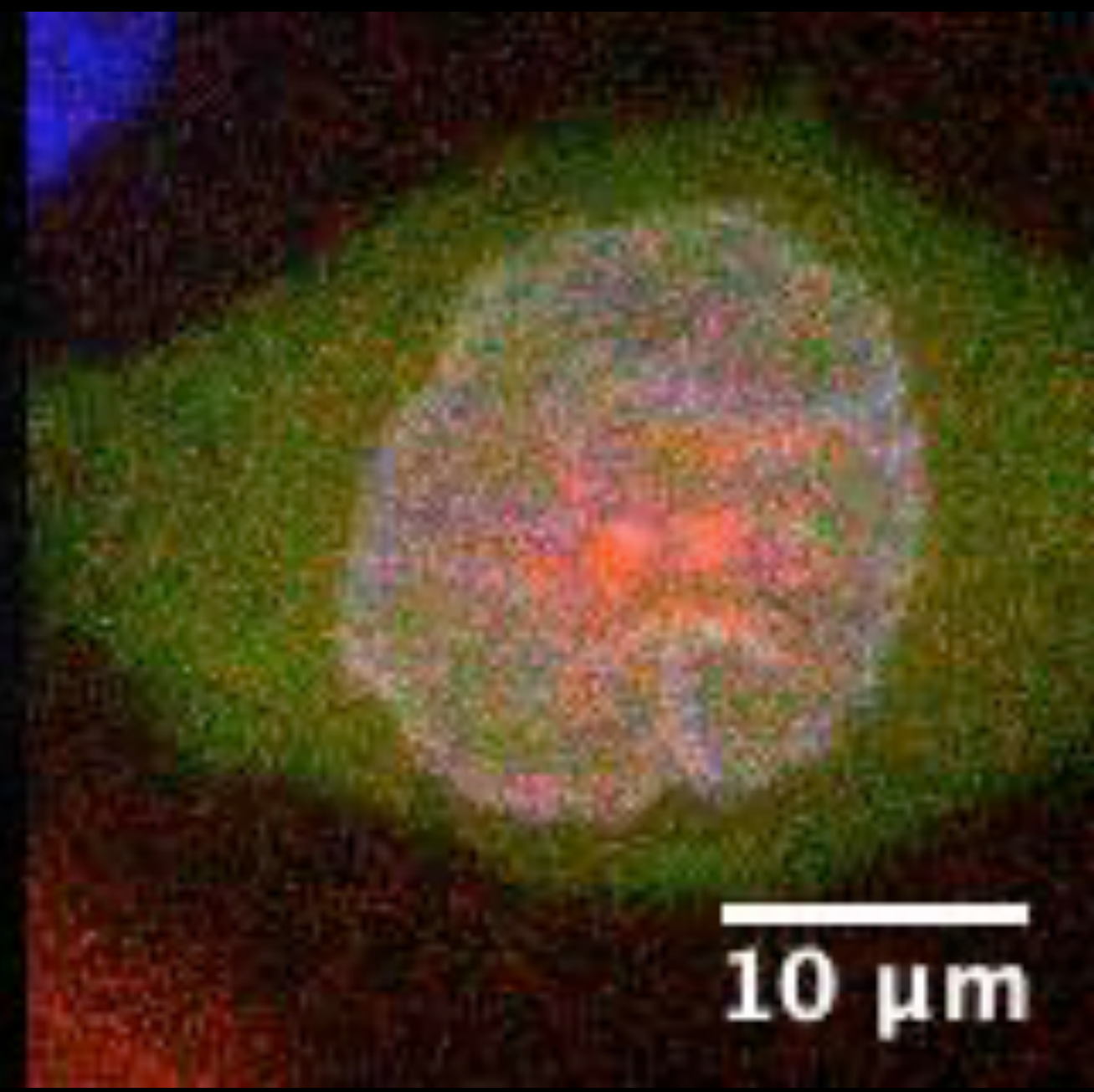
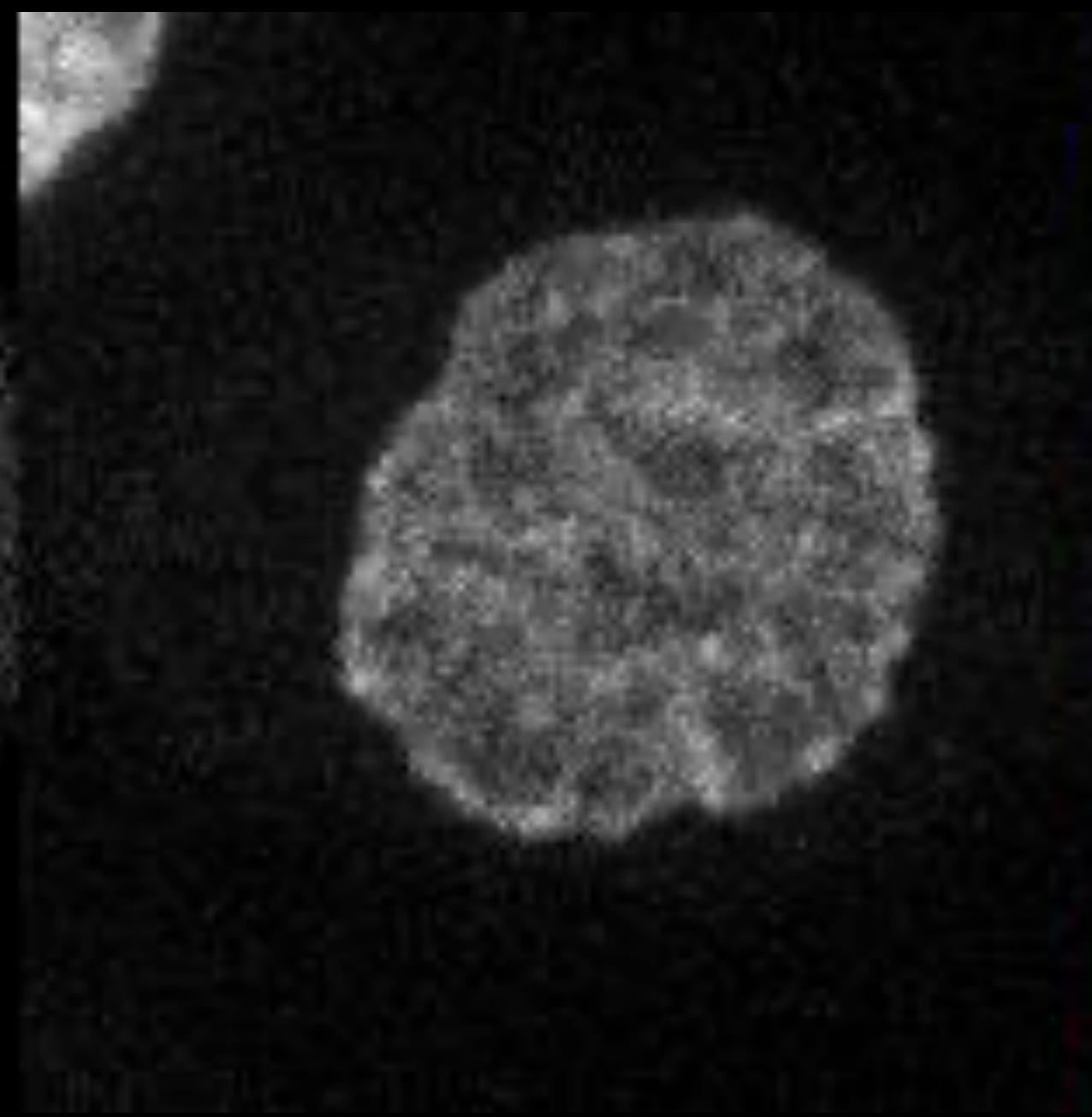
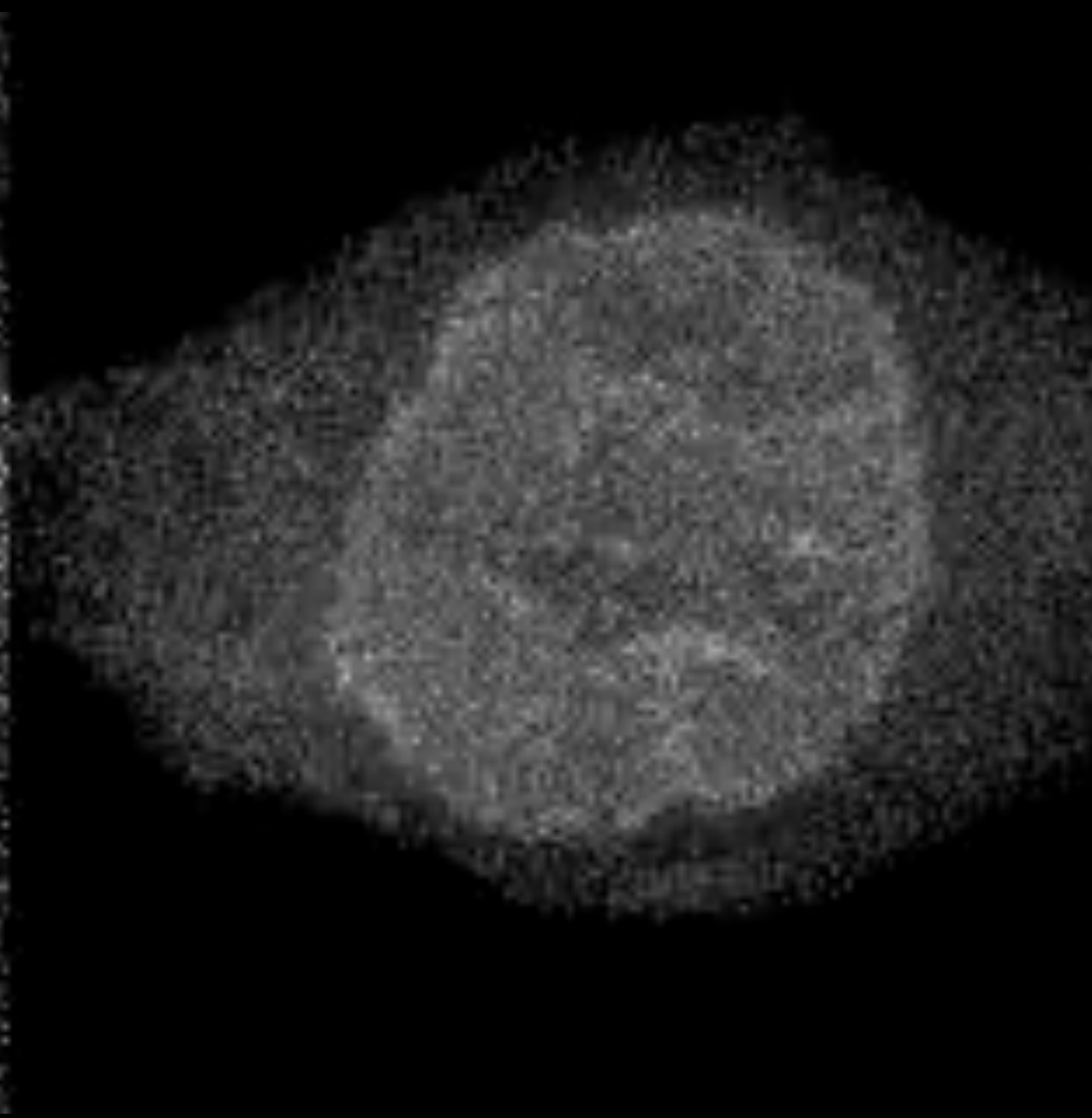
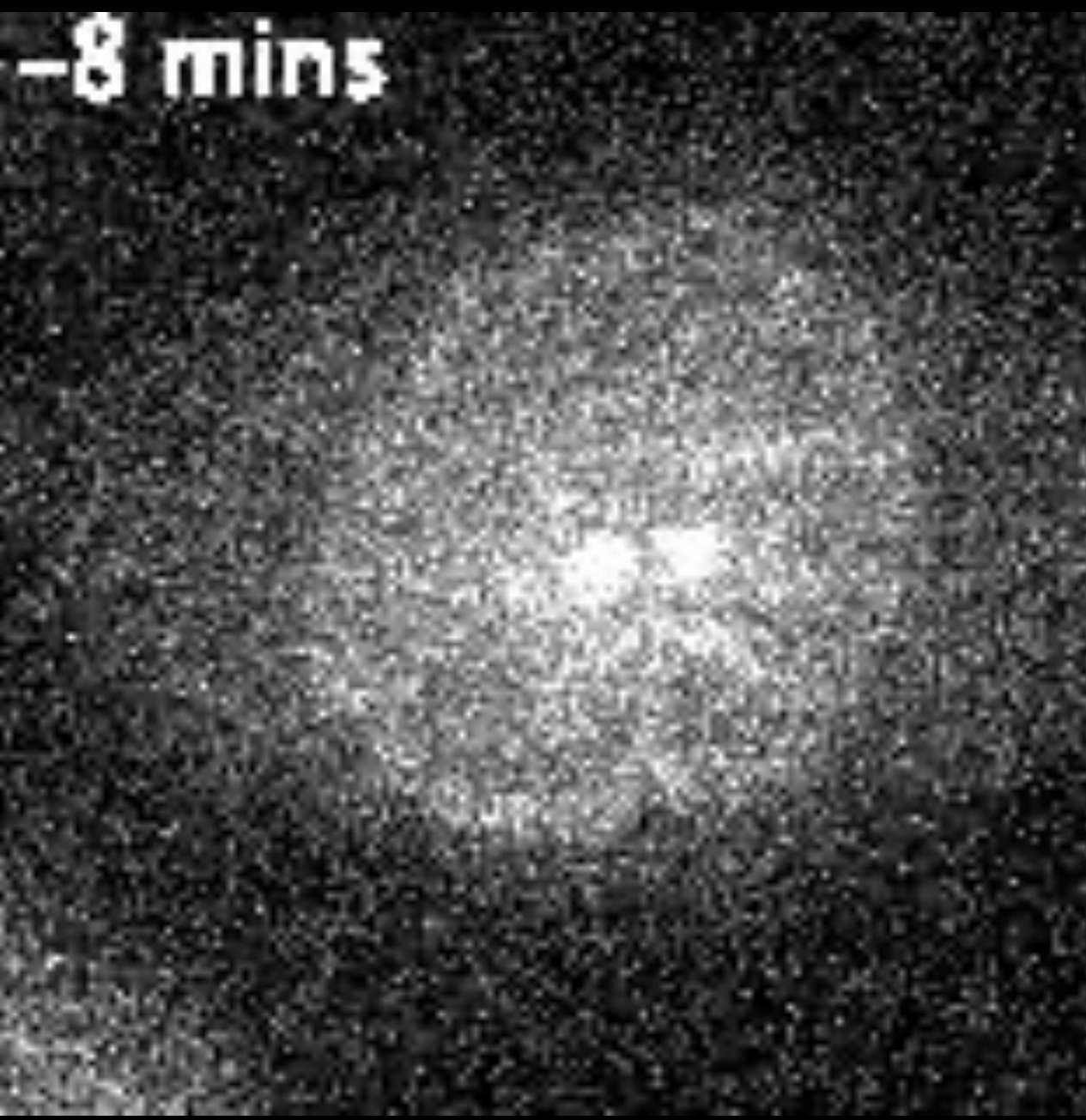
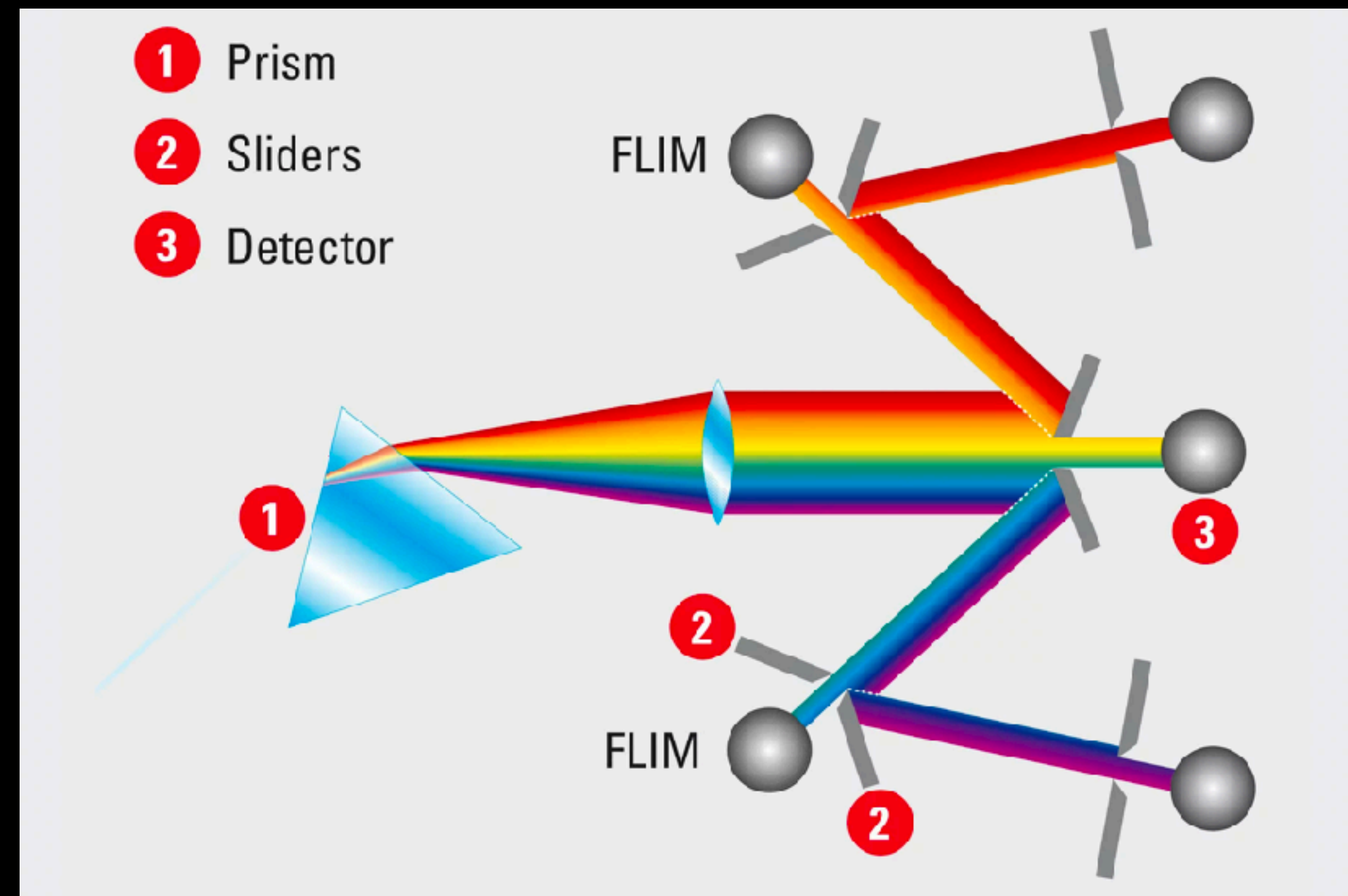
Leica SP8 and SP8 FALCON overview

- Leica SP8 with white light laser and LAS X Navigator, 2 x HyDs, 2 x PMTs and dual external fluorescence lifetime detectors (photon-counting)
- Leica SP8 FALCON, 2 PMTs and 3 HyDs, fully integrated lifetime detection and resonant scan mode for super-fast imaging
- These systems offer sensitivity and huge spectral flexibility
- Easy to configure light paths
- FRET, FLIM, FRAP
- Simple large area multi-FOV imaging and seamless stitching



Spectral imaging

Rapid spectral selection using
AOTF and multiple detectors



Multi-channel Imaging

Spectral flexibility

- Setting up light paths has been simplified by software
- Fluorophores of interest can be selected along with the desired detector type
- Users can select their preferred optical strategy
- Scanning strategies -
 - None sequential = all required lasers at once
 - Line sequential = lasers pulsed and lines scanned with subset of lasers to prevent cross detection but detector windows not moved
 - Frame/stack sequential = each channel scanned separately lasers and detector windows optimised for the channel. Useful with partially overlapping spectra but slow.

