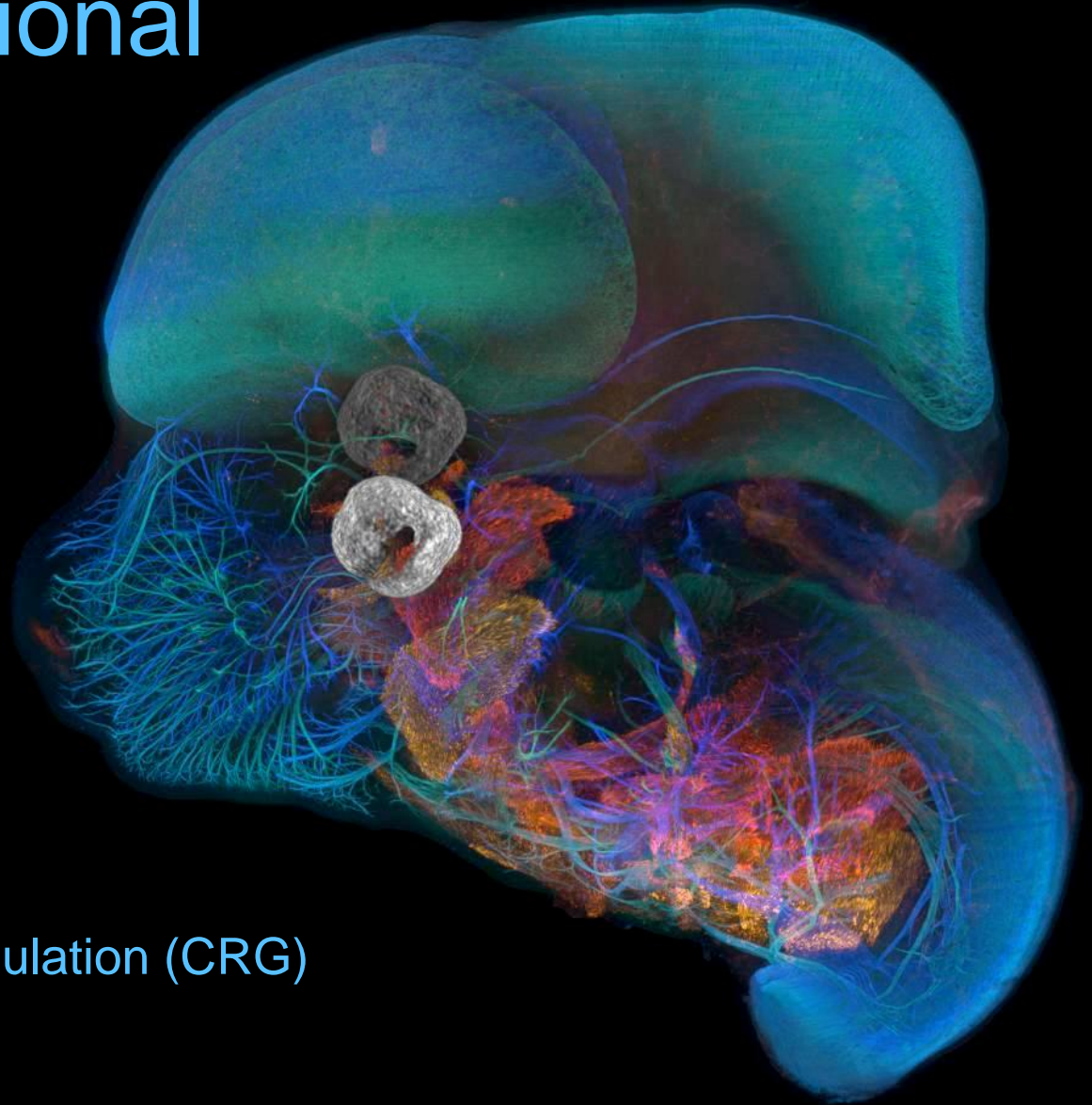# Multidimensional Mesoscopic Biological Imaging

Jim Swoger

Centre for Genomic Regulation (CRG)
Barcelona, Spain
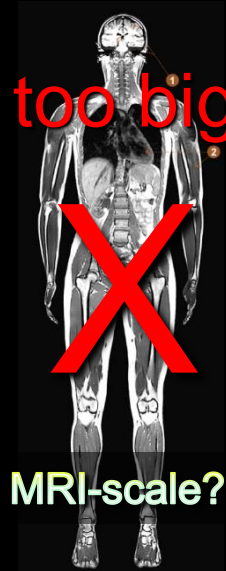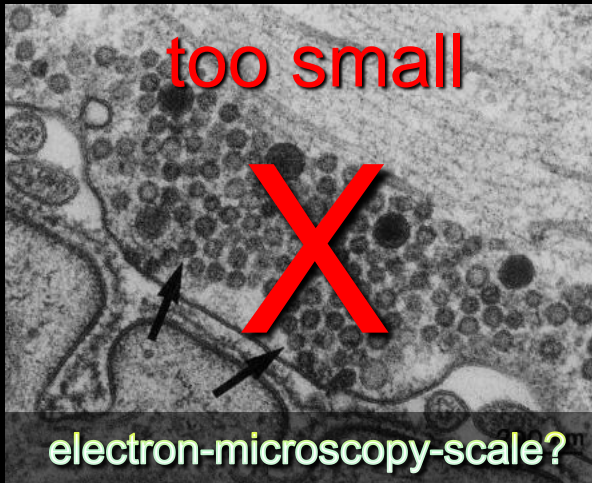
Sept. 15, 2016

"Optical Foundations of Full Parallax Imaging", Valencia, Spain

Background: Mesoscopic Imaging

What do we want to look at?

50 µm – 15 mm

too small

electron-microscopy-scale?

too big

MRI-scale?

meso-scopic structures?

What would we like to see?

Cellular details throughout *intact organs/organisms*

What tools do we develop?   *SPIM & OPT*

Outline

Introduction to Microscopy

Selective Plane Illumination Microscopy (SPIM)

    How it Works

    Live Applications

    Optical Clearing & Fixed Sample Applications
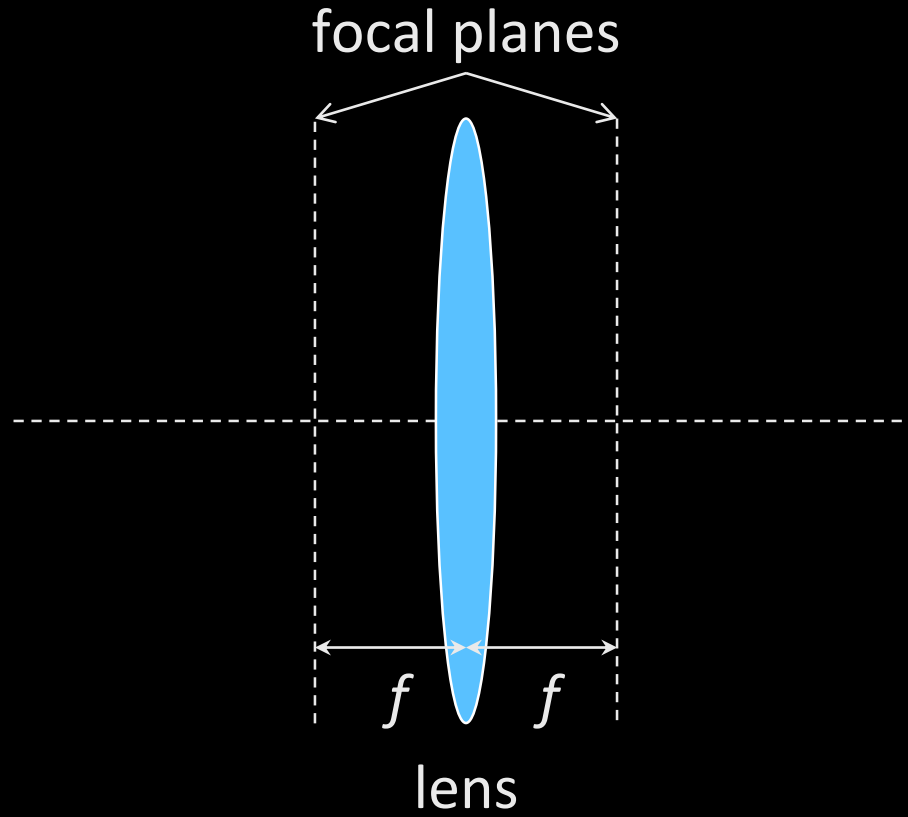
Optical Projection Tomography (OPT)
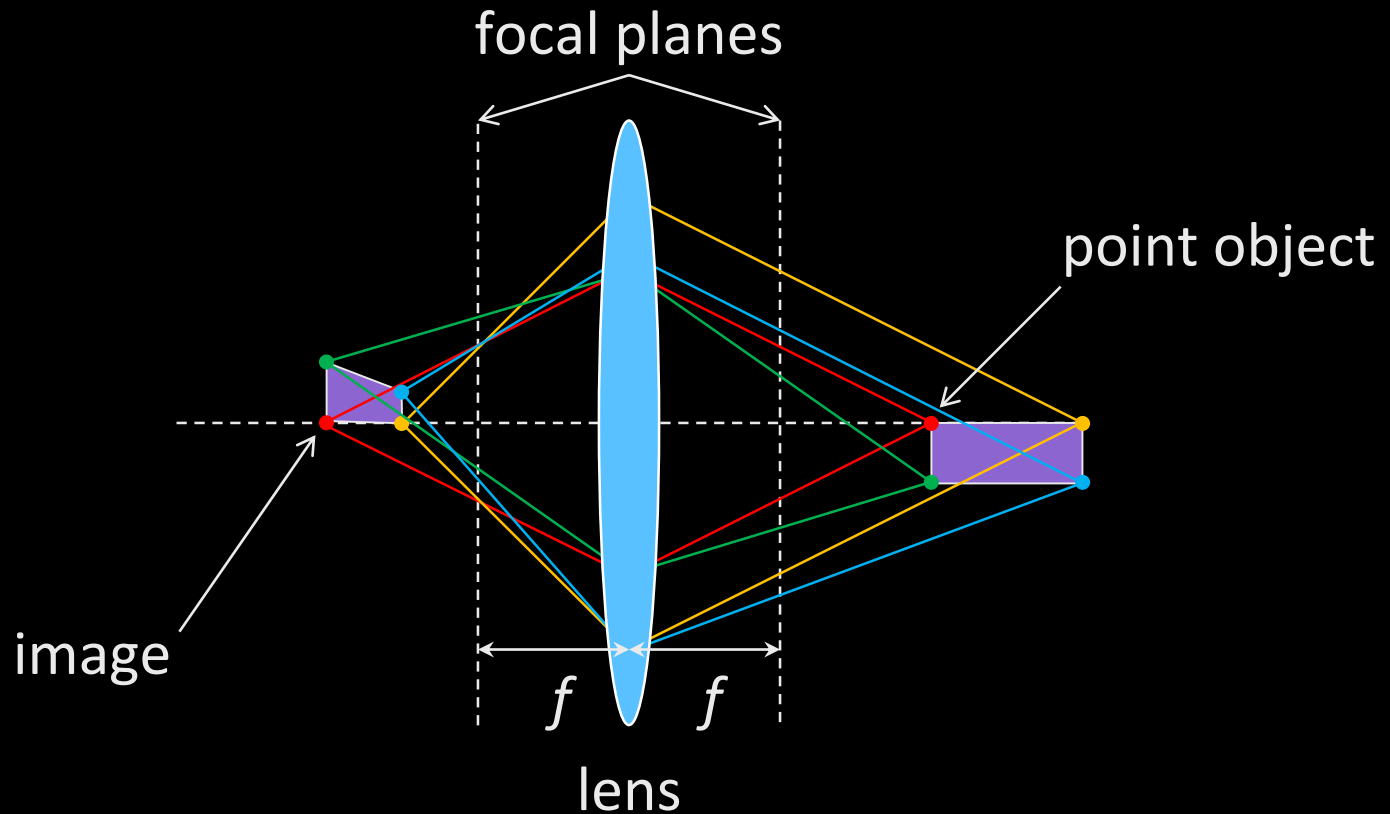
    How it Works

    Applications

The OPTiSPIM

    Applications

Frontiers in Mescopic Imaging

Introduction: A Simple Magnifier
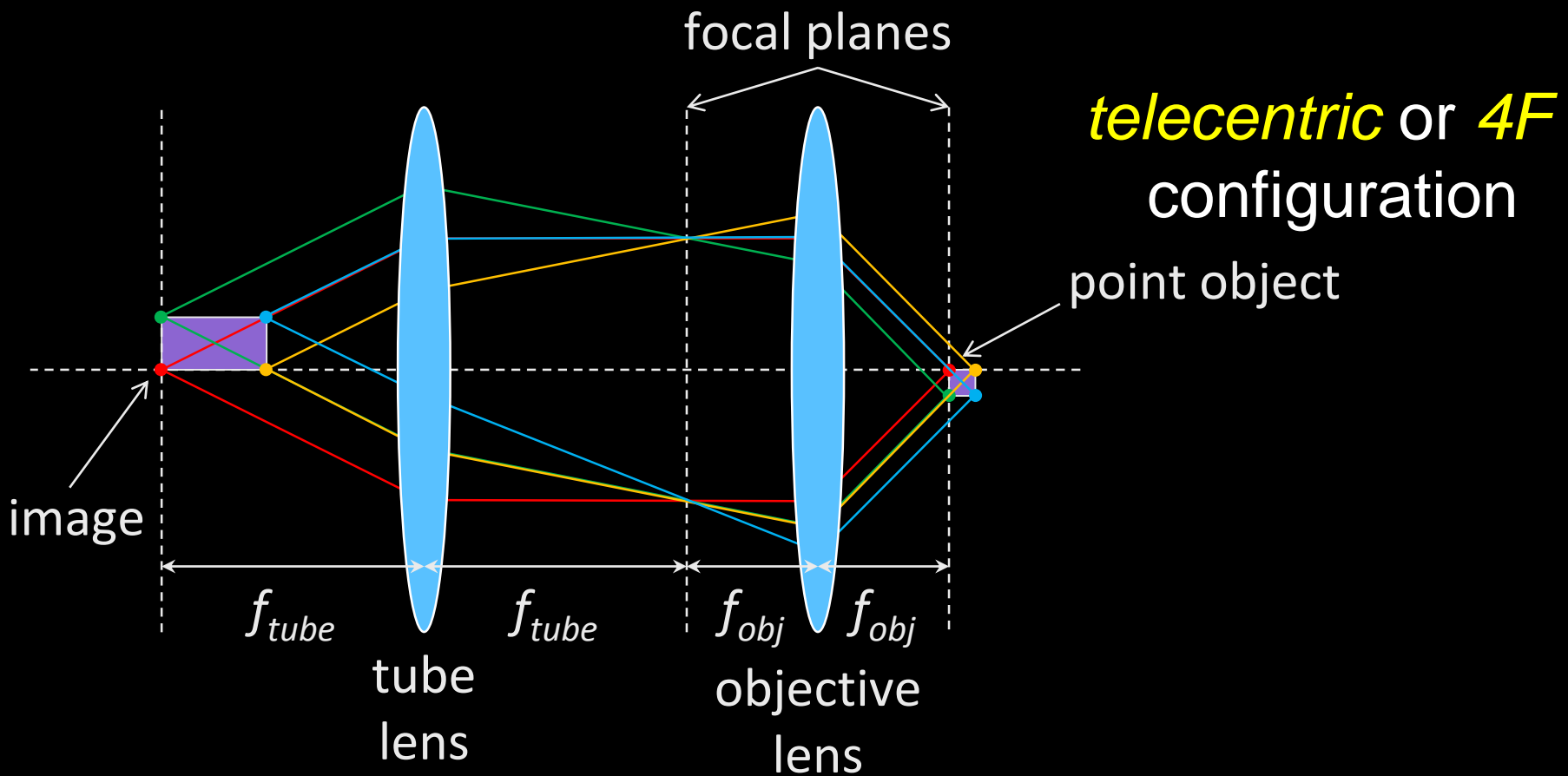
focal planes

lens

f f

Introduction: A Simple Magnifier

focal planes

point object

image

f f

lens

Magnification depends on axial position ☹

Introduction: A Widefield Microscope - *Magnification*

focal planes

telecentric or *4F* configuration

point object

image

f_{tube} f_{tube} f_{obj} f_{obj}

tube lens   objective lens

Magnification is position-independent ☺

$$M = f_{tube} / f_{obj}$$

Introduction: A Widefield Microscope - *Resolution*

point-spread function (PSF)

focal planes

Δx

Δz

telecentric or *4F* configuration

Numerical Aperture

$NA = n_i \sin\alpha$

α

tube lens

objective lens

How do we determine the *resolution* (*Δx* & *Δz*)?

Introduction: A Widefield Microscope - *Resolution*

point-spread function (PSF)

focal planes

telecentric or *4F* configuration

Δx

Δz

α

Numerical Aperture

$NA = n_i \sin\alpha$

tube lens

objective lens

How do we determine the *resolution* (Δx & Δz)?

Introduction: A Widefield Microscope - *Resolution*

point-spread function (PSF)

focal planes

Δx

Δz

α

tube lens

objective lens

telecentric or *4F* configuration

Numerical Aperture

$NA = n_i \sin\alpha$
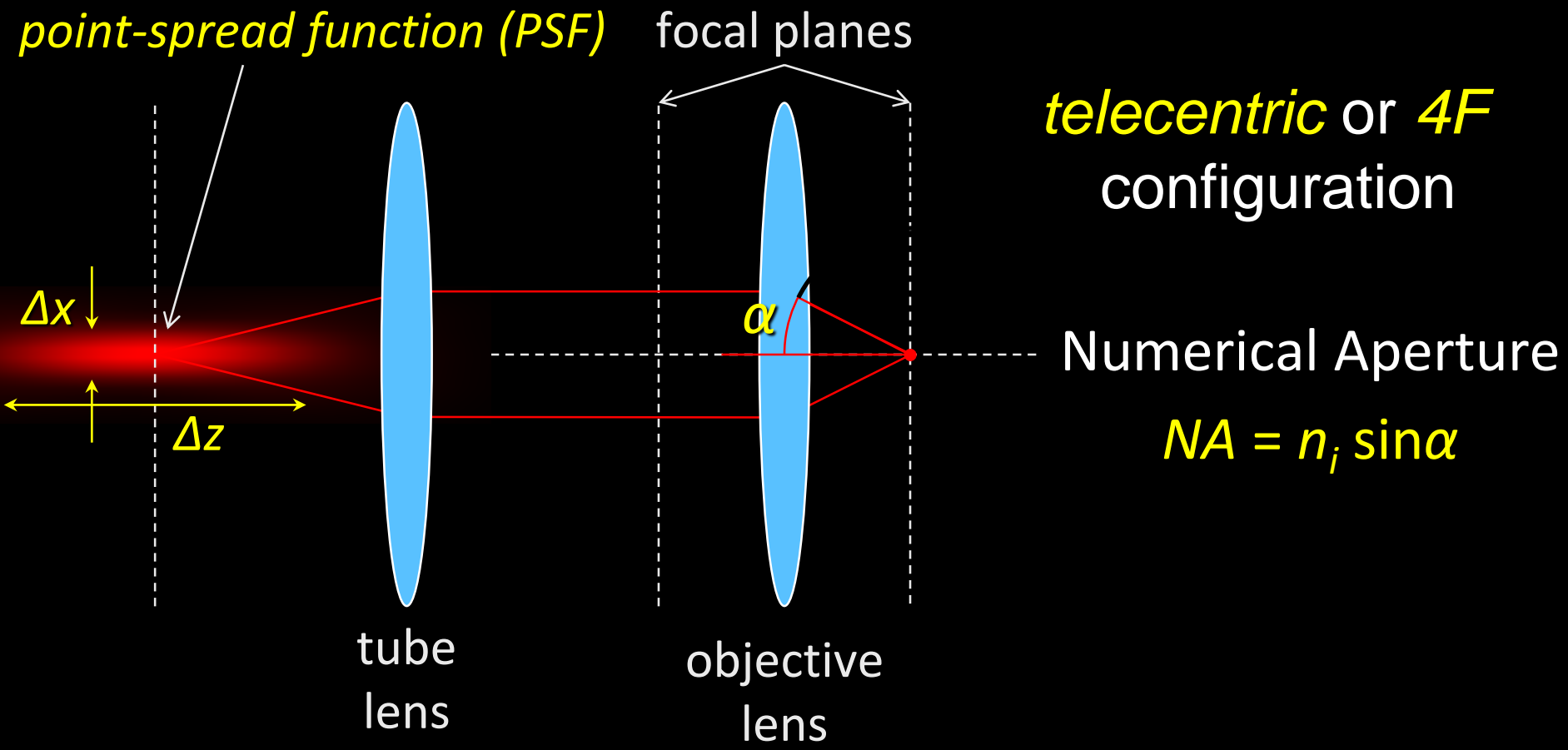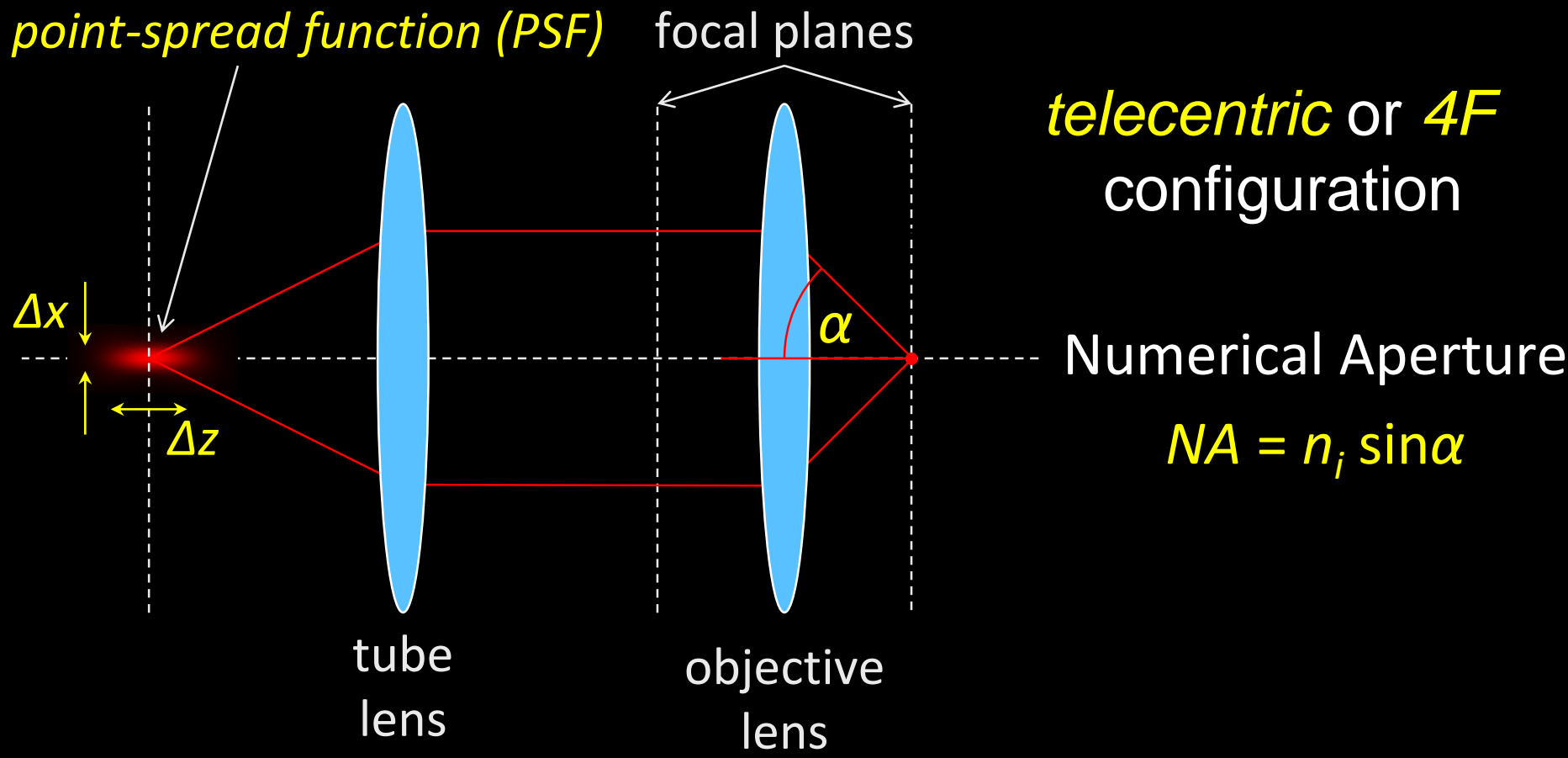
How do we determine the *resolution* (Δx & Δz)?

