# Advancements in Super-resolution Microscopy on the UTHSC Campus

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Advanced Imaging Core (AIC)

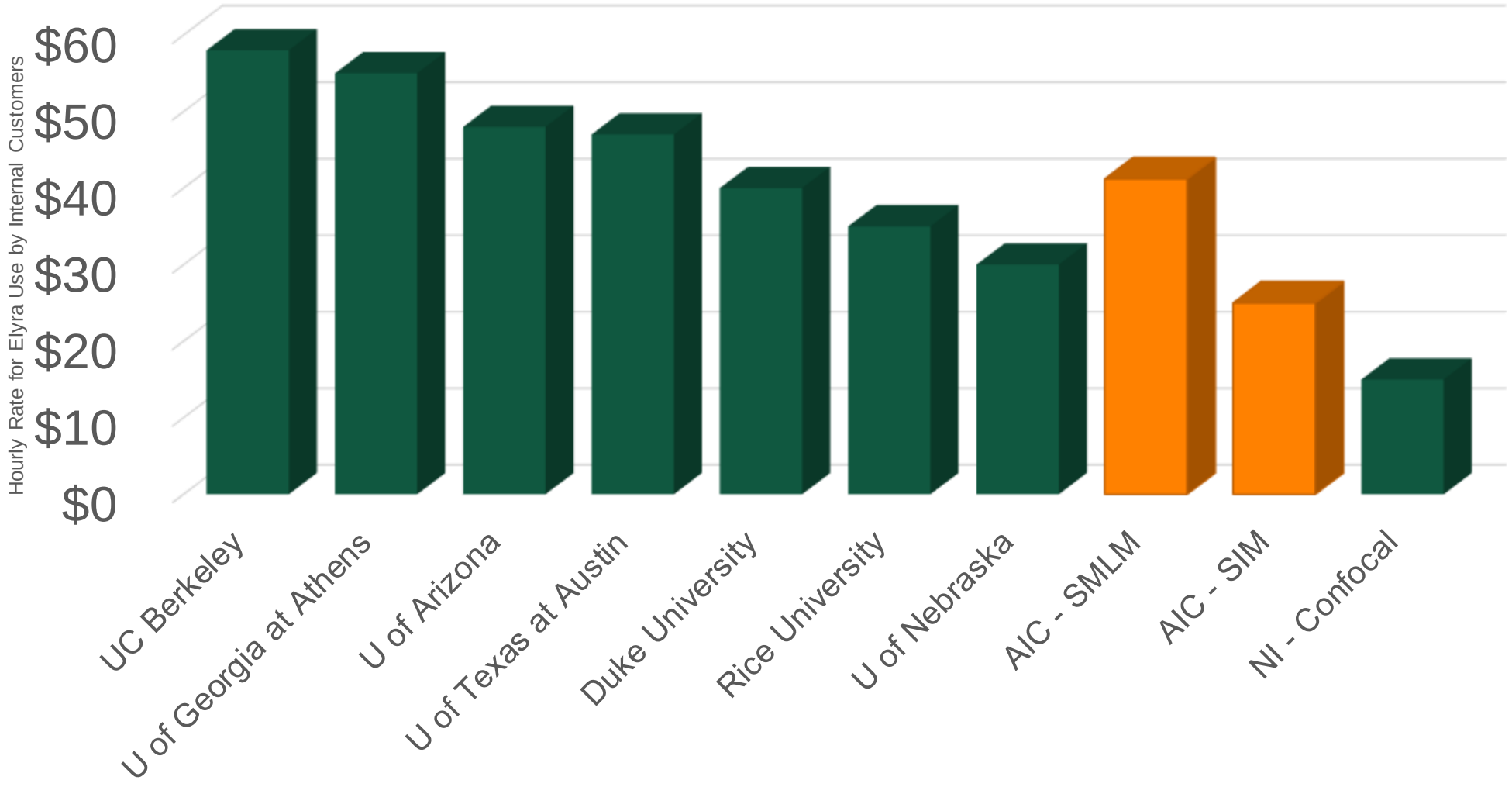
UTHSC RESEARCH

# Advanced Imaging Core

- Opened in January 2021
- Located in Johnson Building, Room 311
- Scheduling through iLab
- **No more Consultation fees!**

Zeiss Elyra 7

# Advanced Imaging Core



# Super-resolution Microscopy (SRM)

- What is super-resolution microscopy?
  - **SRM** is a series of light microscopy techniques that can **overcome the diffraction limit on resolution.**

# Super-resolution Microscopy (SRM)

- Diffraction limit on resolution

$$d = \frac{\lambda}{2NA} \approx 250 \text{ nm}$$

d = maximum resolving distance

$\lambda$ = excitation wavelength

NA = numerical aperture of objective

# Super-resolution Microscopy (SRM)

- Diffraction limit on resolution

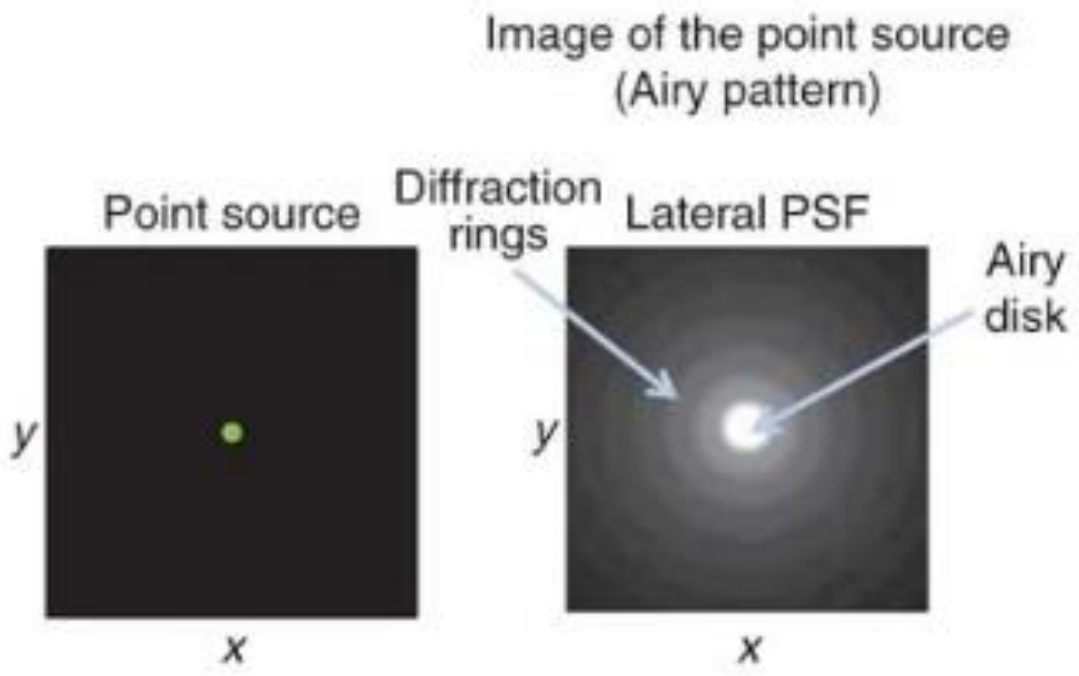
$$d = \frac{\lambda}{2NA} \approx 250 \text{ nm}$$

d = maximum resolving distance

$\lambda$ = excitation wavelength

NA = numerical aperture of objective

- Point spread function (PSF)