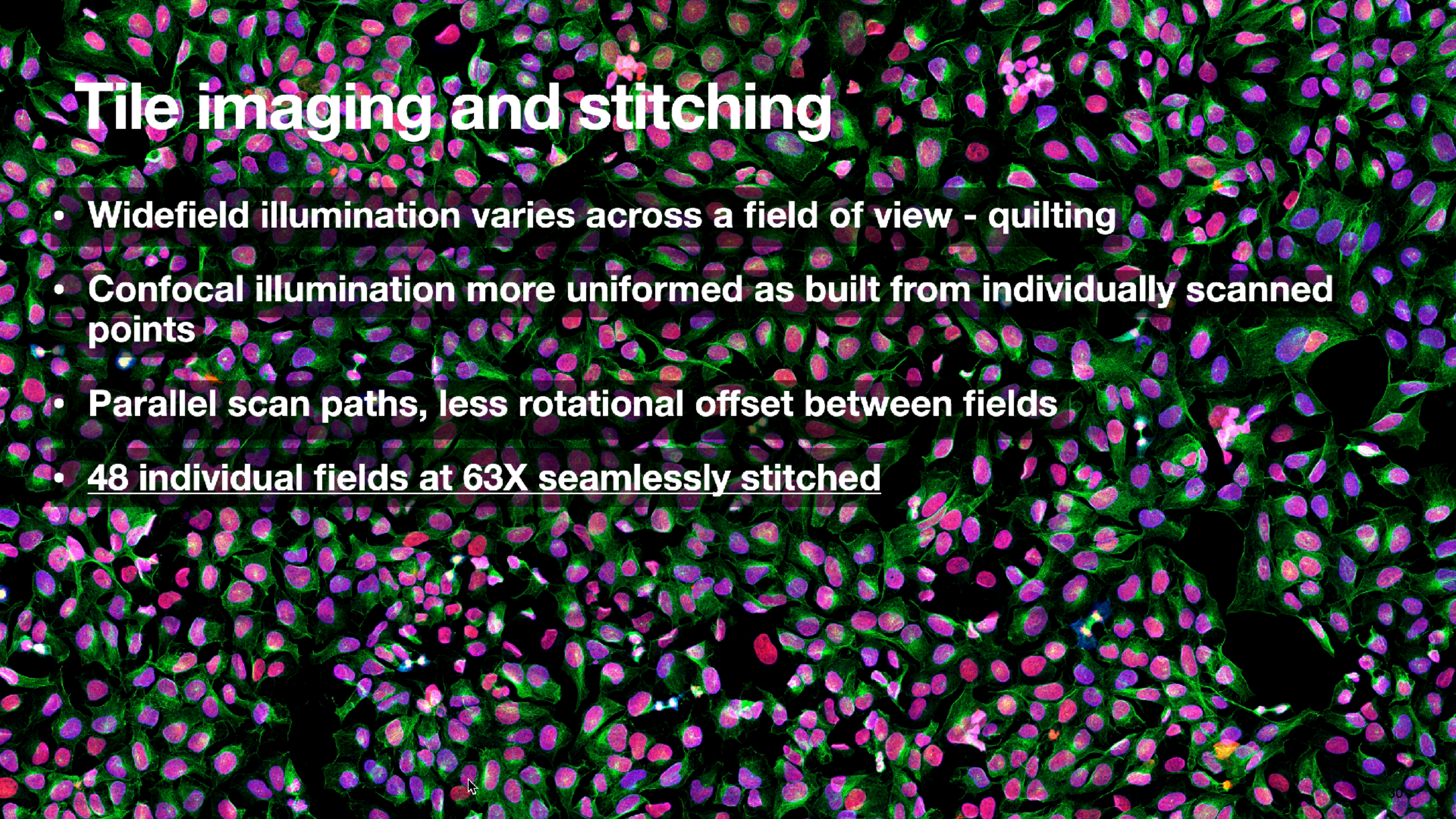
The screenshot displays a software interface for configuring multi-channel imaging. At the top, a list of dyes is shown: DAPI, ALEXA 488, and Mito Tracker Red. Each dye has a corresponding color swatch and a dropdown menu for selecting the detector type (HyD or PMT). A red dashed box highlights the detector selection area, and a red arrow points to the 'HyD' option for Mito Tracker Red. Below the dye list, five scanning strategies are presented, each with a table of dye yield and crosstalk, and a spectral plot showing the relative intensity of the dyes. The strategies are: 1. None sequential (all dyes at once), 2. Line sequential, 2 sequences (DAPI and Mito Tracker Red first, then ALEXA 488), 3. Frame or stack sequential, 2 sequences (DAPI and Mito Tracker Red first, then ALEXA 488), 4. Line sequential, 3 sequences (DAPI, then ALEXA 488, then Mito Tracker Red), and 5. Frame or stack sequential, 3 sequences (DAPI, then ALEXA 488, then Mito Tracker Red). Each strategy includes 'Edit ...' and 'Apply' buttons.

Tile imaging and stitching

- Widefield illumination varies across a field of view - quilting
- Confocal illumination more uniform as built from individually scanned points
- Parallel scan paths, less rotational offset between fields
- 48 individual fields at 63X seamlessly stitched

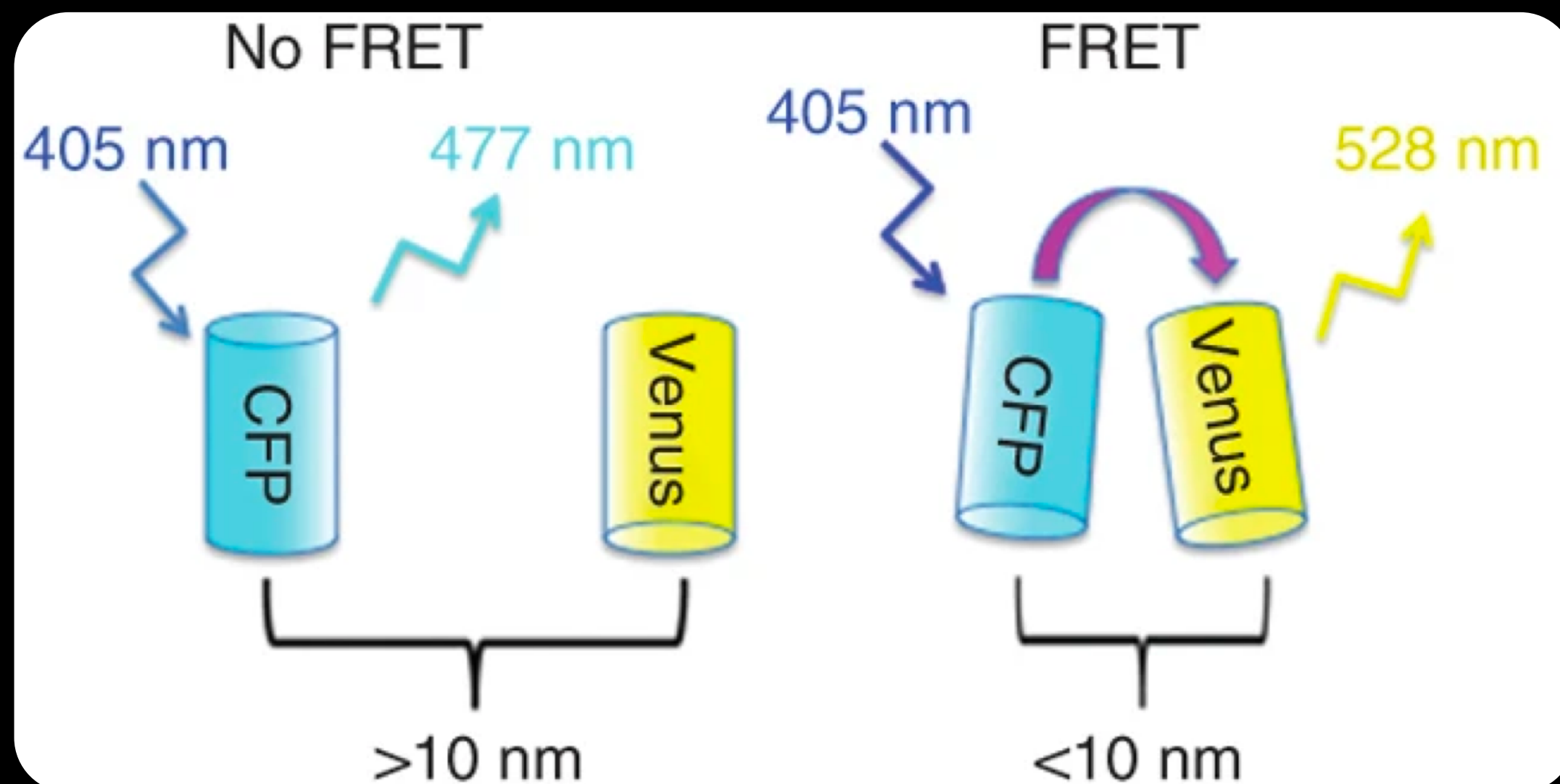
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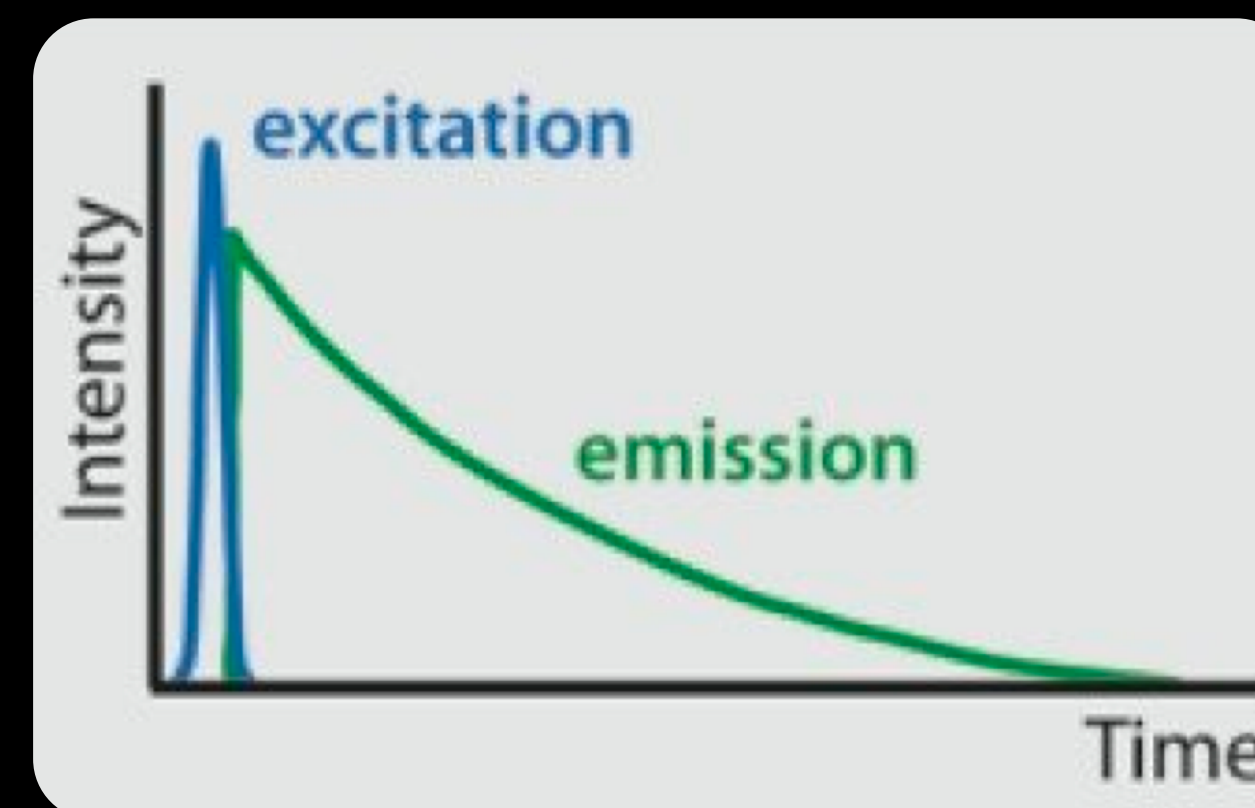


Biosensors - FRET & FLIM

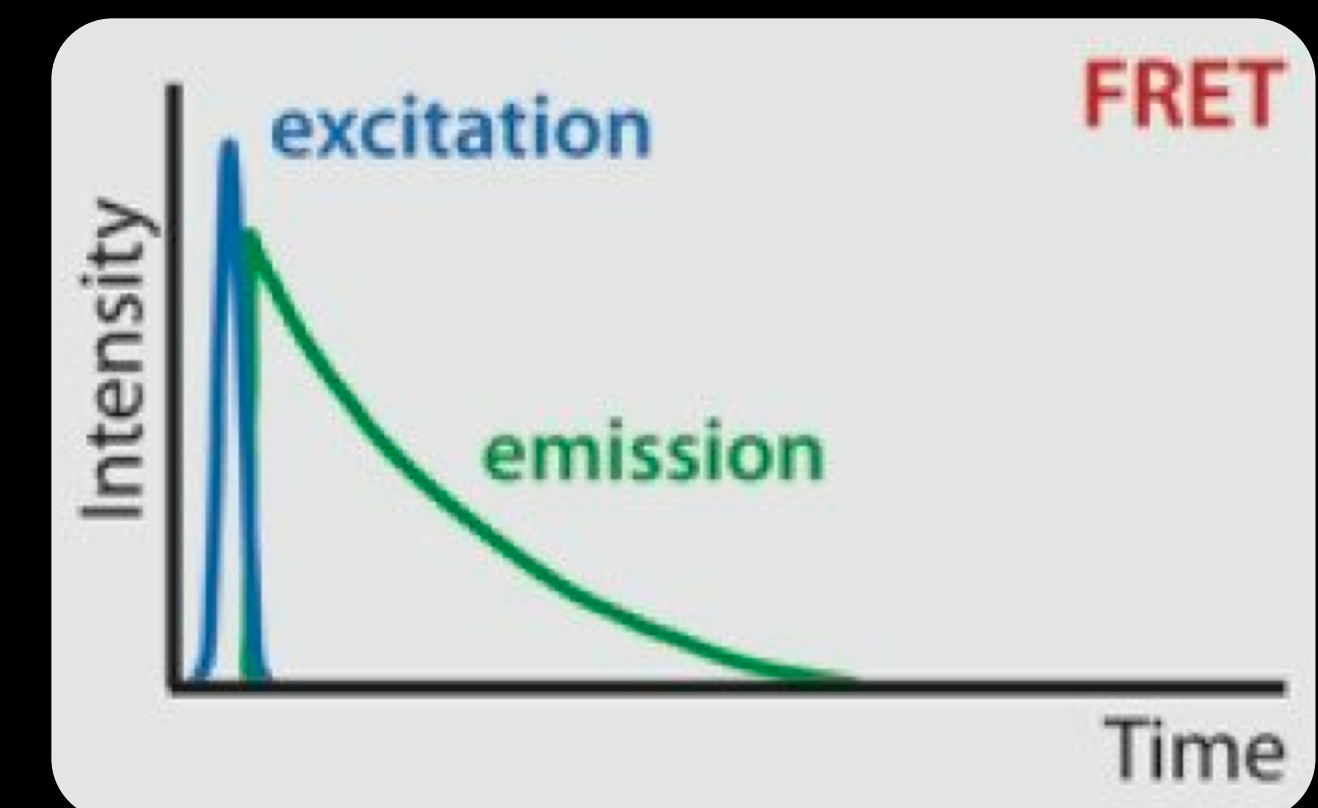
- When a fluorophore is excited it emits light in a longer wavelength (Stokes Shift)
- If another fluorophore is within 10 nm of the emitting molecule (the donor) and can be excited by the wavelength of the emitted photon this molecule can be excited directly (acceptor)
- The excited acceptor then emits a photon of longer wavelength
- We can use this to study molecular interactions
- FLIM offers a more quantitative option by observing changes in the donor lifetime - if FRET is occurring the donor lifetime decreases