$$\Delta x \approx 0.61 \, \lambda \, / \, NA \qquad\qquad \Delta z \approx n_i \, \lambda \, / \, NA^2$$

Introduction: *Non-uniform Illumination*

point-spread function (PSF)   focal planes

Δx

Δz

non-uniform illumination of the sample

We can improve the resolution of a microscope by using non-uniform illumination.

tube lens

objective lens
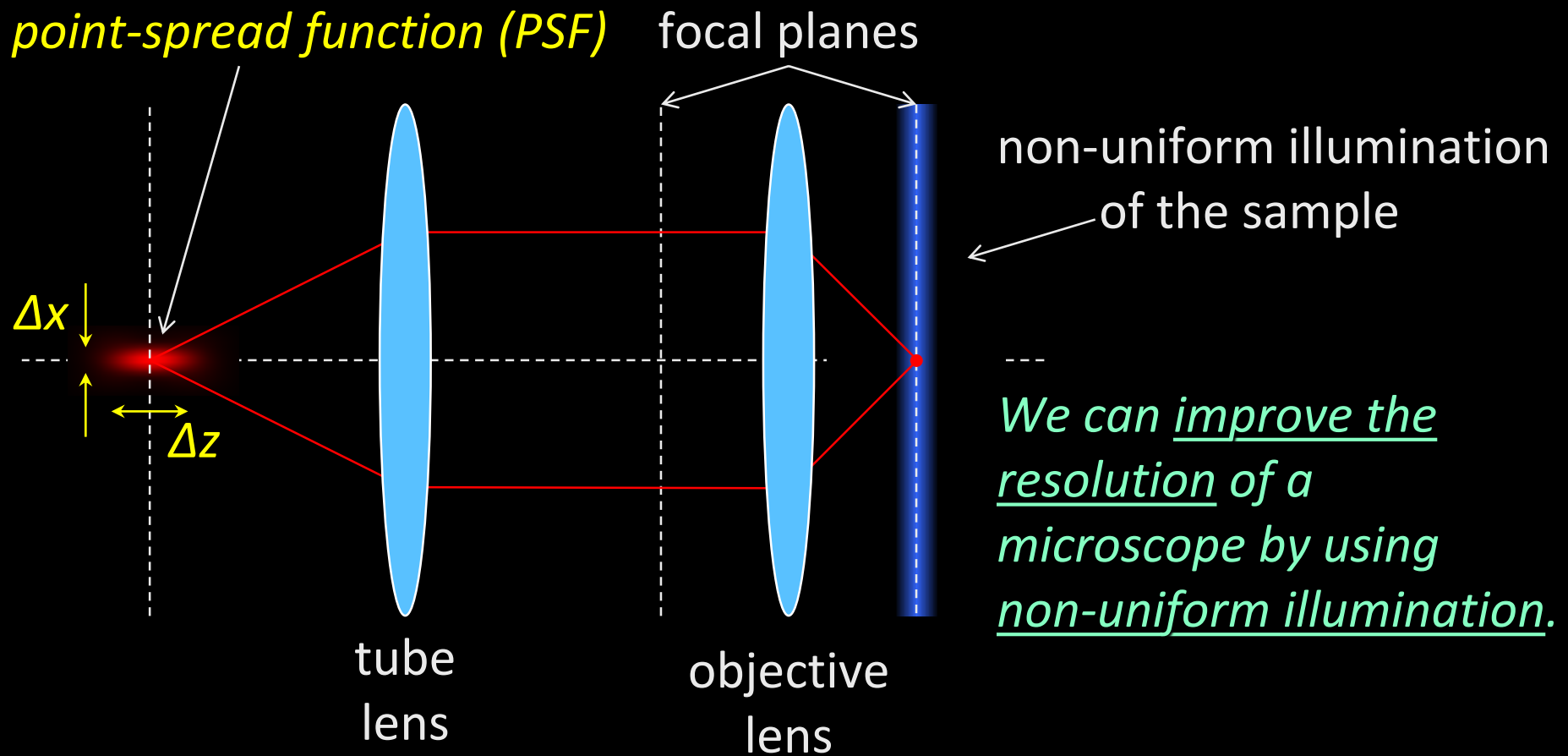
PSF = probability density of detecting emission from a point source

×

probability density of exciting the point source

Introduction: *Summary*
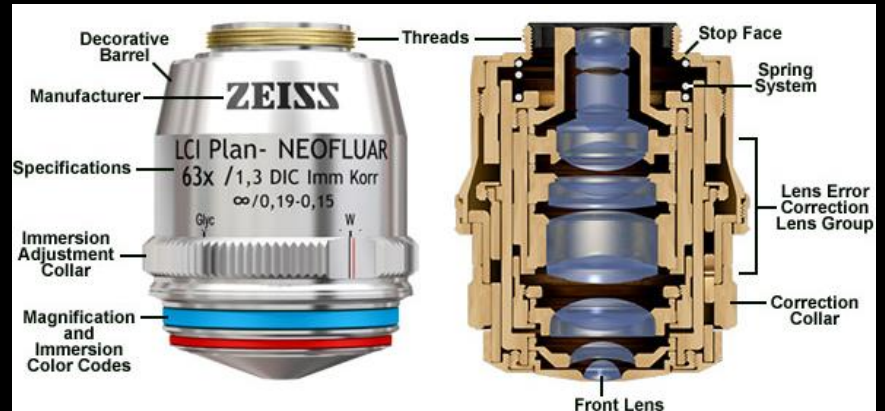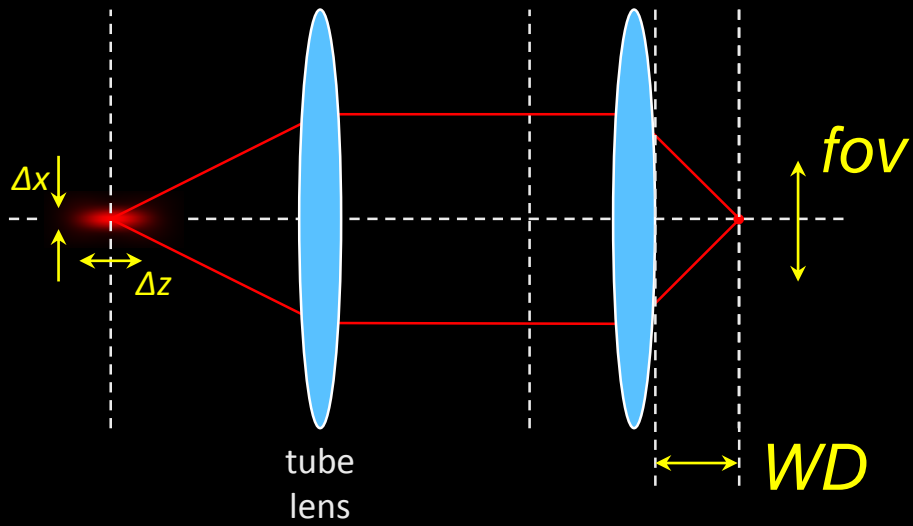
Telecentric (4F) for quantitative imaging

Magnification $M = f_{tube} / f_{obj}$

Resolution $\Delta x \approx 0.61 \, \lambda / NA$, $\Delta z \approx n_i \, \lambda / NA^2$

Working Distance, *WD*:
mechanical distance from objective lens to focal plane

Field of View, *fov*:
size of the region seen by a camera (or eyepiece)

Outline

Fluorescence Contrast

time delay (1-5ns), incoherent emission

spectral discrimination

high specificity – antibodies, fluorescent proteins

excellent signal-to-background
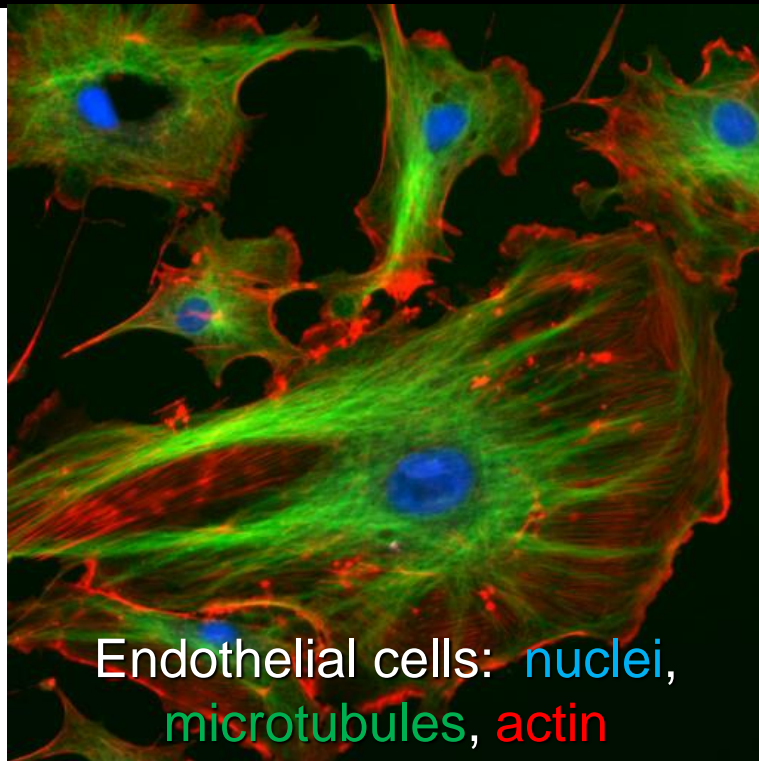
Endothelial cells: nuclei, microtubules, actin

Aequoria victoria, biofluorescent jellyfish

411   452   492   533   574   614   655

Wavelength (nm)

scein (FITC)
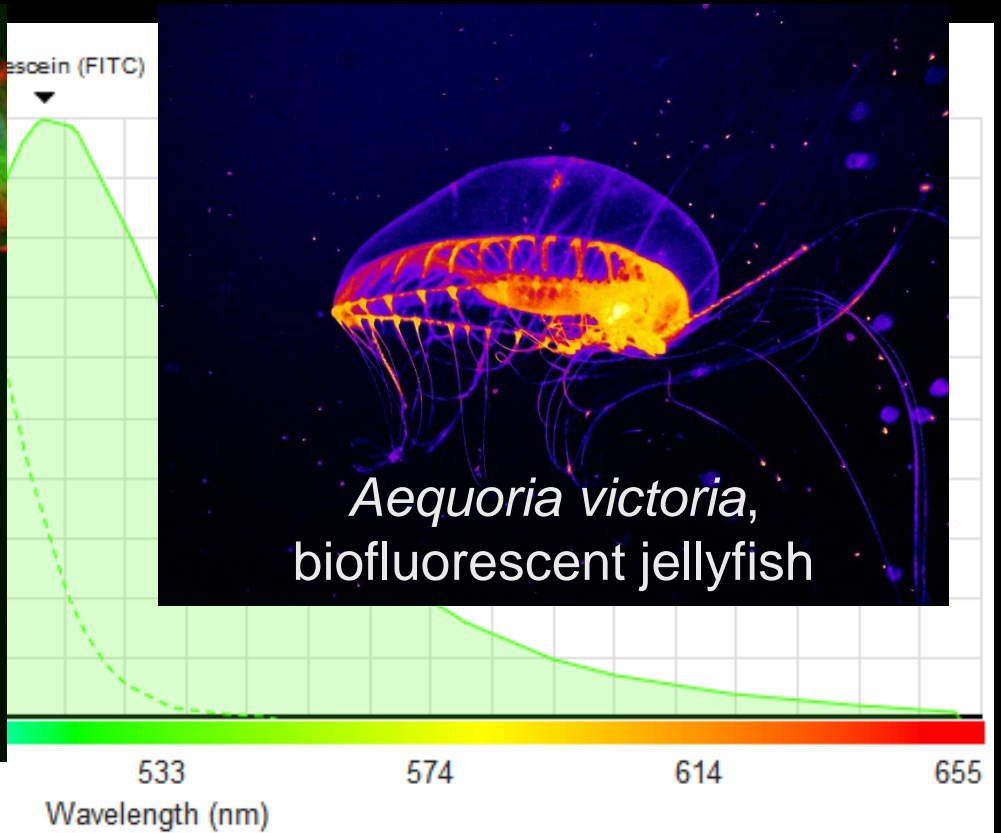
SPIM: Introduction

fluorescence

SPIM = Selective Plane Illumination Microscopy

scanning the sample thru the light sheet builds up the 3D voxel data

thin light sheet

sample

emission filter

scan

camera

cylindrical lens

lenses

input laser beam
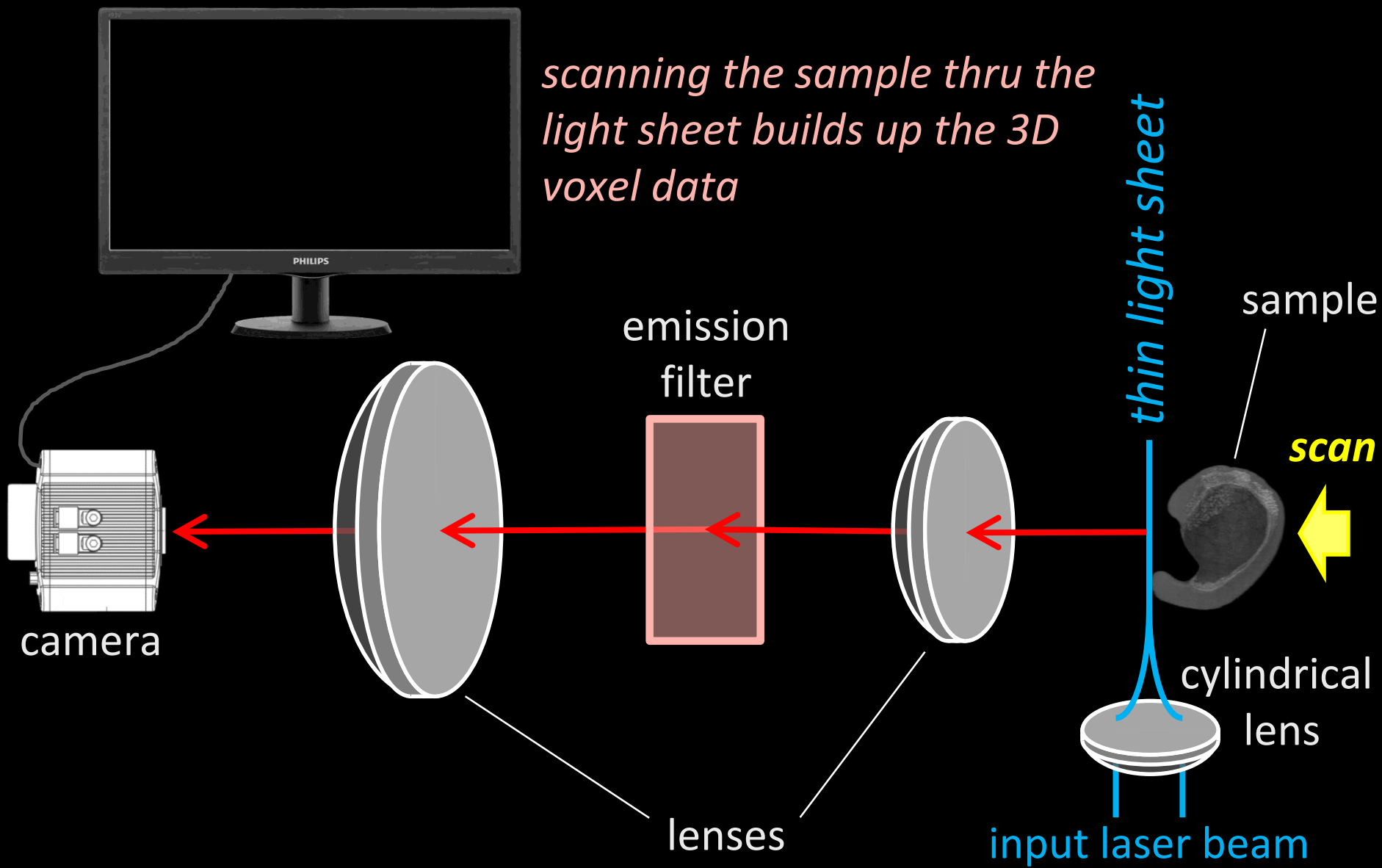
SPIM: Introduction   *fluorescence*

SPIM = Selective Plane Illumination Microscopy

Detection PSF

objective lens:

high NA → high resolution

long WD → large samples

Illumination PSF

cylindrical lens:

high NA → thin light sheet

BUT: low NA → large field of view

long WD → large samples

System PSF = PSF$_{ill}$ × PSF$_{det}$

lateral resolution: objective lens

axial resolution: cylindrical lens

long WDs → large samples

thin light sheet

sample

scan

objective lens

cylindrical lens

input laser beam
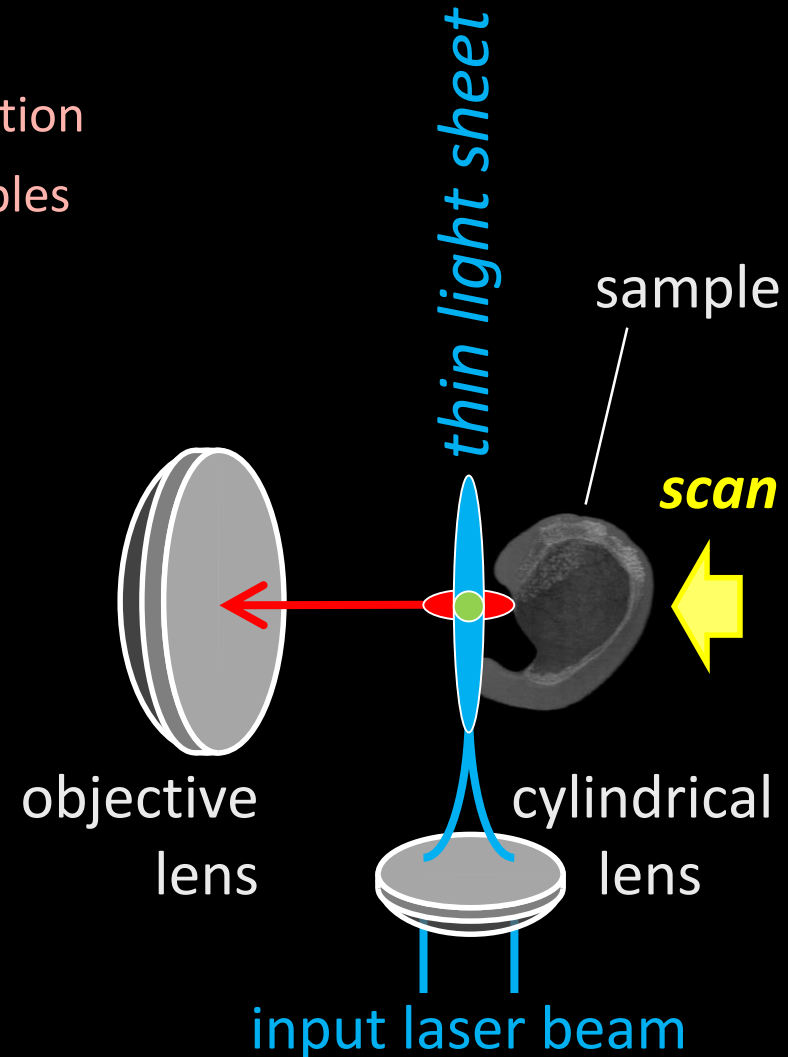
SPIM & Optical Sectioning

