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UNIVERSITY
INTEGRATED CELLULAR
IMAGING CORE

INTRODUCTION TO IMMUNOFLUORESCENCE

Emory ICI µConference
August 26, 2026

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Immuno-fluorescence

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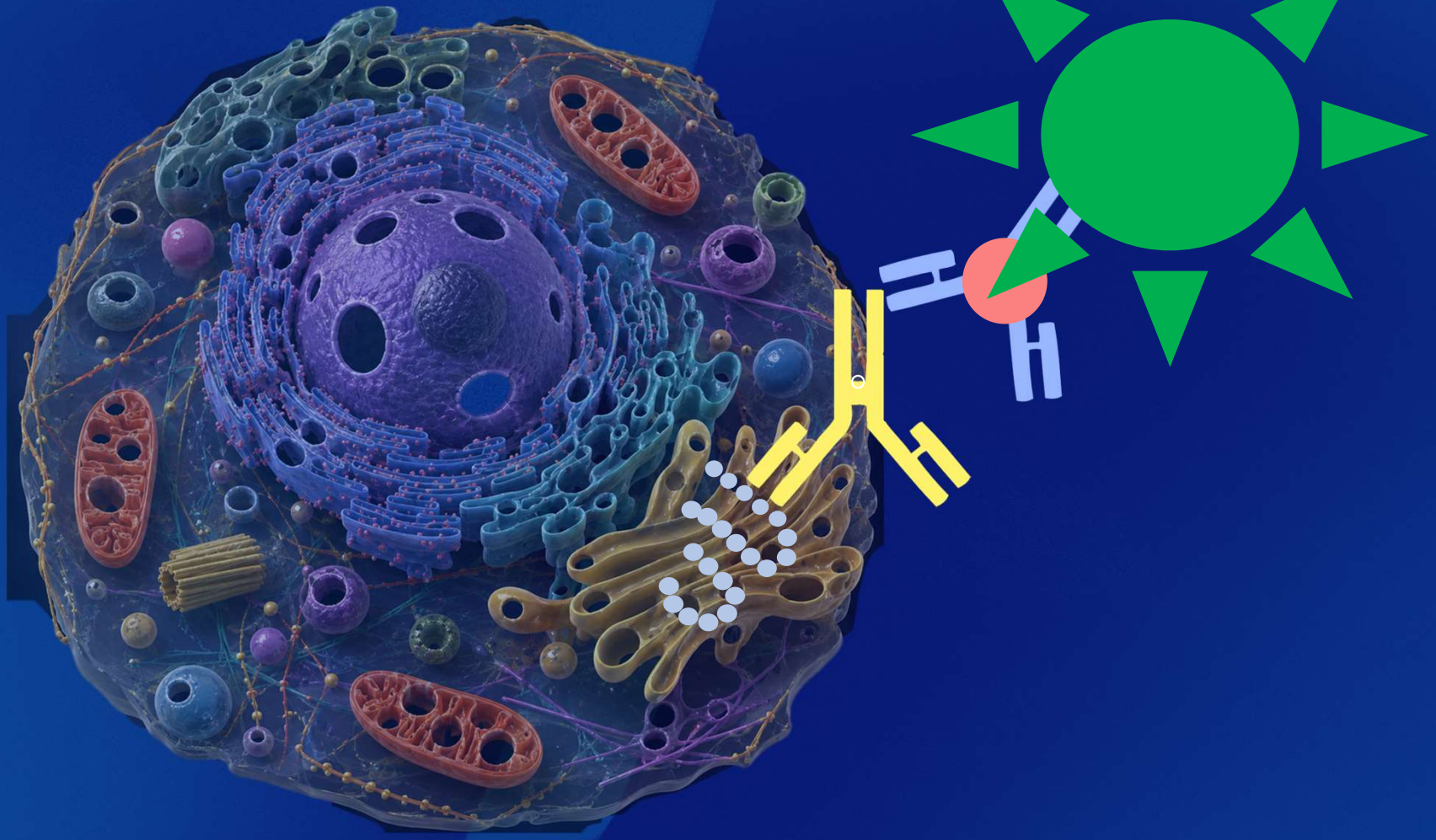

Antibodies

Fixation and Permeabilization

FFPE (Parrafin) vs OCT

Mounting specimens

Fluorophore Wavelengths

Antibodies for whole tissue imaging

Antibodies for STED

Why Your Flow Panel Usually Fails on the Microscope

Live Cell Dyes

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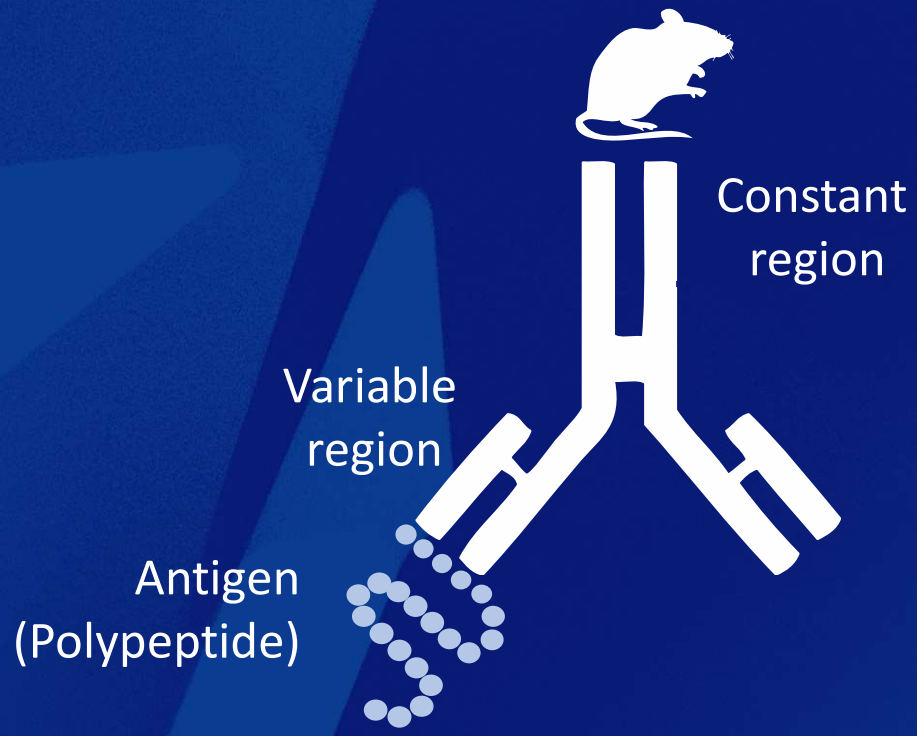
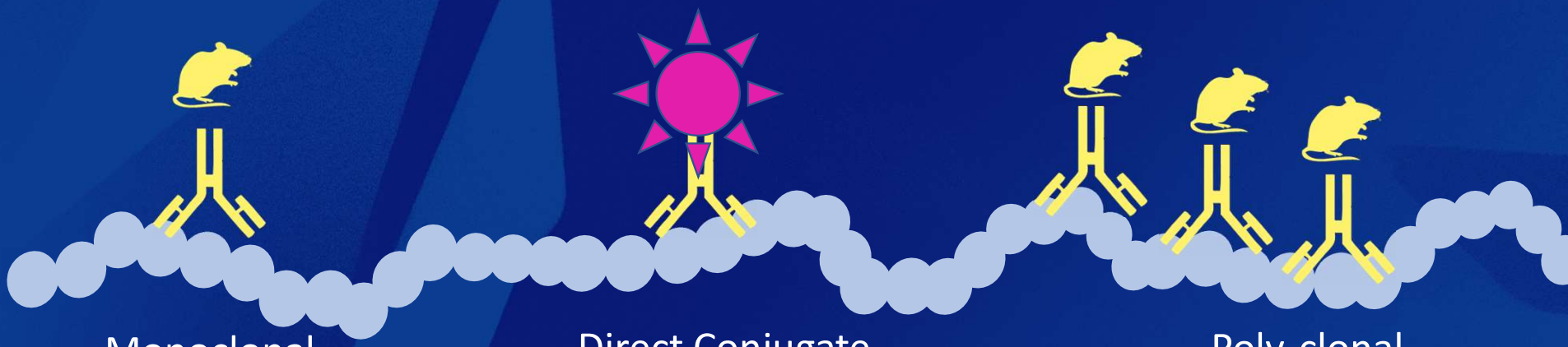
What is an antibody?



Monoclonal vs. Polyclonal vs Direct Conjugate

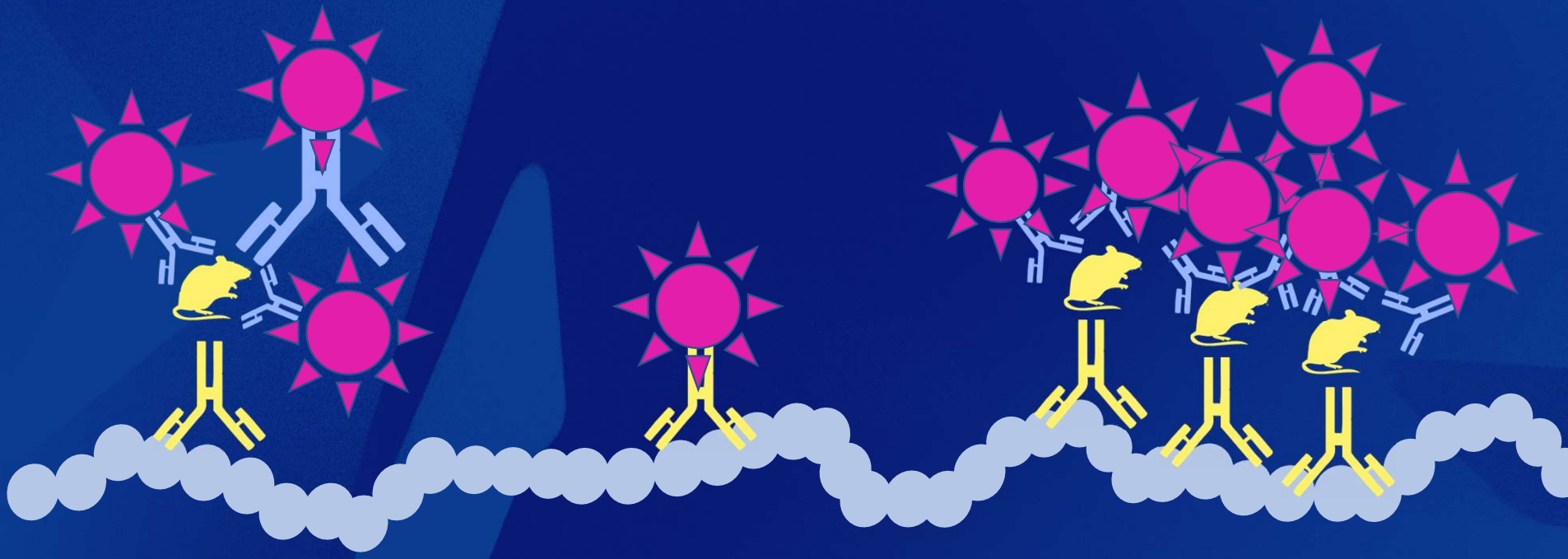


Monoclonal
1 single epitope

Direct Conjugate
1 single epitope

Poly-clonal
Multiple Epitopes

Secondary antibodies amplify the signal



When do you use a secondary? Vs Direct Conjugate?

