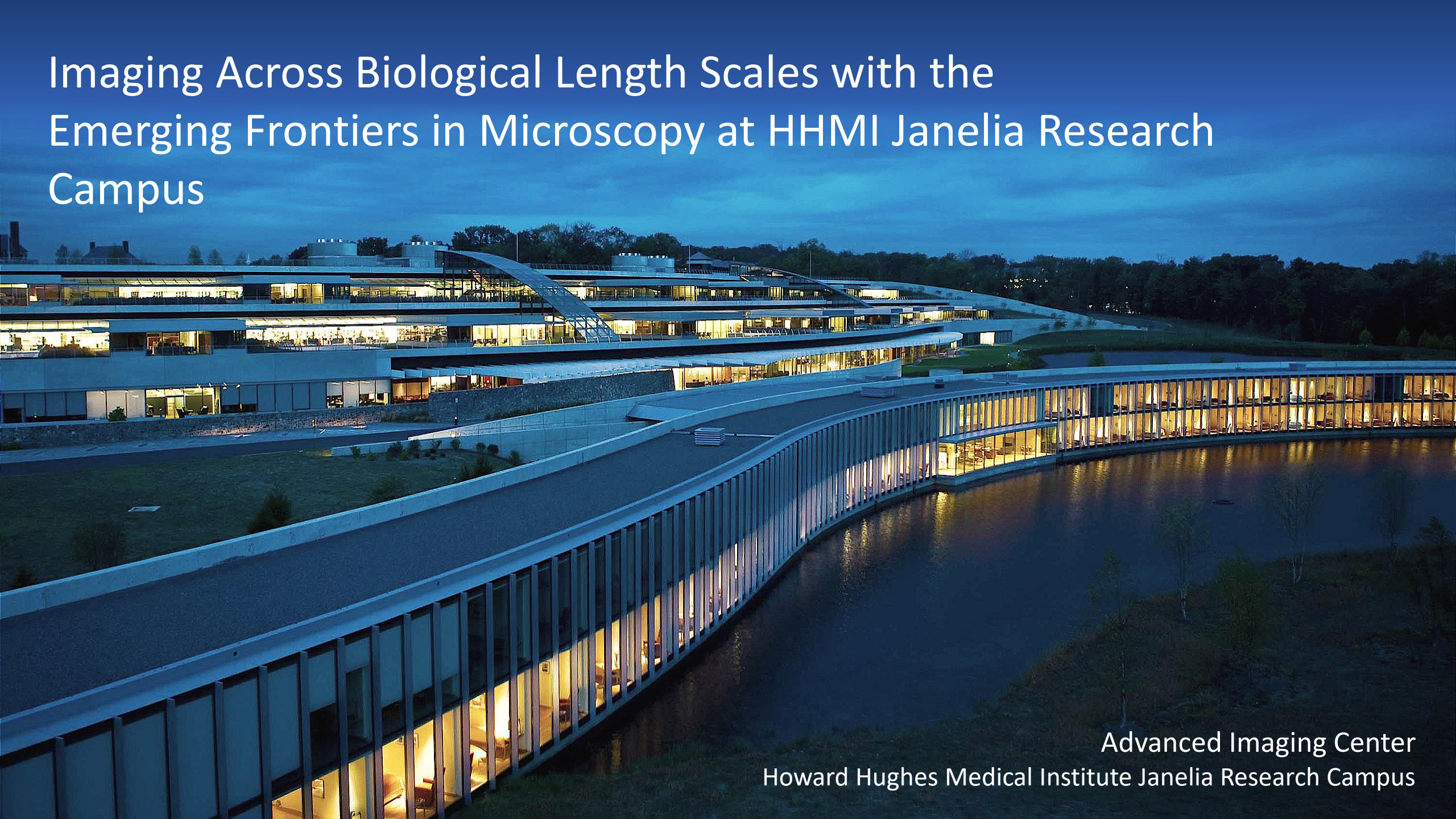
Imaging Across Biological Length Scales with the Emerging Frontiers in Microscopy at HHMI Janelia Research Campus

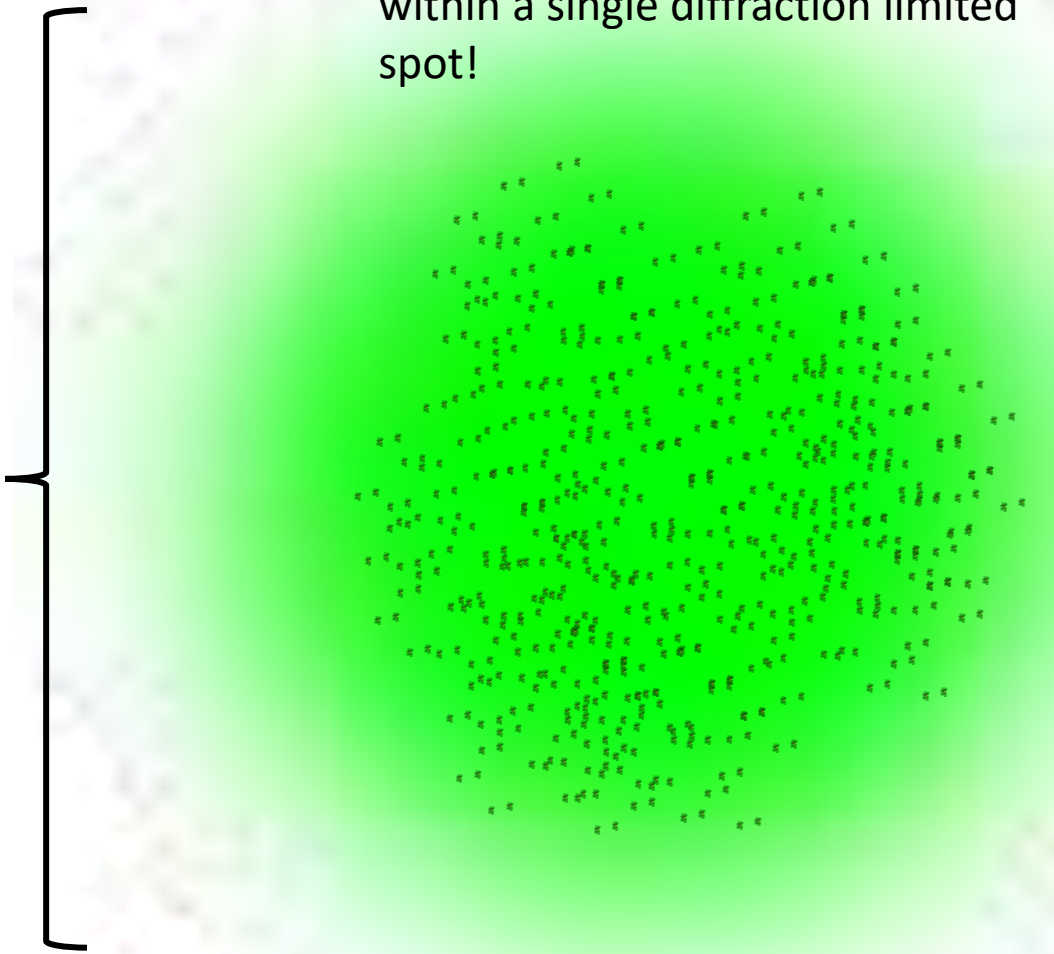


Advanced Imaging Center
Howard Hughes Medical Institute Janelia Research Campus

Resolution limit

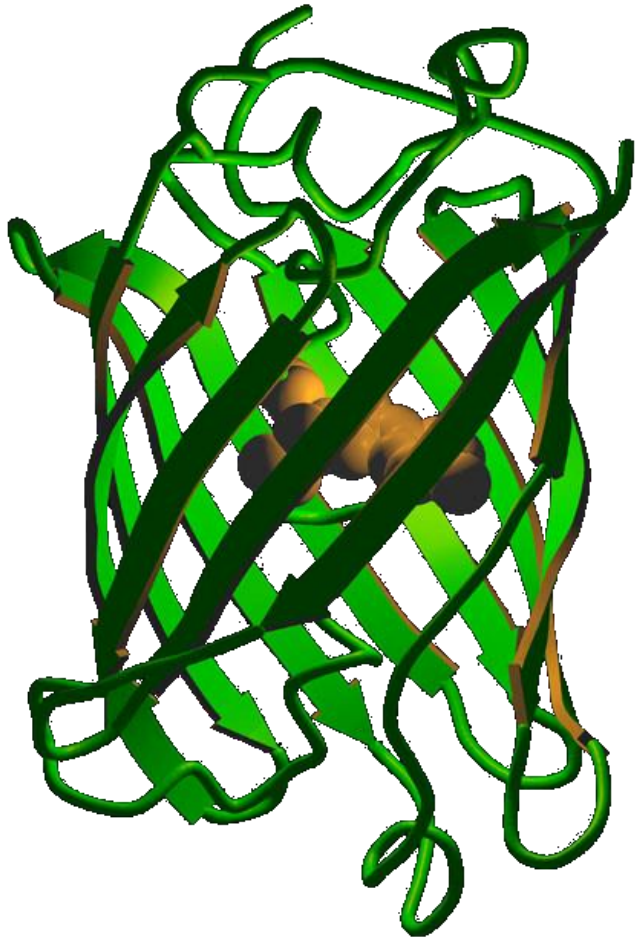
Many thousands of proteins can fit within a single diffraction limited spot!

Resolution Limit



300nm

One GFP Molecule



2-4nm

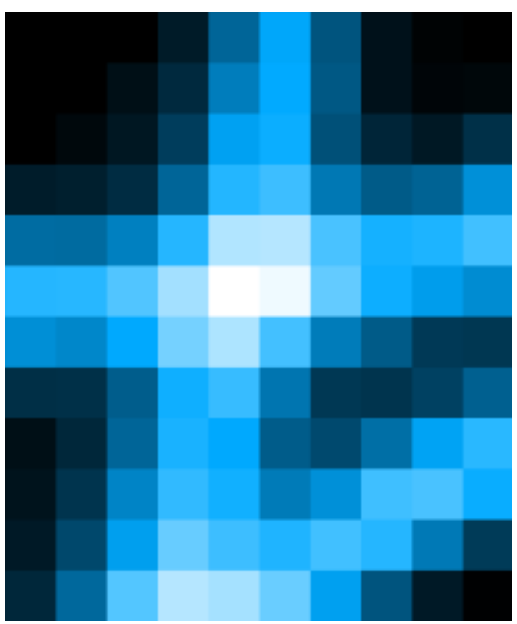
Proposed method for molecular optical imaging

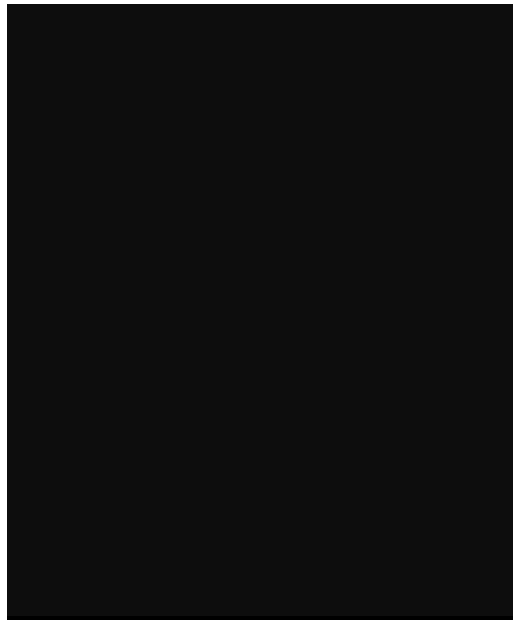
E. Betzig

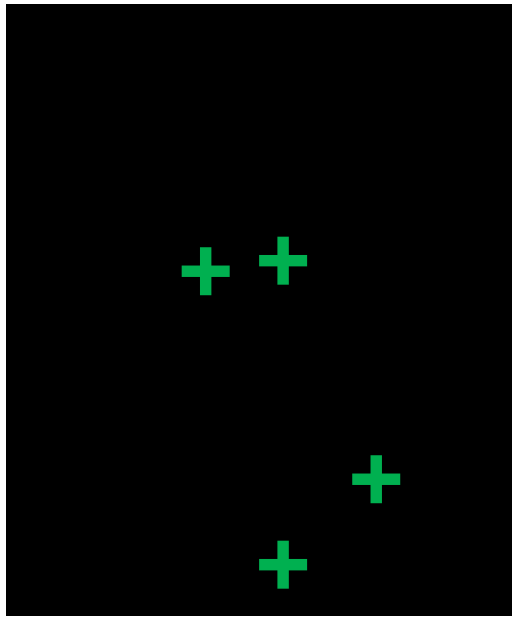
NSOM Enterprises, 17 Webster Drive, Berkeley Heights, New Jersey 07922

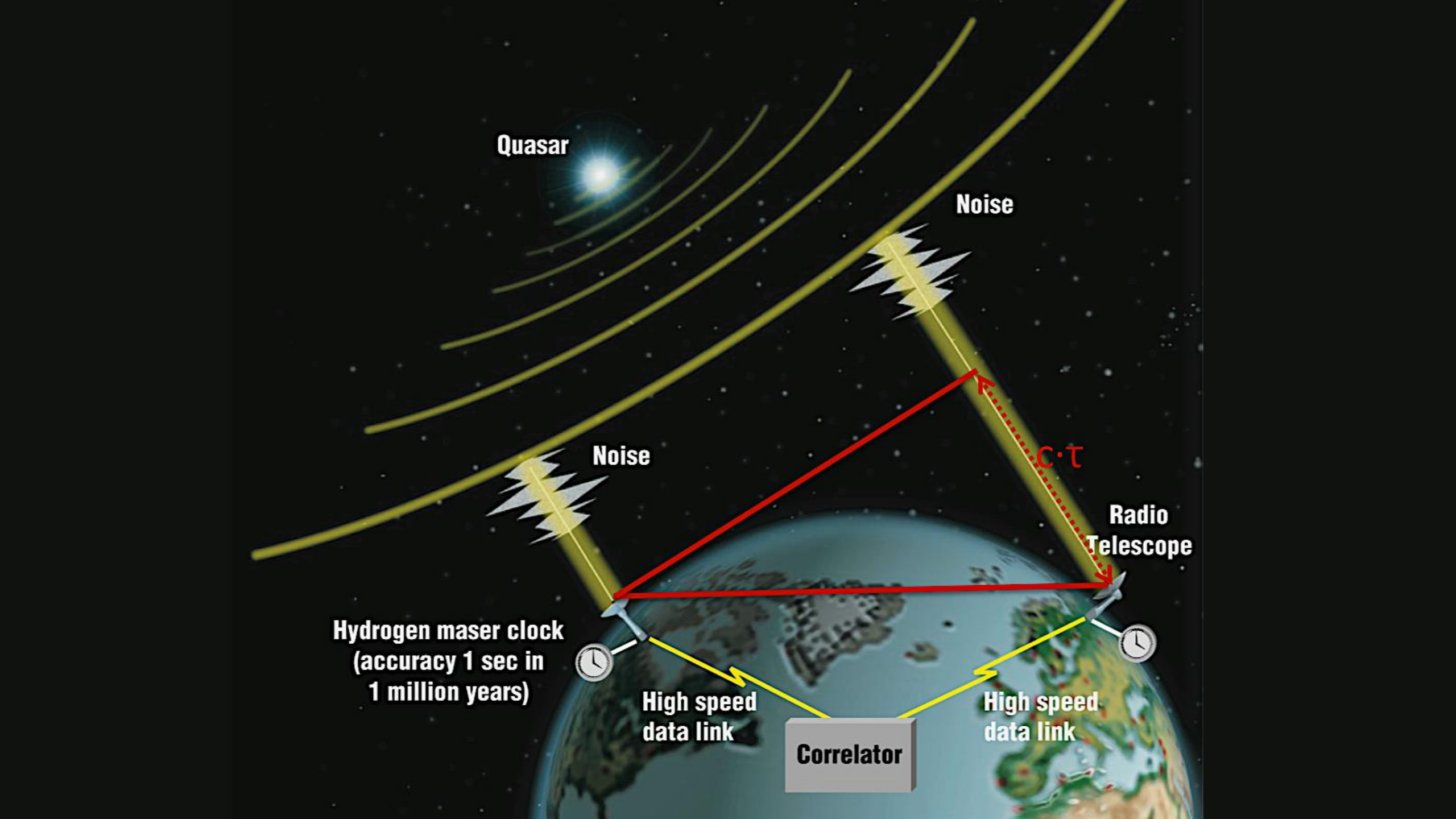
Received September 20, 1994

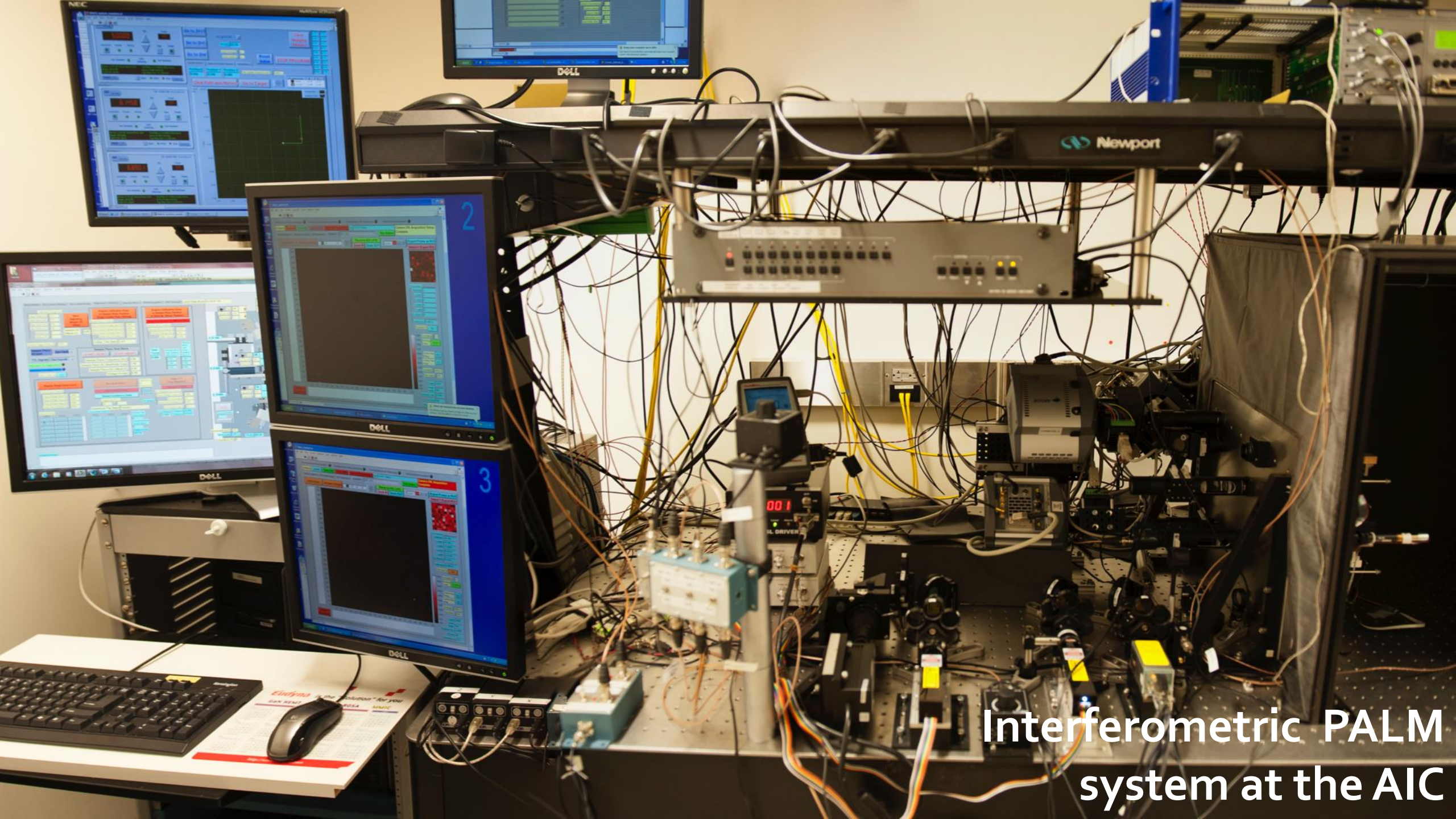
We can resolve multiple discrete features within a focal region of m spatial dimensions by first isolating each on the basis of $n \geq 1$ unique optical characteristics and then measuring their relative spatial coordinates. The minimum acceptable separation between features depends on the point-spread function in the $(m + n)$-dimensional space formed by the spatial coordinates and the optical parameters, whereas the absolute spatial resolution is determined by the accuracy to which the coordinates can be measured. Estimates of each suggest that near-field fluorescence excitation microscopy/spectroscopy with molecular sensitivity and spatial resolution is possible.