Medaka embryo, GFP labeled muscle max-value projections

1550 µm
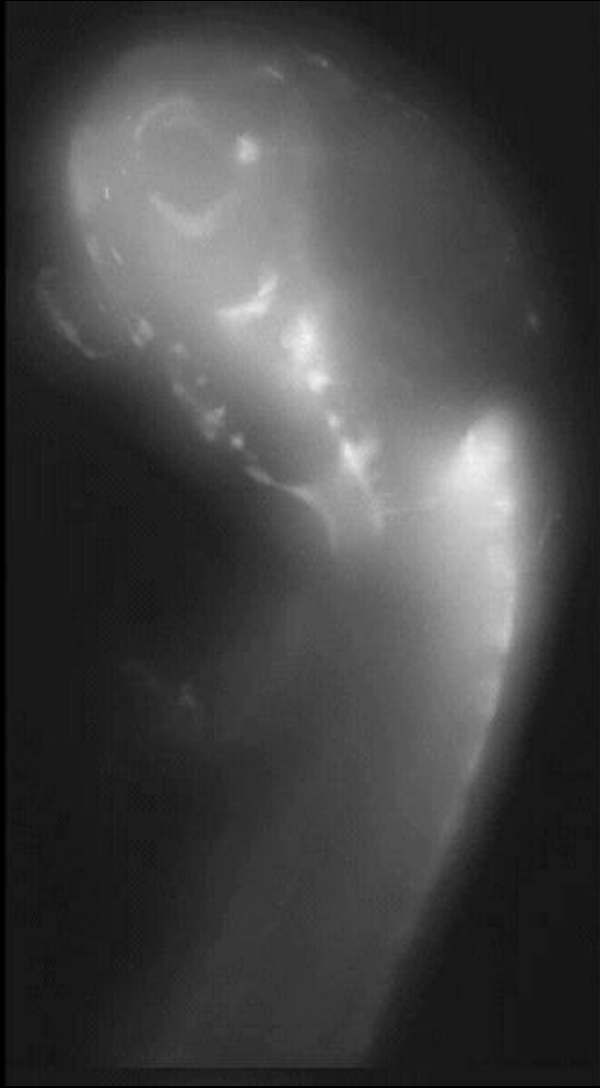
uniform illumination
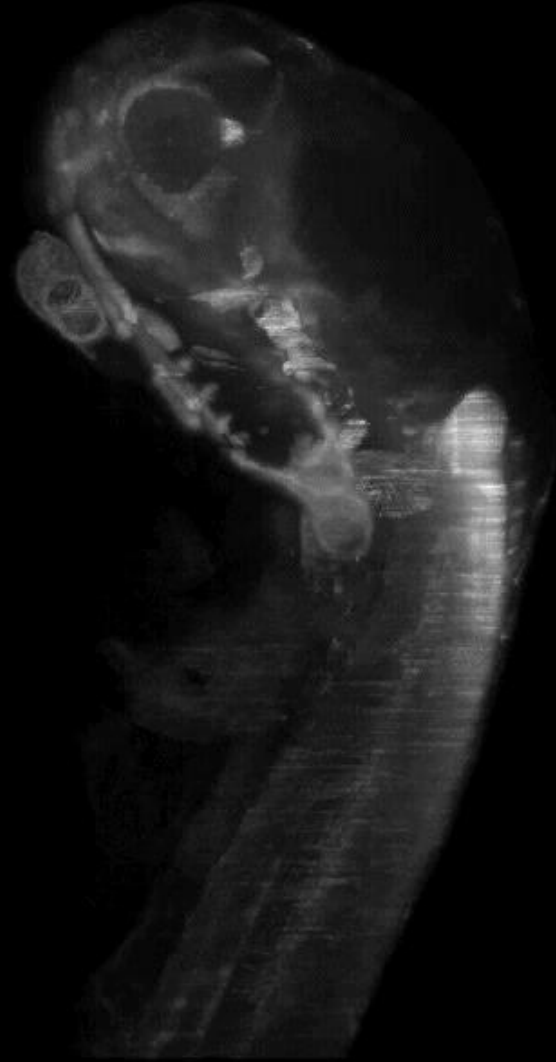
light-sheet illumination

SPIM – Zebrafish – Lateral Line Imaging

A Very Brief Introduction

The Lateral Line:

- mechanosensory organ

- clusters of ciliated sensory patches - neuromasts

- *vibrations in water → neuronal signals*

A Very Brief Introduction

30 hpf

primordium

proneuromast
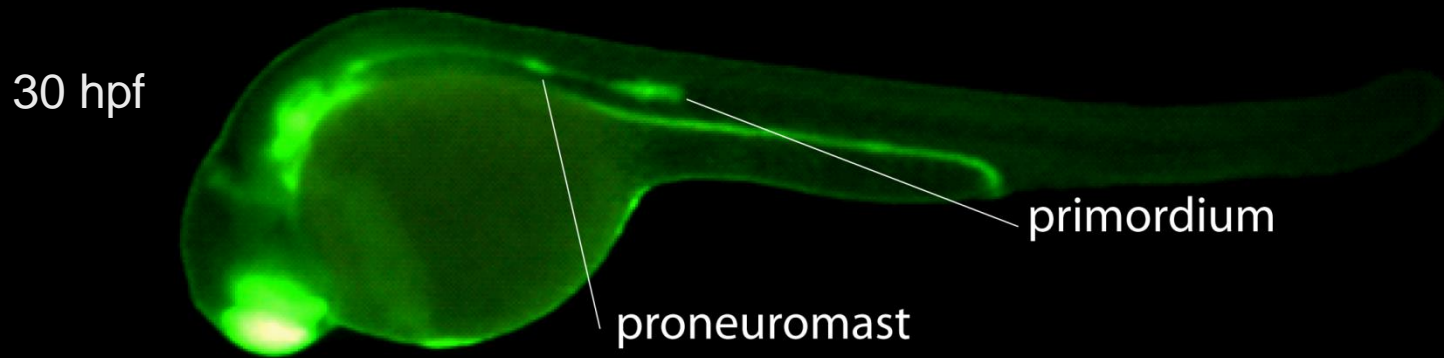
48 hpf

pLL = posterior lateral line

SPIM – Zebrafish – Lateral Line Imaging

Primordium Migration

bright-field stereo microscope image
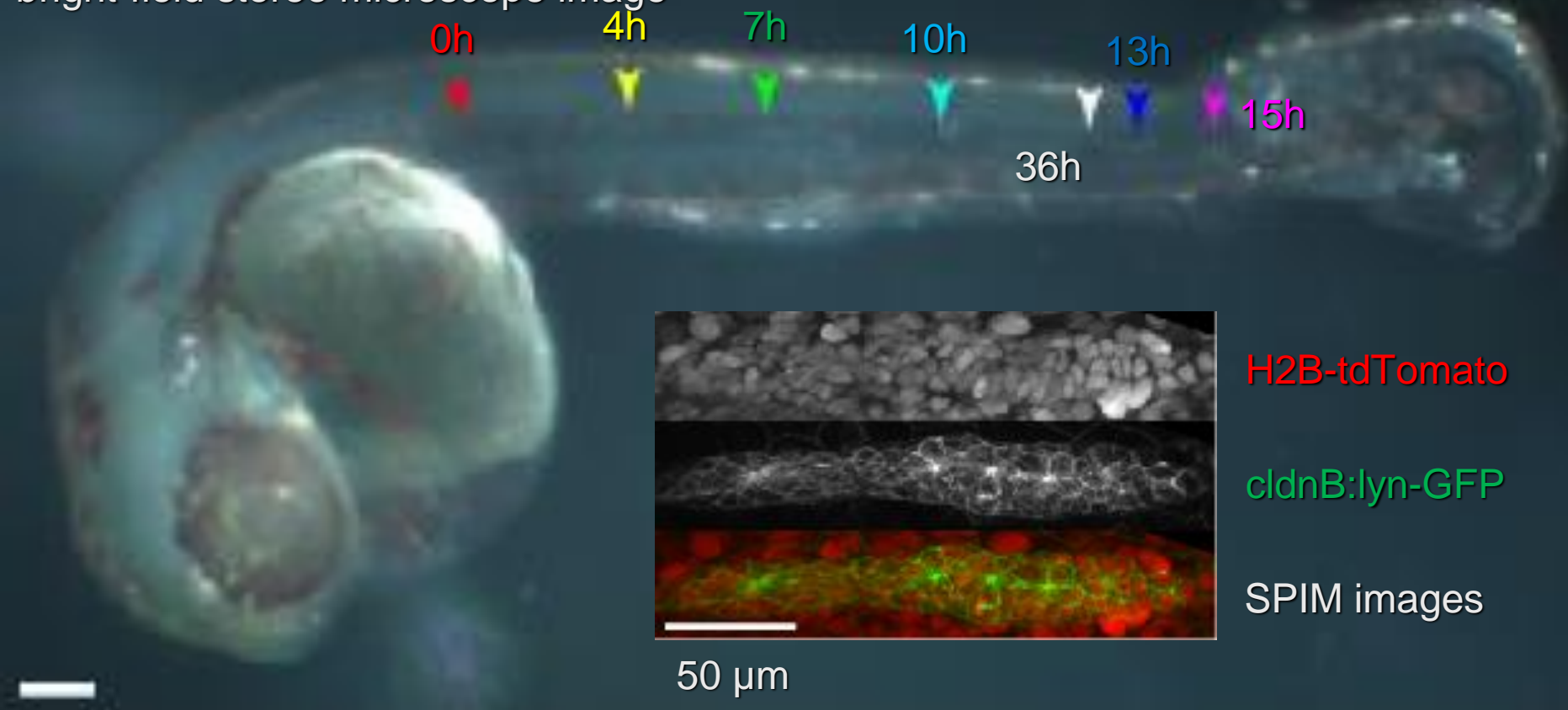
0h 4h 7h 10h 13h 15h

36h

H2B-tdTomato

cldnB:lyn-GFP

SPIM images

50 µm

100 µm

SPIM – Zebrafish – Lateral Line Imaging

Primordium Migration

0.00 h

nato

GFP

50 µm

100 µm
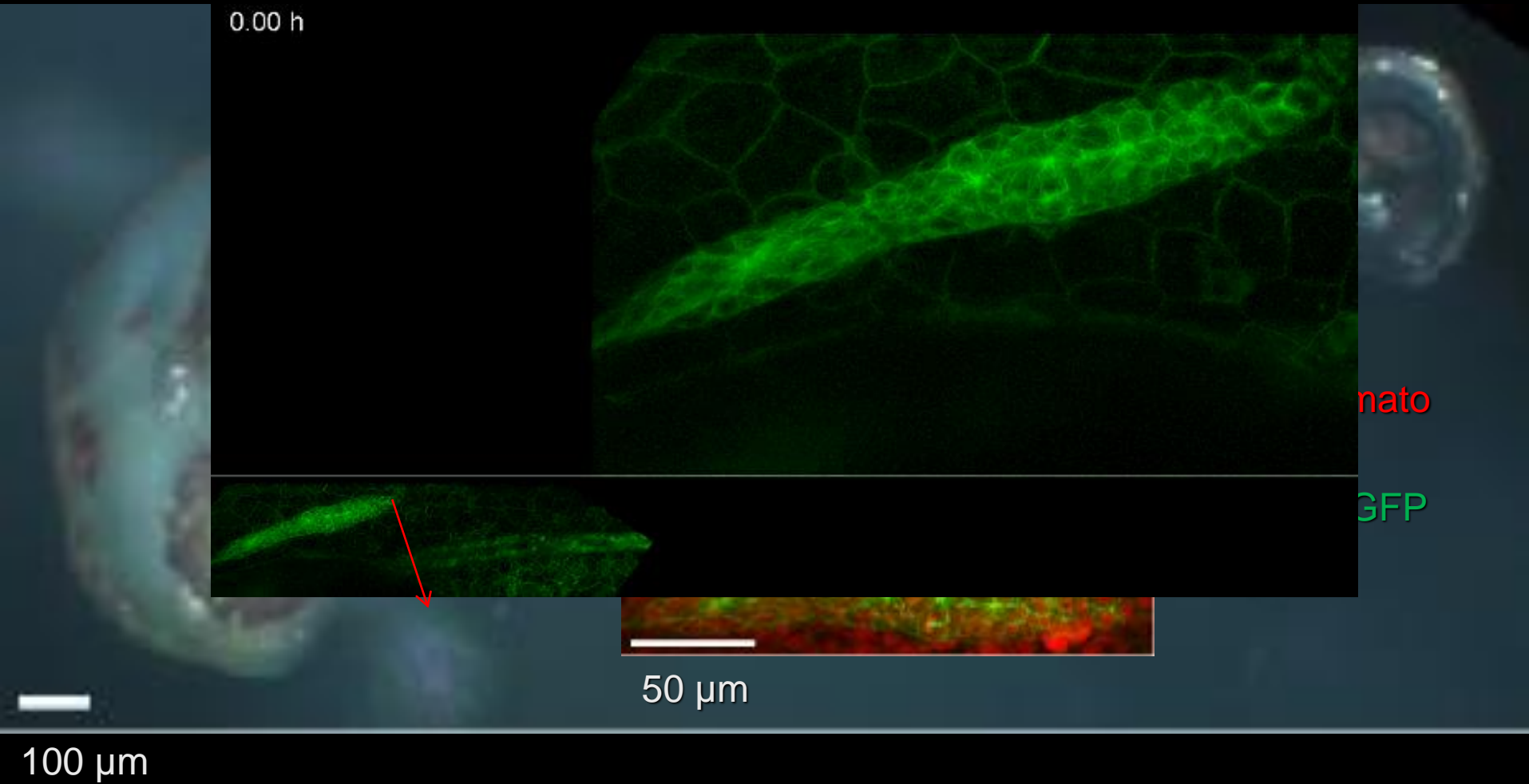
SPIM – Zebrafish – Lateral Line Imaging

Retrospective Lineaging

28.23 h

0h

6h

19h

28h

50 µm

20h 52m

C1.2

20h 56m

C1.2

21h 00m

C2.2

C2.1

20 µm

C1.2.1

C1.2.2
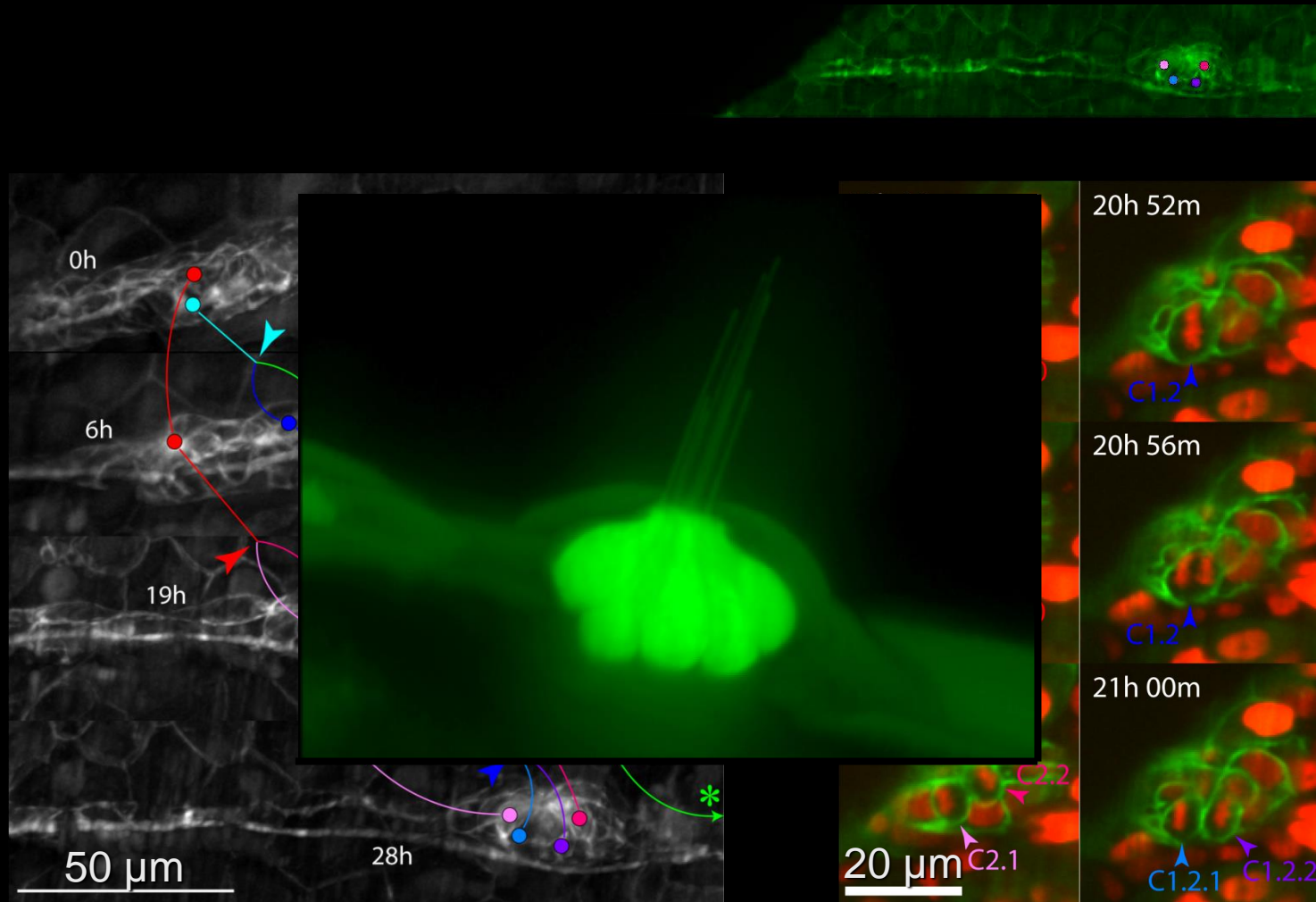
SPIM – Zebrafish – Lateral Line Imaging