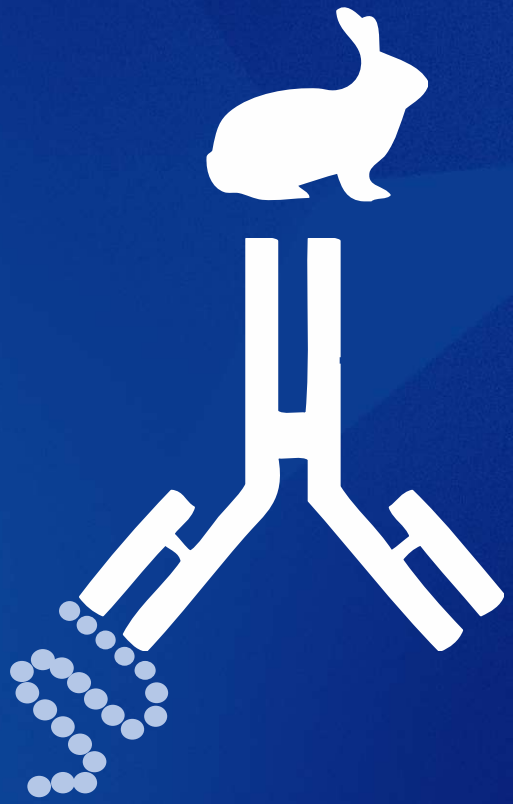
Flow Cytometry- Direct Conjugate
Huge Cleared Tissue-Direct Conjugate
>5 Channals-Direct Conjugate

Everything else use a secondary. It amplifies the signal and is standard.

Antibody spec sheet

Rabbit conserved region

APPROVED FOR IF



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Product Information

Anti-Actin
produced in rabbit, affinity isolated antibody

Catalog Number **A2066**

Product Description
Anti-Actin is produced in rabbit using C-terminal actin fragment (C11 peptide attached to Multiple Antigen Peptide (MAP) backbone) as immunogen. The sequence is Ser-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-Cys-Phe. Affinity isolated antibody is obtained from rabbit anti-actin antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to actin.

Anti-Actin specifically stains typical stress fibers in cultured chicken fibroblasts using indirect immunofluorescent labeling and specifically localizes actin by immunoperoxidase labeling of formalin-fixed, paraffin-embedded human or animal tissue sections following enzymatic unmasking. In immunoblotting, the product localizes actin in many species ranging from human skeletal muscle to amoeba. The product recognizes the 42 kDa actin band using immunoblotting with human or animal tissue extracts.

The antibody is useful for studying actin structure and function, and to probe binding sites of actin-binding proteins.

Actin, a highly conserved protein, is a major component of both the cytoskeletal and contractile structures in all cell types. It varies in amount, being related to the type of differentiation and to the functional state of cells and tissues. Actin can be found in two different forms of aggregation, the globular or the fibrillar state, and at least six distinct isoforms occur in vertebrates. The actins exhibit over 90% sequence homology, but each isoform has a unique NH_2-terminal sequence. The isoforms are comprised of three α actins (skeletal, cardiac, smooth), one β actin (β-non-muscle) and two γ actins (γ smooth muscle and γ non-muscle). Difficulties have been encountered in producing polyclonal antibodies due to the highly conserved nature of actin. Because the amino acid sequence of the C-terminal region is the same for almost all actins, this antibody has been raised using a synthetic peptide corresponding to the C-terminal 11 residues.

Epitope

Reagents
Supplied in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as preservative.

Protein Concentration: 0.4-0.8 mg/ml by absorbance using $E^{1\%}_{280}$ = 14.0 (prior to the addition of BSA).

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile
Indirect immunofluorescence: a working dilution of at least 1:40 was determined using Chicken fibroblasts.

IF concentration 1:40

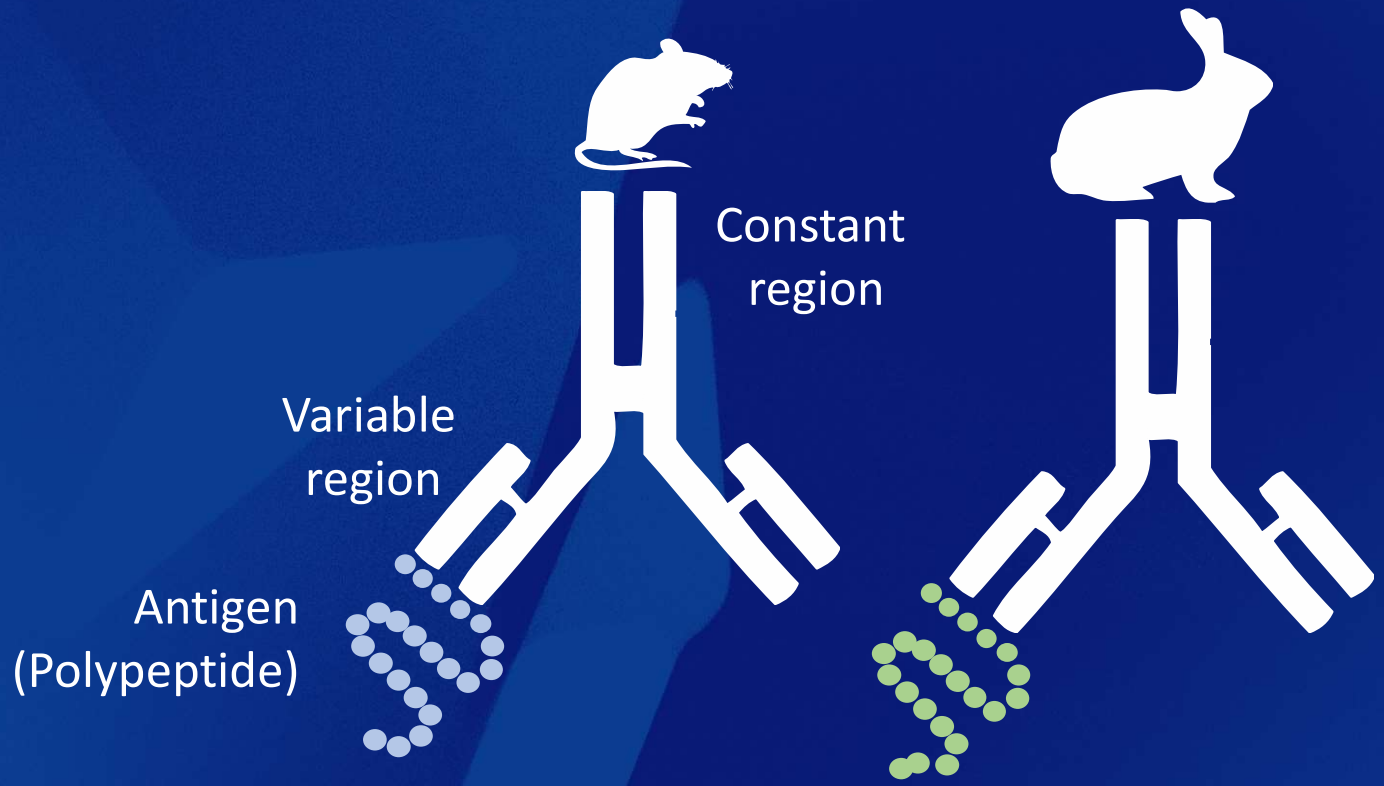
Immunohistochemistry: a working dilution of at least 1:25 was determined using formalin-fixed paraffin embedded human or animal tissue.

Immunoblotting: a working dilution of at least 1:100 was determined using chicken gizzard extract.