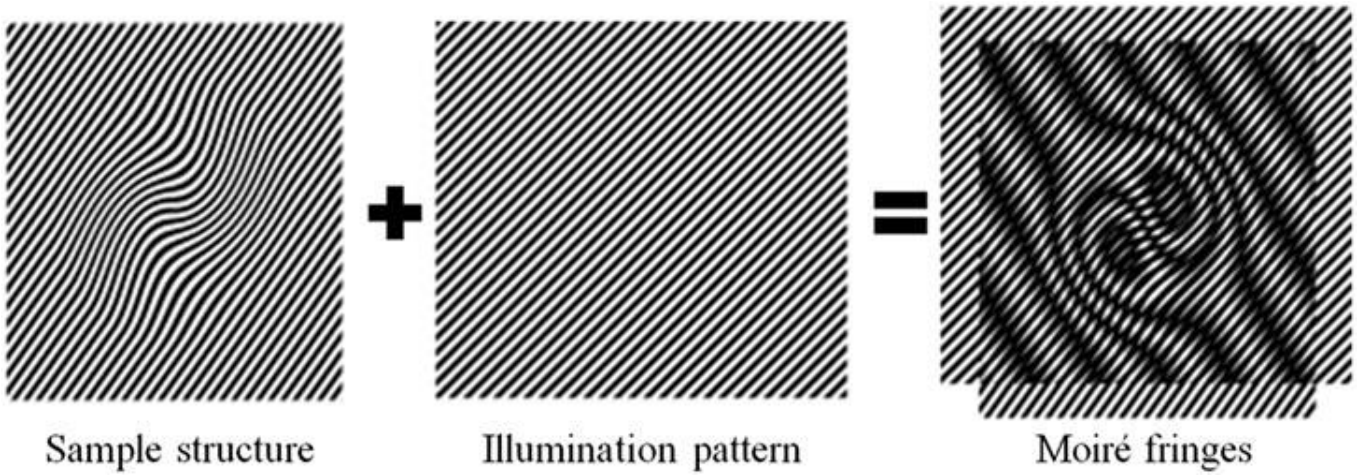

Cole, R., Jinadasa, T. & Brown, C. Measuring and interpreting point spread functions to determine confocal microscope resolution and ensure quality control. *Nat Protoc* **6**, 1929–1941 (2011).

# Super-resolution Microscopy (SRM)

- SRM Techniques Used in the AIC
  - Structured Illumination Microscopy (SIM)
  - Single-Molecule Localization Microscopy (SMLM)

# Structured Illumination Microscopy (SIM)

- Principle: Exciting the sample with a **known illumination structure** produces an **interference pattern** (moiré fringes). This interference pattern can be used to computationally recover sub-resolution information about the sample structure.
- Closest to confocal microscopy
- Max. resolution up to 120nm



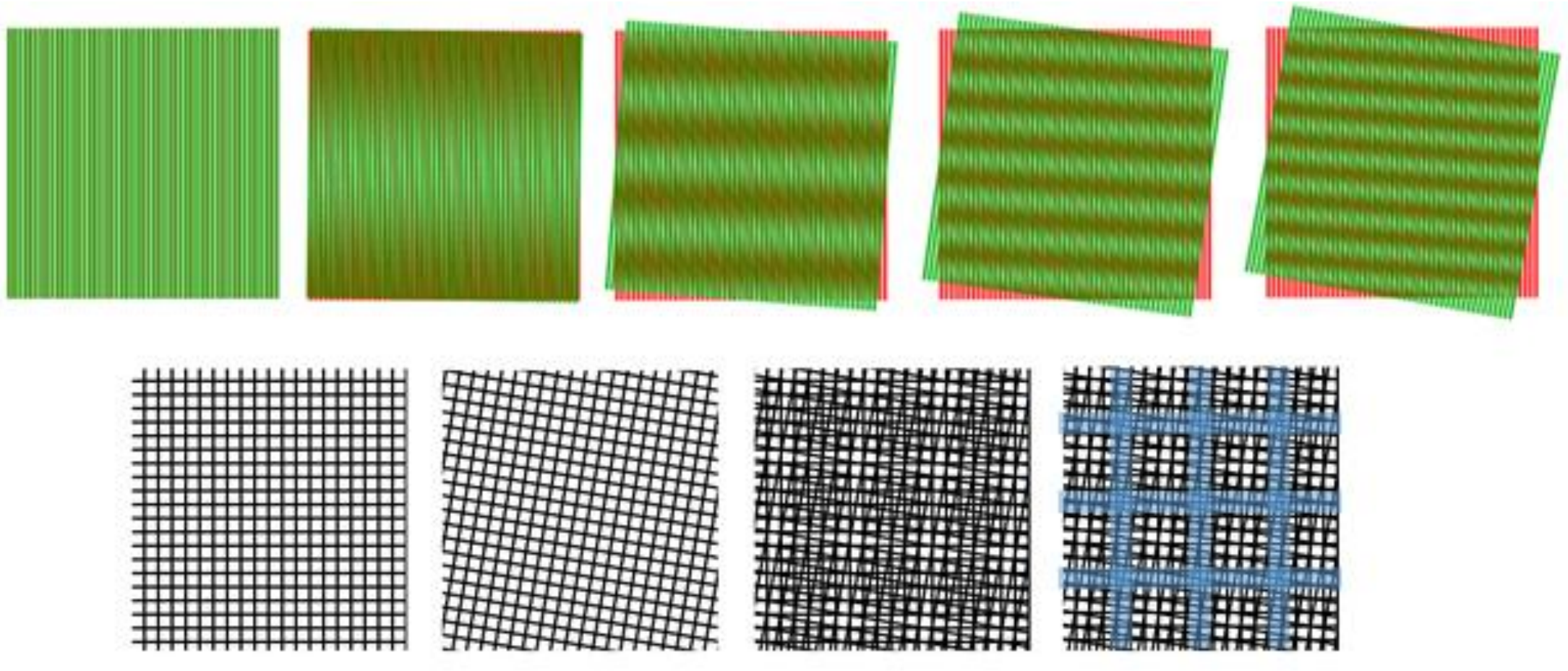

Zhao T, Wang Z, Chen T, Lei M, Yao B, Bianco PR. Advances in High-Speed Structured Illumination Microscopy. *Front. Phys.* **9** (2021)

# Structured Illumination Microscopy (SIM)

- Grating placed between sample and excitation light, creating a striped or lattice pattern of illumination
- Grating position changes multiple times for each scan, with the emitted fluorescence recorded at each position (phase images)