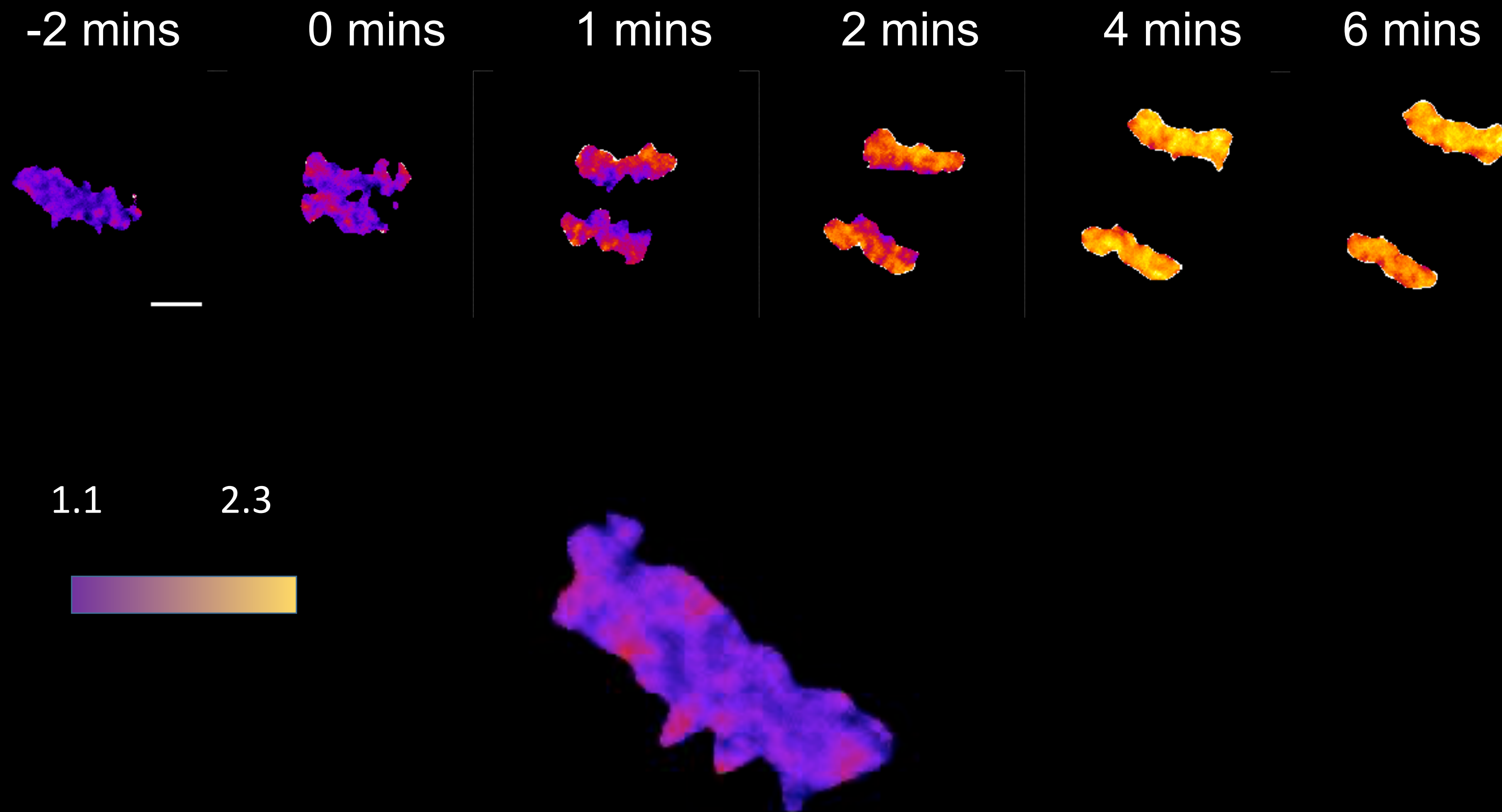
Donor only



Donor with acceptor



Measuring dephosphorylation by phosphatase using FLIM-FRET

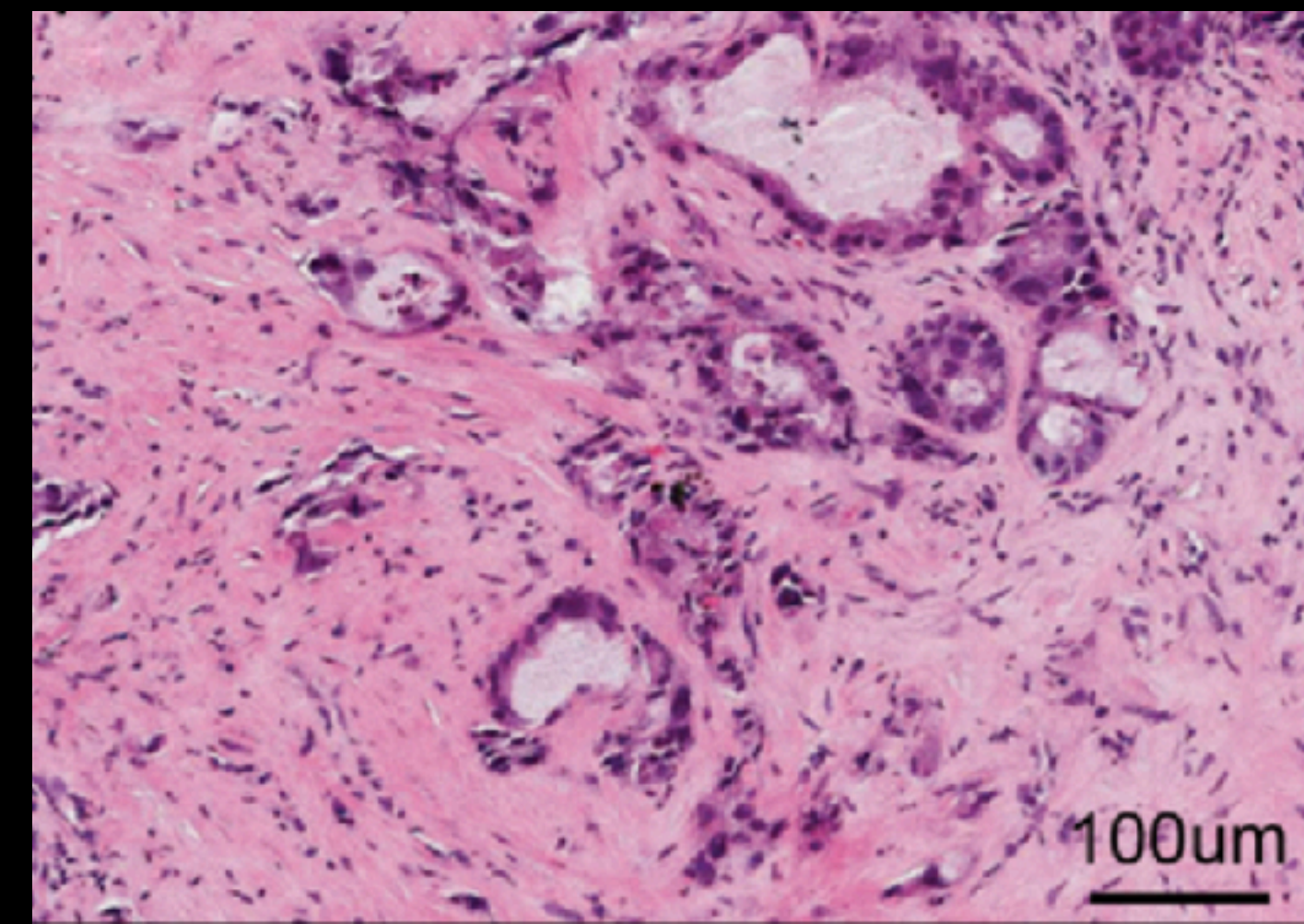
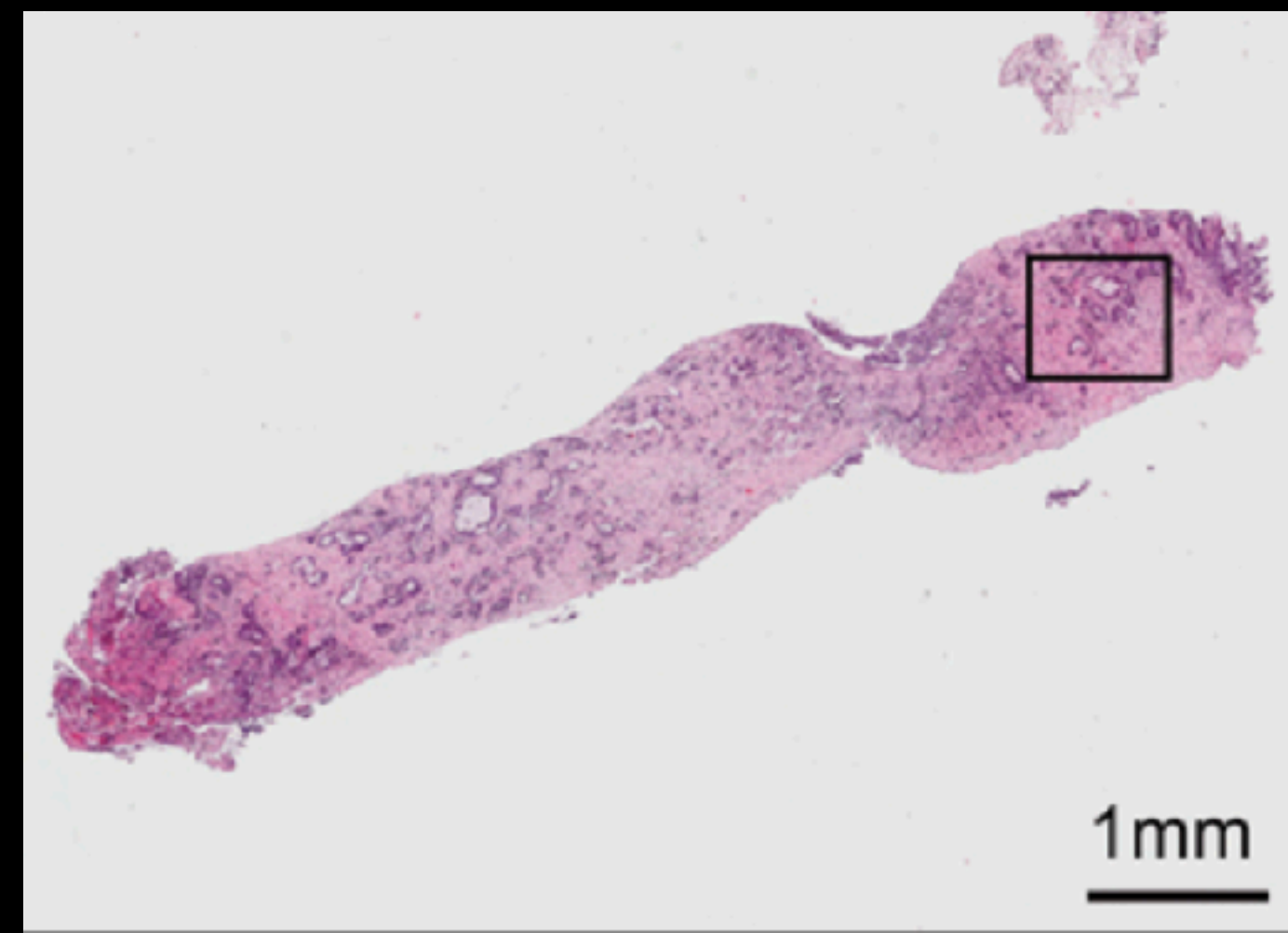
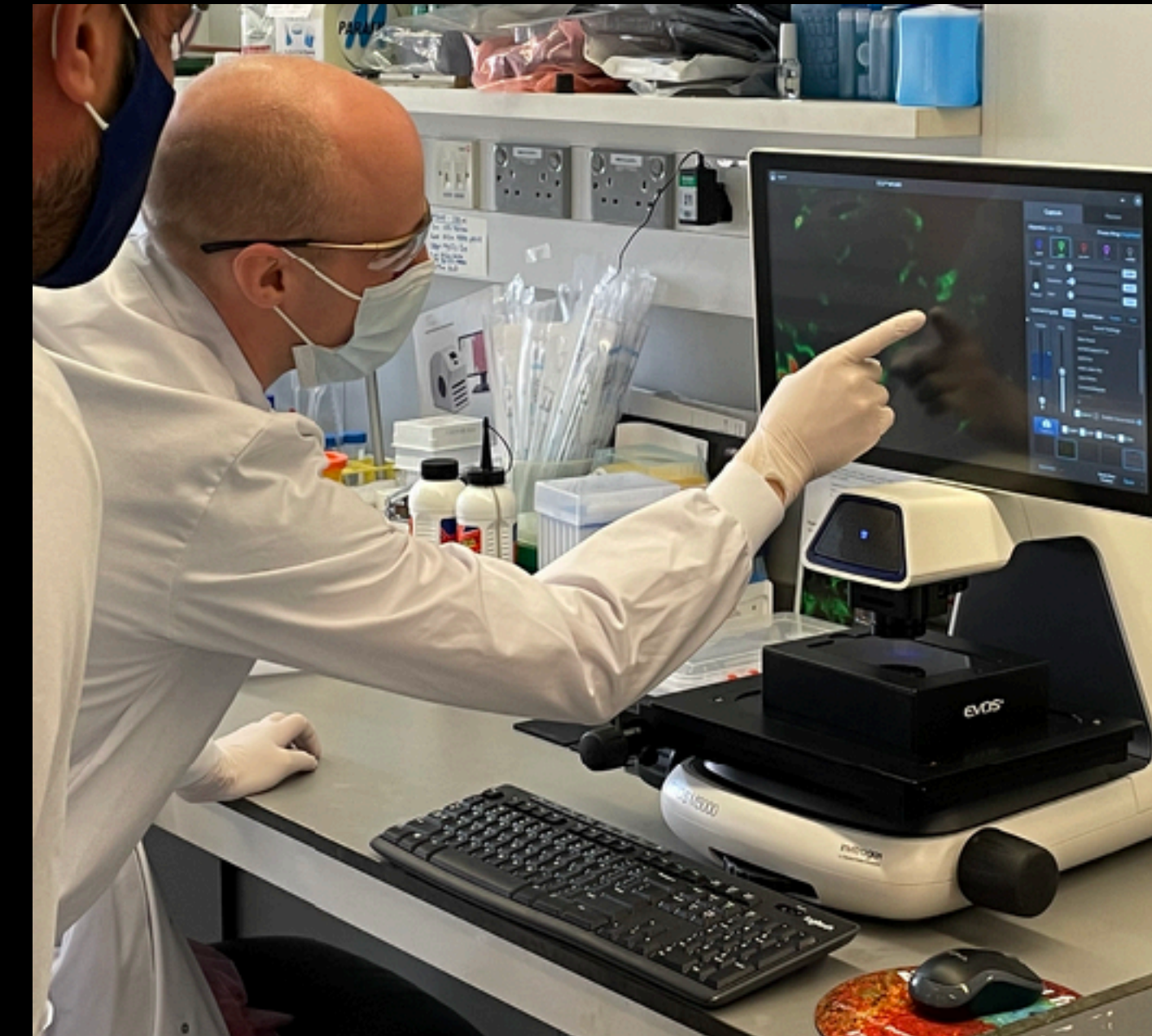
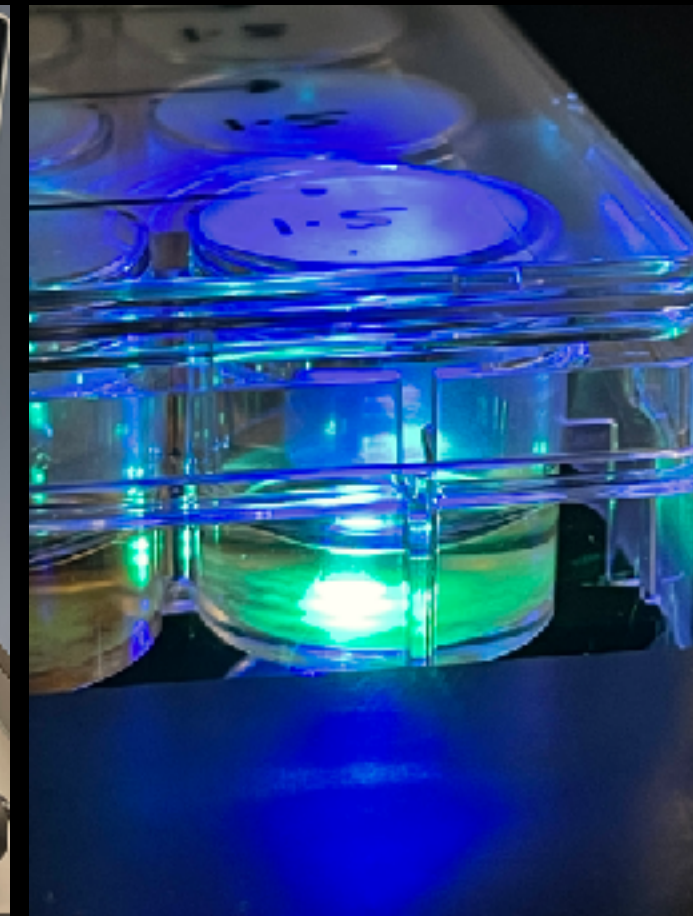
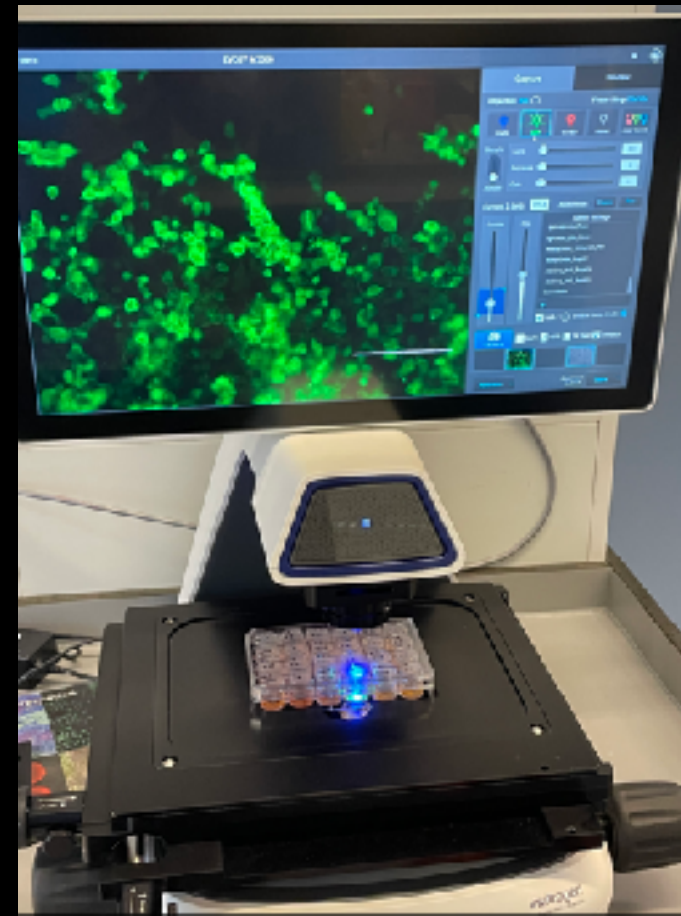