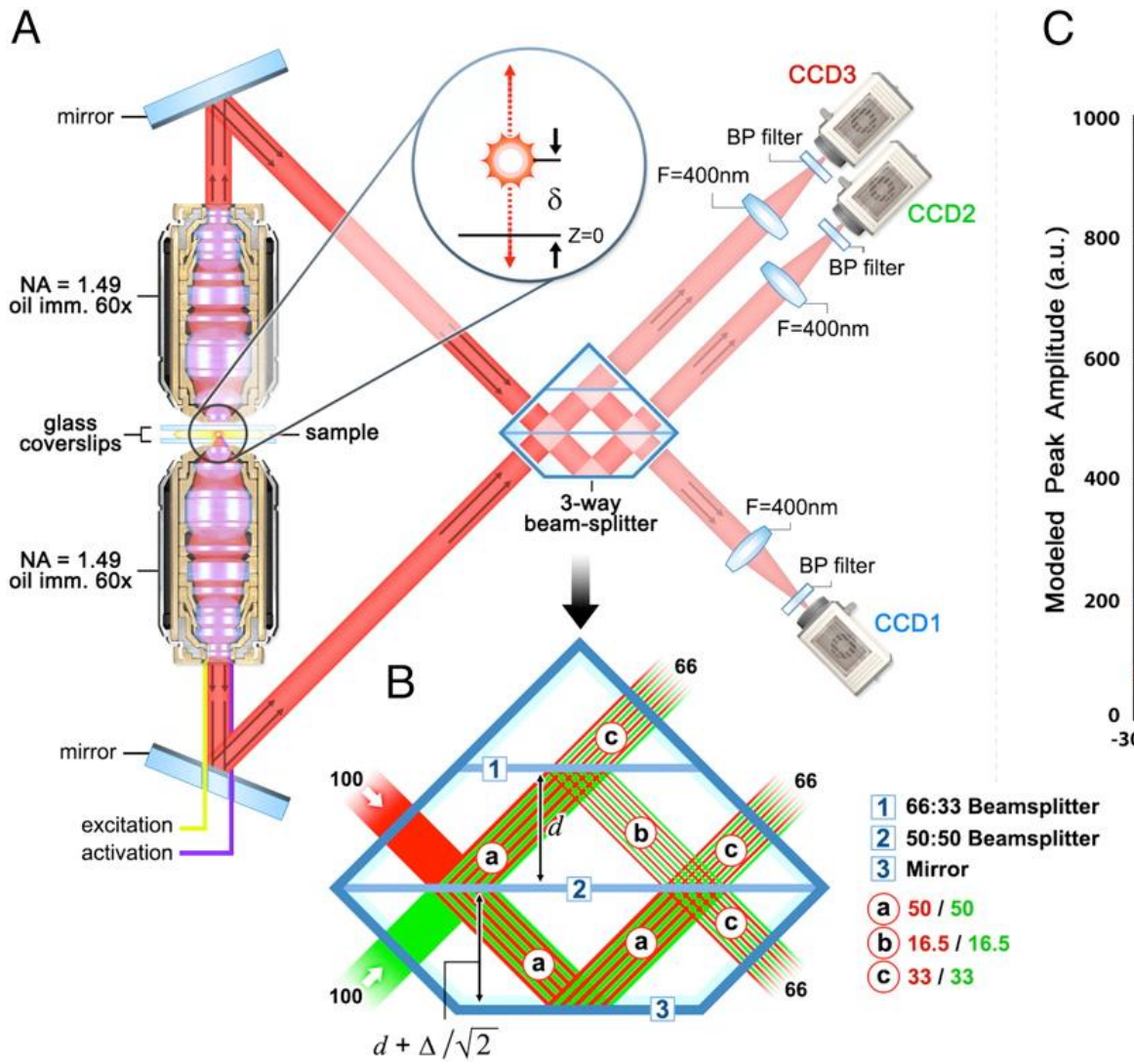


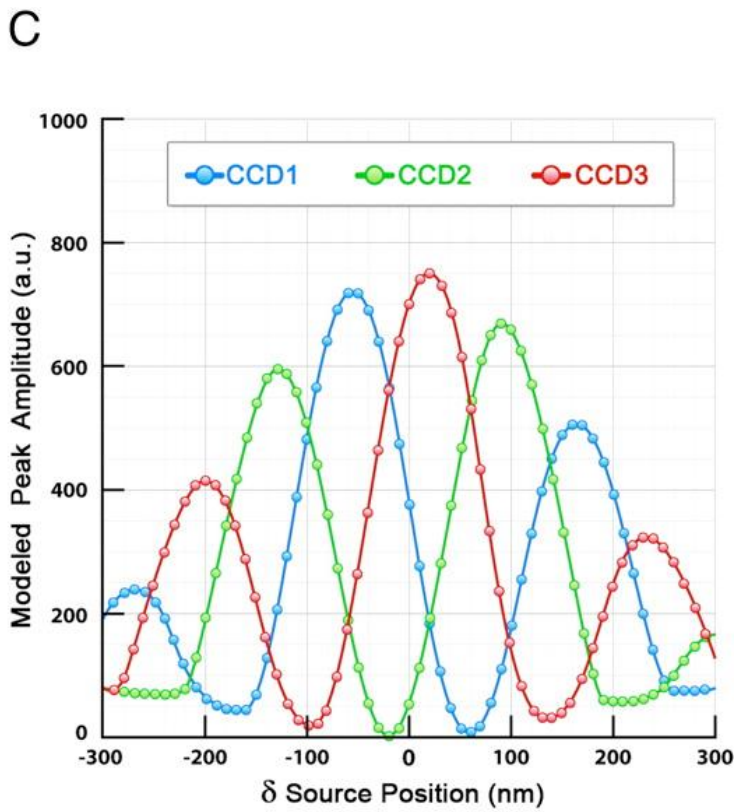

Quasar
Noise
Noise
c·τ
Radio
Telescope
Hydrogen maser clock
(accuracy 1 sec in
1 million years)
High speed
data link
Correlator
High speed
data link

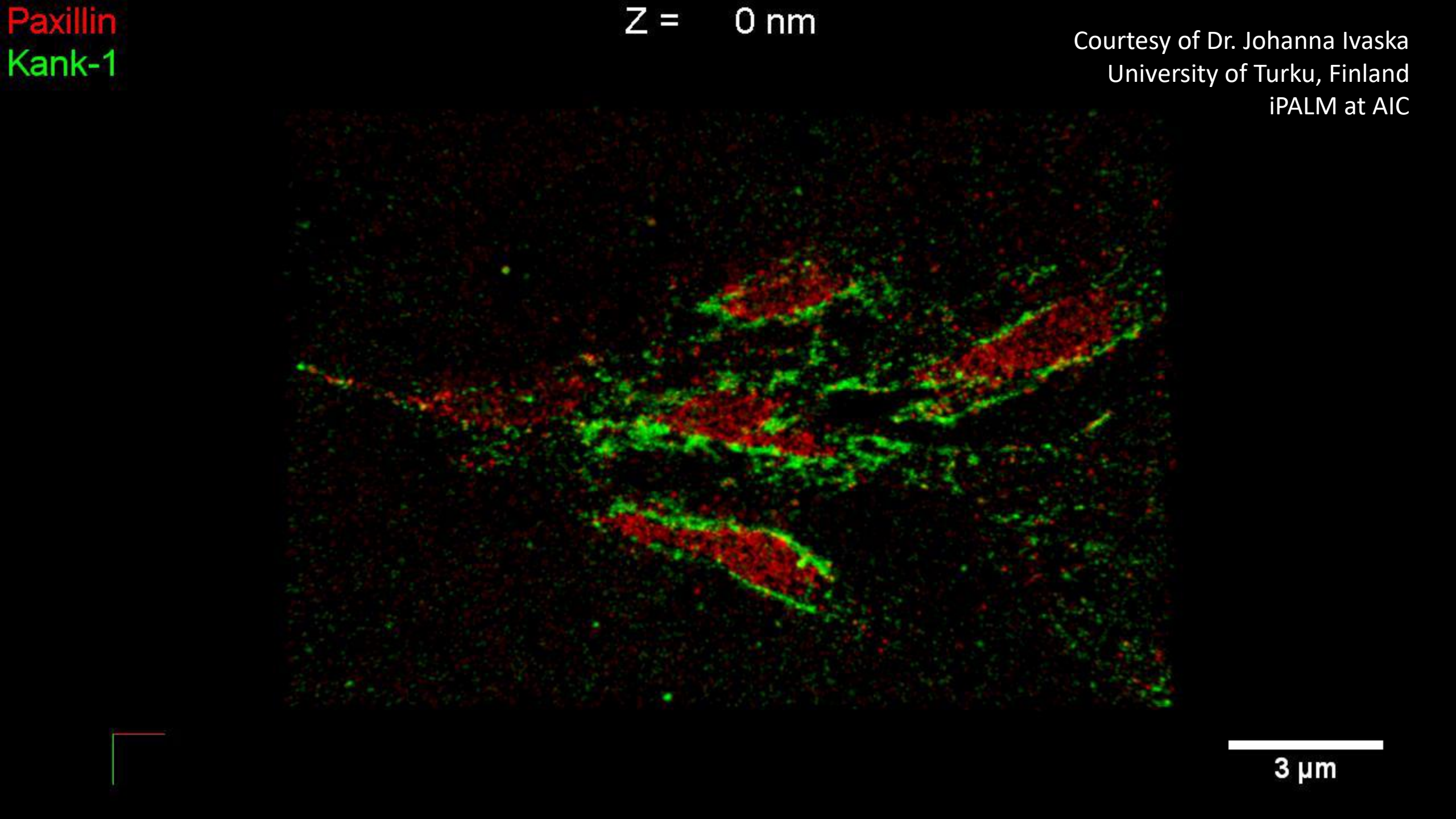


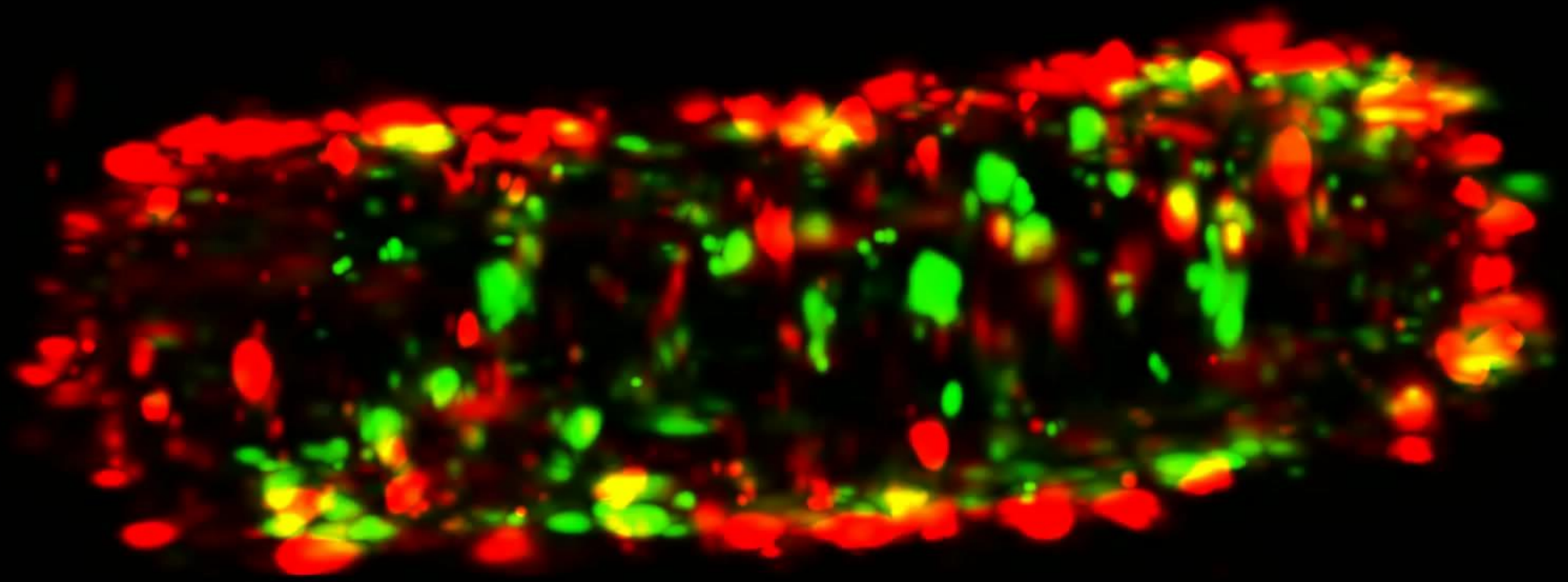
Interferometric PALM system at the AIC

Interferometric PALM (iPALM)



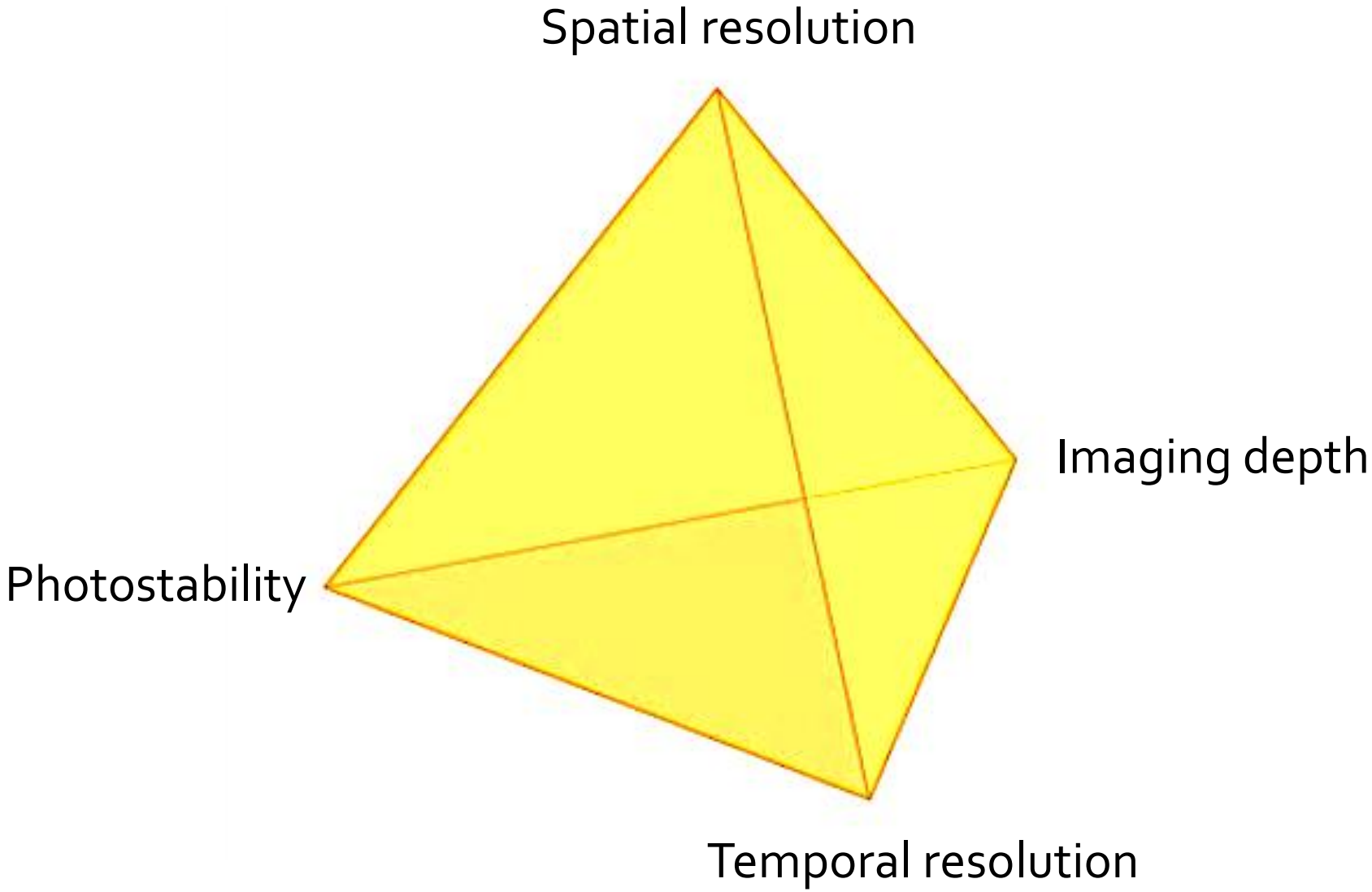



Paxillin
Kank-1
Z = 0 nm
Courtesy of Dr. Johanna Ivaska
University of Turku, Finland
iPALM at AIC
3 µm



Resolving the inner and outer membranes of a single bacterium on iPALM

Courtesy of Dr. Phil Klebba
Kansas State University
iPALM at AIC

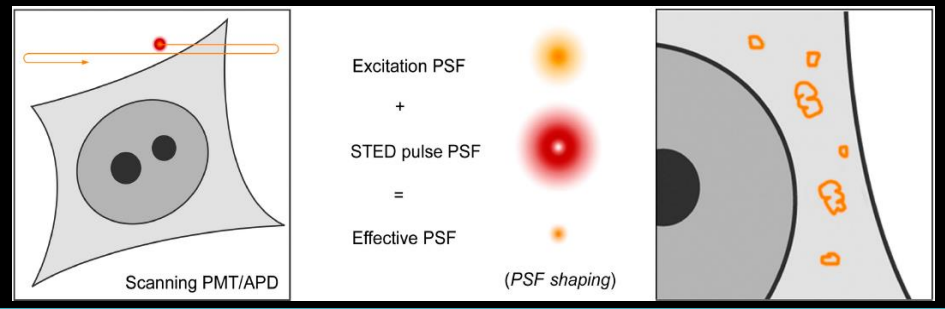
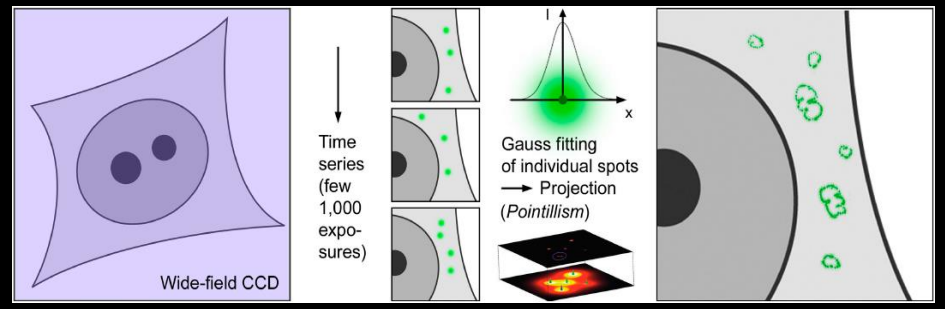
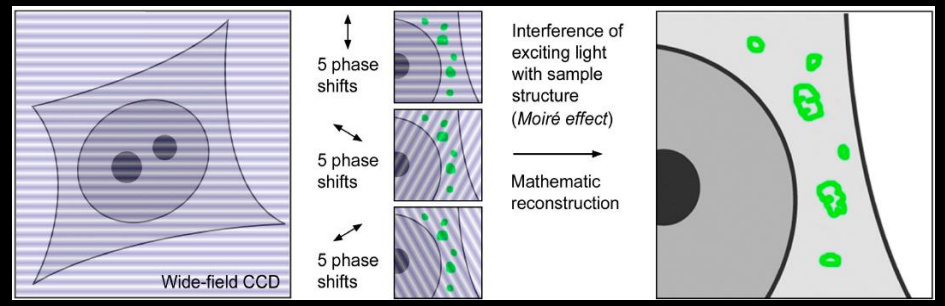

Spatial resolution
Imaging depth
Photostability
Temporal resolution

Pros and Cons of Current Super-Resolution Techniques

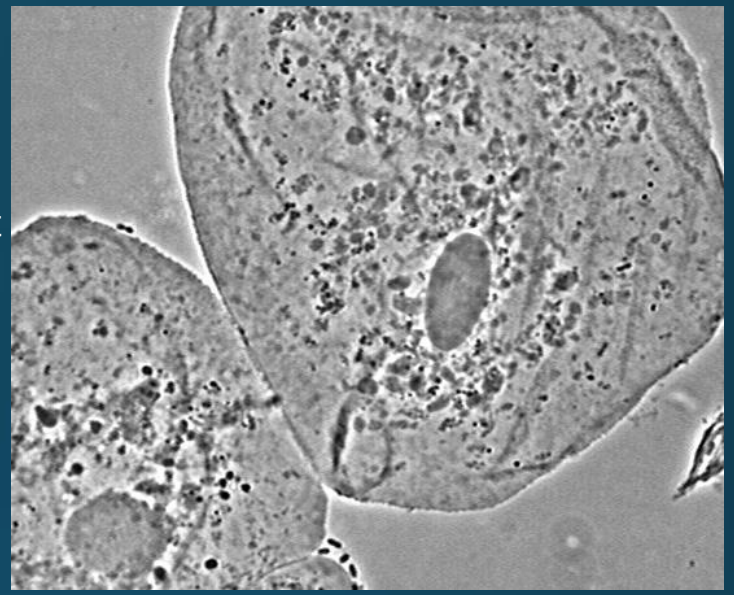
Nyquist Criterion:

N -fold resolution increase in ***D*** dimensions → N^D -fold more photons collected

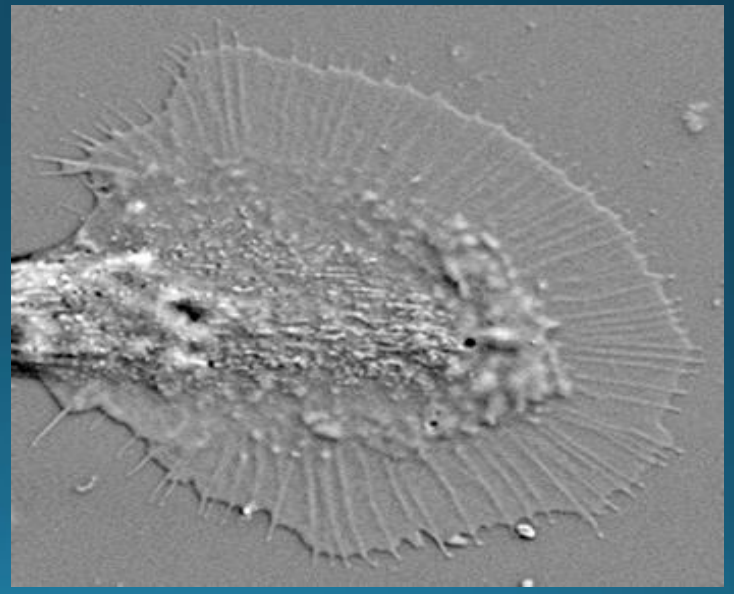
L. Schermelleh, *et al, J. Cell Biol.* (2010)

		Resolution (nm)	N^D	Intensity (W/cm²)	Acquisition Time (sec)
STED / RESOLFT		***xy*: 20 nm**	100	10^4 - 10^7	>60
		***xyz*: 30 nm**	1,070		~ 1,000
Localization		***xy*: 20 nm**	100	10^3 - 10^4	>20
		***xy*: 10 nm, *z*: 20 nm**	14,400		1,500
SIM		***xy*: 100 nm**	4	10 - 10^2	0.1 - 1
		***xy*: 100 nm, *z*: 360 nm**	8		~ 10

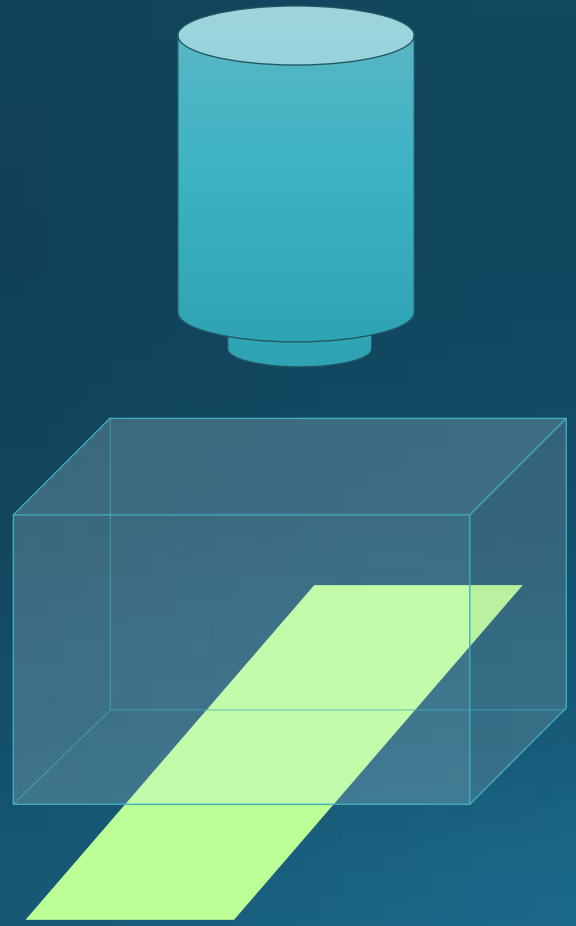
Phase contrast



DIC

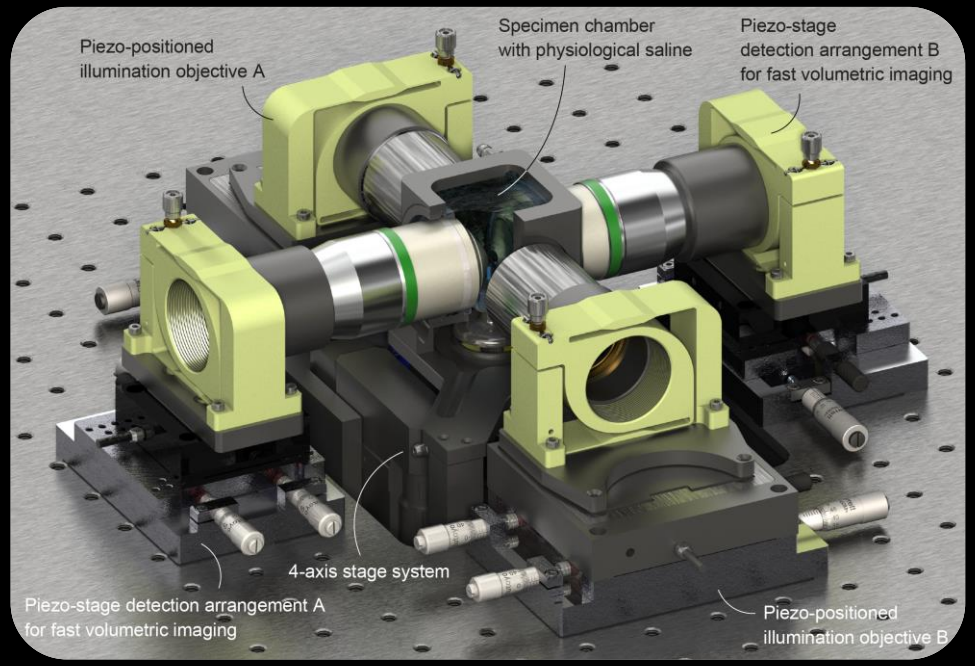


Light sheet/plane illumination microscopy



Widefield light collection lens
No photon wasted due to pinhole rejection

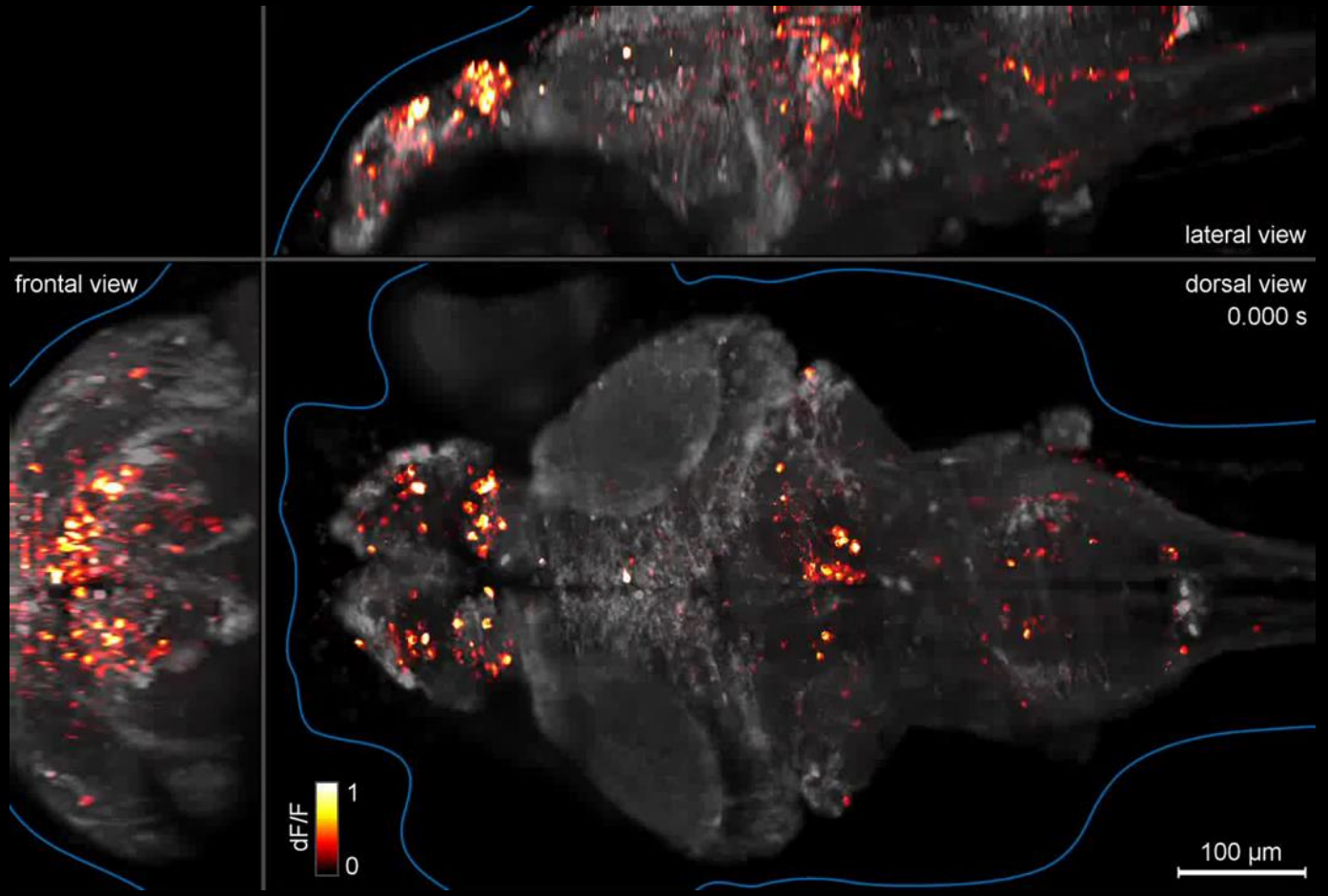
Only the plane that is being imaged is illuminated