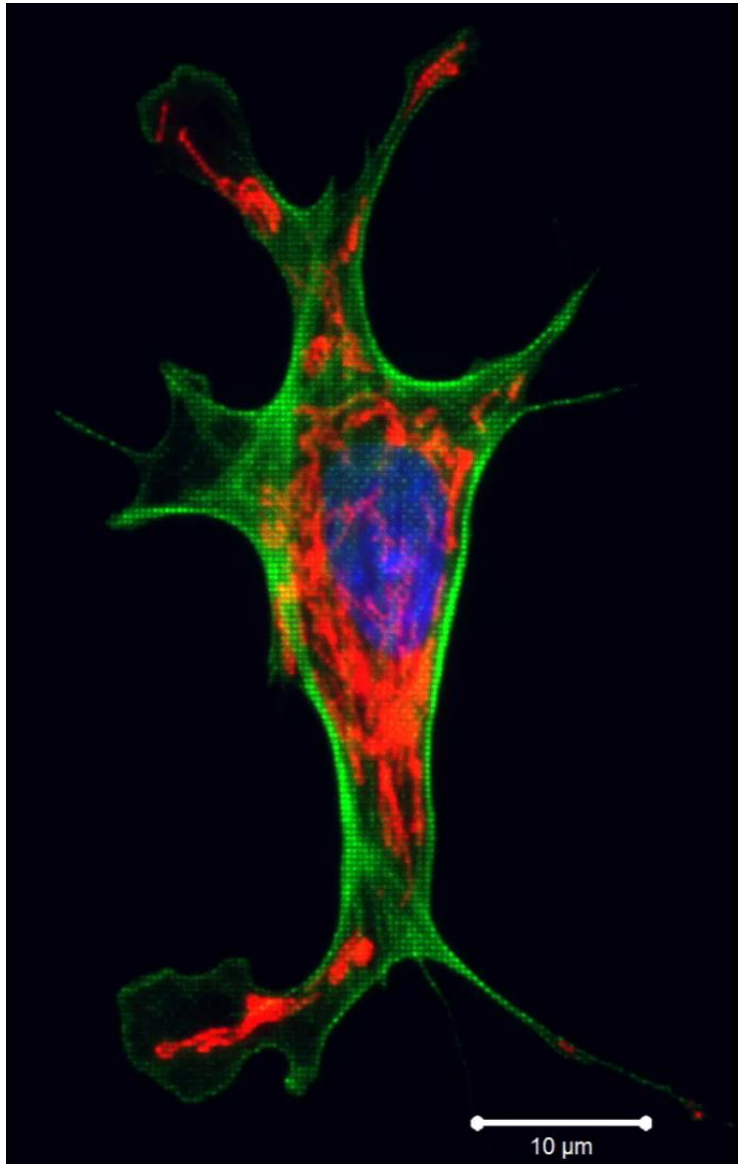

Zeiss, Zen Black

# Structured Illumination Microscopy (SIM)



https://www.photometrics.com/learn/super-resolution-microscopy/introduction-to-sim-and-isim

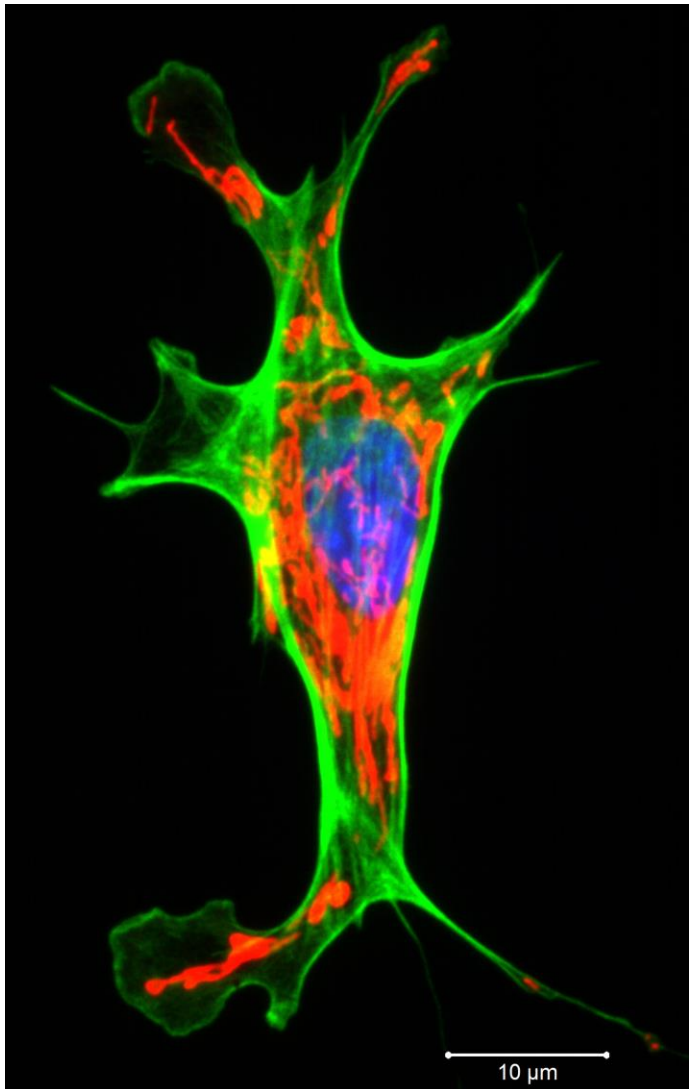
# SIM Example



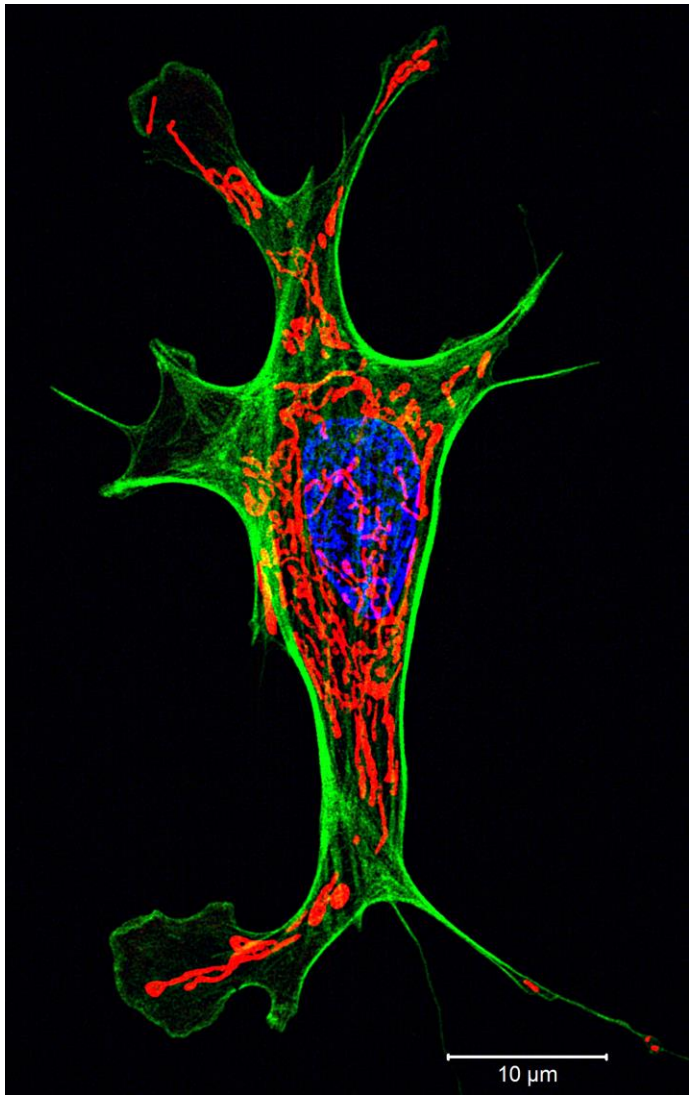

BPAEC with MitoTracker Red CMXRos, AlexaFluor 488 Phalloidin, and DAPI.