Summary

- 4D data sets, sub-micron resolution up to 70 hours

- Retrospective lineage tracing for organogenesis studies

- Relies on the high SNR and low photo-toxicity of SPIM

SPIM: Drosophila Dorsal Closure

Spinning Disc Confocal
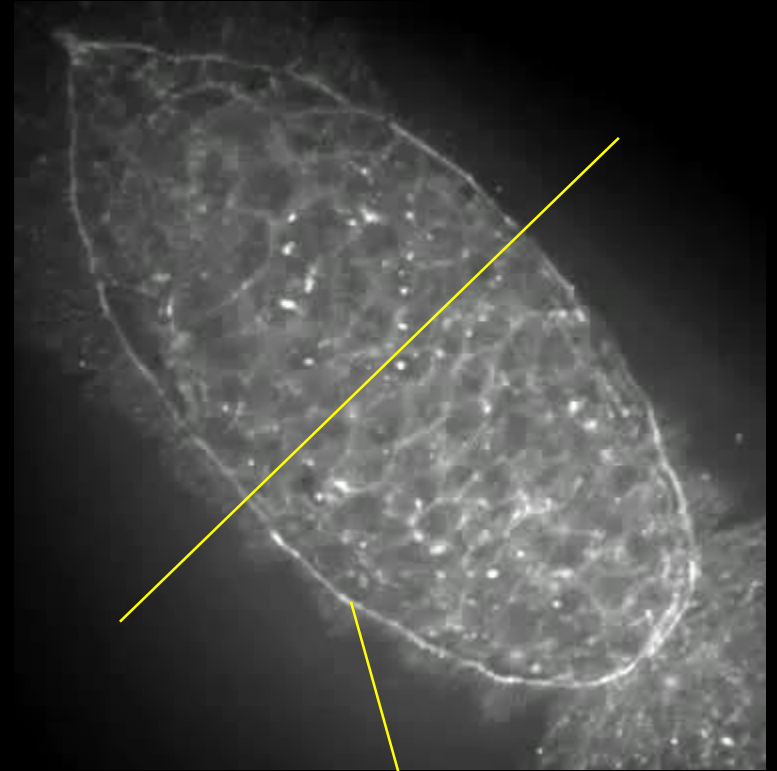
sqh-Moe-GFP

actin cable

SPIM: Drosophila Dorsal Closure
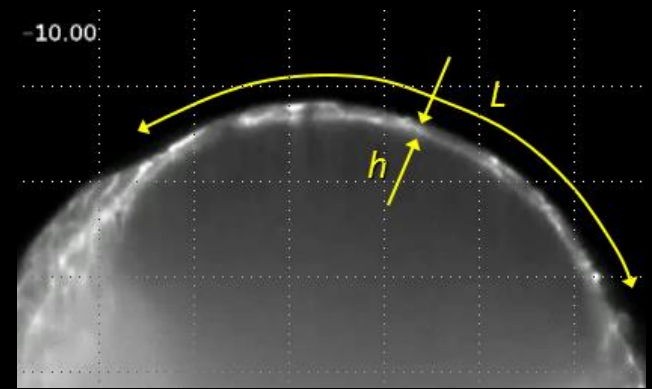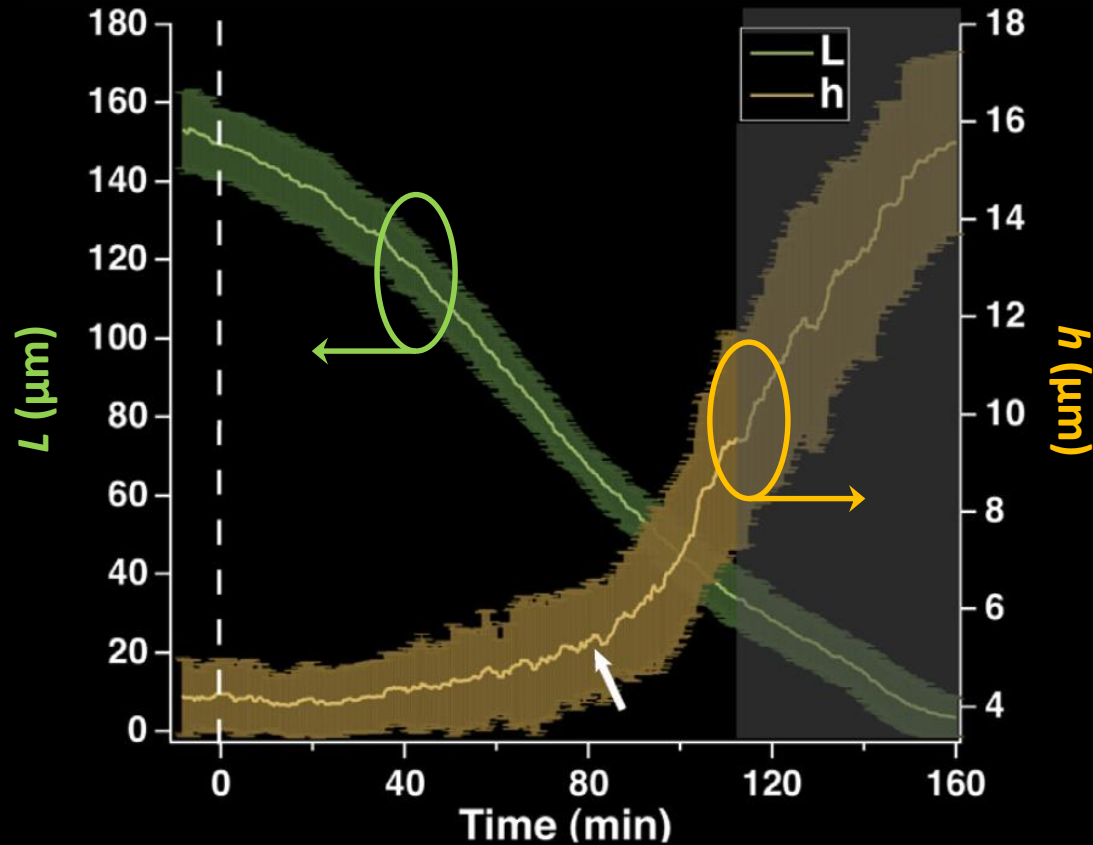
dorsal closure begins ~80 min *before* apico-basal elongation

cells decrease in volume, rather than just change shape

SPIM: Drosophila Dorsal Closure

quantitative multi-modal imaging

+

mathematical modelling of forces acting on cells

+

laser cutting, …

Before dorsal closure: Tensions are balanced

Epidermis   AS Cell   P   A

Actin cable tension

cell volume decrease

→ contractile force

→ tissue shrinkage
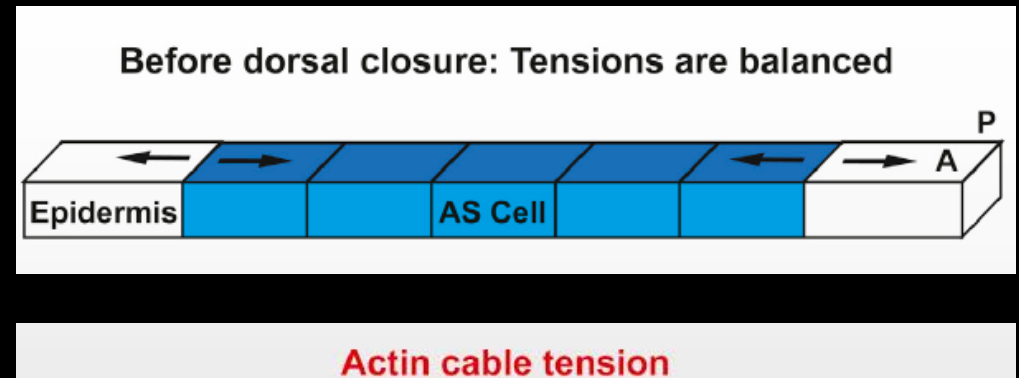
Optical Clearing of Fixed Tissue

mouse embryo, embedded in agarose gel

in H_2O
"natural"
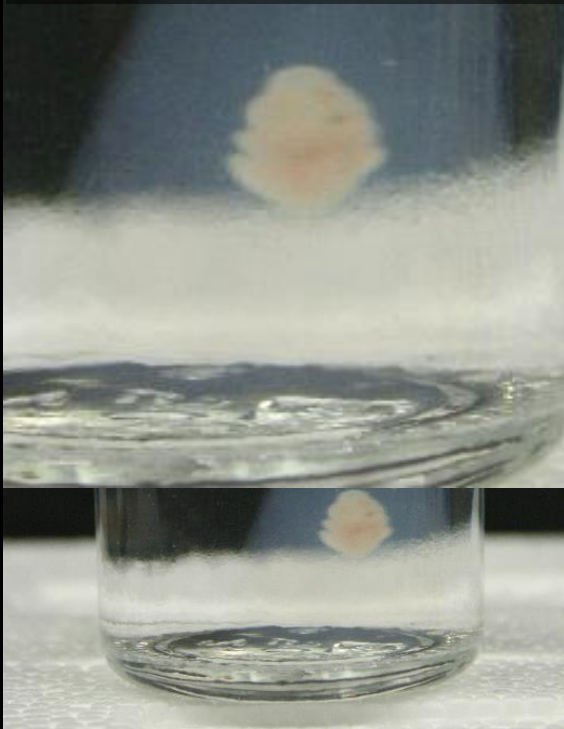
in CH_3OH
dehydrated
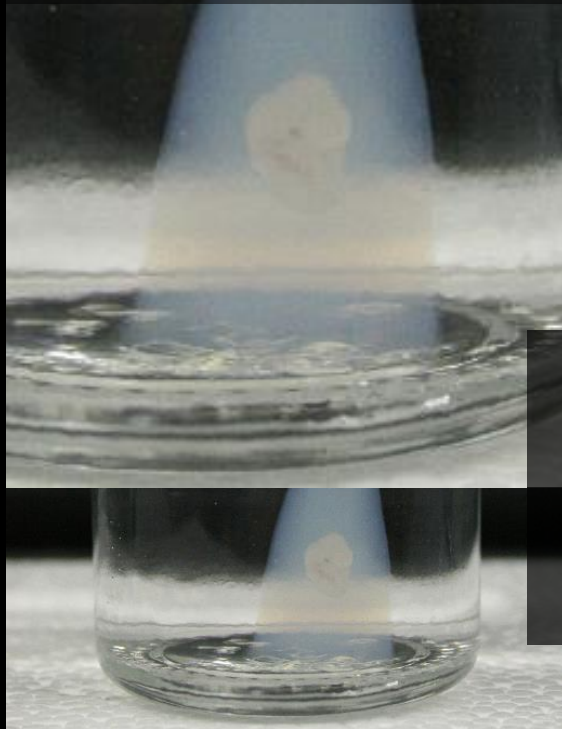
in BABB*
optically cleared
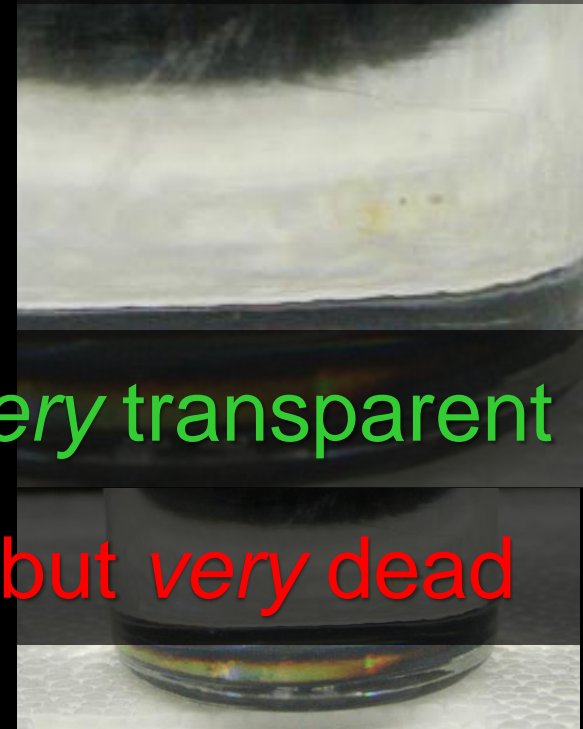
very transparent
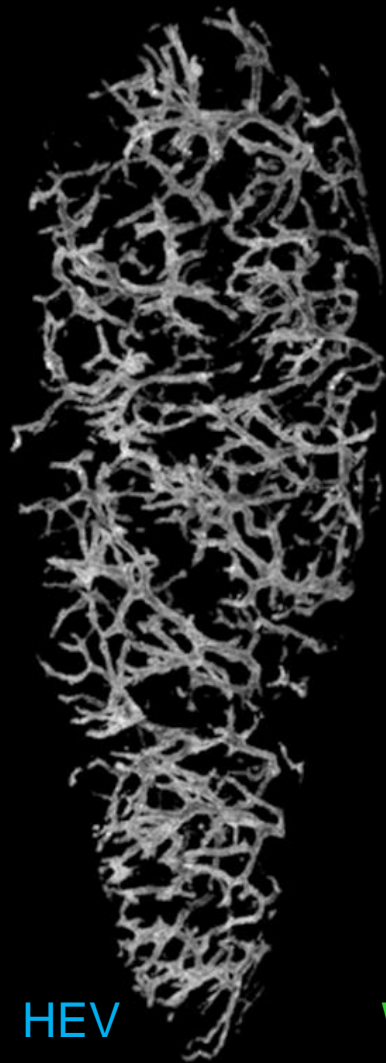
but *very* dead

* 1:2, benzyl alcohol : benzyl benzoate

Immunology

The Lymphatic Network
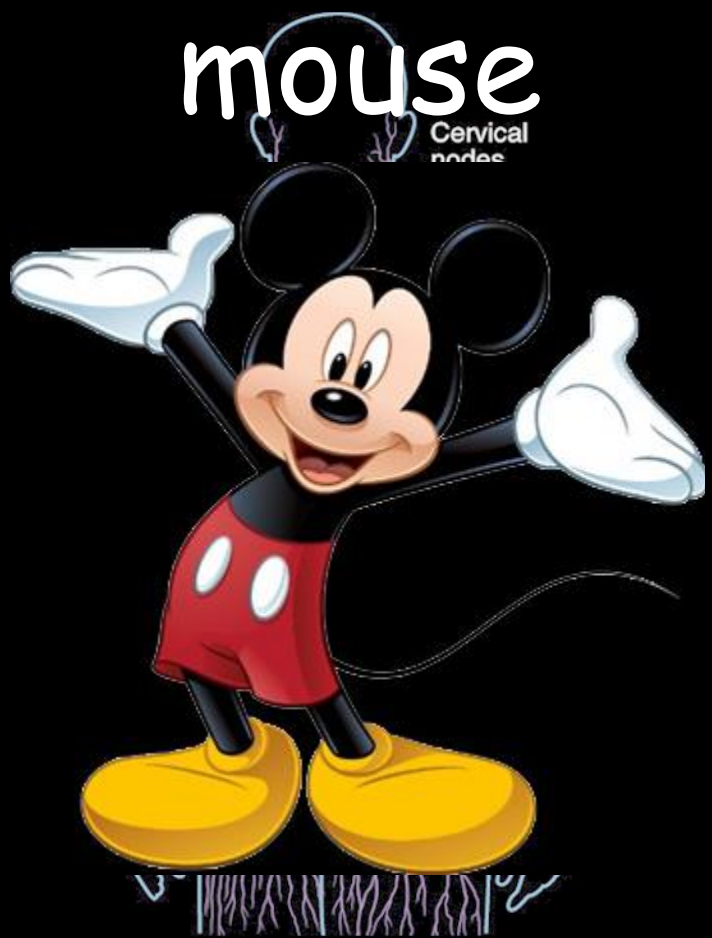
Lymph Node Schematic

Immunology: 3D Spatial Quantification

HEV   WT B-cells   CXCR5$^{-/-}$ B-cells   Overlay
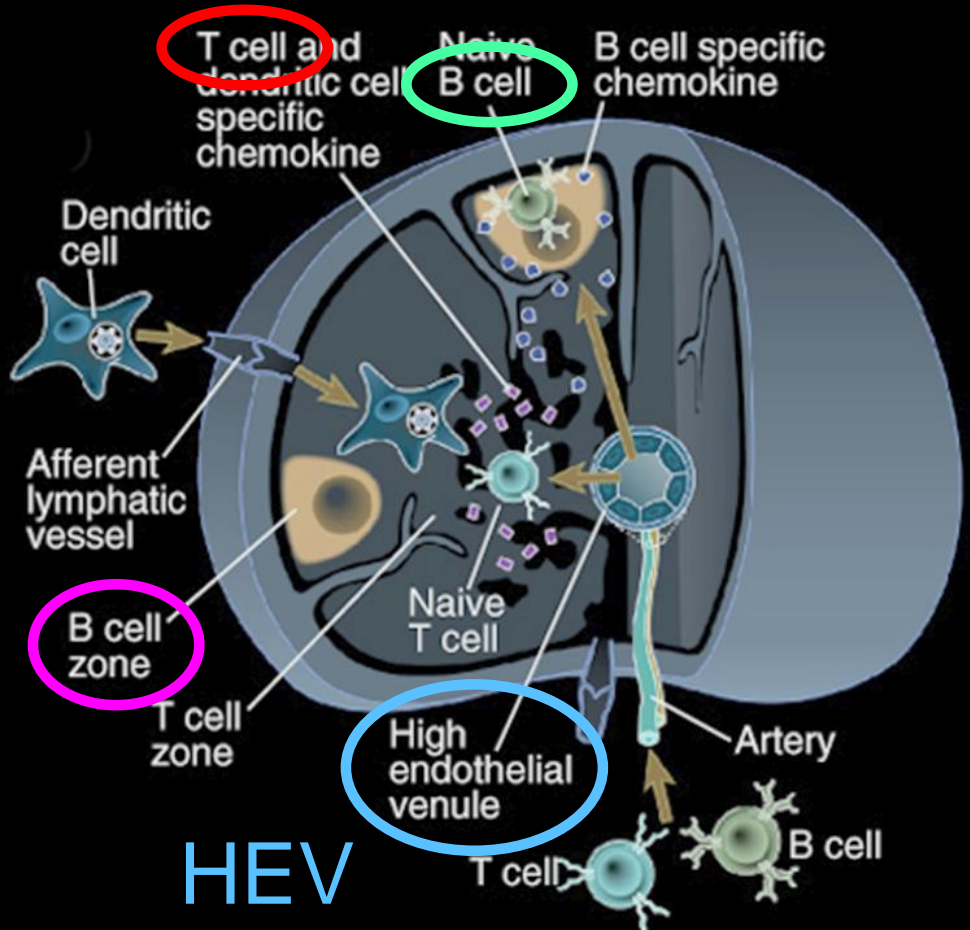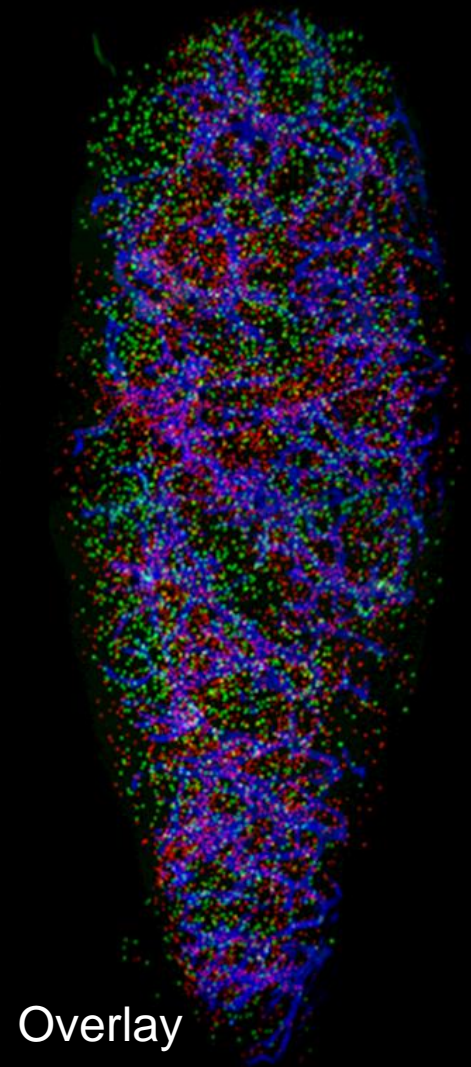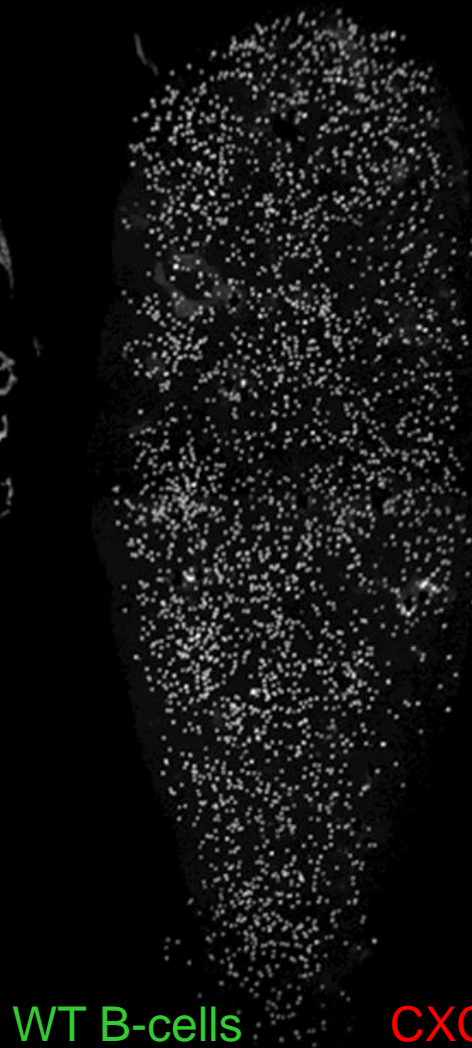
Immunology: 3D Spatial Quantification

3D Data

HEV = entry-way of B-cells into LN
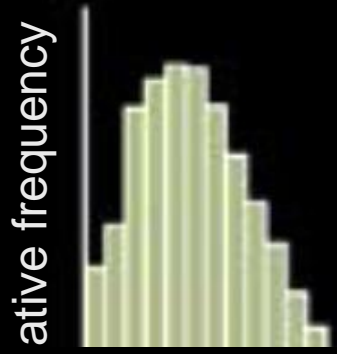
B-cells migrate to B-cell zones