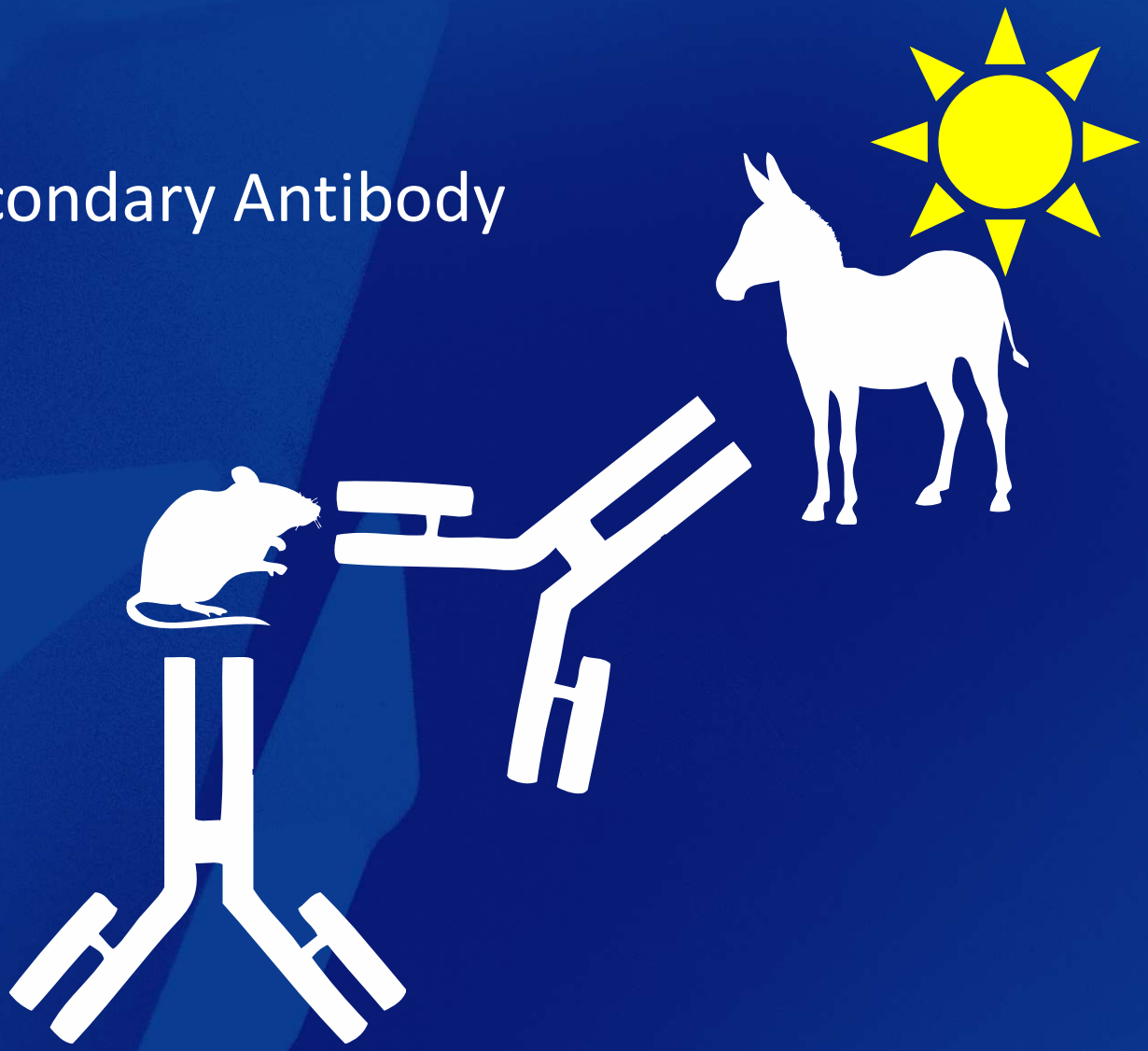
Note: In order to obtain best results, it is recommended that each individual user determine their optimum working dilution by titration assay.

DS,KAA,PHC 10/12-1

Optimize separately than together



Secondary Antibody



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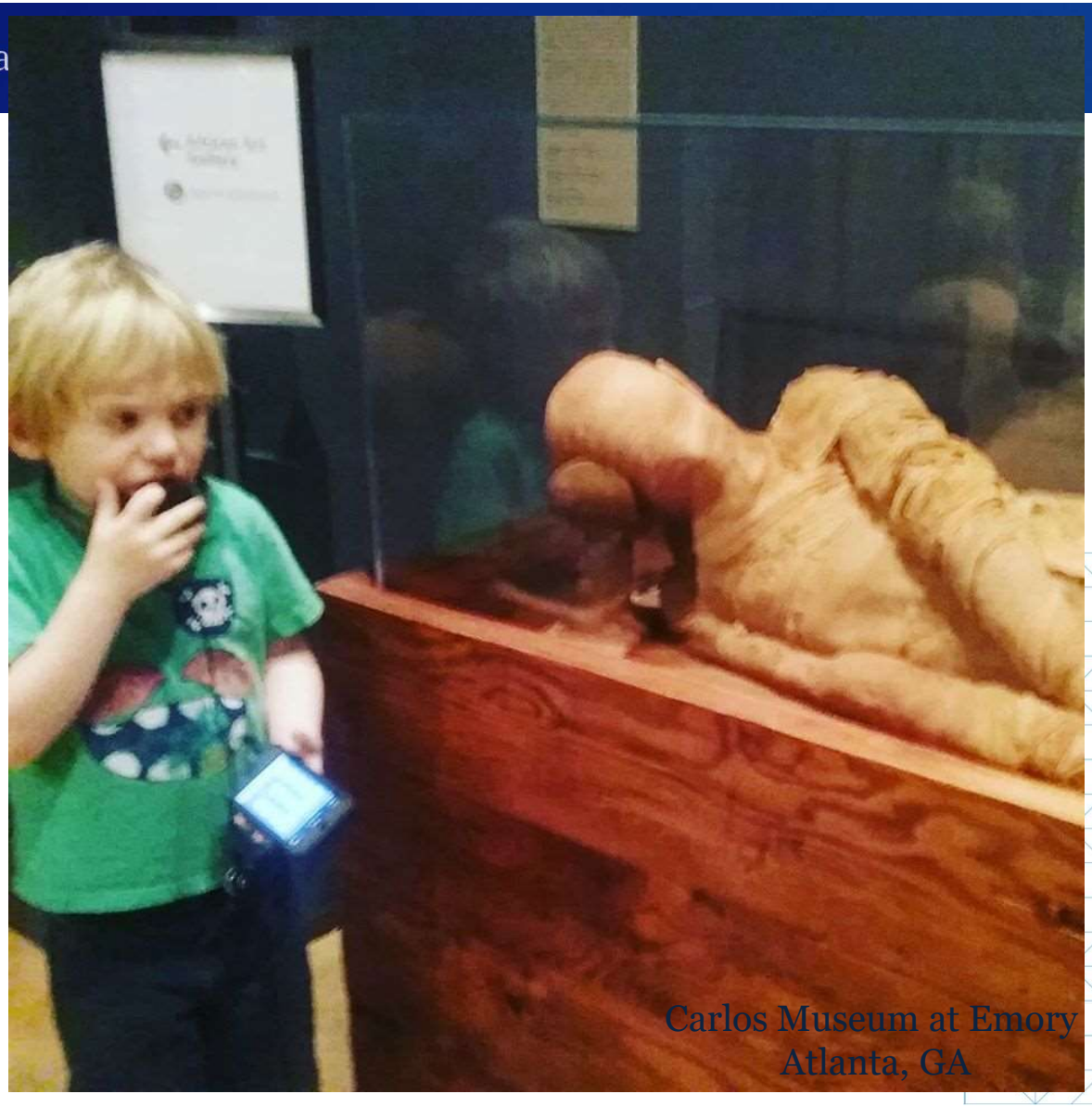
Fixation
The process of preserving a specimen, protein or biological tissues.



Carlos Museum at Emory
Atlanta, GA



Methanol, ethanol and acetone removes water from the sample causing precipitation of proteins and extraction of lipids.

Causes cell shrinkage and disruption of organelles.
Great for cytoskeletal components.

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Formaldehyde preserves the cellular structure by crosslinking proteins via methylene bridges. The number of bridges depends on the formaldehyde concentration, pH, temperature and fixation time. Can cause conformational changes

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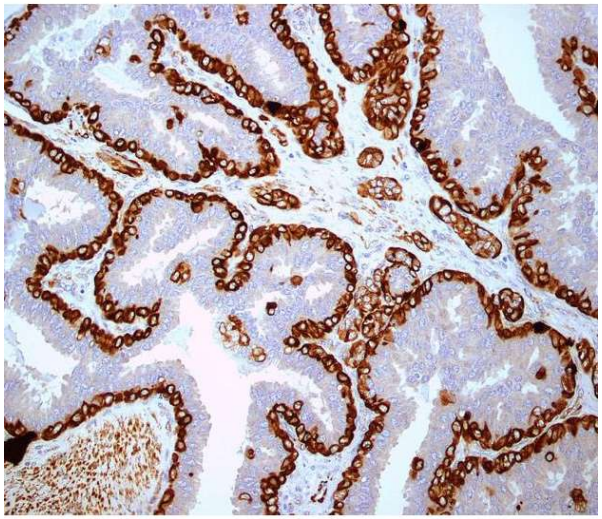
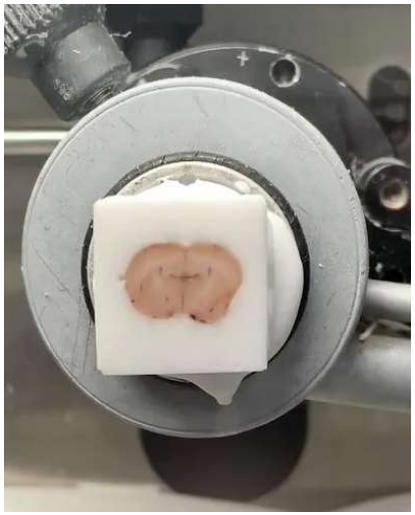
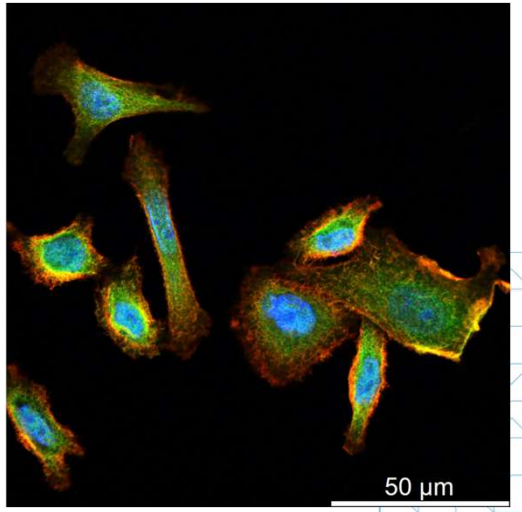
Formalin-Fixed Paraffin-Embedded (FFPE) tissue (wax) vs OCT (frozen)



IHC enzymatic stains: H&E

Requires a COLOR Camera





Samples are Flash frozen in OCT solution

Cryo-sectioned

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Formalin-Fixed Paraffin-Embedded (FFPE) tissue (wax) vs OCT (frozen)

	FFPE	OCT
Storage	Stable for years at room temperature	Requires continuous freezer storage (-20°C to -80°C)
Antigen Accessibility	Formalin cross-linking can mask epitopes	Epitopes remain more intact and accessible
Antigen Retrieval	Requires heat-induced antigen retrieval (HIER) with citrate or EDTA buffers	No antigen retrieval required
Immunofluorescence Optimization	autofluorescence and masked epitopes	easier optimization with stronger signal
Signal Quality	reduced signal intensity	brighter, higher-quality fluorescence
Morphology	Excellent tissue architecture	may have freezing artifacts
Processing Time	Longer processing	Rapid processing and sectioning
Best Applications	Clinical pathology, long-term archiving, histology	Immunofluorescence IHC