# SIM Example

Raw Data Composite




SIM Processed Image




BPAEC with MitoTracker Red CMXRos, AlexaFluor 488 Phalloidin, and DAPI

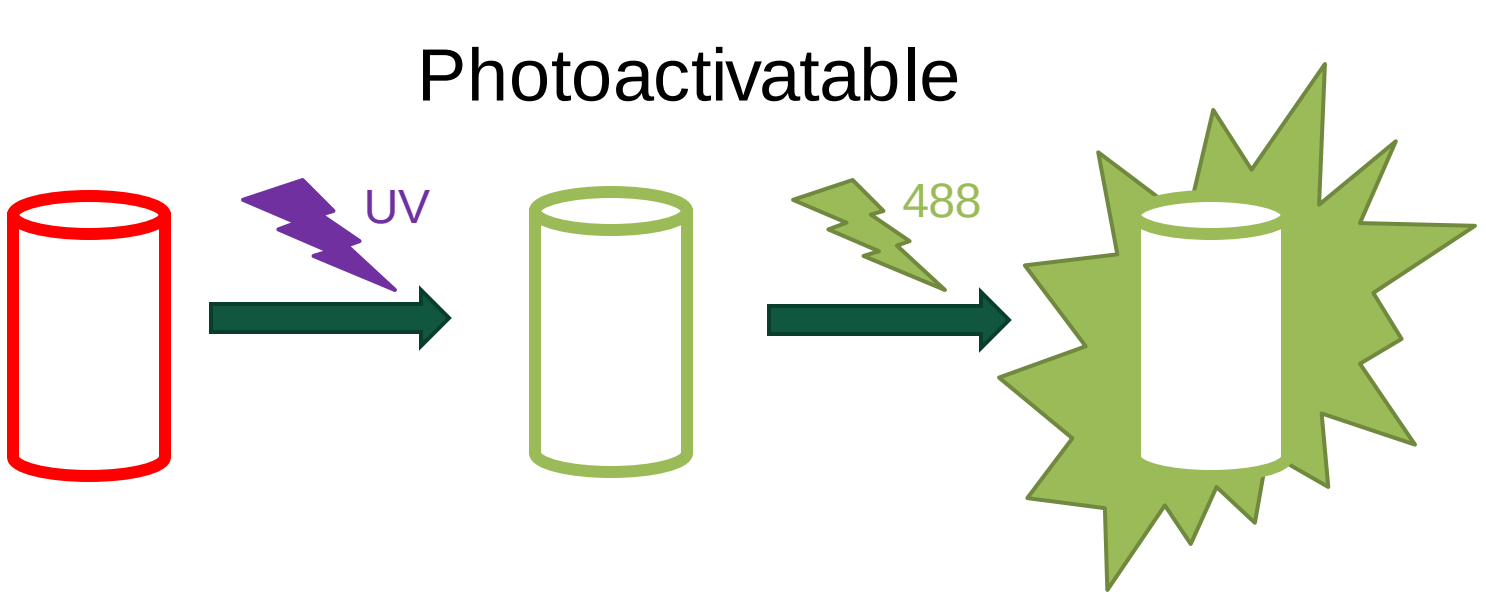
# Single-Molecule Localization Microscopy

- Principle: The localization of single fluorescent molecules (fluorophores or fluorescent proteins) can be precisely determined if the **PSFs do not overlap**.
- Max. resolution up to 20nm
- dSTORM/PALM/DNA-PAINT: “**Blinking**” of fluorescent molecules
  - Large time series experiment (**min. 10k-15k images**)

# PALM

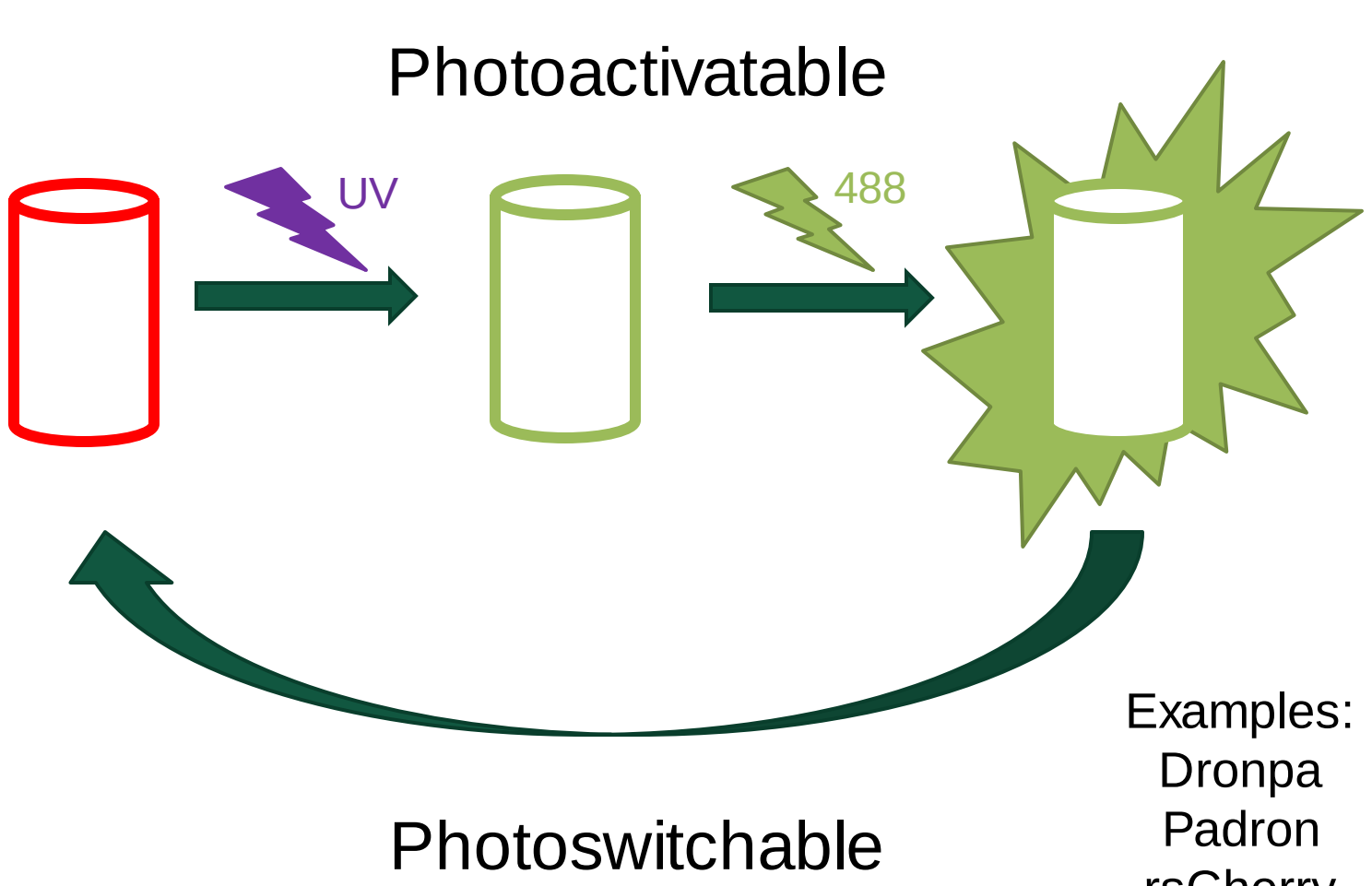
- Photoactivatable Localization Microscopy
- Live cell technique
- Fluorescent proteins requiring activation
  - Photoactivatable
  - Photoswitchable
  - Photoconvertible

