Imaging cleared tissue with light-sheet microscopy

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THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

Huge thanks to

Organizing gurus
Marc Schneeberger Pane
Han Tan

iDISCO conversations
Nico Renier
Zhuhao Wu
Alba Vieites Prado

Access to microscope
BIRC – Alison North
Miltenyi Biotec

Access to software
Oxford Instruments - Imaris

Funding & hosting
Kavli Neural System Institute
The Rockefeller University

THE KAVLI FOUNDATION

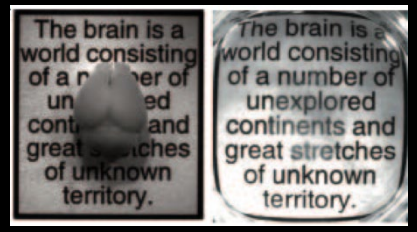
SCIENTIA · PRO · BONO · HUMANI · GENERIS
The Rockefeller University
1901

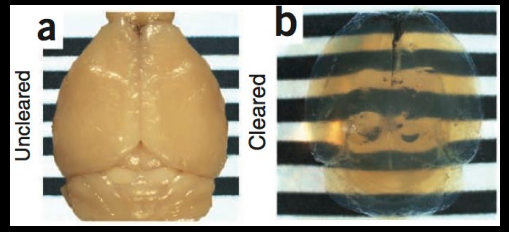
Miltenyi Biotec

OXFORD INSTRUMENTS | IMARIS

Workflow

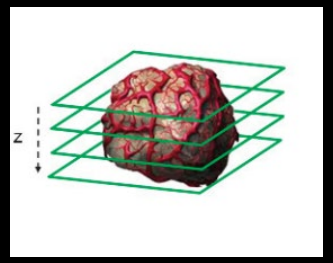
Sample preparation
days - weeks



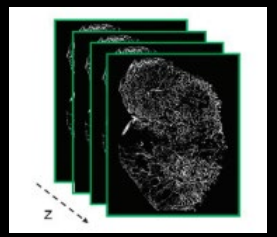


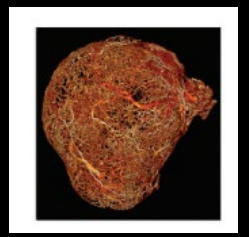
Sample mounting
minutes

Sample imaging
minutes - hours

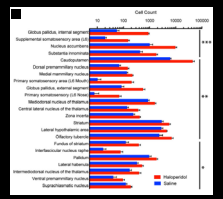


Data visualization
minutes – tens of minutes



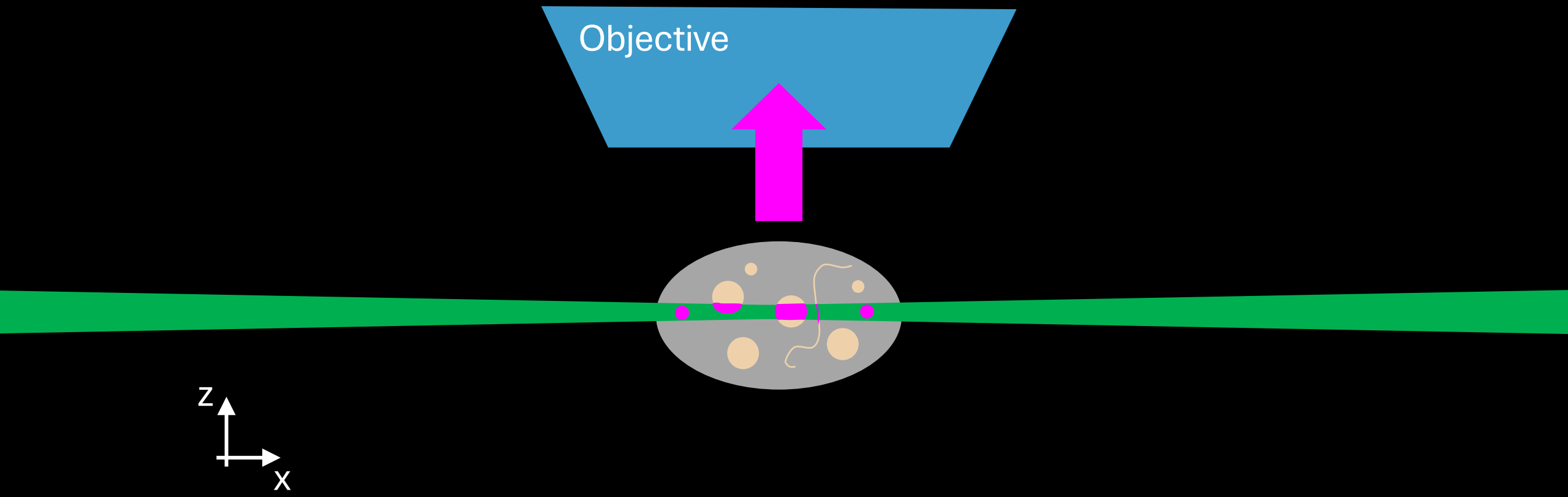


Data analysis
hours - months



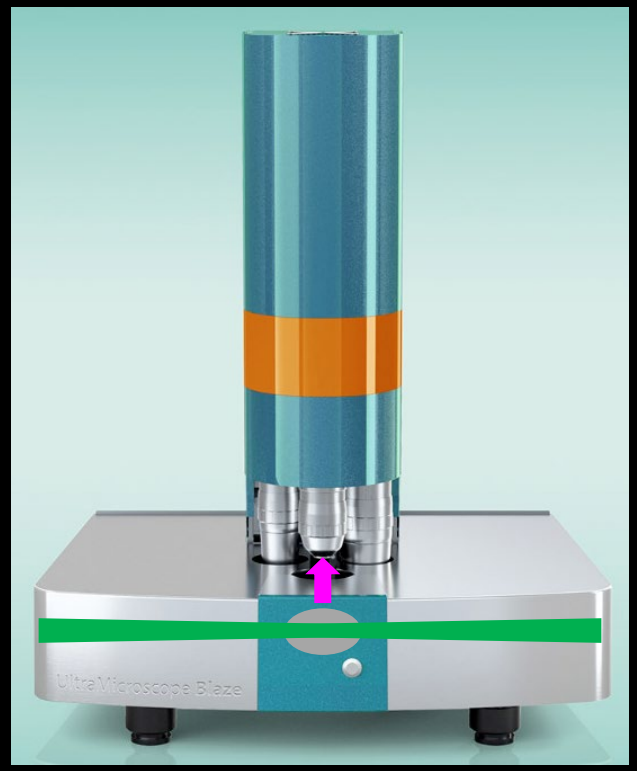
Data management
tens of GBs – thousands of GBs

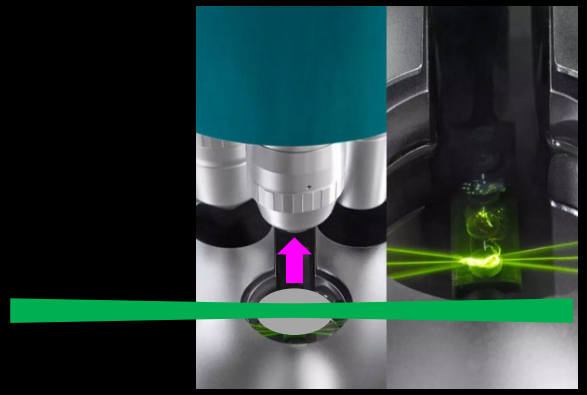
Light-sheet microscopy



- Sample with fluorophores
- Laser sheet
- Detection orthogonal to sheet

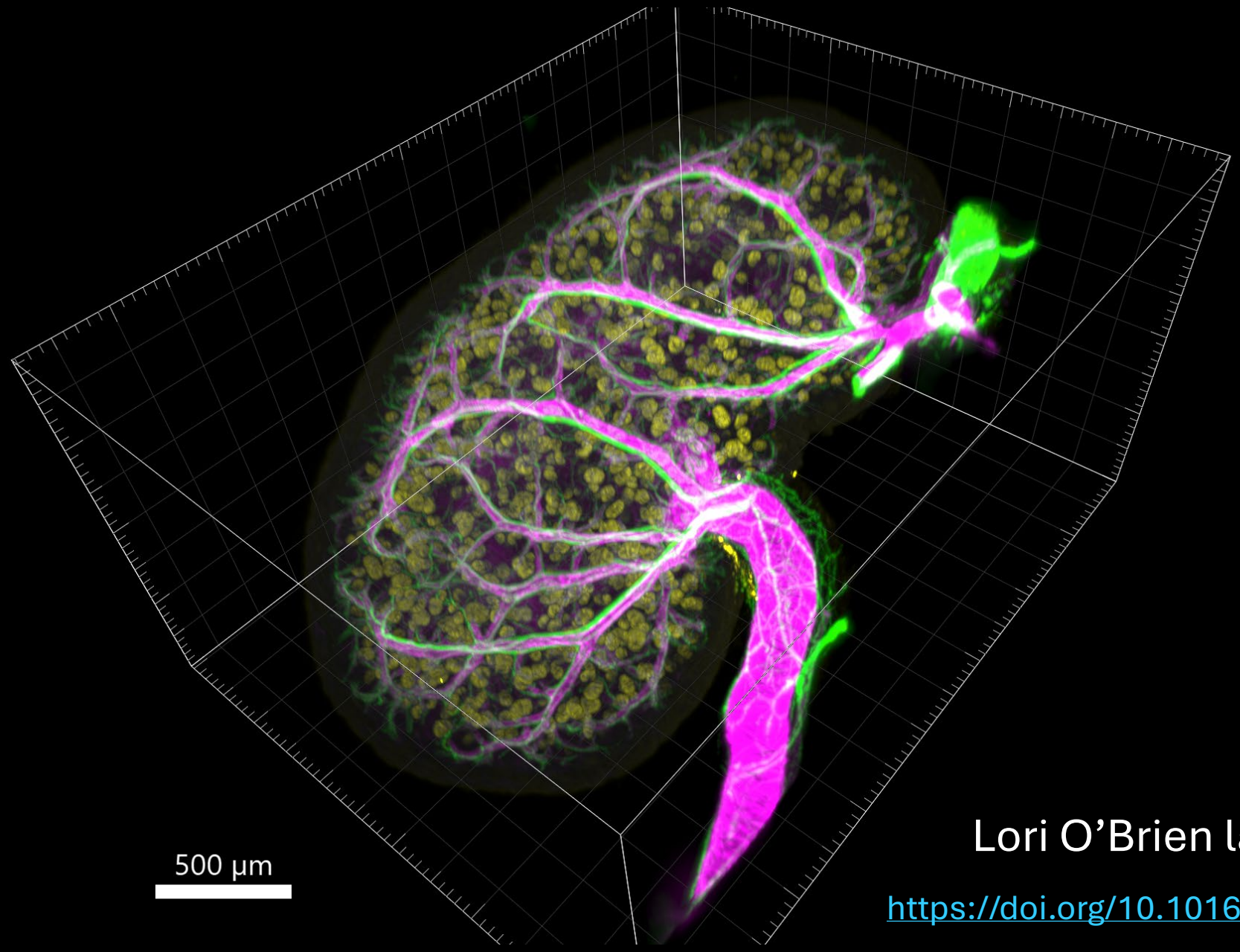
Microscope we will have for the workshop





Miltenyi UltraMicroscope Blaze
with Lightspeed mode

We reconstruct a sample in 3D from optical sections



Lori O’Brien lab, UNC-CH

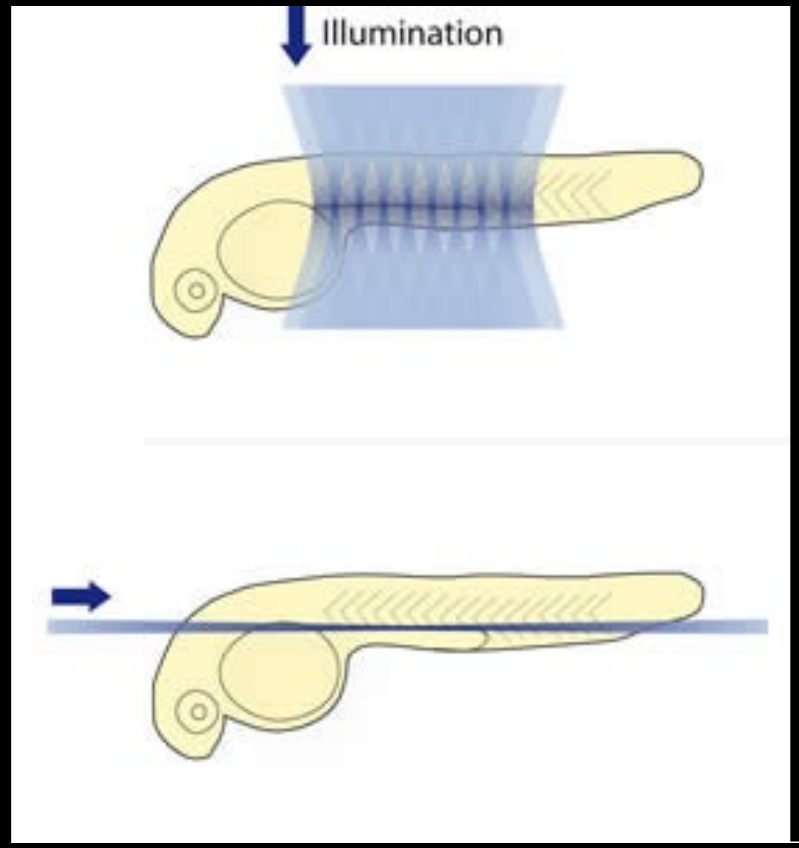
https://doi.org/10.1016/j.celrep.2024.114860

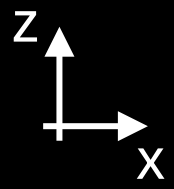
Why light-sheet microscopy at all?

Compared to confocal, light-sheet is:

Faster

Less damaging





Modified from Leica Microsystems

Things you can do to improve your imaging

Before

During

After

Things you can do to improve your imaging

Before

During

After

Use a clearing method that works

Find something that

- Has been used for similar tissues
- Not just in the lab that created it
- With minimal equipment and fuss (avoid $$$)

iDISCO+ and AdipoClear perform well in many cases

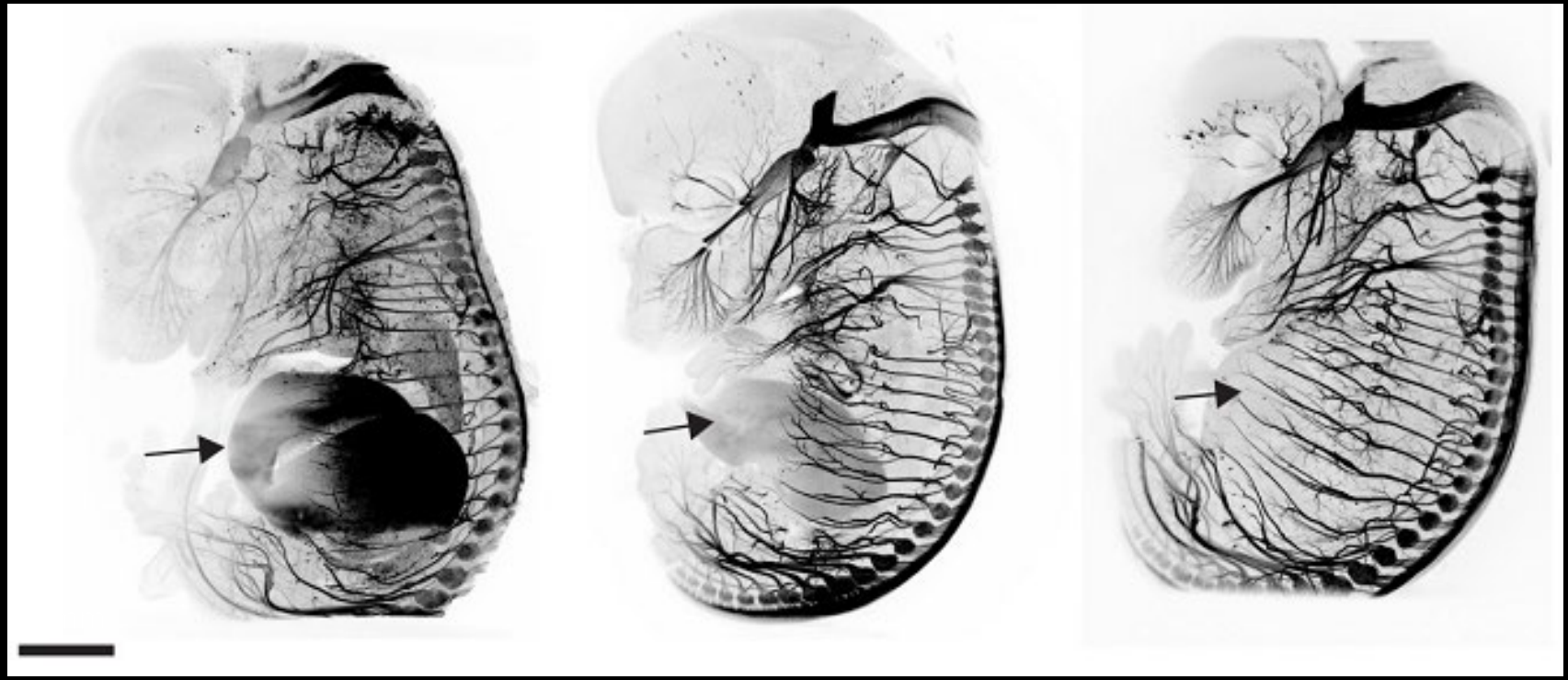
Do not waste your time reinventing the wheel

Labeling – (far) redder is better

AlexaFluor 488
Ex 488; Em 525/50

AlexaFluor 568
Ex 561; Em 595/40

AlexaFluor 647
Ex 640; Em 680/30



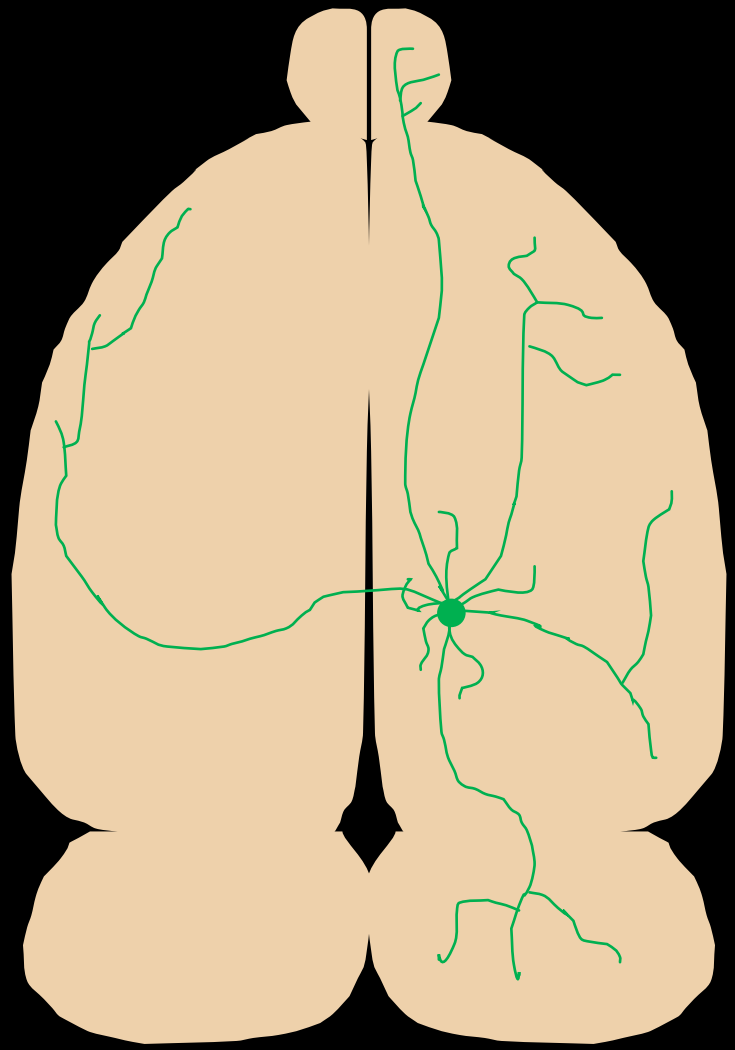
Autofluorescence

Scattering

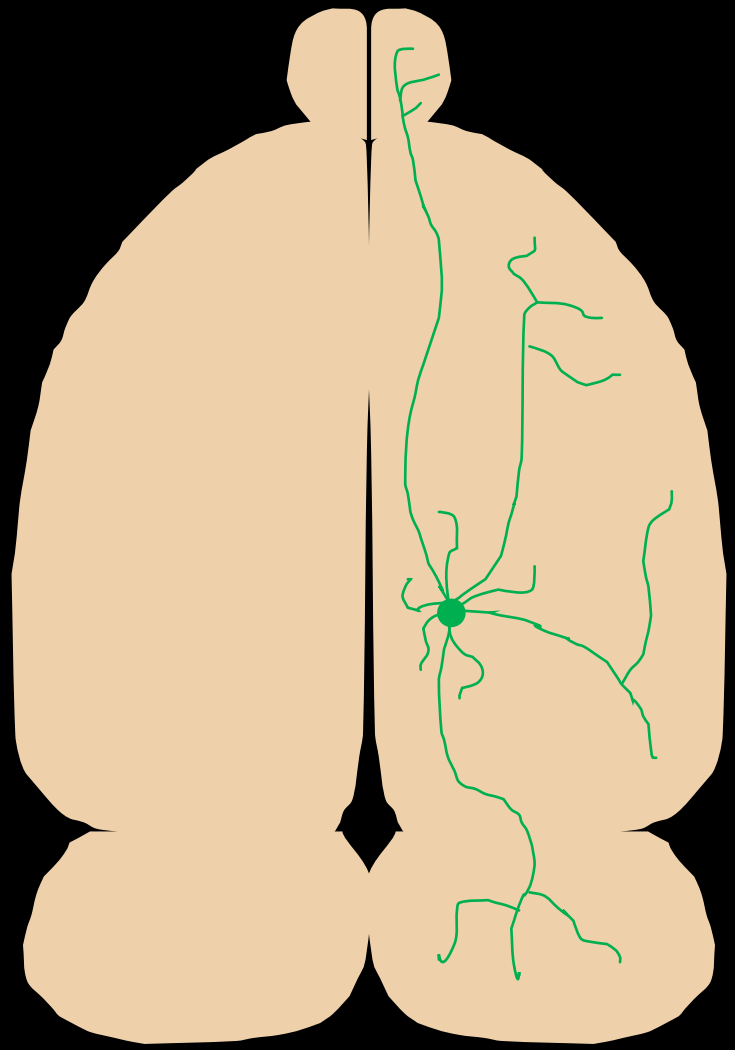
Absorbtion

https://doi.org/10.1016/j.cell.2014.10.010

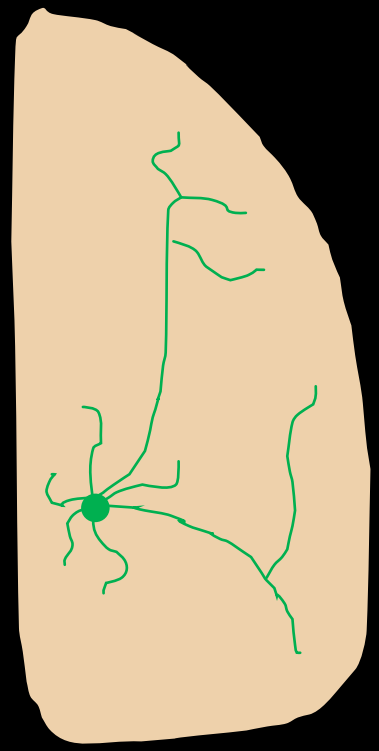
Trim based on your biology



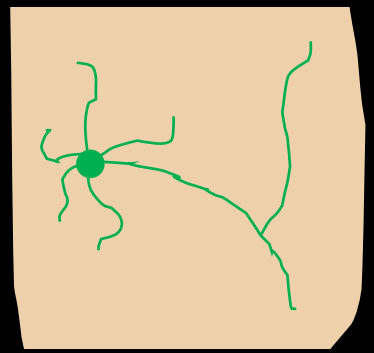
Trim based on your biology



Trim based on your biology



Trim based on your biology



Trim based on your biology

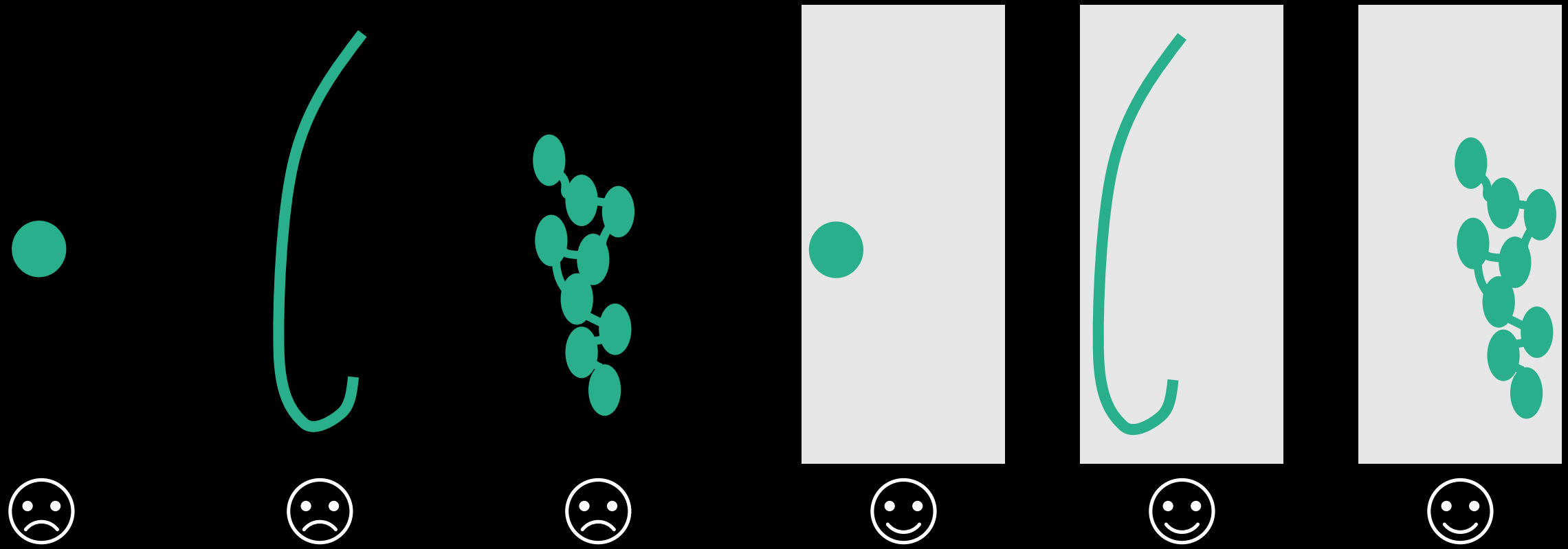
Small samples

- Stain faster
- Require less antibody
- Clear faster
- Are easier to image (usually)

Use agarose for small or uneven samples

A block of agarose is easier to mount if samples are:

- very small: *eyeballs, organoids, organs from embryos,…*
- have very uneven dimensions: *tubes, sheets,…*
- very soft and/or have irregular shape: *pancreas,…*



Use agarose for small or uneven samples

Recomendations:

- Mount in agarose before the clearing module
- Avoid big temperature differences:
  - cool down agarose (but still liquid)
  - warm up sample
- Avoid bubbles
- Cut to size once agarose is solid

Video tutorial : https://dx.doi.org/10.3791/58271-v

Plan ahead: what will you do when you get data?

¿ Looking vs measuring ?

¿ How will you look?

¿ What will you measure?

¿ How will you measure?

¿ How will you move data ?

¿ How will you store data ?