Analyze B-cell to HEV 3D distances
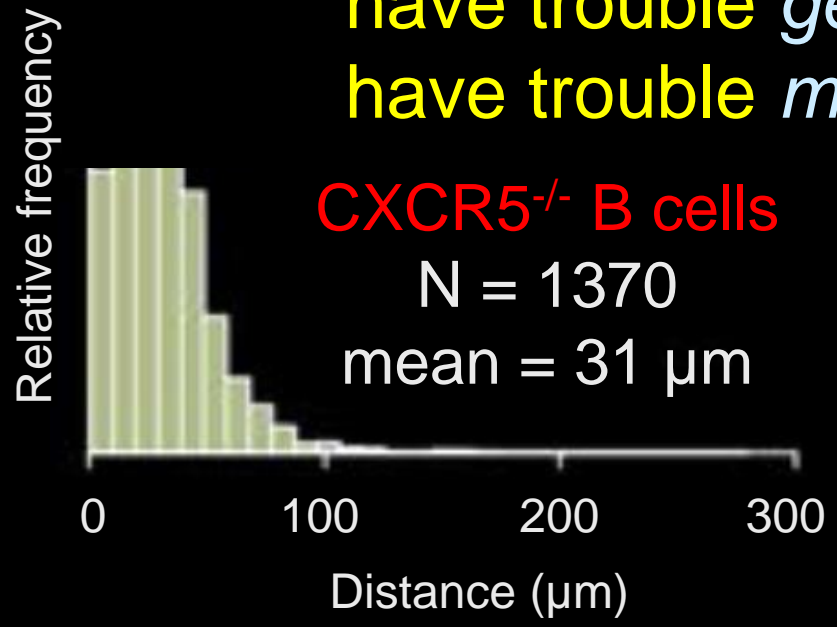

Overlay

Immunology: 3D Spatial Quantification

3D Data
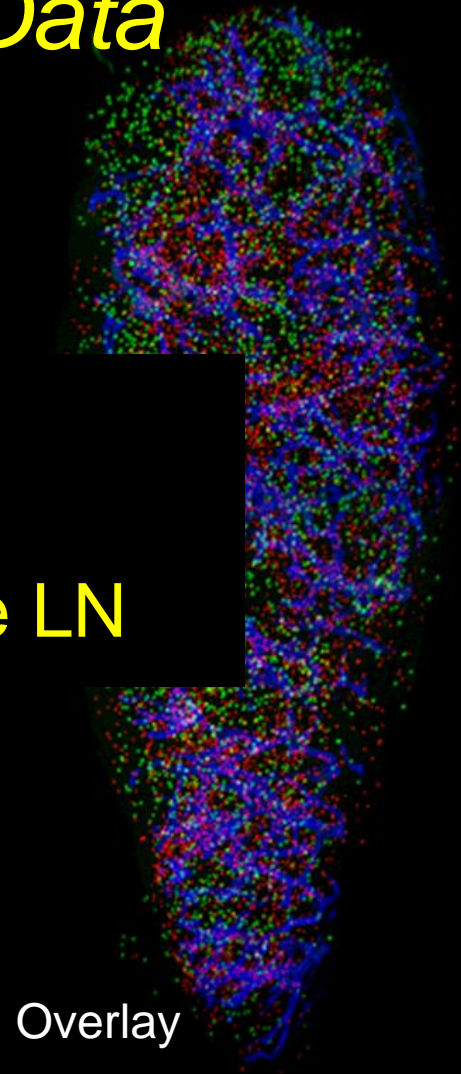

WT B cells
N = 6820
mean = 61 μm

CXCR5$^{-/-}$ B cells:
    have trouble *getting to* the LN
    have trouble *moving within* the LN
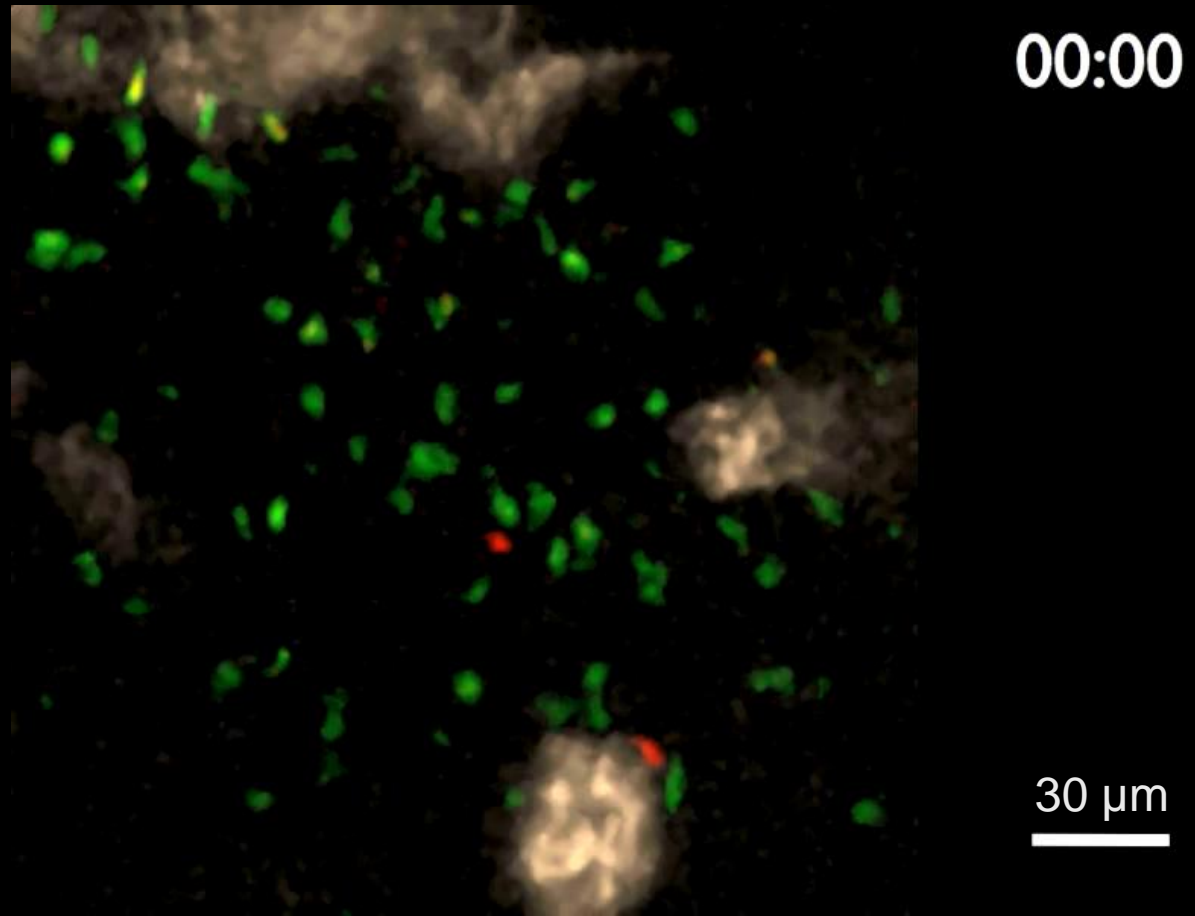
CXCR5$^{-/-}$ B cells
N = 1370
mean = 31 μm

Relative frequency

Distance (μm)

Overlay

Mayer *et al*, *Computation & Mathematical Methods in Medicine*, 2012

Immunology: Intravital Multi-photon Microscopy
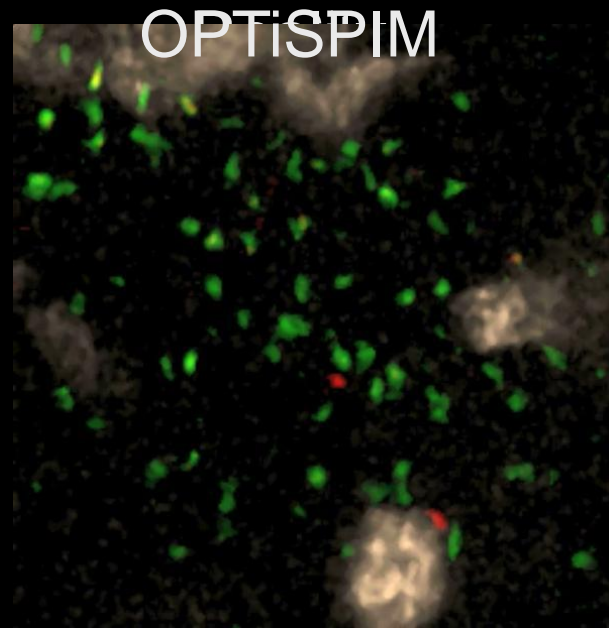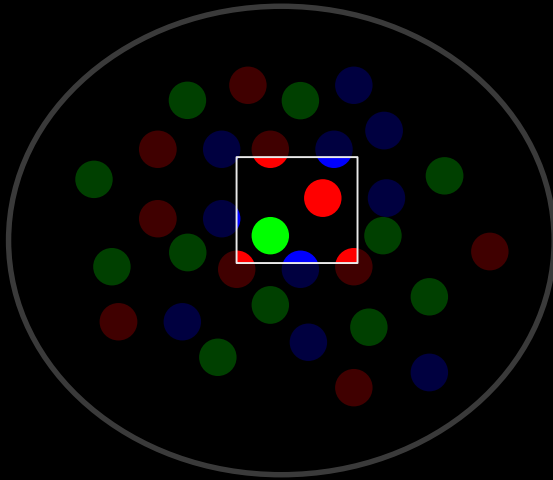

HEV
WT T-cells
mutant T-cells

Dynamics!

but…

00:00

30 µm

Faroudi *et al*, *Blood* **116**(25) pp. 5536-5547 (2010)

Immunology: Intravital Multi-photon Microscopy

Multi-photon Model System

OPTiSPIM

- no or limited 3D spatial information on whole-organ level
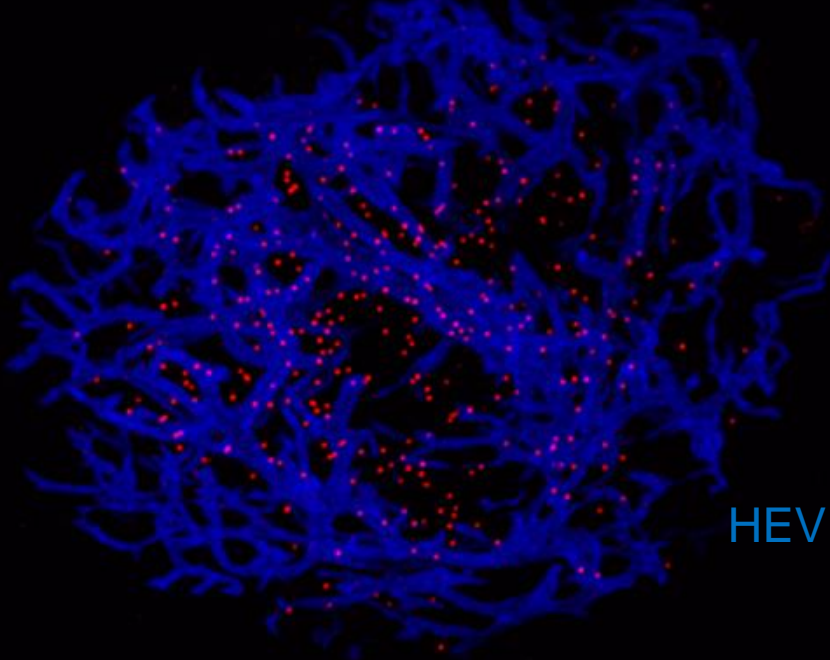
- supra-physiological #s of cells used

Complement intravital microscopy with fixed-sample OPTiSPIM:

Comprehensive analysis of *entire lymph nodes* with *single-cell* resolution at *physiological concentrations*
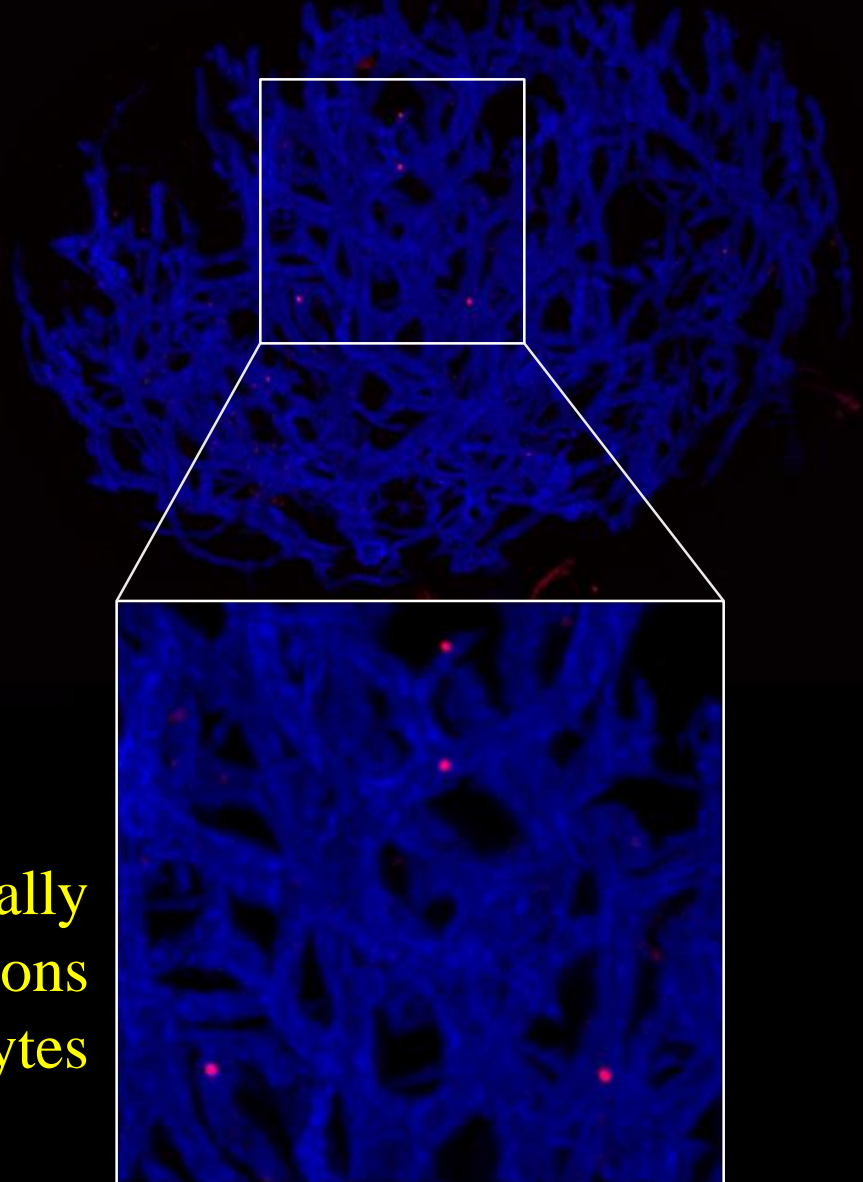
Immunology: SPIM & Rare Cells

1×10^5 T cells injected

1×10^3 T cells injected

HEV

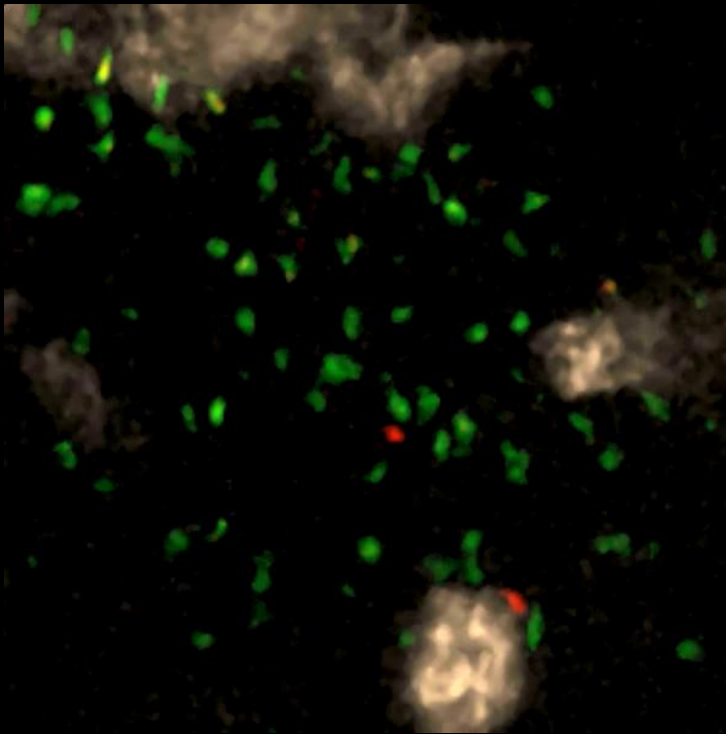concentrations needed
for intra-vital imaging

physiologically
relevant concentrations
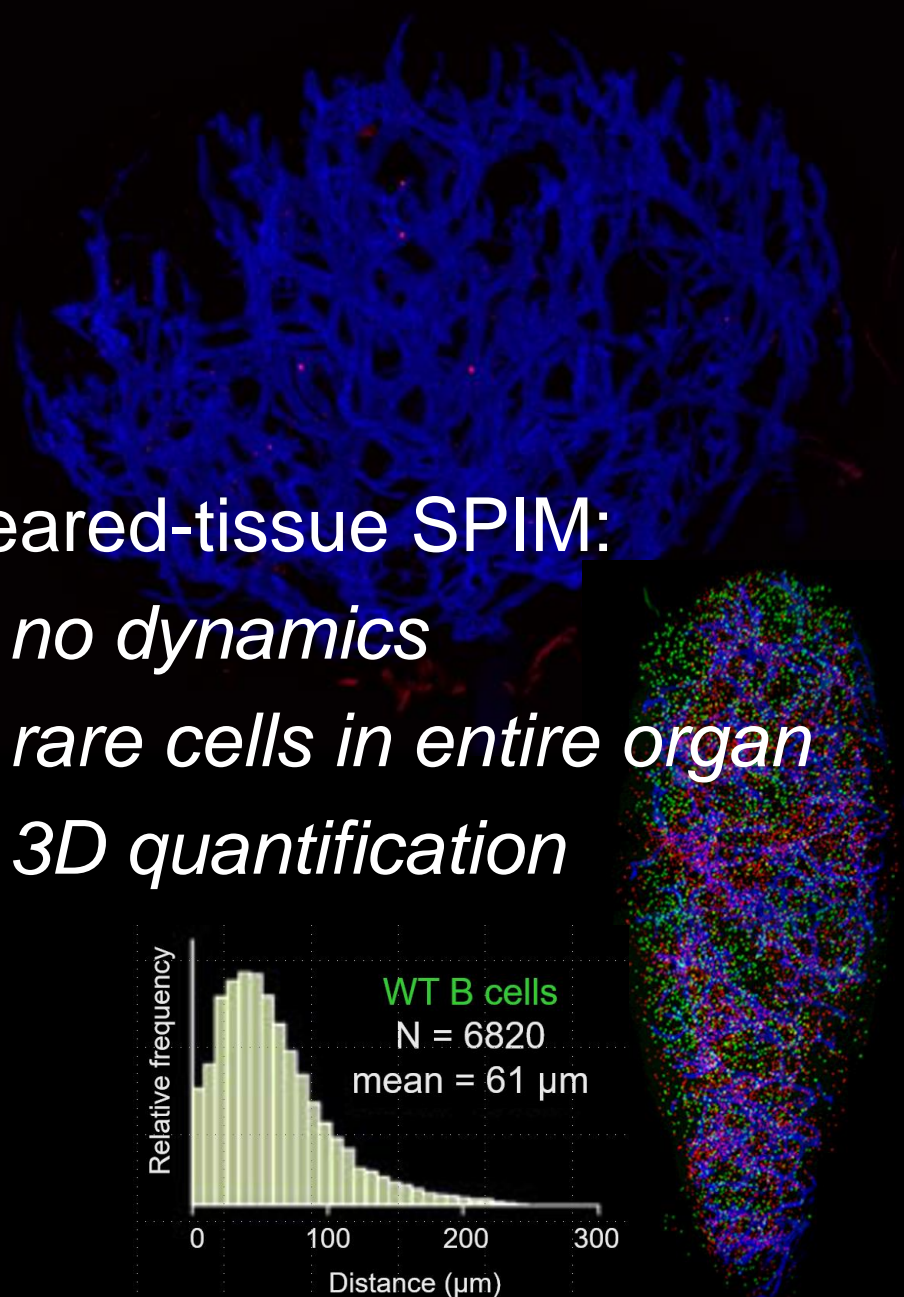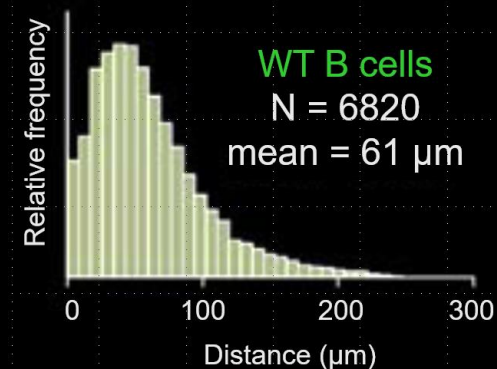for antigen-specific lymphocytes

Immunology: Summary

00:00

cleared-tissue SPIM:

☹ *no dynamics*

☺ *rare cells in entire organ*

☺ *3D quantification*

Intra-vital microscopy:

☺ *dynamics*

☹ *volume imaged*

☹ *cell densities*

WT B cells
N = 6820
mean = 61 μm

Relative frequency

0   100   200   300

Distance (μm)

Outline

Introduction to Microscopy

Selective Plane Illumination Microscopy (SPIM)