# PALM




Examples:
PA-GFP
PAmCherry1
PATagRFP
PAmKate

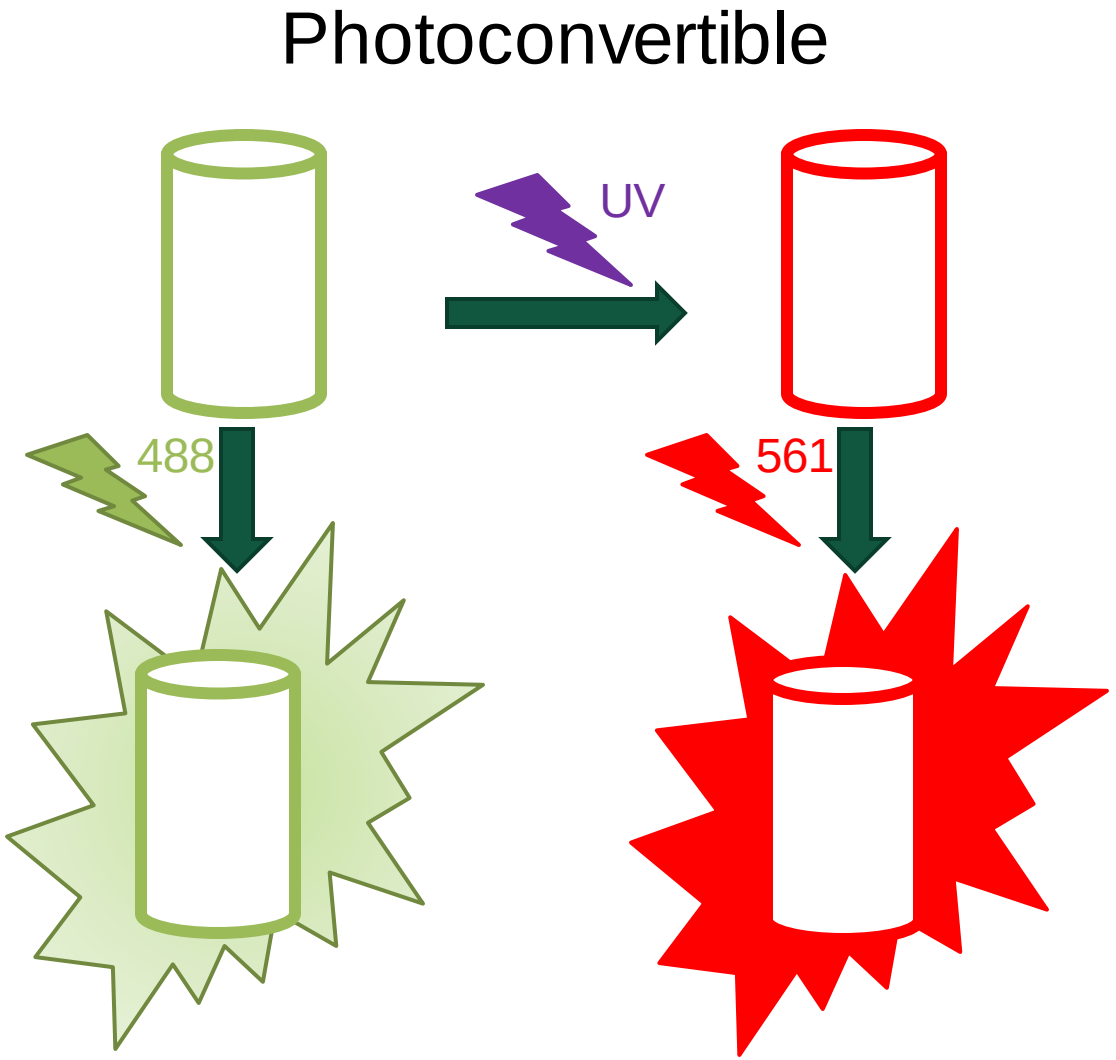
# PALM




Examples:
PA-GFP
PAmCherry1
PATagRFP
PAmKate

Examples:
Dronpa
Padron
rsCherry

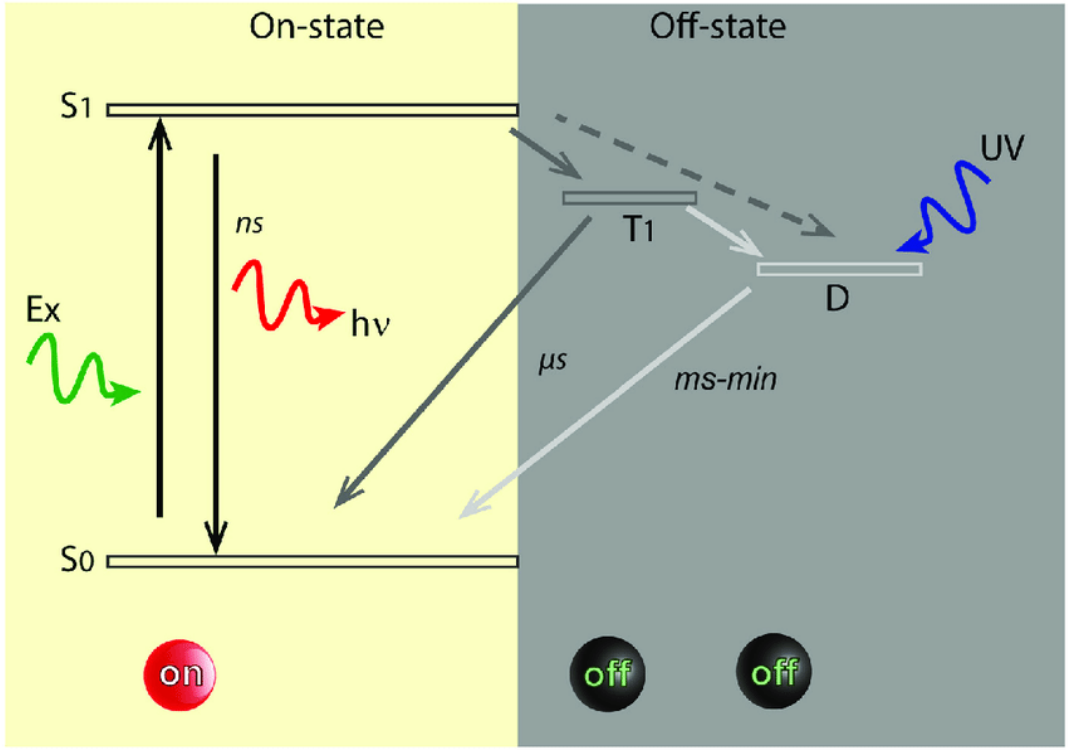
# PALM




Examples:
mMaple
mClavGR2
Dendra2
Kaede

# dSTORM

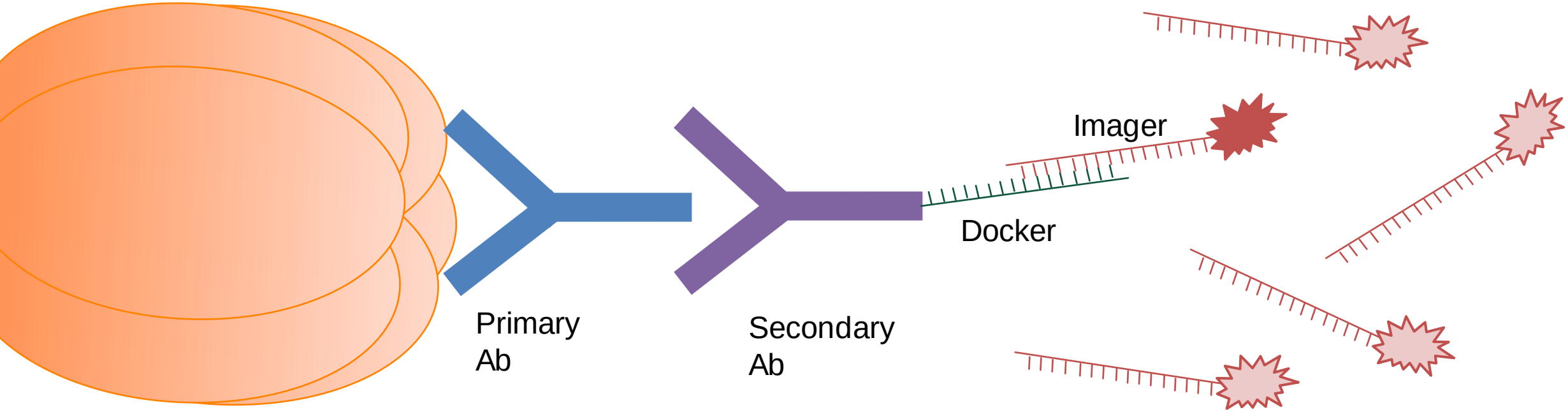
- direct Stochastic Optical Reconstruction Microscopy
- Blinking achieved by **buffer-induced switching** of the fluorophore between On- and Off-states
- Compatible with common fluorescent dyes
