



Bioimage Informatics

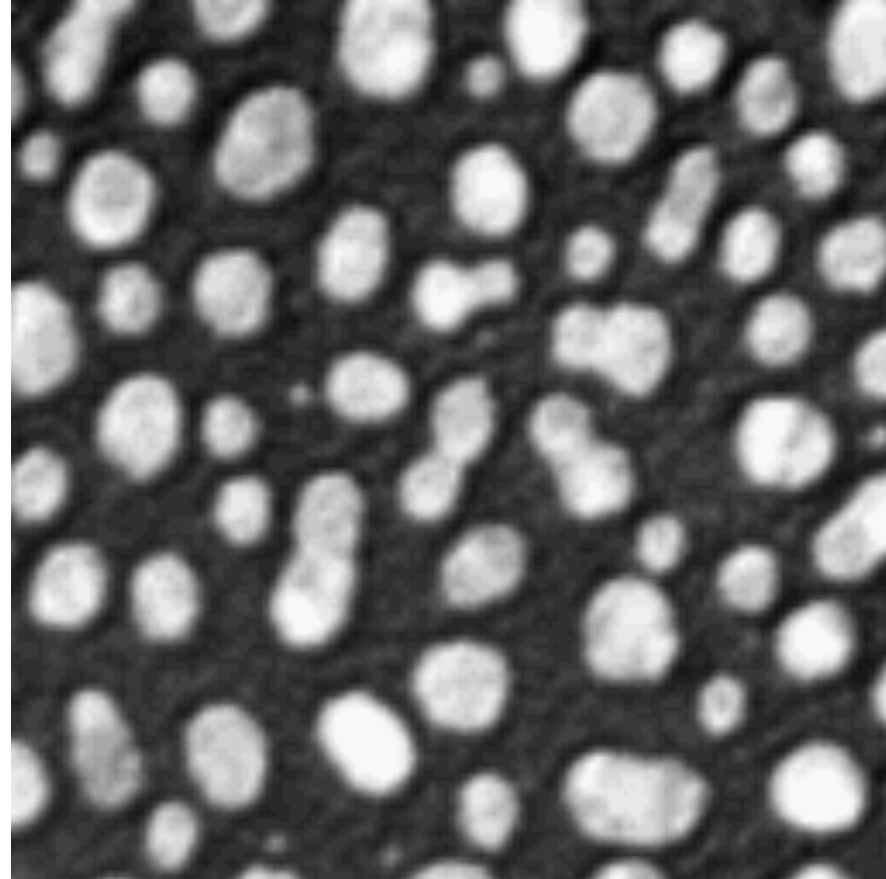
Daniel Sage & Arne Seitz

Course