An Aurora B kinase sensor fused to Histone 3 to tether it to DNA shows reduction in phosphorylation as chromatin moves away from Aurora B which remains on the central spindle during anaphase

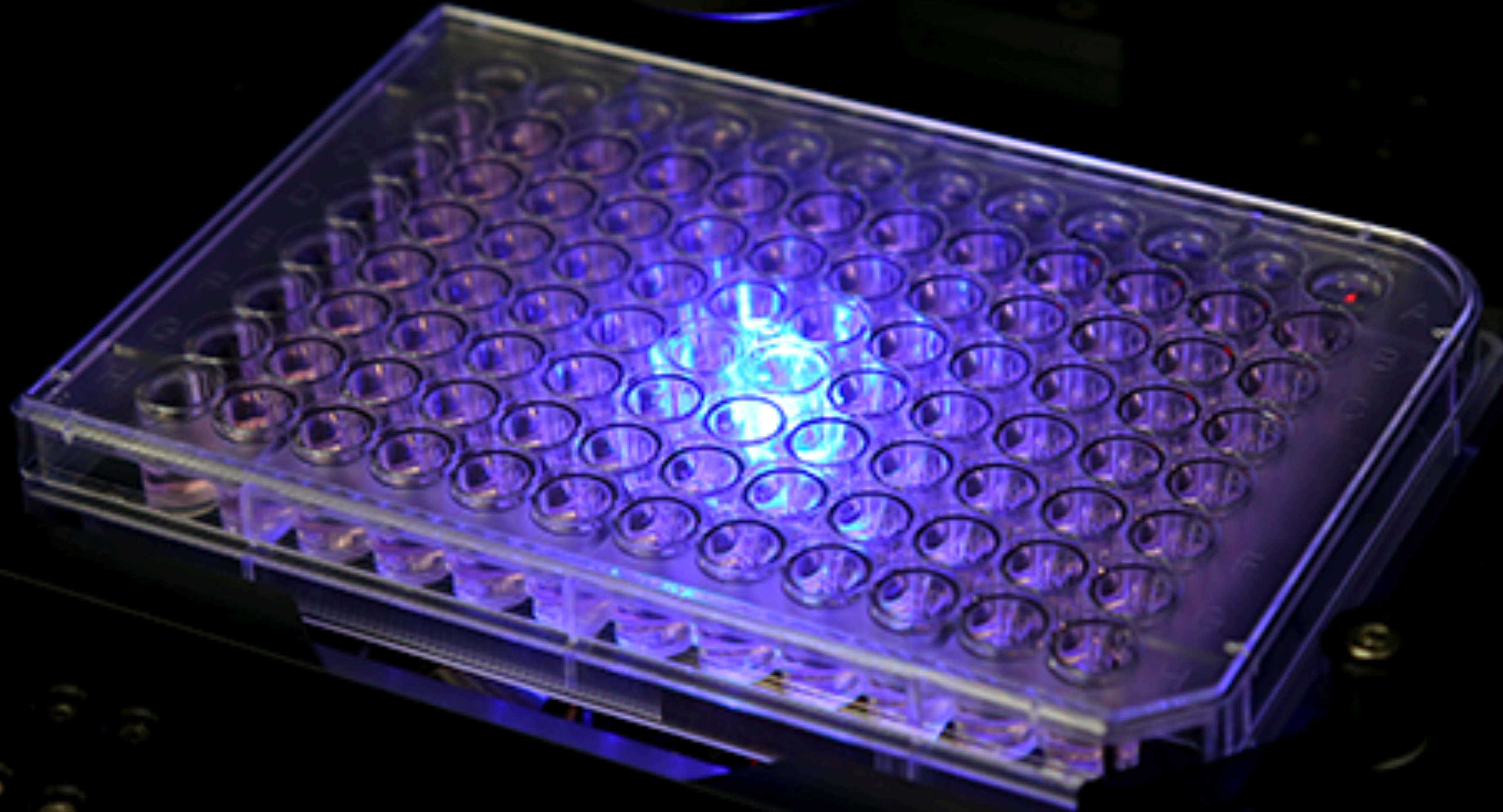
Widefield Imaging

Leica DMI8 and EVOS M5000

- EVOS M5000 easy to use, high sensitivity lab based fluorescent system (4 channels + colour)
- Leica DMI8 advanced widefield
 - 2 cameras - colour + high-sensitivity monochrome
 - Fast tile stitching
 - Fast filter wheel
 - Objectives
 - Air
 - LWD
 - Oil



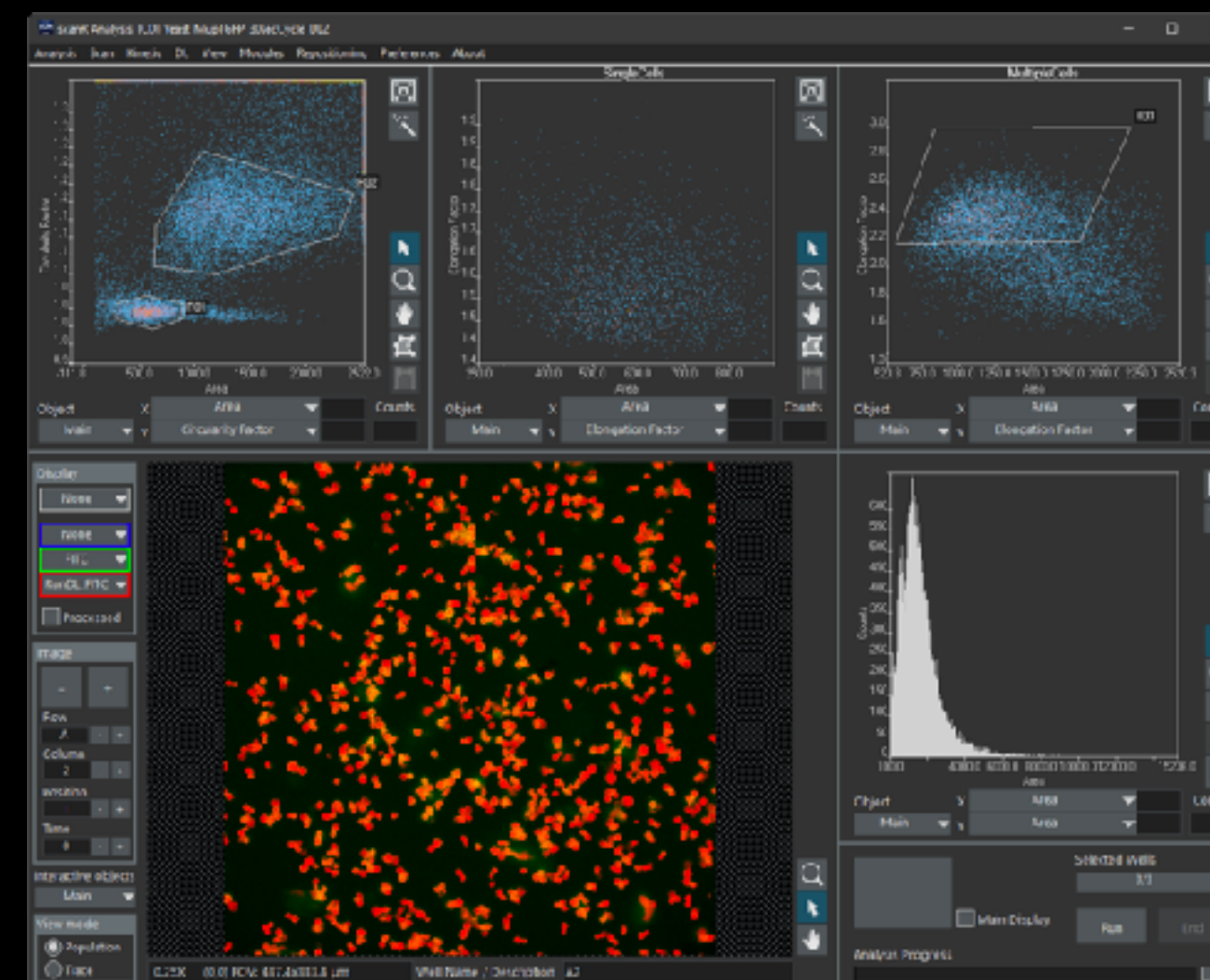
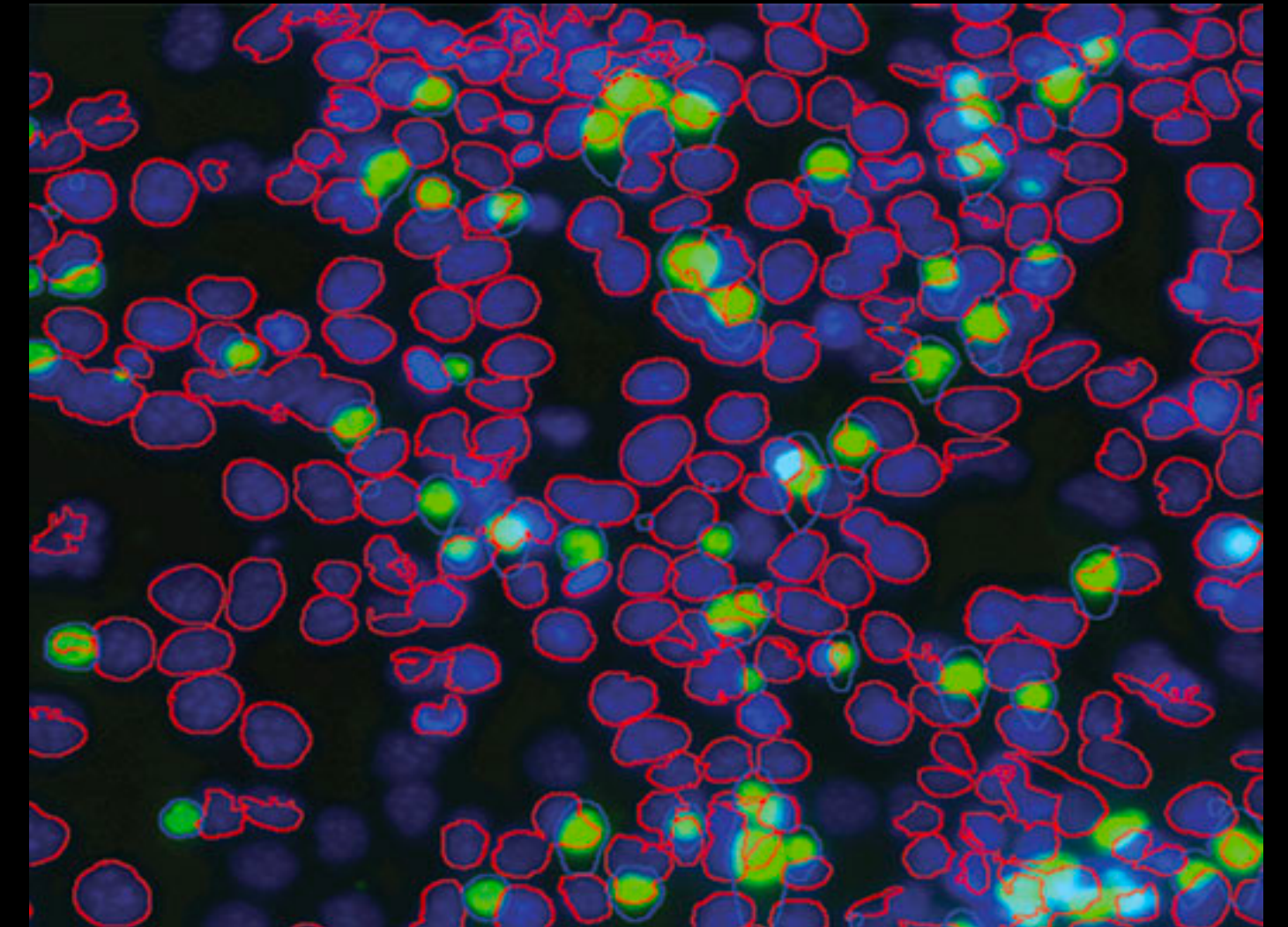
High content screening



High content imaging and analysis

Olympus ScanR

- ScanR - first high-content screening system with confocal and super-resolution in Oxford
- Large areas or large format plates (e.g. 96 well) scanning and full population data analysis
- Compatible with live cell imaging and fate map generation
- Deep learning and label free imaging tools
- Intuitive sub-population gating and analysis, similar to FACS



Laser Capture Micro-dissection (LCM)

Zeiss Microbeam

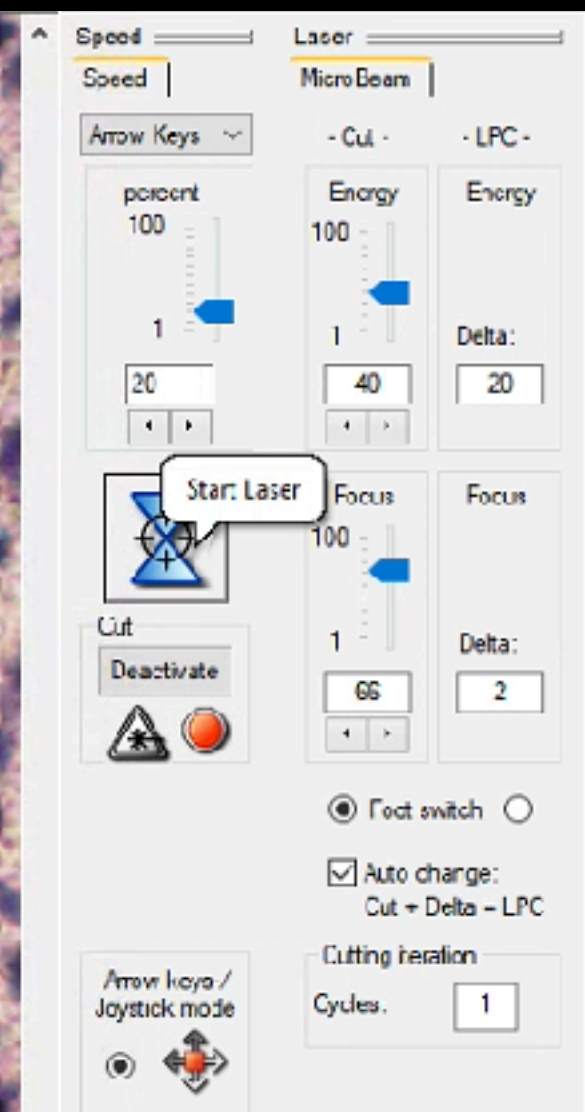
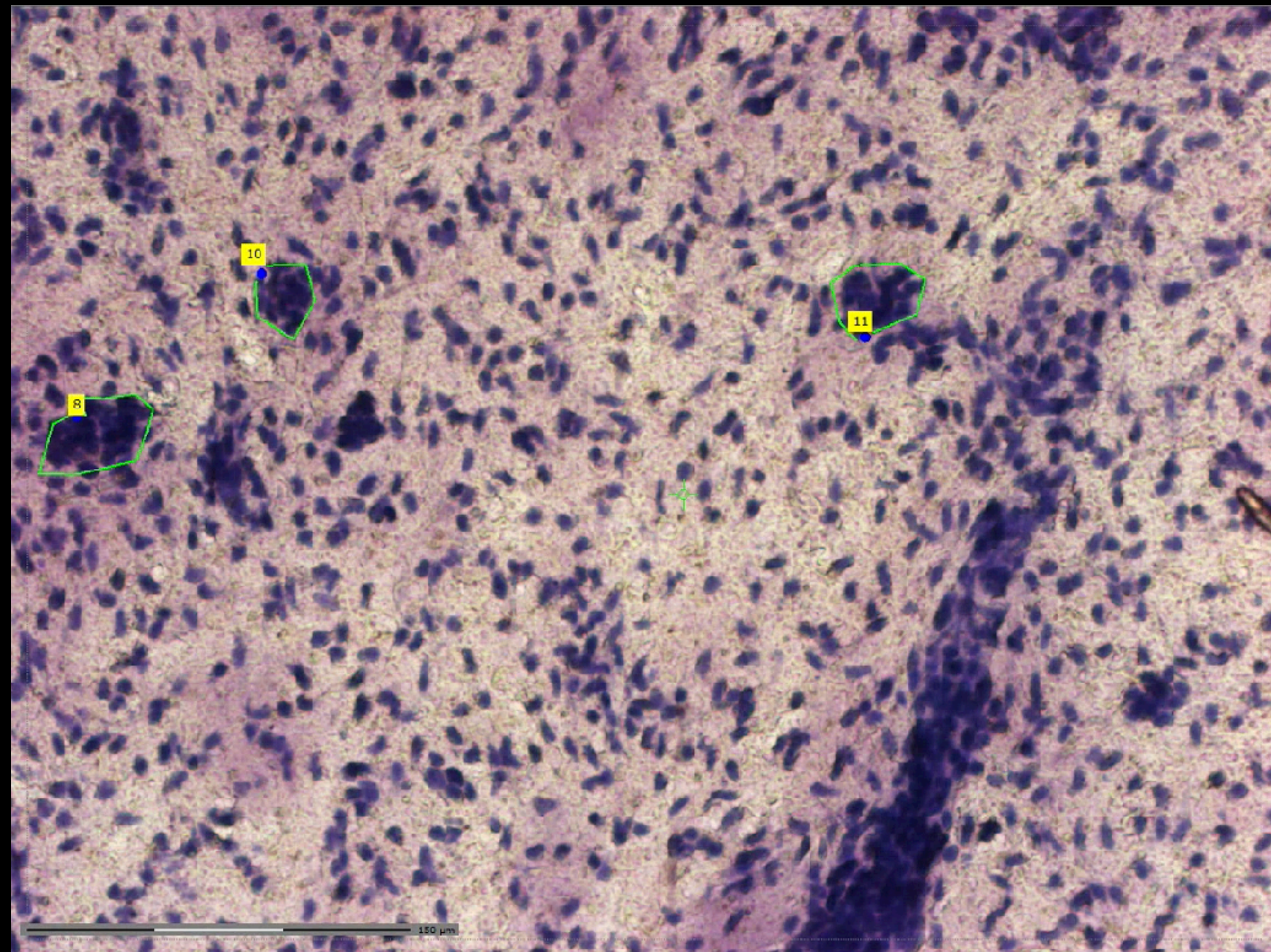
Pulsed LWL-UV laser cuts tissue samples