Thinking this through in advance
will avoid wasting time

If you are not sure:
Talk to your local experts

Things you can do to improve your imaging

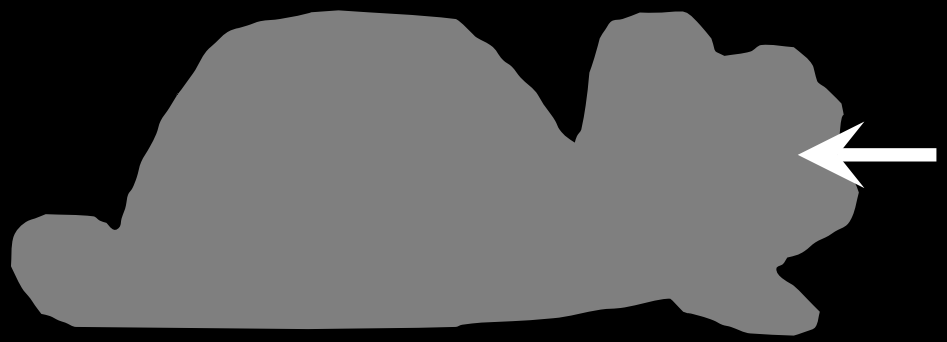
Before

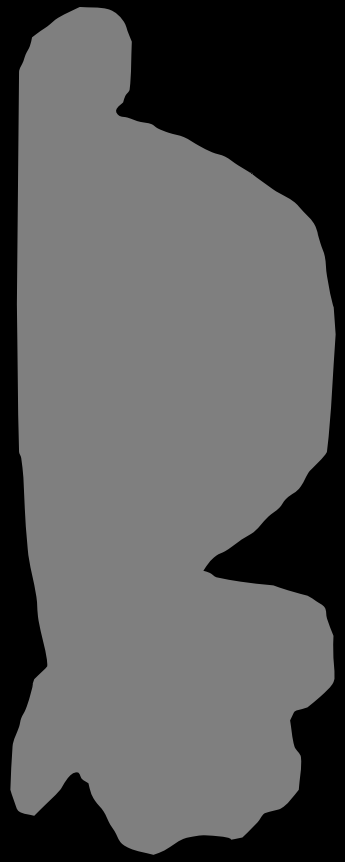
During

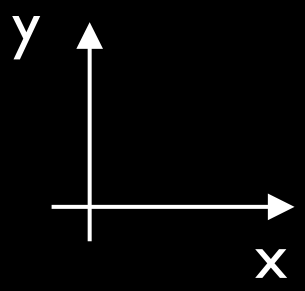
After

Sample orientation has a big effect on image quality

Clearing is
never perfect

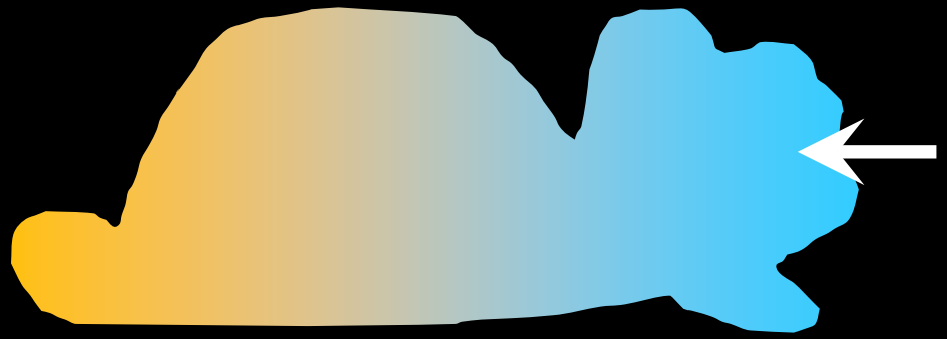






Sample orientation has a big effect on image quality

Clearing is
never perfect



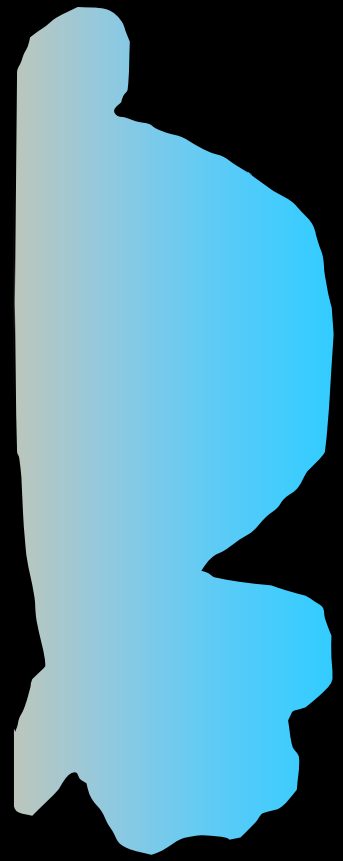
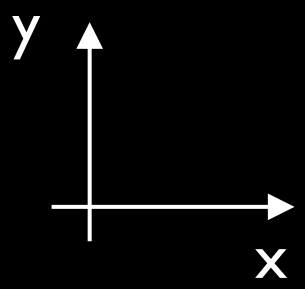


Image quality

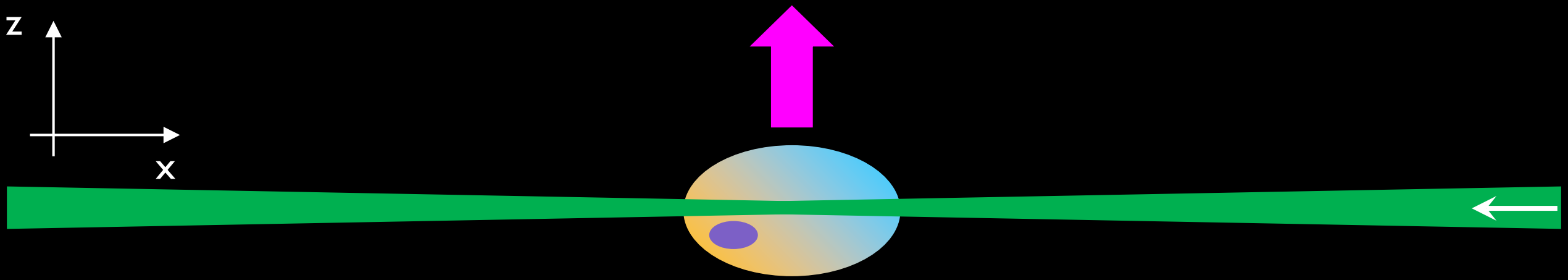
Better

Worse

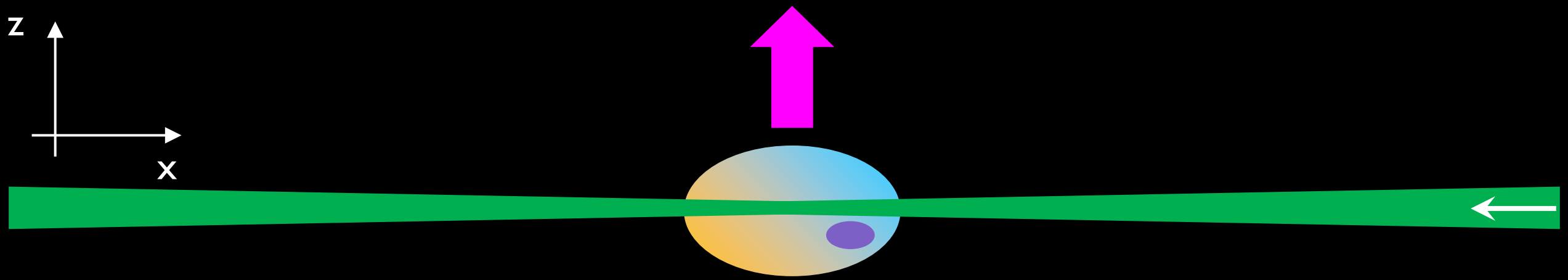


Minimize distance excitation light travels into sample

Sample orientation has a big effect on image quality

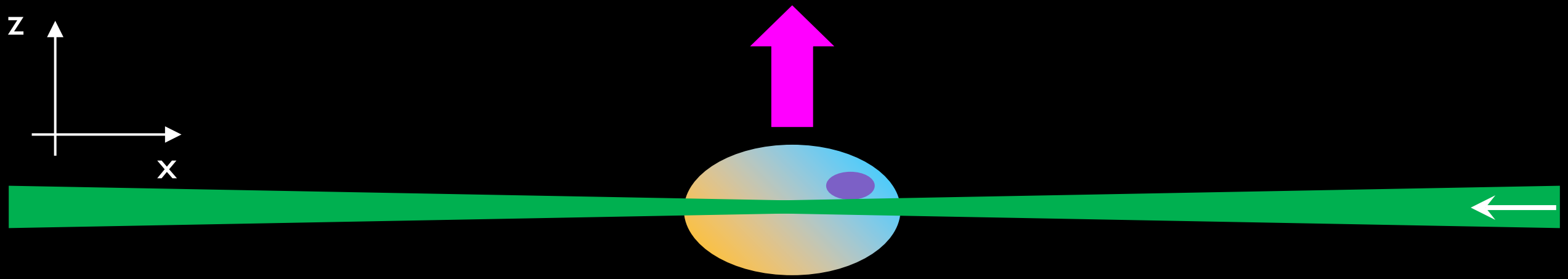


Sample orientation has a big effect on image quality



Minimize distance excitation light travels to your object of interest

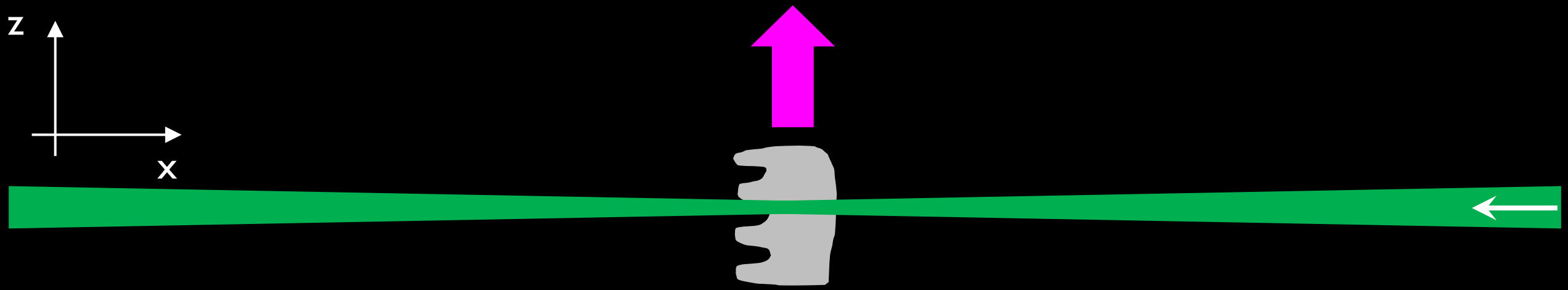
Sample orientation has a big effect on image quality



Minimize distance excitation light travels to your object of interest

Minimize distance emission light travels from your object of interest

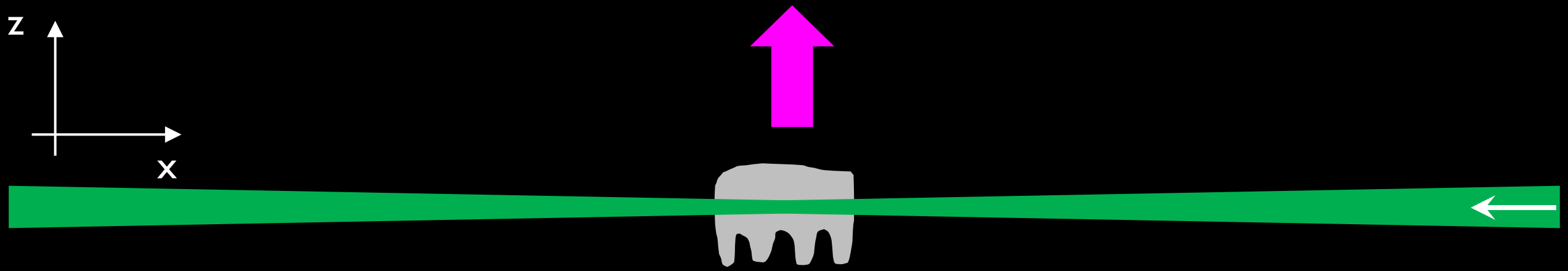
Sample orientation has a big effect on image quality



Minimize distance excitation light travels to your object of interest

Minimize distance emission light travels from your object of interest

Sample orientation has a big effect on image quality

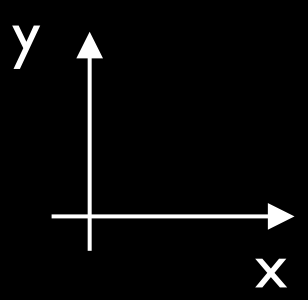


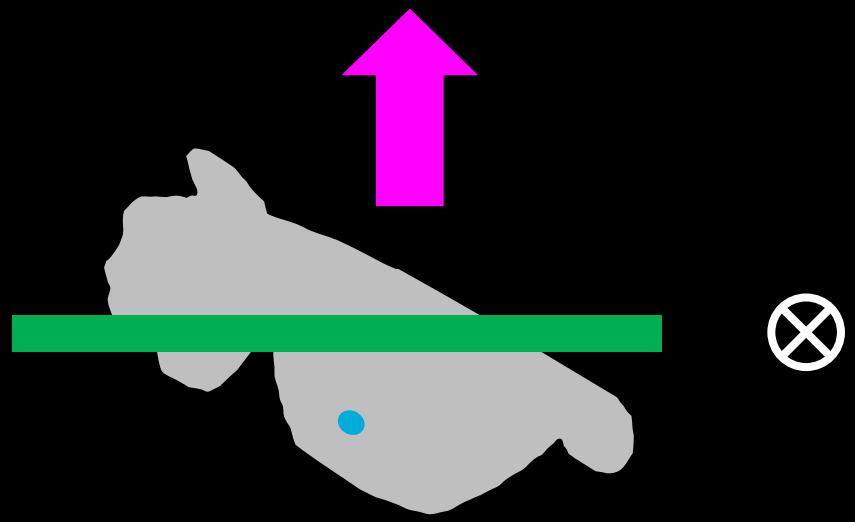
Minimize distance excitation light travels to your object of interest

Minimize distance emission light travels from your object of interest

Maximize resolution in plane of interest by putting it in XY orientation

Sample orientation has a big effect on image quality



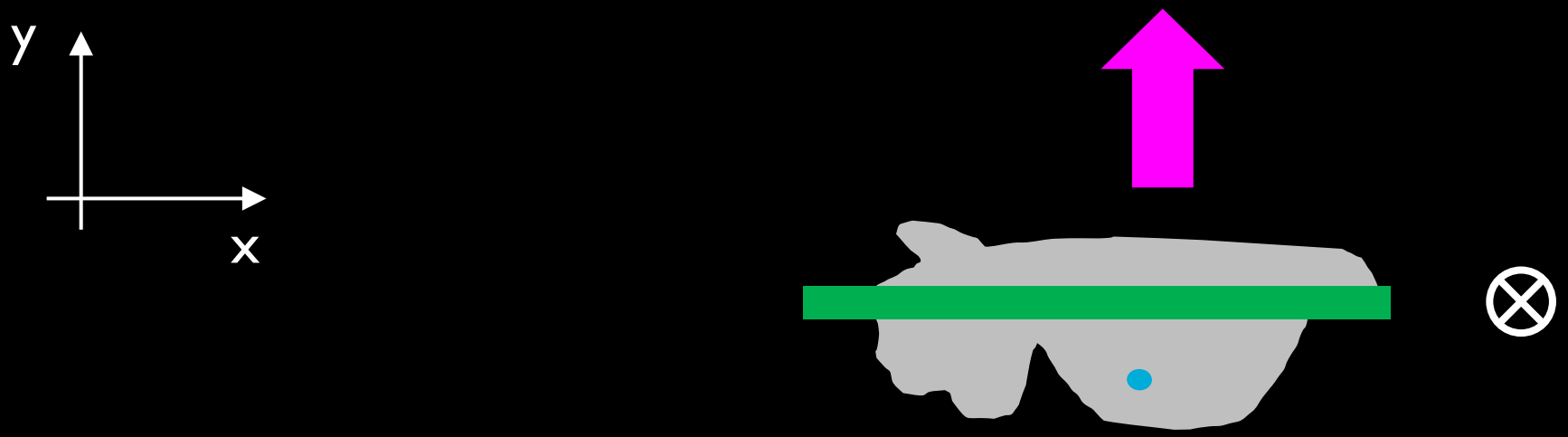


Minimize distance excitation light travels to your object of interest

Minimize distance emission light travels from your object of interest

Maximize resolution in plane of interest by putting it in XY orientation

Sample orientation has a big effect on image quality



Minimize distance excitation light travels to your object of interest

Minimize distance emission light travels from your object of interest

Maximize resolution in plane of interest by putting it in XY orientation

Put a classical histological sectioning plane in XY, XZ or YZ

Match your resolution to your question

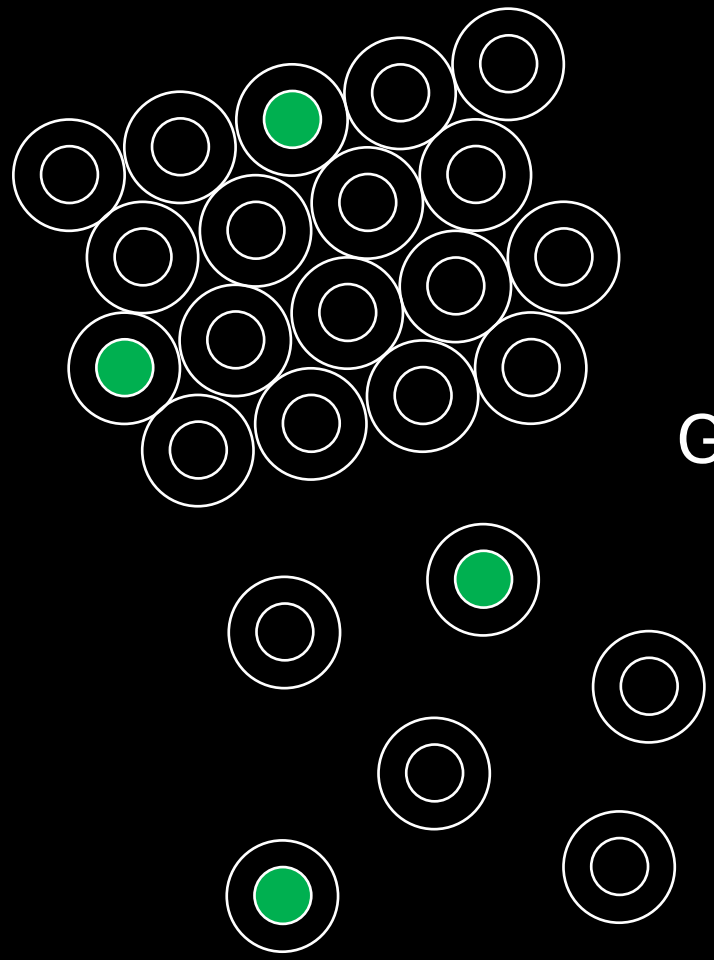
Do not overestimate required resolution
This common mistake wastes time and money