Temporal resolution: 800 x 400 x 200 μm^3 at **5 Hz**

Spatial resolution: **400 nm** laterally, **1.8 µm** axially

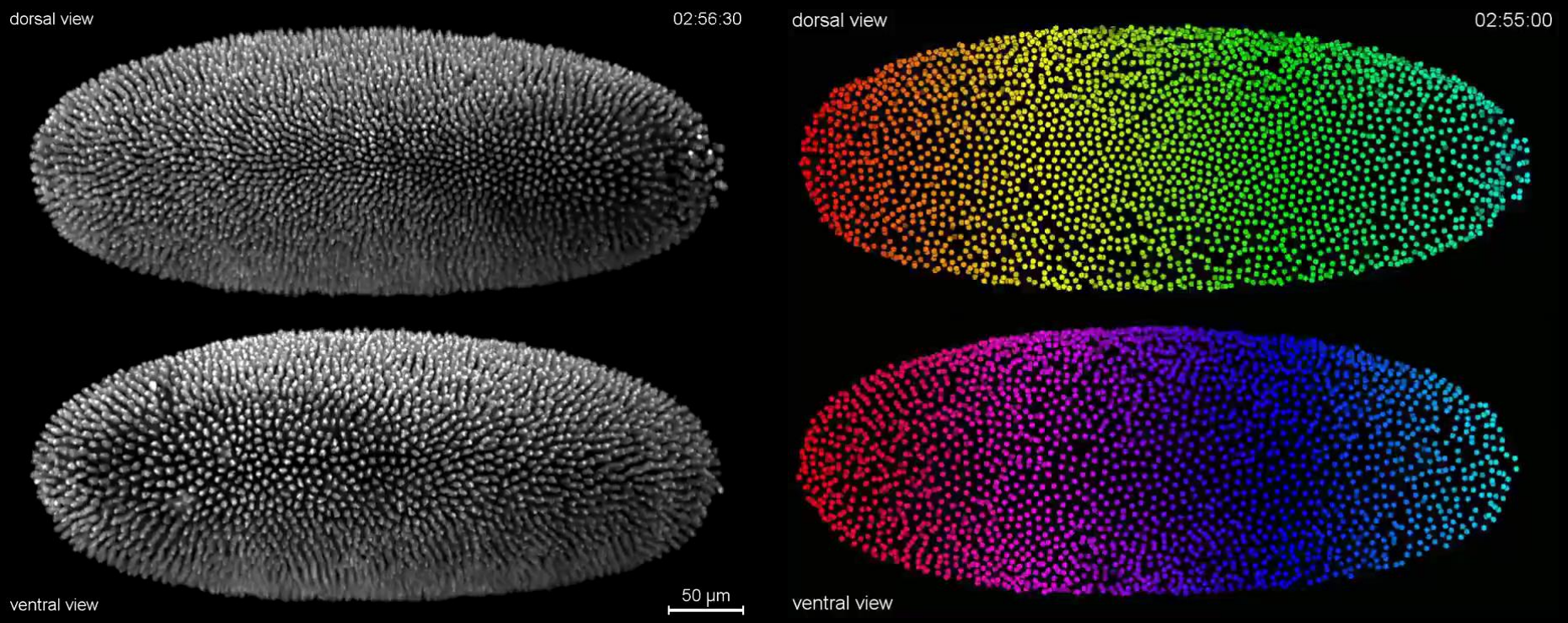
175 million voxels per second



Neuronal signaling in whole zebrafish brain

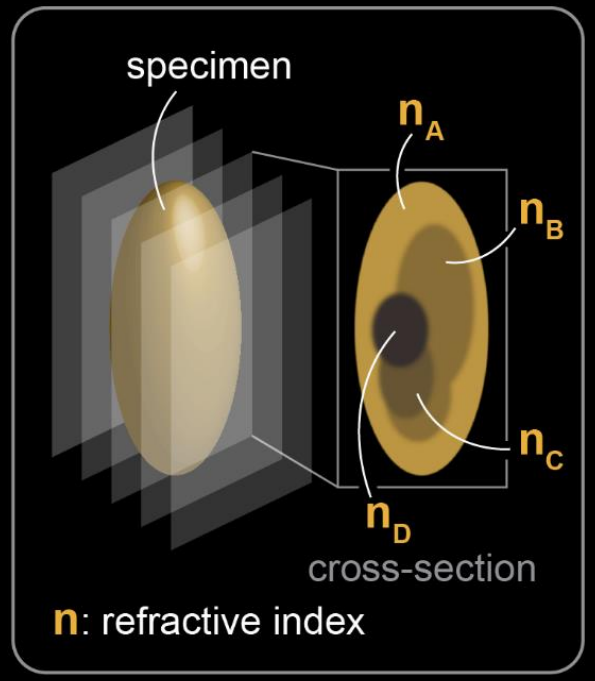
Misha Ahrens and Philipp Keller
HHMI Janelia Research Campus
Nature Methods 10: 413-420, 2013

Imaging high scattering sample: Drosophila embryogenesis

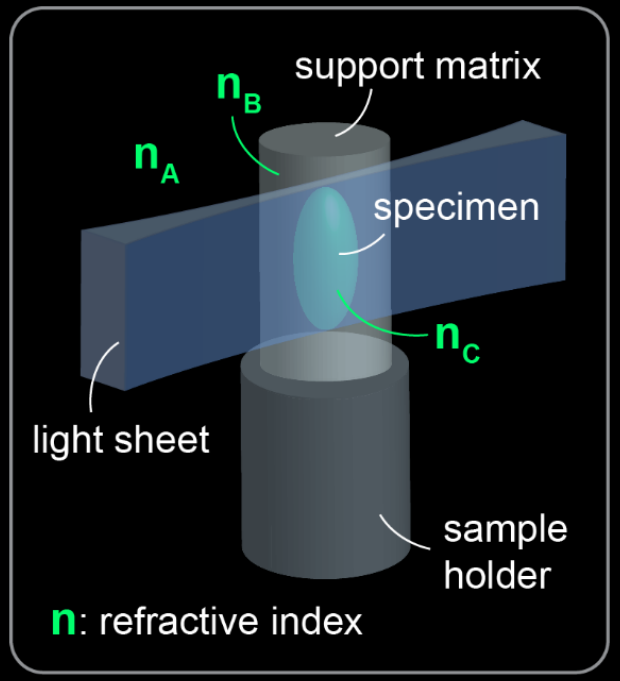


Philipp Keller
HHMI Janelia Research Campus

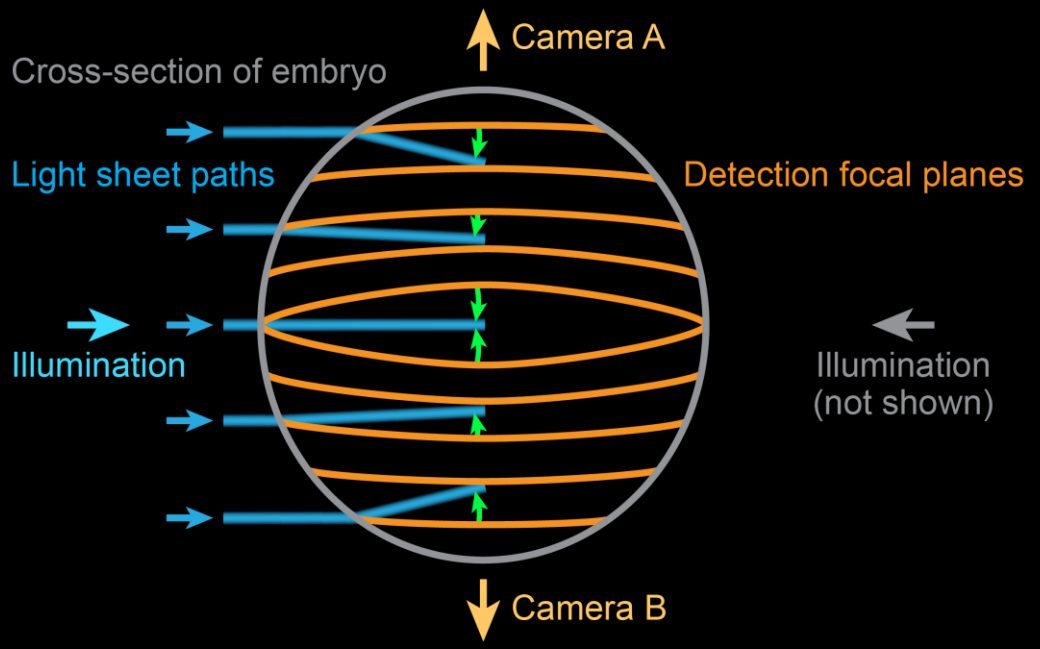
Optical heterogeneity of sample



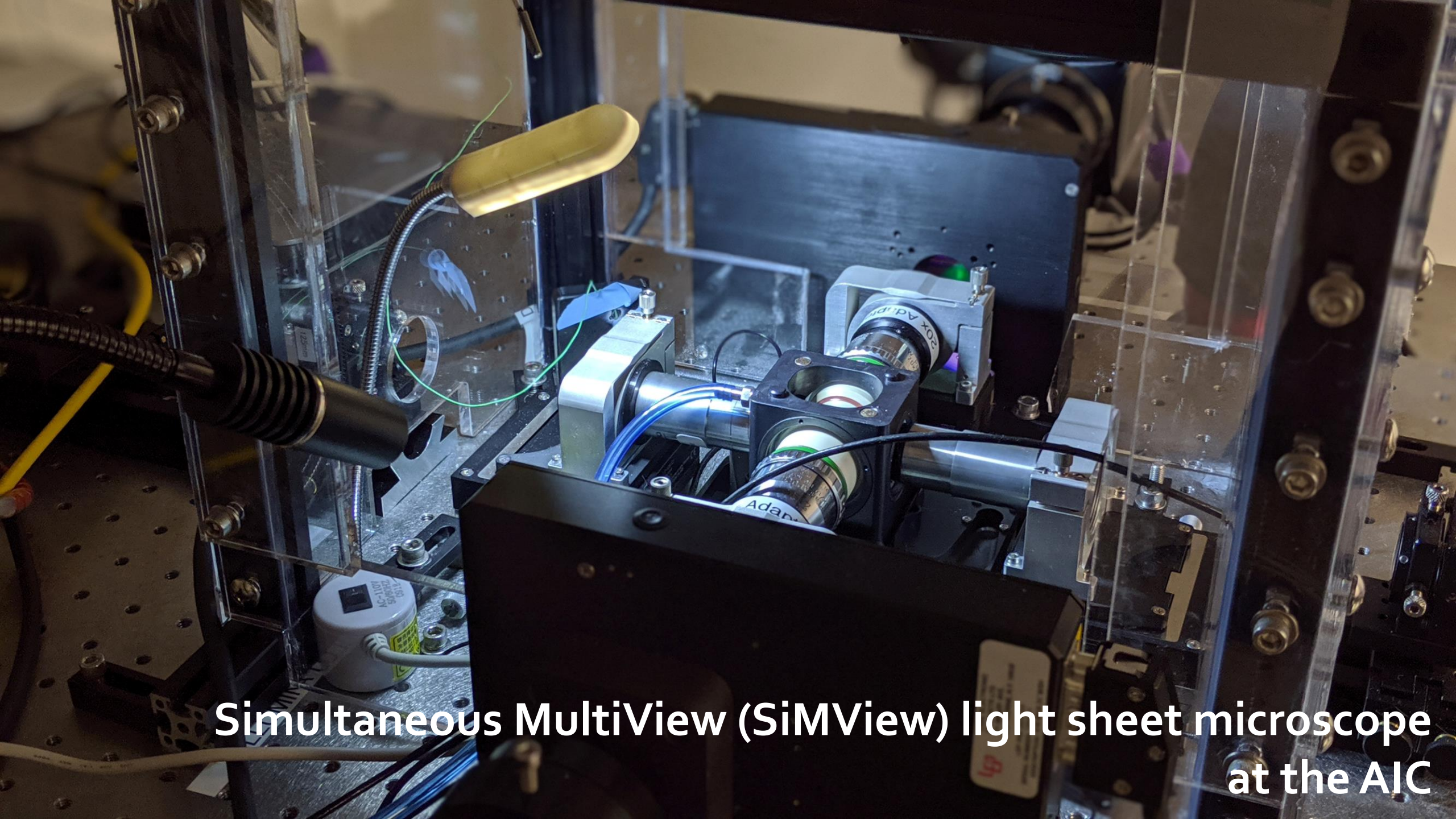
Optical heterogeneity of environment



Optical challenges in light-sheet imaging of large specimens



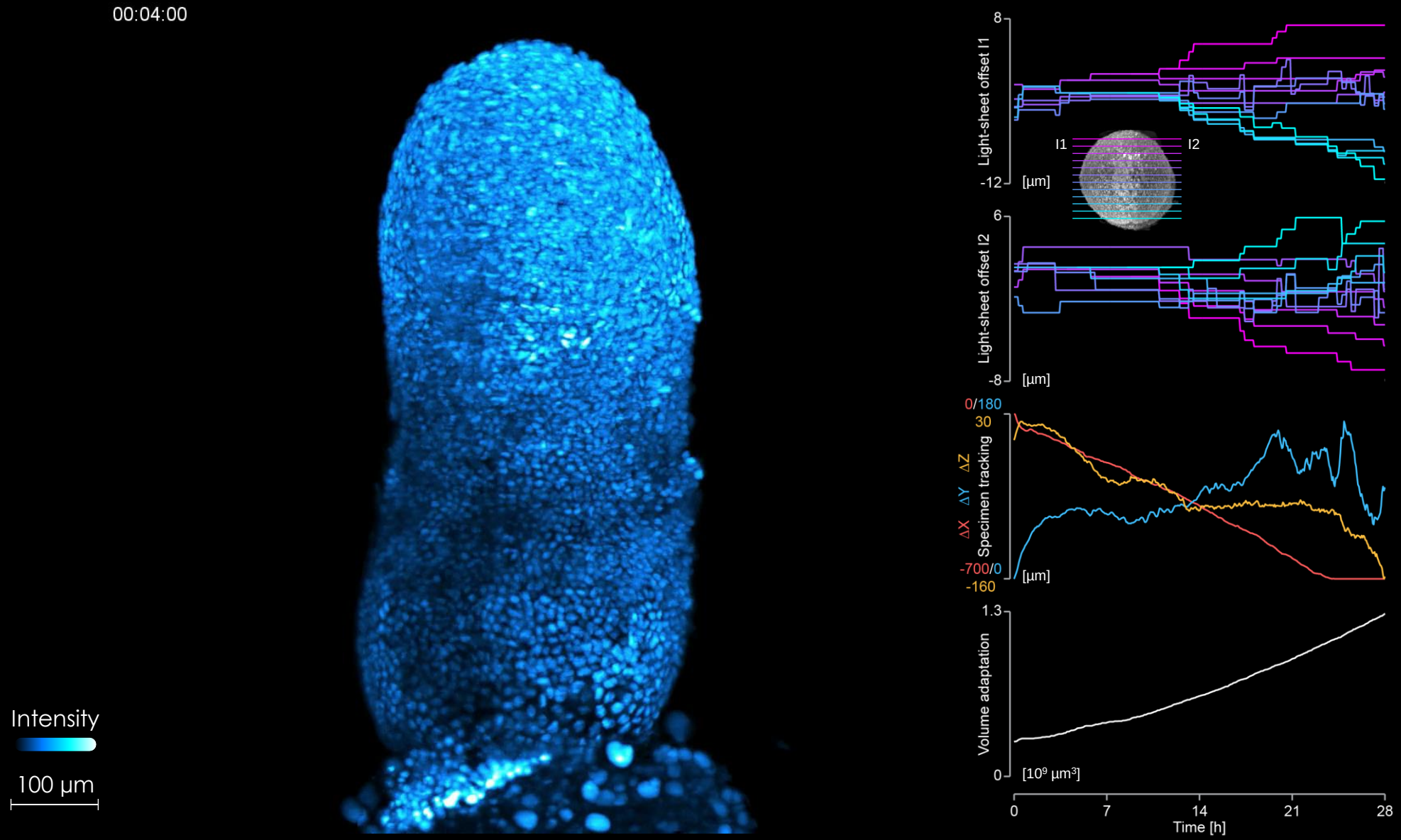
Spatial mismatches in illumination and detection processes



Simultaneous MultiView (SiMView) light sheet microscope at the AIC

Projection of imaging volume

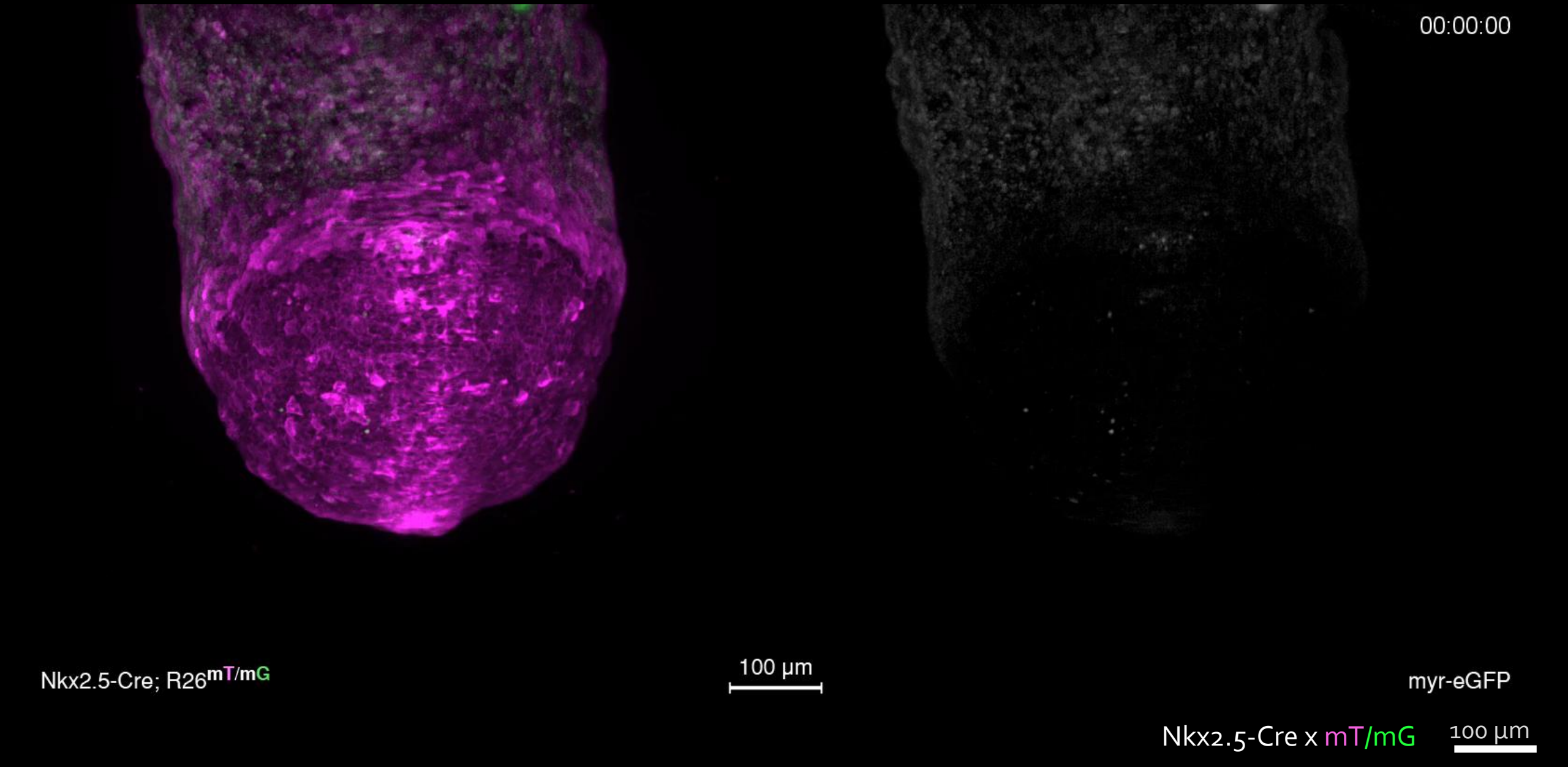
Real-time optimization of imaging conditions