Defocused pulse moves sample into collection cap

Robomover upgrade can accommodate up to 96 collection caps

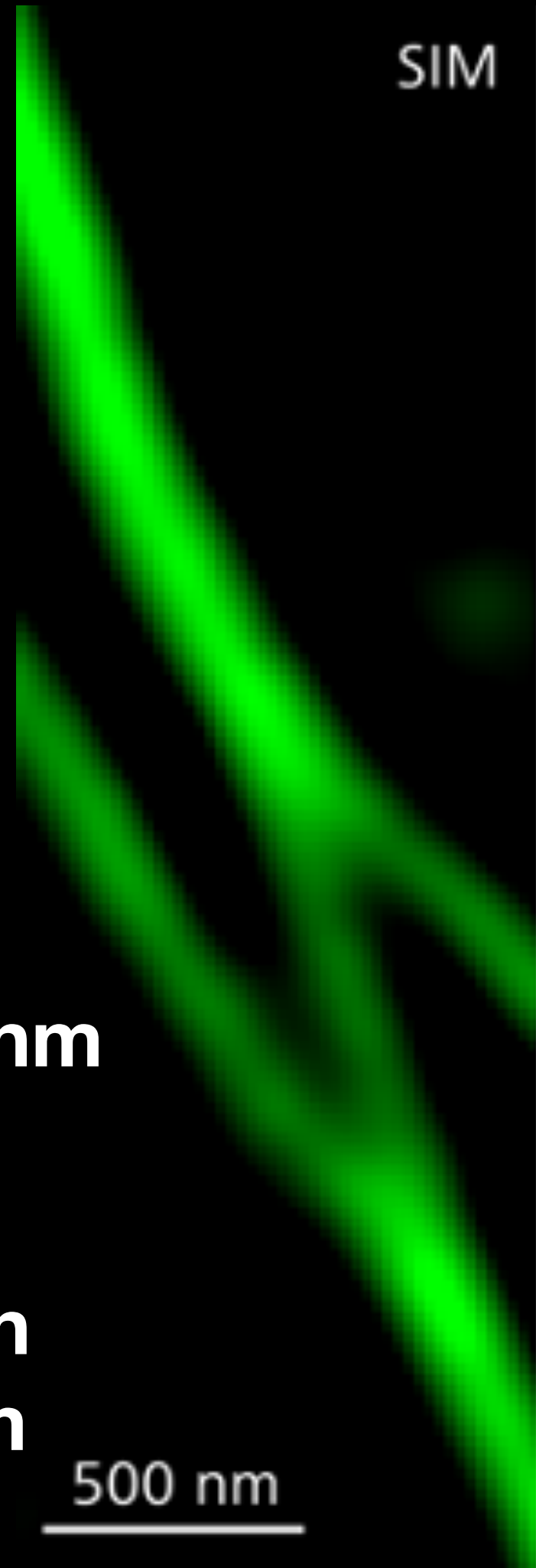
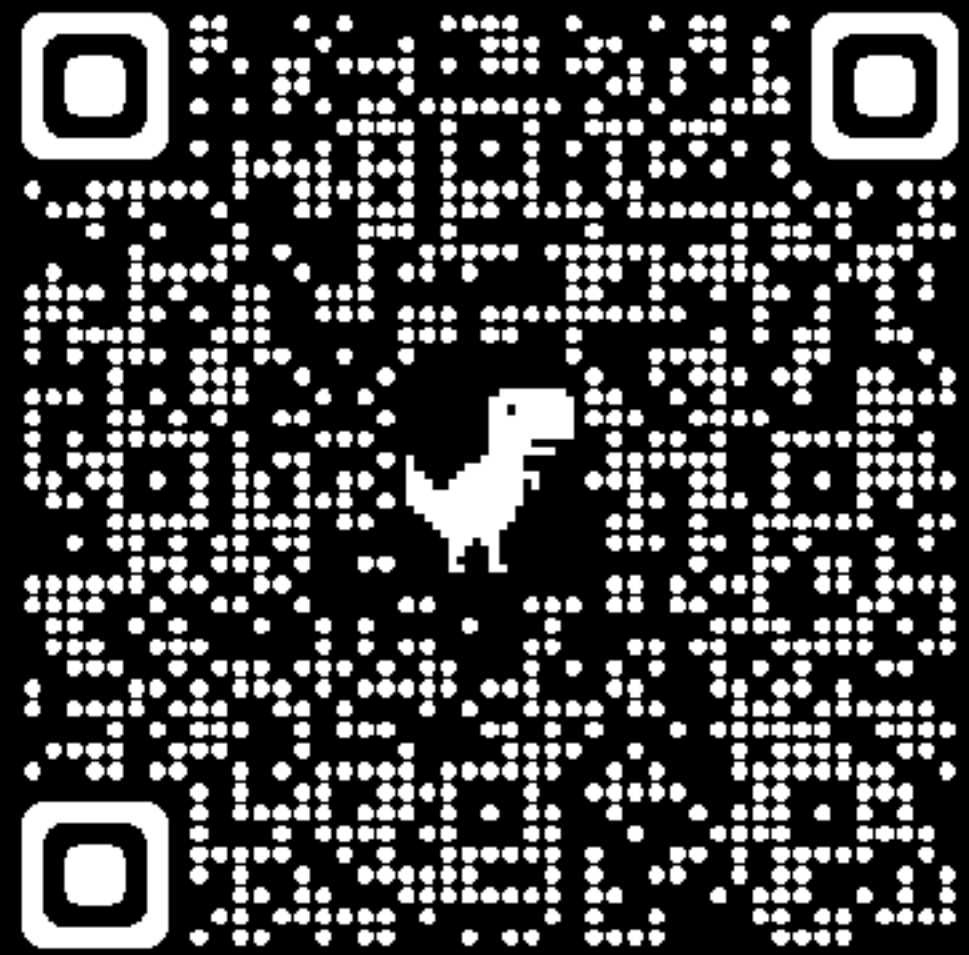
Monochrome camera and fluorescence now available

Downstream proteomic, transcriptomic or genomic sample analysis

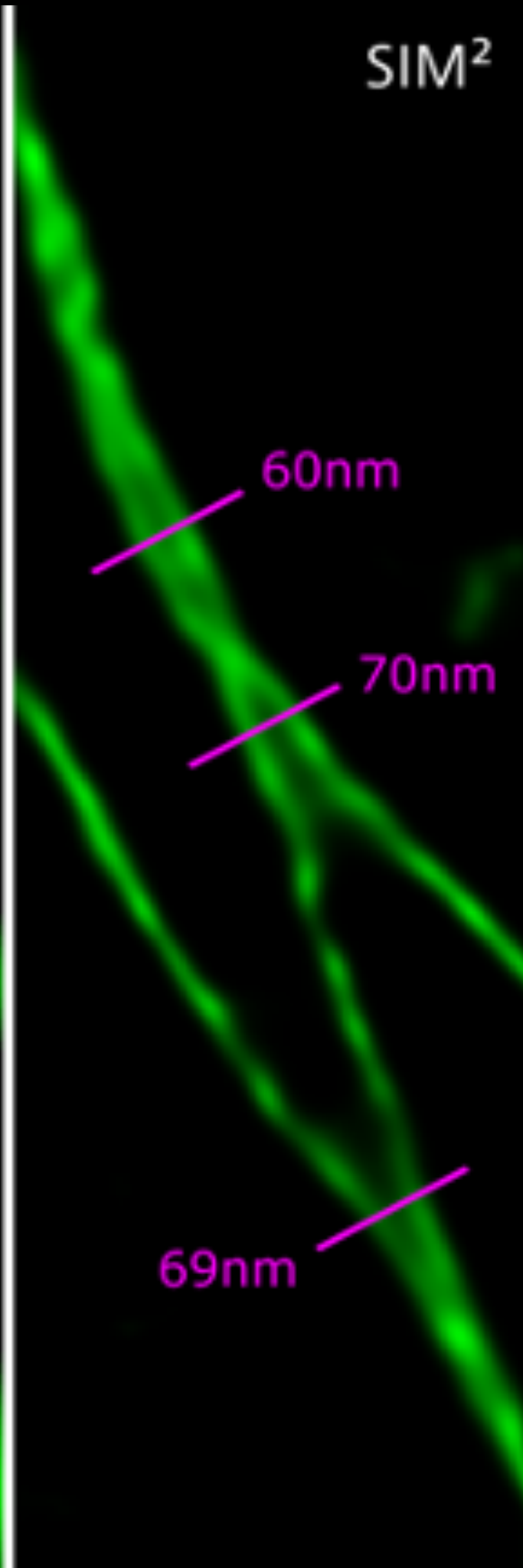


Coming soon - Zeiss Elyra 7 - Lattice SIM

Super-fast super-resolution



SIM

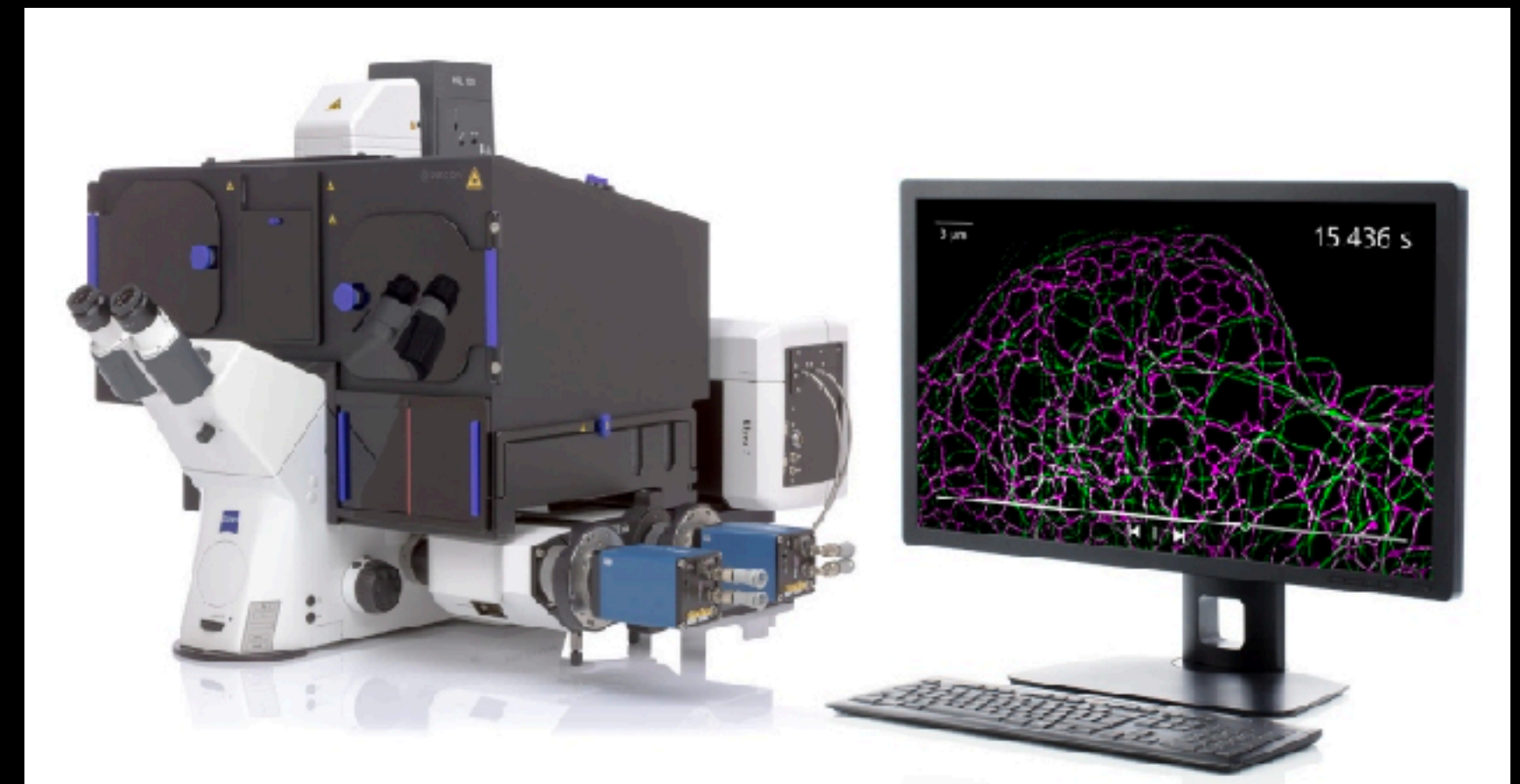
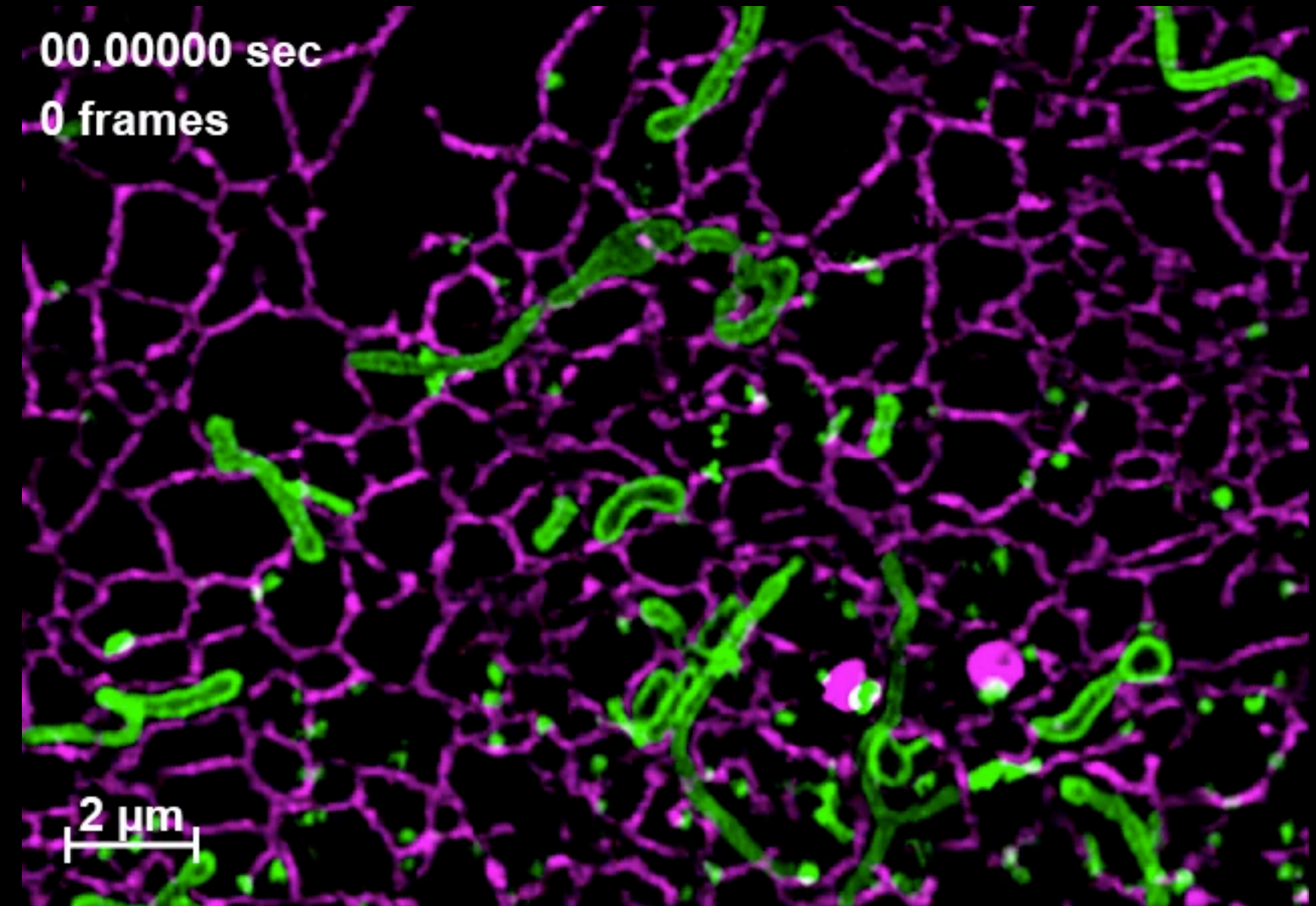
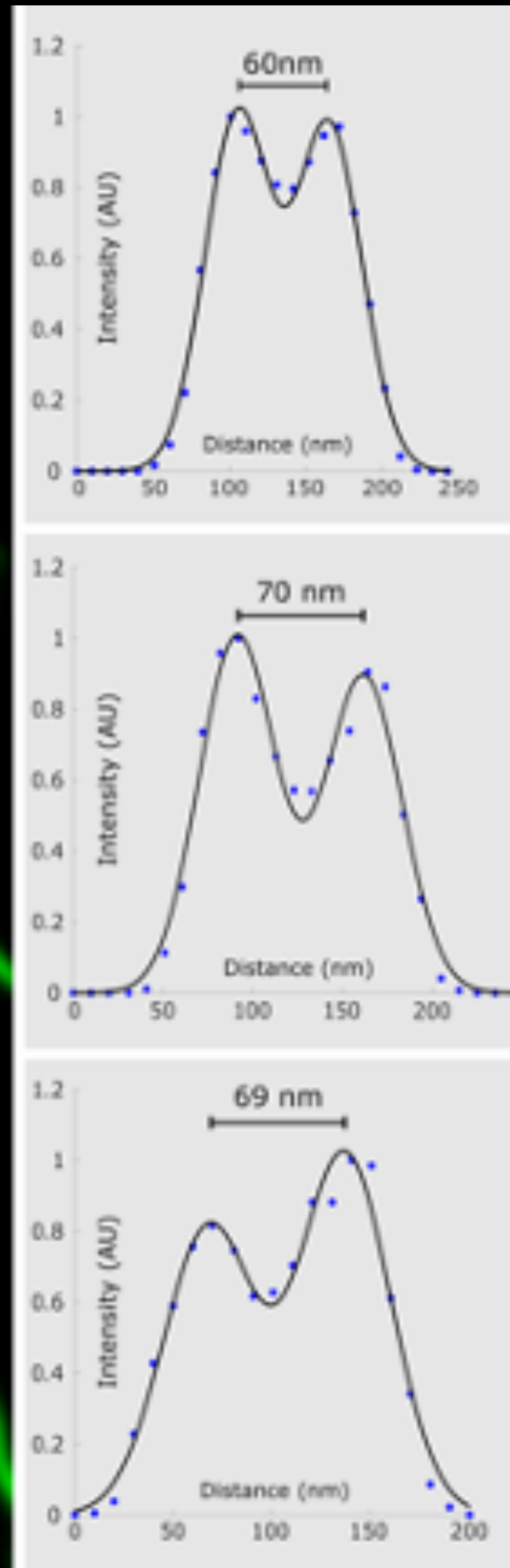