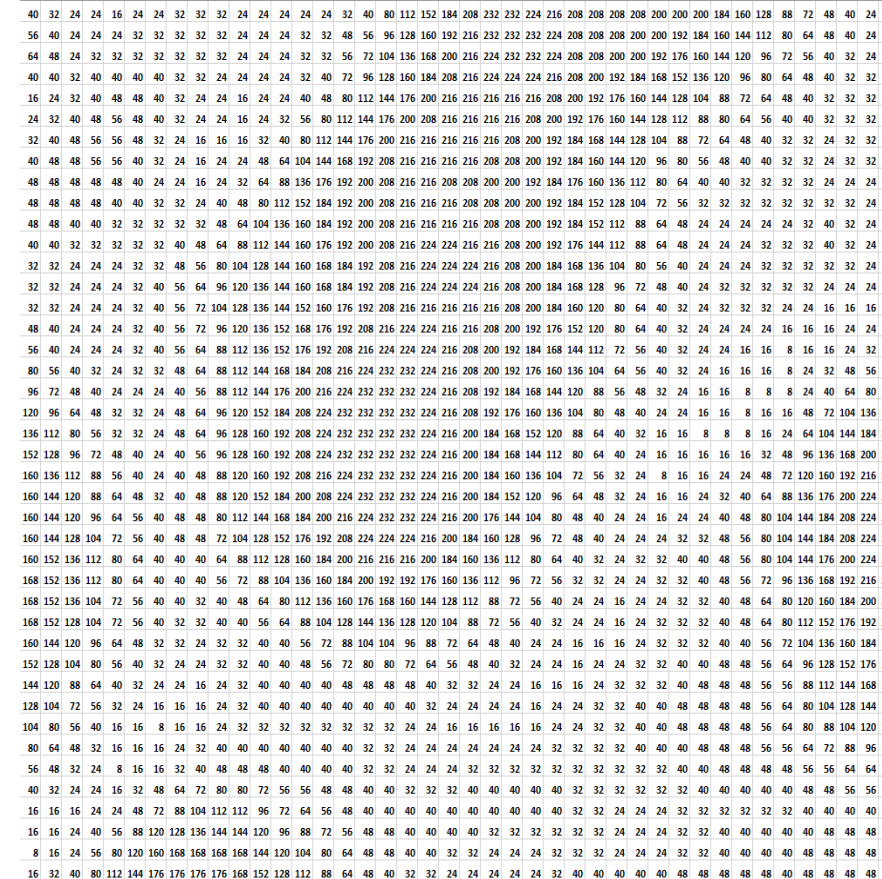
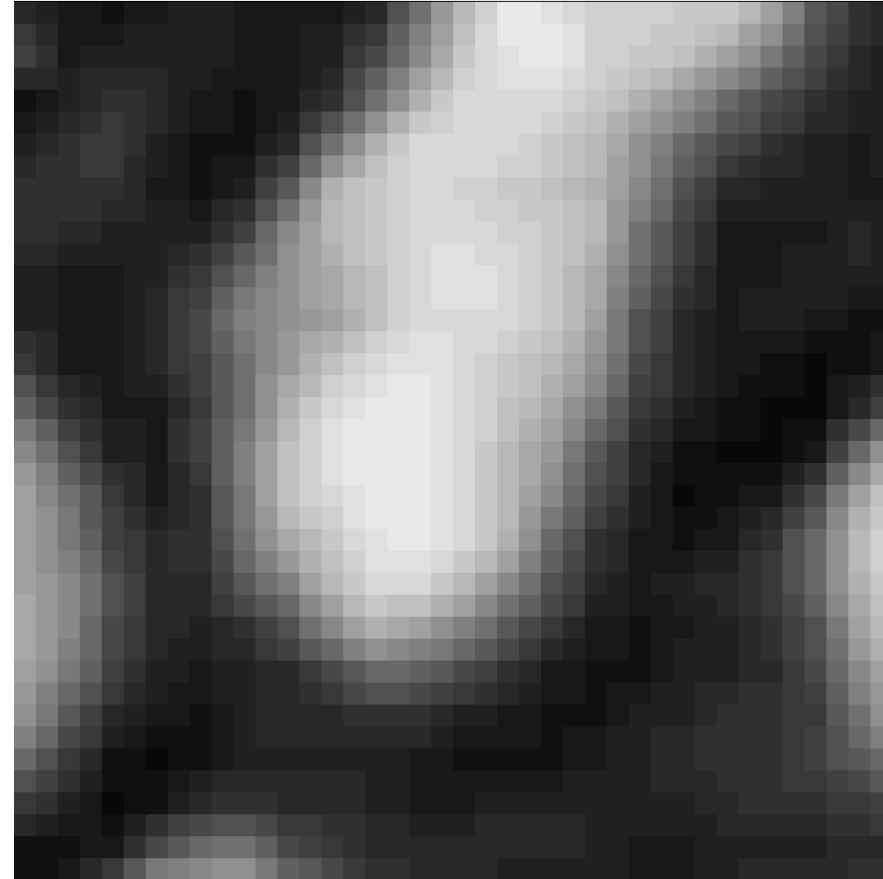
Digital Images