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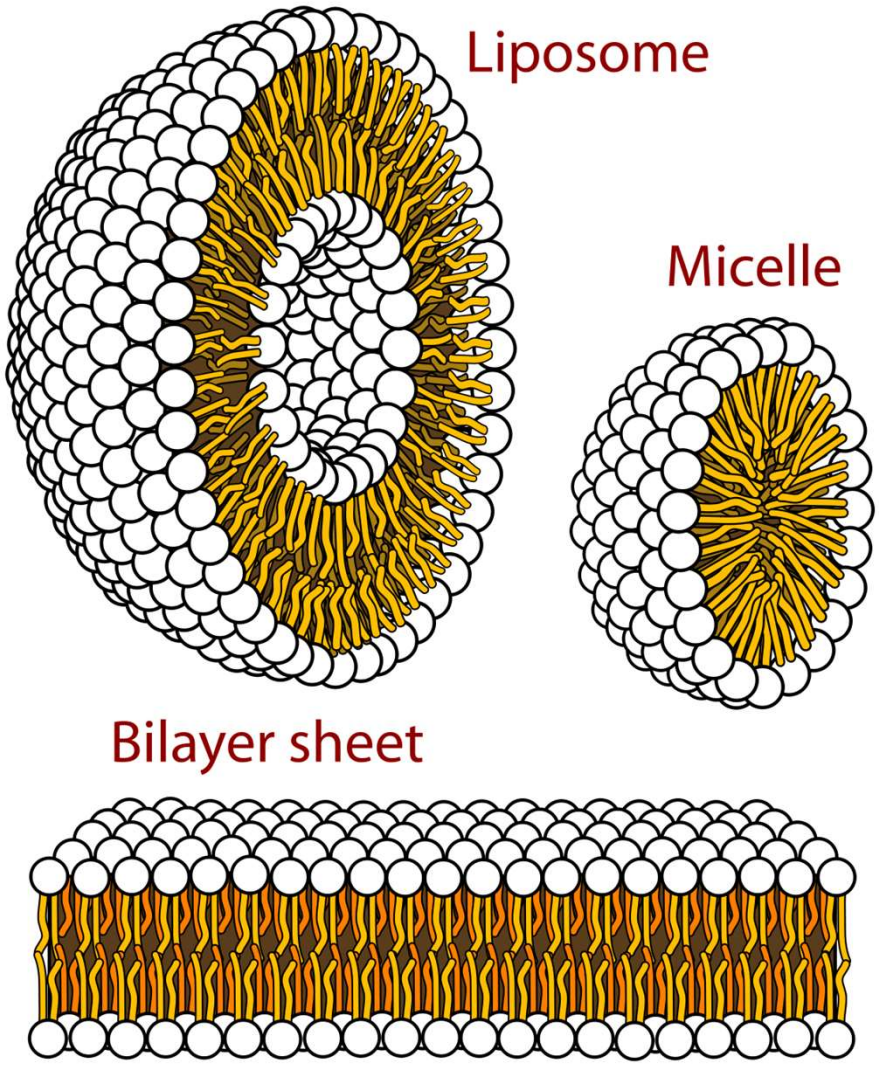
Permeabilization:
Disrupting the plasma membrane so reagents can be taken in



https://www.britannica.com/

Get yourself some detergent!

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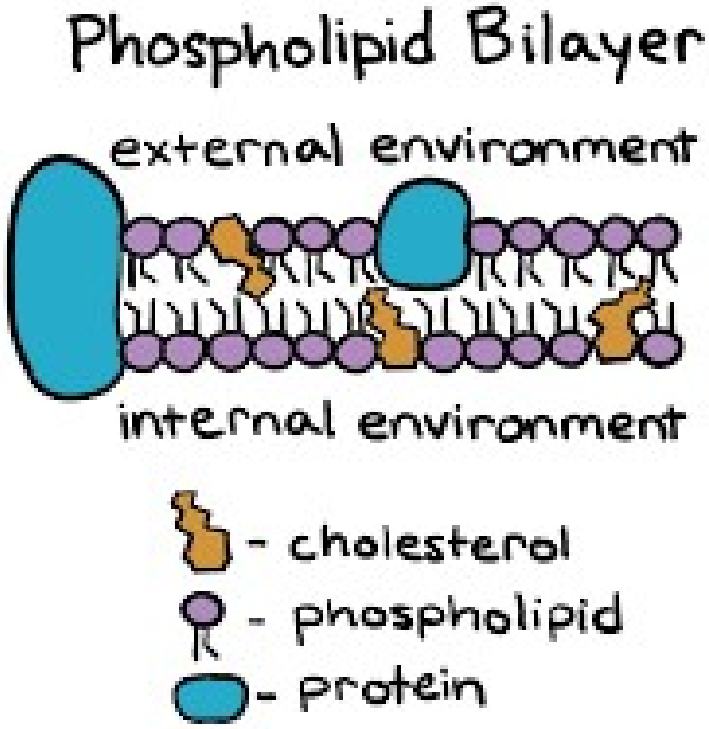
Permeabilization

Type	Chemicals
ionic	sodium dodecyl sulfate (SDS), deoxycholate, cholate, sarkosyl
non-ionic	Triton X-100, DDM, digitonin, tween 20, tween 80
zwitterionic	CHAPS
chaotropic	urea

Nonionic detergents permeabilize membranes by solubilizing and extracting lipids in a nonselective manner, resulting in possible coextraction of hydrophobic integral membrane proteins. Good for permeabilizing the nuclear membrane.



MATER METHODS
2013;3:163

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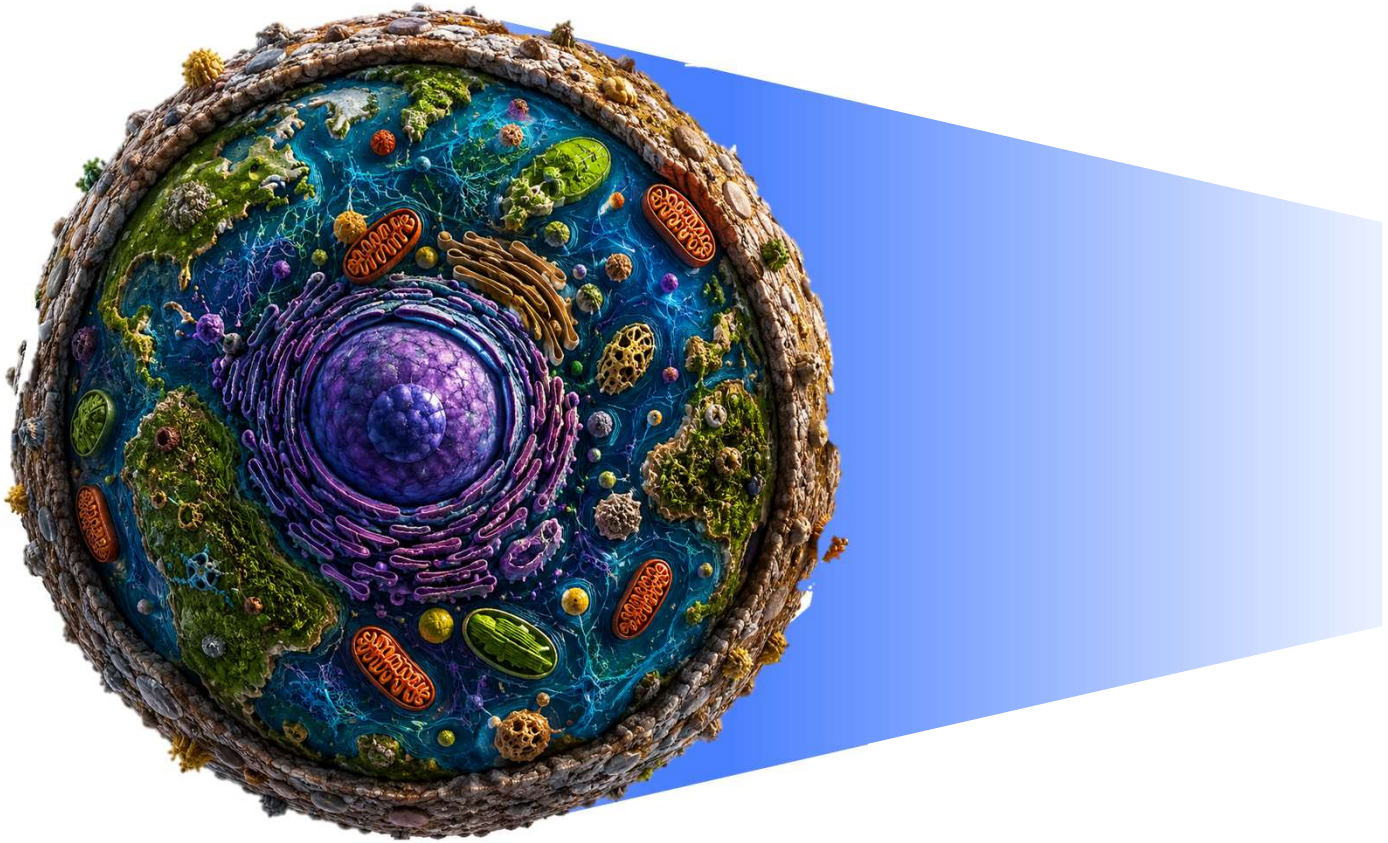
FLOW Cytometry does not use permeabilization