Adaptive imaging of mouse post-implantation development

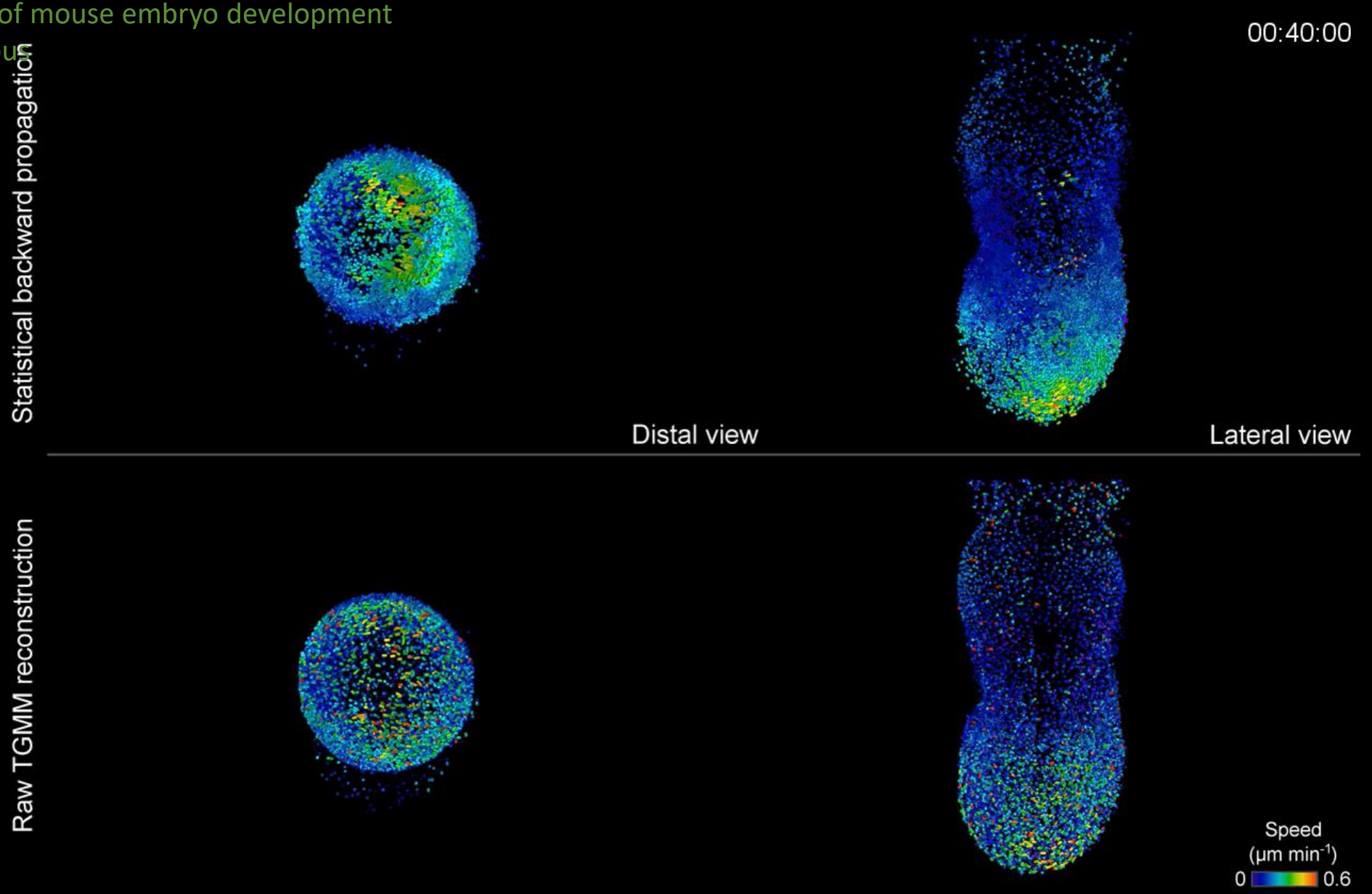
McDole *et al*. **Cell**, 175: 859, 2018

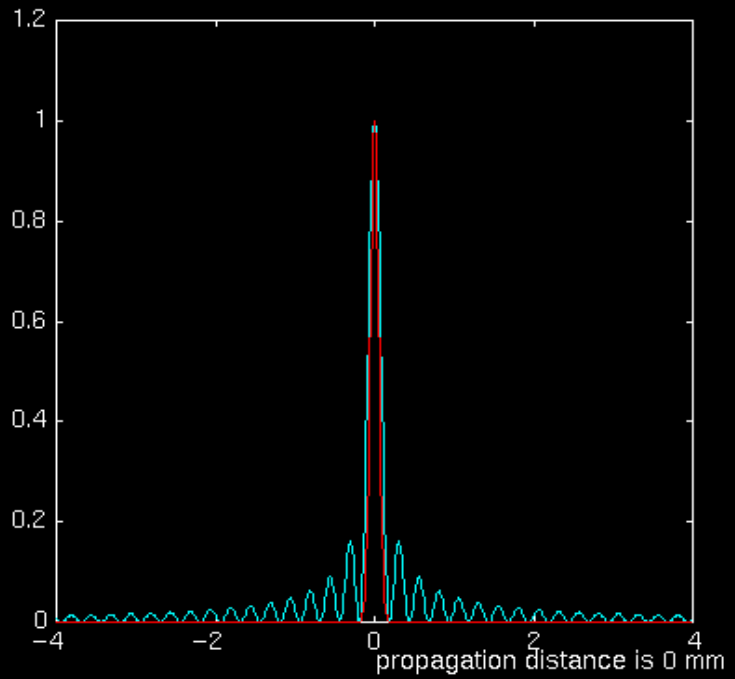
Visualizing early heart formation



Putting it all together: vector flow of every cell in mouse embryo

SiMView light sheet imaging of mouse embryo development
HHMI Janelia Research Campus





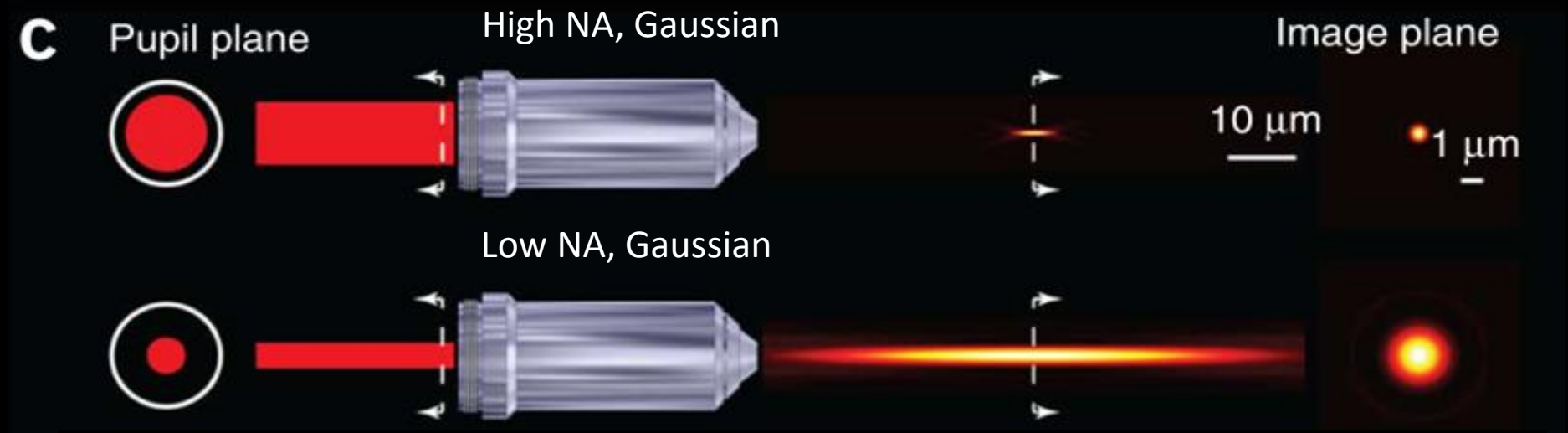
Gaussian beam profile during propagation

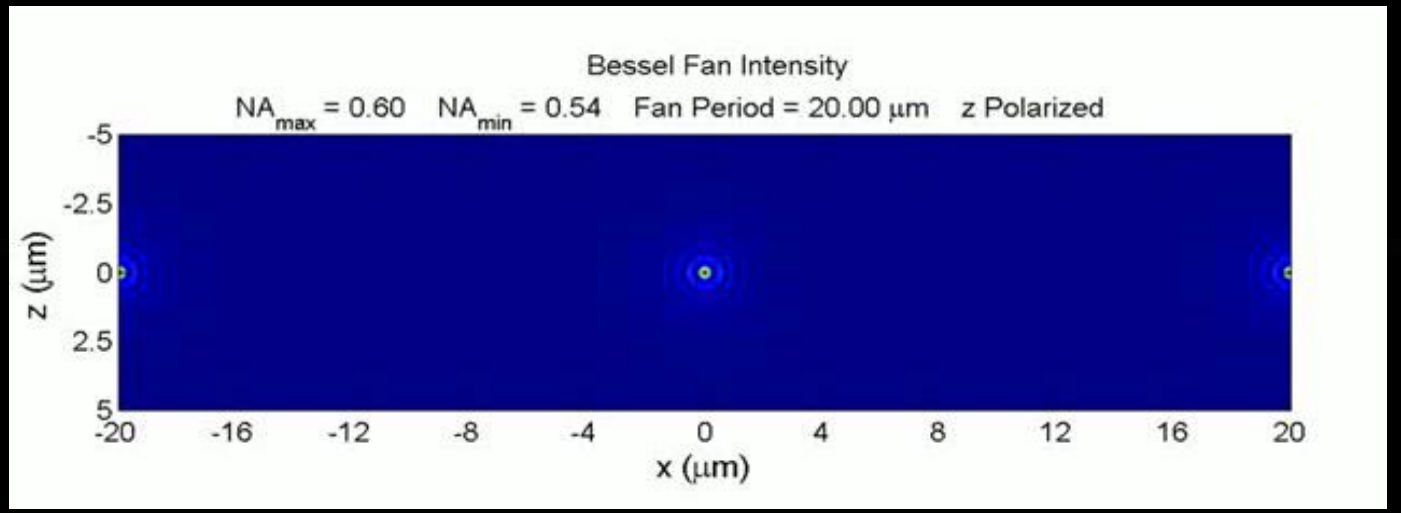
Bessel beam profile during propagation

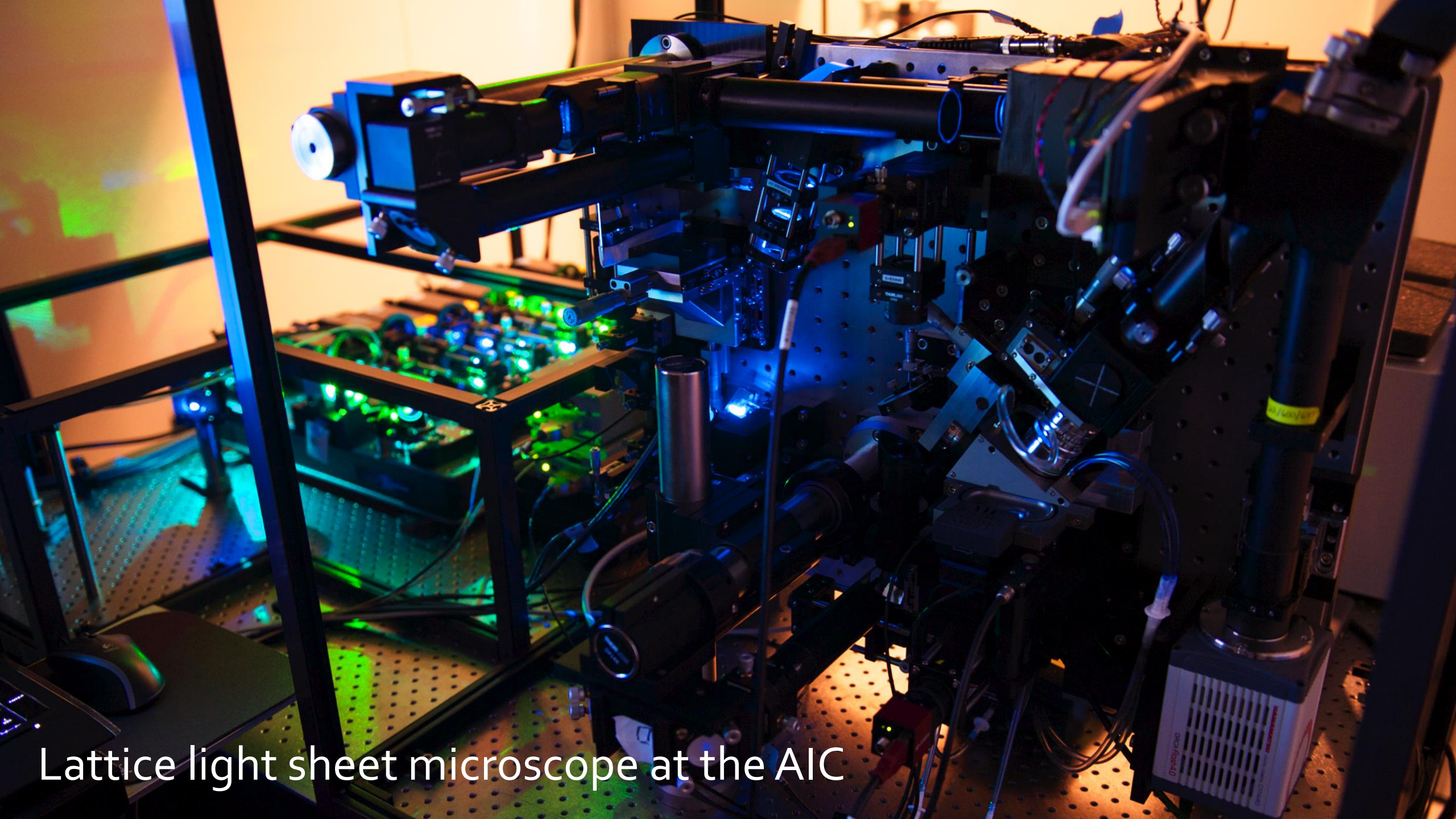
Gaussian beam profile during propagation

Bessel beam profile during propagation

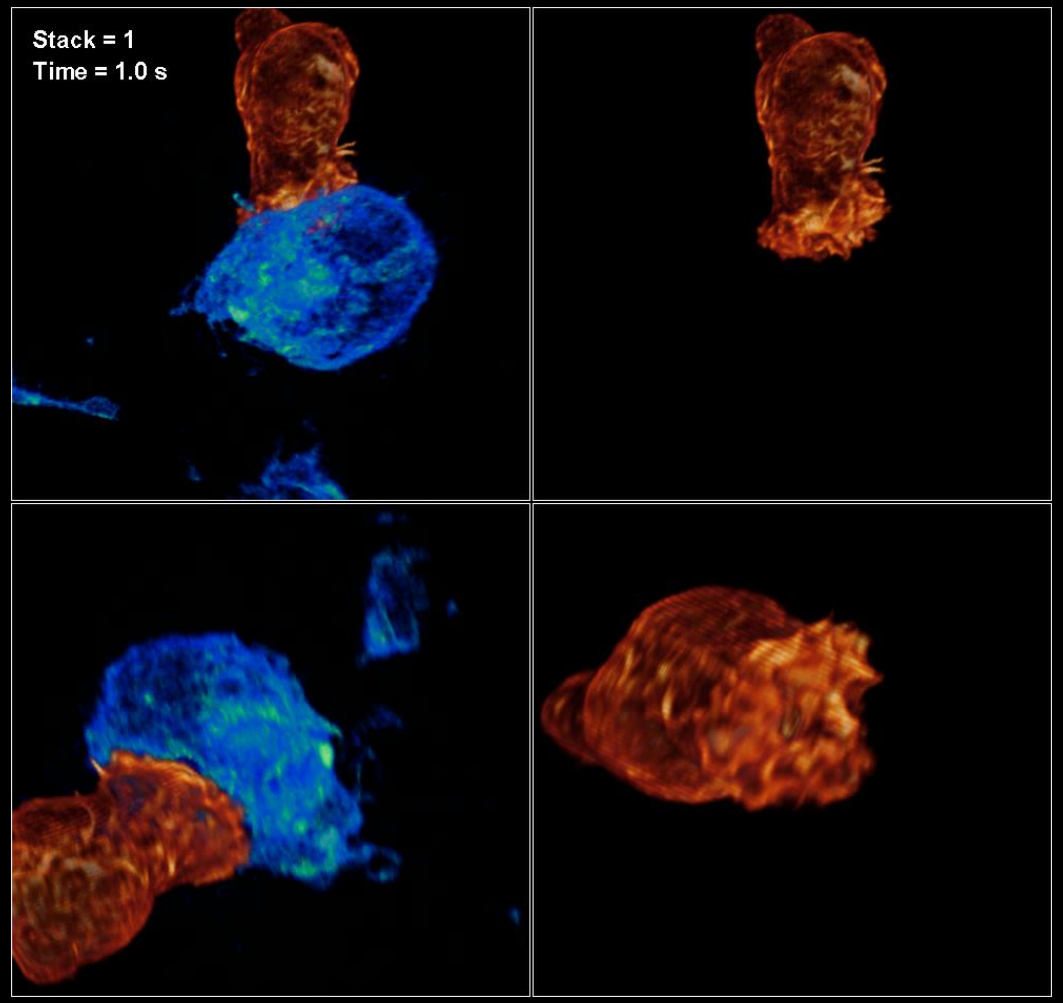
Gao, et al. Nat Prot 2014





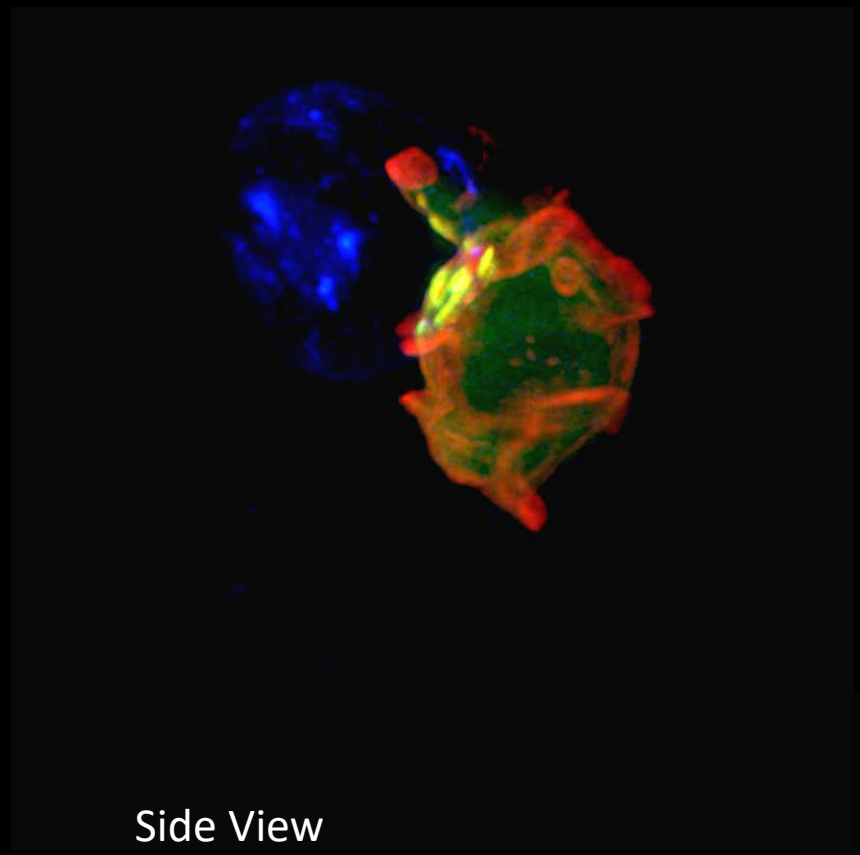


Lattice light sheet microscope at the AIC



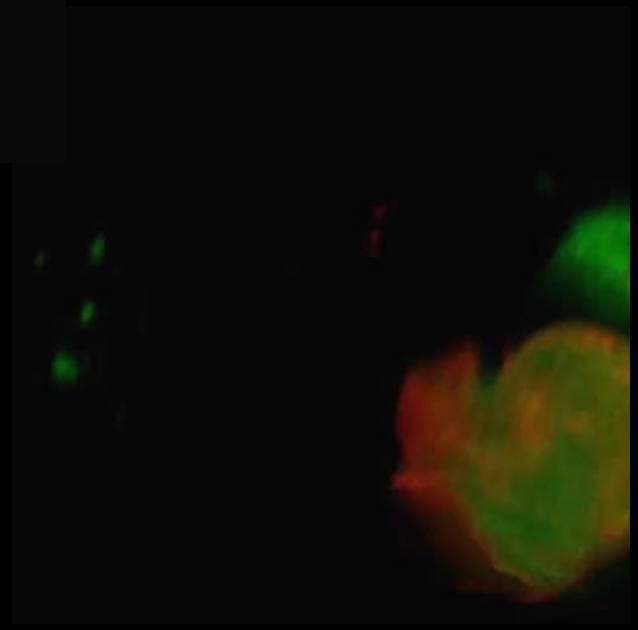
T cell (orange, actin) forming immunological synapse with an antigen presenting cell (blue)