To determine necessary resolution
Spacing is more important than size

Spacing is more important than size

Count sparse subset of cells

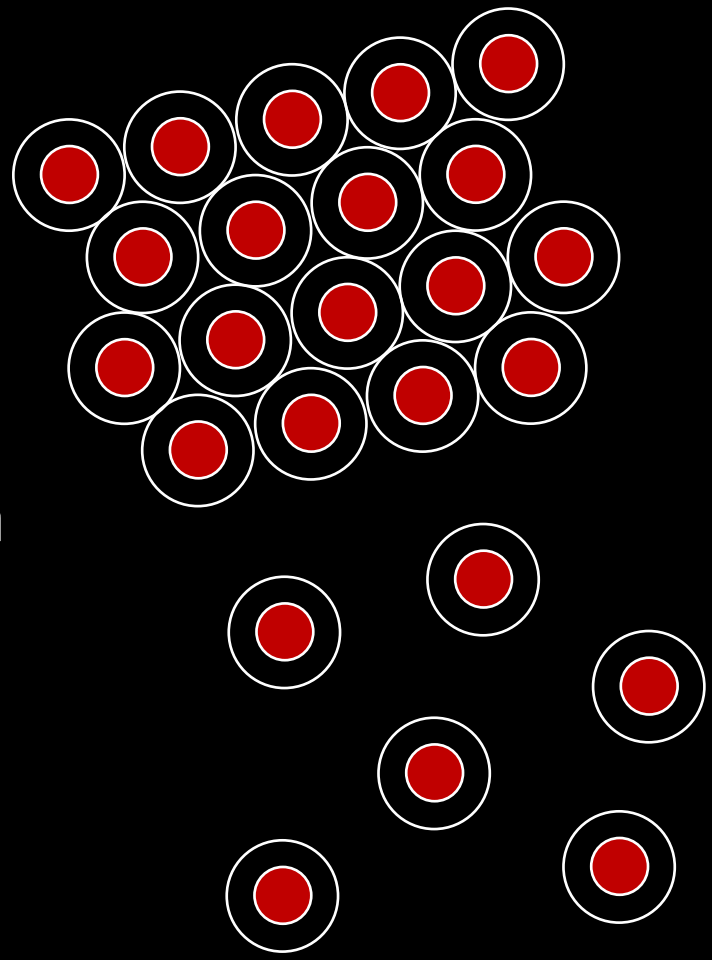
Example: cFos+



Ground truth

Count all cells

Example: TO-PRO3



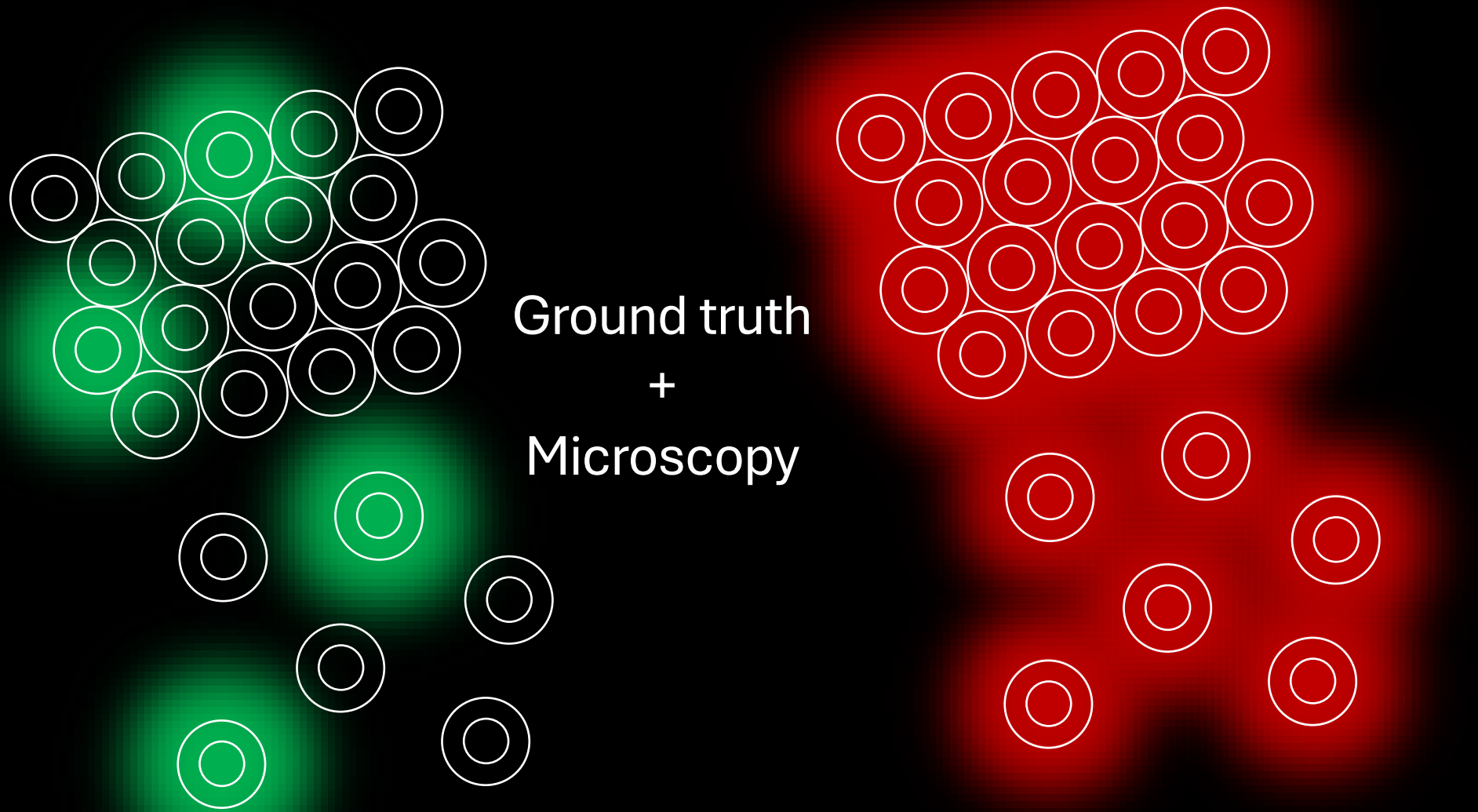
Spacing is more important than size

Count sparse subset of cells

Example: cFos+

Count all cells

Example: TO-PRO3



Ground truth

+

Microscopy

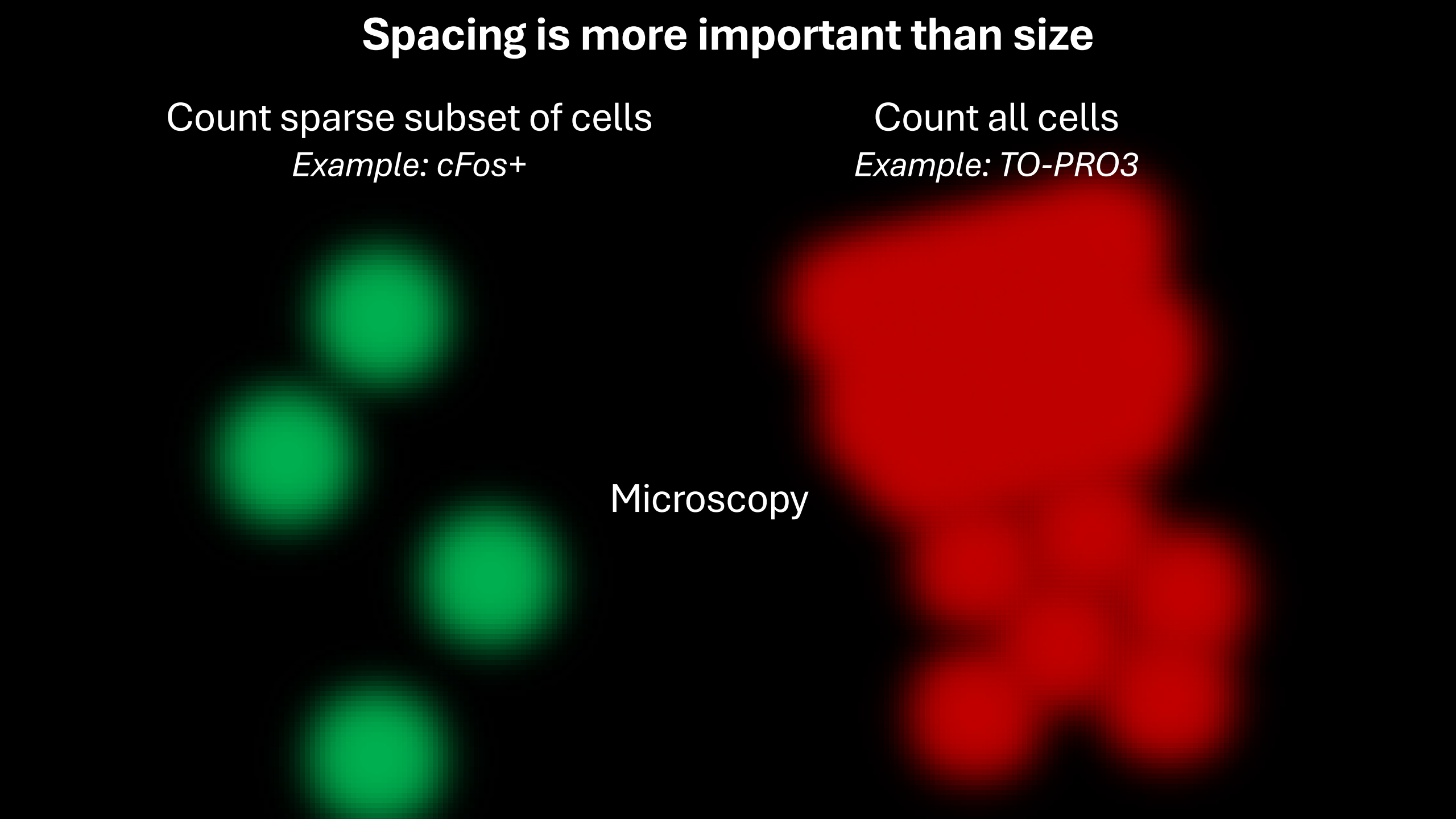
Spacing is more important than size

Count sparse subset of cells

Example: cFos+

Count all cells

Example: TO-PRO3



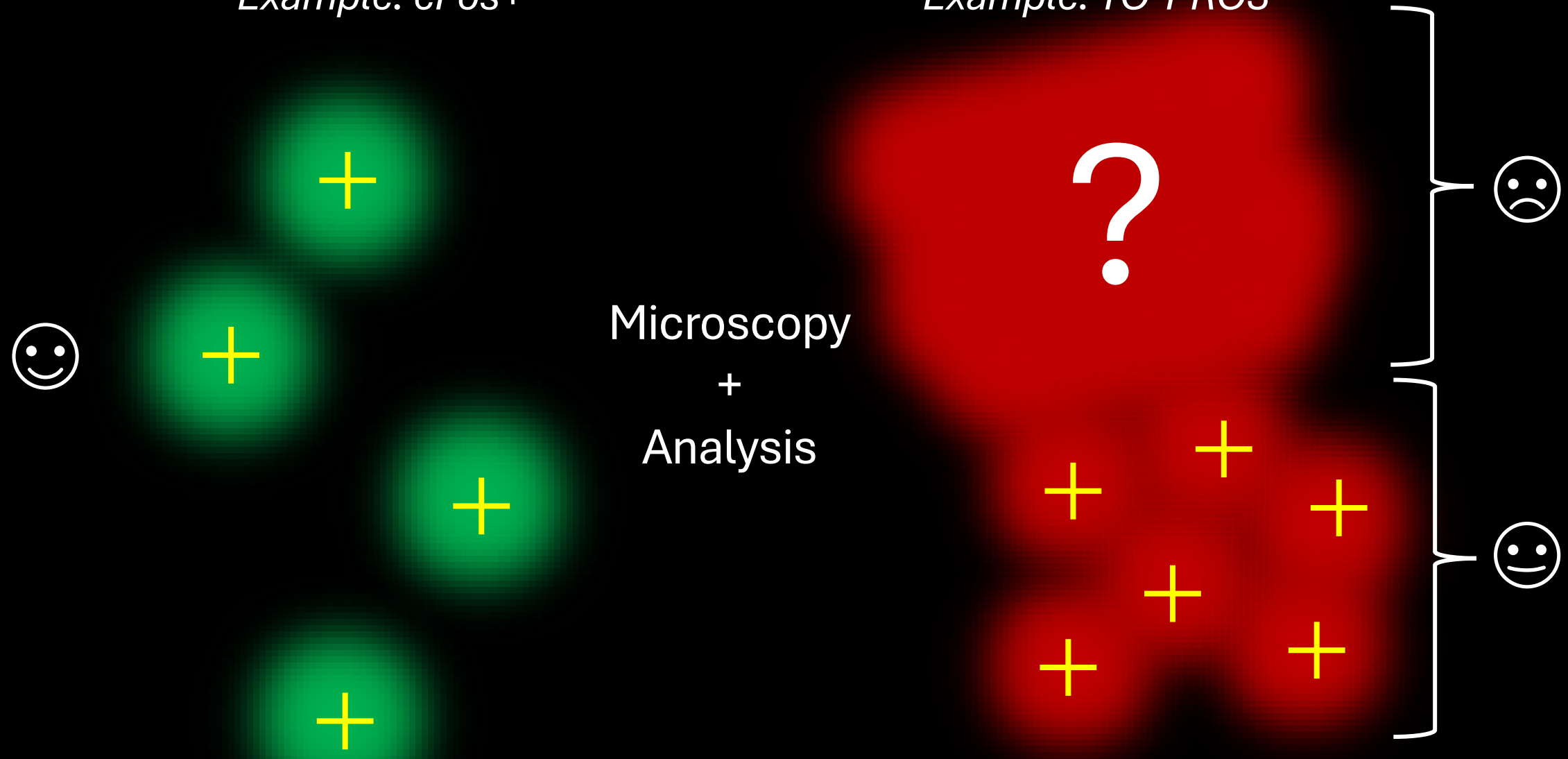
Spacing is more important than size

Count sparse subset of cells

Example: cFos+

Count all cells

Example: TO-PRO3



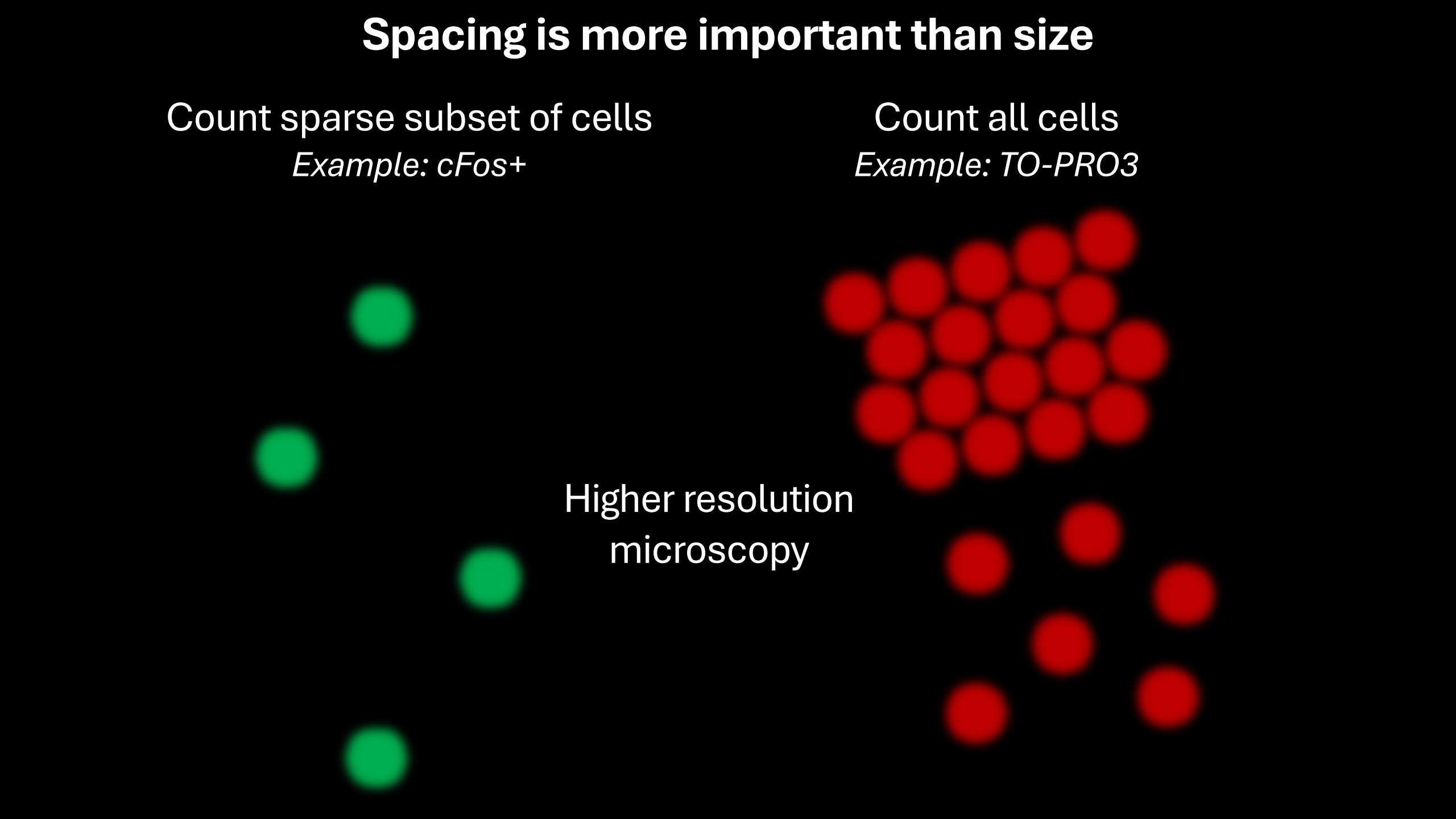
Spacing is more important than size

Count sparse subset of cells

Example: cFos+

Count all cells

Example: TO-PRO3



Higher resolution microscopy

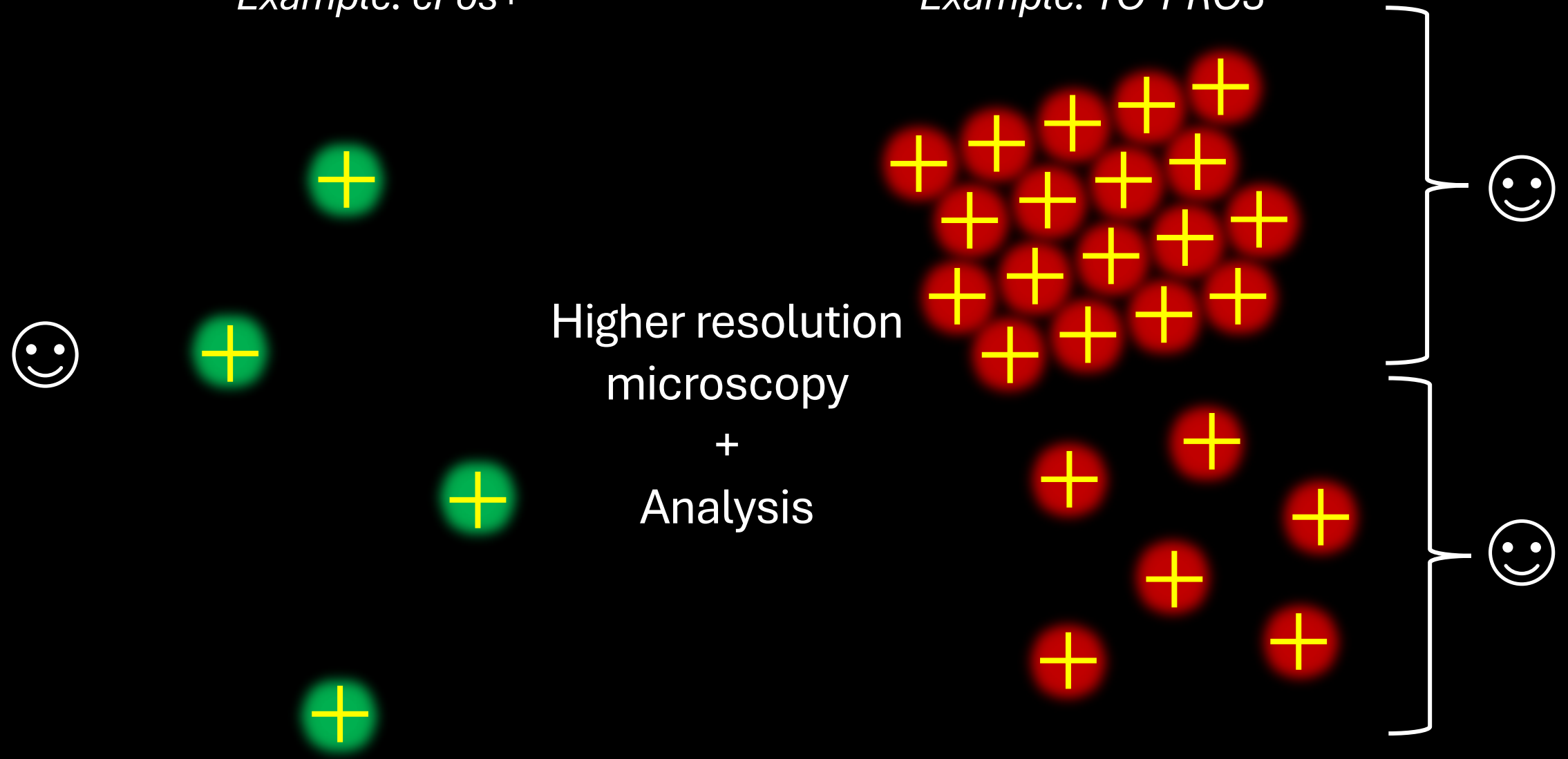
Spacing is more important than size

Count sparse subset of cells

Example: cFos+

Count all cells

Example: TO-PRO3



Higher resolution microscopy + Analysis

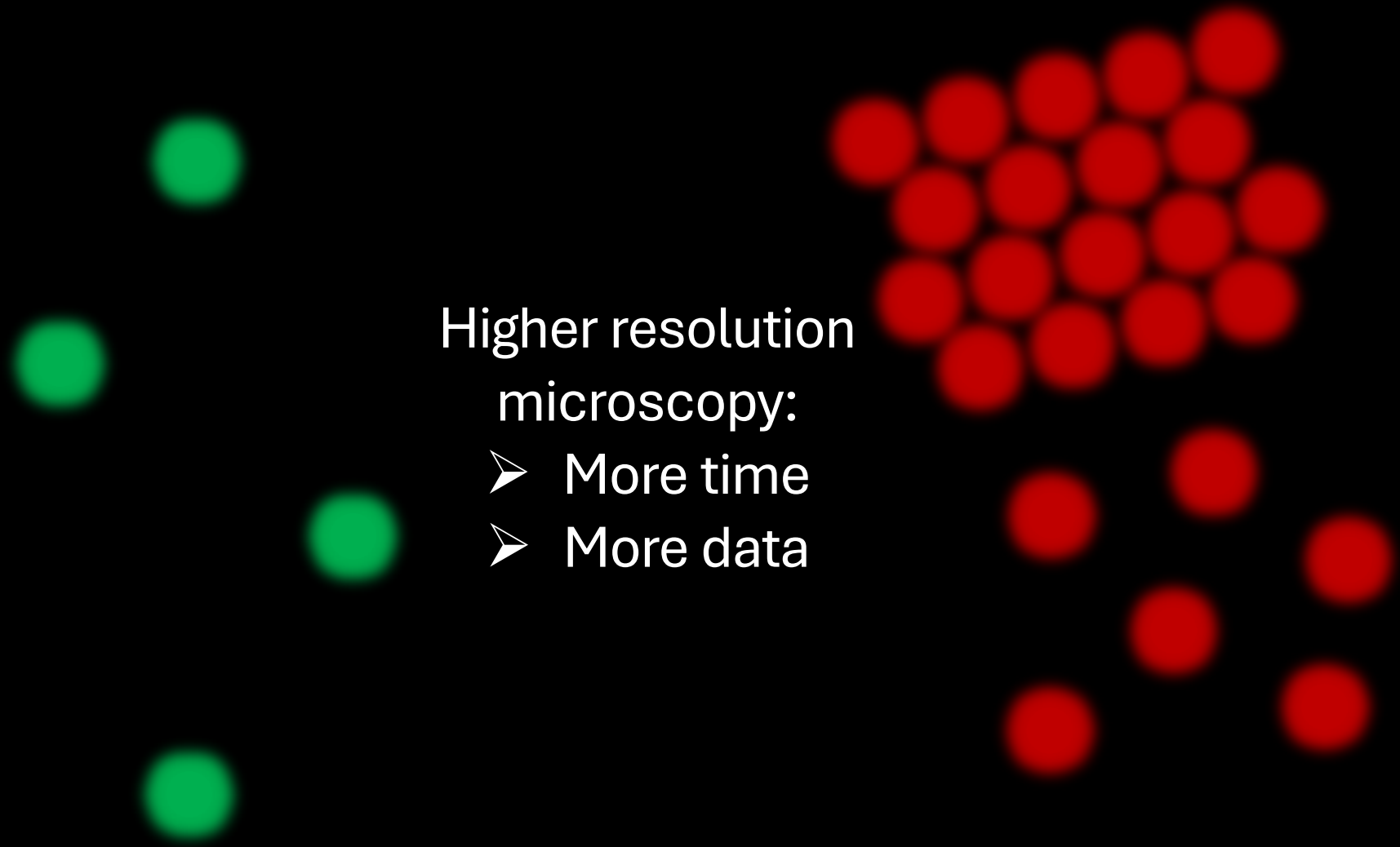
Spacing is more important than size

Count sparse subset of cells

Example: cFos+

Count all cells

Example: TO-PRO3



Higher resolution microscopy:

- More time
- More data

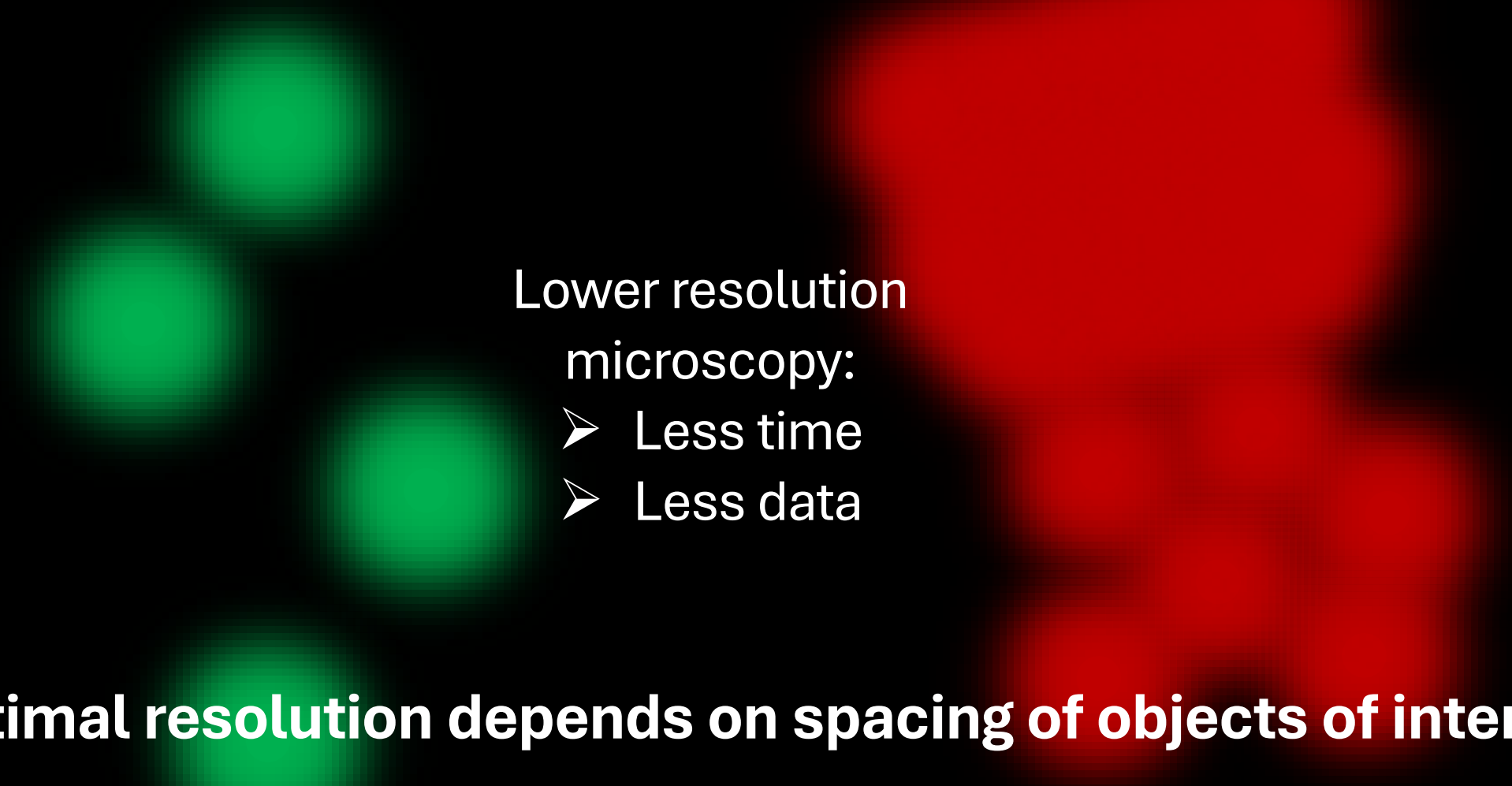
Spacing is more important than size

Count sparse subset of cells

Example: cFos+

Count all cells

Example: TO-PRO3



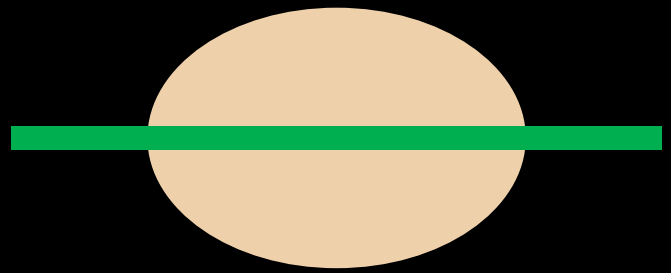
Lower resolution microscopy:

- Less time
- Less data

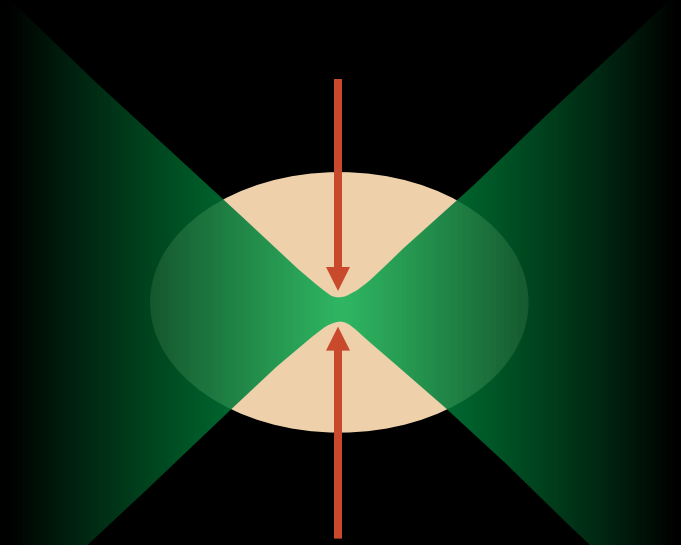
Optimal resolution depends on spacing of objects of interest

Sheet shape is a key parameter

Idea

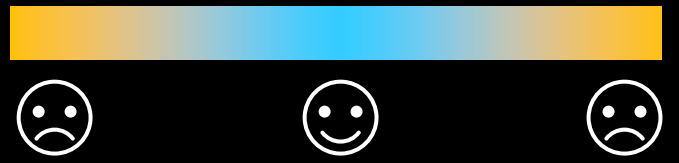


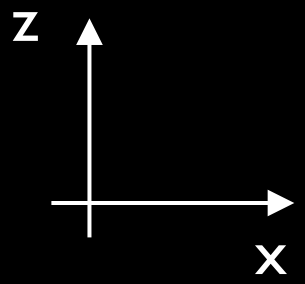
Reality



“Waist”

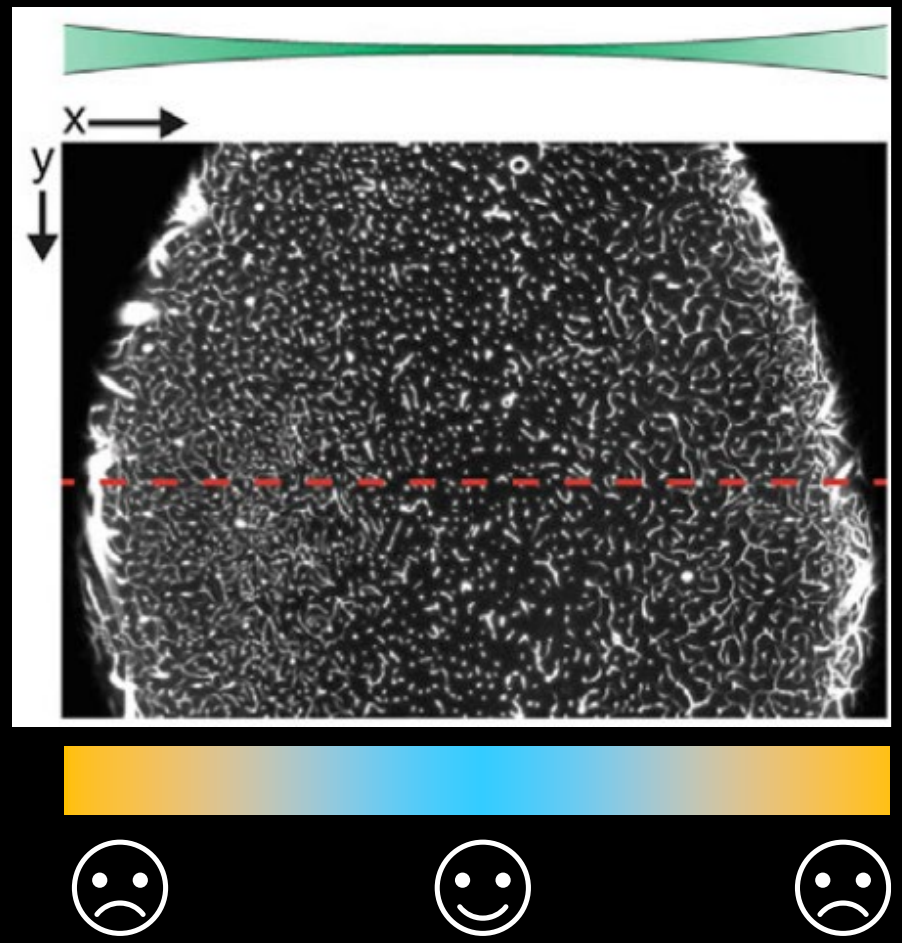
Resolution in Z and quality





Sheet shape is a key parameter

Vasculature in mouse brain



Z resolution and quality

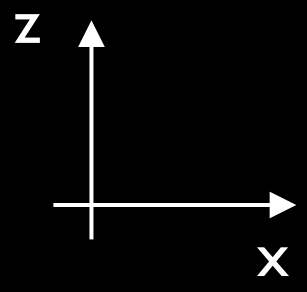
Nico Renier lab

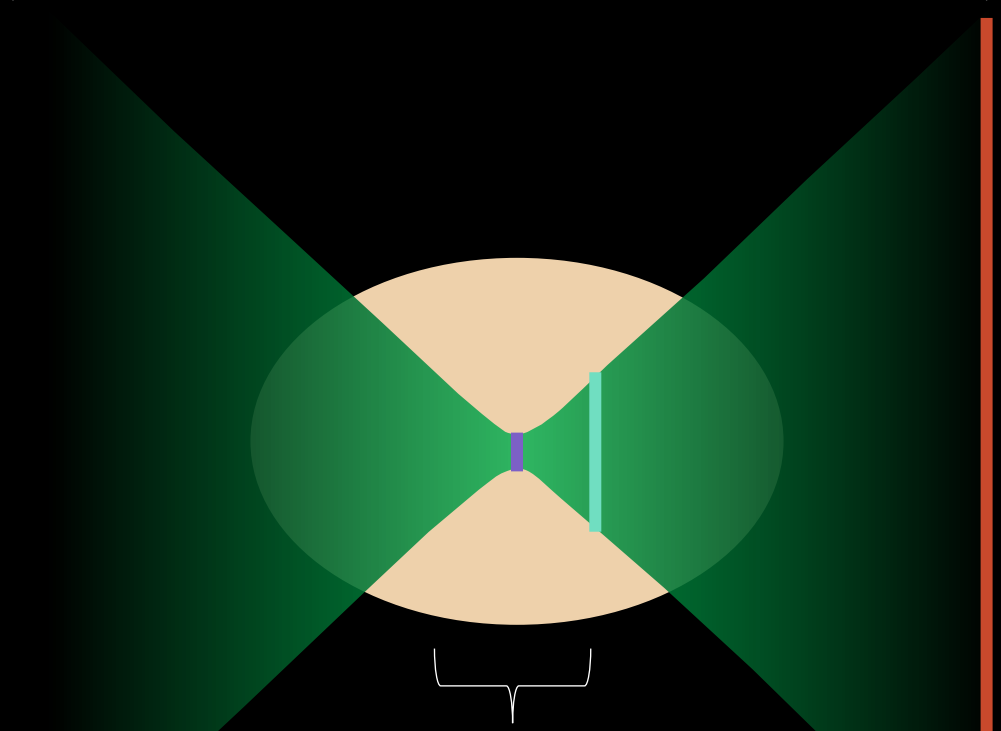
https://doi.org/10.1016/j.cell.2020.01.028

The magnitude of this problem depends on size of field of view

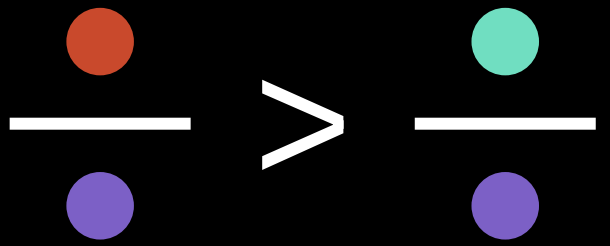
Low zoom or no cropping

Effect is smaller with:
- High Zoom
- Cropping





High zoom
Cropping

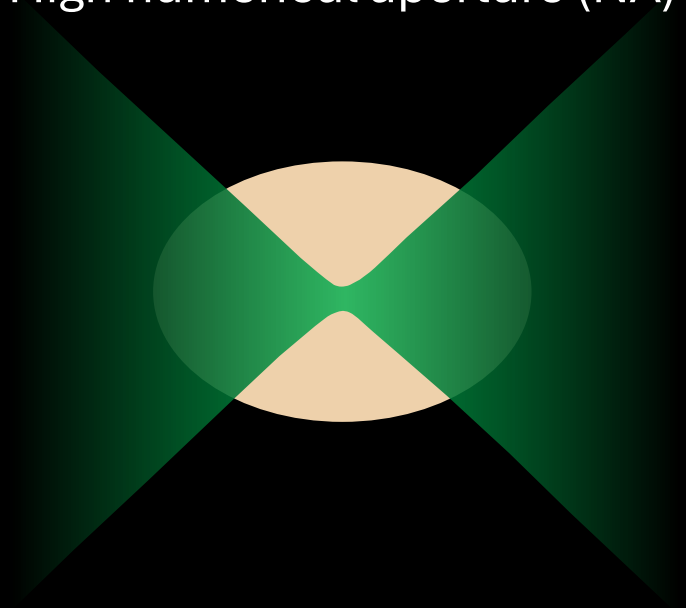


How to deal with uneven sheet shape

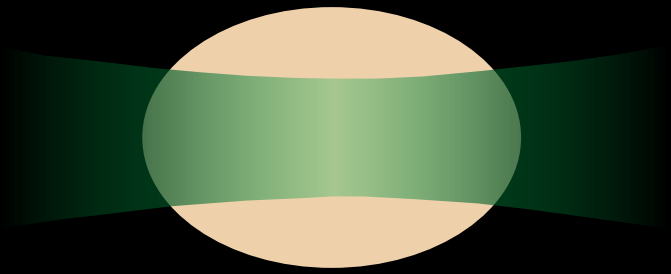
Option #1

Change the shape

High numerical aperture (NA)