Digital Images

Image



Raster 2D Array



Picture



Observation

Discrete Functions

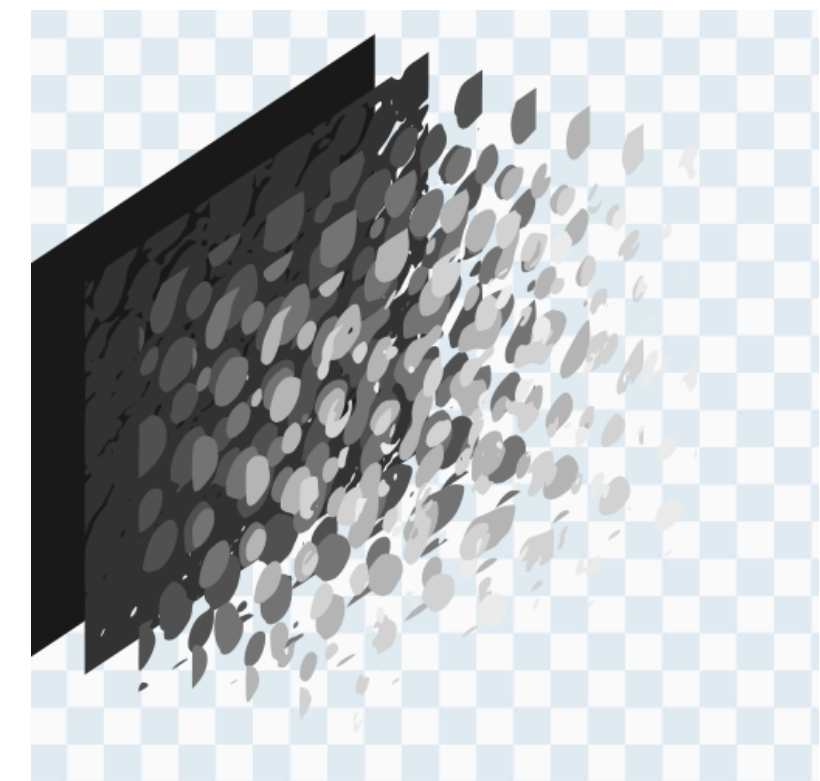
$$f : \Omega^2 \longrightarrow R$$

$$(x, y) \longmapsto f(x, y)$$

Spatial domain Ω
coordinates (x, y)