  - Alexa Fluors 488, 555, 647
  - Cy3B, Cy5



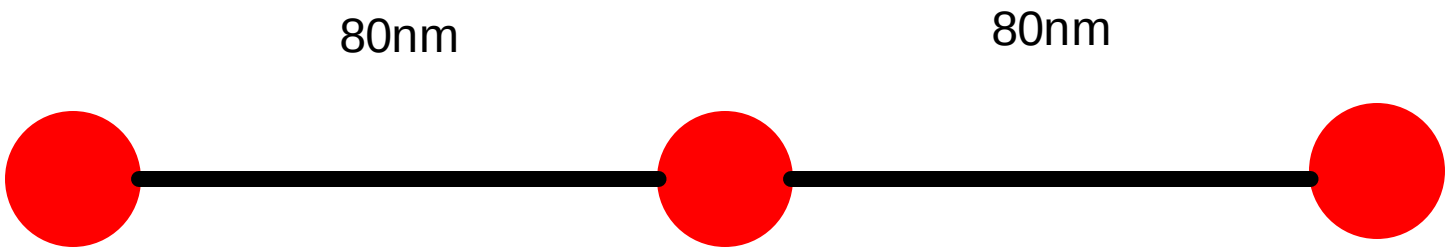

Nahidiazar L, Agronskaia AV, Broertjes J, van den Broek B, Jalink K. Optimizing Imaging Conditions for Demanding Multi-Color Super Resolution Localization Microscopy. PLoS One. 2016 Jul 8;11(7)

# DNA-PAINT

- DNA points accumulation for imaging nanoscale topography
- Blinking achieved through **transient binding** of a fluorescently-labeled, freely-diffusing oligonucleotide (imager strand) to one targeting a protein of interest (docker strand)