Low numerical aperture (NA)

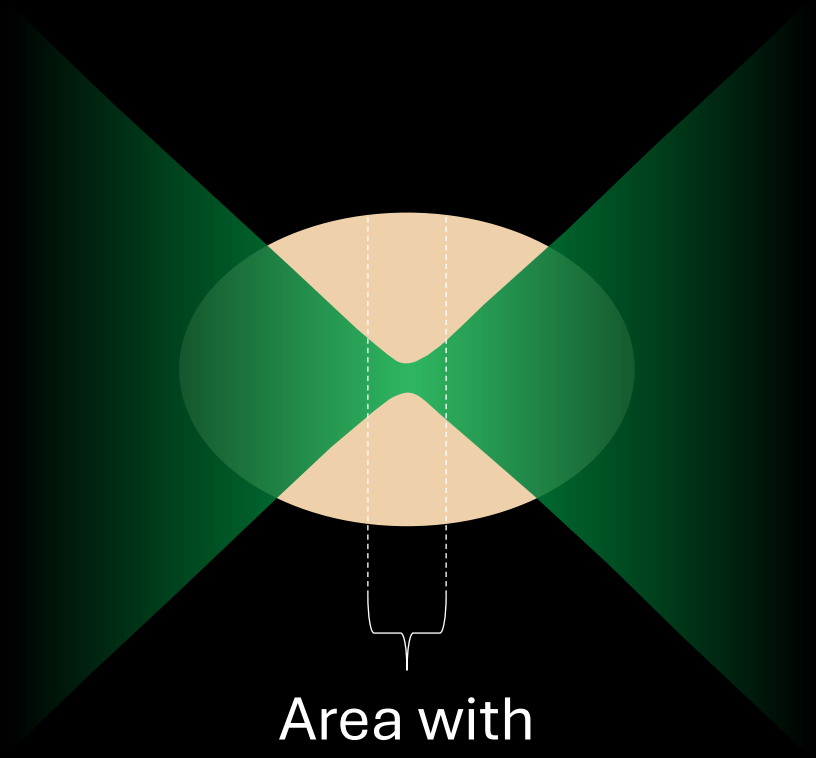


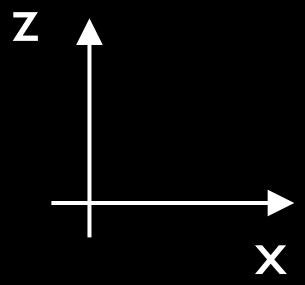
- Worse Z resolution at waist
- Less laser power
- More even Z resolution
- Does not take longer*