400 stacks, 1.27s/2-color stack



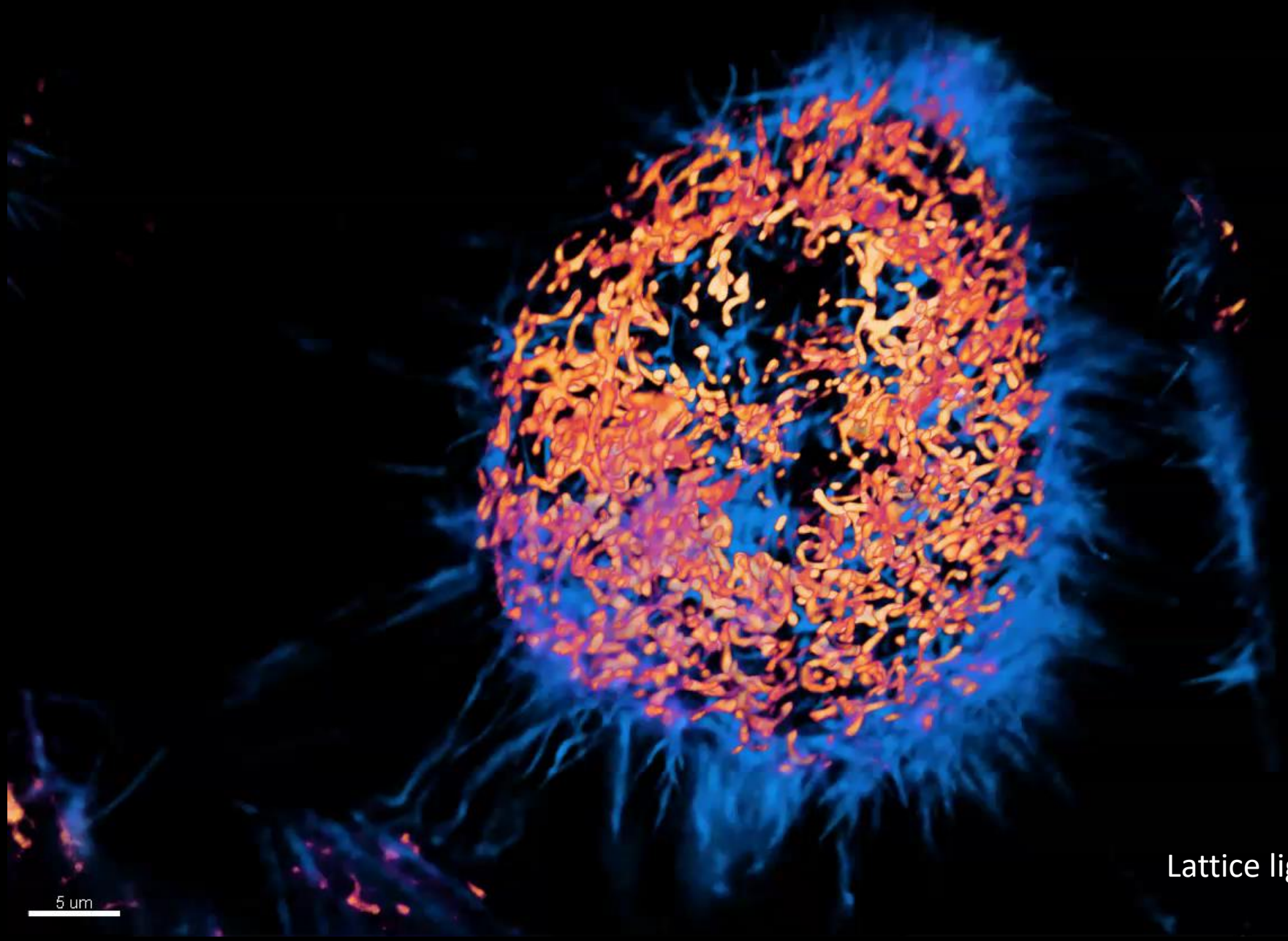
Side View

En face View

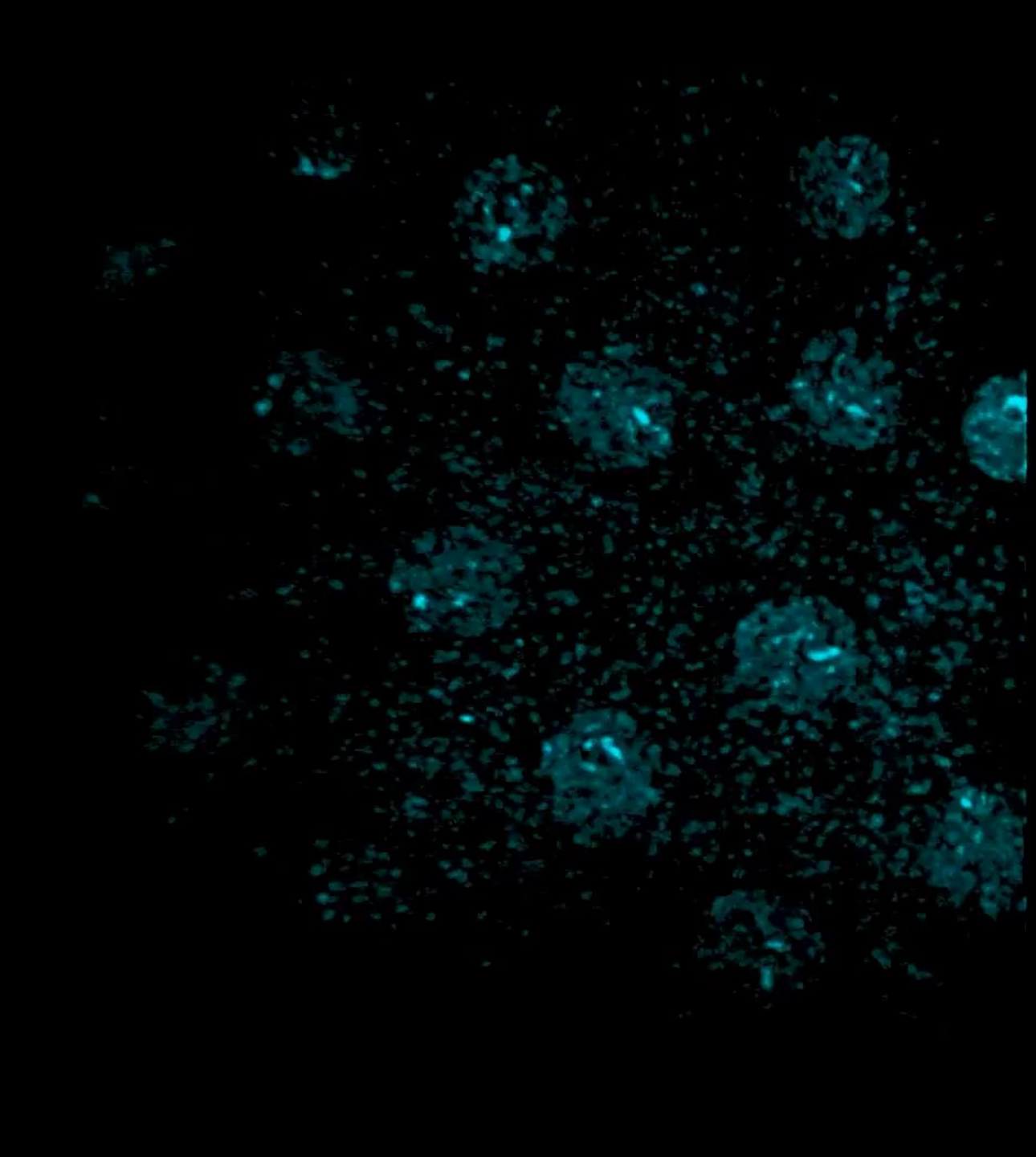


Courtesy of Dr. Lakshmi Bagolapalan
NIH
Lattice light sheet microscope at AIC

Mitochondrial segregation during HeLa cell division

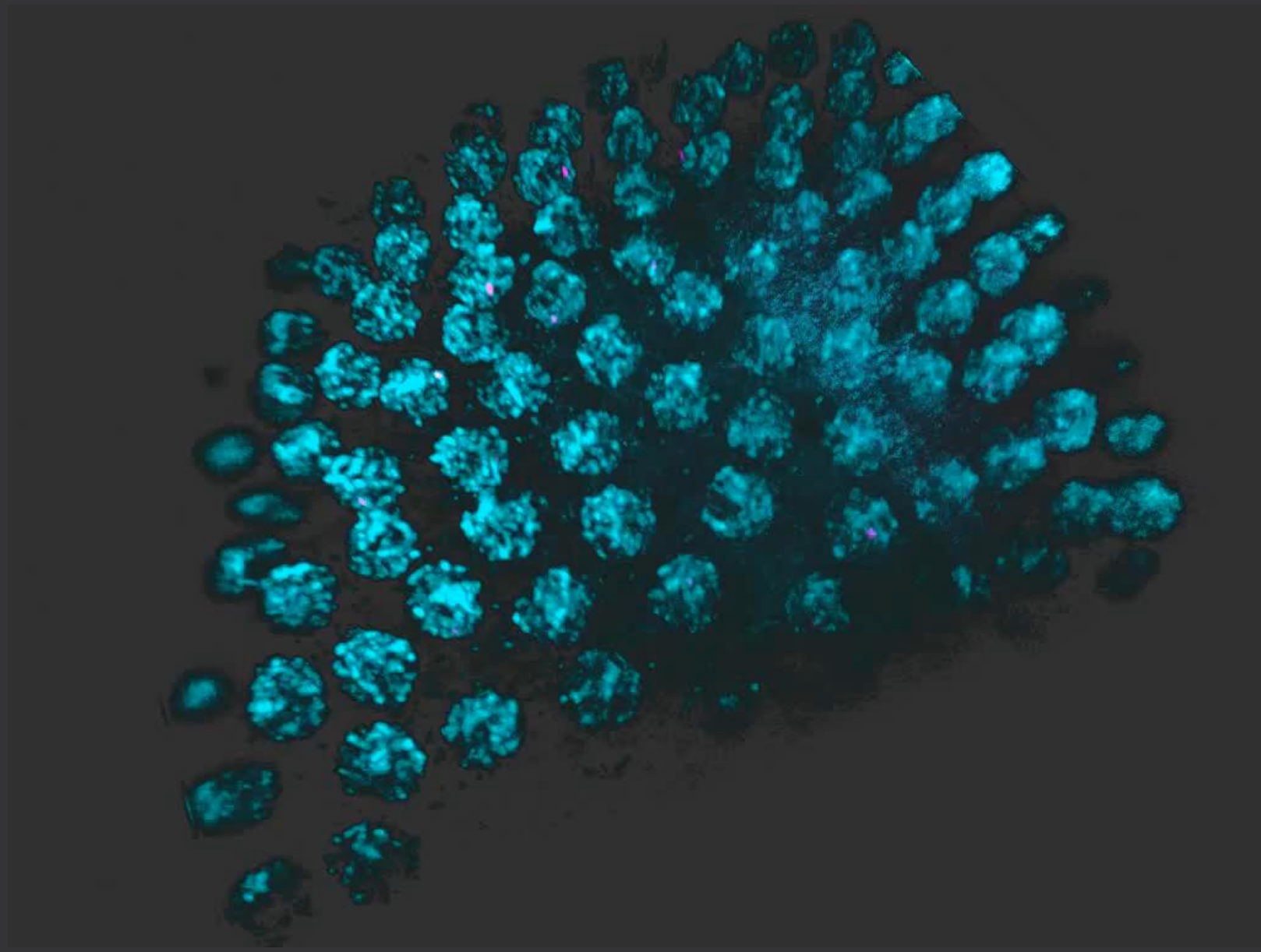


Courtesy of Dr. Erika Holzbaur
University of Pennsylvania
Lattice light sheet microscope at Janelia

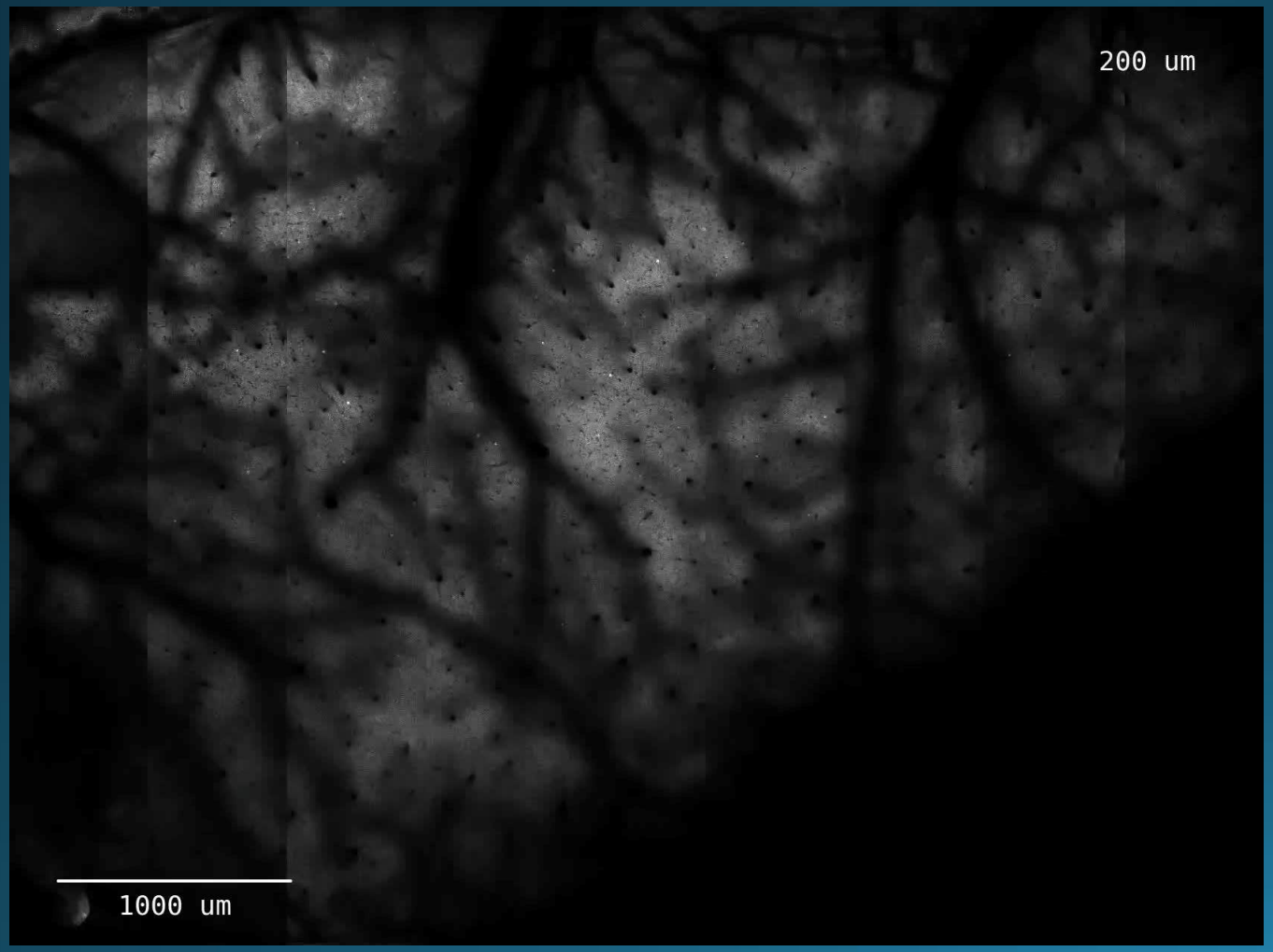


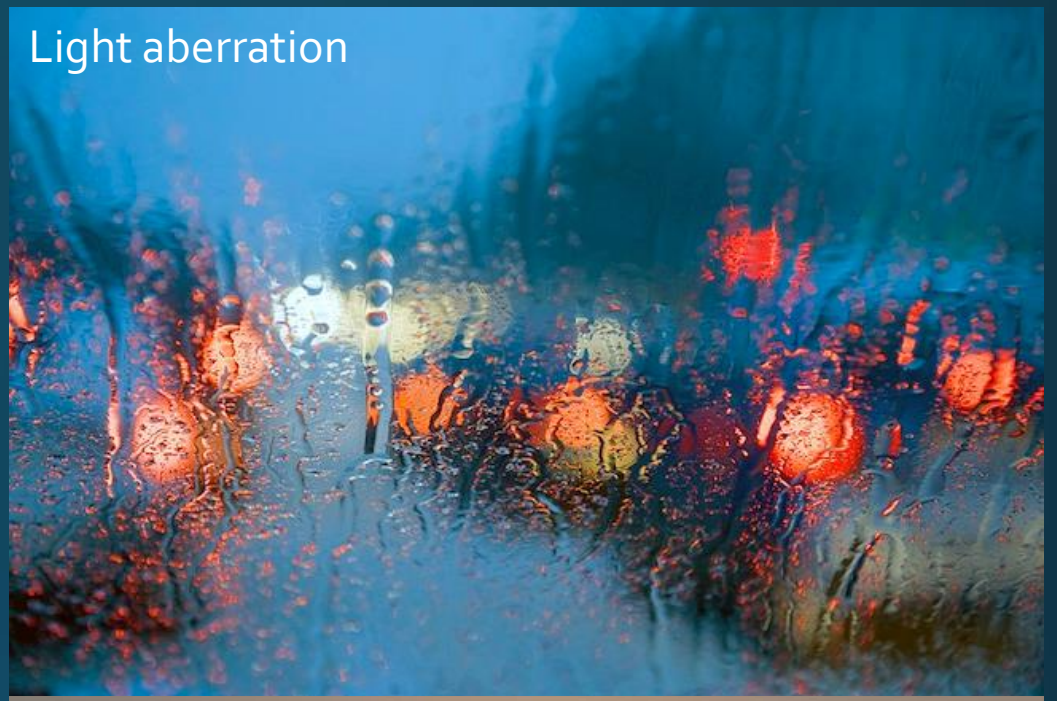
Synchronized cell division in Drosophila

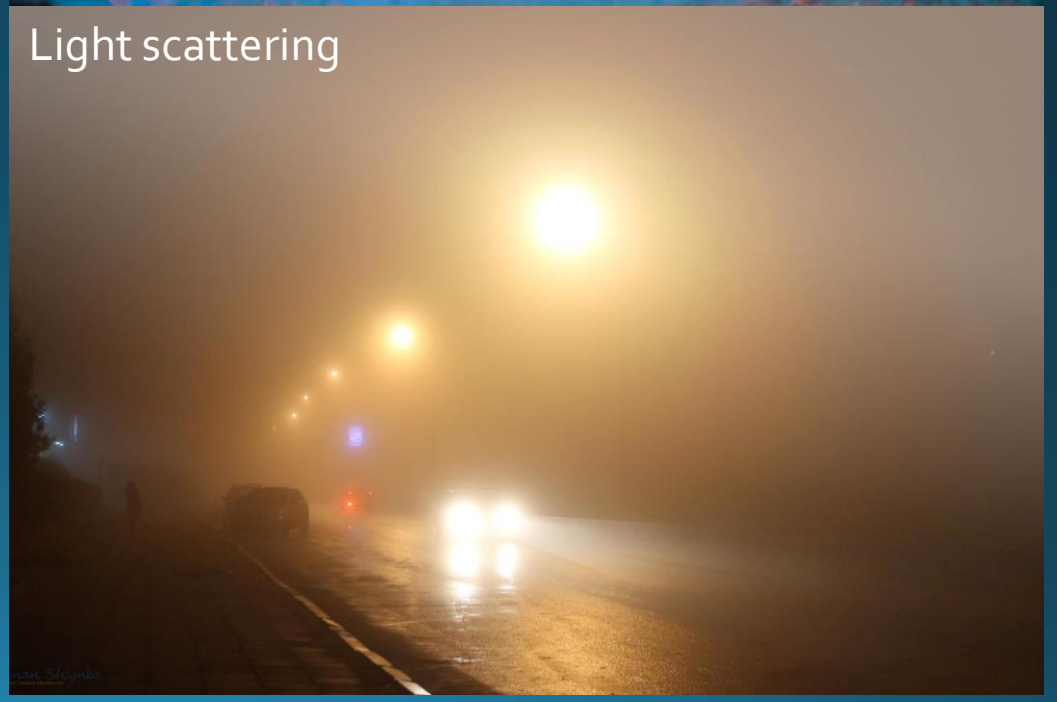
Courtesy of Dr. Edouard Bertrand
Lattice light sheet microscopy at the AIC
IGMM-CNRS,
Montpelier, France



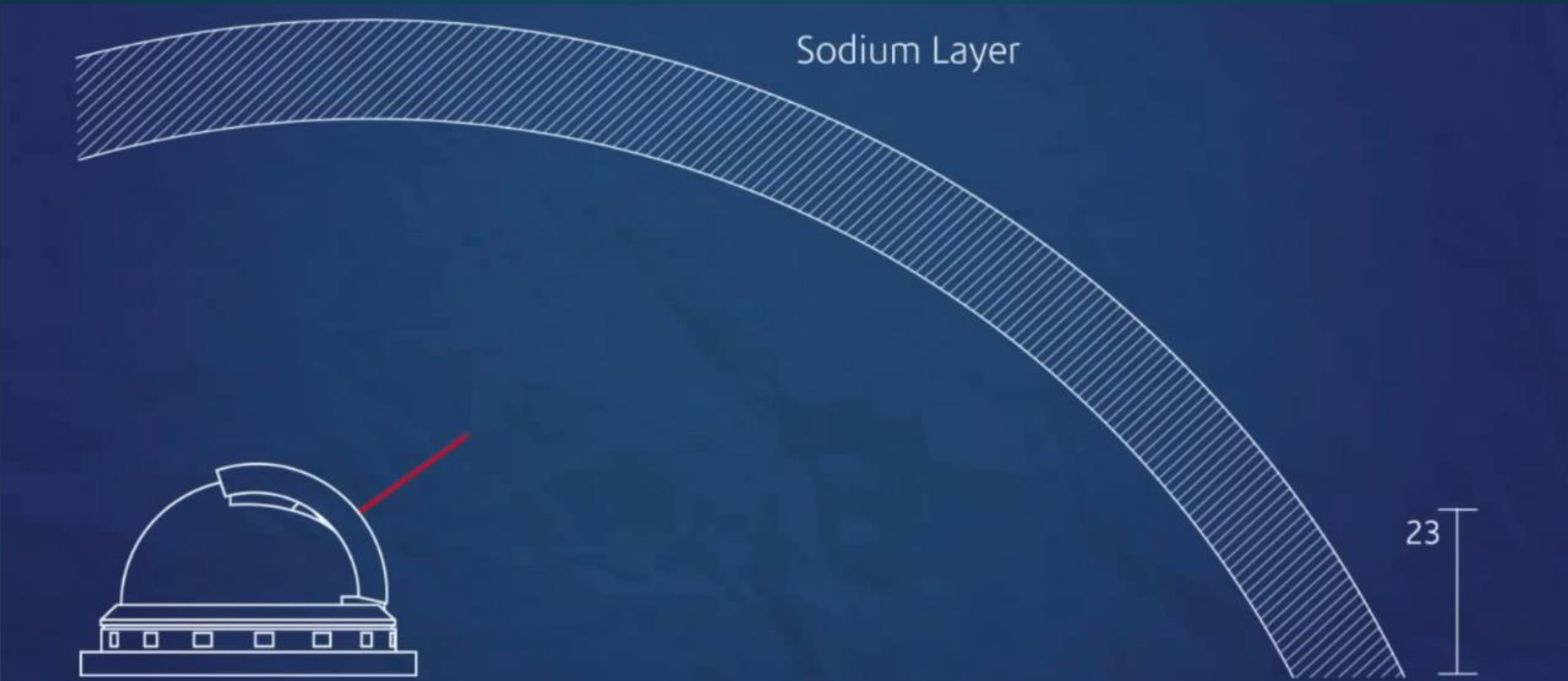
The hurdle of imaging with increasing penetration depth