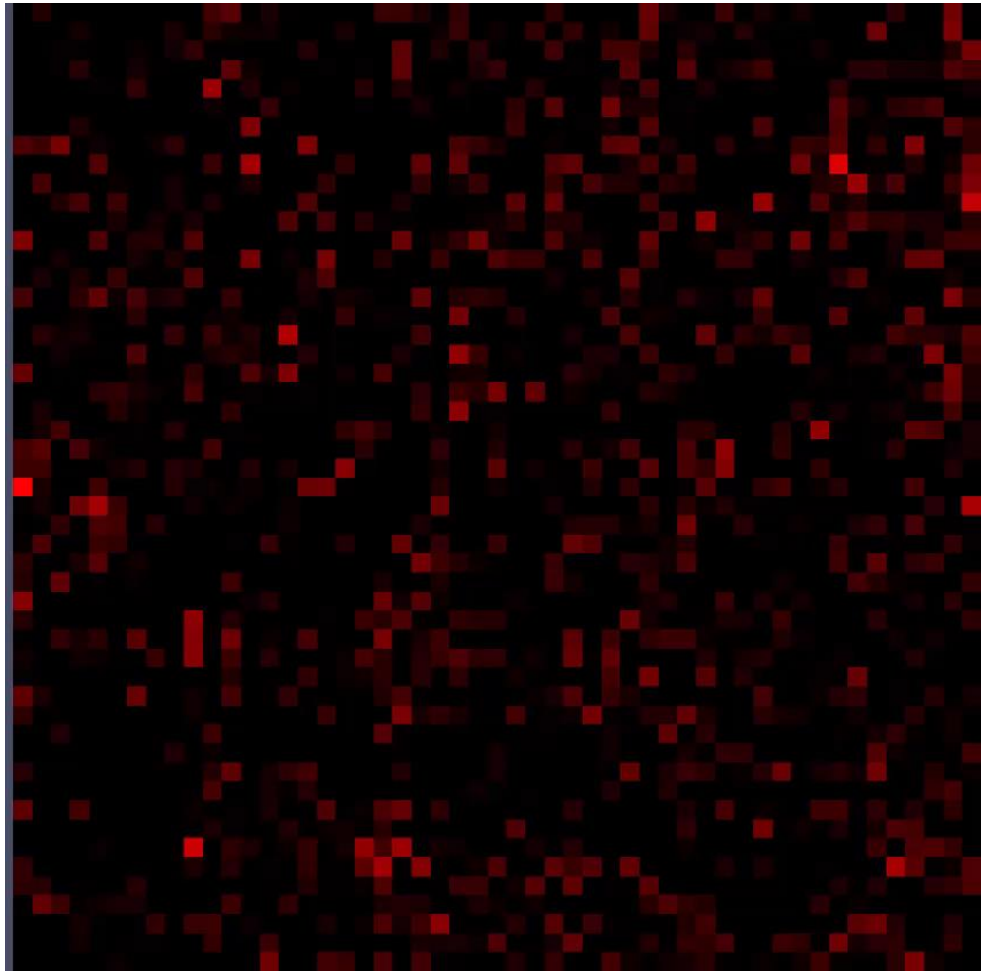


# DNA-PAINT Example – Nanoruler Slide



# DNA-PAINT Example – Nanoruler Slide

Raw Data Time Series



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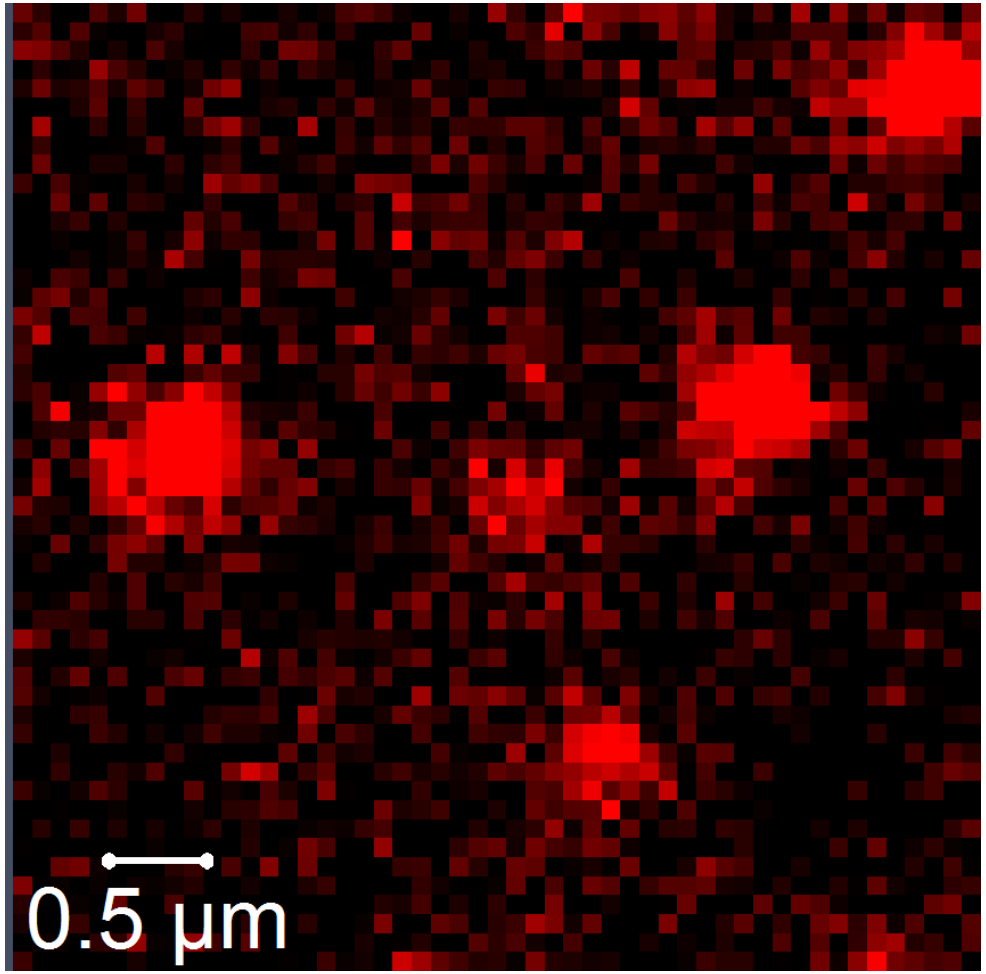
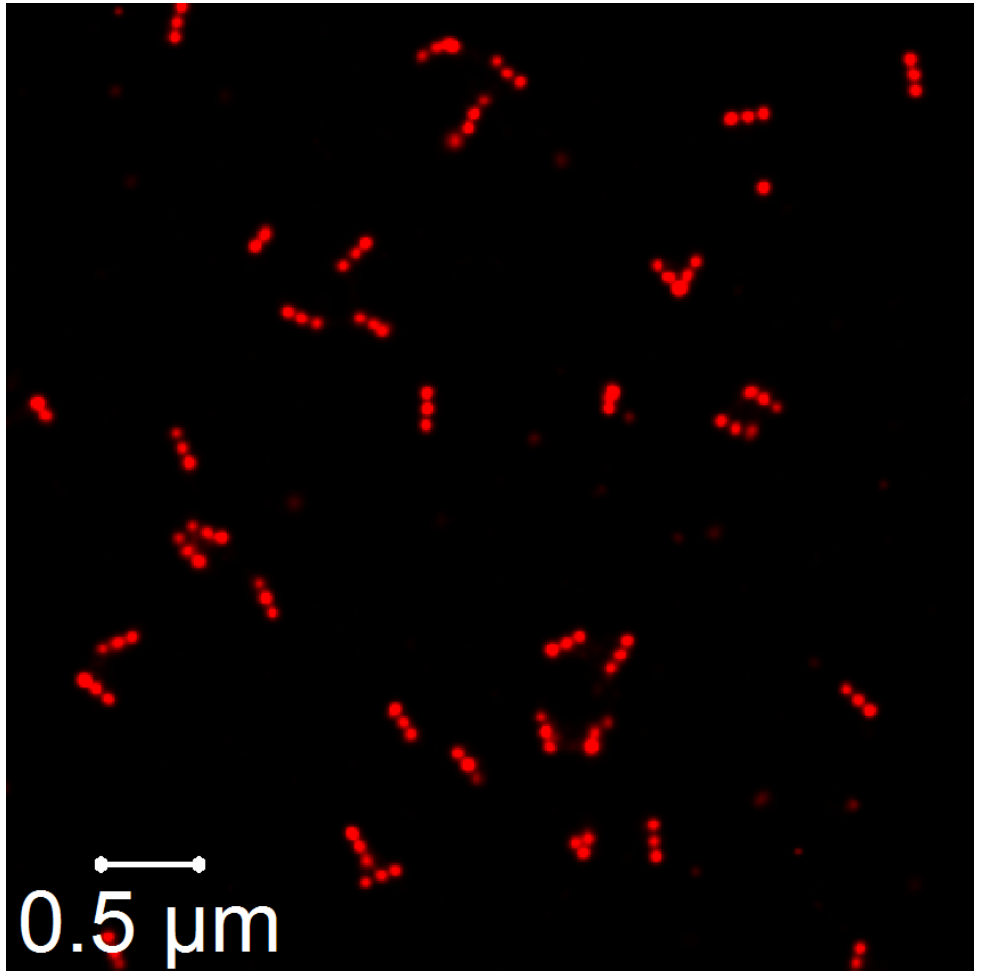