How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

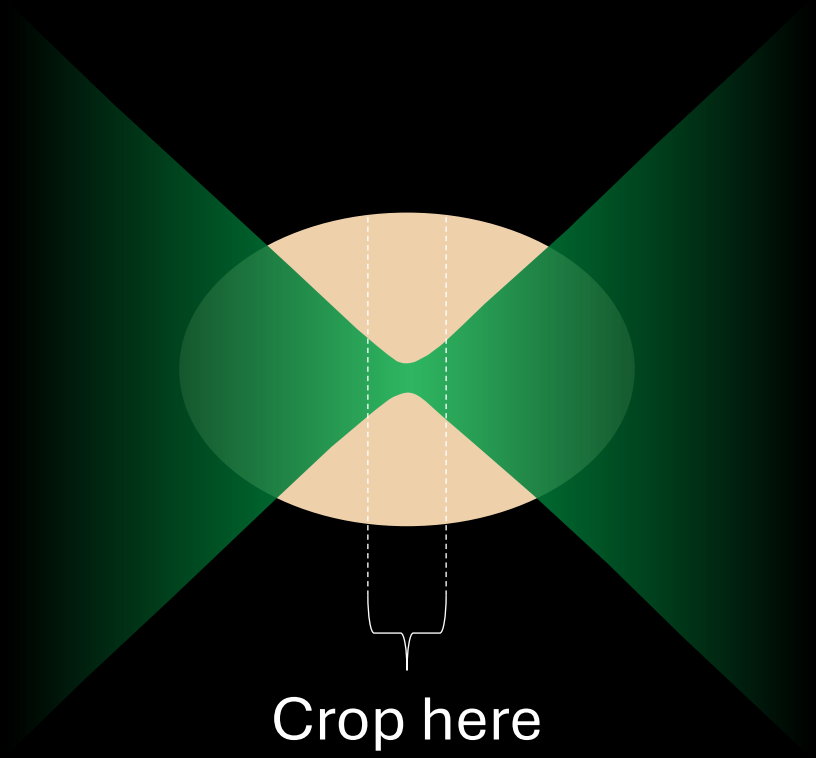


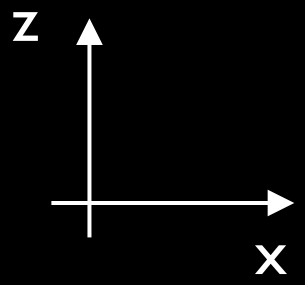


How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

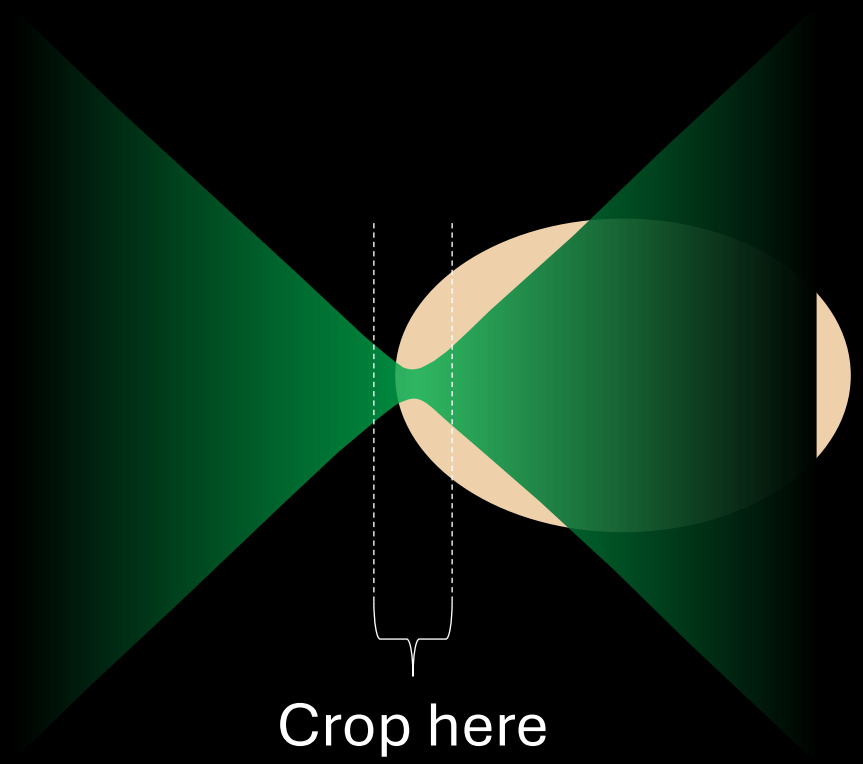


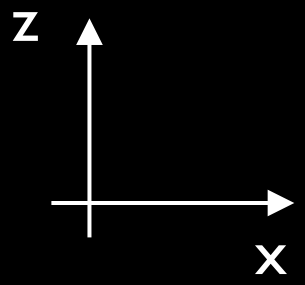


How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

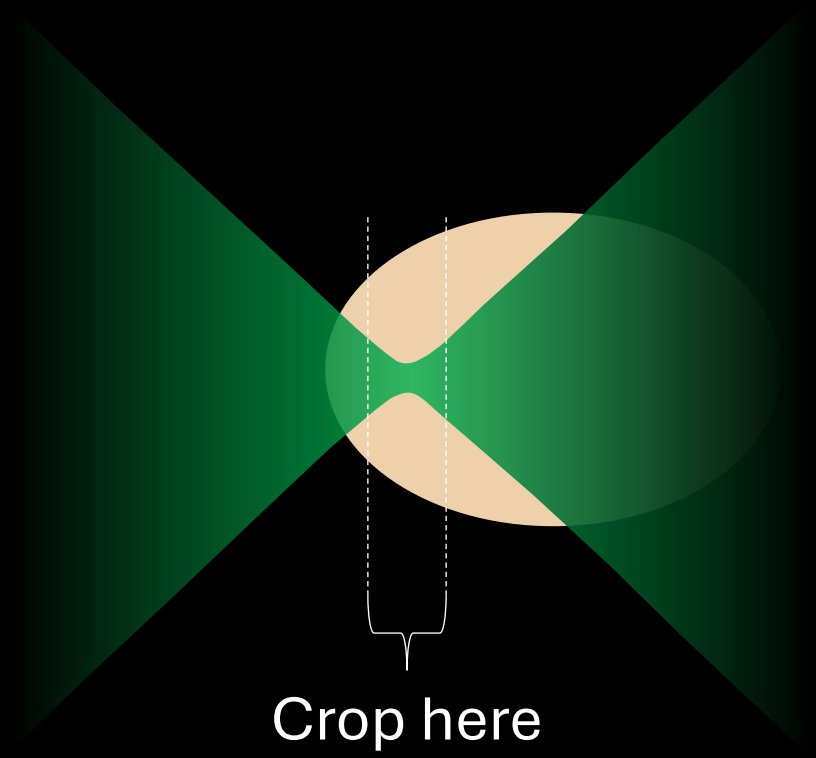


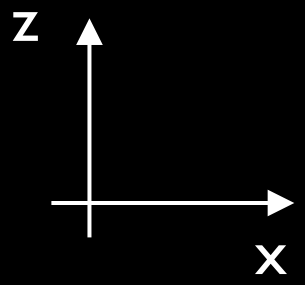


How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

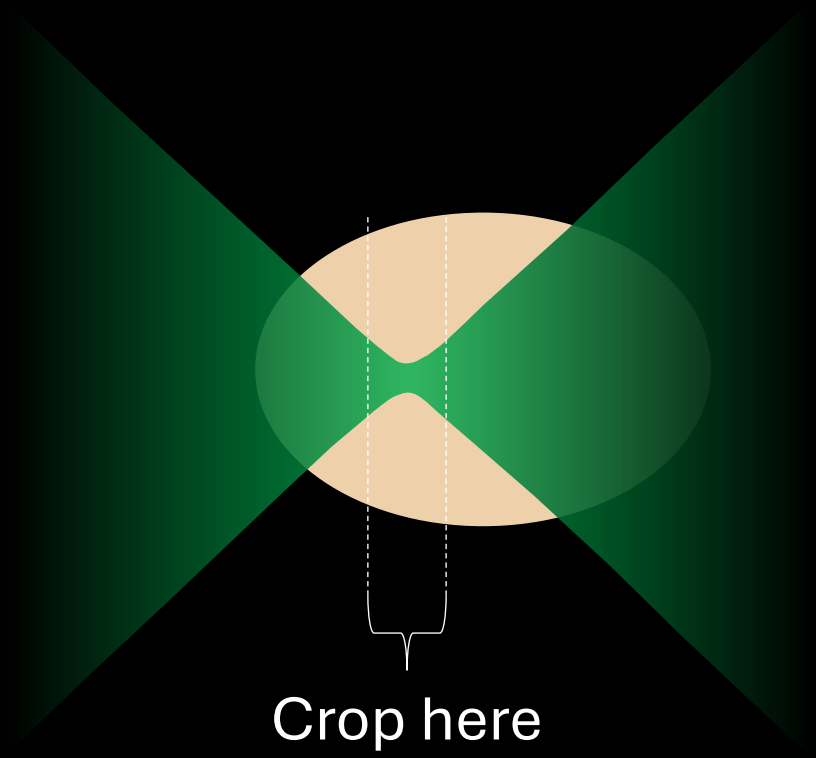


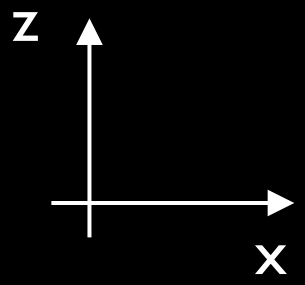


How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

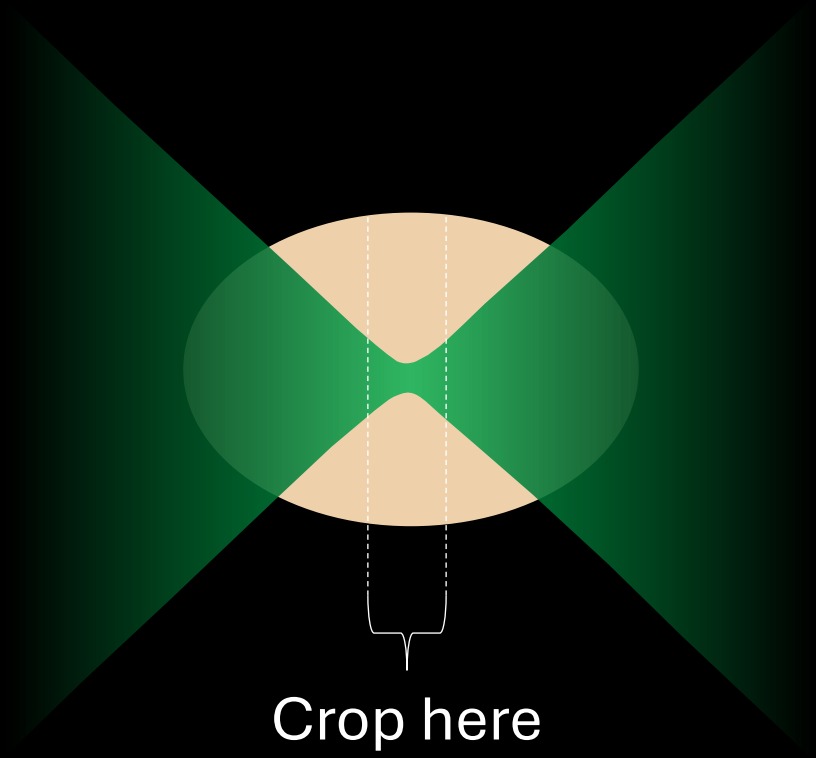


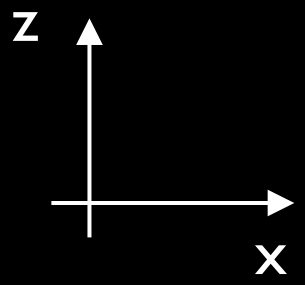


How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

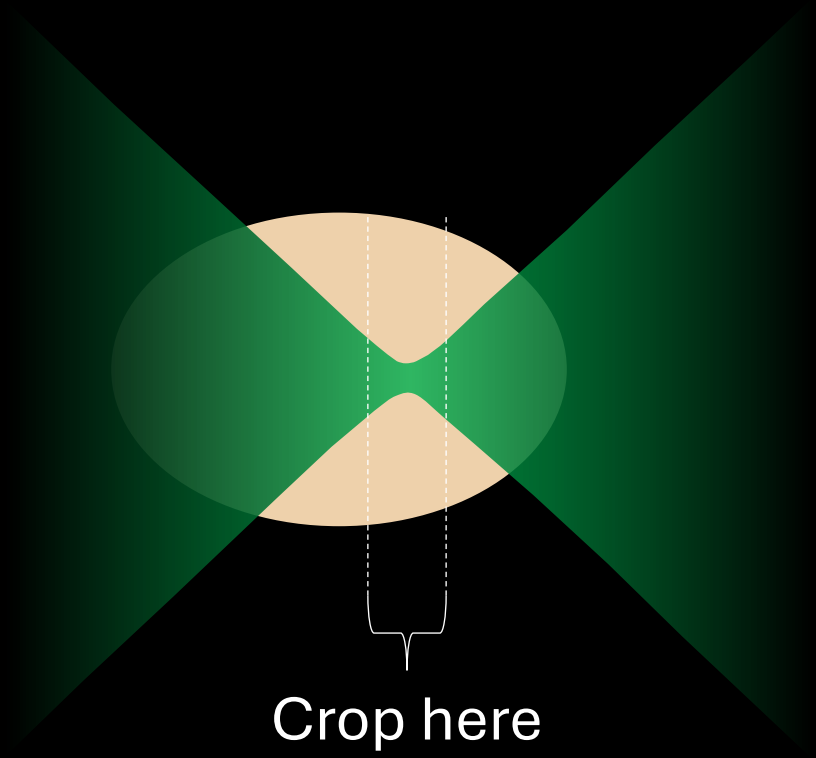


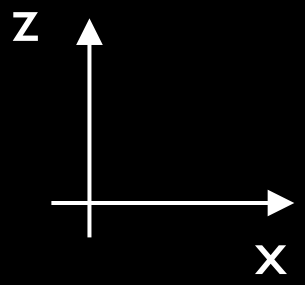


How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

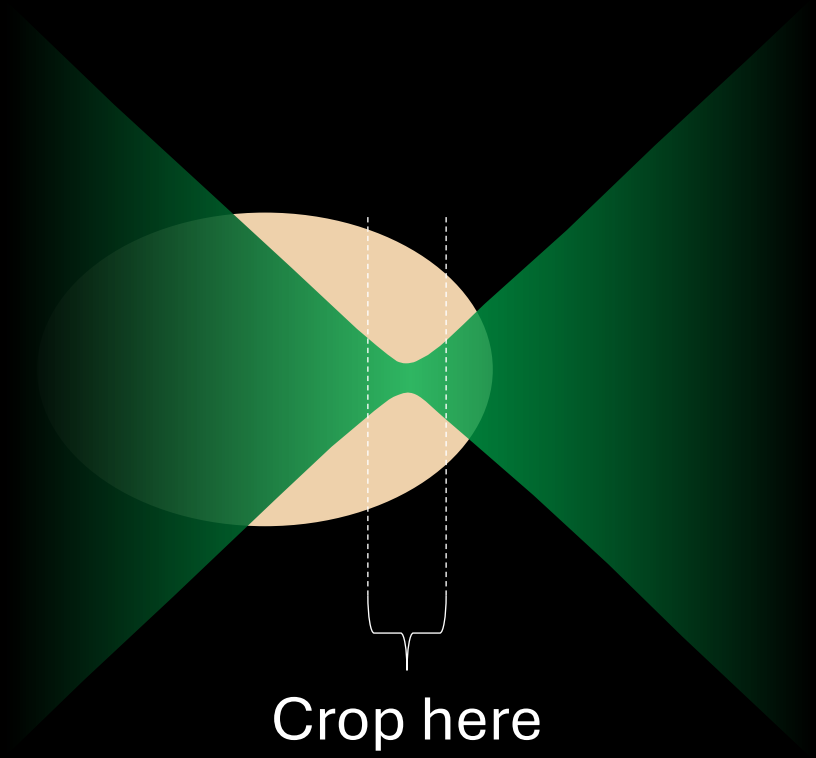


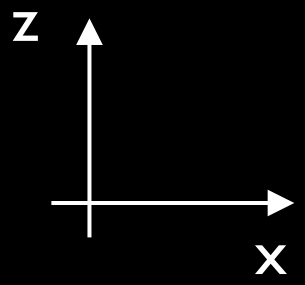


How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

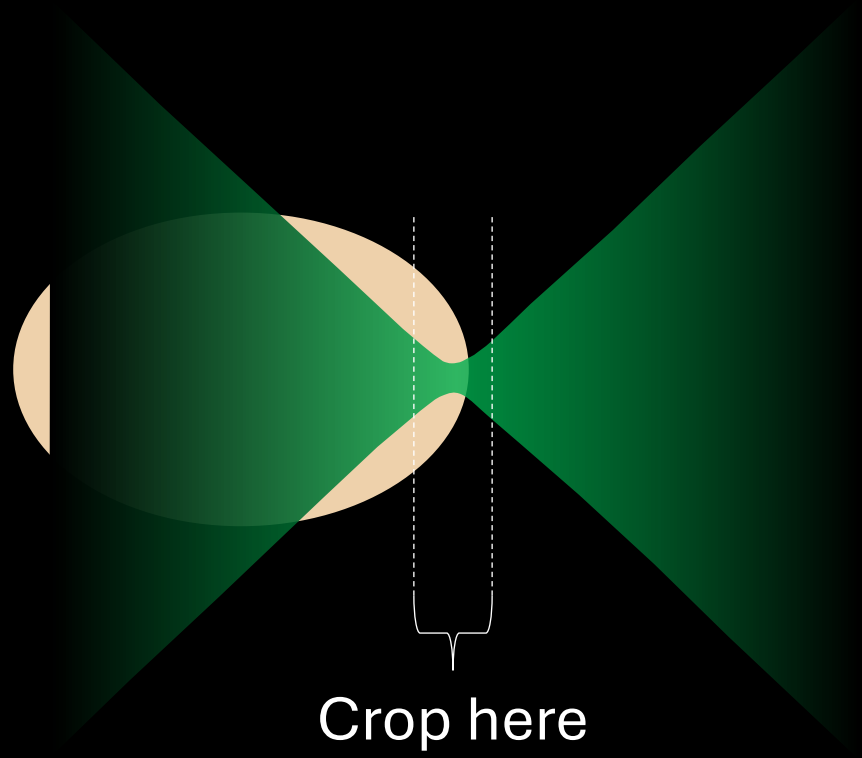


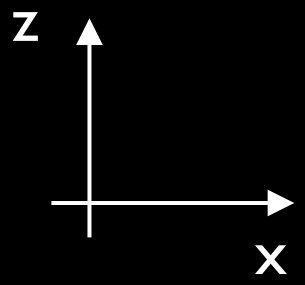


How to deal with uneven sheet shape

Option #2

Crop + tile + stitch





How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

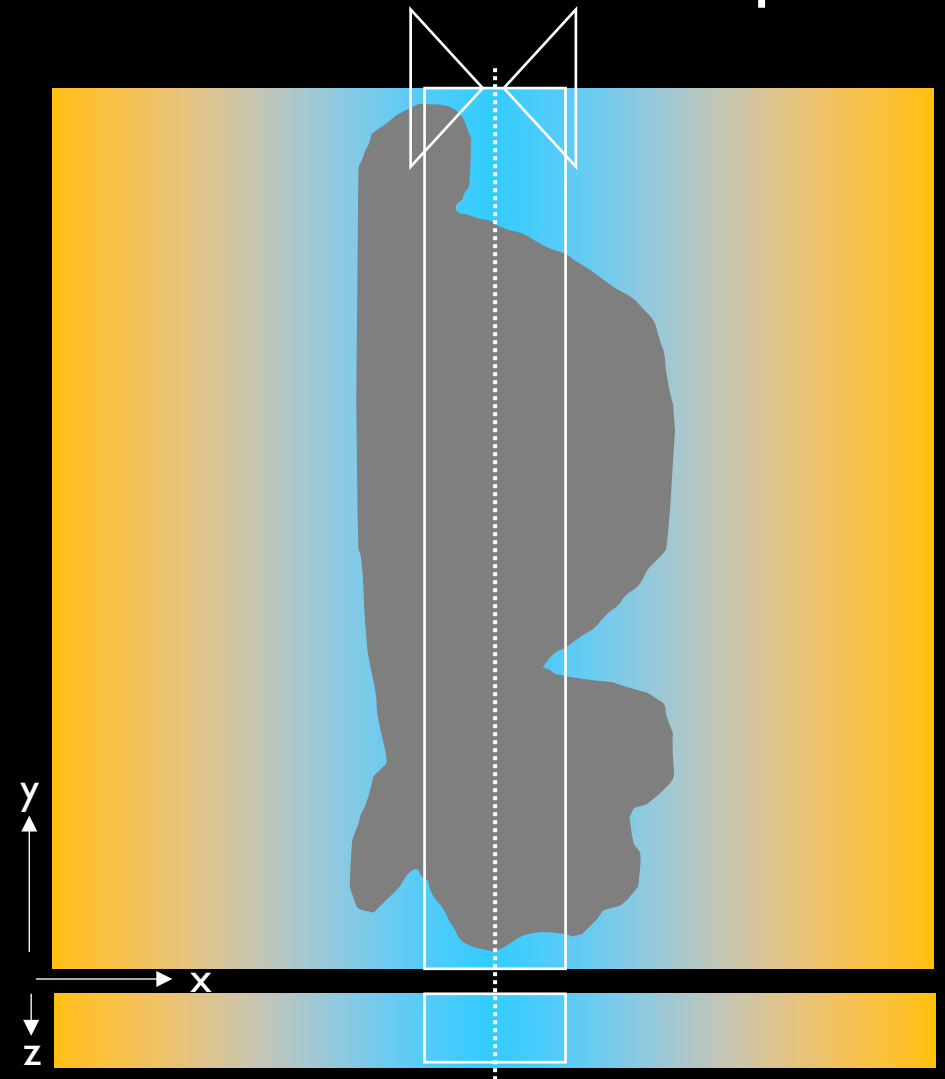


Image quality

Better

Worse

How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

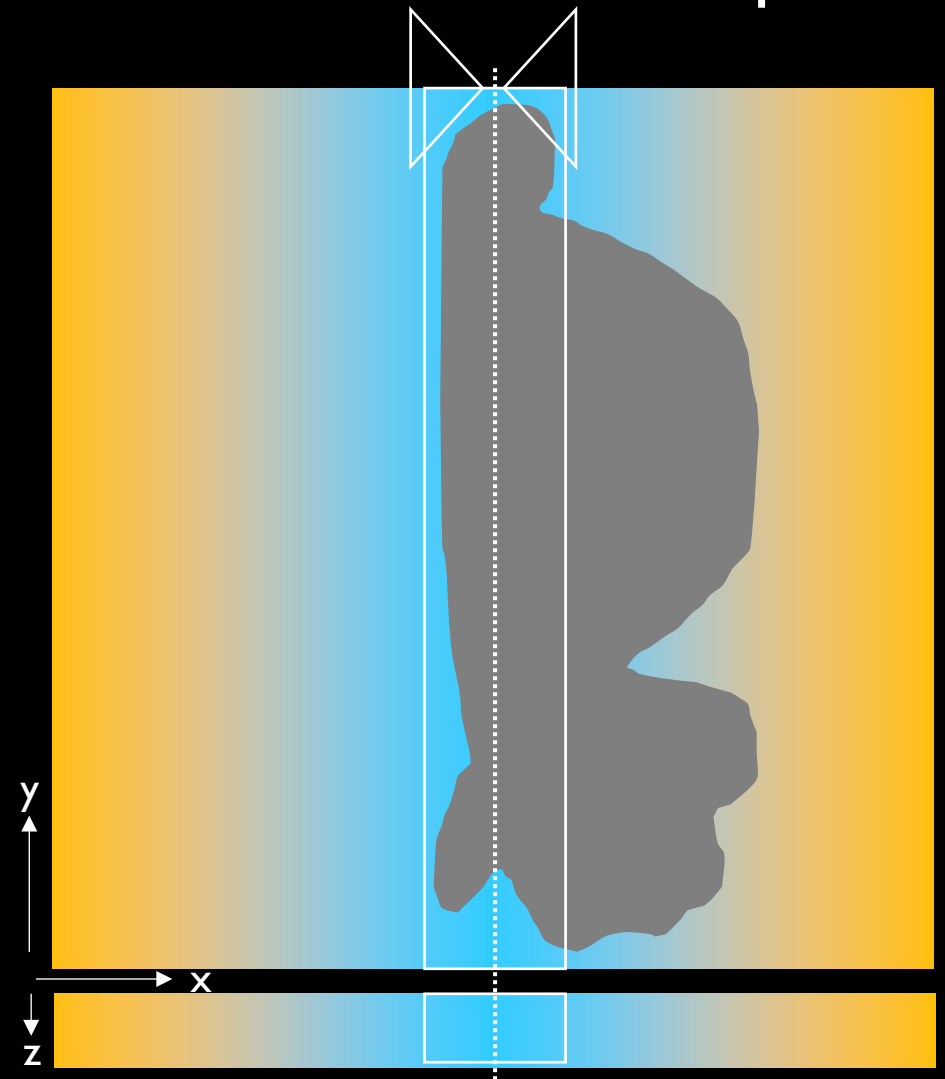


Image quality

Better

Worse

How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

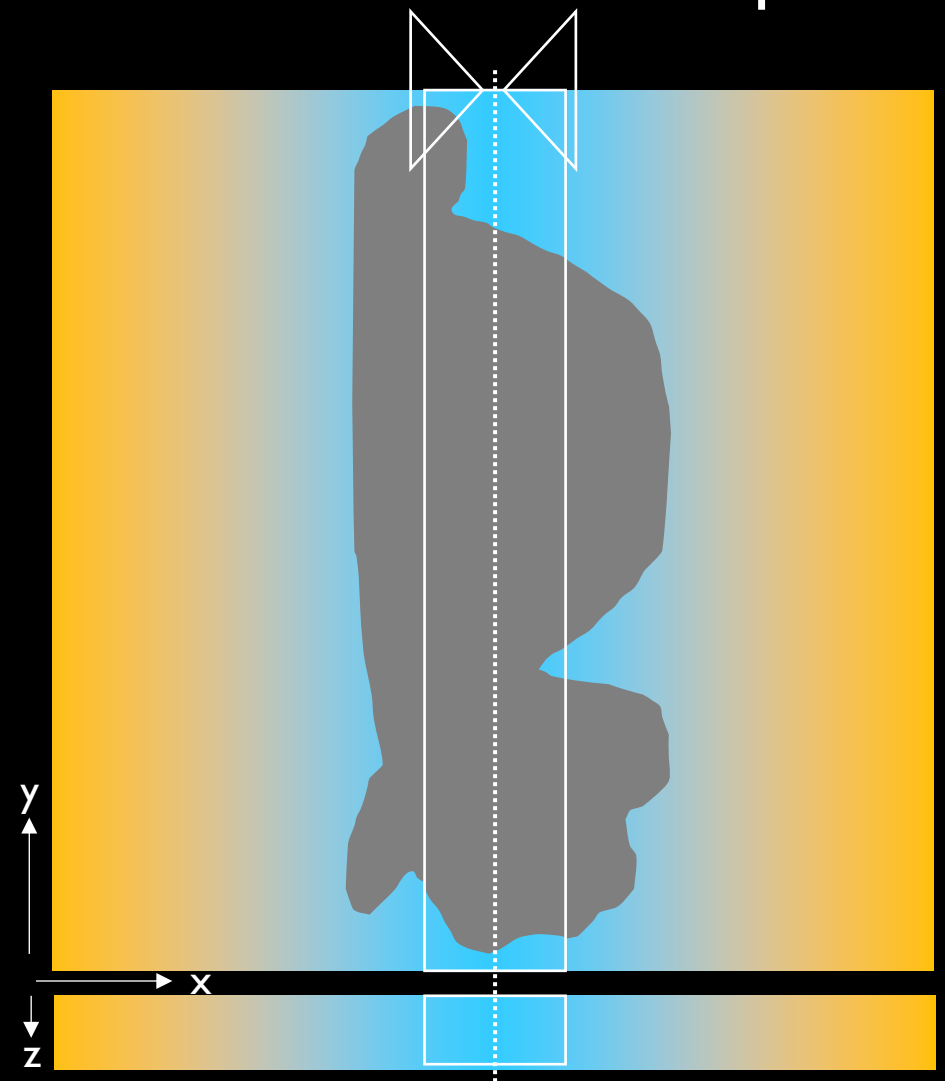


Image quality

Better

Worse

How to deal with uneven sheet shape

Option #2

Crop + tile + stitch

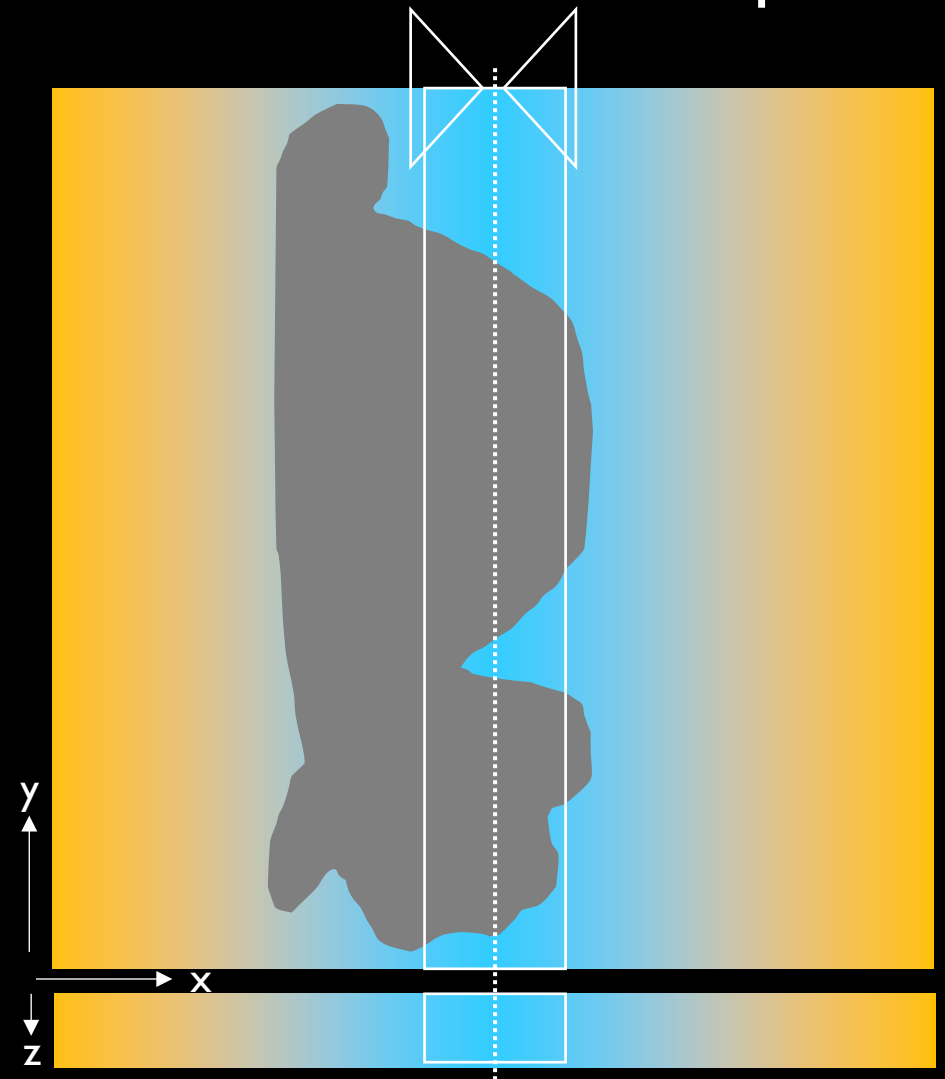


Image quality

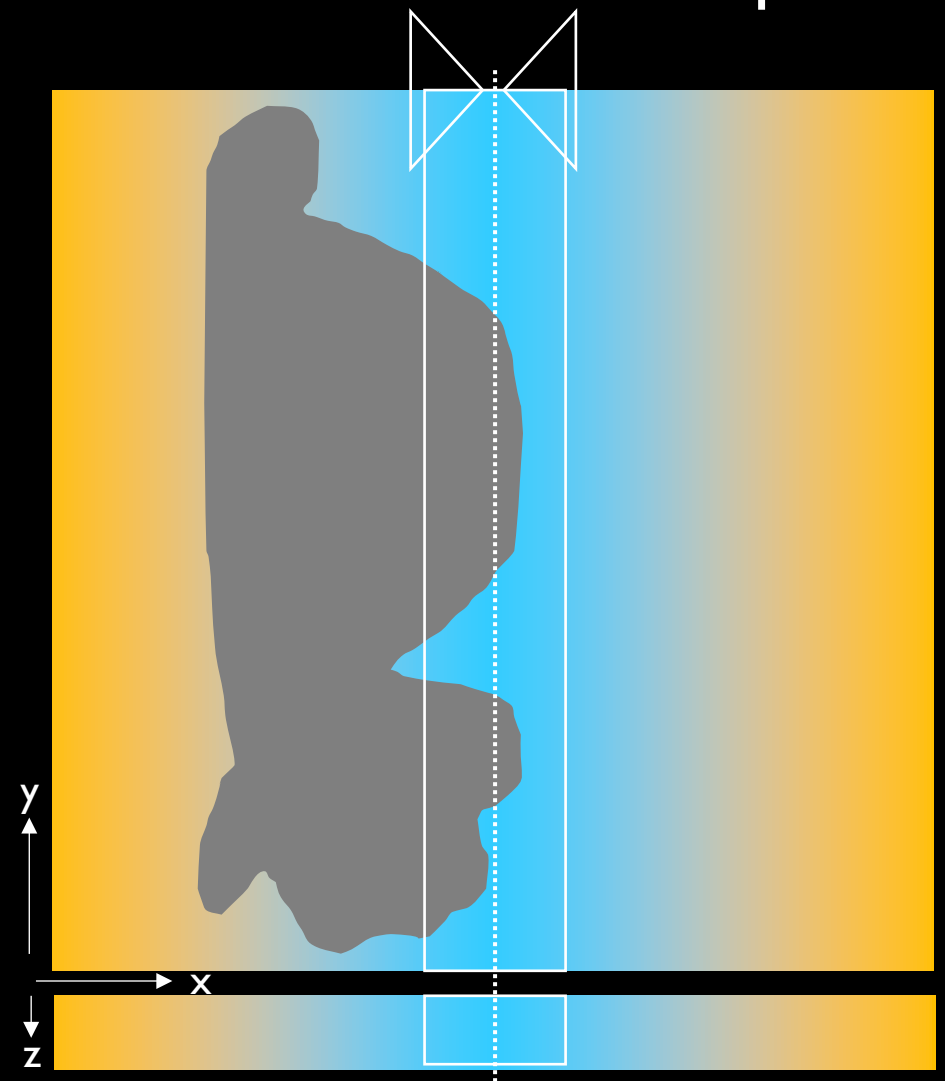
Better

Worse

How to deal with uneven sheet shape

Option #2

Crop + tile + stitch



- Higher, even Z resolution
- Takes longer
- Stitching can be hard
- Requires excellent sheet alignment