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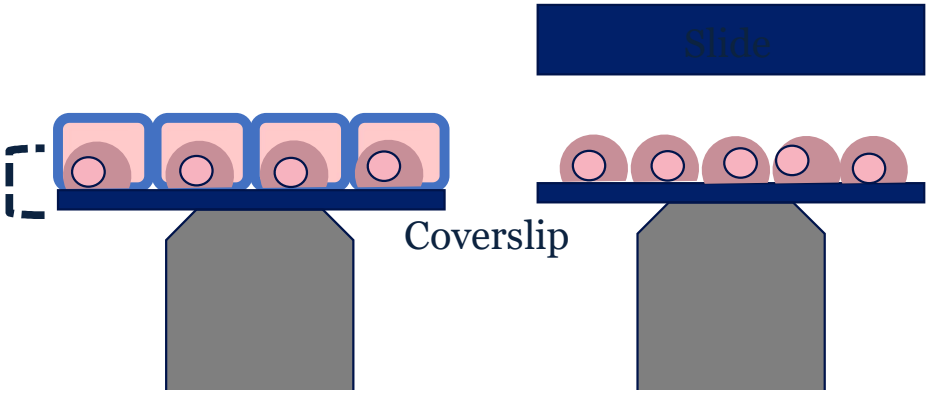
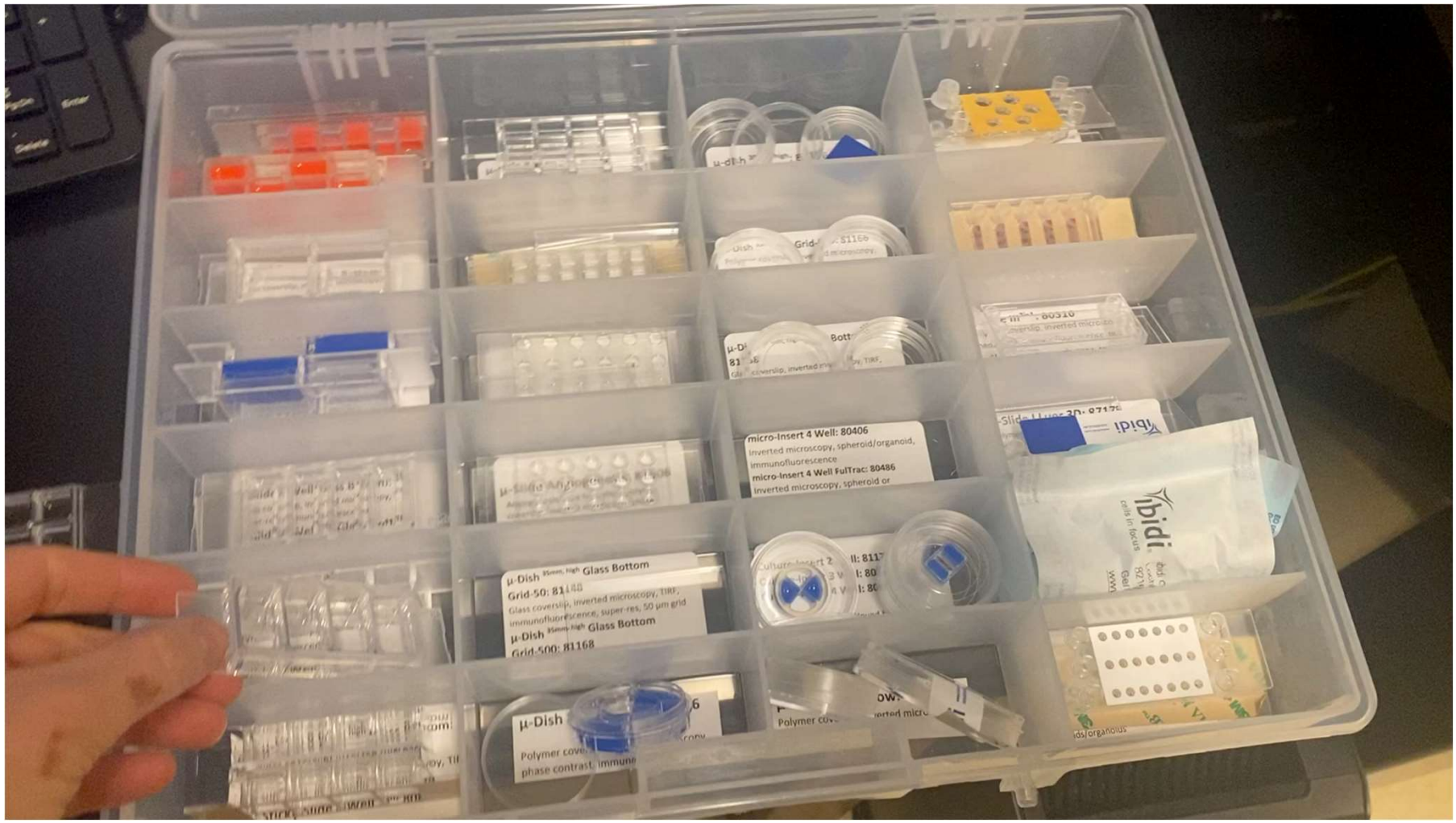
Cells and tissues must be mounted on coverslips

Working Distance





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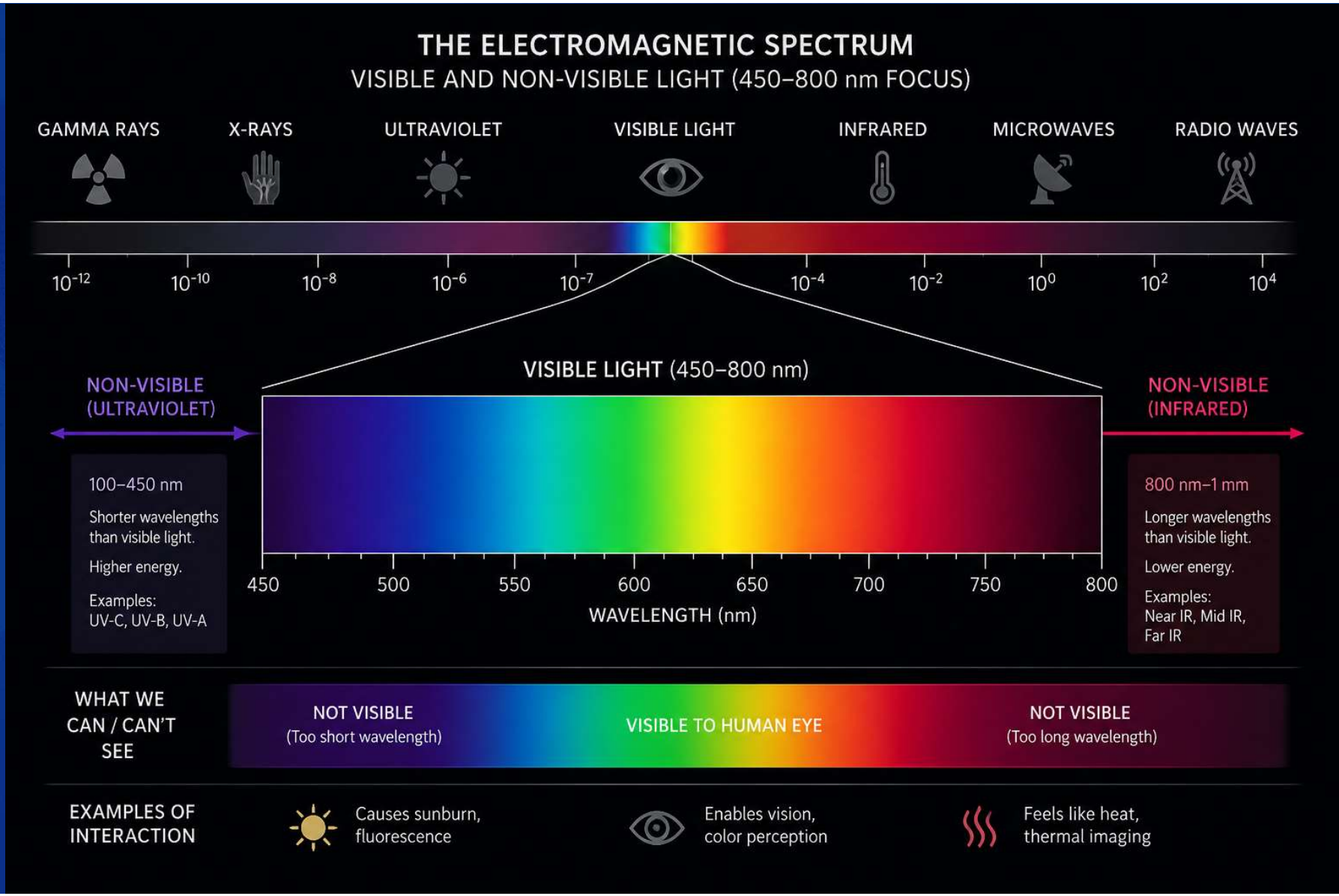
405/ DAPI