Value range R
bits depth

Vectorized Objects





Source of Data

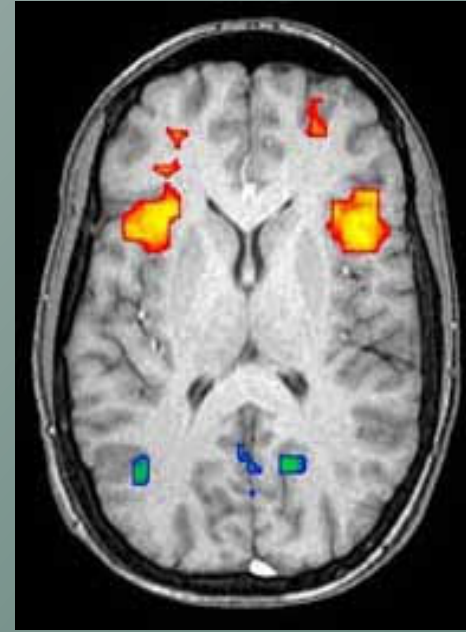
SCIENCE



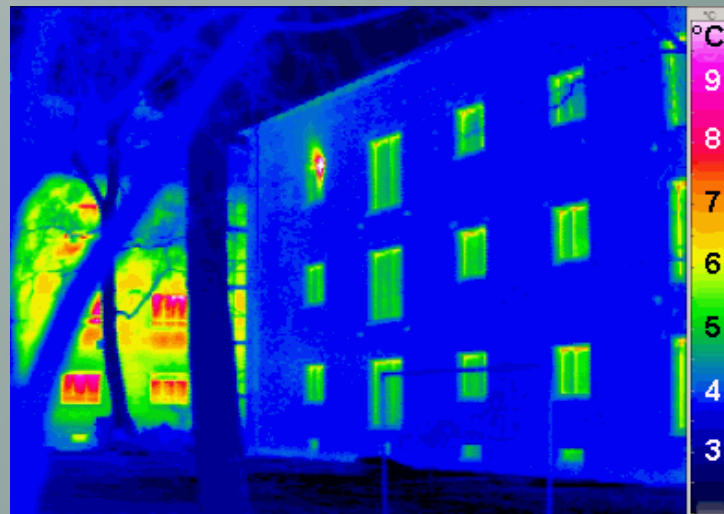
Photography



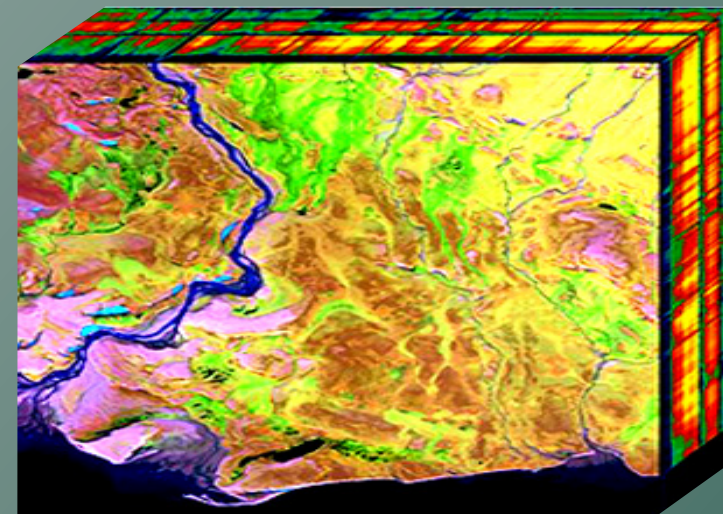
X-rays



fMRI



Thermal Imaging



Spectral Imaging

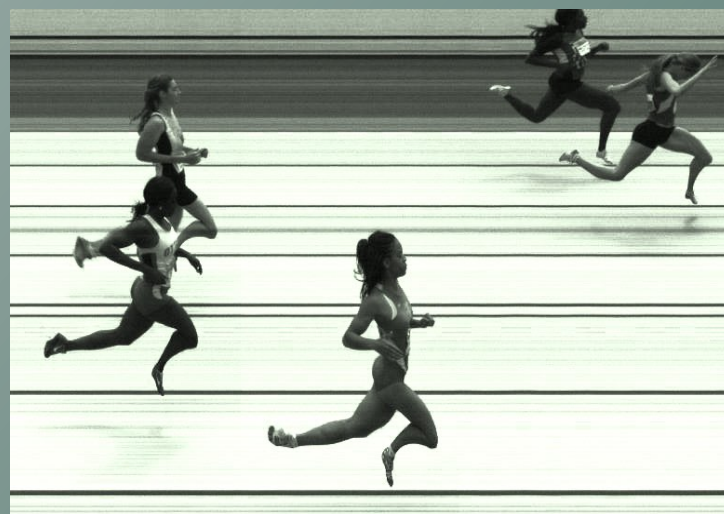
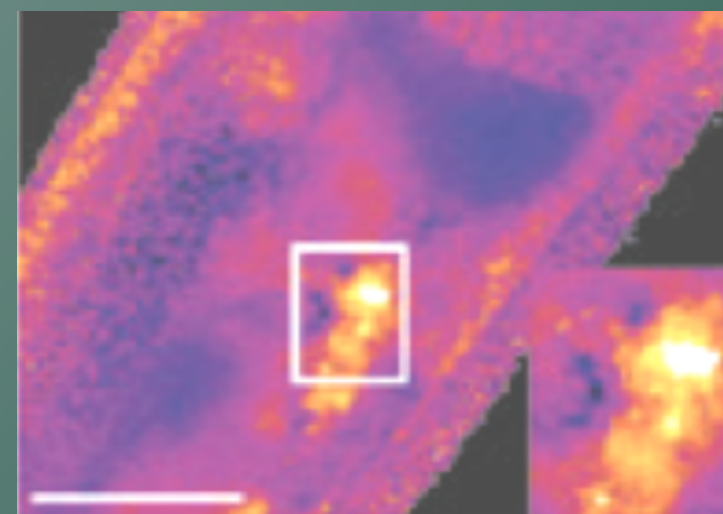


Photo-finish (XT)



Map of a computed variable

MICROSCOPY

Light

- Incoherent light: visible, UV, IR
- Fluorescence signal, FRET, FLIP
- Phase recording: Phase Contrast, DIC
- Quantitative phase imaging, digital holography (DHM)

Non-optical

- Electron Microscopy (EM)
- Cryo-electron Microscopy
- Atomic Force Microscopy (AFM)

Computational data

- Optical Coherence Tomography (OCT)
- Structured Illumination Microscopy (SIM)
- Single-Molecule Localization Mic. (PALM/STORM)

Correlative Technique

- Correlative Light-Electron Microscopy (CLEM)