The Blaze does this very quickly

Image quality

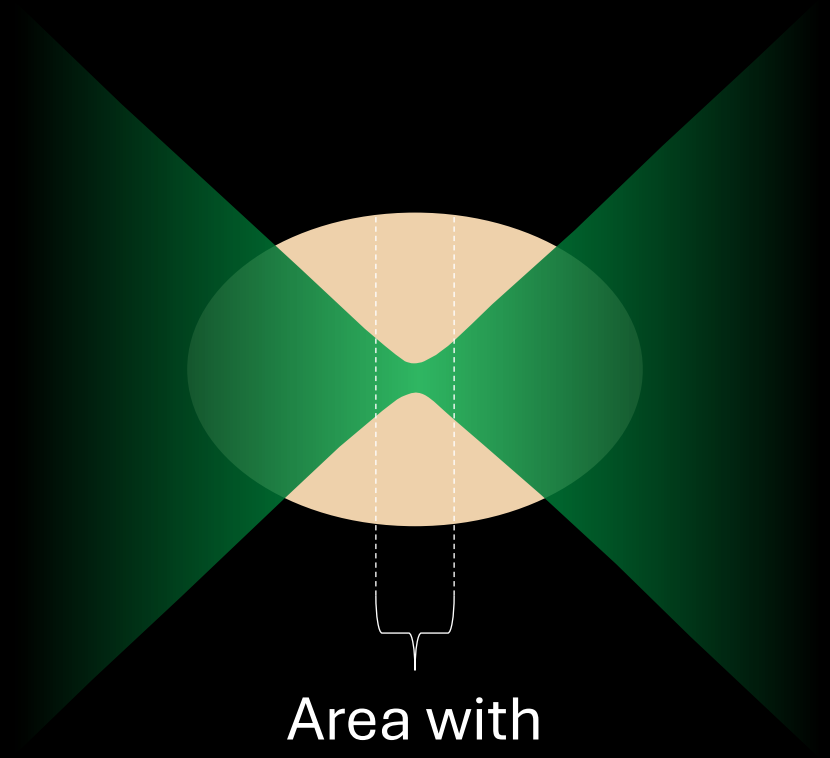
Better

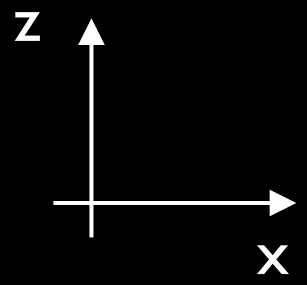
Worse

How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

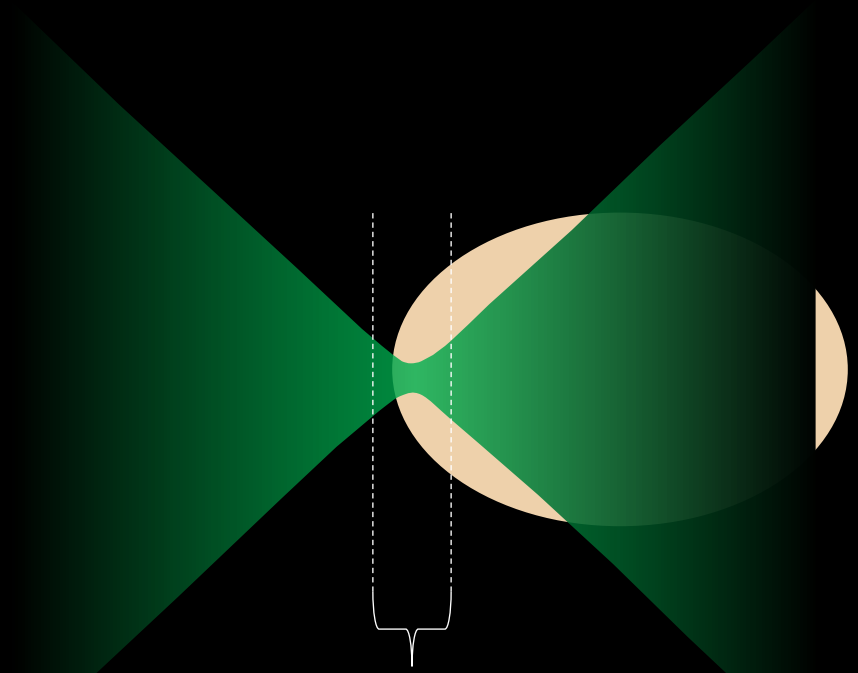


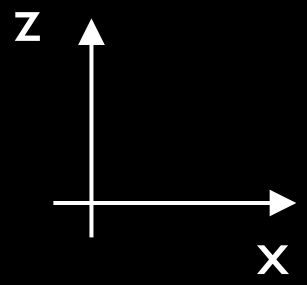


How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

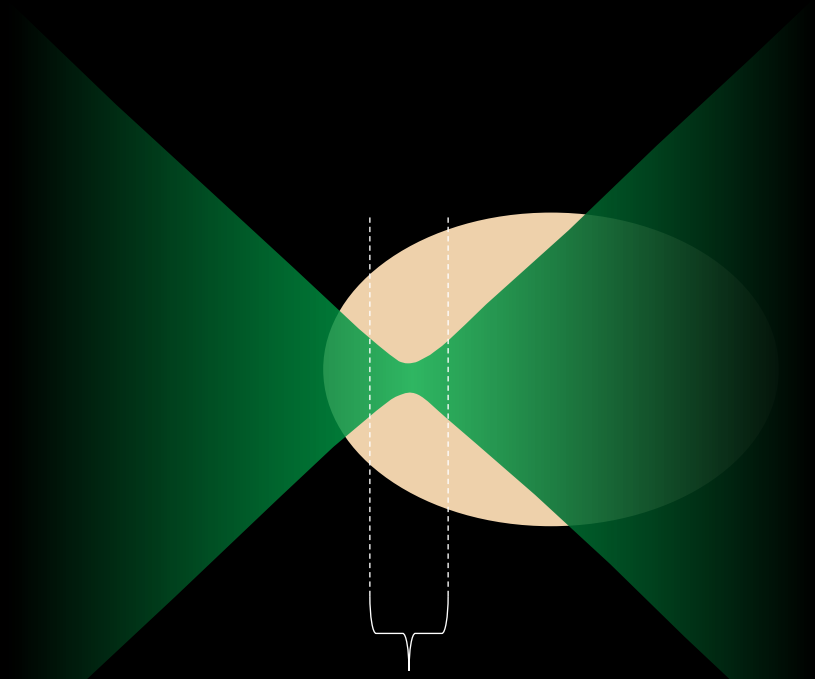


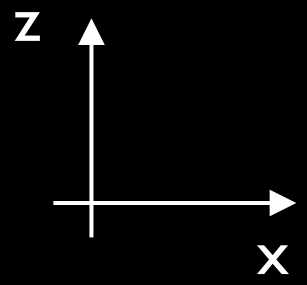


How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

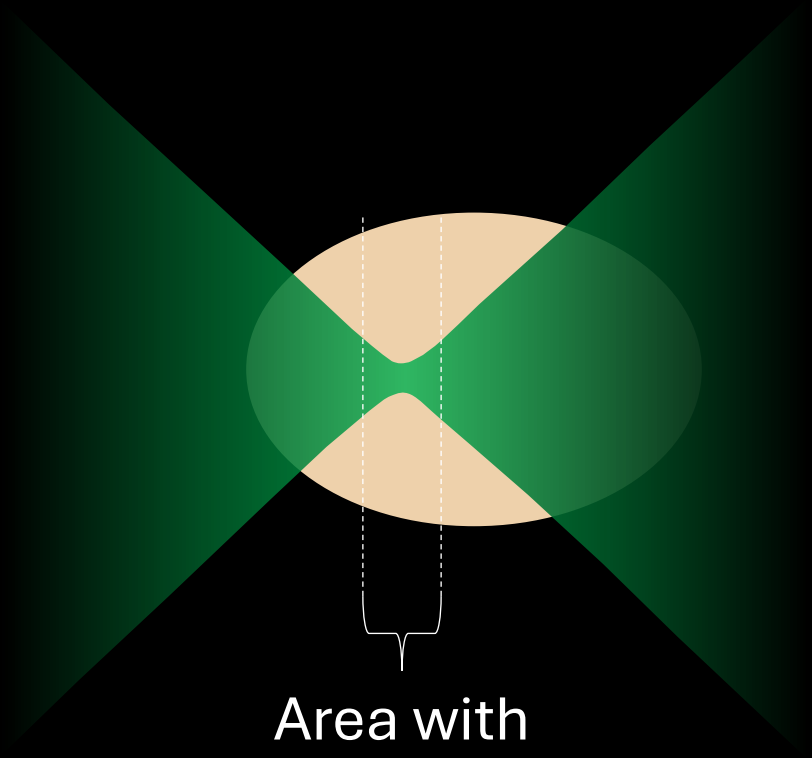


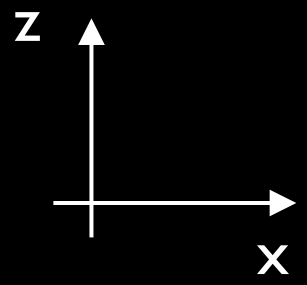


How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

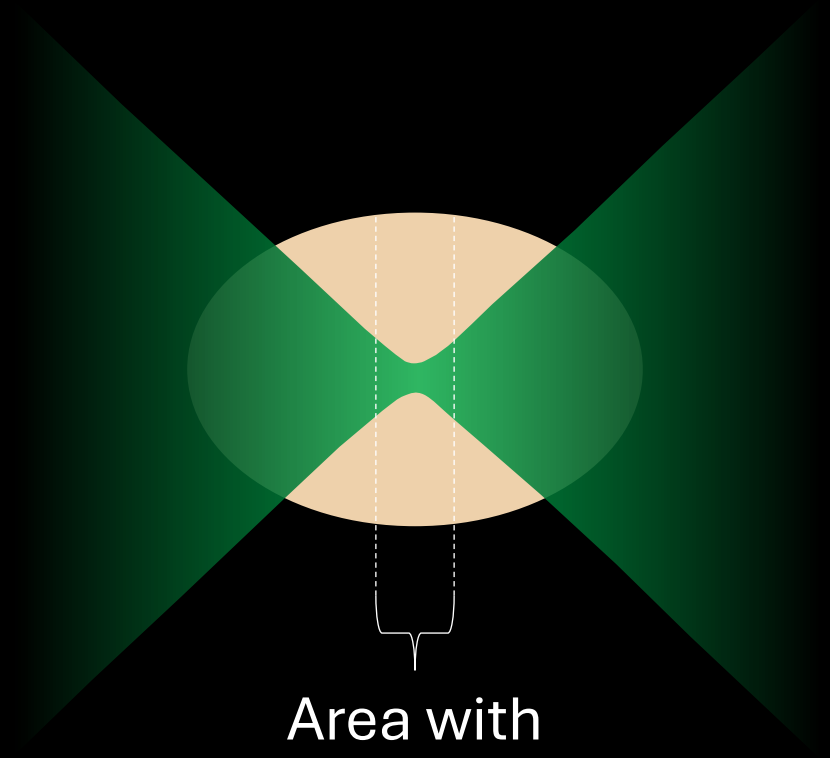


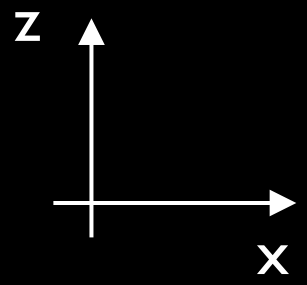


How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

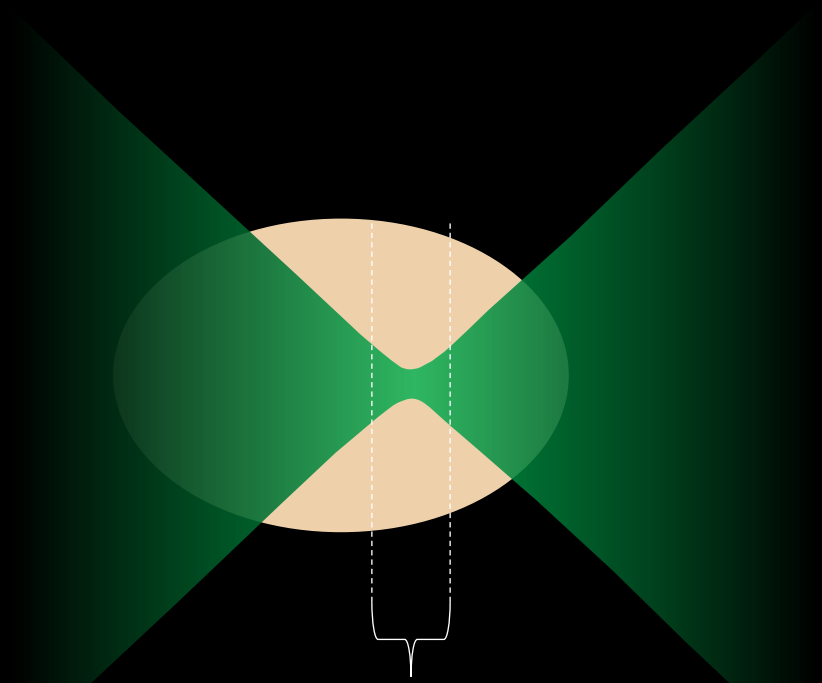


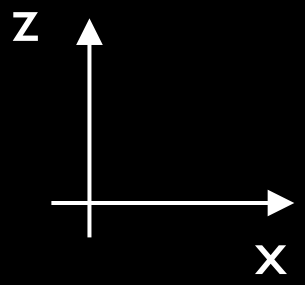


How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

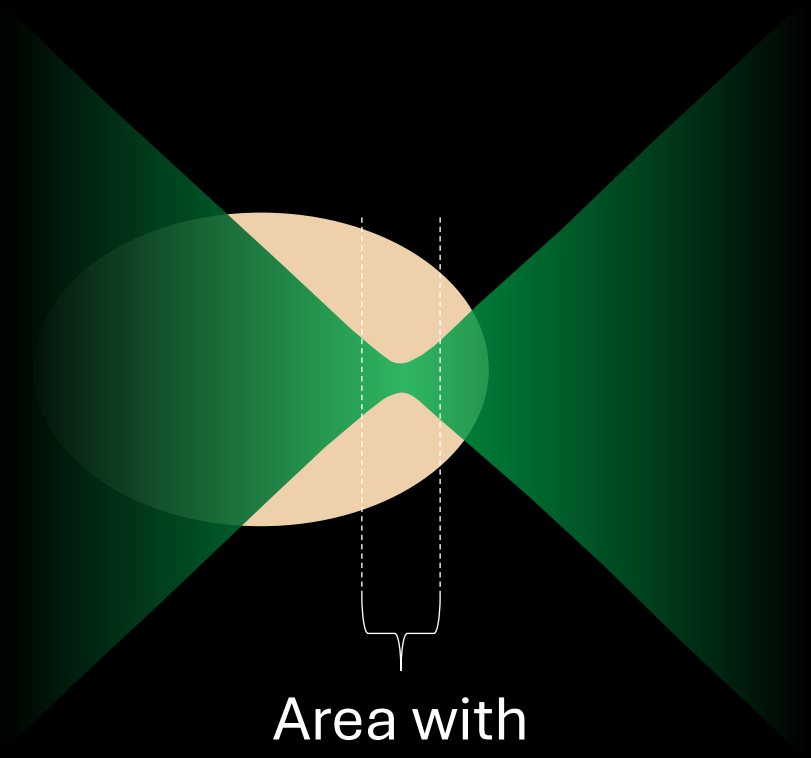


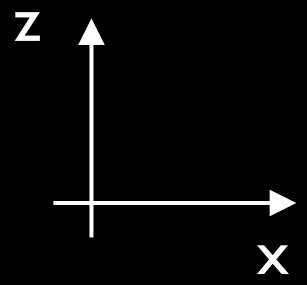


How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

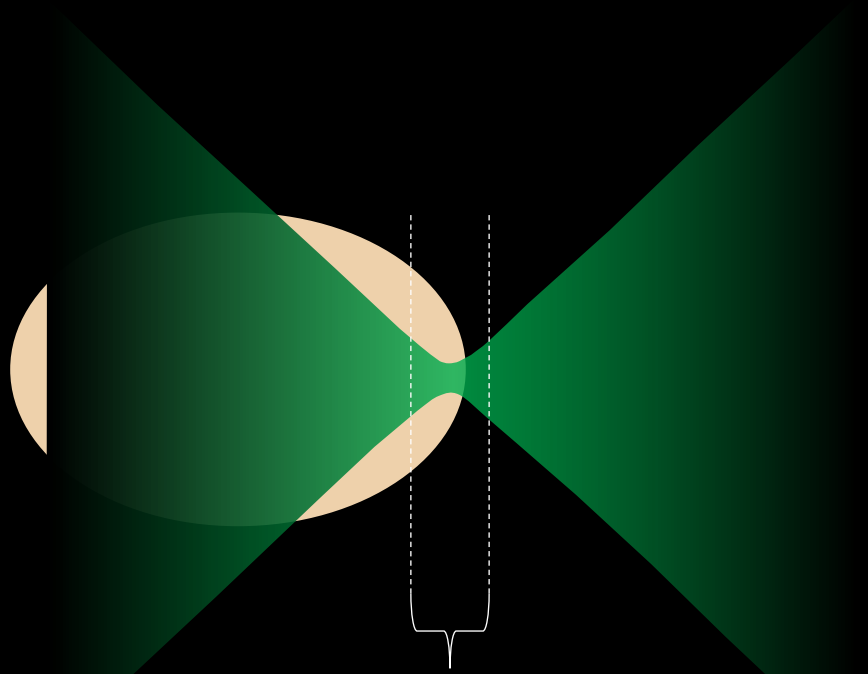


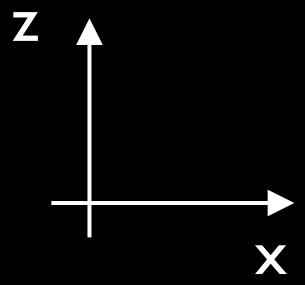


How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images





How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

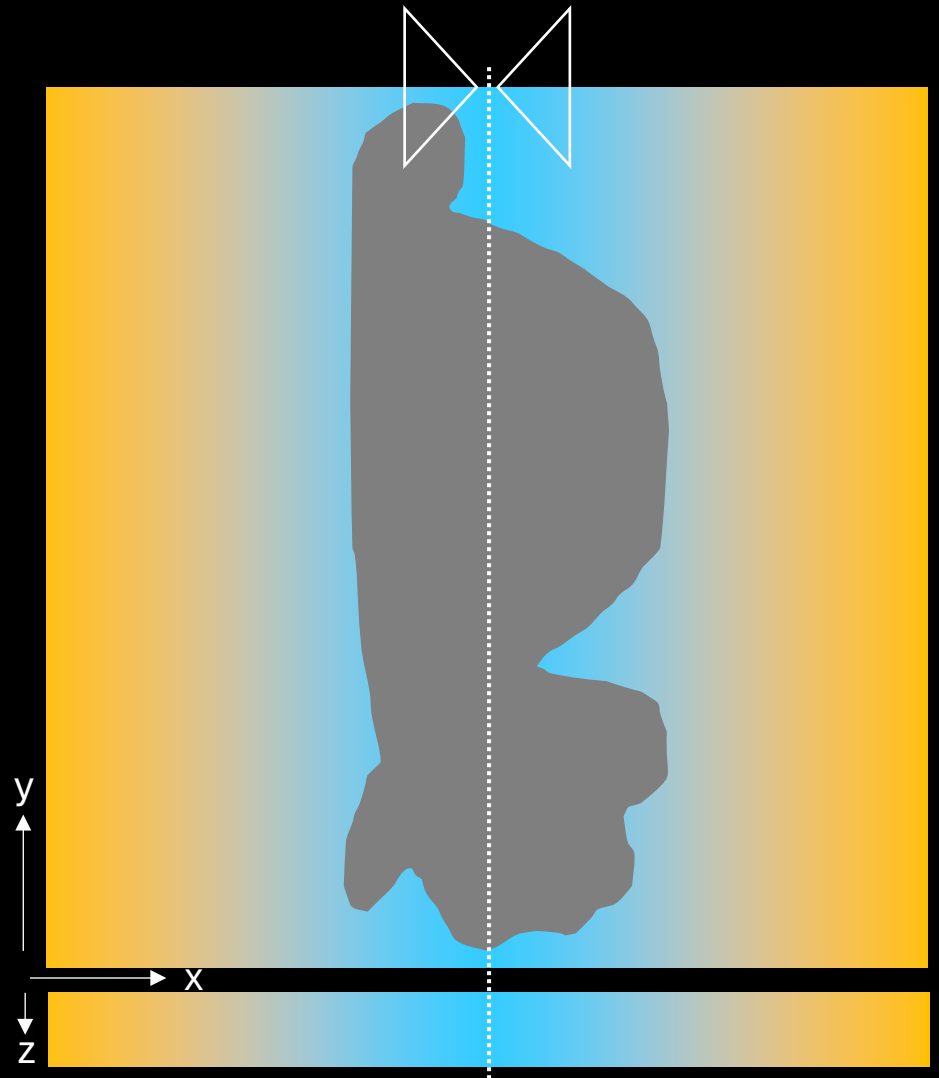


Image quality

Better

Worse

How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

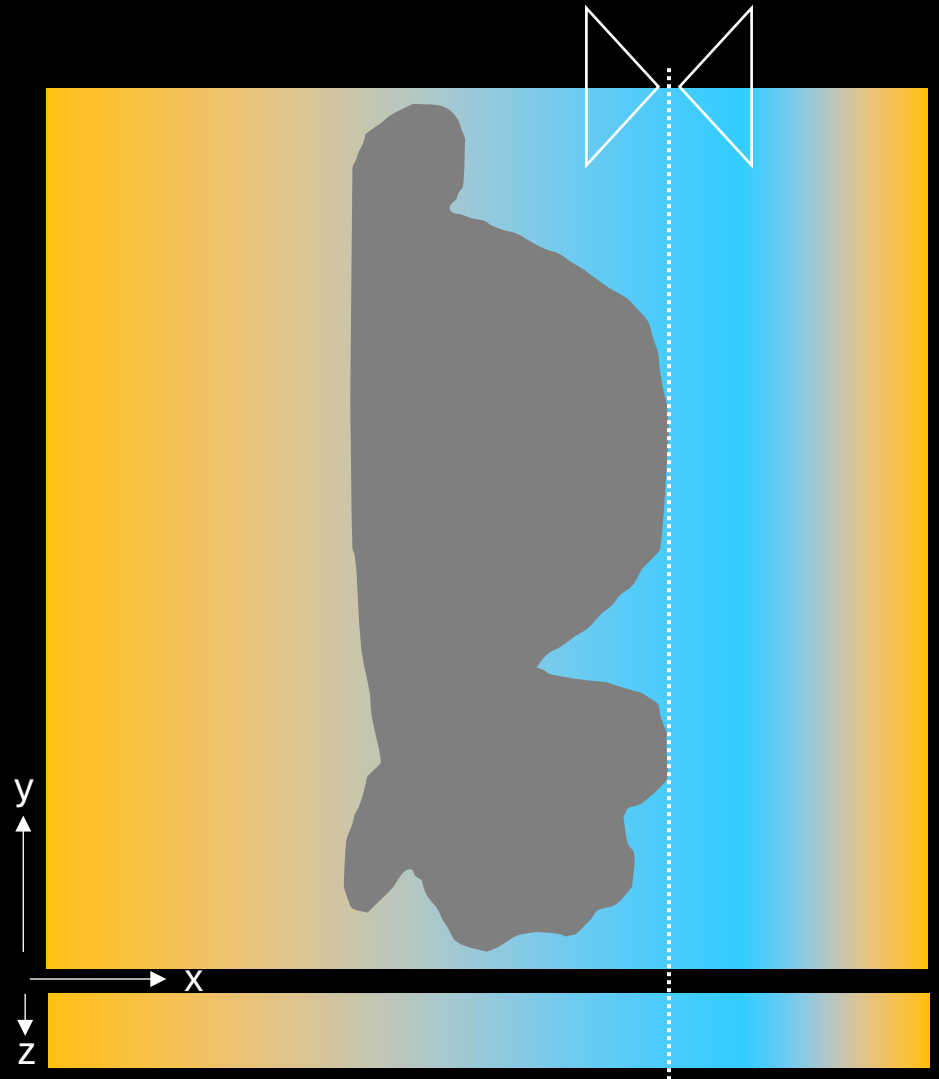


Image quality

Better

Worse

How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

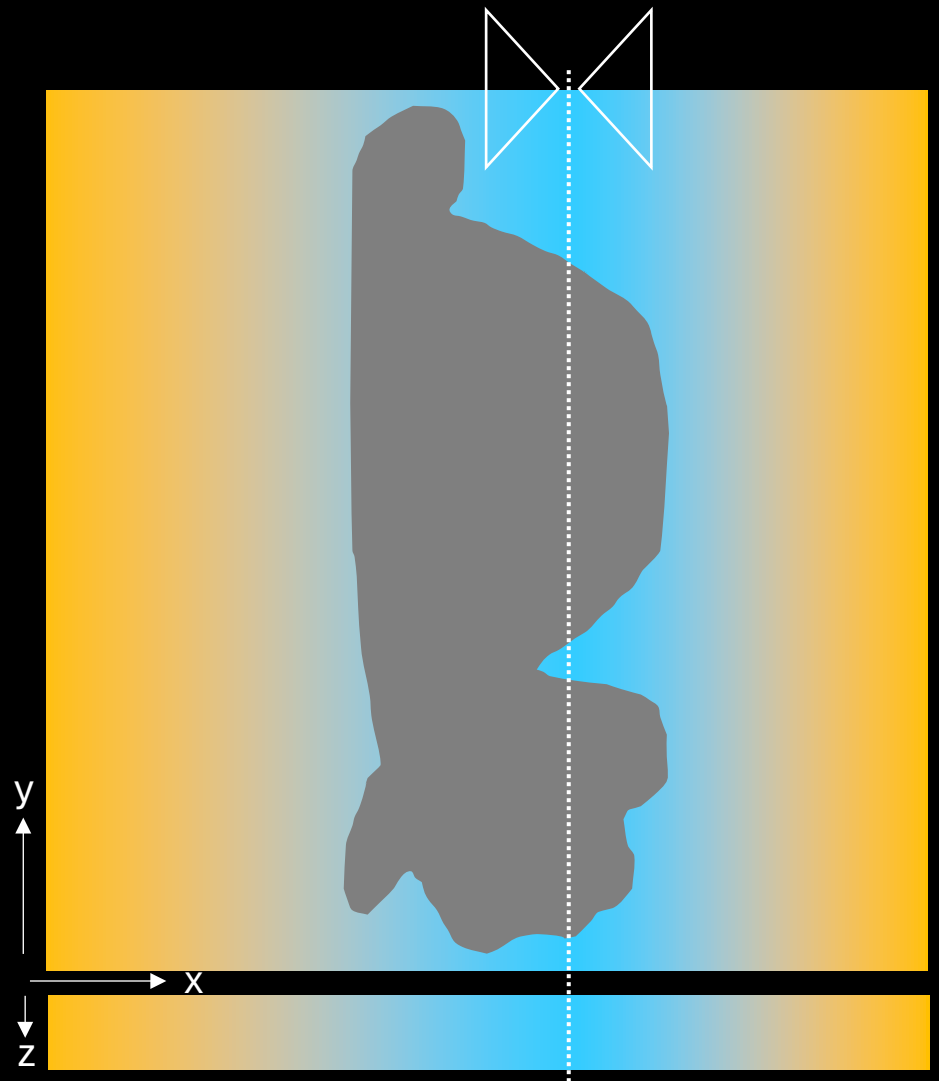


Image quality

Better

Worse

How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

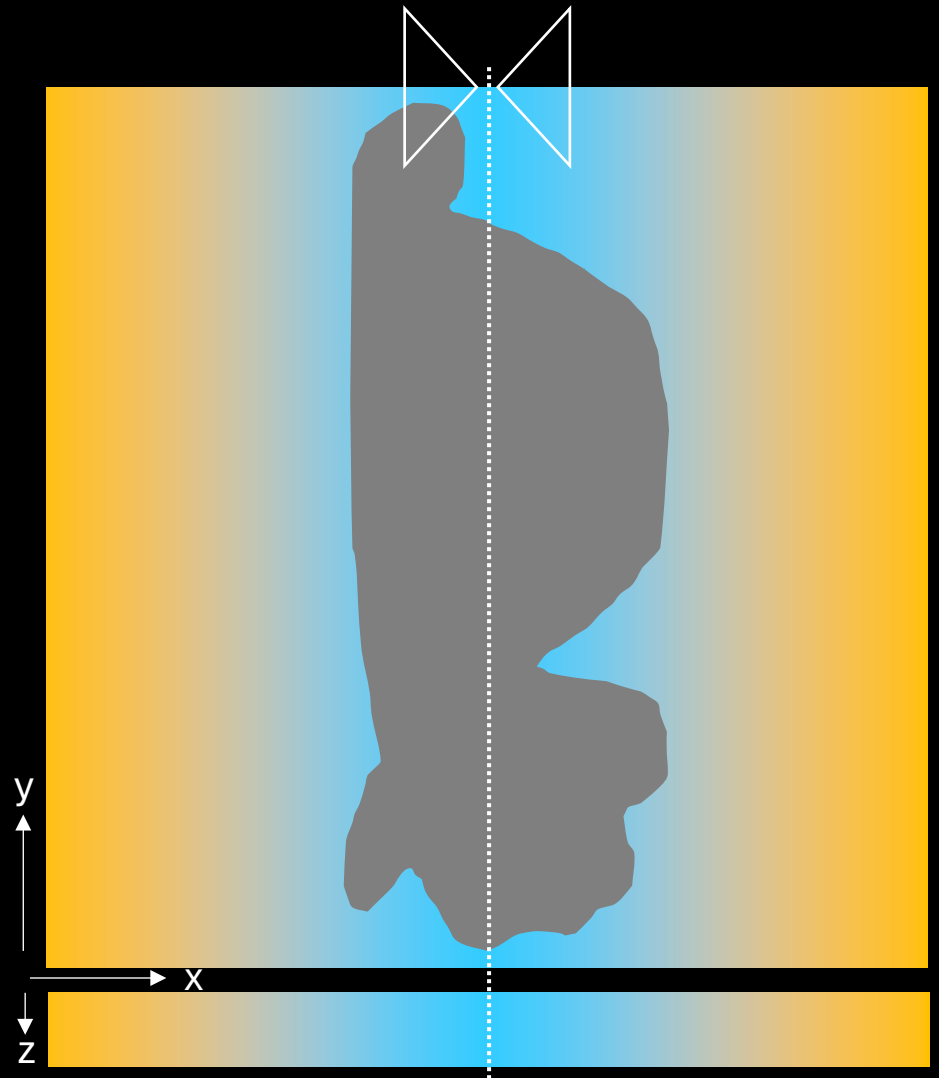


Image quality

Better

Worse

How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images

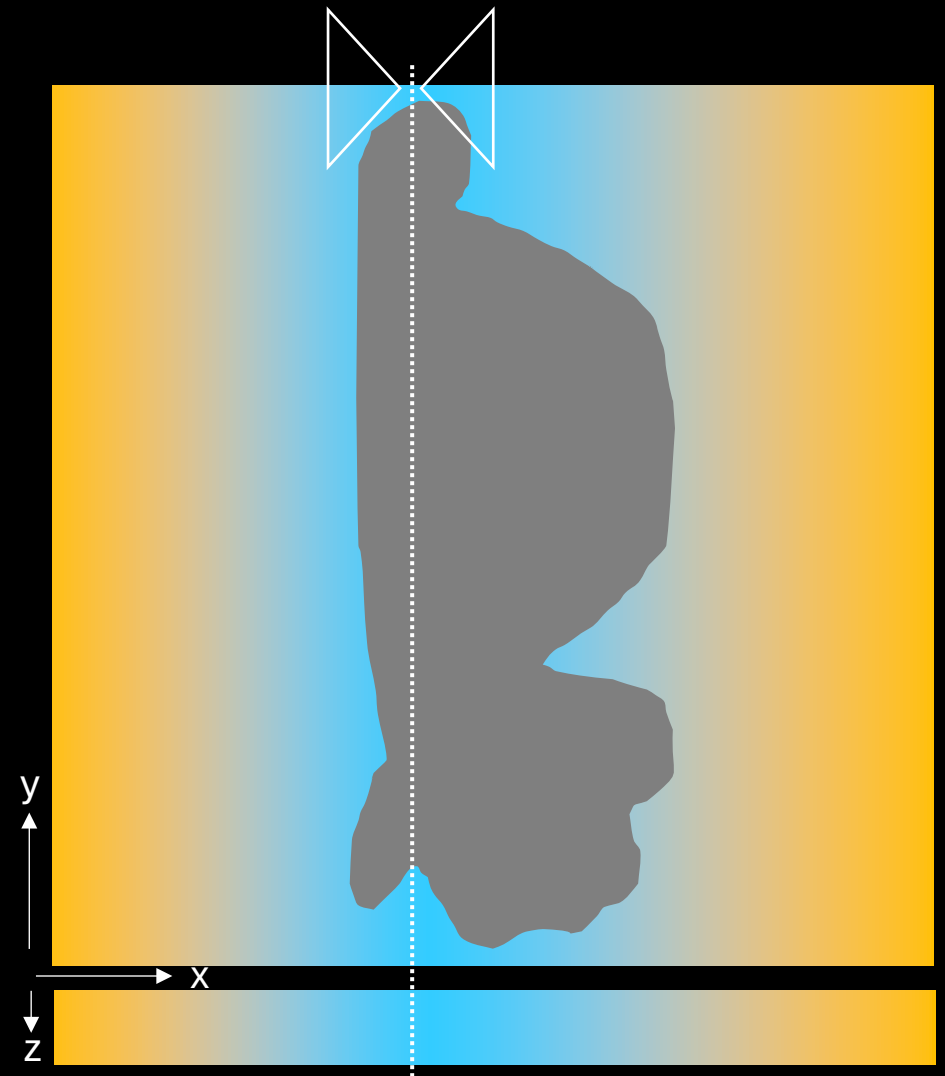


Image quality

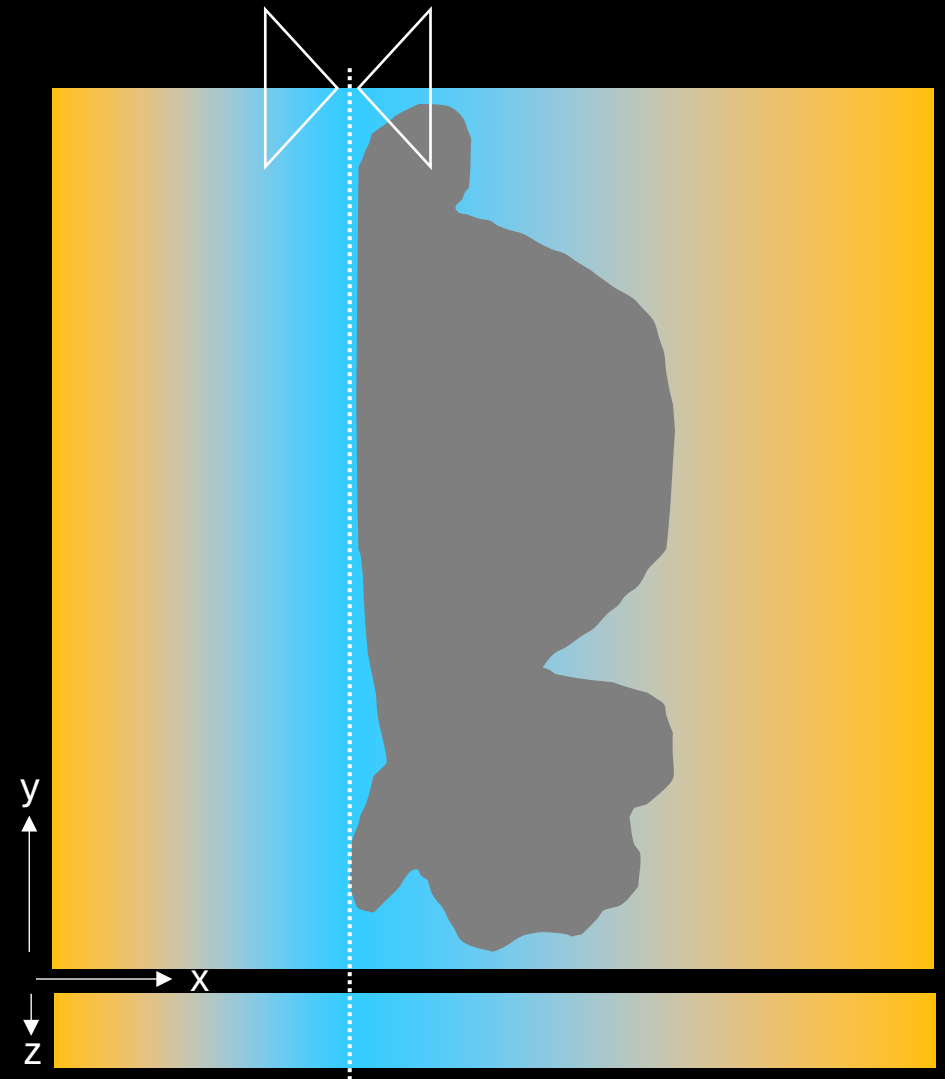
Better

Worse

How to deal with uneven sheet shape

Option #3

Dynamic focus: Move sheet waist, combine multiple images



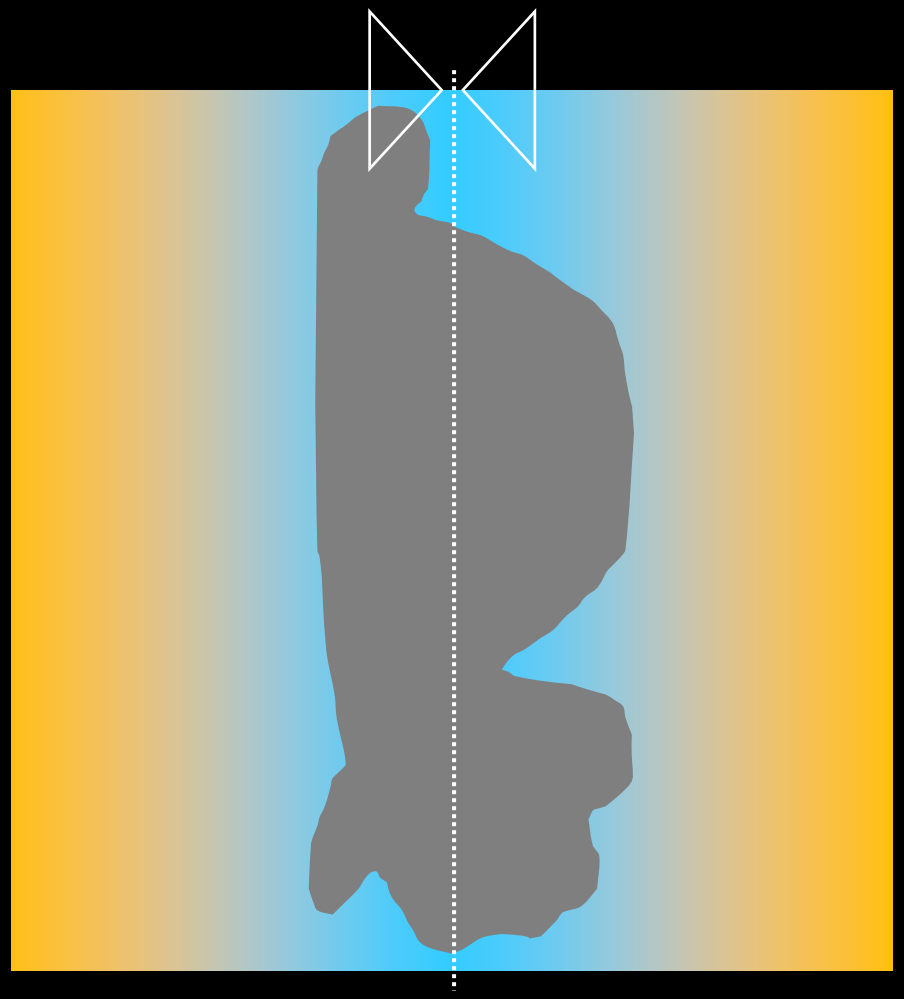
- Higher, even Z resolution
- Takes much longer
- Combining images can be challenging
- Requires excellent sheet alignment

Image quality

Better

Worse

Comparison of options to deal with sheet shape



Change shape - lower NA

Dynamic Focus

Crop + Tile + Stitch

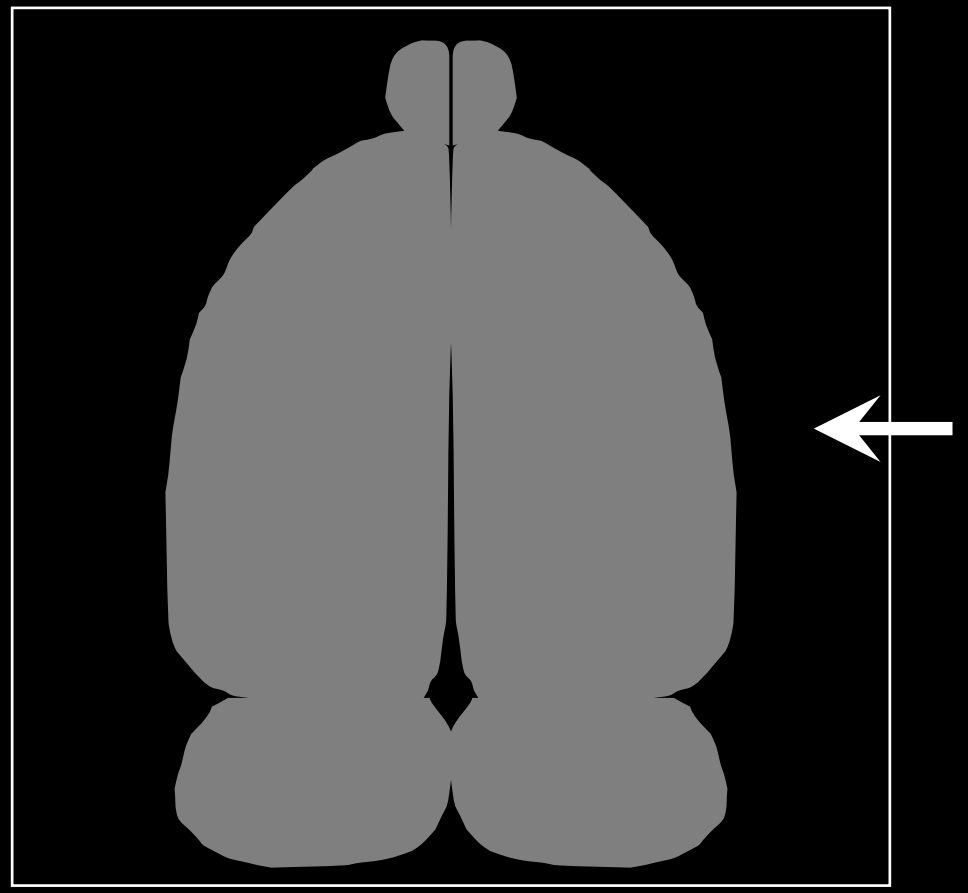
Speed

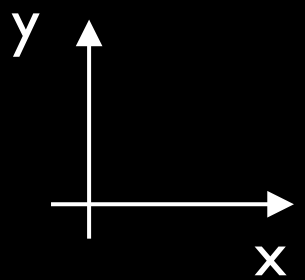
Quality

y

x

Dealing with the challenge of bigger samples





Single sheet illumination

Dealing with the challenge of bigger samples

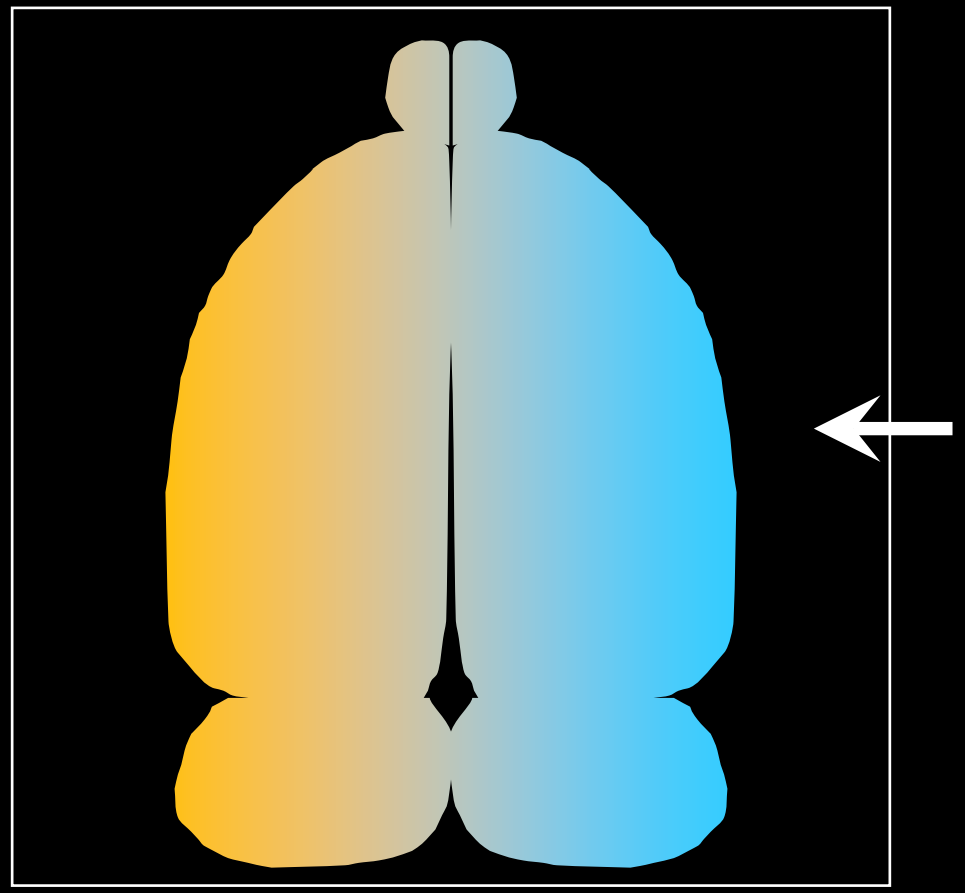
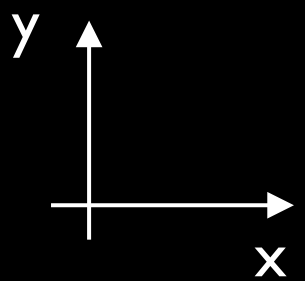