SIM²

60nm

70nm

69nm



Resolution down to 60nm
Up to 255 fps
2 cam simultaneous
Easy sample navigation
dSTORM/SMLM ~30nm

500 nm

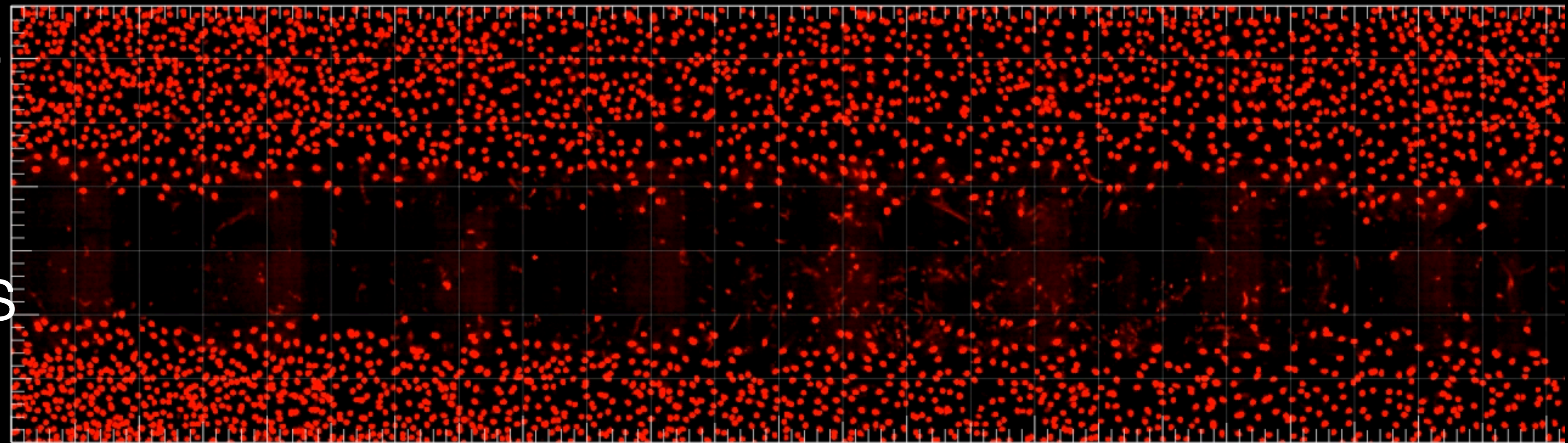
Image analysis



Migration tracking

Scratch assay

- 4x10 fields stitched in each well of a 24 well plate
- 15 minute interval for 24 hours
- Big data sets! Cells tracked using IMARIS
- Ensemble and individual statistics exportable



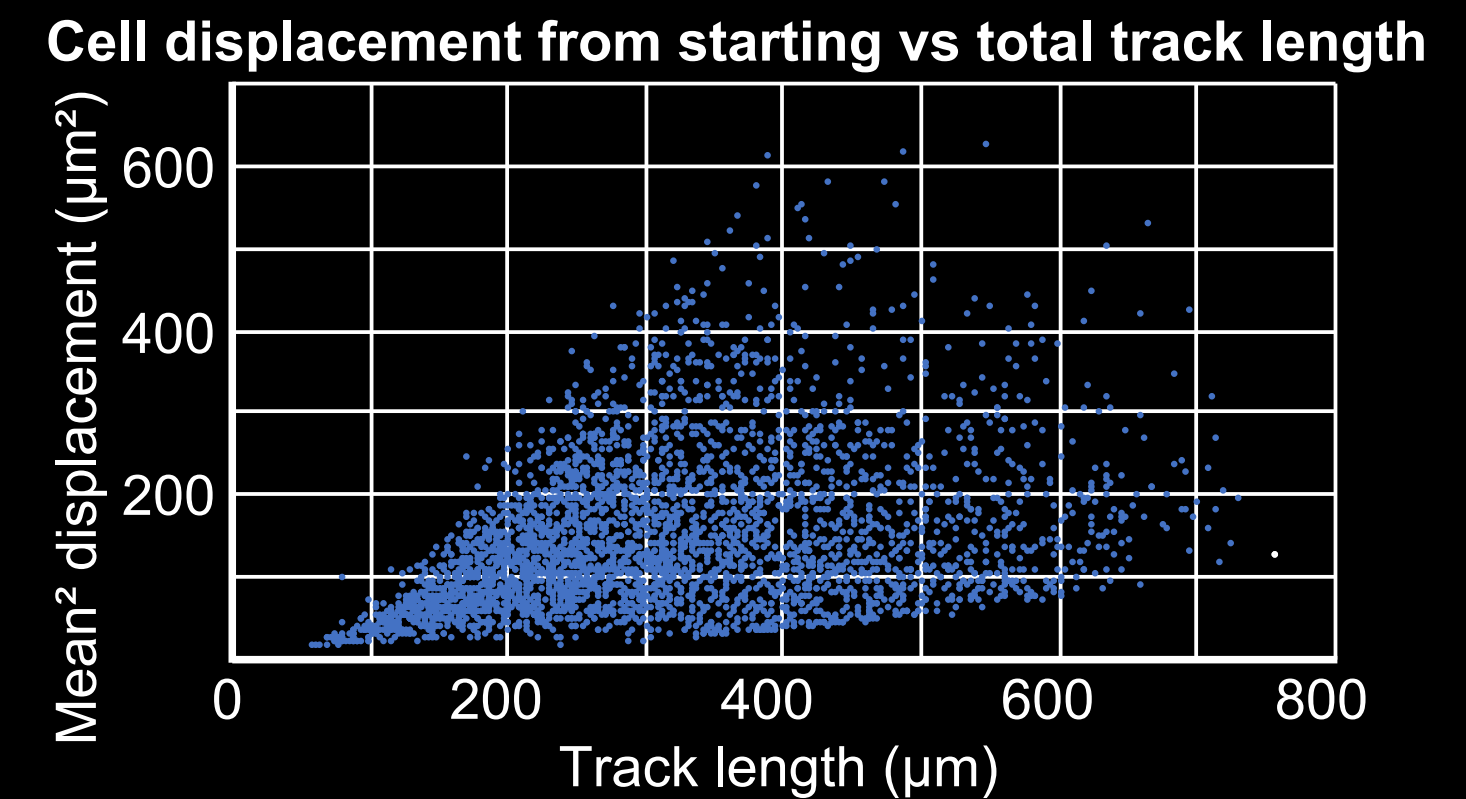
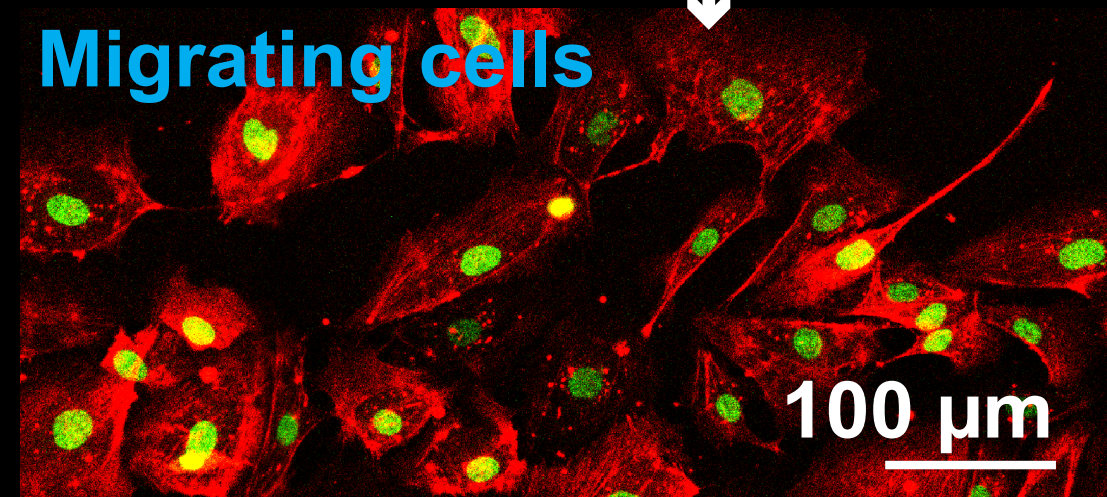
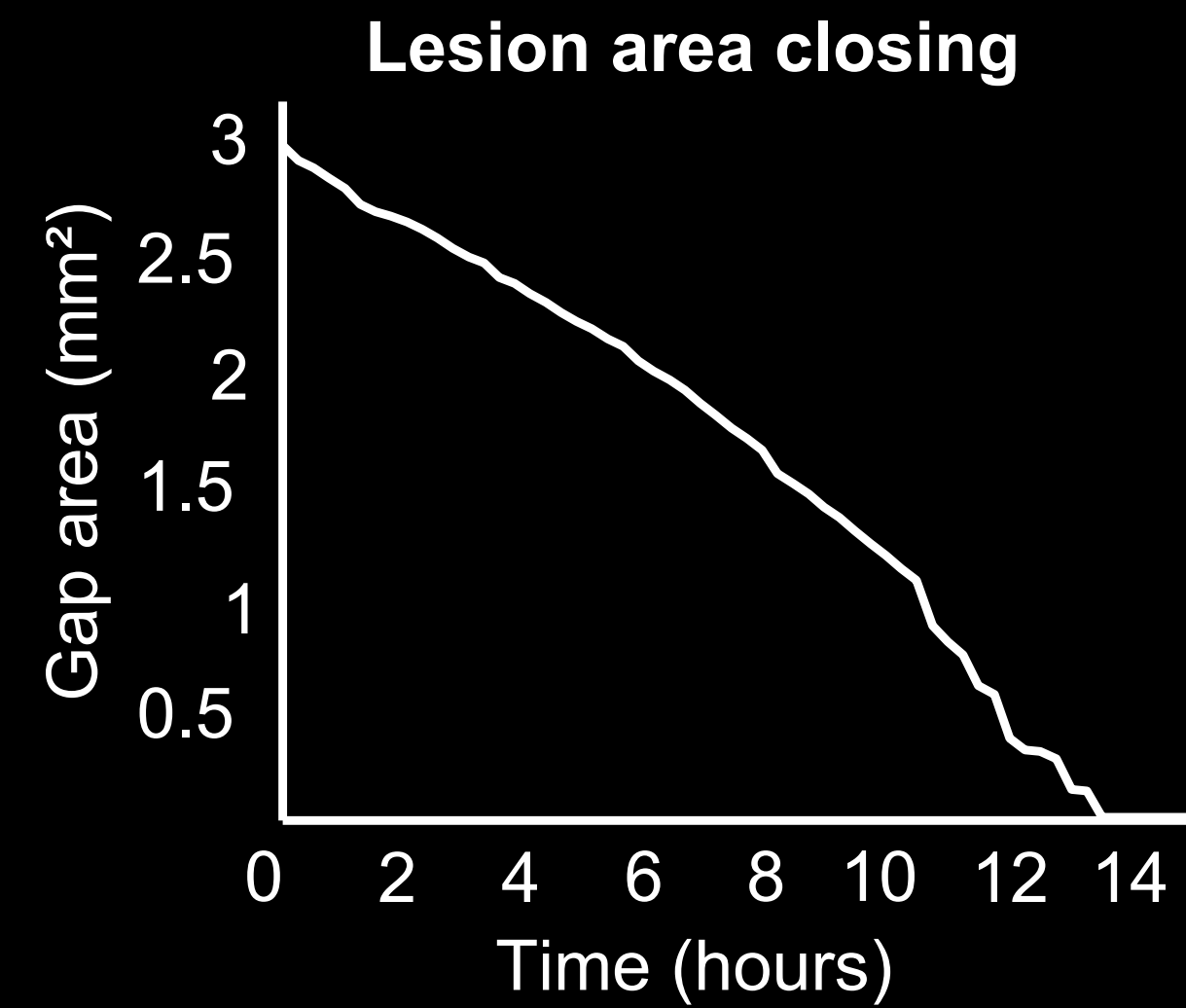
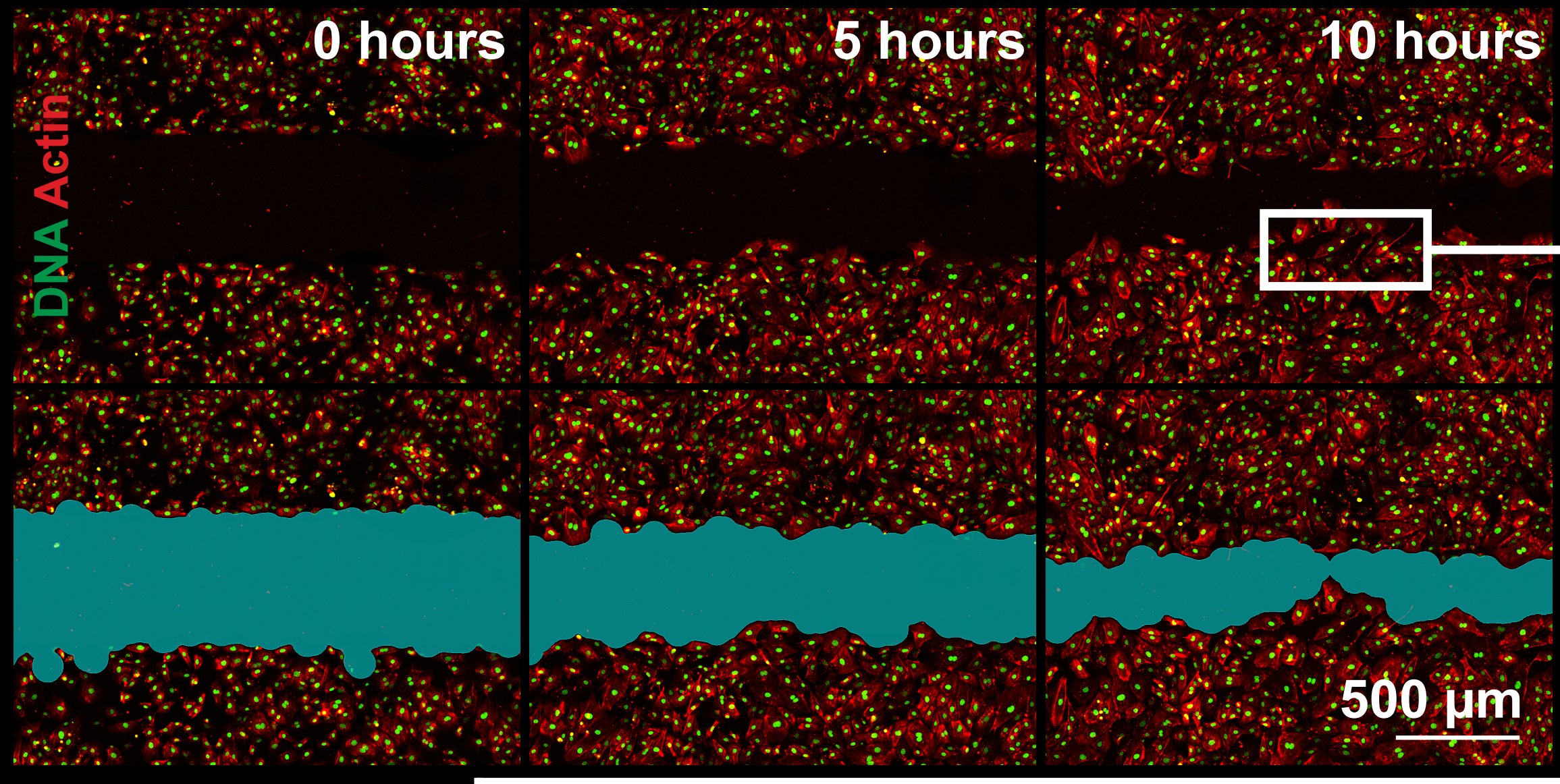
400 μ m

0:00:00.000

Data collected with Tom Hiron & Jiahao Jiang

Cell migration analysis

Scratch assay



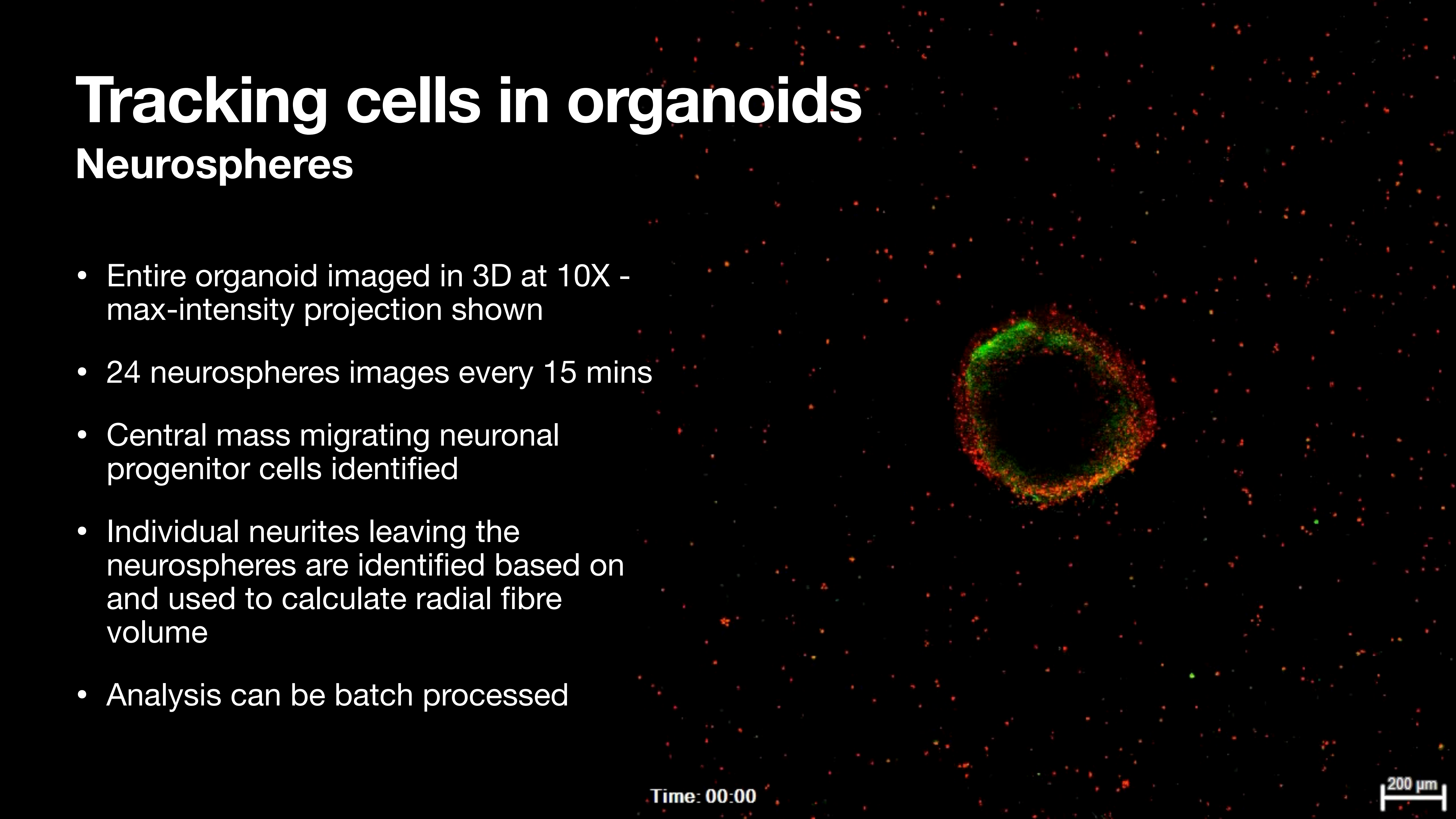
Tracking cells in organoids

Neurospheres

- Entire organoid imaged in 3D at 10X - max-intensity projection shown
- 24 neurospheres images every 15 mins
- Central mass migrating neuronal progenitor cells identified
- Individual neurites leaving the neurospheres are identified based on and used to calculate radial fibre volume
- Analysis can be batch processed