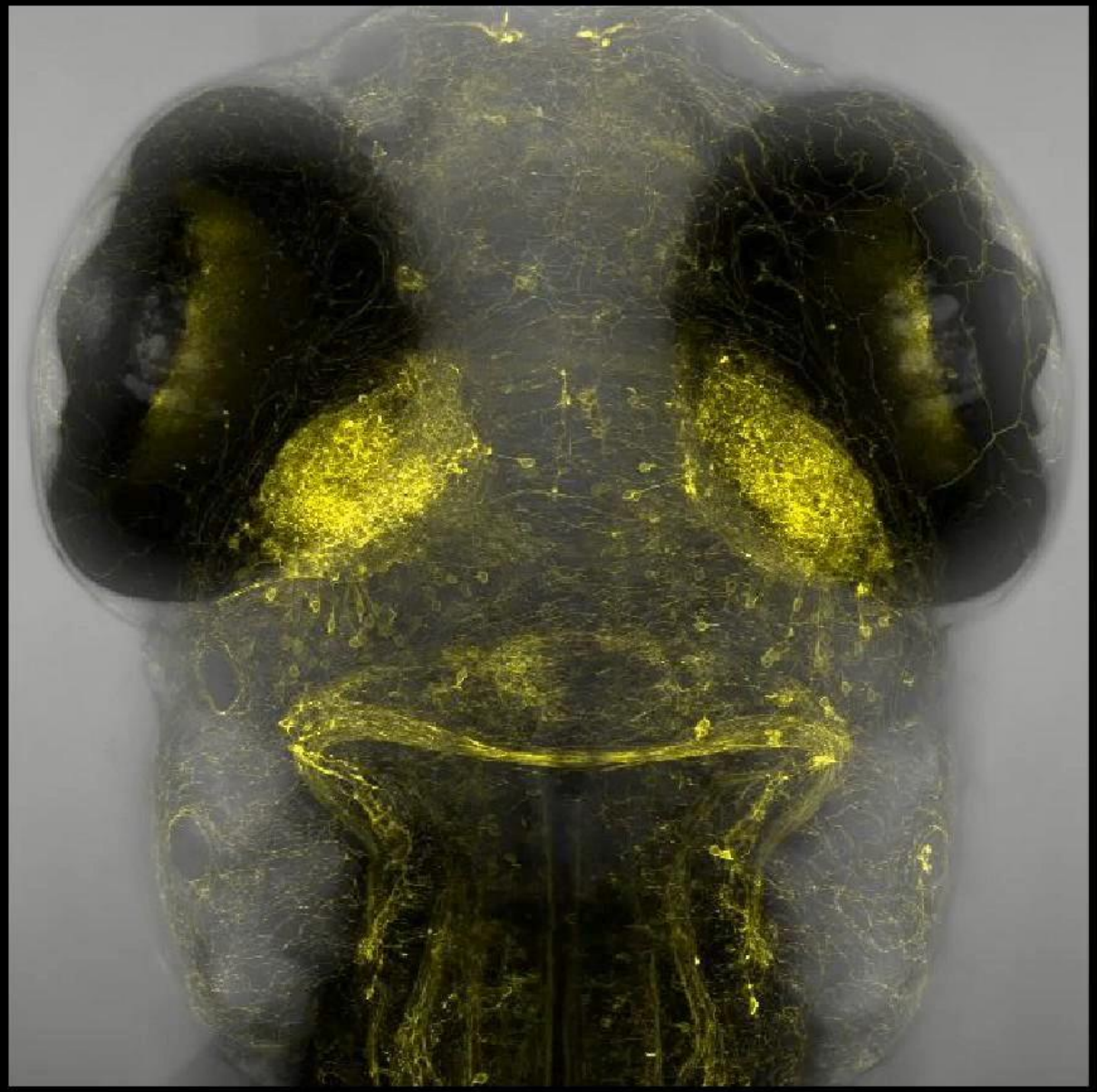


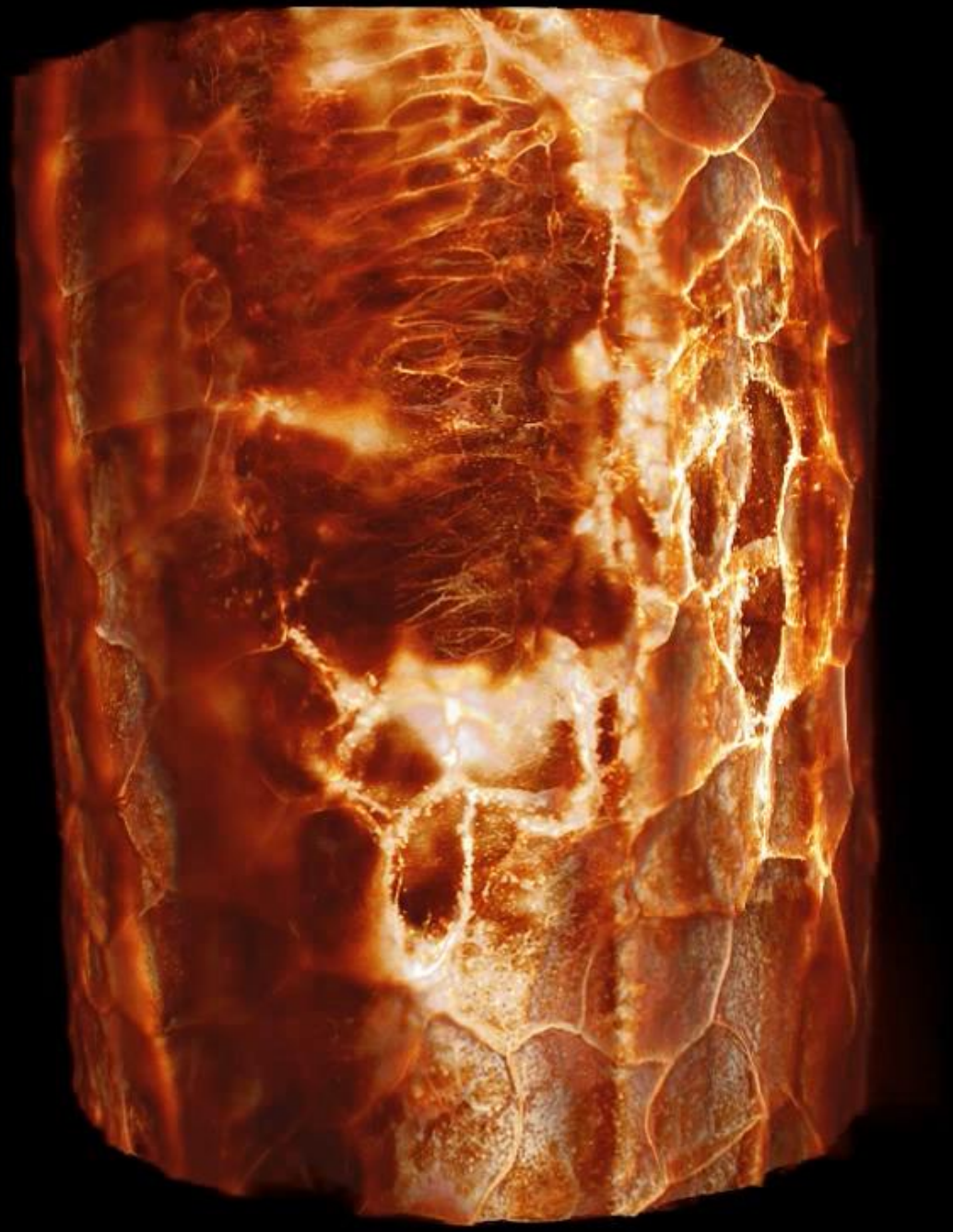




Adaptive Optics

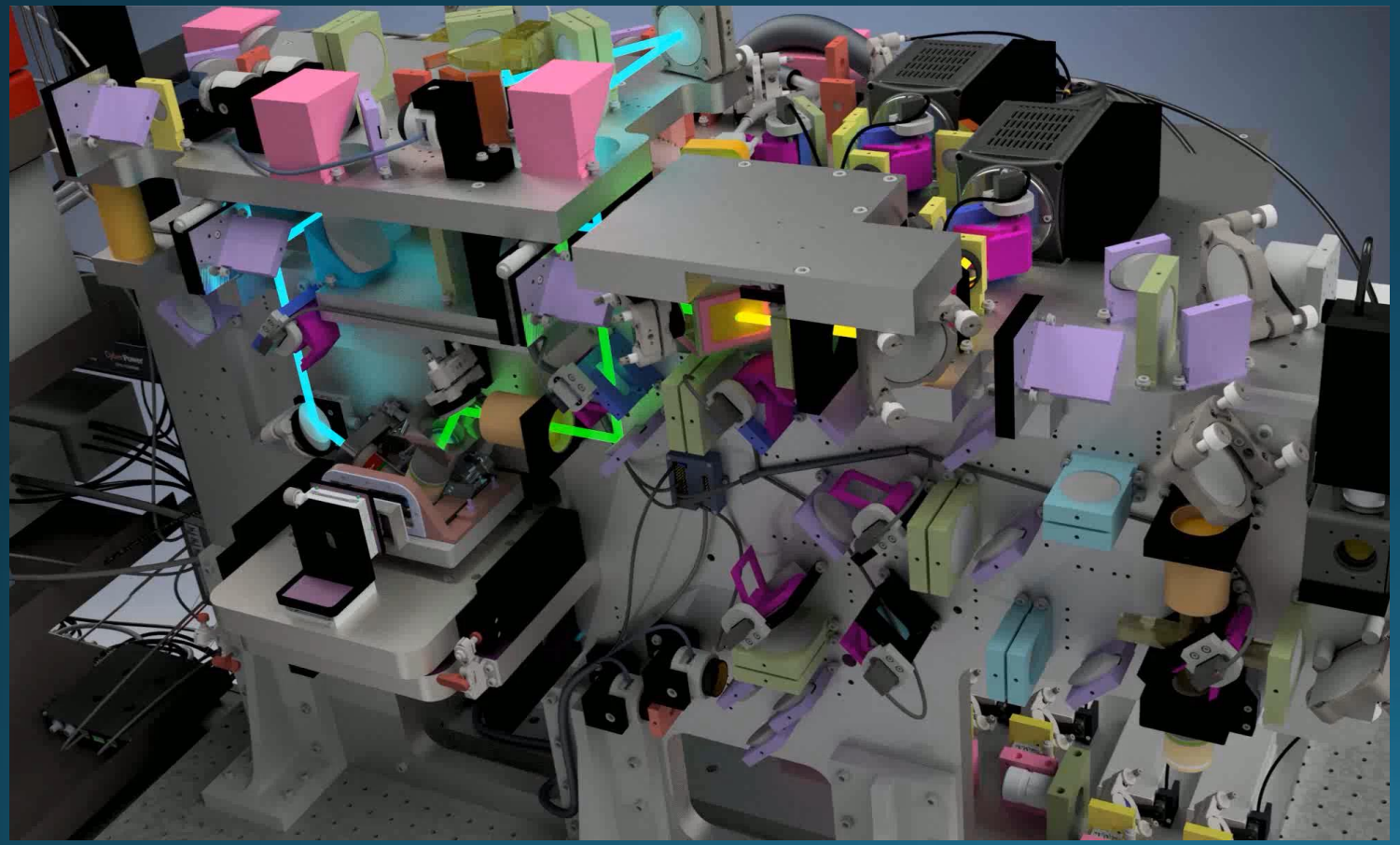






170 X 185 X 135 µm
96 hpf zebrafish embryo

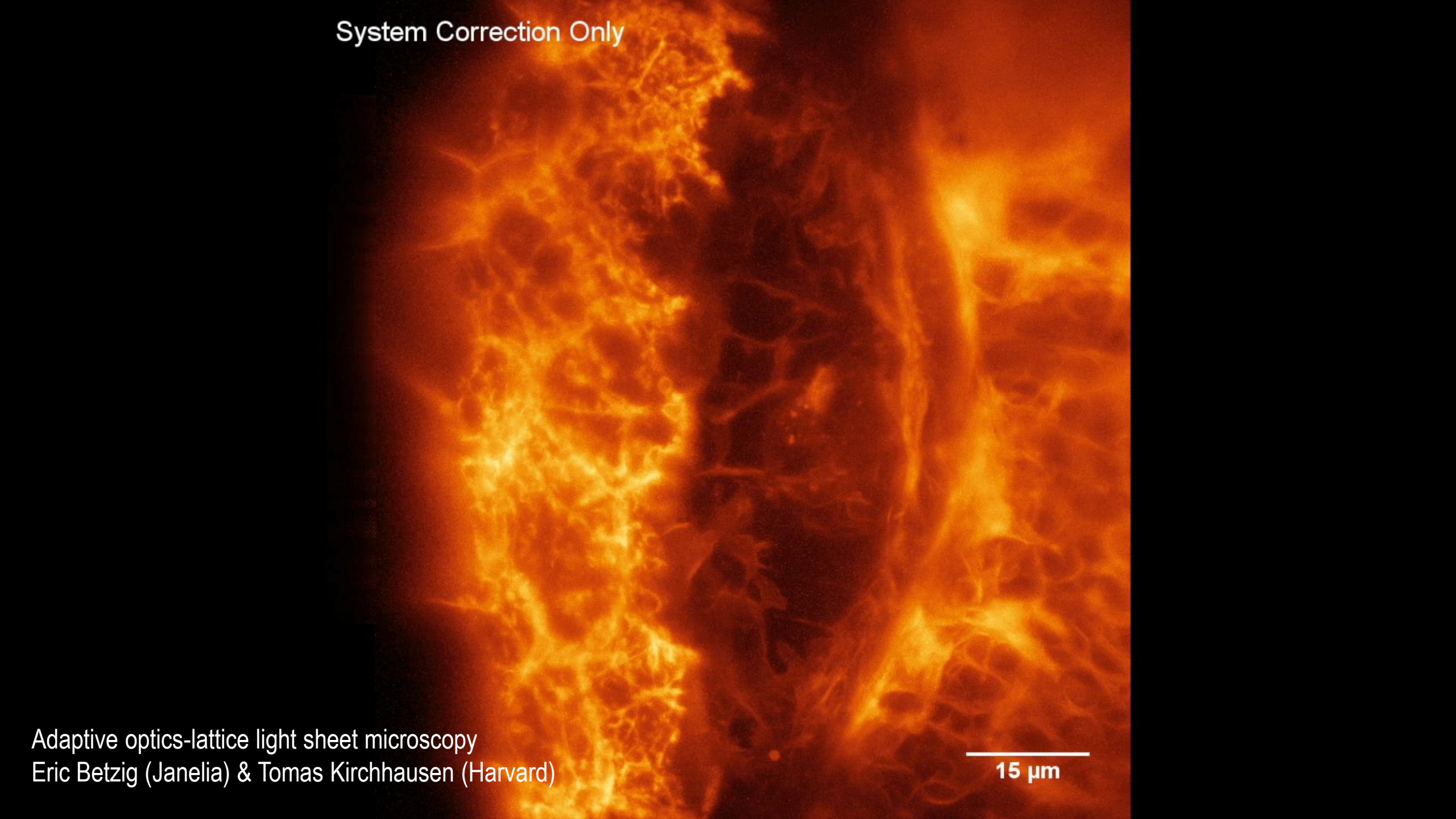
MOSAIC: Multimodal Optical System with Adaptive Imaging Correction



Operational modes:

- Gaussian or lattice light sheet
- TPE Gaussian or Bessel light sheet
- Multifocal photon reassignment
- 3D structured illumination
- 3D single molecule localization
- TPE point or Bessel resonant scan
- TPE photon reassignment
- 3D phase imaging
- Expansion lattice light sheet
- Patterned photostimulation

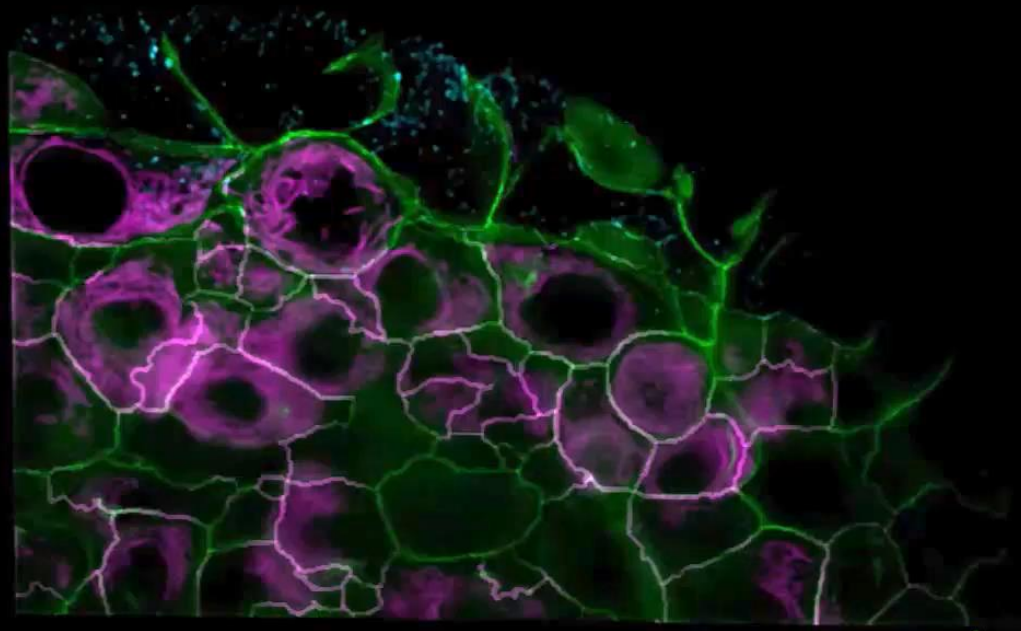
All with adaptive optics



Adaptive optics-lattice light sheet microscopy
Eric Betzig (Janelia) & Tomas Kirchhausen (Harvard)

Zebrafish 14-16 hfp

Courtesy of: Drs Eric Betzig, *Janelia Research Campus,*
and Tomas Kirchhausen & Gokul Upadhyayula, *Harvard Medical School*



membrane-Citrine
cis-golgi: gm130-mNeonGreen
ER: sec61-tRFP-t
MitoTracker: Deep Red FM Dye

10 um

Focused Ion Beam SEM at the AIC

Remove ~2-10 nm with Ga Ion Beam

Image with Scanning electron microscope

Repeat…

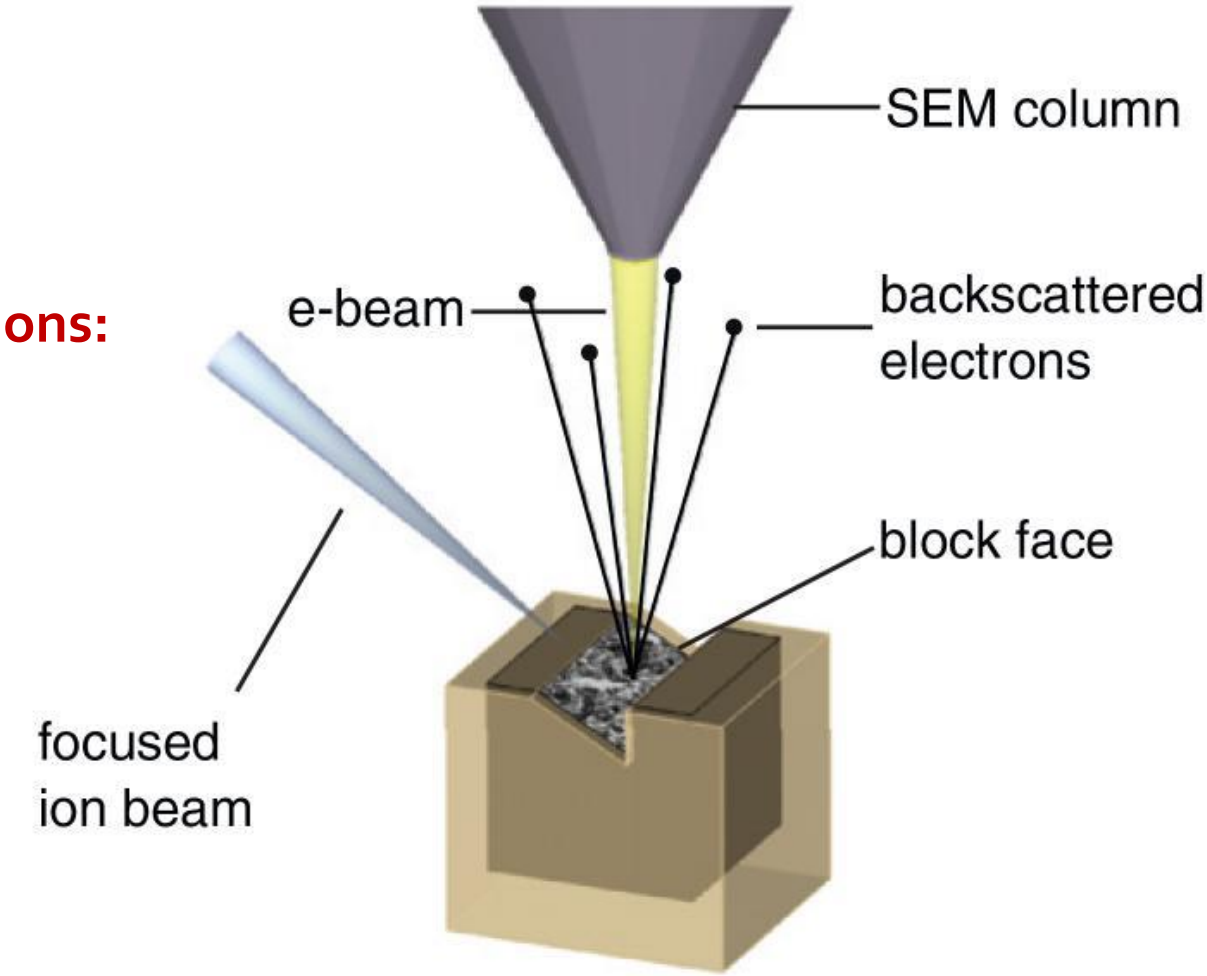
But commercial FIB-SEMs have severe limitations:

Interrupts: Destructive and Unforgiving

- FIB Source reheat ~ 3 days
- Room Temperature Fluctuations ~ weekly
- Utility failure ~ monthly
- Component Failure ~ quarterly

Restart: Transient Data Loss

- Uneven milling
- Defocus loss



Focused Ion Beam SEM at the AIC

Custom Control Acquisition Hardware/Software

- Pausing on any system issue e.g.:
  - Temperature excursion, Milling current jump, Focus shift, etc.
- Long term optimization:
  - Focus, Stigmation, Drift
- Automated Seamless Restart:
  - Predictive correction of restart transients
  - Enables nights sleep, no data degradation or loss

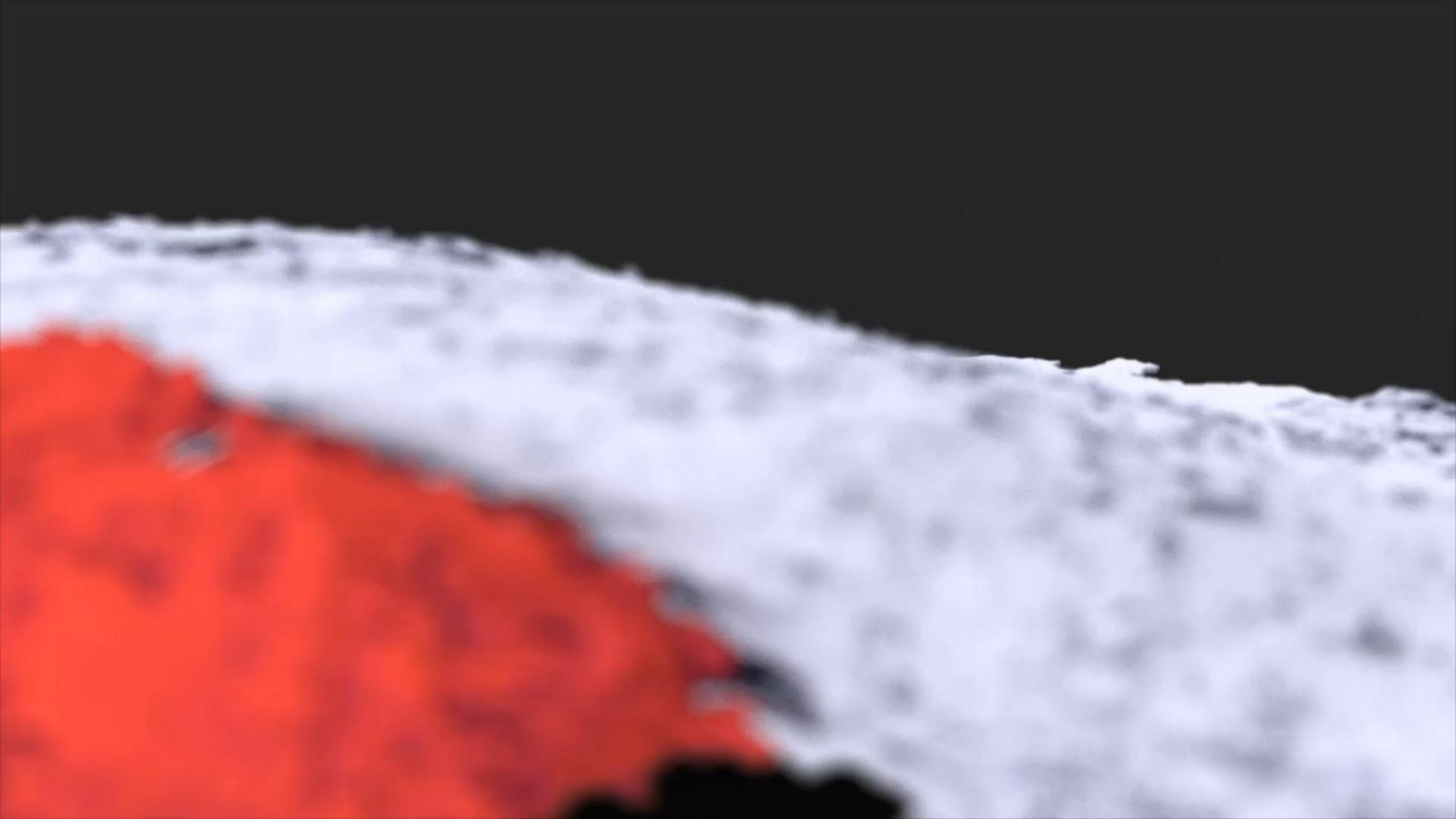
Cross integrate best technologies:

- SEM and FIB columns from different vendors

Custom Feedback on ion beam milling current

- nm position control on milling
- Automated seamless restart

Stabilized and Fault Tolerant Room Environment, Utilities

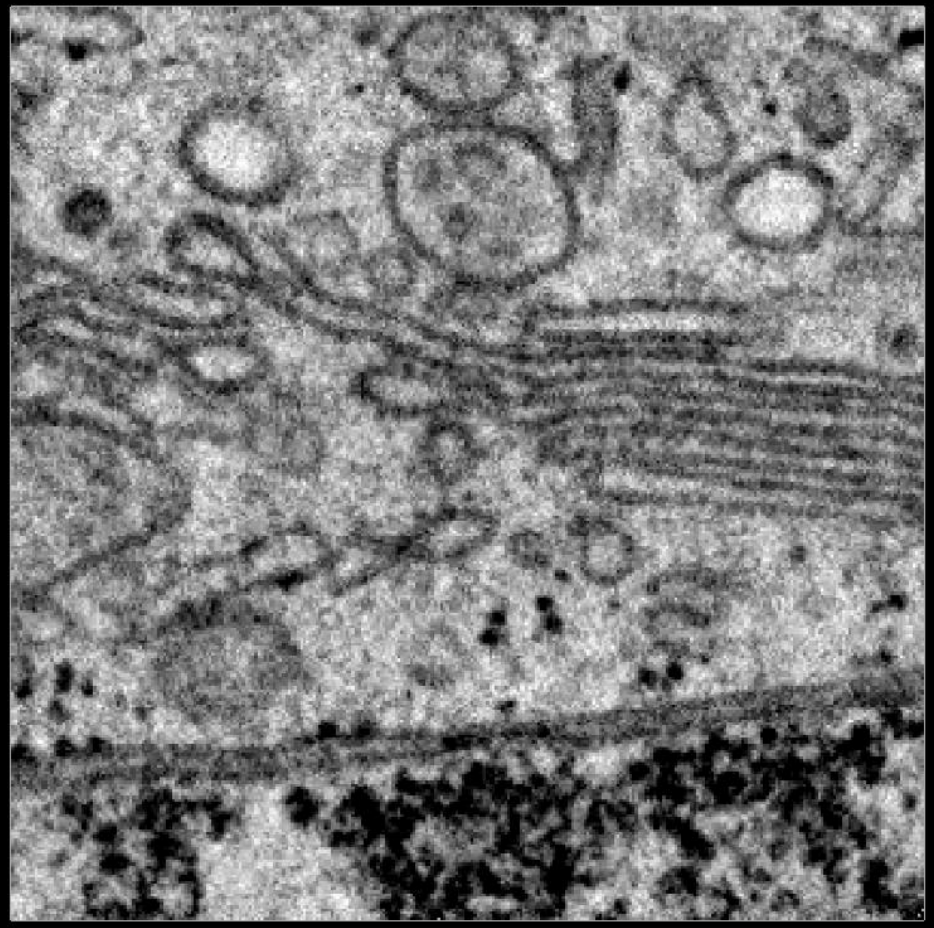


HeLa Cell (manual segmentation)

1.2 μm x 1.2 μm x 0.9 μm volume (1.27 μm^3)

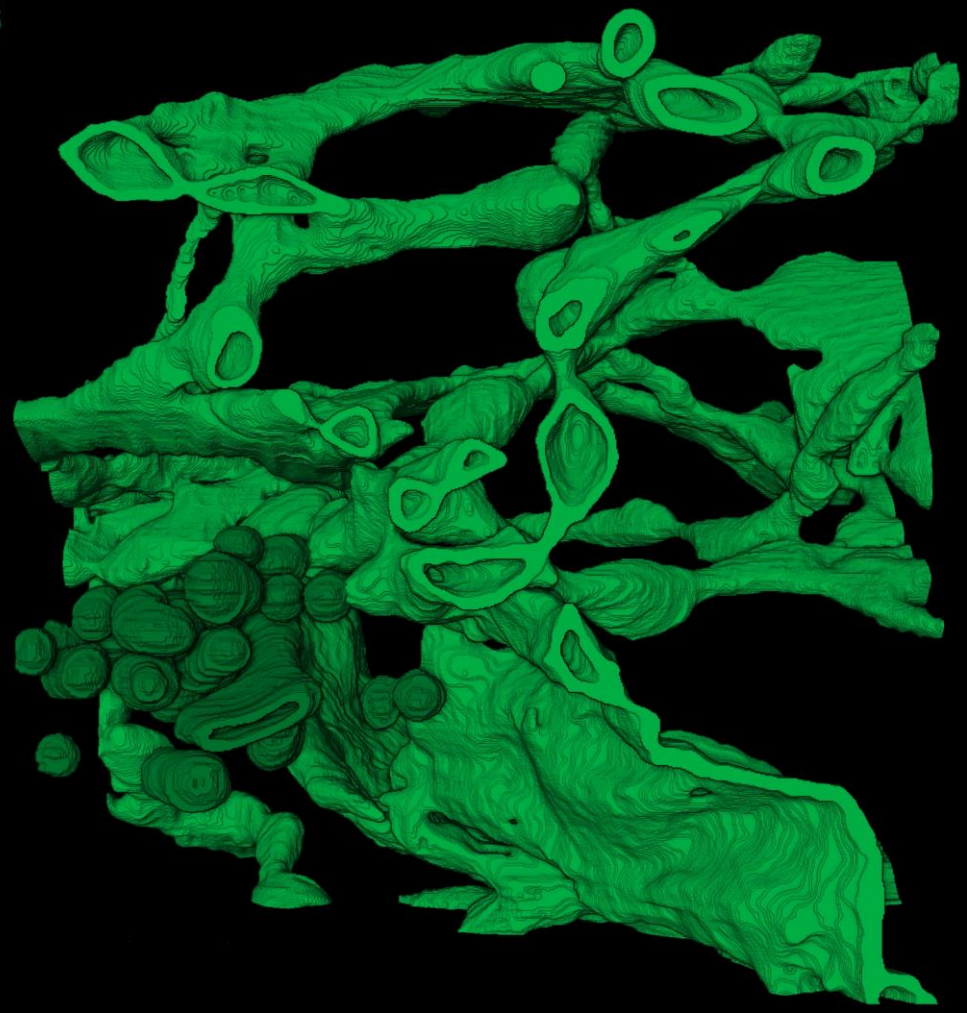
4 nm x 4 nm x 4 nm voxels

300 x 300 x 225 pixels



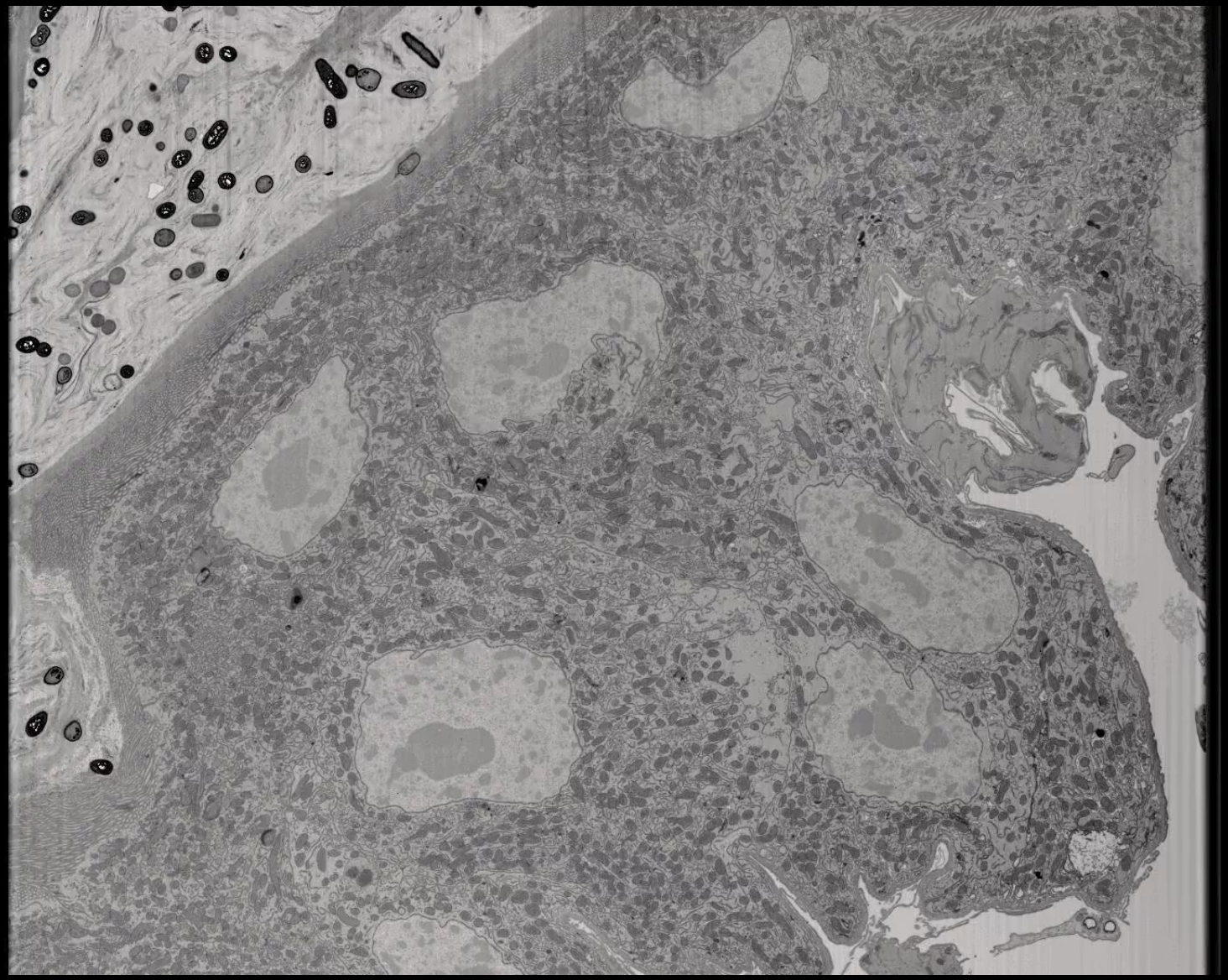
ER

ERES



Oocyst of *Plasmodium falciparum* inside the mosquito gut

Courtesy of Dr. Flaminia Catteruccia
FIB-SEM at the AIC
Harvard University




Janelia Research Campus
River Creek Club
The National Conference Center
Izaak Walton League of America...
Lansdowne
Belmont Country Club
Seneca
Darnestown
North Potomac
Rockville
Leisure World
Norbeck
Spencerville
Cloverly
Burtonsville
West Laurel
Colesville Park
Layhill
Aspen Hill
Colesville
Fairland
Layhill South
Wheaton-Glenmont
Rollins Park
Calverton
Ammendale
Beltsville
Hillandale
Kemp Mill
White Oak
Garrett Park
The Mansion At Strathmore
North Bethesda
Lowes Island
Travilah
Maryland
Virginia
Ashburn
Potomac Falls
Shady Oak
Westfield Montgomery
Potomac
Chevy Chase View
Washington DC Temple - The Church...
Broadlands
Waxpool
Sterling
Great Falls
Great Falls Park, Virginia
Huntington Terrace
Chevy Chase
Bethesda
Silver Spring
Adelphi
Greenbelt
Berwyn Heights
College Park
Langley Park
Greenbelt Park
Oak Grove
Bear Island
Cabin John
Herndon
Dulles
Dulles International Airport
Reston
Friendship Village
Friendship Heights
District of Columbia
Chillum
University Park
Riverdale Park
New Carrollton
Hyattsville
East Riverdale
Hattontown
Floris
Wolf Trap
Odricks Corner
Brookmont
Mt Rainier
Bladensburg
Landover Hills
The Barns At Wolf Trap
McLean
Northwest Washington
Smithsonian National Zoological Park
Columbia Heights
Cheverly
Greater Landover
Steven F. Udvar-Hazy Center
South Riding
Oak Hill
Tysons
Pimmit Hills
Woodland Acres
Washington
Northeast Washington
FedExField
Lees Corner
Vienna
NoMa
Conklin
Chantilly
Dunn Loring
Cherrydale
The White House
Pepper Mill Village
Capitol Hill
Capitol Heights
Schneider Crossroads
Oakton
Falls Church
Virginia Square
Arlington
Navy Yard
Walker Mill
Merrifield
The Pentagon
Southwest Washington
Southeast Washington
Coral Hills
Ellanor C. Lawrence Park
West Falls Church
Seven Corners
Pentagon City
Aurora Highlands
Barry Farm
District Heights
Cub Run Stream Valley Park
Mantua
Strathmeade Springs
Bailey's Crossroads
Lake Barcroft
Joint Base Anacostia-Bolling
Suitland-Silver Hill
Forestville
Legato
Fairfax
Centreville
Annandale
Alexandria West
Potomac Yard
Del Ray
Hillcrest Heights
Morningside
Marlow
EagleBank Arena

Visitor Program at AIC

Cost of experiment covered by AIC

Lodging cost will be 100% covered by HHMI Janelia

Advanced Imaging Center Total Programmatic Support

How the AIC maintains ~85% experimental success rate

Technical consultation
AIC

IACUC/IBC review
IBC, Vivarium, EH&S, Cell & Mol. Biol. Facilities

Travel arrangement/visa application
Lab Coordinator, Visitor Program

Sample shipment and receiving
EH&S, Cell Culture Facility

Lodging
Visitor Program, Lab Coordinator

Vivarium/model organism handling
Vivarium, Drosophila Resources

Pre-arrival sample preparation
Cell Culture, Histology Facilities

cDNAs with FPs/model organisms
Molecular Biology Facility/Various labs

Imaging probes
Luke Lavis and Loren Looger's Labs

Microscope time
AIC

Imaging assistance
AIC

Image processing and analysis
AIC

Call-for-Proposals

Announced ~ twice a year

Two-tier Review Process

First Tier: Technical assessment by the AIC team

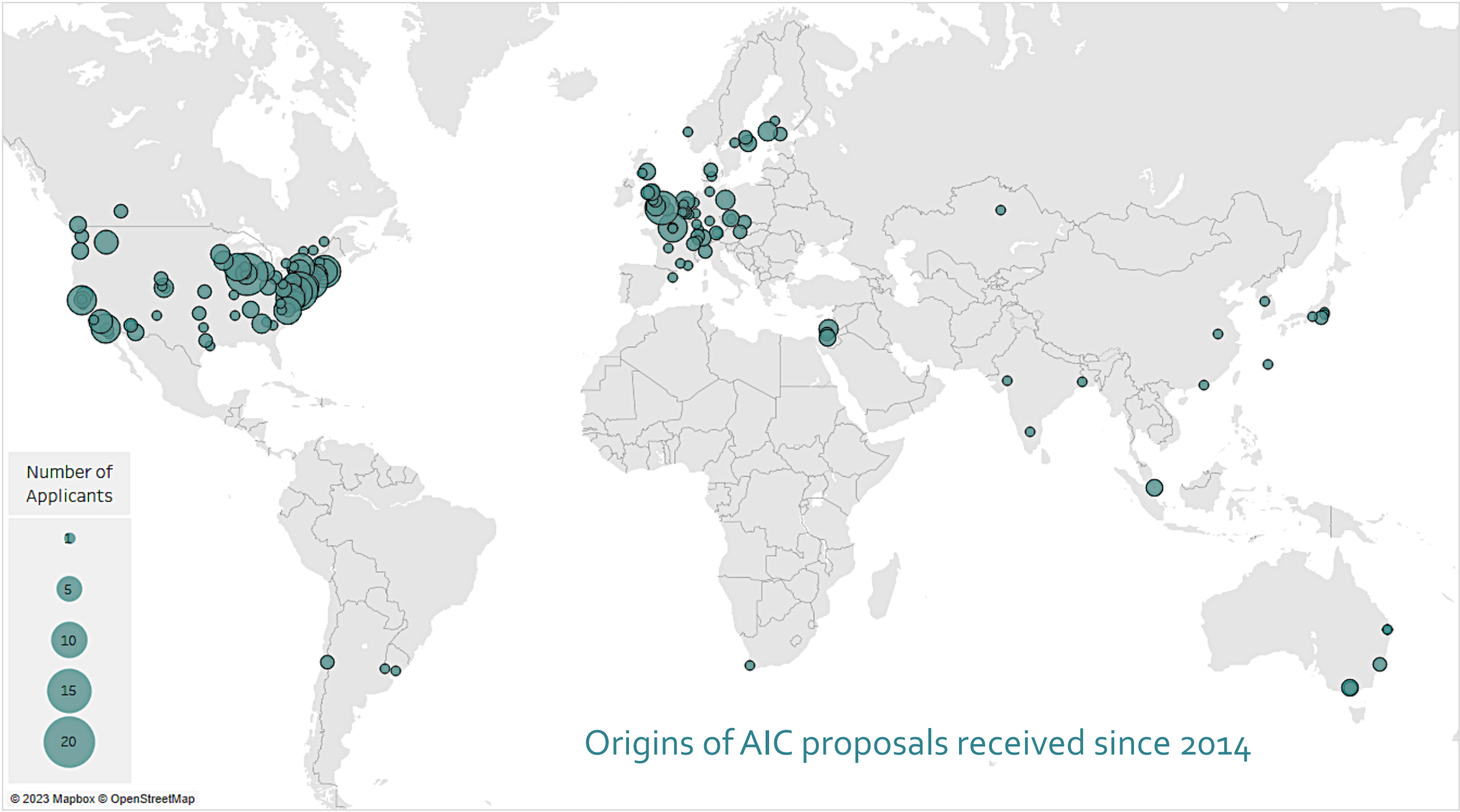
Pass/Fail grade will be assigned

- Can the question be addressed by commercial instruments?
- Can the AIC instrument(s) deliver the expected results?
- Can the specimen be safely handled at Janelia?

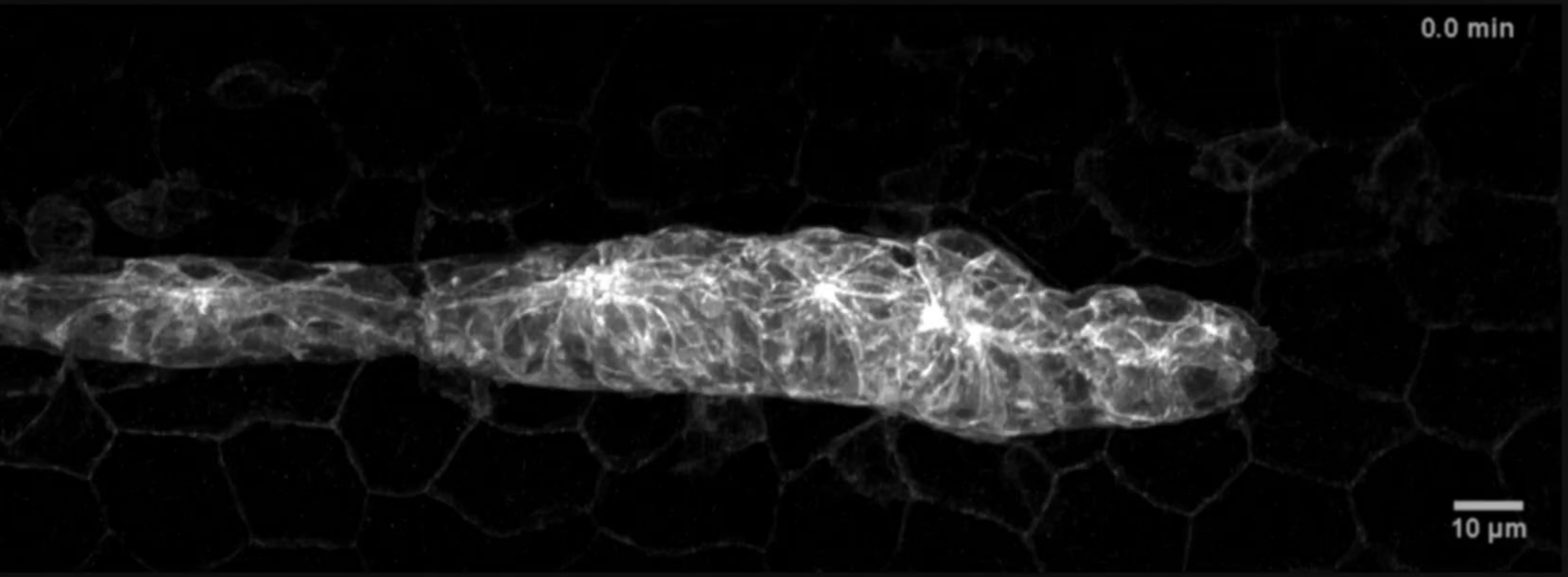
Second Tier: Peer Review by HHMI and external reviewers and Moore Foundation

Tiered priority score will be assigned

- How well the scientific rationale supports the project
- How the experimental question justifies the use of the AIC instruments
- Discussion of potential problems and alternative approaches
- Quantitative approach
- Amount of time required in the AIC to meet the project goals
- Personnel



Origins of AIC proposals received since 2014

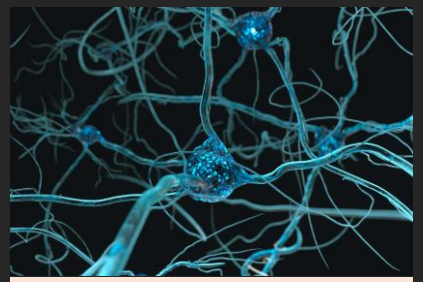

0.0 min
10 µm

Africa Microscopy Initiative

AMI Imaging Center

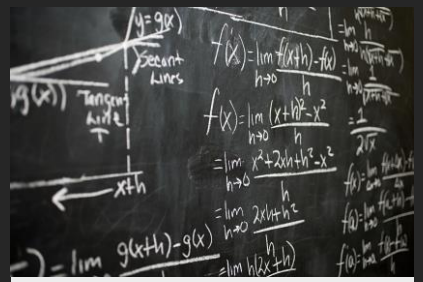
- All expense-paid (including costs of imaging, travels and lodging) imaging center for African scientists working in Africa
- Located at the University of Cape Town, accessible through peer-reviewed proposals
- Zeiss' basic to advanced microscopy systems, as well as TissueGnostics slide scanner
- Fully supported by technical staff; local research infrastructure available to visitors

Project Incubator



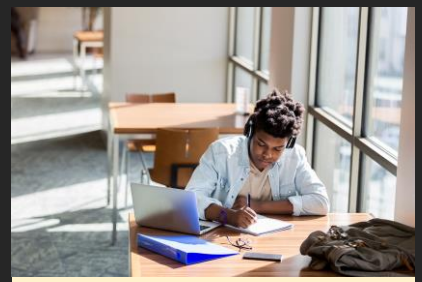
- The AMI imaging center also serves as project incubator
- Allow potentially high impact projects time and resource to develop
- Help pave the way for the continuation of such projects at the imaging centers at Janelia and EMBL, e.g.