How it Works

Live Applications

Optical Clearing & Fixed Sample Applications

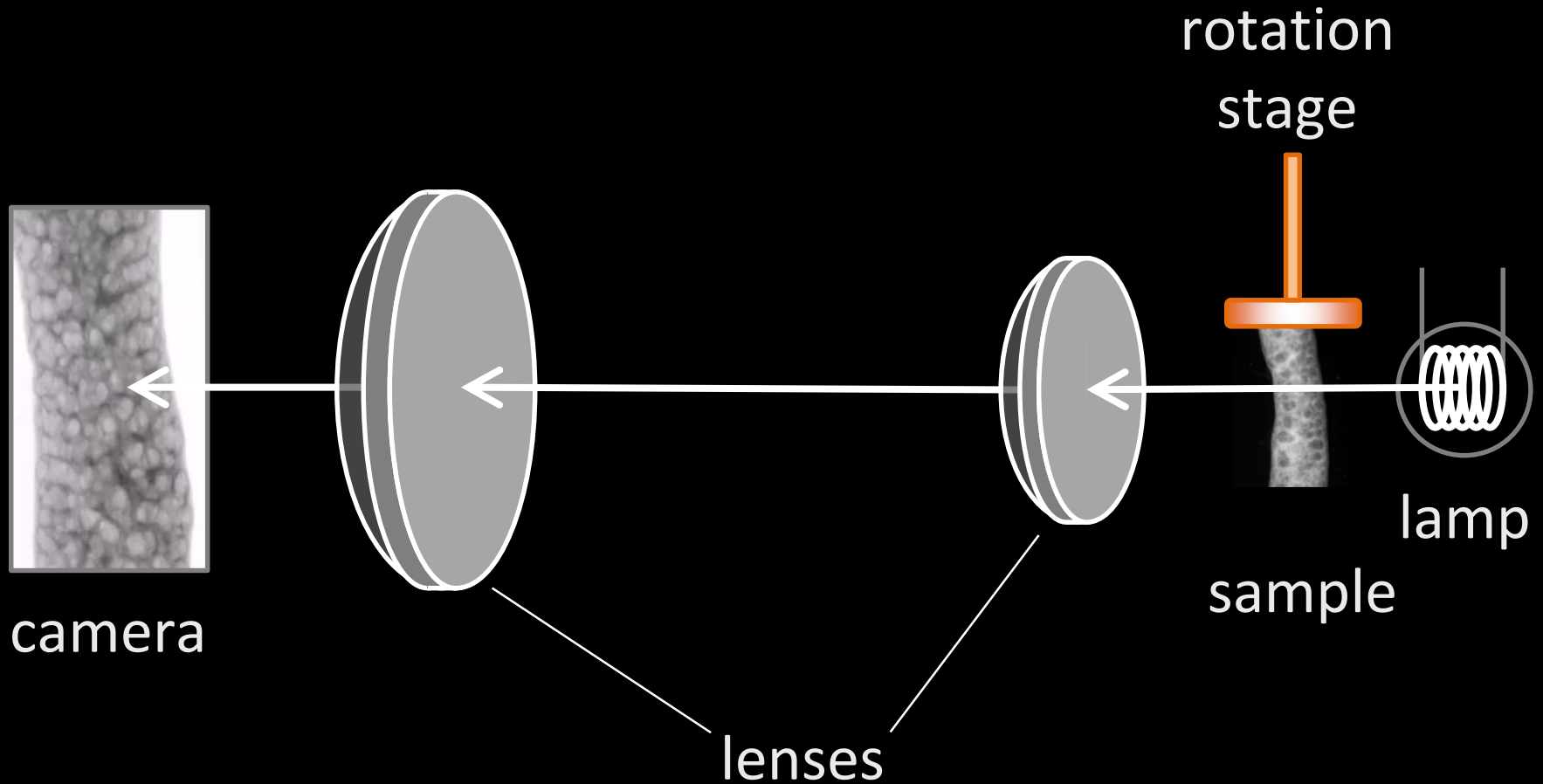## Optical Projection Tomography (OPT)

How it Works

Applications
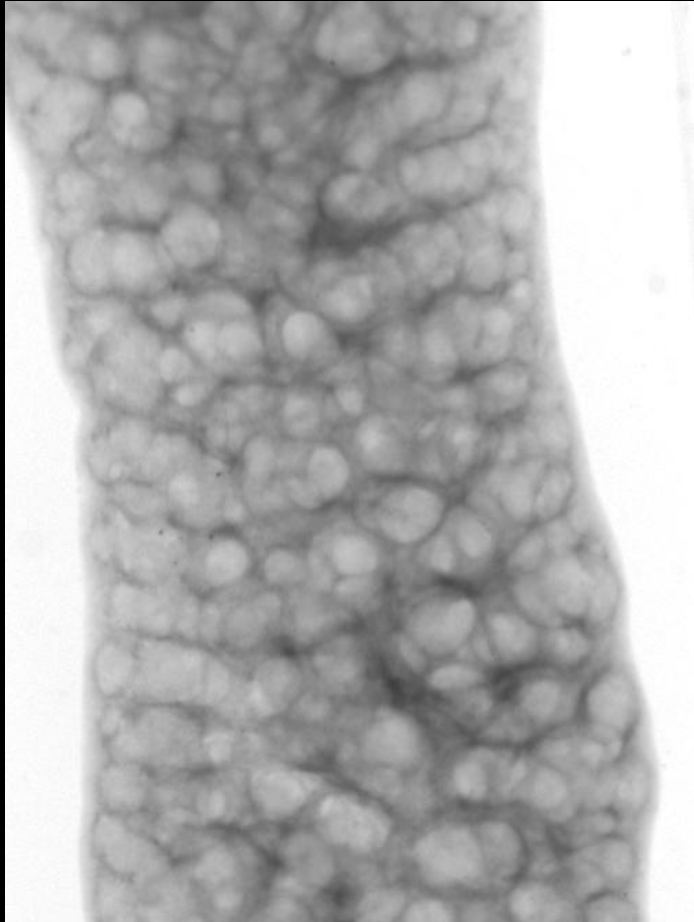
The OPTiSPIM

Applications

Frontiers in Mescopic Imaging

OPT: Transmission

OPT = Optical Projection Tomography

rotation stage

lamp

sample

camera

lenses

OPT: 3D Reconstruction via Back-Projections

record views from 360°

intensity line profile

mouse spleen
raw OPT projection
(inverted contrast)

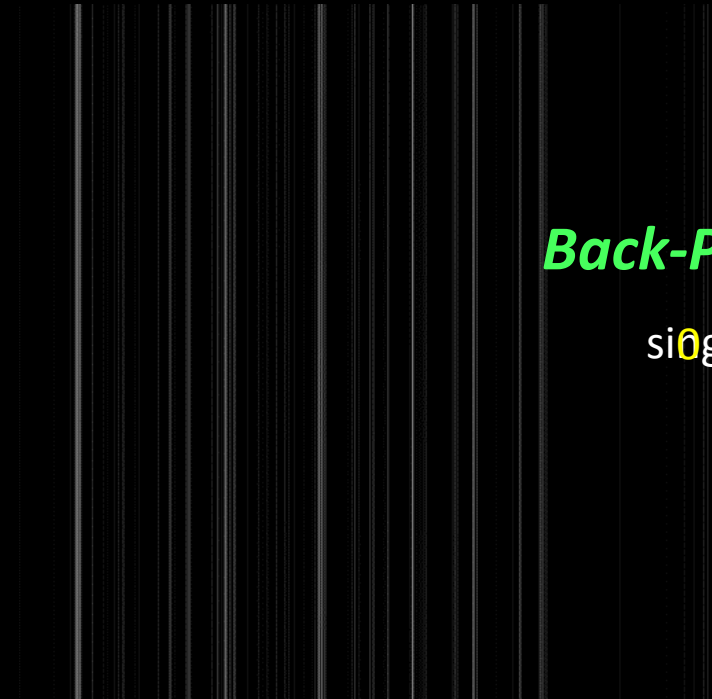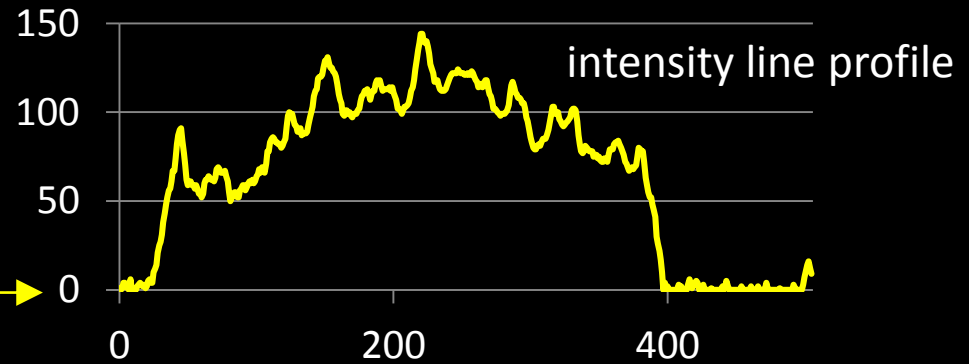# OPT: 3D Reconstruction via Back-Projections

record views from 360°

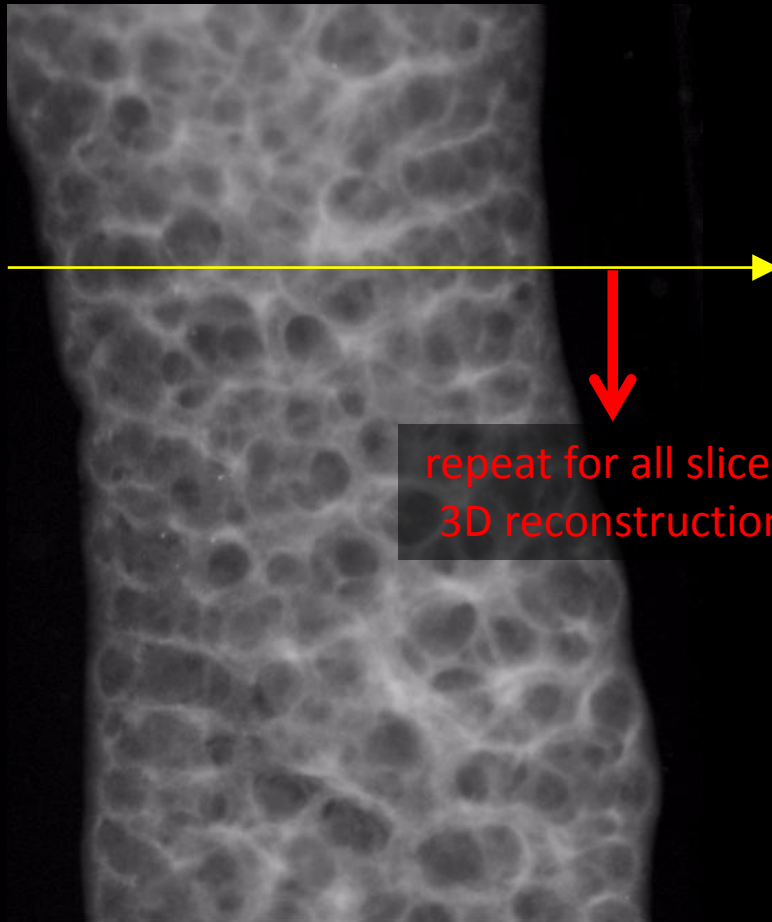mouse spleen
raw OPT projections
(inverted contrast)

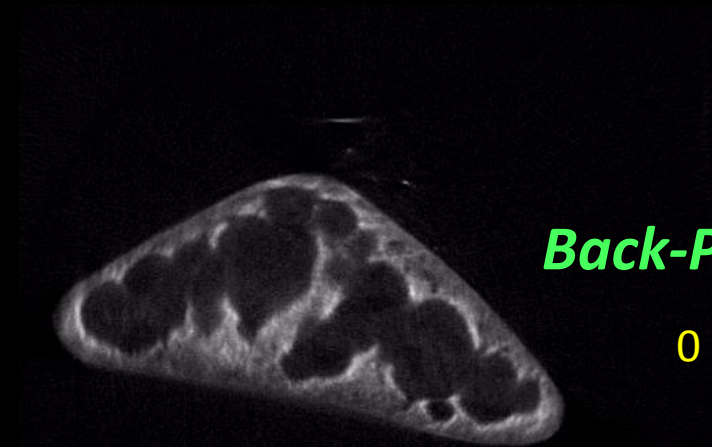intensity line profile

Back-Project

single view 0   360°

OPT: 3D Reconstruction via Back-Projections

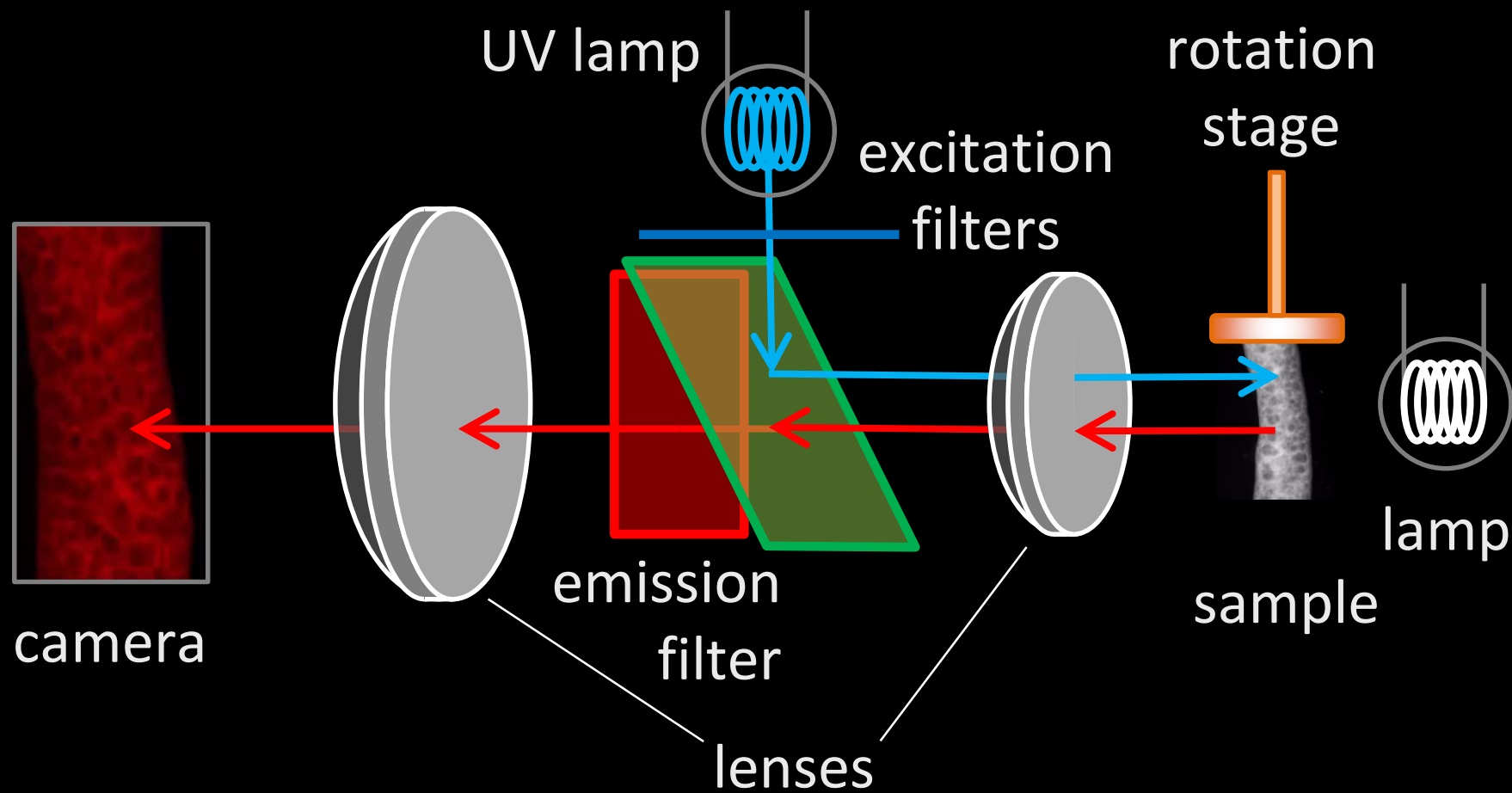*record views from 360°*

repeat for all slices:
3D reconstruction

Back-Project

0 - 360°

mouse spleen
raw OPT projections
(inverted contrast)

OPT: 3D imaging of *fluorescence* & *transmission*

UV lamp

rotation stage

excitation filters

camera

emission filter

lenses

sample

lamp

reconstruct fluorescence as transmission
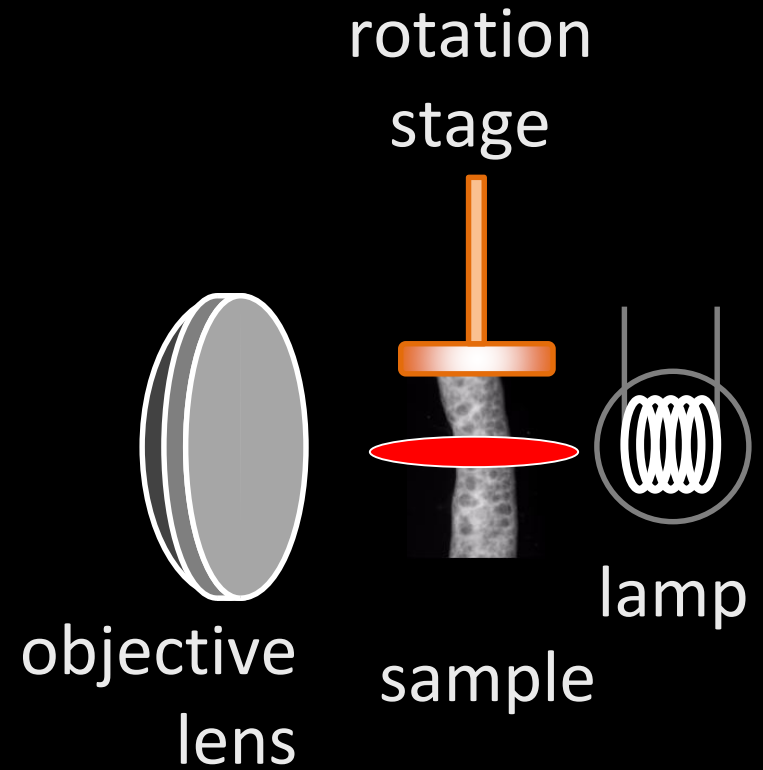
Point-spread Function (PSF)

objective lens:

high NA → high resolution

BUT: low NA → large depth of field
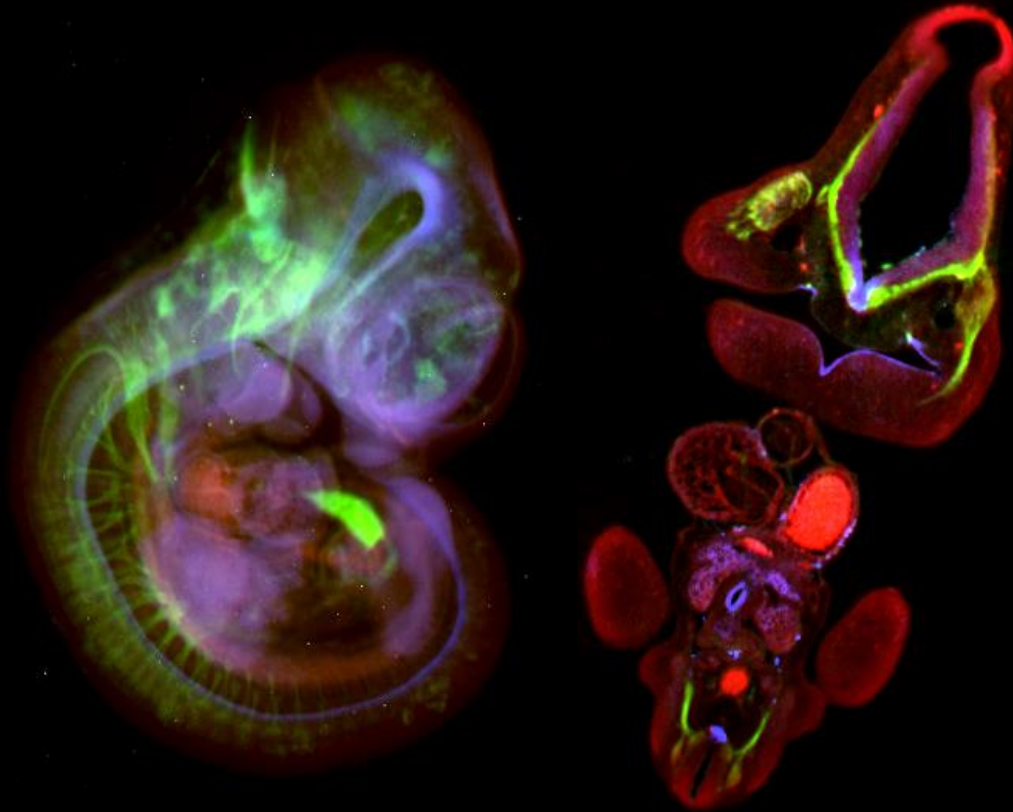
long WD → large samples

rotation
stage

objective
lens

sample

lamp

OPT: Fluorescent Mouse Embryo

Multi-Channel Fluorescence OPT

E10.5 mouse embryo

Red: autofluorescence
Blue: HNF3β, Alexa 488
Green: neurofilament, Cy3

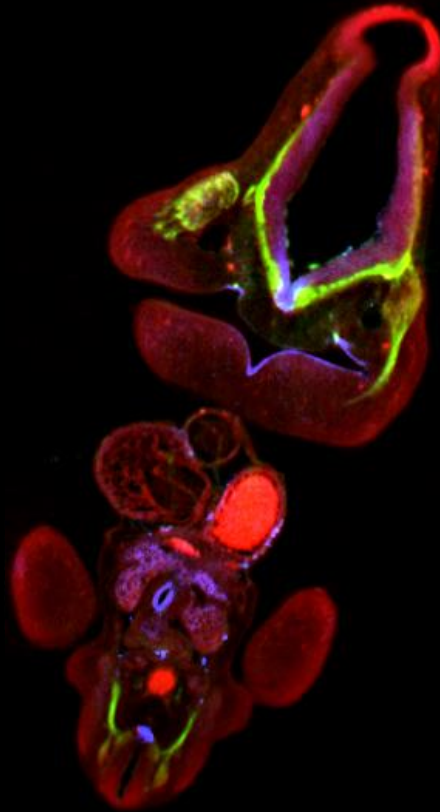*antibody labelling*

Raw Projection   Reconstruction

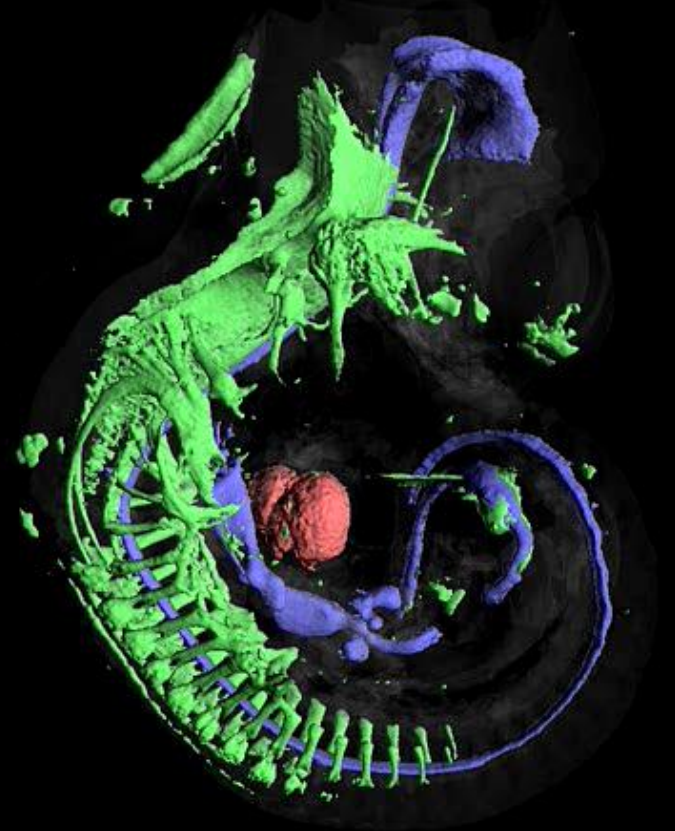# OPT: Fluorescent Mouse Embryo

Multi-Channel Fluorescence OPT

E10.5 mouse embryo

Raw Projection   *Reconstruction*   *3D Rendering*

OPT: Fluorescence + Transmission

Fetal mouse fore-limbs

Yellow: cartilage, alcien blue (TRANS)