488/FITC

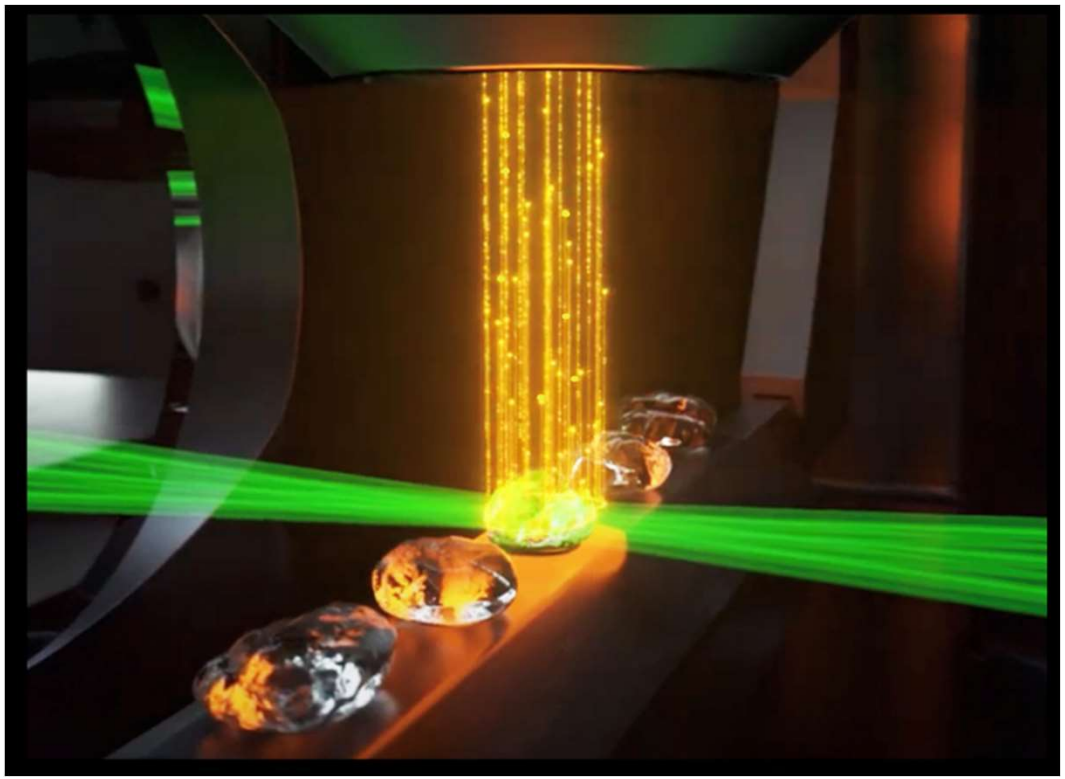
568/TRITC

635/CY5

750 NEAR IR



IR RED (640→ 750) for light sheet microscopy



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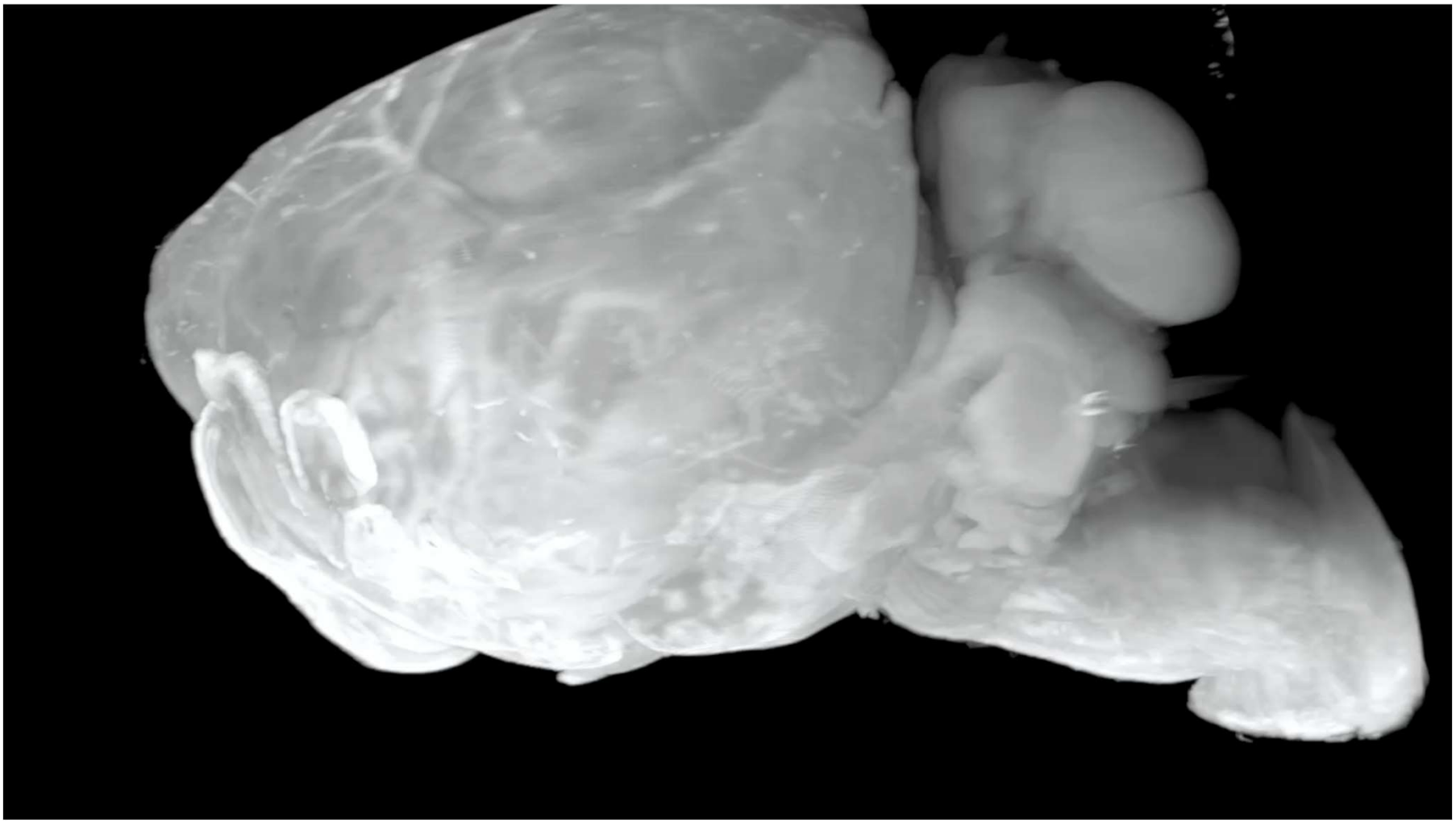

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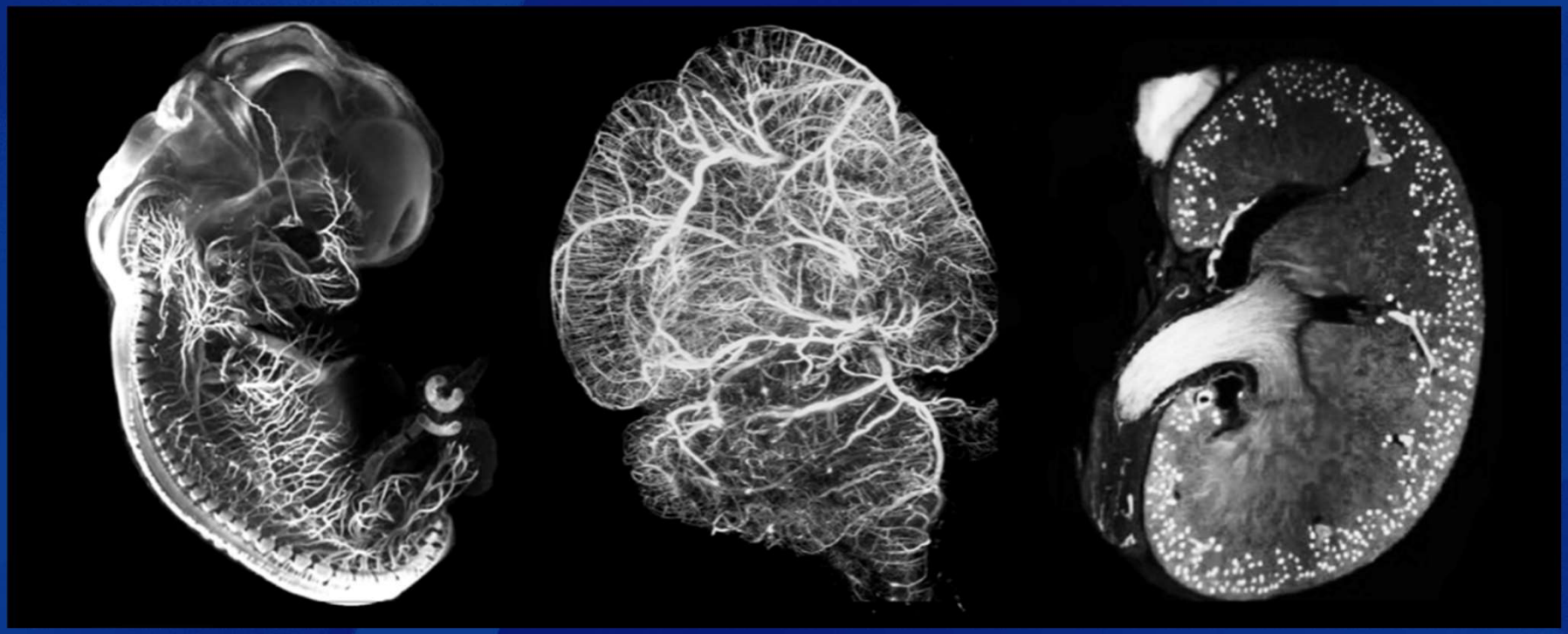
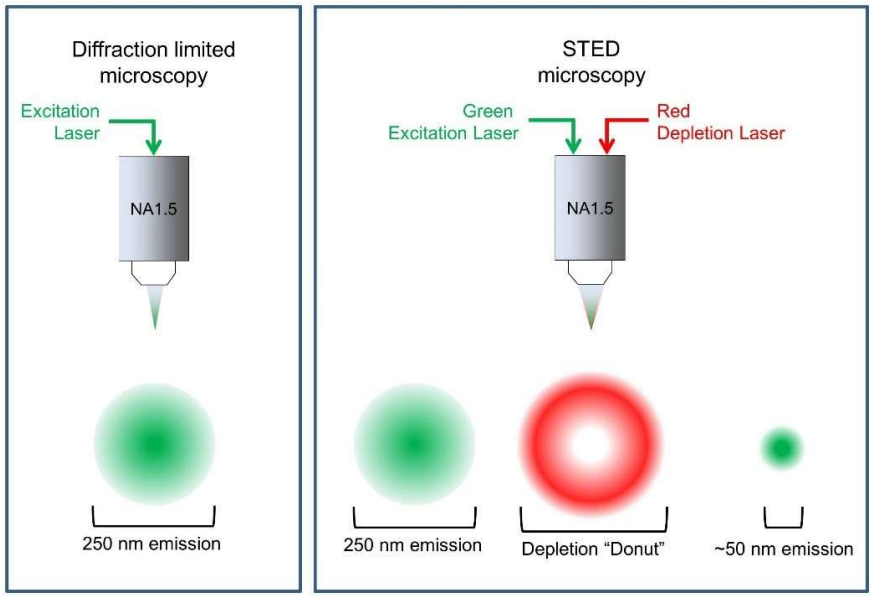
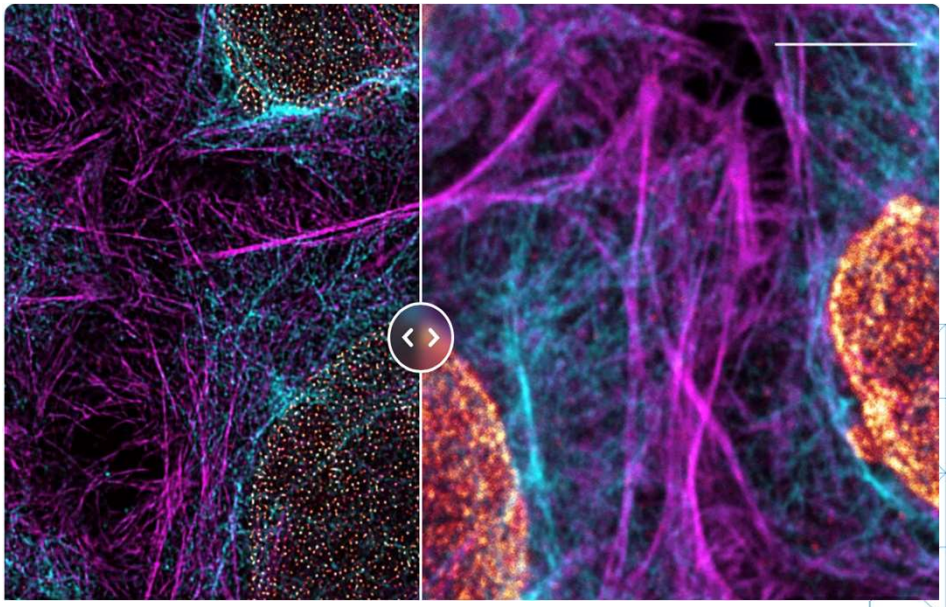
3D vALIDATED ANTIBODIES