Time: 00:00

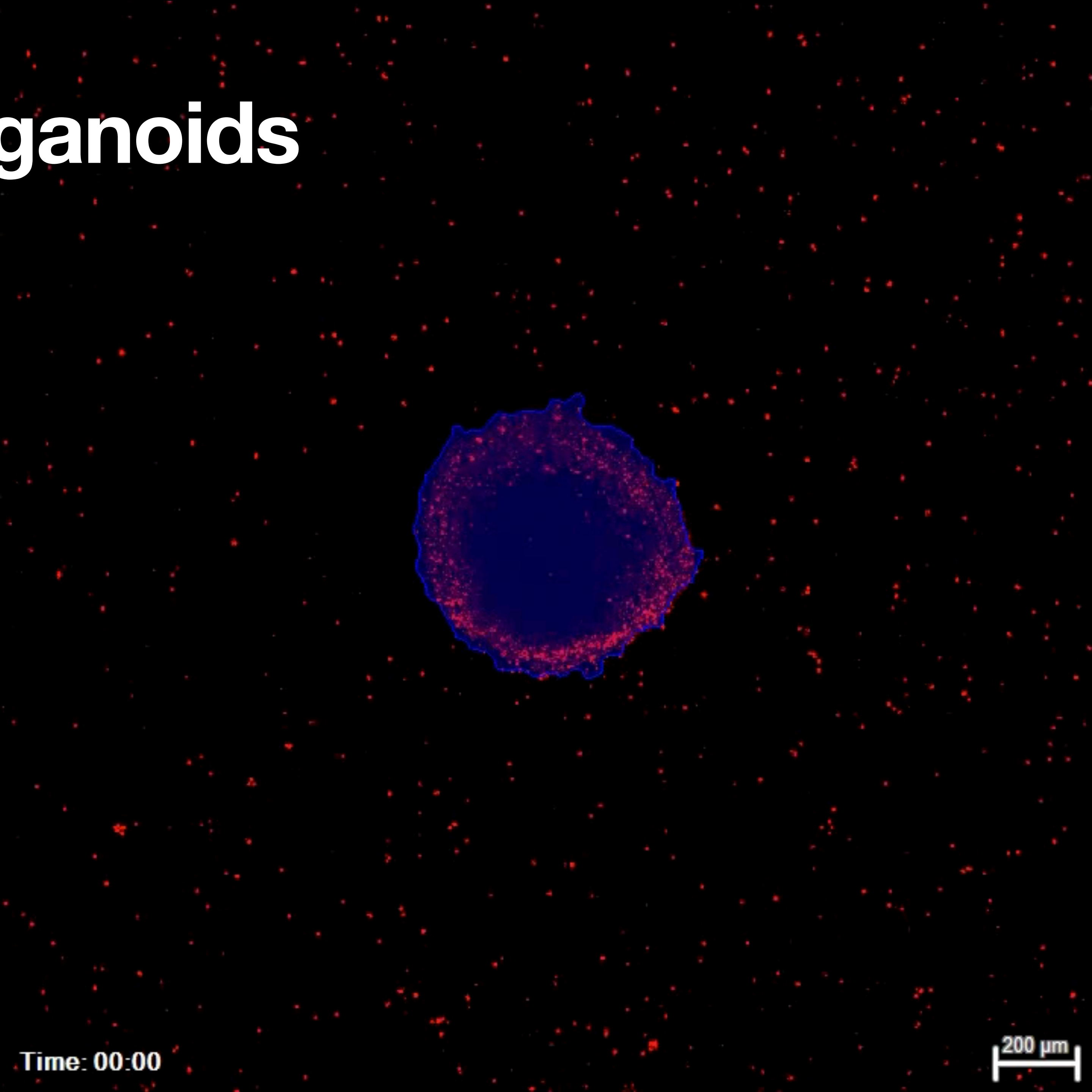
200 μ m



Tracking cells in organoids

Neurospheres

- Migrating neuronal progenitor cells identified and region defined



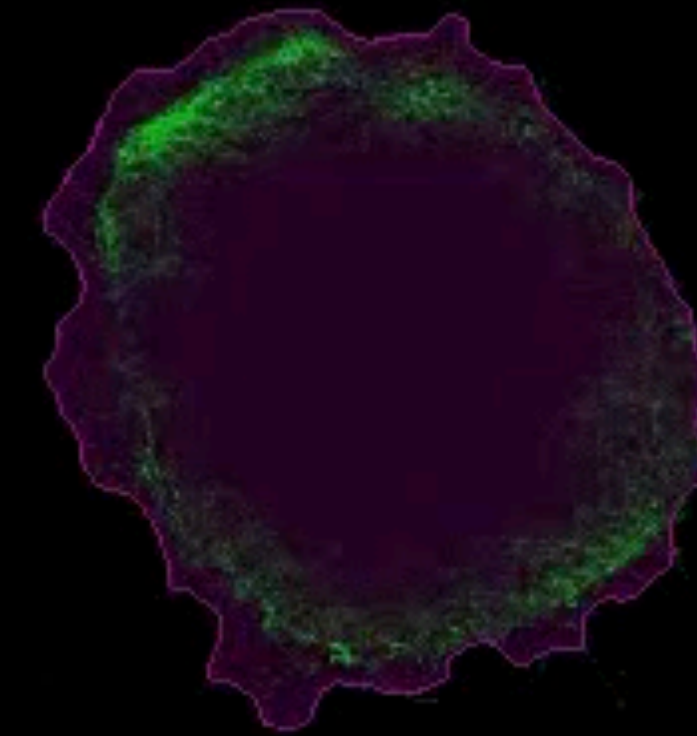
Time: 00:00

200 μm

Tracking cells in organoids

Neurospheres

- Individual neurites leaving the identified leaving neuronal progenitor cells and used to calculate radial fibre volume



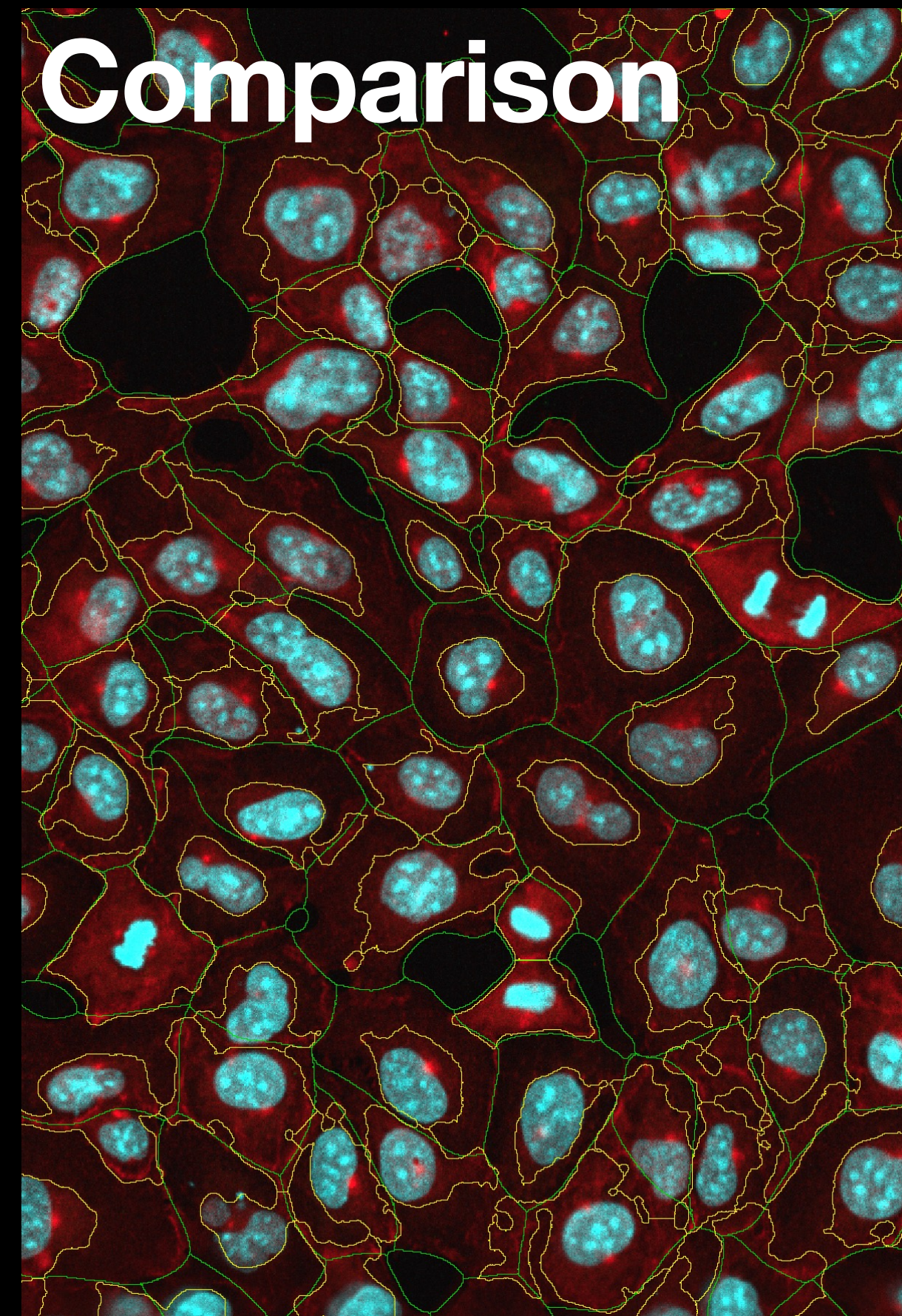
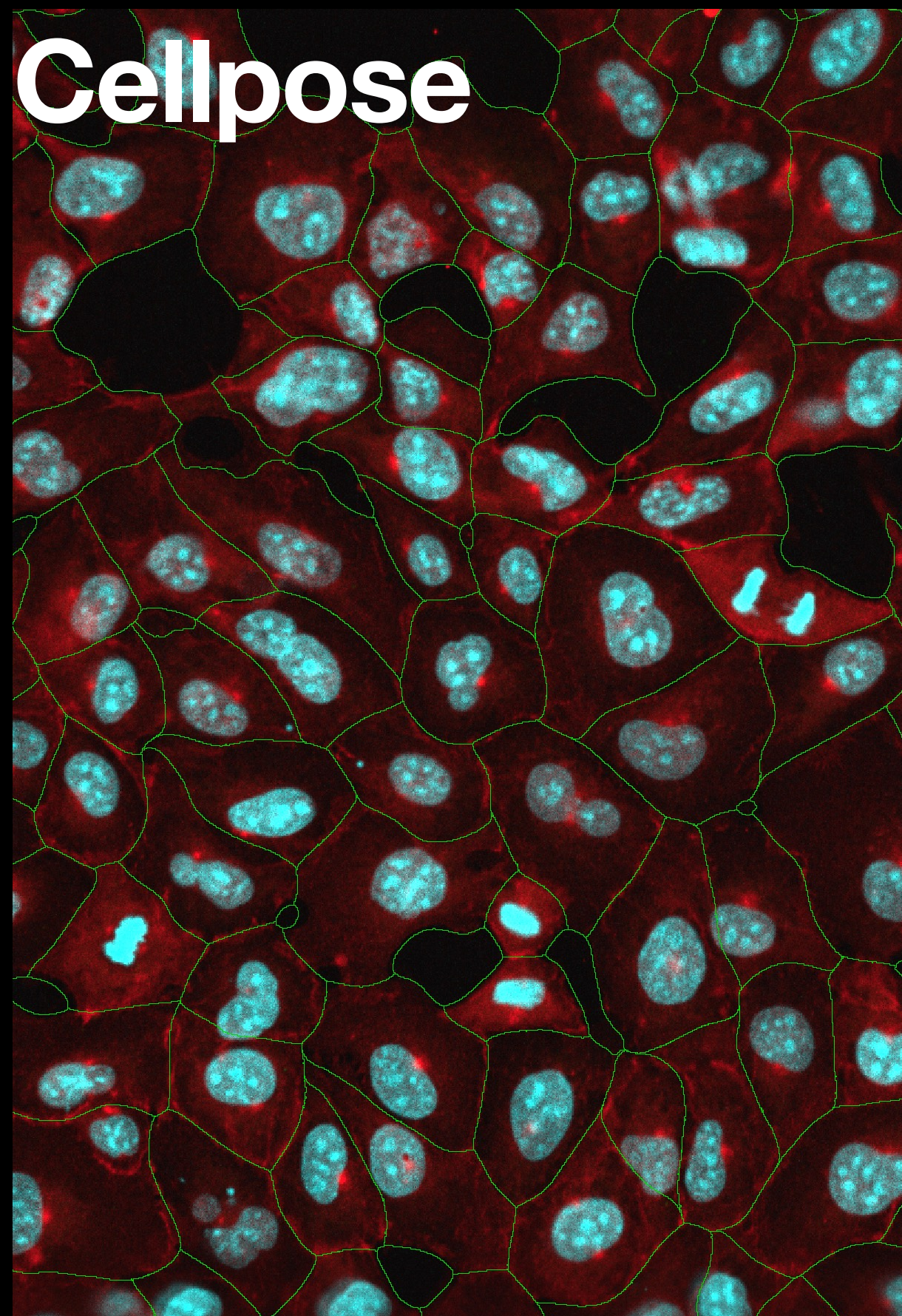
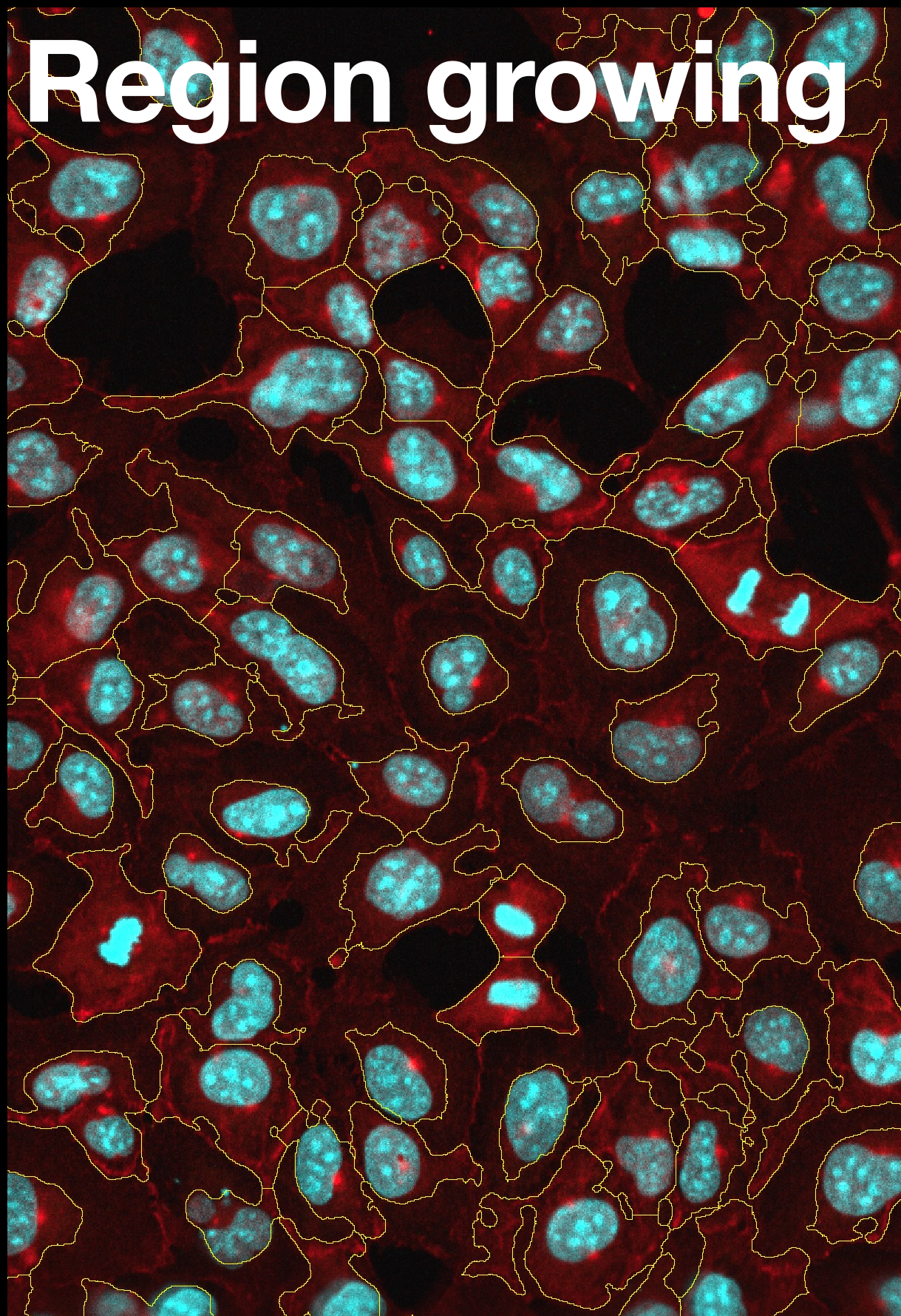
Time: 00:00