Imaging Africa



- ***Imaging Africa***: an all expense-paid, continent-wide microscopy workshop
- Repeatedly over-subscribed by 25-30-fold capacity
- Will rotate the workshop venue through Africa
- Planning regional satellite workshops with focused scientific themes

Webinars



- ***Microscopy Matters*** webinar series
- Short and focused microscopy-related topics
- Presented mainly by African imaging scientists for the African communities

PiTCH

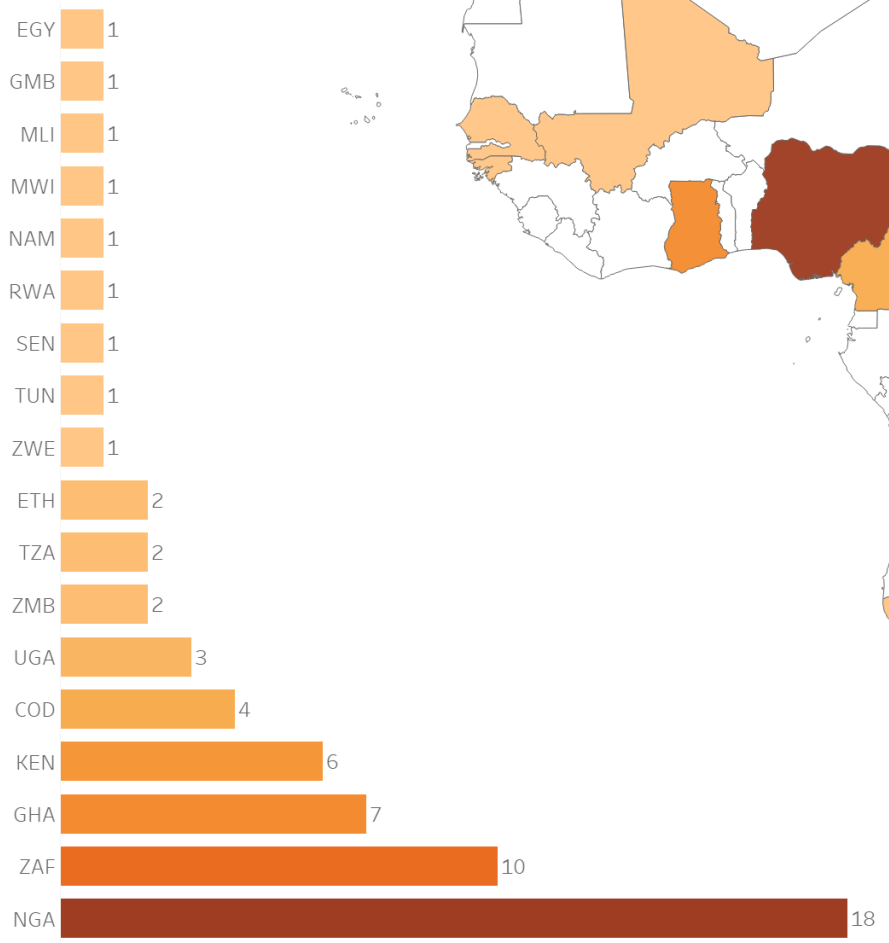
- ***Partners in Teaching*** (PiTCH)
- Fellowship program opened to imaging scientists worldwide. Successful African candidates will be paired with their international counterparts
- Each pair will be coached by a mentor on how to organize and teach imaging workshop
- The pair will offer all-expenses-paid workshops at the home institution of the African partner

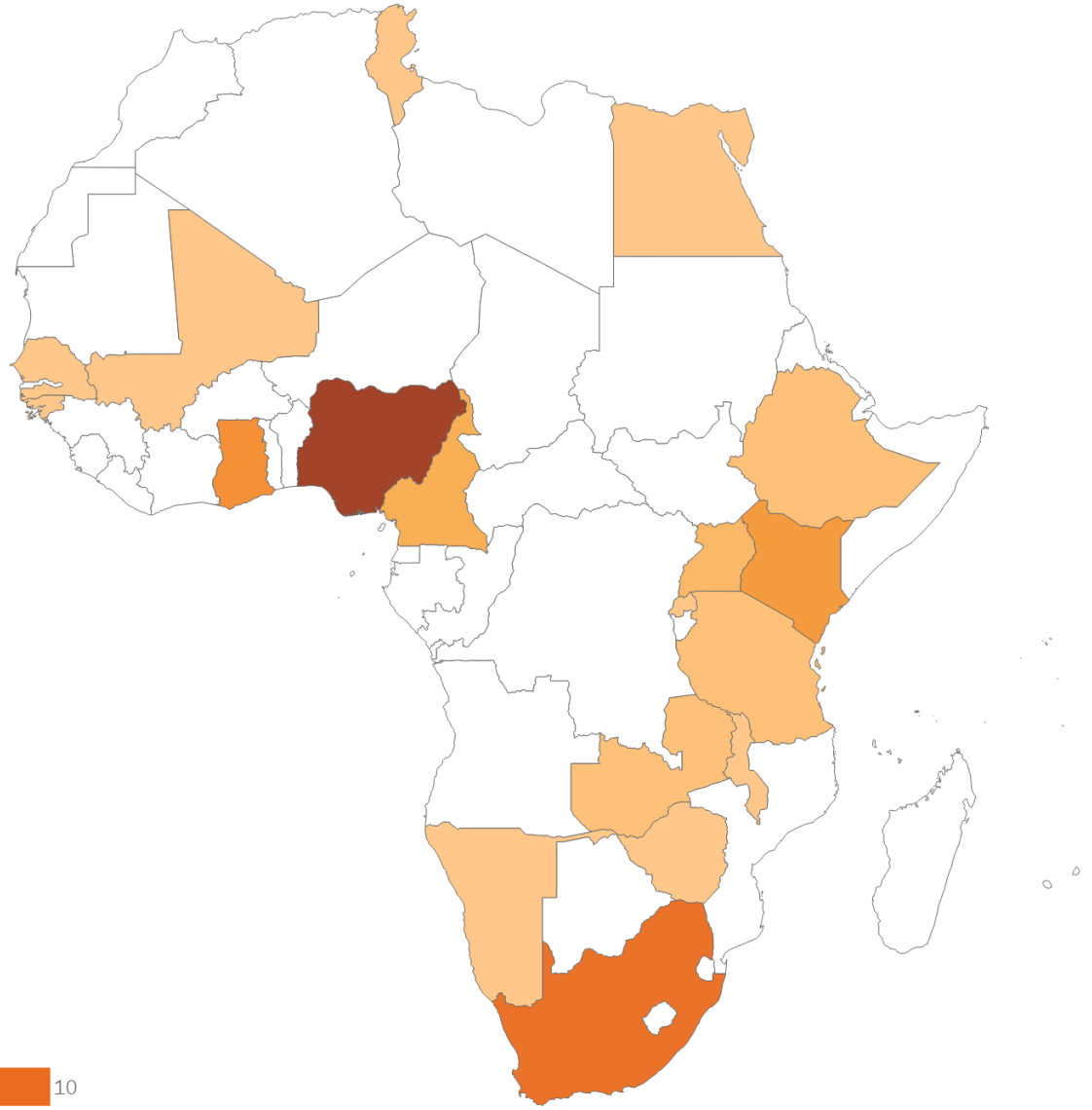
PEER

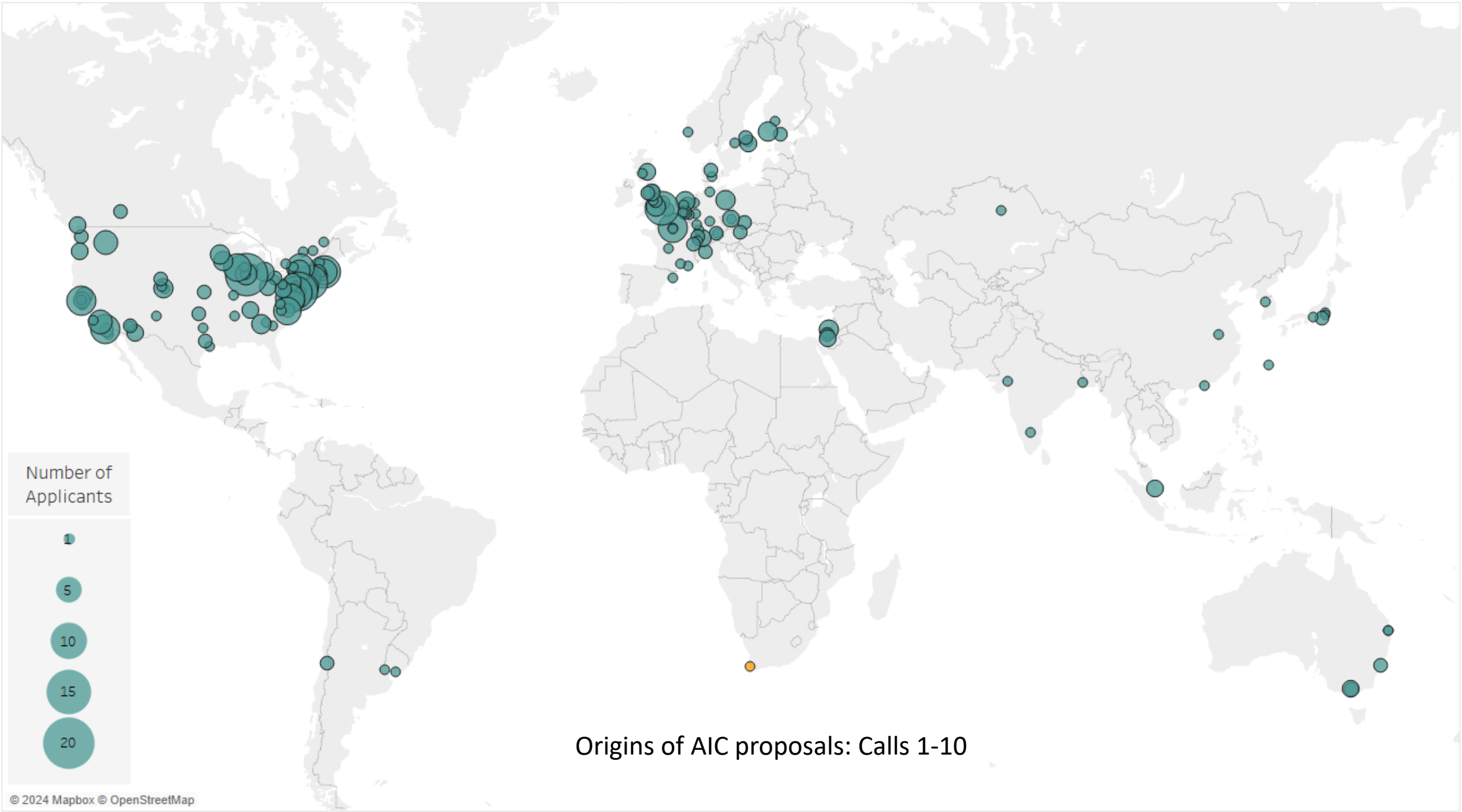
- ***Program for Equipment Exchange and Reutilization*** (PEER)
- Working with industry partners to bring pre-owned, traded-in instruments to Africa, through peer-review application process
- Recipient institution must commit two full-time employees to receive full training
- Instruments must be set up as a core facility at the recipient institution

Response to the first proposal call from the AMI Imaging Centre

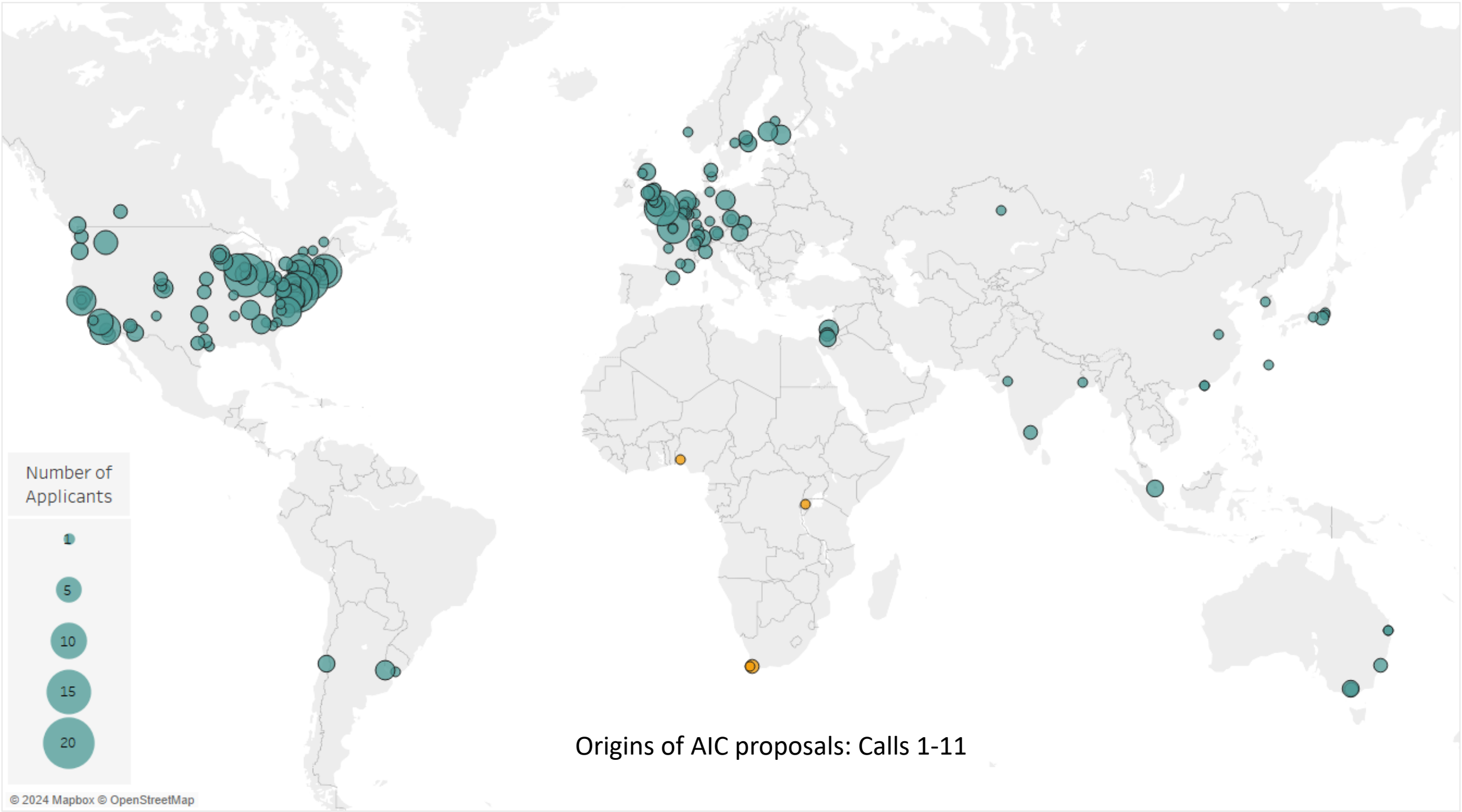
63 proposals from 18 countries



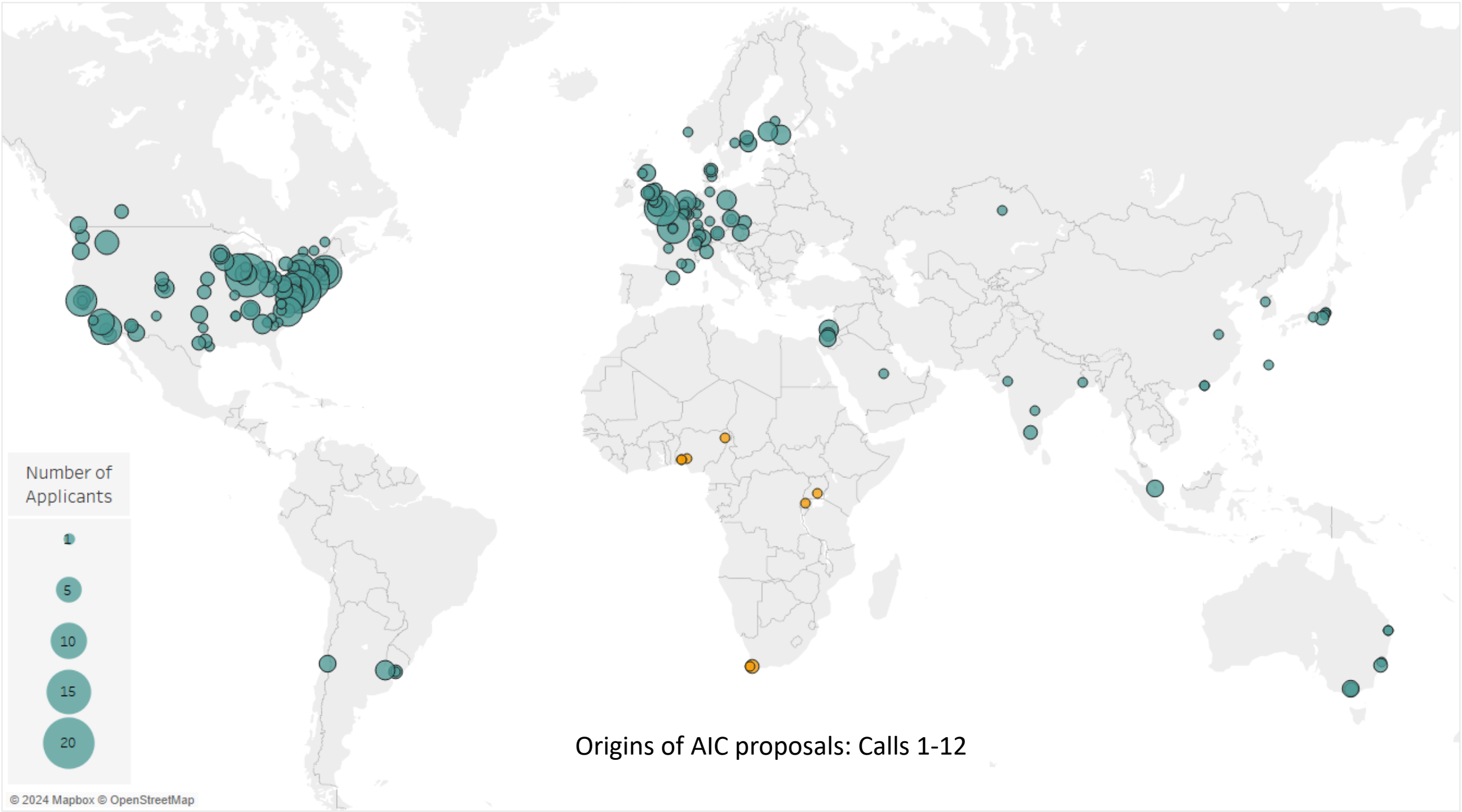




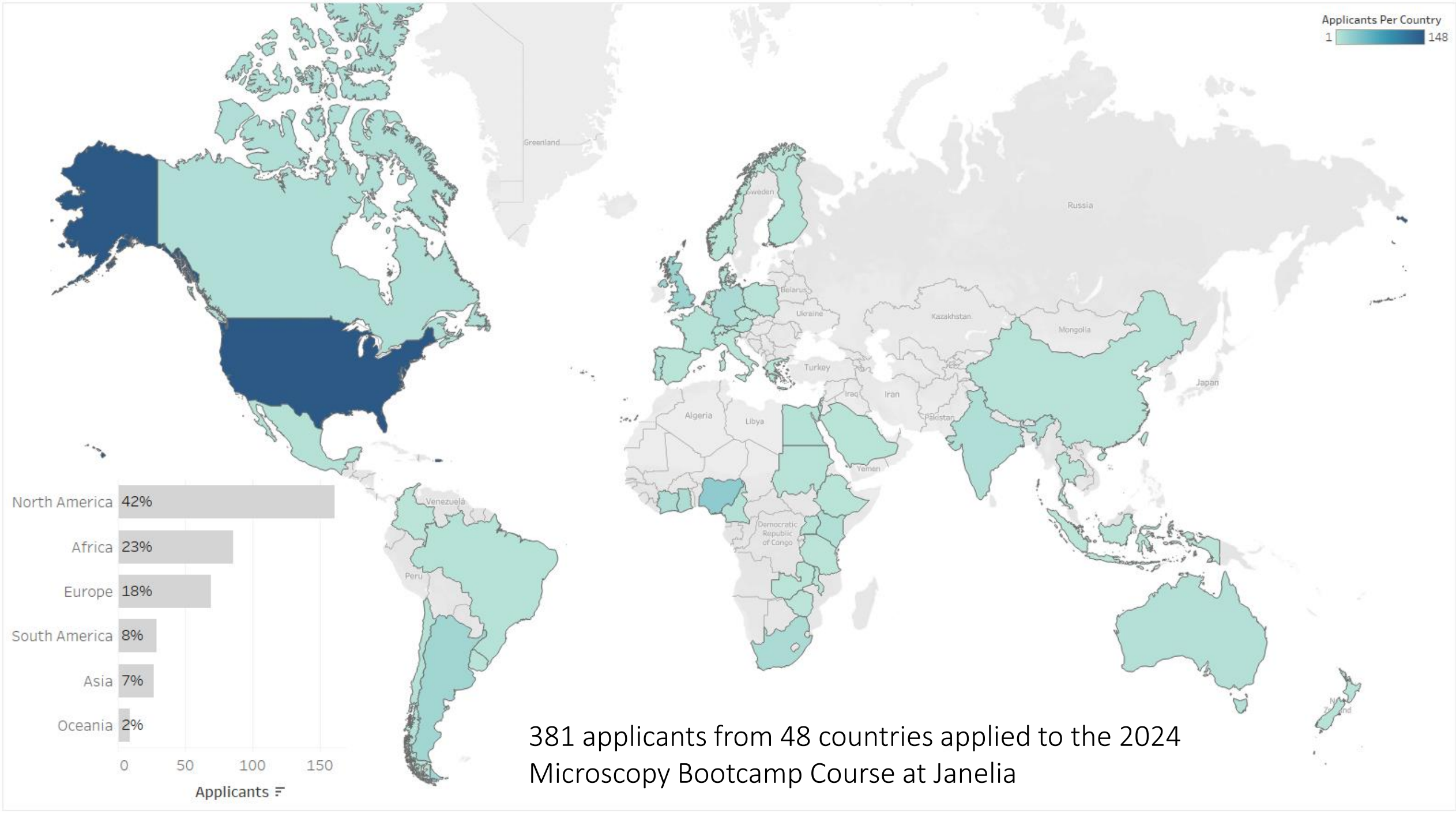
Origins of AIC proposals: Calls 1-10



Origins of AIC proposals: Calls 1-11

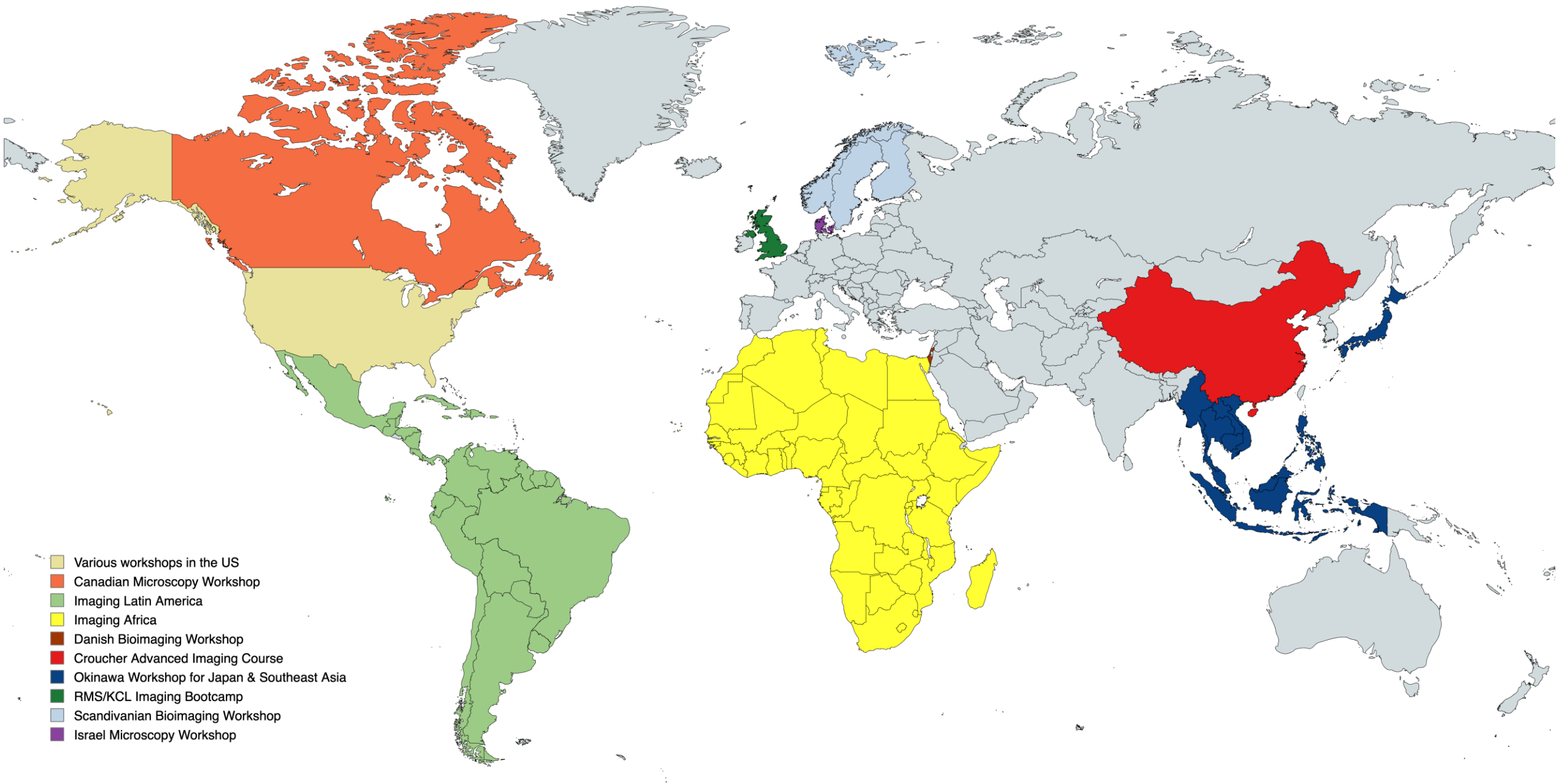


Origins of AIC proposals: Calls 1-12



381 applicants from 48 countries applied to the 2024 Microscopy Bootcamp Course at Janelia

AIC global microscopy outreach programs



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