LOW AND SLOW; VALIDATE WITH MEOH

STED Microscopy Antibodies





abberior STAR	Absorption max. [nm]	Emission max. [nm]	STED light [nm]	Brightness [$M^{-1}cm^{-1}$]	Fluorescence Lifetime [ns]
abberior STAR ORANGE	589	616	750 - 800	52,250	4.5
abberior STAR RED	638	655	750 - 800	66,000	3.4

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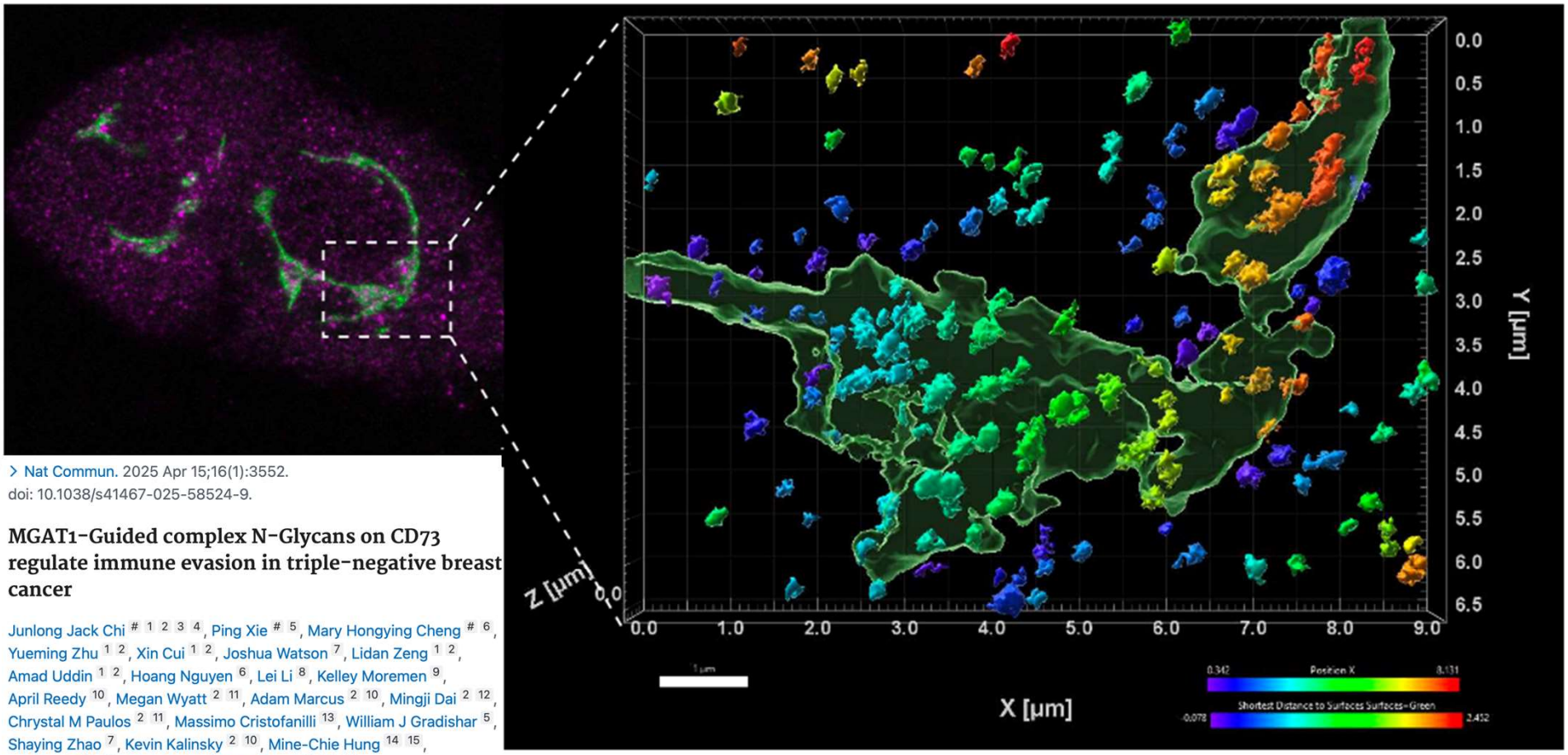
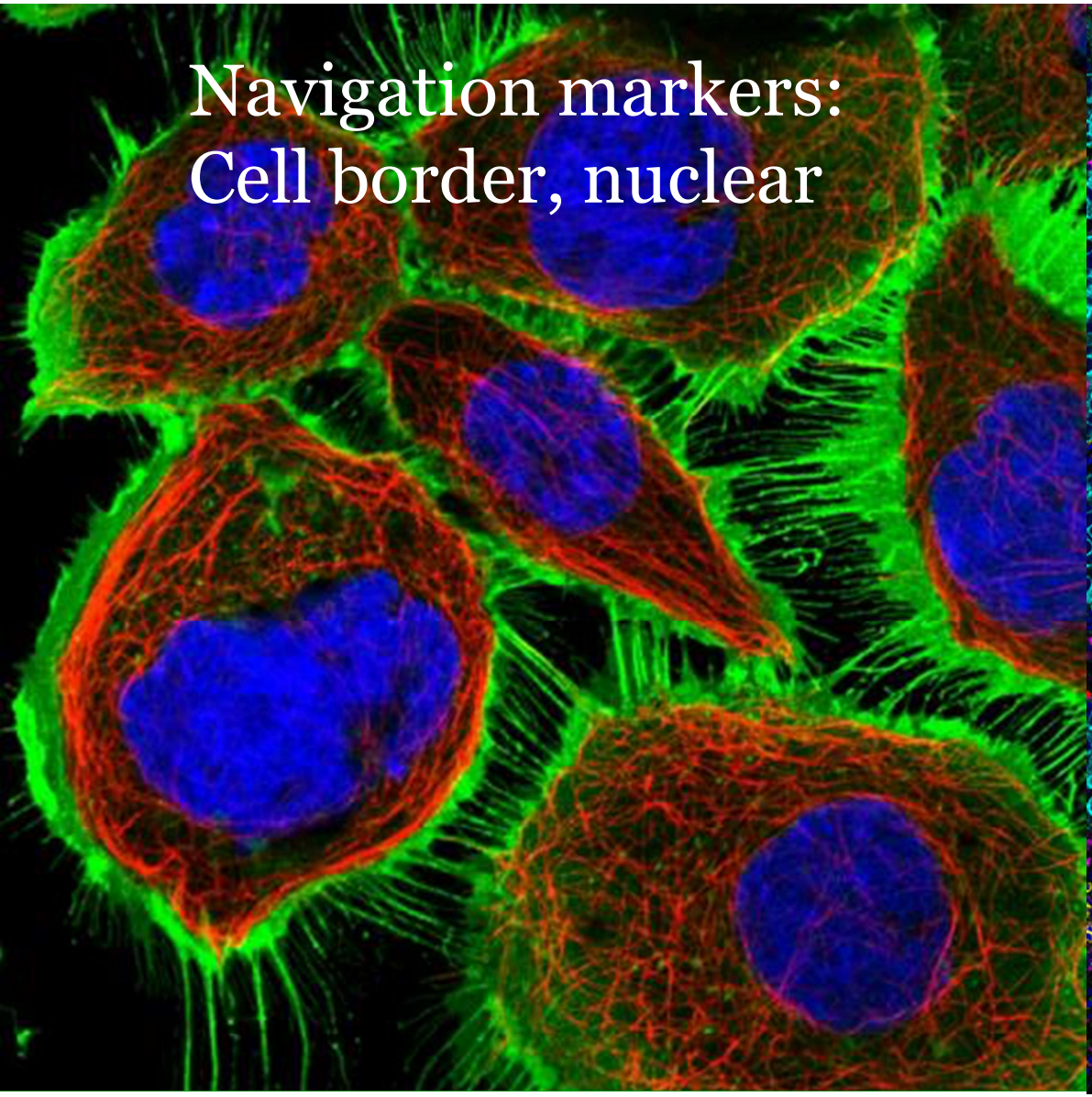


› Nat Commun. 2025 Apr 15;16(1):3552.
doi: 10.1038/s41467-025-58524-9.