200 μm

2D segmentation using AI

Region growing vs Cellpose AI cellular segmentation

Cellpose is a pre-trained generalist deep learning neural network



3D segmentation of nuclei in ROI using AI

Immune infiltrate visualisation in pancreatic tissue - segmentation in Arivis using CellPose

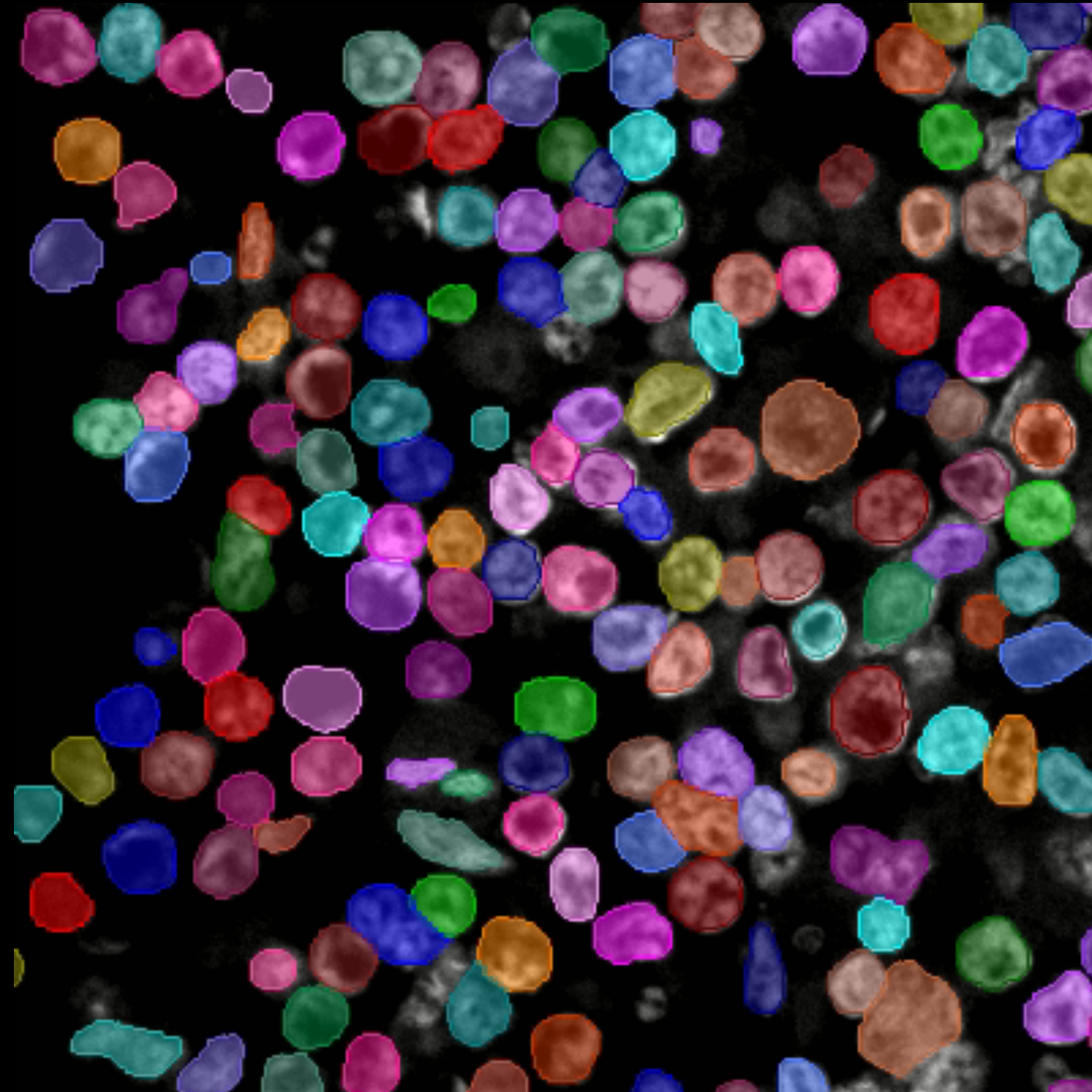
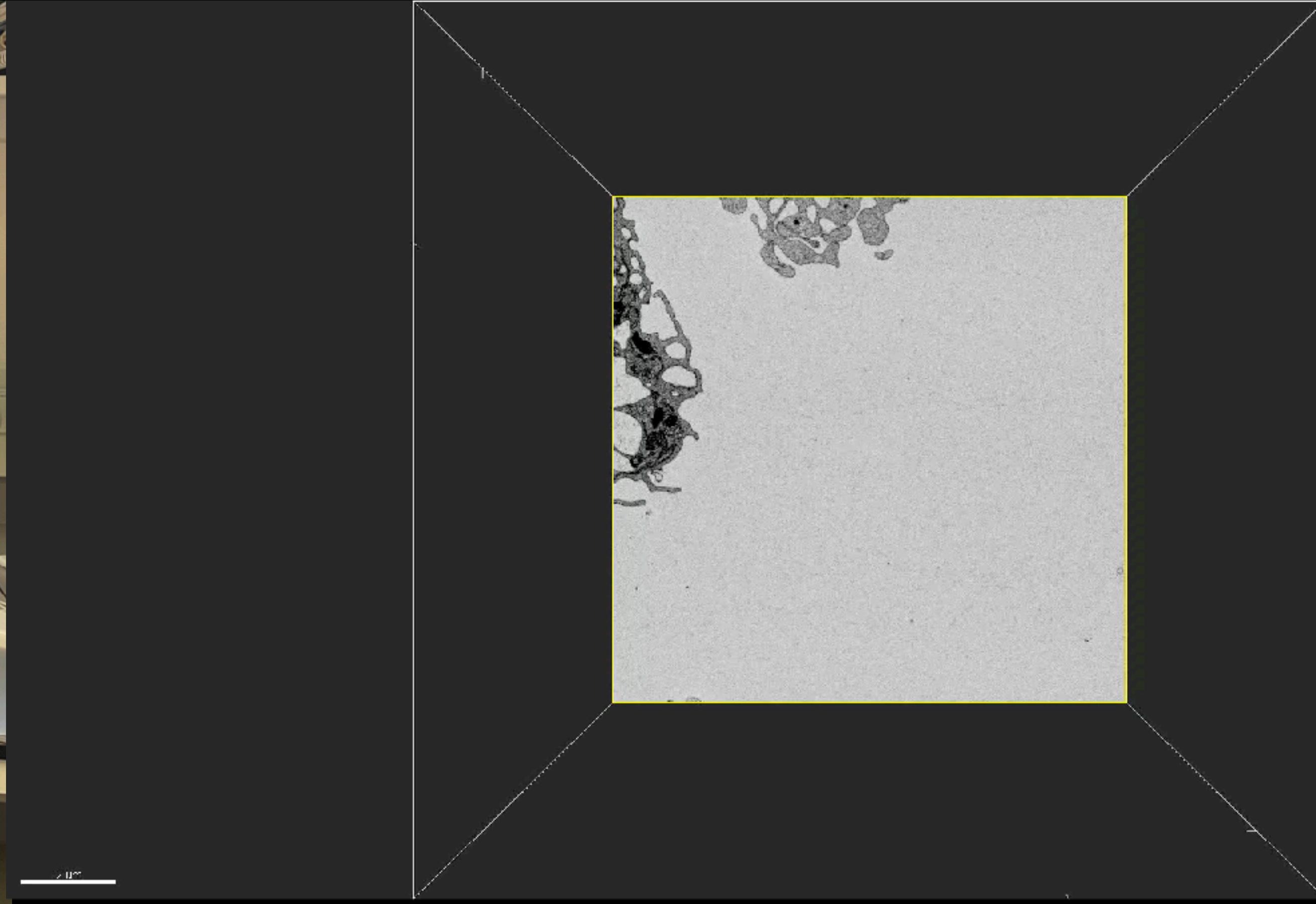
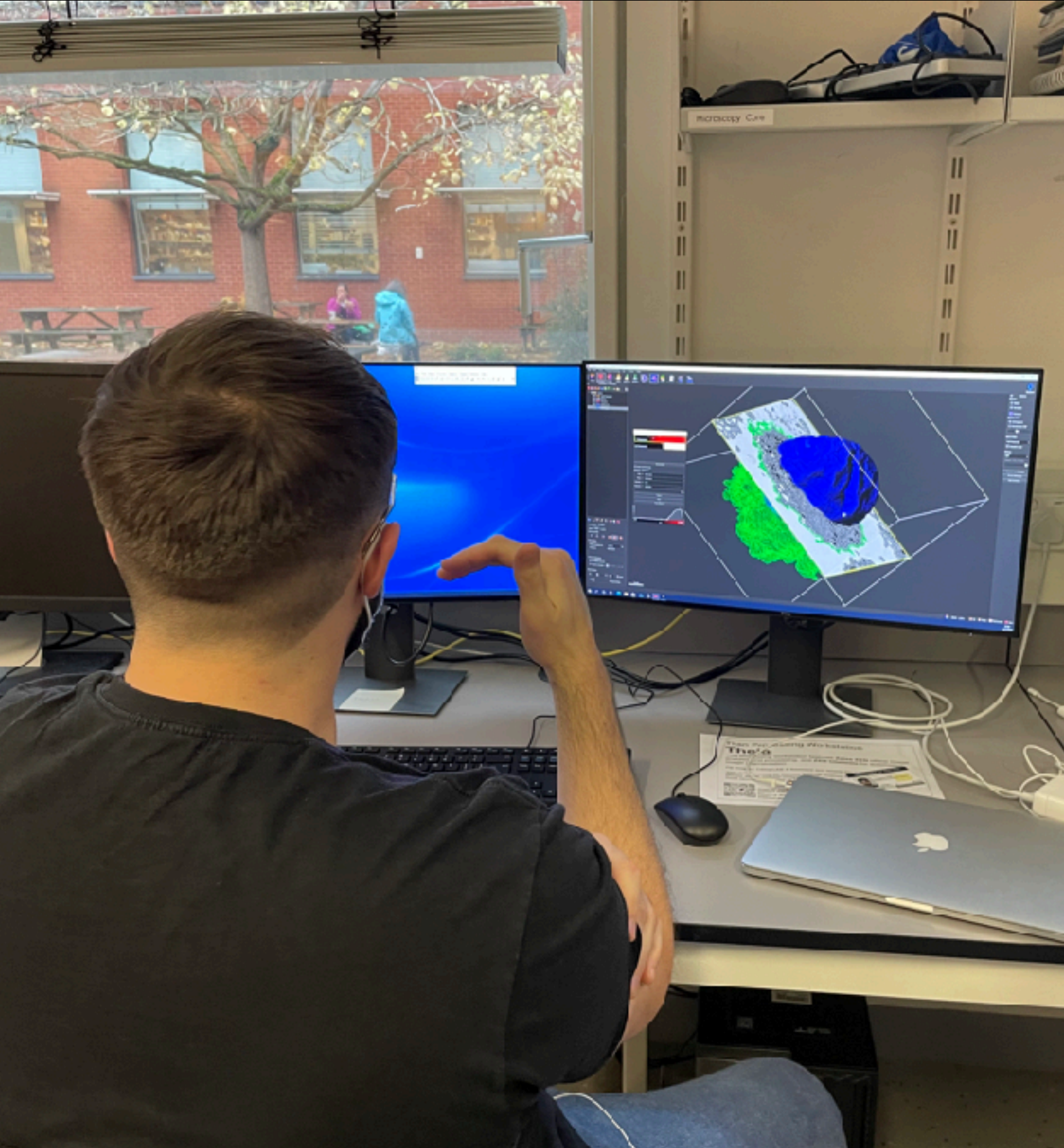


Image thanks to Felicia Anna Tucci

3D visualisation & analysis

ARIVIS & Imaris

- Compatible with most 3D data sets
- Volumetric segmentation and analysis



3D visualisation & analysis

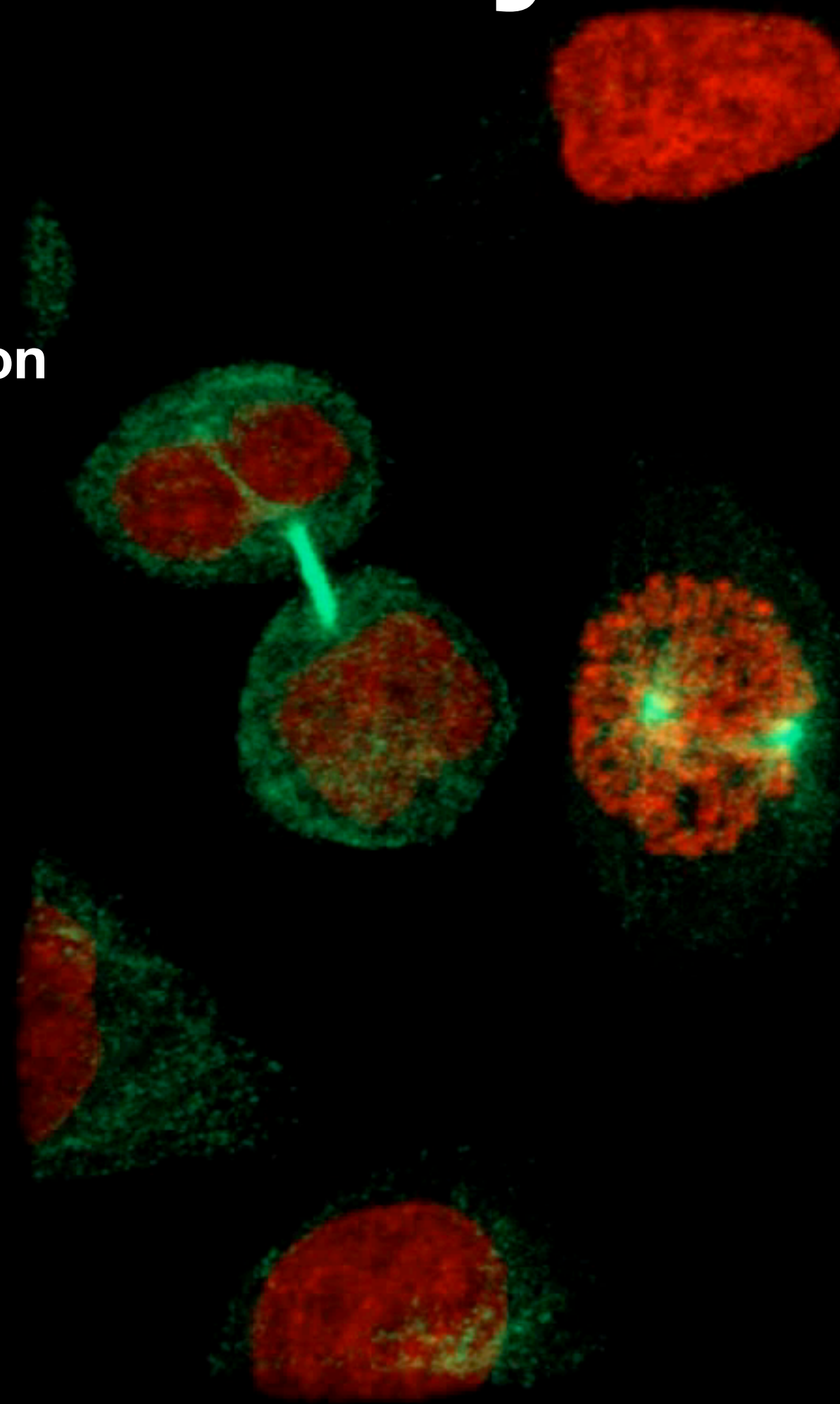
Zeiss ZEN Blue

Seamless workflow with Zeiss acquisition

Image processing tools - e.g. deconvolution

Easy 3D rendering

Batch processing and analysis pipelines



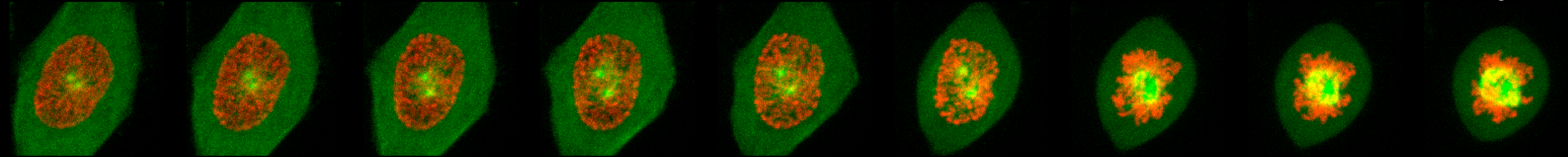
Instruments and imaging modalities available

Microscope	Type	Resolution	Sensitivity	Speed	Spectral Flexibility	Live cell	Specialist applications
LSM 900 Airyscan 2	Point scanning confocal	+++	+++	+++	+++	+++	Live cell, Super res (120nm), Large sample scanning
SP8 WLL	Point scanning confocal	++	++	++	+++	++	FLIM, FRAP, FCS Complex spectral separation (5/6 channel experiments)
SP8 FALCON	Point scanning confocal	++	++	++	+++	++	Live cell, FLIM, FRAP, FCS
SpinSR SoRa	Spinning disk confocal	+++	++	+++	++	+++	High content, Rapid live cell, Super res (120nm), Rapid large sample scanning
DMI-8	Widefield/TIRF	+	+++	+++	++		Dual cam: Colour and fluorescent imaging, Rapid large sample scanning
ELYRA PS1	Widefield/TIRF/STORM	+++	++	+	++	+	STORM super res (30-40nm)
EVOS M5000	Widefield	+	++	+	++		Quick colour and fluorescent imaging of most sample formats

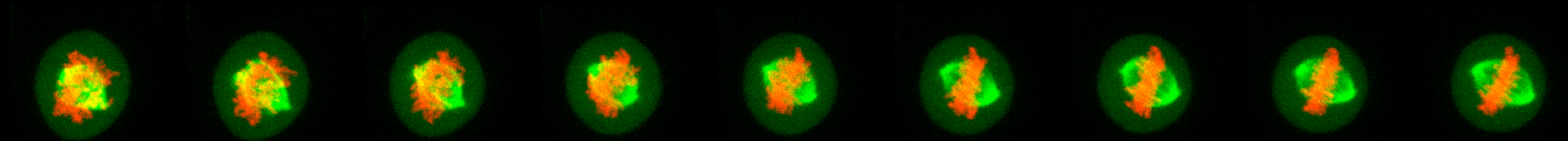


cellular imaging

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Thank you



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