Blue: mineralized, alizarin red (FLUOR)

Yellow: green autoFLUORescence

Blue: muscle, Xgal, green dye (TRANS)

Niamh Nowlan, CRG, Barcelona

Outline

The OPTiSPIM

UV lamp

excitation filters

rotation + translation stage

OPT + SPIM

camera

lenses

emission filter

sample

lamp

emission filter

translation stage
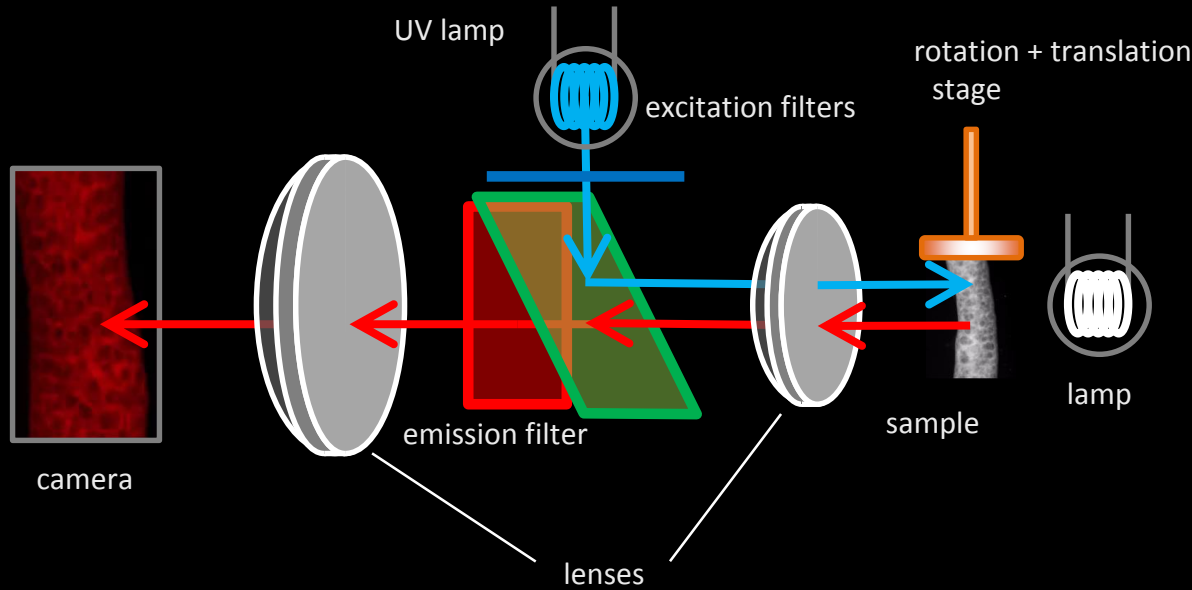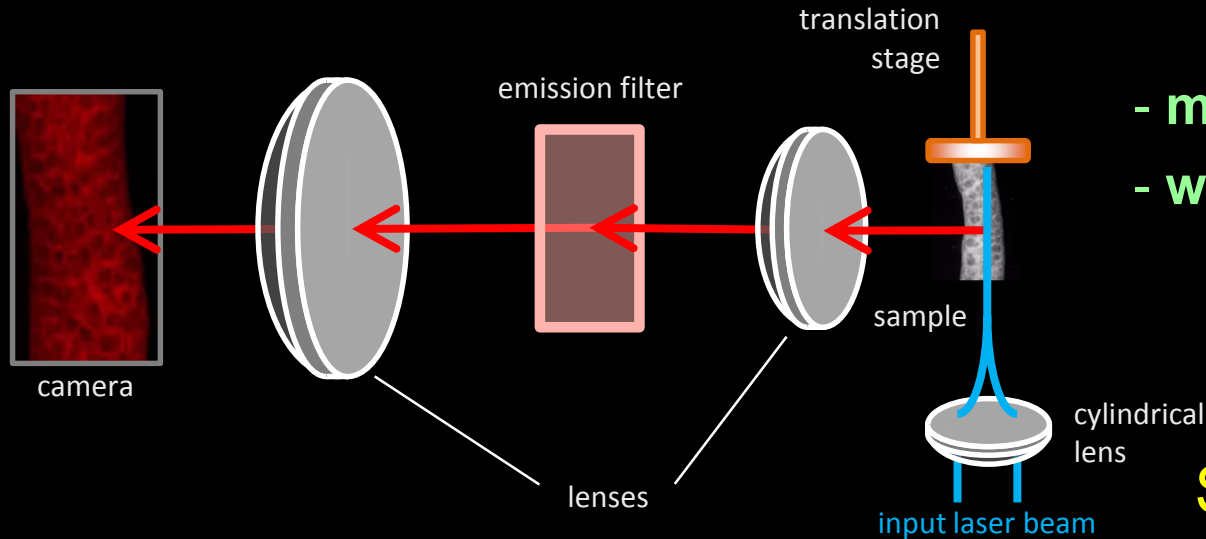
- **many shared components**
- **why not combine?**
  1 multi-modal instrument

camera

lenses

sample

cylindrical lens
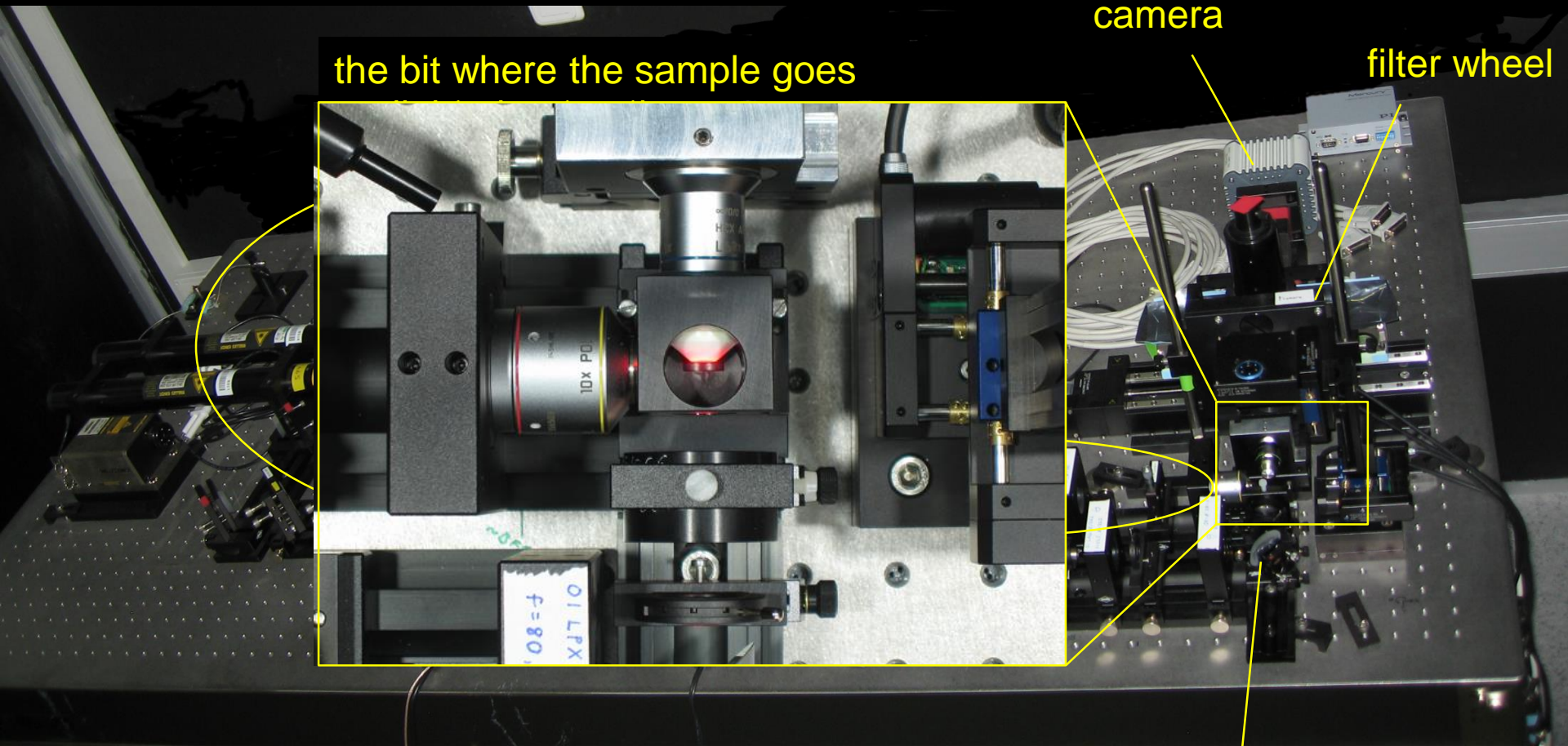
input laser beam
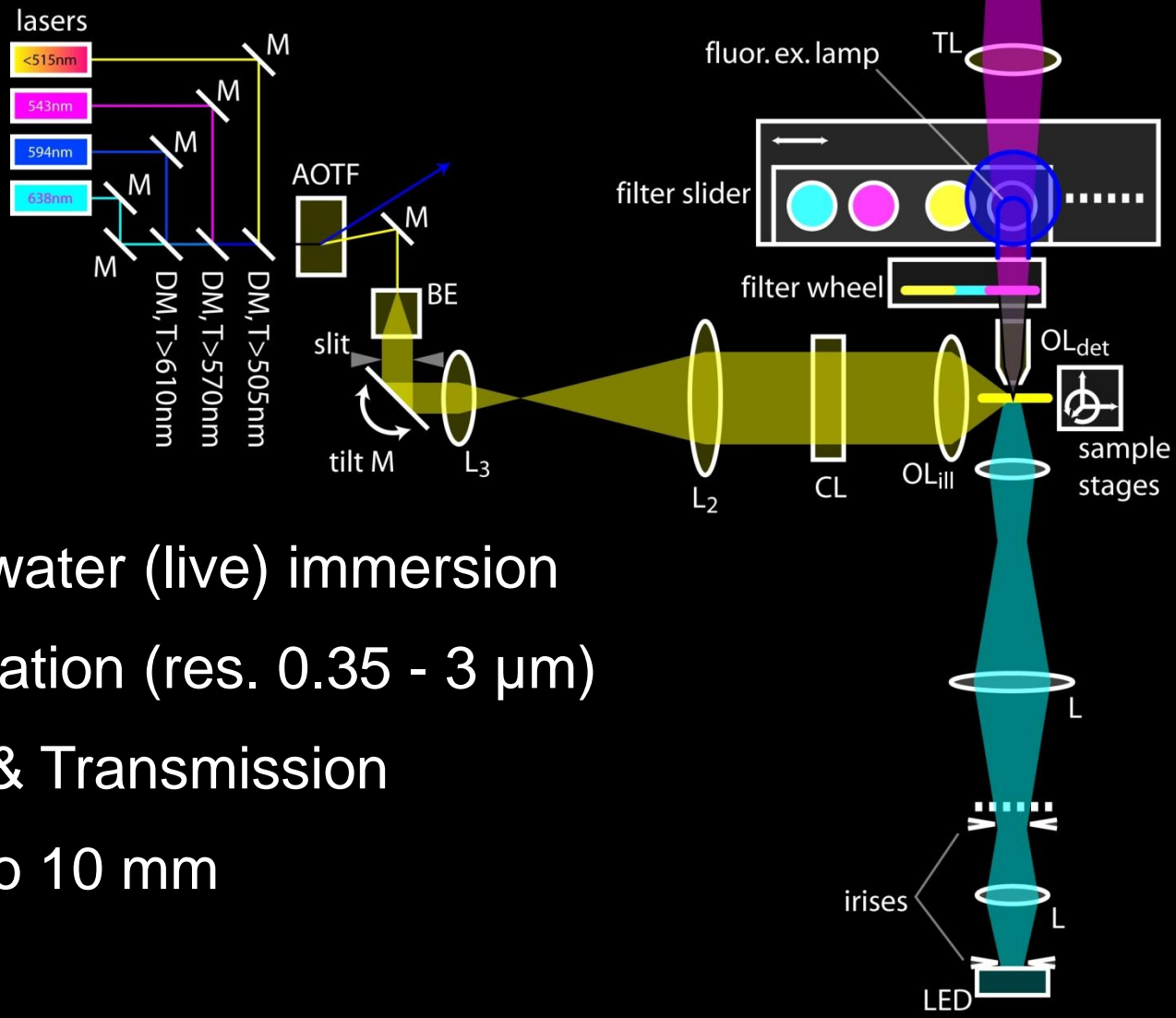
SPIM

The OPTiSPIM

- cleared (fixed) or water (live) immersion

- 5x to 63x magnification (res. 0.35 - 3 μm)

- 3D Fluorescence & Transmission
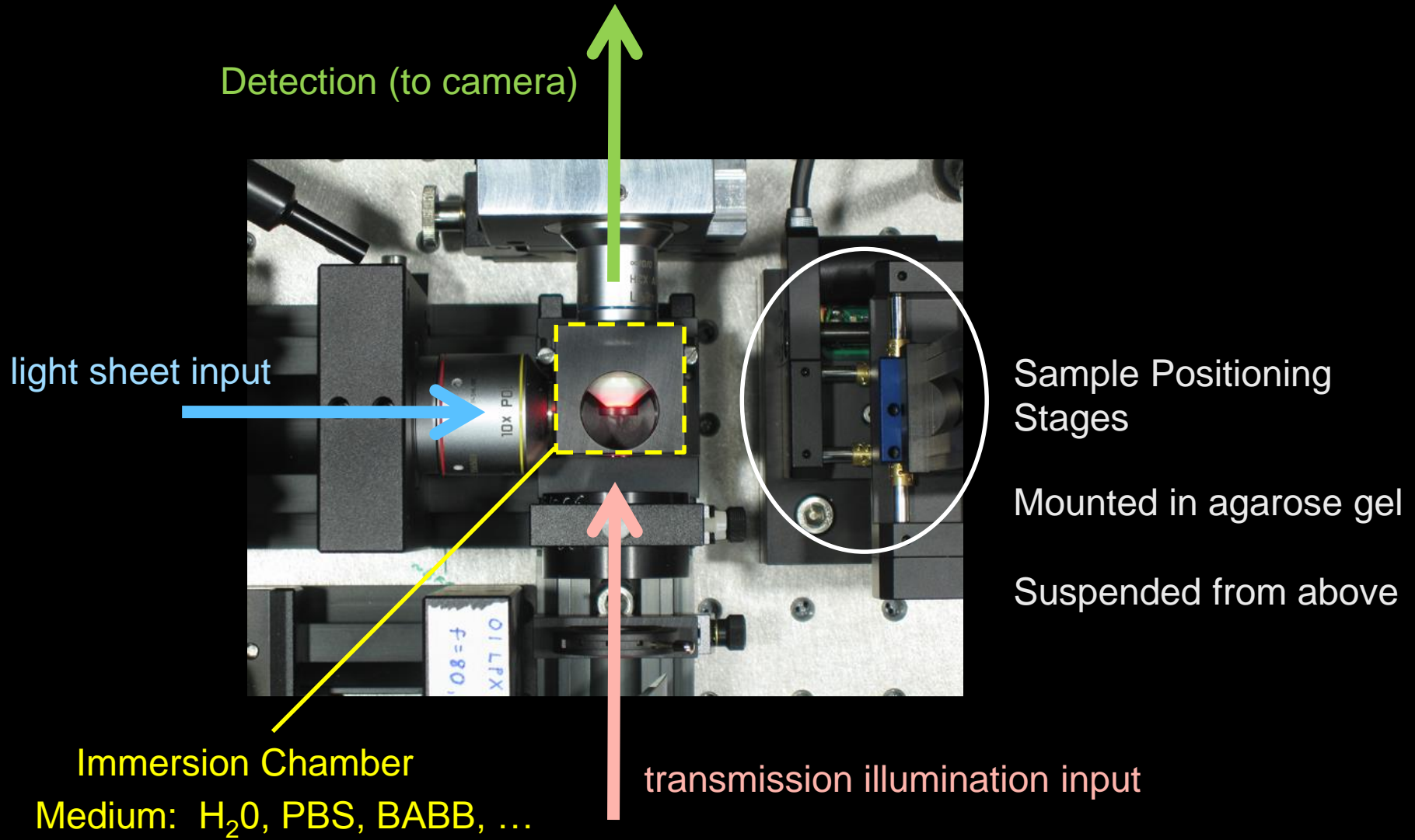
- samples: 50 μm to 10 mm