Image quality

Better

Worse



Single sheet illumination

Dealing with the challenge of bigger samples

Option

1) Take image with right sheet

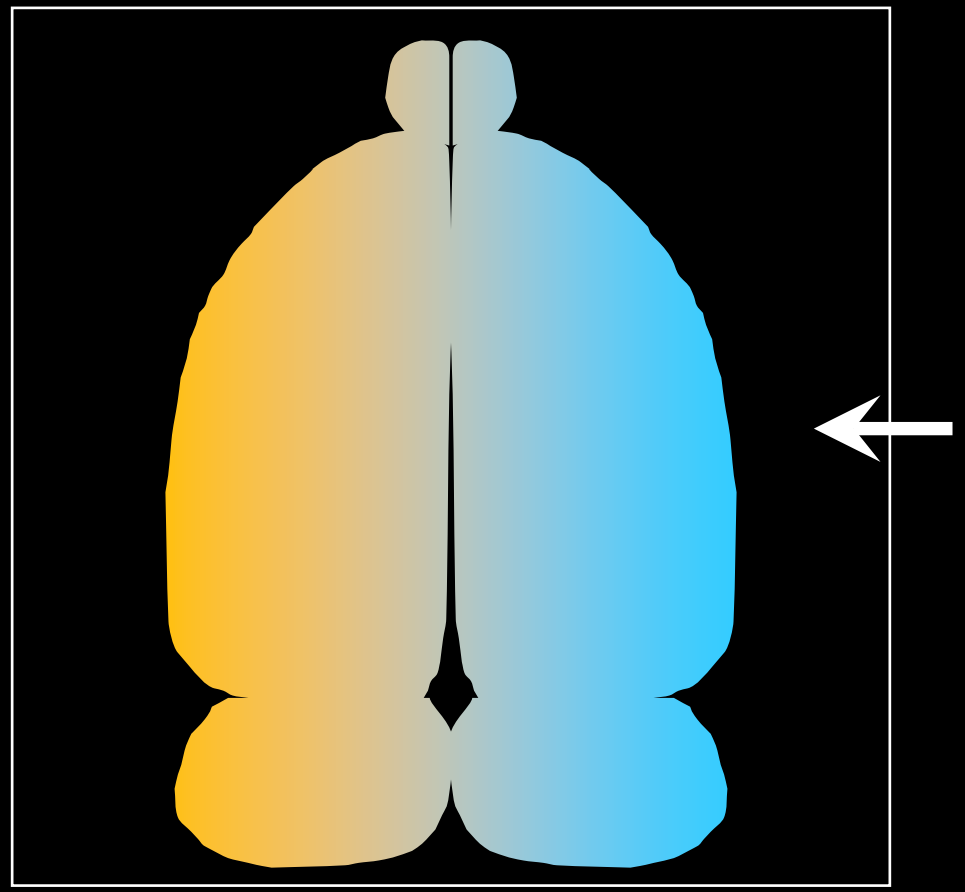
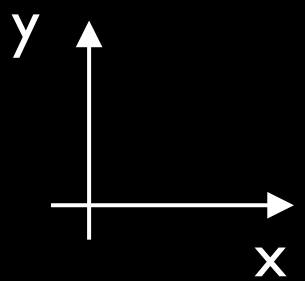


Image quality

Better

Worse



Dealing with the challenge of bigger samples

Option

1) Take image with right sheet
2) Take image with left sheet

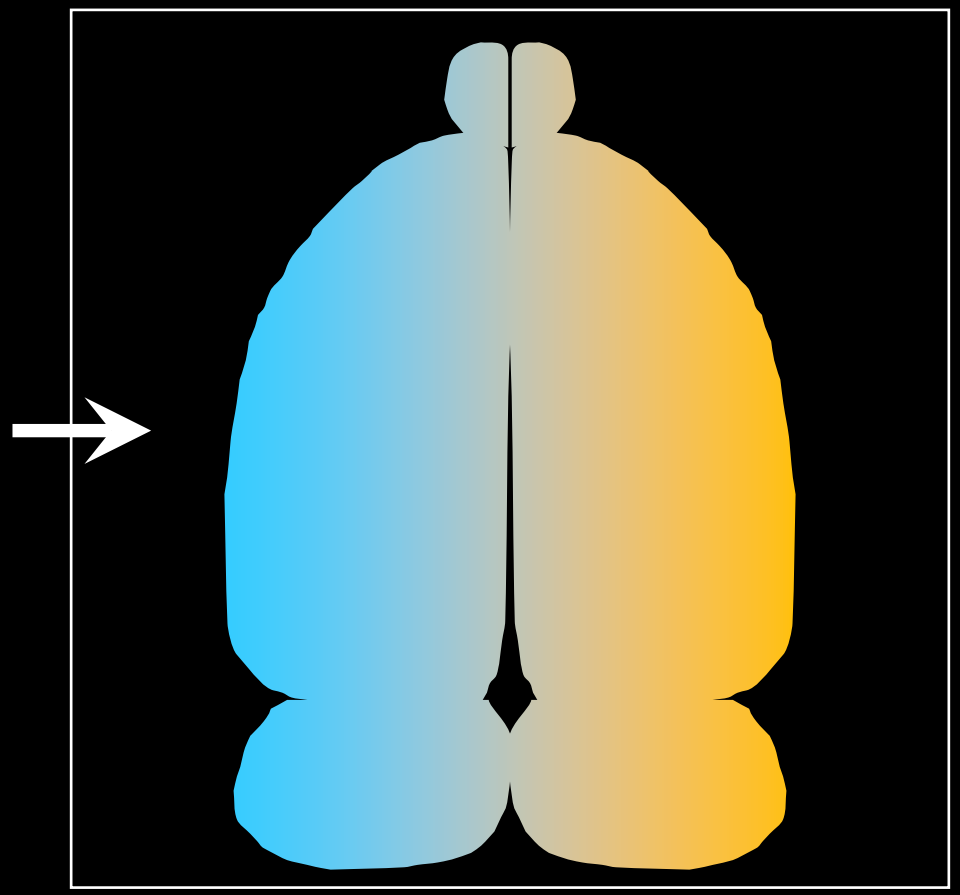
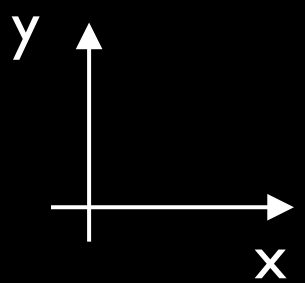


Image quality

Better

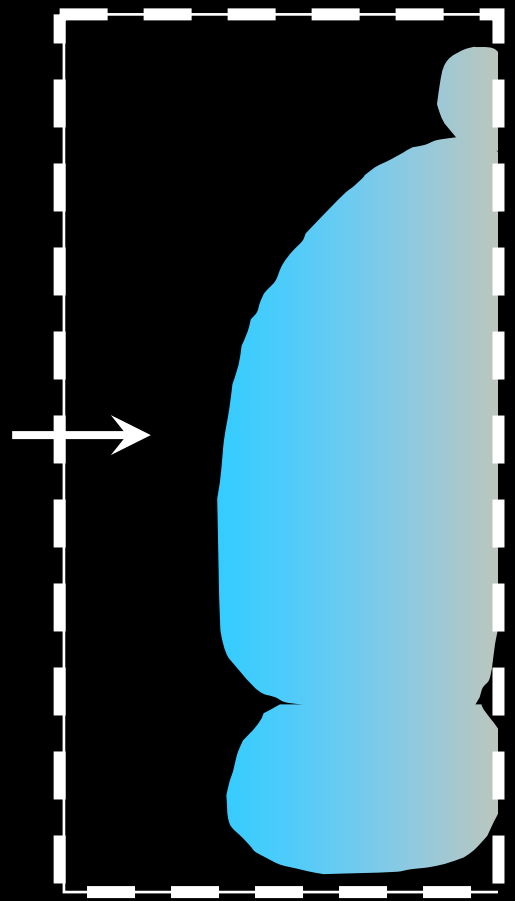
Worse

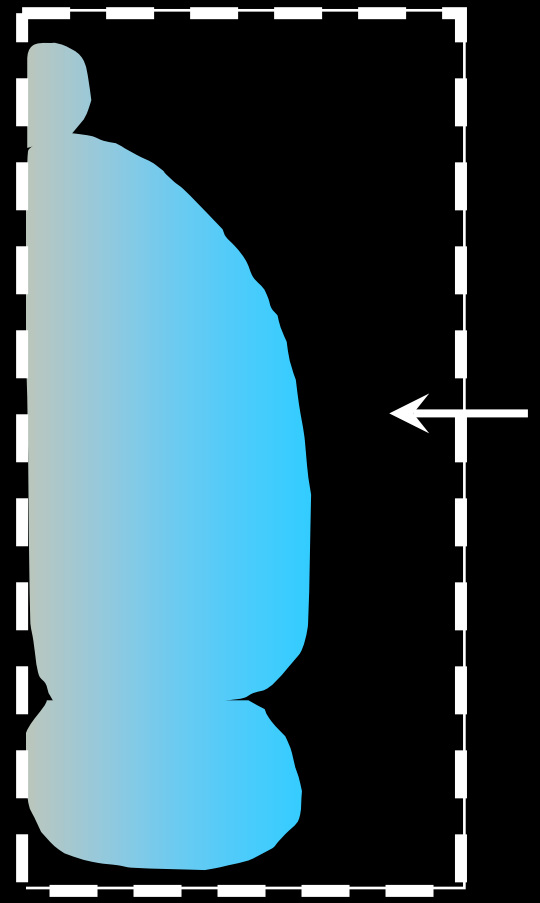


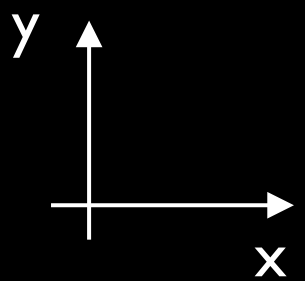
Dealing with the challenge of bigger samples

Option

1) Take image with right sheet
2) Take image with left sheet
3) Crop left side of left image
   and right side of right image



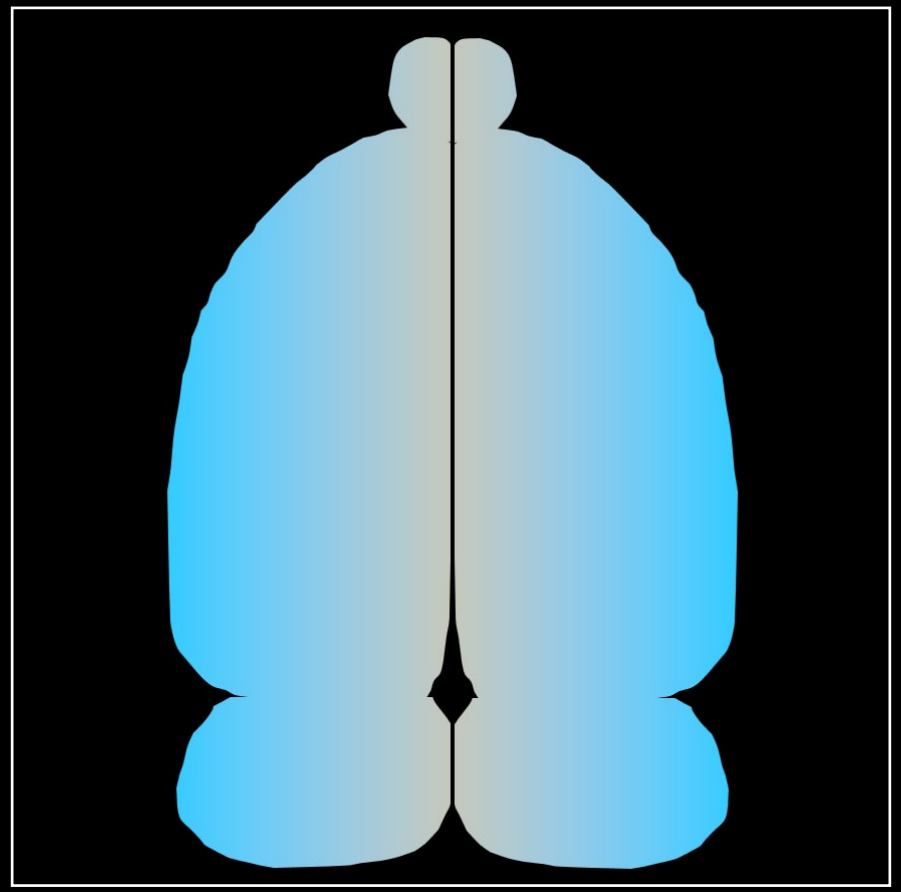


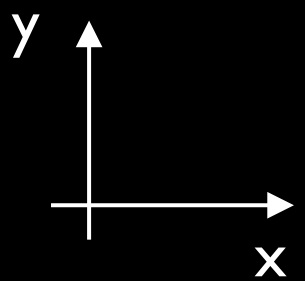


Dealing with the challenge of bigger samples

Option

1) Take image with right sheet
2) Take image with left sheet
3) Crop left side of left image and right side of right image
4) Merge subimages

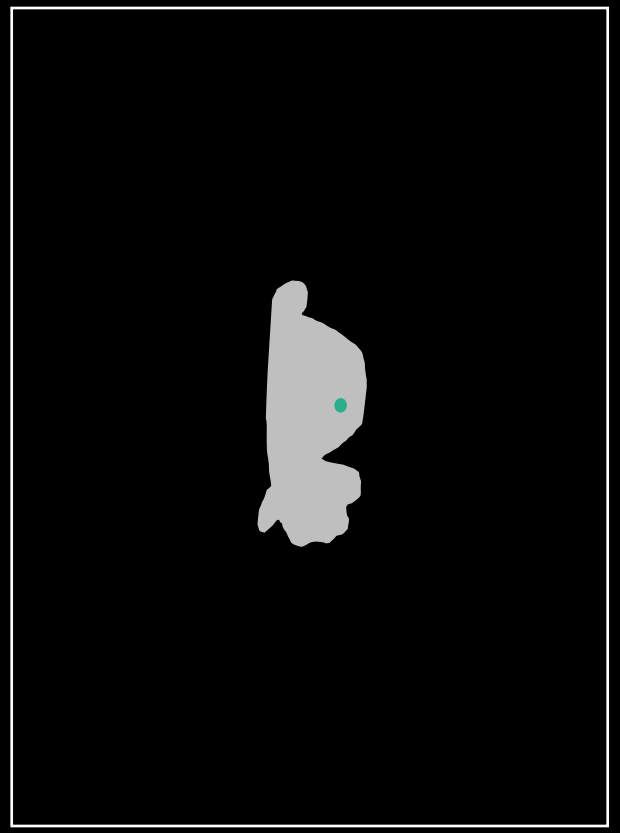


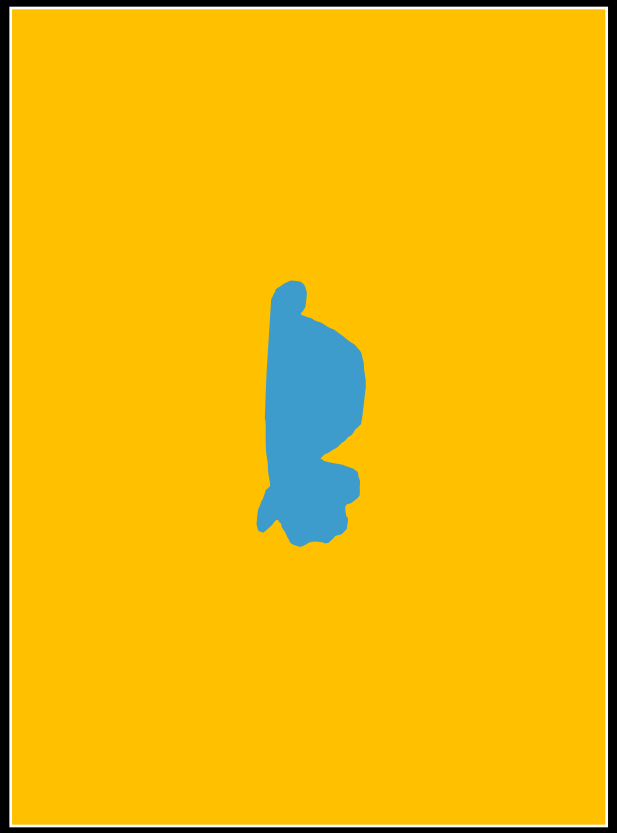


How to set laser power and exposure times

	Explore sample	Take data
Laser power	Low	High
Exposure time	Long	Short
	Low bleaching	High speed

Avoid taking empty pixels



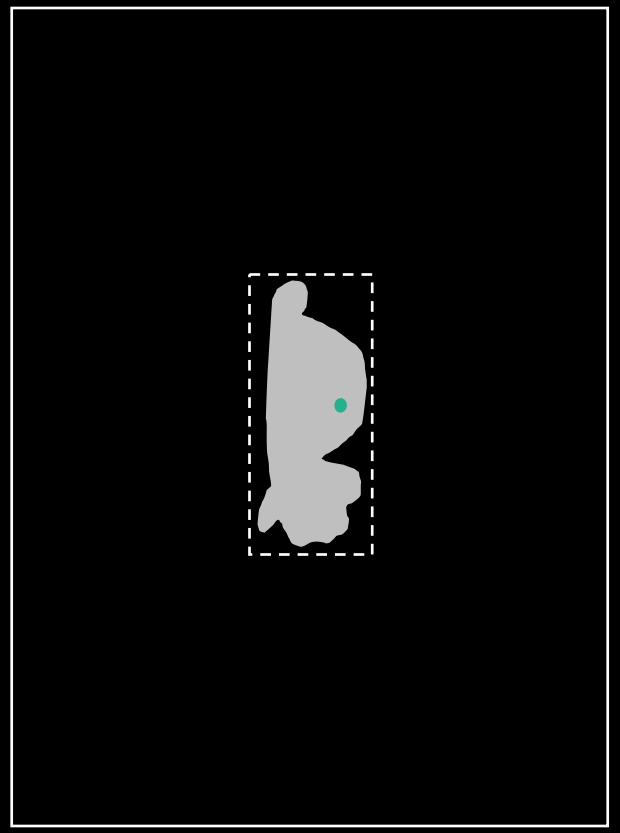


Pixels

informative << non informative

Avoid taking empty pixels

Option 1: crop before acquiring



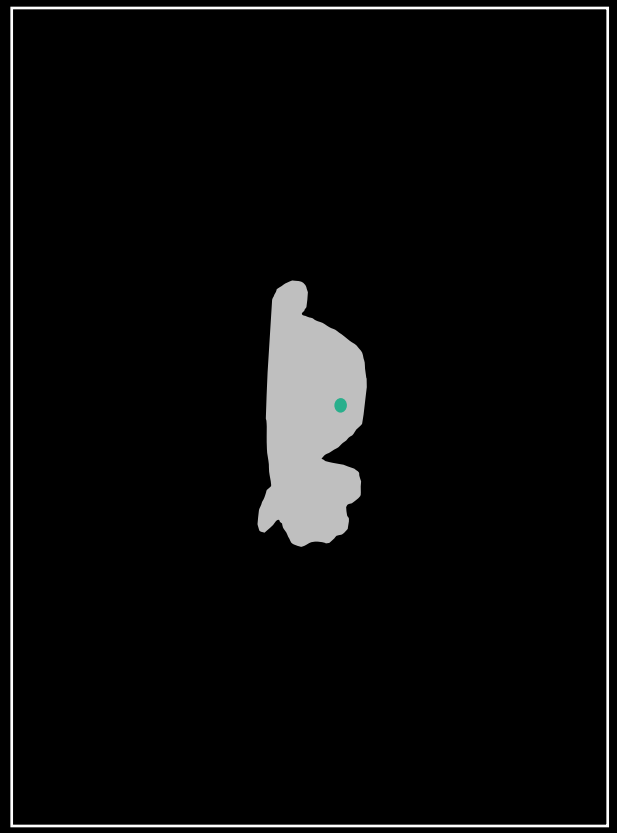
Pixels

informative > non informative

- Faster
- Less data
- Lower resolution

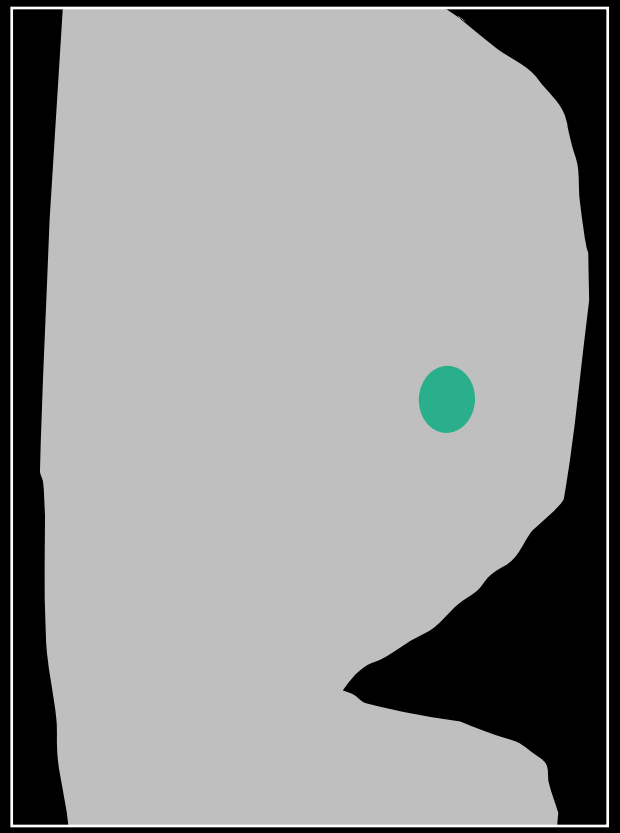
Avoid taking empty pixels

Option 2: increase magnification



Avoid taking empty pixels

Option 2: increase magnification

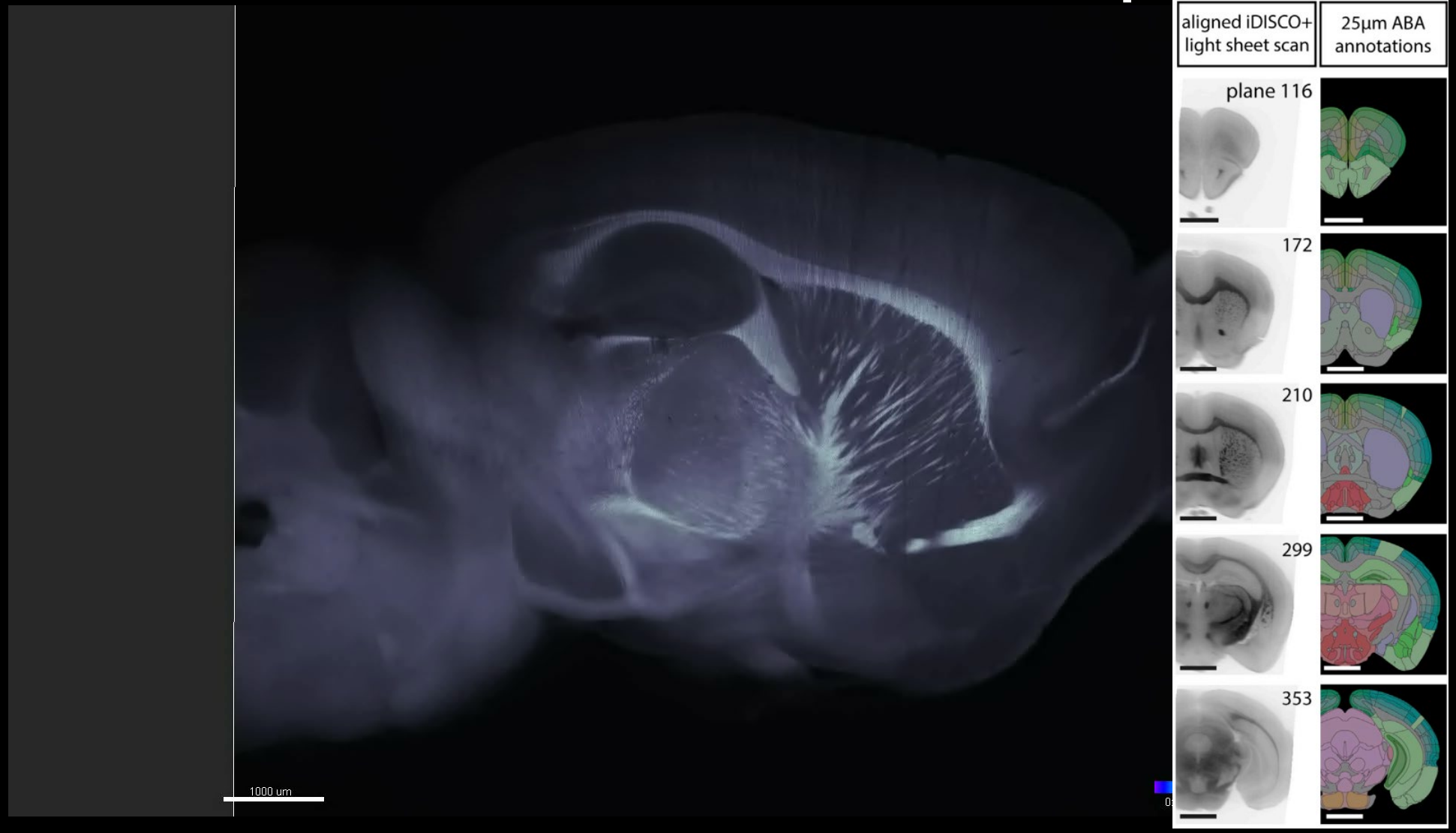


Pixels

informative > non informative

- Slower
- More data
- Higher resolution

Use autofluorescence as a map



Equivalent of counterstain – without need to counterstain

iDISCO-treated brain
No antibodies
Ex 488; Em 525/50

Data courtesy of Nicolas Renier

Start by taking a low resolution image

Typical conditions:

- Low mag
- No tiling, everything in a single field
- Illumination from one side
- Low sheet NA: sheet waist thick and even
- Spacing in Z ≥ 5 μm

This will allow you to:

- Quickly check sample quality
- Find areas of interest
- Find the best sample orientation

Things you can do to improve your imaging

Before

During

After

Always check XZ with Imaris Viewer

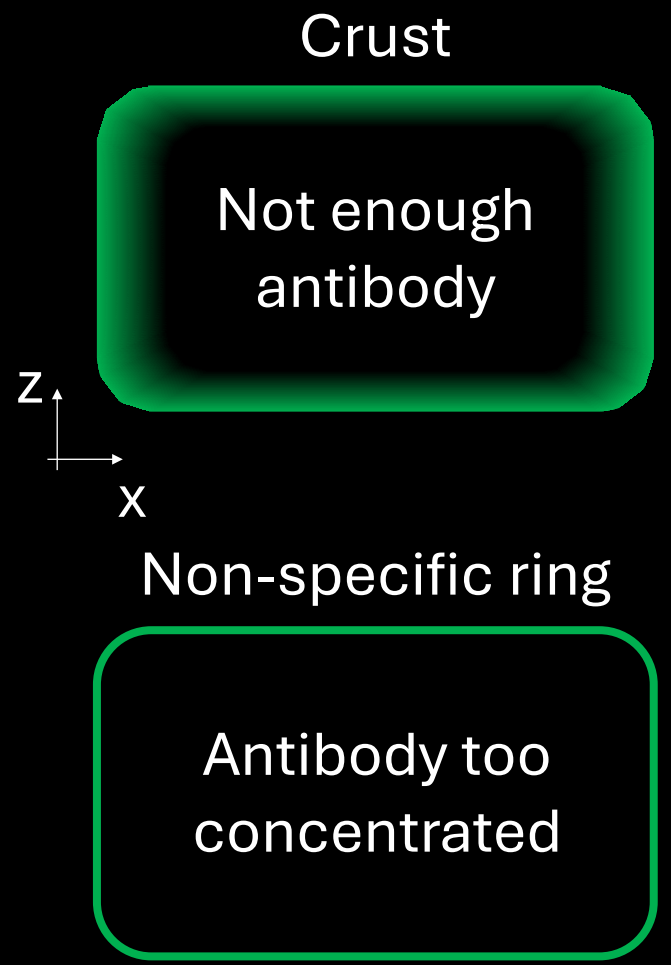
Cannot miss:

- Poor clearing

Always check XZ with Imaris Viewer

Cannot miss:

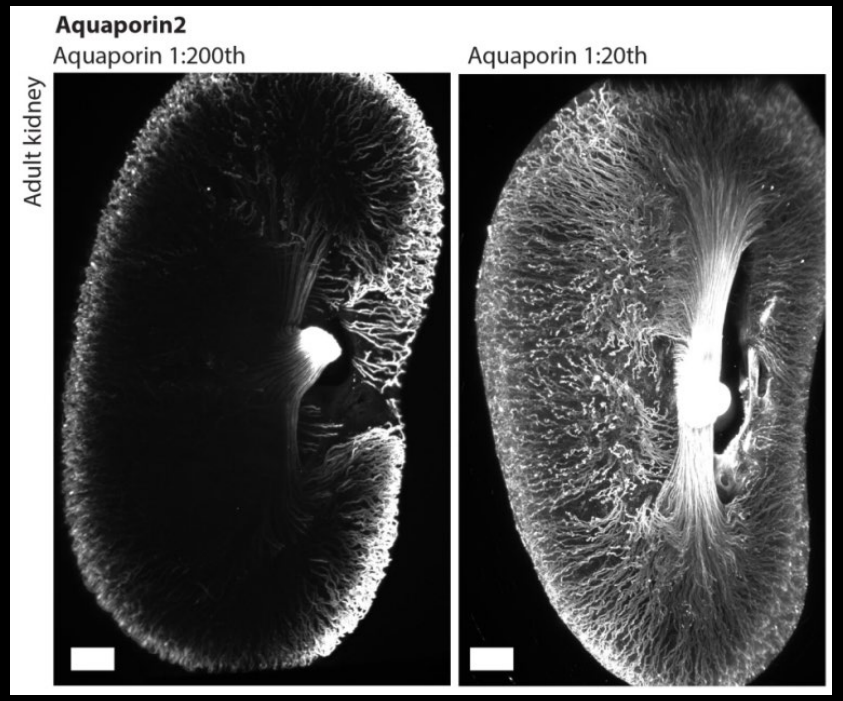
- Poor clearing
- Poor staining



Always check XZ with Imaris Viewer

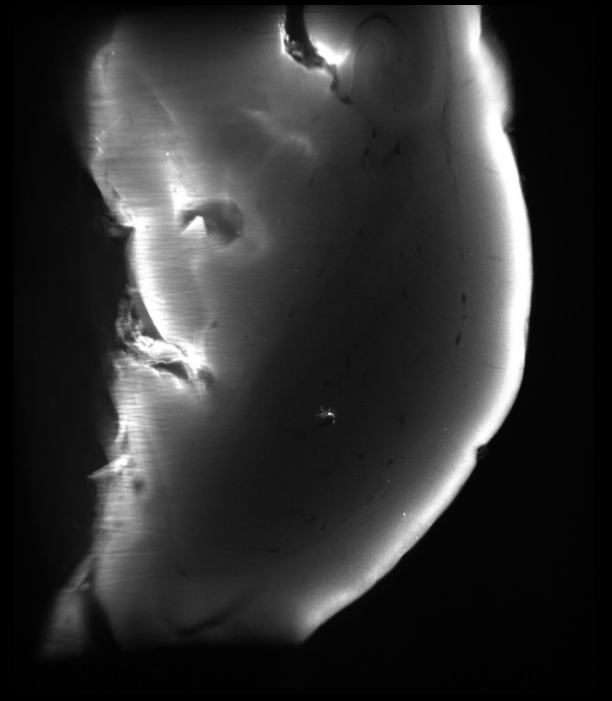
Crust

Not enough antibody



Non-specific ring

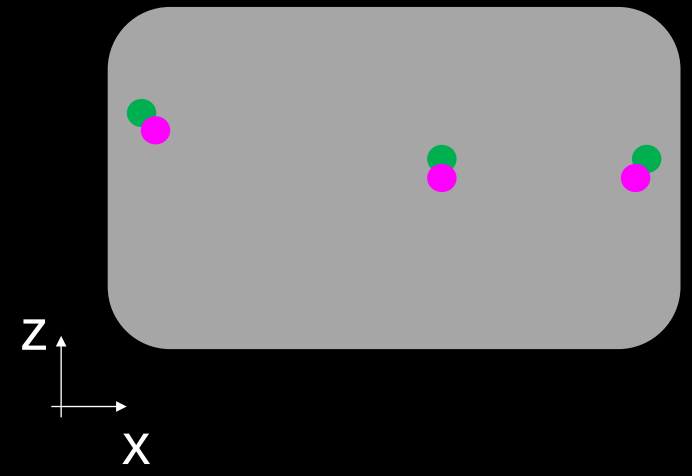
Antibody too concentrated



Always check XZ with Imaris Viewer

Cannot miss:

- Poor clearing
- Poor staining
- Chromatic aberrations

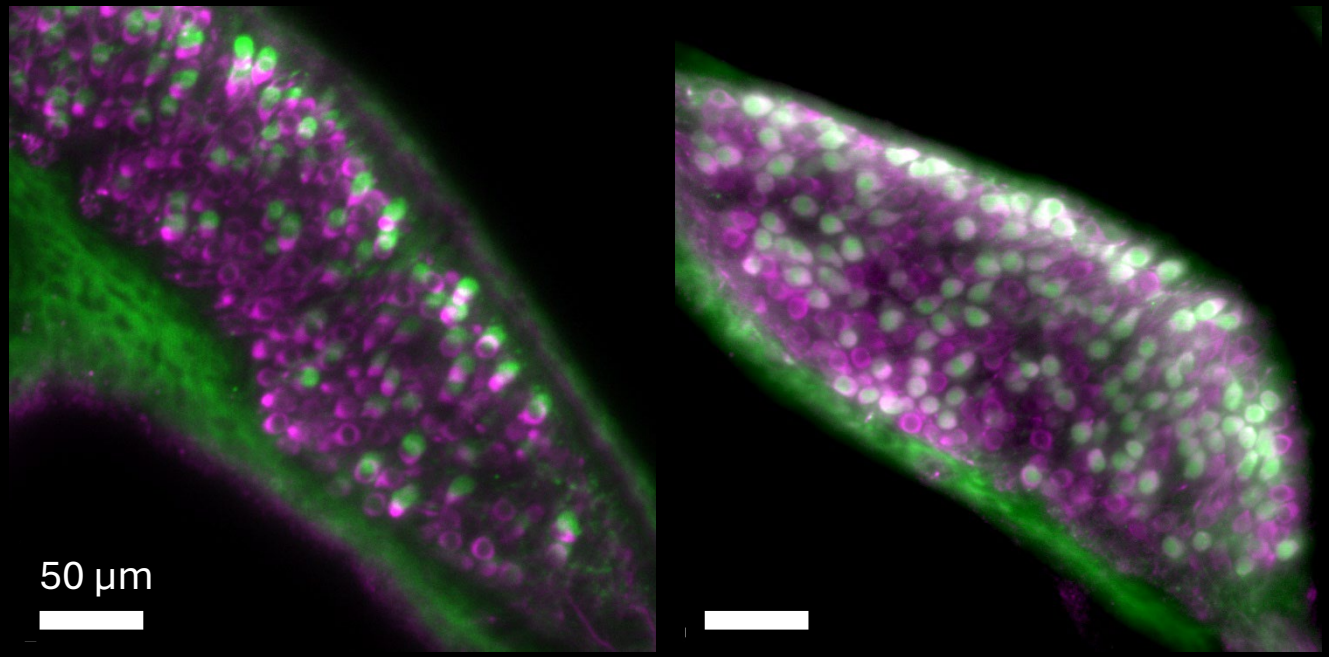


Example of chromatic aberrations

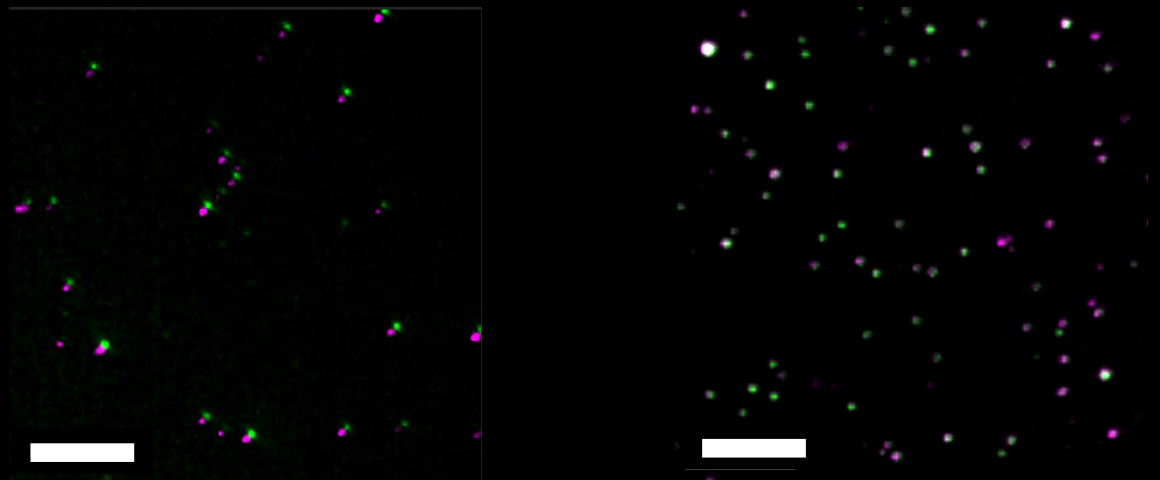
Microscope #1

Microscope #2

Sample



Beads



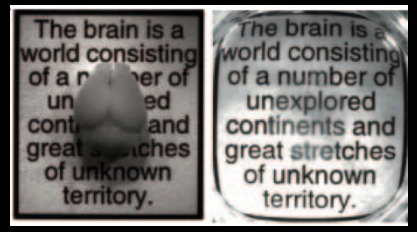
y

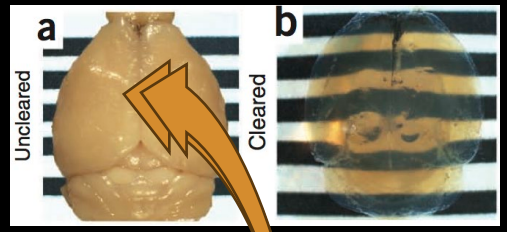
x

Data from
Ken Hutson

Visualize, analyze, change conditions

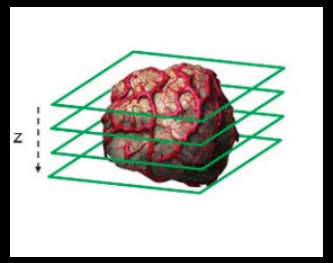
Sample preparation
days - weeks



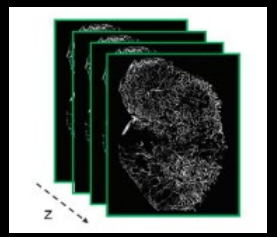


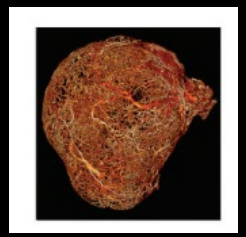
Sample mounting
minutes

Sample imaging
minutes - hours

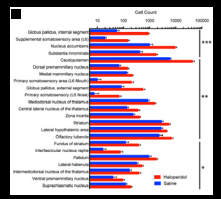


Data visualization
minutes – tens of minutes





Data analysis
hours - months

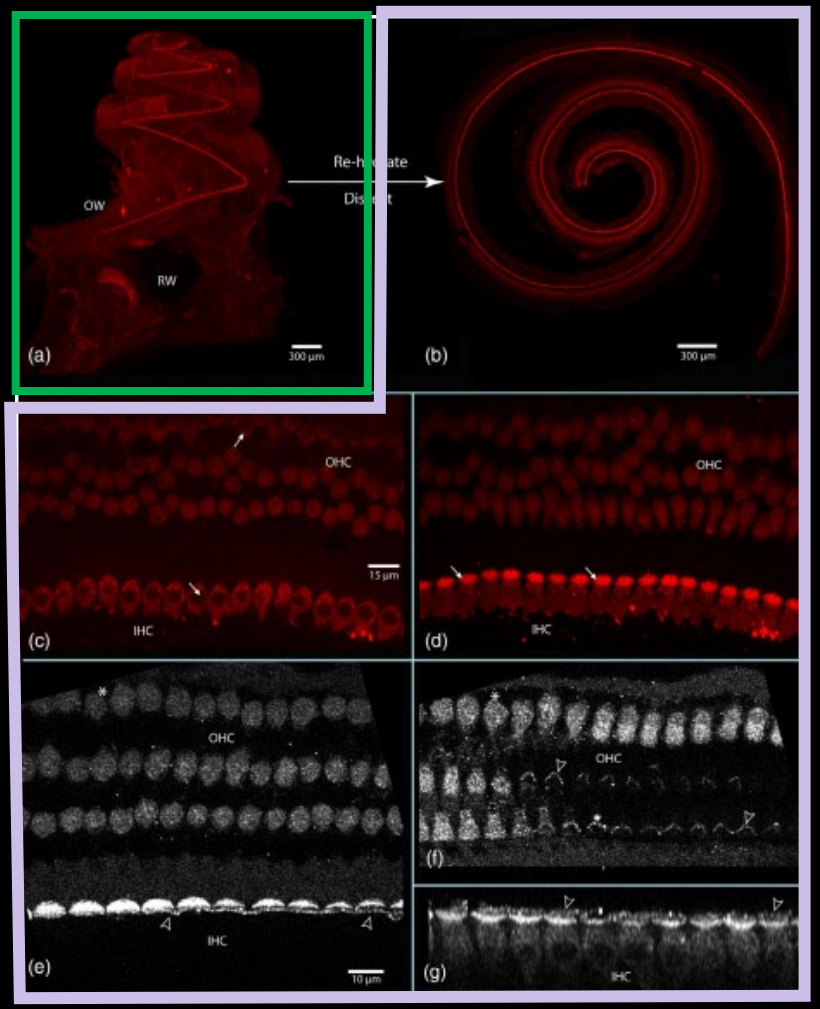


Iterate to improve

a[illegible]a management
tens of GBs – thousands of GBs

Consider rehydrating and cutting

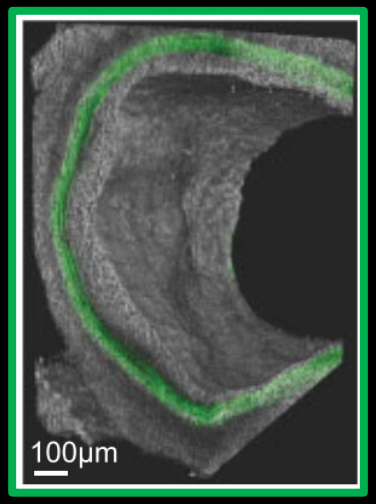
Confocal for higher resolution

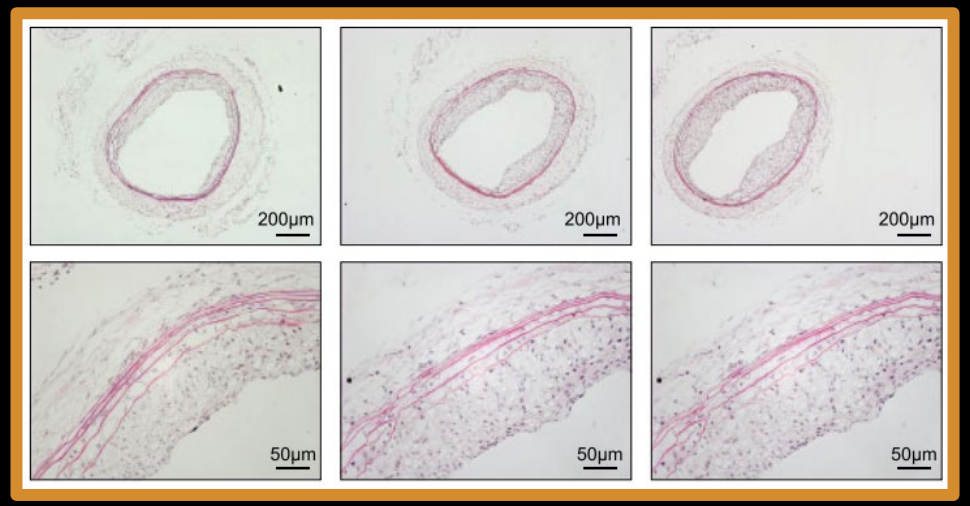


Gerbil cochlea (Fitzpatrick)

https://doi.org/10.1002/cne.24977

Pathology with H&E

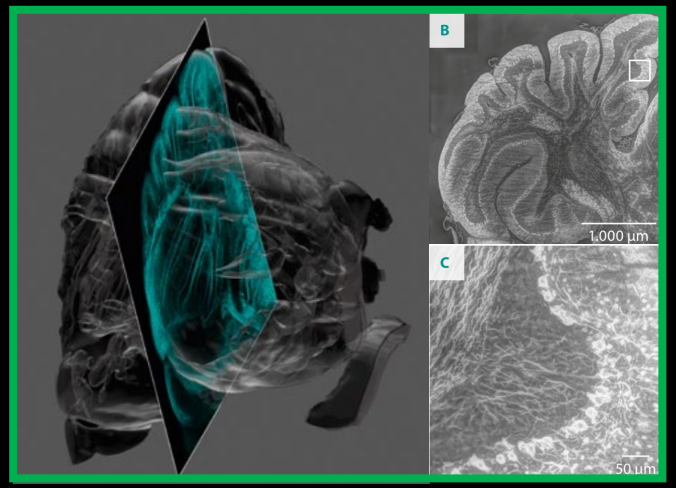


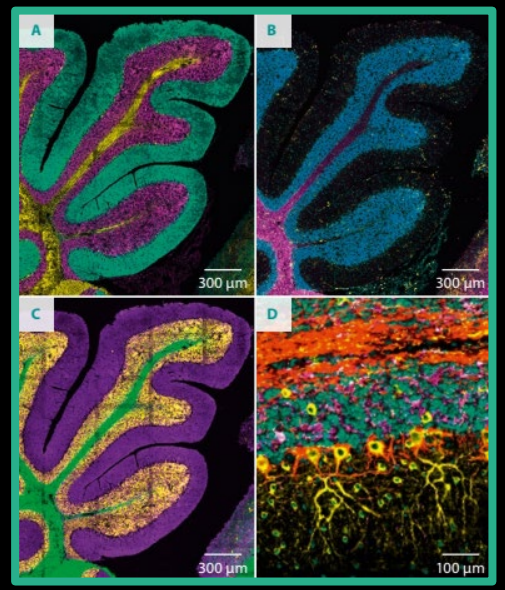


Rat carotid artery (Bahnson)

https://doi.org/10.1093/cvr/cvaa037

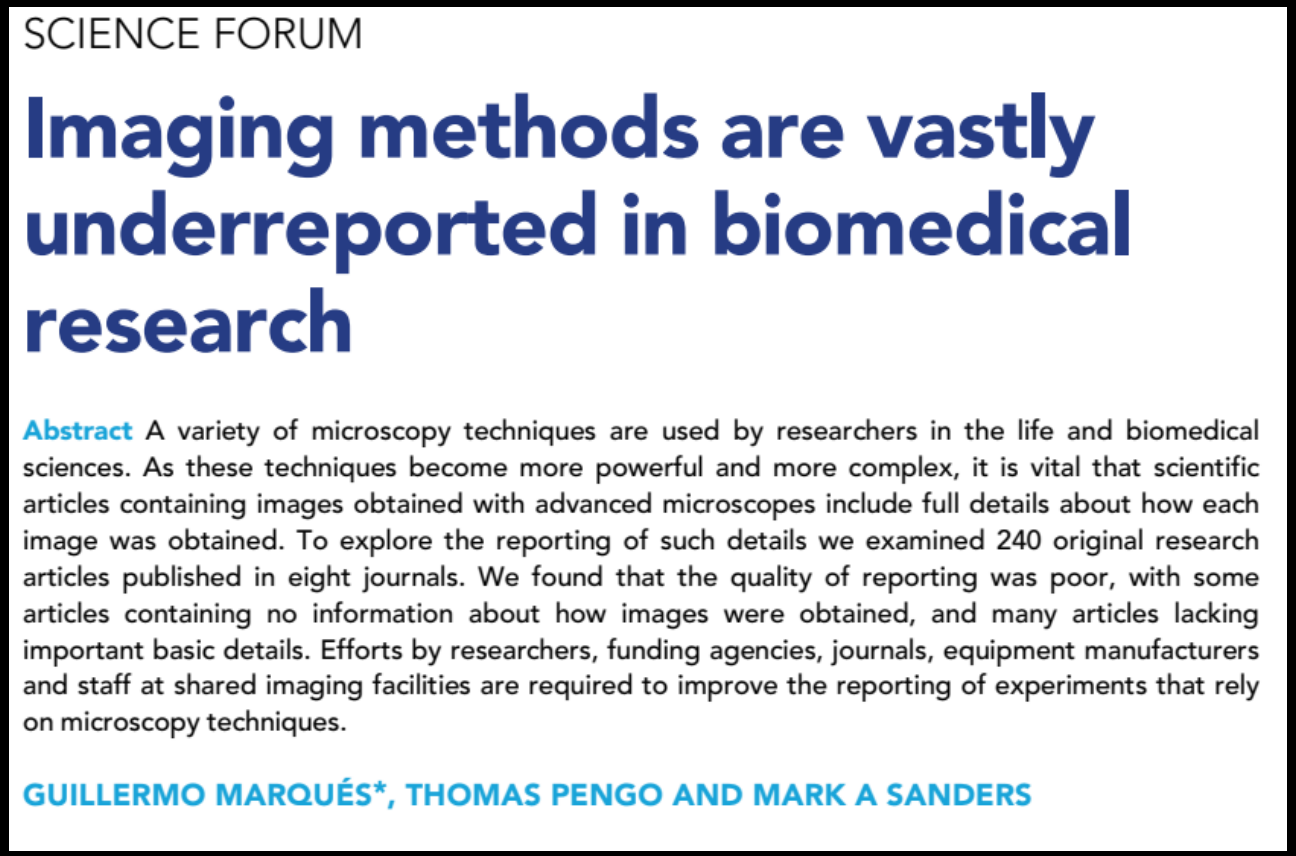
Spatial biology





Cerebellum (Miltenyi)

Explain what you did



SCIENCE FORUM

Imaging methods are vastly underreported in biomedical research

Abstract A variety of microscopy techniques are used by researchers in the life and biomedical sciences. As these techniques become more powerful and more complex, it is vital that scientific articles containing images obtained with advanced microscopes include full details about how each image was obtained. To explore the reporting of such details we examined 240 original research articles published in eight journals. We found that the quality of reporting was poor, with some articles containing no information about how images were obtained, and many articles lacking important basic details. Efforts by researchers, funding agencies, journals, equipment manufacturers and staff at shared imaging facilities are required to improve the reporting of experiments that rely on microscopy techniques.

GUILLERMO MARQUÉS*, THOMAS PENGO AND MARK A SANDERS

DOI: https://doi.org/10.7554/eLife.55133

Only 17% of high-profile papers had a minimally acceptable description of what they did

Explain what you did

This Ertürk paper is a good example

Shrinkage-mediated imaging of entire organs and organisms using uDISCO

Chenchen Pan[1,2,6], Ruiyao Cai[1,2,6], Francesca Paola Quacquarelli[1,6], Alireza Ghasemigharagoz[1], Athanasios Lourbopoulos[1], Paweł Matryba[1,5], Nikolaus Plesnila[1–3], Martin Dichgans[1–4], Farida Hellal[1,3] & Ali Ertürk[1–3]

[1]Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig Maximilians University of Munich (LMU), Munich, Germany. [2]Graduate School of Systemic Neurosciences (GSN), Munich, Germany. [3]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany. [4]German Center for Neurodegenerative Diseases (DZNE, Munich), Munich, Germany. [5]Current address: Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland. [6]These authors contributed equally to this work. Correspondence should be addressed to A.E. (ali.ertuerk@med.uni-muenchen.de).

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https://doi.org/10.1038/nmeth.3964

Explain what you did

Supplementary Table 2: Imaging specifications

FIGURES		SYSTEM	OBJECTIVE SPECIFICATIONS				ACQUISITION PARAMETERS			
			Magnification	NA	RI	WD	Zoom	Image pixel size	z-step	imaging depth
Figures										
1	a	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	8µm	1mm
	g	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	2µm	2.0-2.2mm
2	c	AxioZoom	Zeiss 1X	0.25	1.0	56mm		6.49µmx6.49µm	n.a.	n.a.
	d	UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	0.63	5.16µmx5.16µm	8µm	3D reconstruction
	e	UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	0.63	5.16µmx5.16µm	8µm	1mm
	f	UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	0.63	5.16µmx5.16µm	8µm	3mm
	g	UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	0.63	5.16µmx5.16µm	8µm	5mm
	h	UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	0.63	5.16µmx5.16µm	10µm	projection of 8mm scan
	i,j	UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	3.2	1.015µmx1.015µm	4µm	projection of 8mm scan
3	c	AxioZoom	Zeiss 1X	0.25	1.0	56mm		6.49µmx6.49µm	n.a.	n.a.
	d,e,f	AxioZoom	Zeiss 1X	0.25	1.0	56mm		1.42µmx1.42µm	n.a.	n.a.
	j,k,l,m	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	20µm	projection of 10mm scan
4	a,c	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	4µm	entire scan projection
	d	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	4µm	1.8mm
	e	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	4µm	2.0mm
	f	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	4µm	3.0mm
	g	UM II	Zeiss 20x	1.0	1.45	5.6mm	2	0.1625µmx0.1625µm	2µm	500µm
5	b	UM II	4X corr.	0.28	1.56	10mm	0.63	2.56µmx2.56µm	16µm	1.5-3mm
		UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	0.63	5.16µmx5.16µm	16µm	3D reconstruction
6	virus b,c,d,e,f	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	12µm	entire scan projection
	mouse l,m	LSM880	Leica 25x	0.95	1.33	2.5mm		0.2µmx0.2µm	3µm	450-480µm, 600-630µm
	human u	LSM880	Leica 25x	0.95	1.33	2.5mm		0.2µmx0.2µm	3µm	270-282µm
Sup. Figures										
S1	c	AxioZoom	Zeiss 1X	0.25	1.0	56mm		6.49µmx6.49µm	n.a.	n.a.
	d,e,f,g	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	n.a.	n.a.
S2		UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	12µm	2.5mm
S5		UM II	2X corr.	0.14	1.56	10mm		3.25µmx3.25µm	8µm	0.5mm, 2.5mm
S6		UM II	2X corr.	0.14	1.56	10mm		3.25µmx3.25µm	8µm	0.5mm, 2.5mm
S8		AxioZoom	Zeiss 1X	0.25	1.0	56mm		5.68µmx5.68µm	n.a.	n.a.
S9	a,c,e	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	4µm	0.44mm
	b,d,f	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	4µm	0.66mm
S10	a-f	UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	0.63	5.16µmx5.16µm	4µm	0.66mm
S11	a,c	UM II -MVX10	Oly. 2X	0.5	1.33/1.56	6mm	0.63	5.16µmx5.16µm	4µm	3D reconstruction
S12		AxioZoom	Zeiss 1X	0.25	1.0	56mm		5.68µmx5.68µm	n.a.	n.a.
S13		LSM880	Leica 25x	0.95	1.33	2.5mm		0.183µmx0.183µm	3-4µm	0-100µm
S14		LSM880	Leica 25x	0,95	1.33	2.5mm		0.183µmx0.183µm	3-4µm	0-100µm
S17	a	UM II	2X corr.	0.14	1.56	10mm		3.25µmx3.25µm	6µm	10mm
	b,c,d,e	UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	6µm	10mm
S19	h	LSM880	Leica 25x	0.95	1.33	2.5mm		0.67µmx0.67µm	2µm	18-62µm
	i	LSM880	Leica 25x	0.95	1.33	2.5mm		0.43µmx0.43µm	1µm	6-45µm
	small boxes in fig S4h	LSM880	Leica 25x	0.95	1.33	2.5mm		0.16µmx0.16µm	1µm	
	small boxes in fig S4i	LSM880	Leica 25x	0.95	1.33	2.5mm		0.10µmx0.10µm	1µm	
S20		AxioZoom	Zeiss 1X	0.25	1.0	56mm		5.68µmx5.68µm	n.a.	n.a.
S21		UM II -MVX10	Oly. 2X	0.5	1.33/1.56	10mm	0.63	5.16µmx5.16µm	8µm	2.35mm
		UM II -MVX10	Oly. 2X	0.5	1.33/1.56	10mm	0.63	5.16µmx5.16µm	8µm	1.4mm
		UM II -MVX10	Oly. 2X	0.5	1.33/1.56	10mm	0.63	5.16µmx5.16µm	8µm	2.9mm
S22		UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	6µm	1.9mm
S23		UM II	4X corr.	0.28	1.56	10mm		1.625µmx1.625µm	12µm	entire scan projection
S24	d	LSM880	Leica 25x	0.95	1.33	2.5mm		0.2µmx0.2µm	3µm	24-30µm
	e	LSM880	Leica 25x	0.95	1.33	2.5mm		0.2µmx0.2µm	3µm	237-246µm
S25	d	LSM880	Leica 25x	0,95	1,33	2.5mm		0.2µmx0.2µm	3µm	327-387µm
	h	LSM880	Leica 25x	0,95	1,33	2.5mm		0.2µmx0.2µm	4µm	276-292µm
S27	a	UM II	Oly. 25X	0,95	1.31-1.52	4mm		0.26µmx0.26µm	4µm	0.8-1.2mm
	b	UM II	Zeiss 20x	1	1,45	5.6mm		0.325µmx0.325µm	4µm	0.4-0.8mm
	c	UM II	20X corr.	0,45	1,56	5mm		0.325µmx0.325µm	4µm	0.5-1.0mm
	d	UM II	Leica 20X	0,95	1,56	1.8mm		0.325µmx0.325µm	2µm	0.65-1.05mm

Legend of abbreviations

Imaging Systems

UM II	LaVision BioTec - UltraMicroscope II
UM II -MVX	LaVision BioTec - UltramicroscopeM II -MVX10
LSM880	Zeiss confocal LSM880 with Airyscan
AxioZoom	Zeiss AxioZoom EM53/SyCoP3

NA	Numerical aperture
RI	Refractive Index
WD	Working distance
n.a.	not applicable

Objectives

Oly. 2X	Olympus MVPLAPO2XC
2X corr.	Olympus XLFLUOR2x corrected
4X corr.	Olympus XLFLUOR4x corrected
20X corr.	Olympus LUCPLFLN20x corrected
Oly. 25X	Olympus XLPLN25X
Zeiss 1X	Zeiss PlanNEOFLUAR Z 1x
Zeiss 20X	Zeiss Clr Plan-Neofluar 20x
Leica 20X	Leica HCX APO L20×
Leica 25X	Leica HCX IRAPO L 25x

https://doi.org/10.1038/nmeth.3964

Take home:

Focus on what you **need** to answer your biological question

"Quality"
Resolution XYZ
Evenness of Z resolution
Signal-to-noise ratio

Volume

Time

Bleaching

Dataset size

Nothing is free in microscopy

How to reach me

pablo_ariel@med.unc.edu

Microscopy Services Lab
Website:

Useful resources