MGAT1-Guided complex N-Glycans on CD73 regulate immune evasion in triple-negative breast cancer

Junlong Jack Chi [# 1 2 3 4], Ping Xie [# 5], Mary Hongying Cheng [# 6], Yueming Zhu [1 2], Xin Cui [1 2], Joshua Watson [7], Lidan Zeng [1 2], Amad Uddin [1 2], Hoang Nguyen [6], Lei Li [8], Kelley Moremen [9], April Reedy [10], Megan Wyatt [2 11], Adam Marcus [2 10], Mingji Dai [2 12], Chrystal M Paulos [2 11], Massimo Cristofanilli [13], William J Gradishar [5], Shaying Zhao [7], Kevin Kalinsky [2 10], Mine-Chie Hung [14 15], Ivet Bahar [16 17], Bin Zhang [18], Yong Wan [19 20 21]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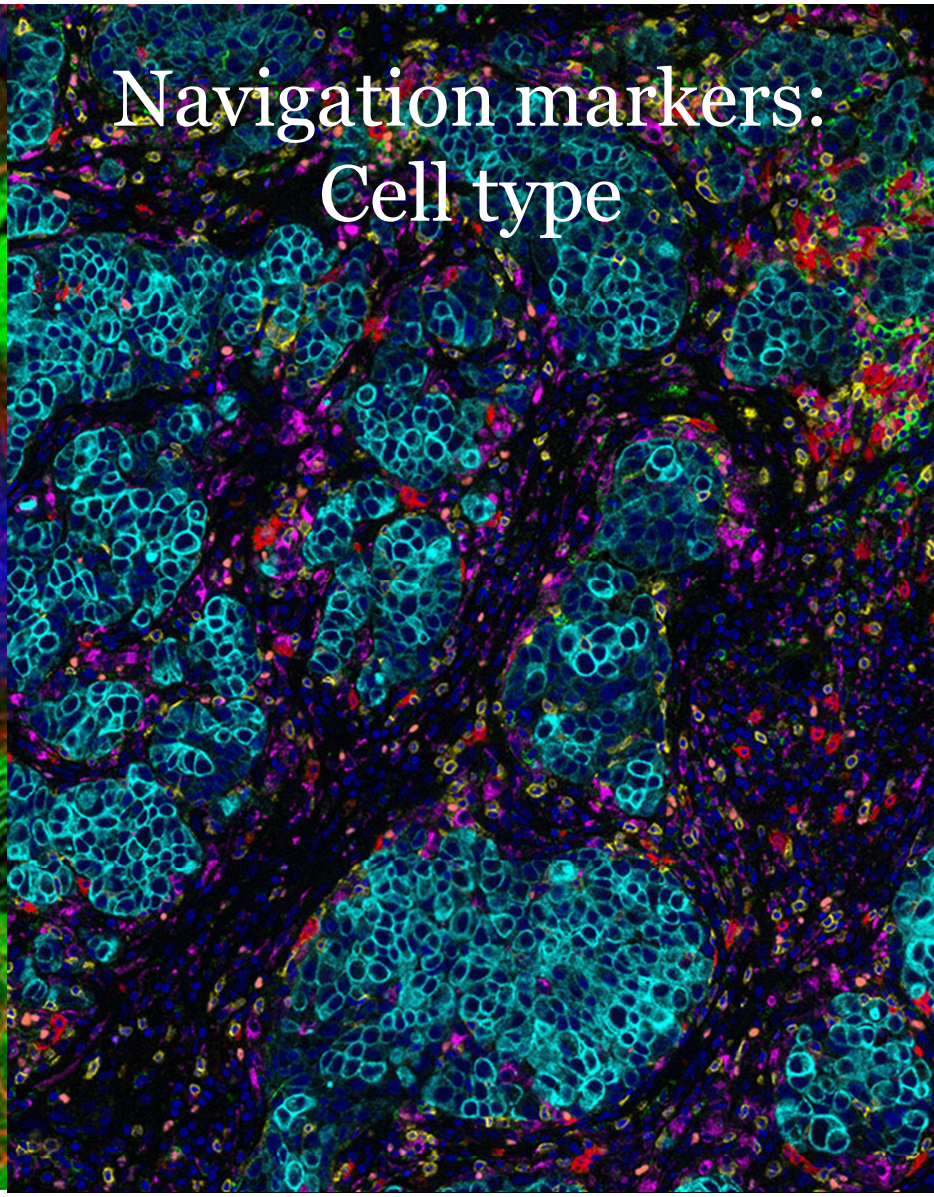
Cell border, nuclear


Navigation markers:
Cell type

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Why Your Flow Panel Usually Fails on the Microscope

Flow cytometry is like taking attendance. "Is the protein present?"
Immunofluorescence is like Google Maps."Exactly where is the protein, and what is it next to?"

Flow Cytometry	Immunofluorescence (IF)
Single cells in suspension	Cells or tissues with preserved architecture
Measures total fluorescence from one cell	Forms an image—requires spatial resolution
Bright fluorophores compensate for lack of amplification	Signal often needs amplification (primary + secondary antibodies)
Most antibodies are directly conjugated	Indirect staining is often preferred
Minimal concern for where proteins are located	Precise localization is the goal
Spectral overlap handled by compensation	Spectral overlap appears as image bleed-through and false colocalization
Usually stains surface proteins (or fixed/permed with optimized kits)	Intracellular targets require optimized fixation and permeabilization

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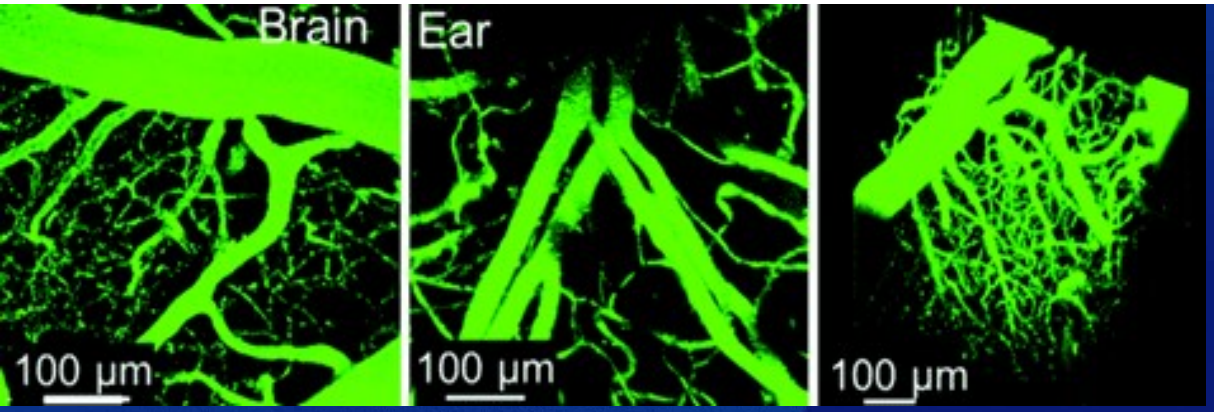
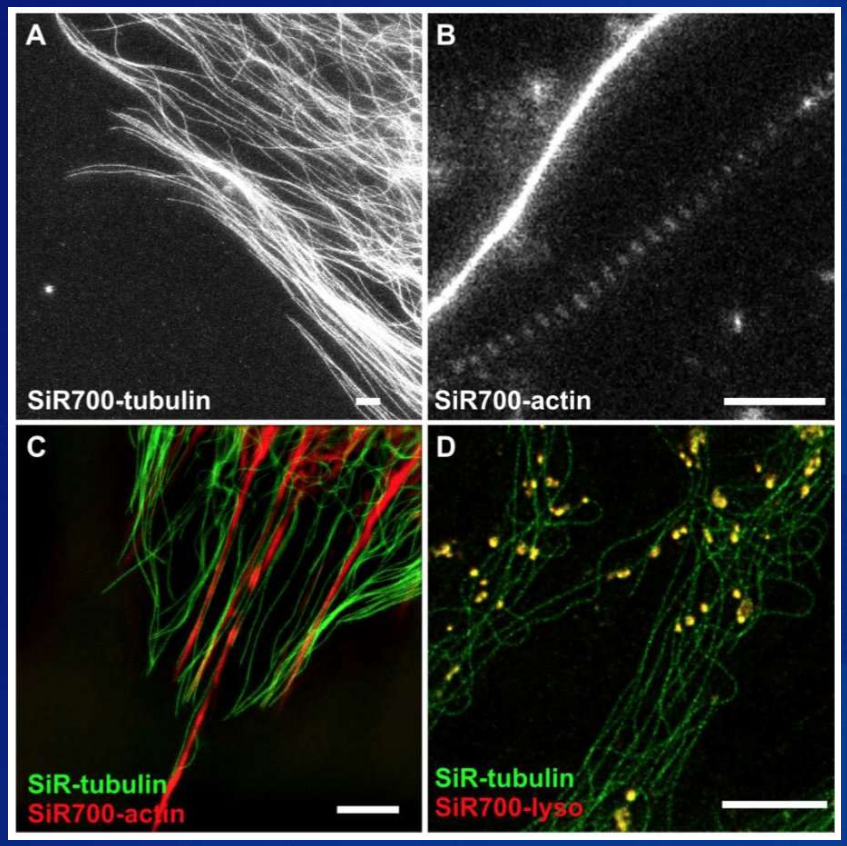
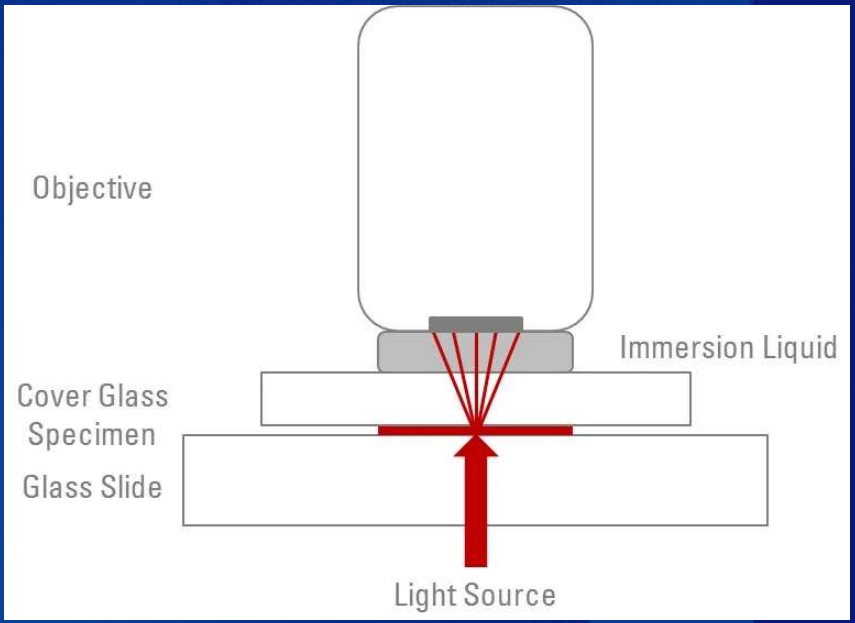


LIVE CELL DYES

Structure	Live/fixed (cells or tissue)
Endoplasmic reticula, smooth and rough (ER)	Live/fixed (aldehyde)
Golgi apparatus	Live/fixed (aldehyde)
F-actin / actin filaments	Fixed (aldehyde)
Lysosome	Live (can be fixed in aldehyde after staining)
Mitochondria	Live (can be fixed in aldehyde after staining)
Nucleolus	Live



Refractive index and Clean glass



Thanks for listening!
Do you have any questions?