The OPTiSPIM

the bit where the sample goes

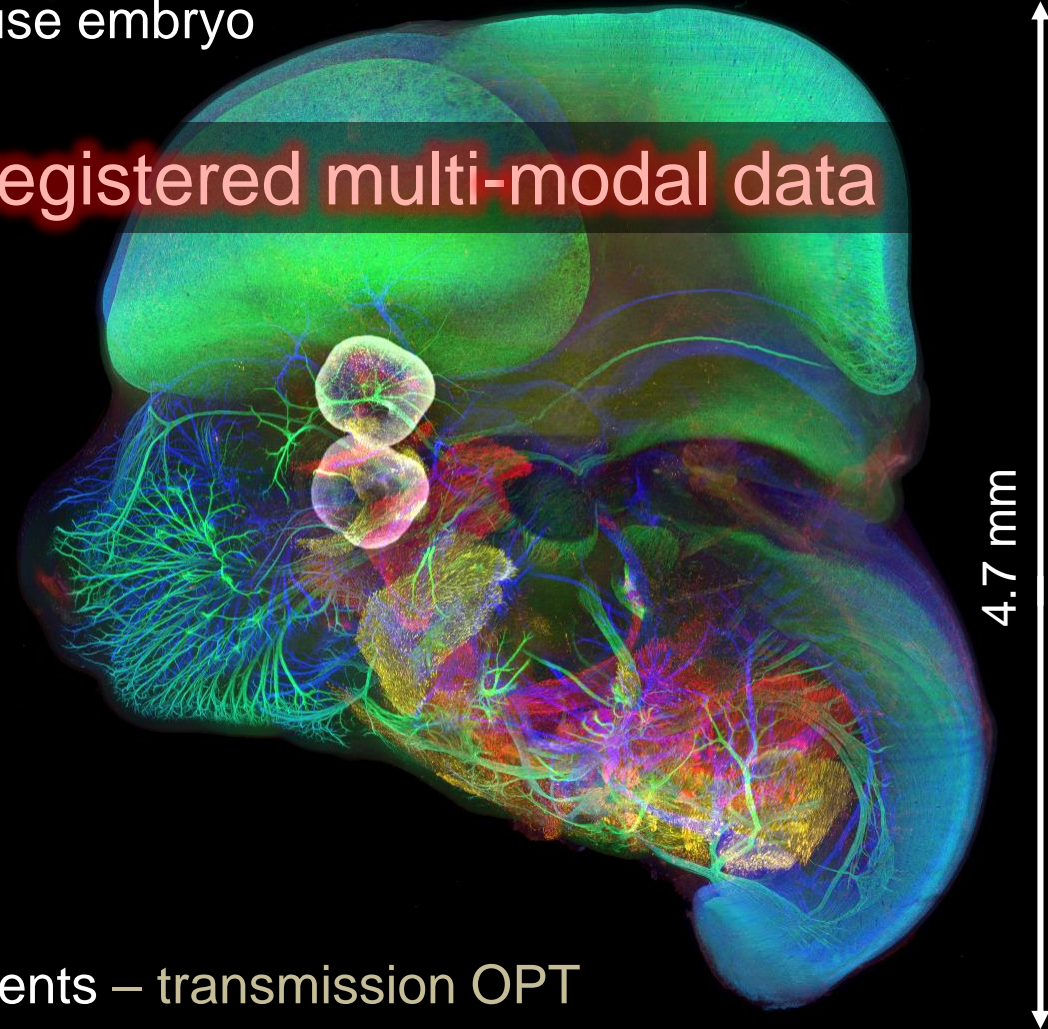camera

filter wheel

transmission illum. optics

The OPTiSPIM

Detection (to camera)

light sheet input

Immersion Chamber
Medium: H_2O, PBS, BABB, …

transmission illumination input

Sample Positioning Stages

Mounted in agarose gel

Suspended from above

Mouse Eyes, Nerves & Muscles

E12.5 mouse embryo

high resolution co-registered multi-modal data

4.7 mm
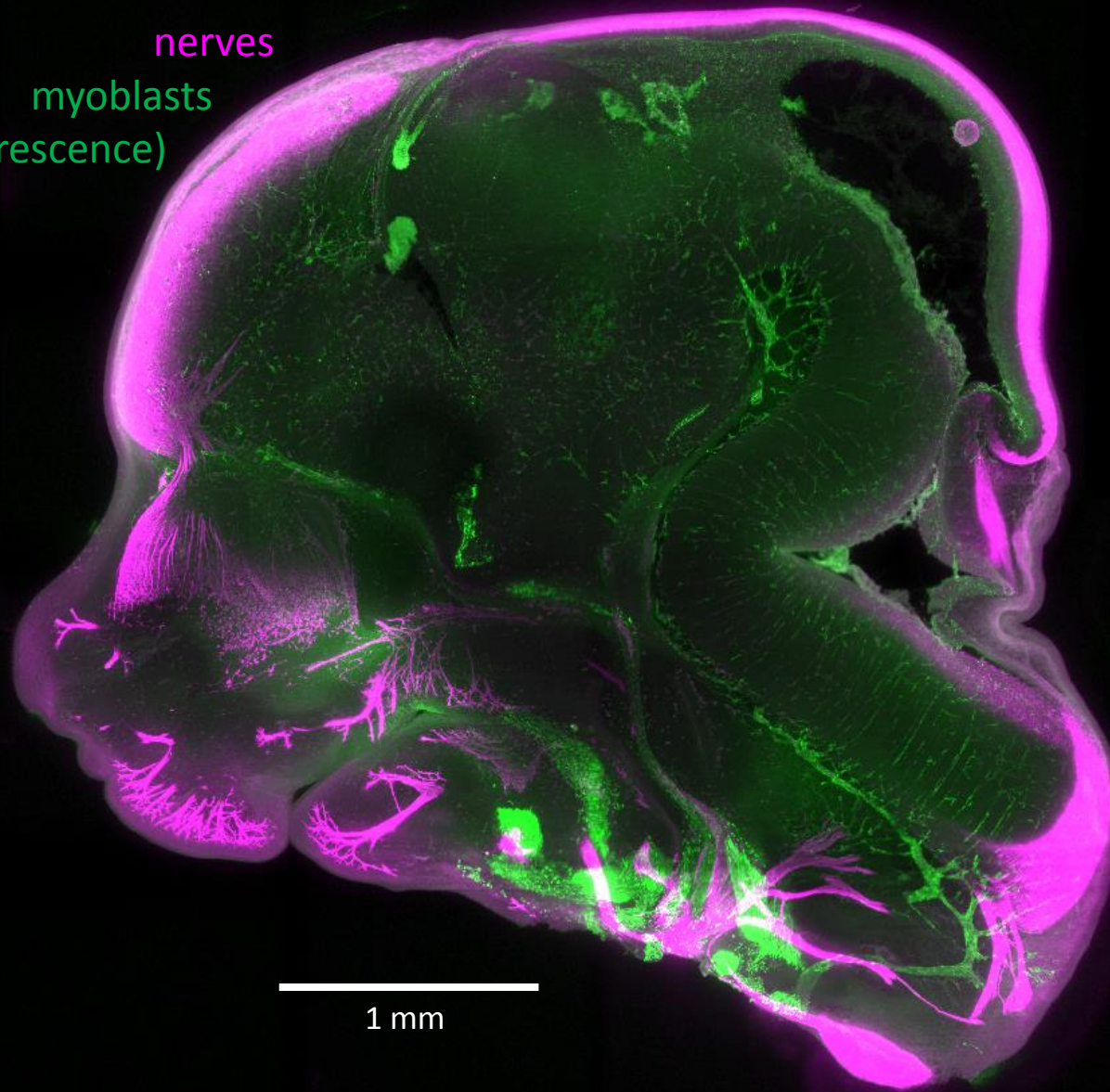
Eye pigments – transmission OPT

Neurofilament – fluorescence SPIM

Myoblasts – fluorescence SPIM

SPIM: High Signal-to-Noise Ratio

nerves

myoblasts

(autofluorescence)

1 mm

max-projection

slab, *dz* = 150 μm
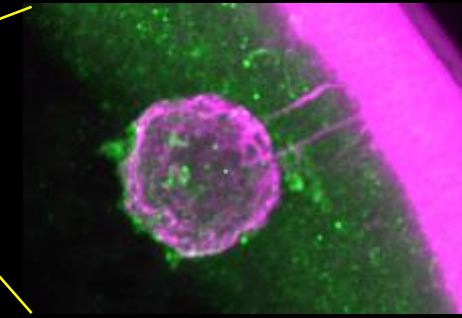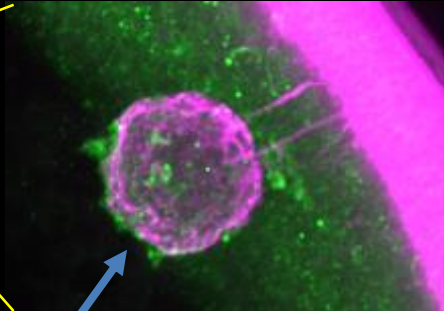
enhanced

contrast

SPIM: High Signal-to-Noise Ratio

nerves

myoblasts
(autofluorescence)

Is this a normal feature in brain development?

slices

1 mm

max-projection

slab, *dz* = 150 μm

enhanced contrast

SPIM: High Signal-to-Noise Ratio

nerves

myoblasts
(autofluorescence)

max-projection

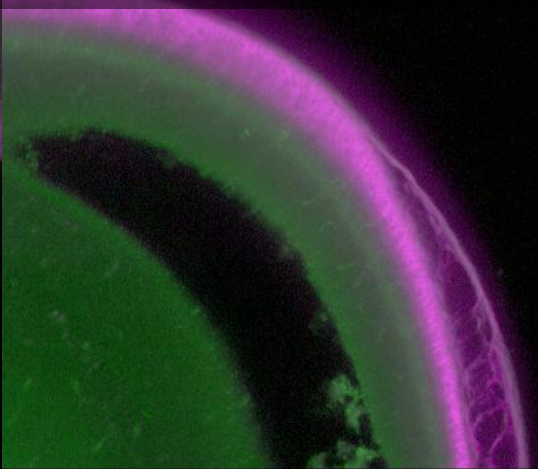*Is this a normal feature in brain development?*

Embryonic Mouse Brain Tumour?

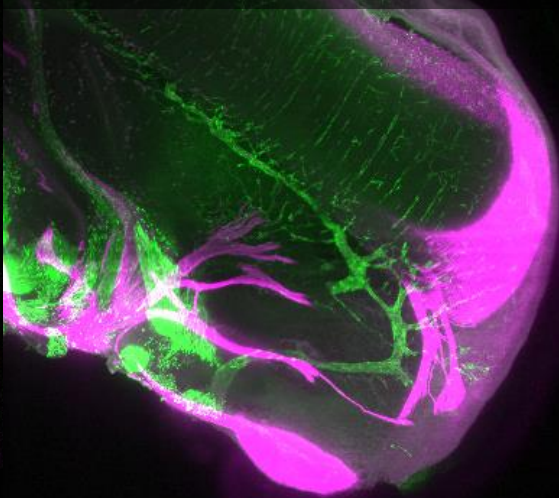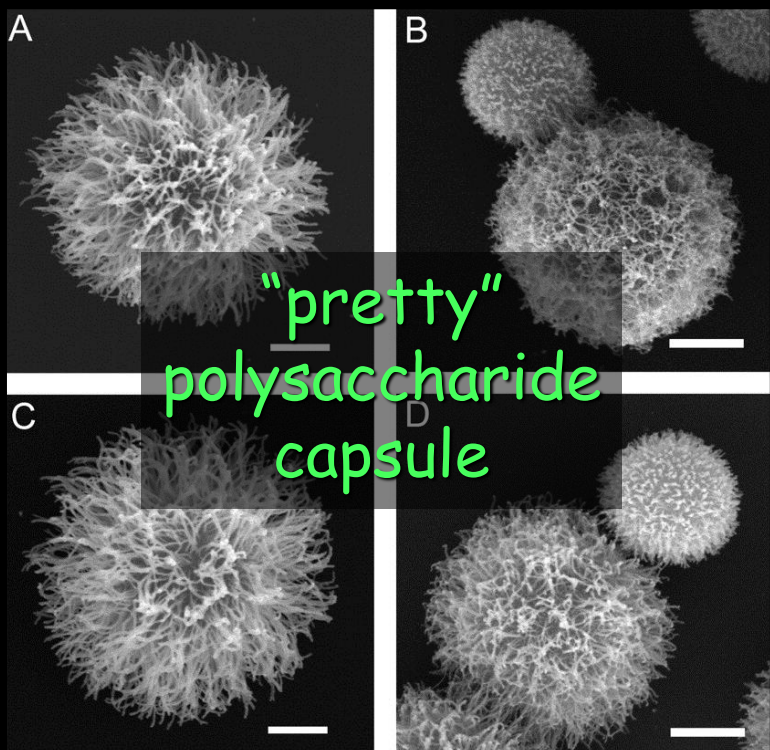slices

max-projection

slab, *dz* = 150 µm

enhanced contrast

slices, another embryo

Infectious Disease: *Cryptococci neoformans*

"pretty" polysaccharide capsule

SEM of *C. neoformans*
scale bars = 2 μm

- usually immune-compromised patients (e.g. AIDS)
- *but*, occasionally healthy people
- skin lesion treatable

ulcerated skin lesion, immunocompetent patient

"not-so-pretty" infection

after 45 days therapy (fluconazole)

van Duin *et al. Antimicrob. Agents Chemother.* **48** (2004)   da Silva *et al. Rev. Inst. Med. Trop. S. Paulo.* **44** (2002)

Infectious Disease: *Cryptococci neoformans*

How do they cross the blood-brain barrier?
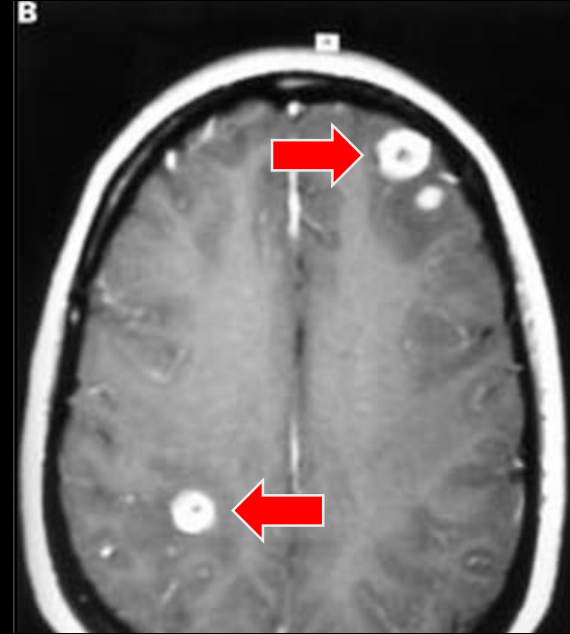
in vivo methods (CT, MRI):   good for *longitudinal studies*

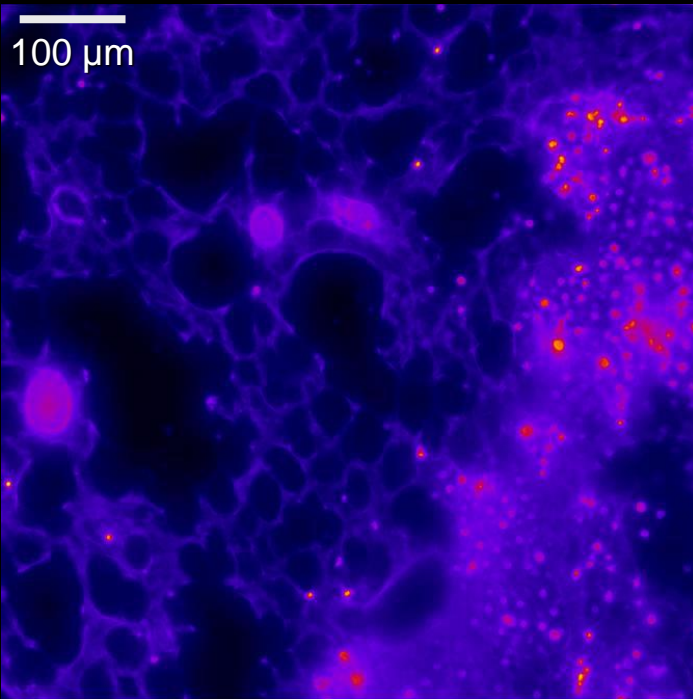But: can't *resolve Cryptococci*
       lack *specific contrasts*

OPTiSPIM for complementary *ex vivo* studies

Grosse *et al. J Neurol Neurosurg Psychiatry* 2001;**70**:113–116

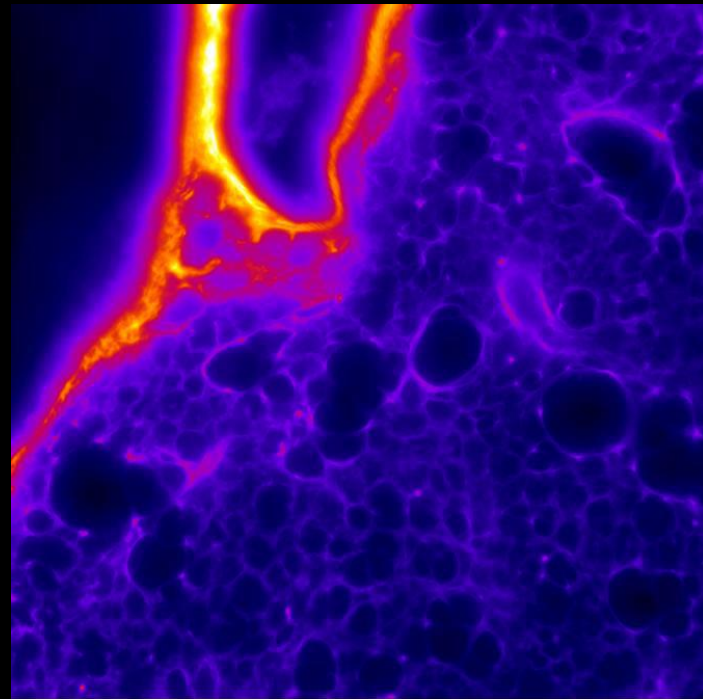# Infectious Disease: OPT of Mouse Lungs

OPT projections

SPIM

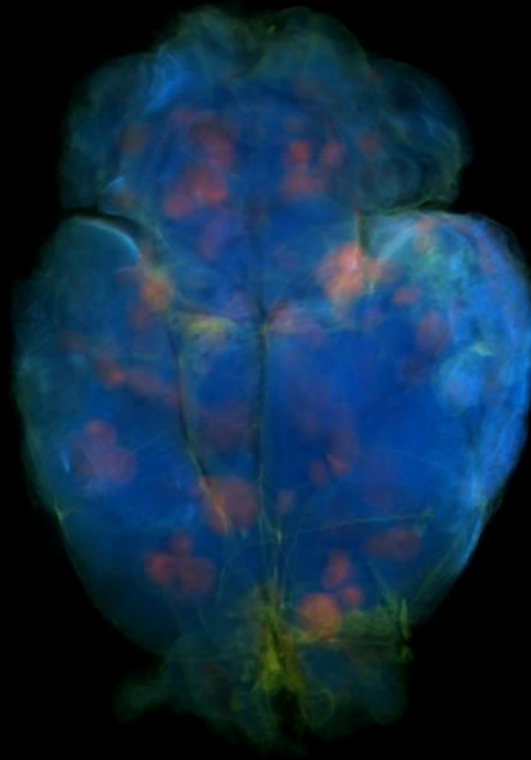SPIM, uninfected

100 µm

max-projections

ex. 638nm, em. 660-740nm

Infectious Disease: OPT of Mouse Brains

transmission projections

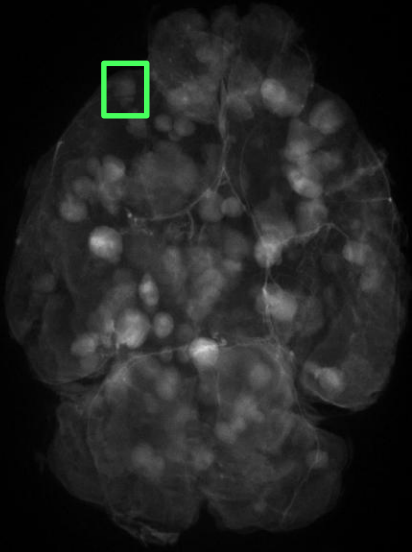**fluorescence projections**

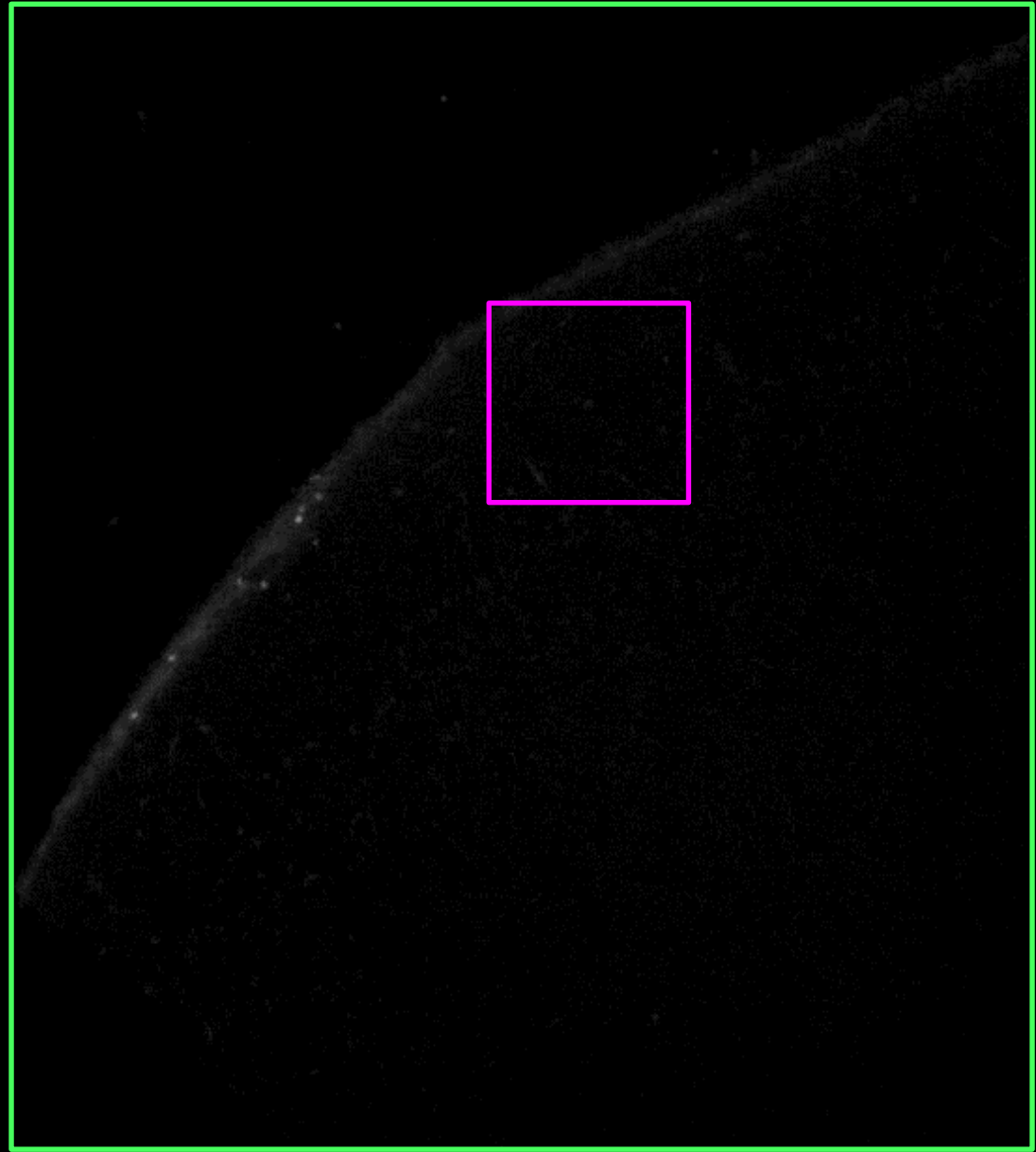**fluorescence reconstructions**

cryptos: ex. 590-650, det. 663-737

ex. 510-520, det. 590-650

ex. 450-490, det. 500-550

Infectious Disease: OPTiSPIM of Mouse Brains

OPT
(crypto
channel)

SPIM

SPIM