Processed Image



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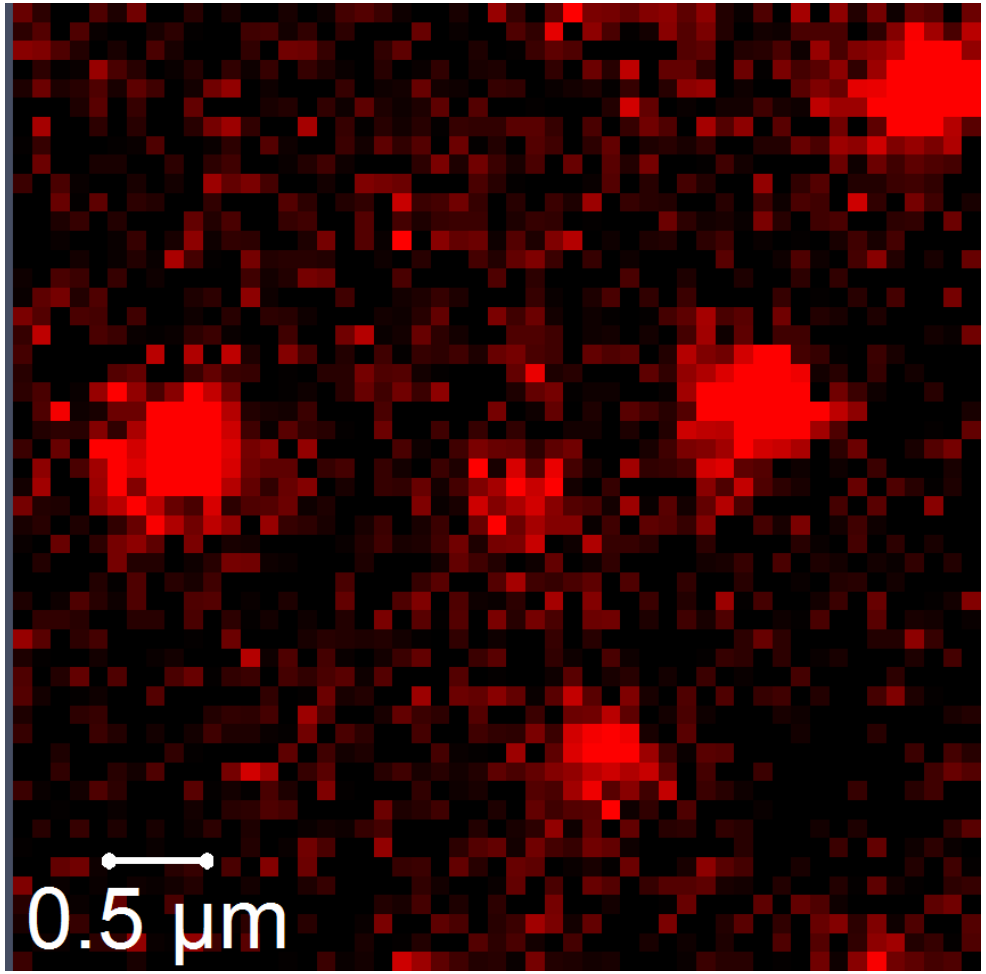


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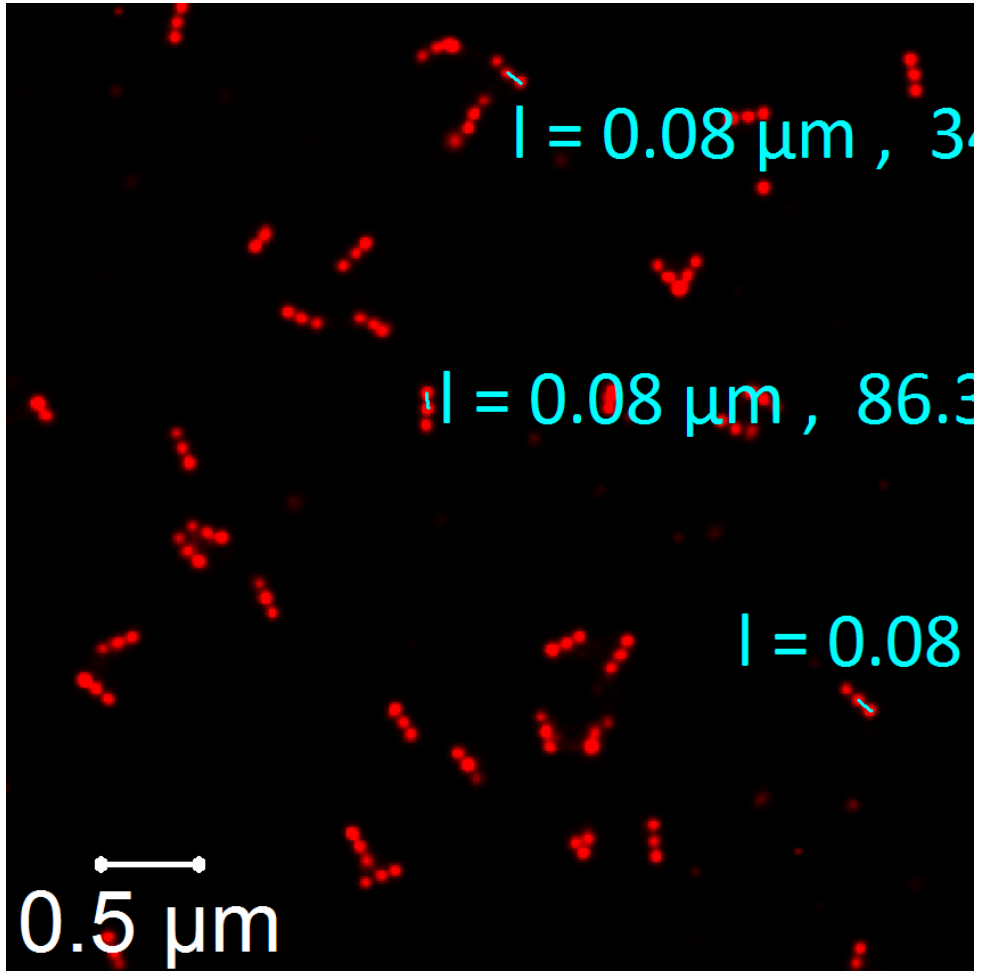

# Conclusions

- The AIC is UT's latest and greatest imaging resource!
- Super-resolution microscopy is super easy (if you have the right equipment, assistance, and training)!
- **AIC DROP IN WEEK!!**
  - May 1st-5th