Data Type

Bit-Depth

Grayscale

Color

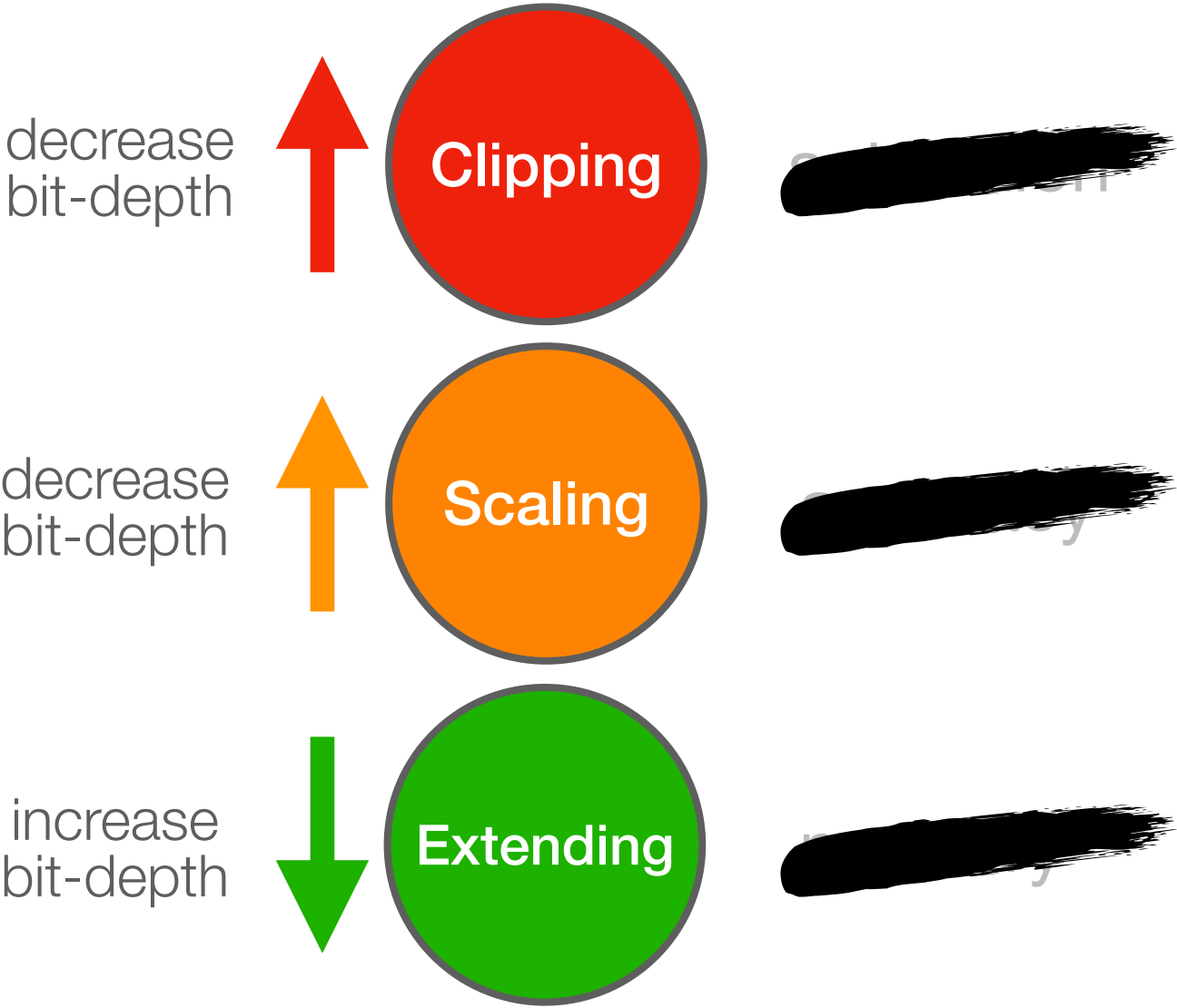
Computational

Ch.	Bit-Depth	Range	Use	numpy	Java	TIF
1	1	0...1	Binary Image: illustration, fax	bool	boolean	
1	8	0...255	Universal: photo, scan print	uint8	byte	
1	14	0...16383	Science, measure, microscopy, PMT			
1	16	0...65535	Highest quality: medecine, astronomy	uint16	short	
3	24	[0...255] ³	RGB, universal: photo, scan, print	uint8[3]	byte[3]	
4	32	[0...255] ⁴	RGBA (alpha -> transparency)	uint8[4]	int	
4	32	[0...255] ⁴	CMYK, digital prepress			
1	32	-2 ³¹ ...2 ³¹ - 1	Map, label	uint32	int	
1	32	± 3.4 10 ³⁸	Floating point	float32	float	
1	64	± 1.8 10 ³⁰⁸	High-precision floating point	float64	double	
2	64	[± 1.8 10 ³⁰⁸] ²	Complex floating point: Fourier coefficient	complex64	double[2]	

Dynamic range in microscopy

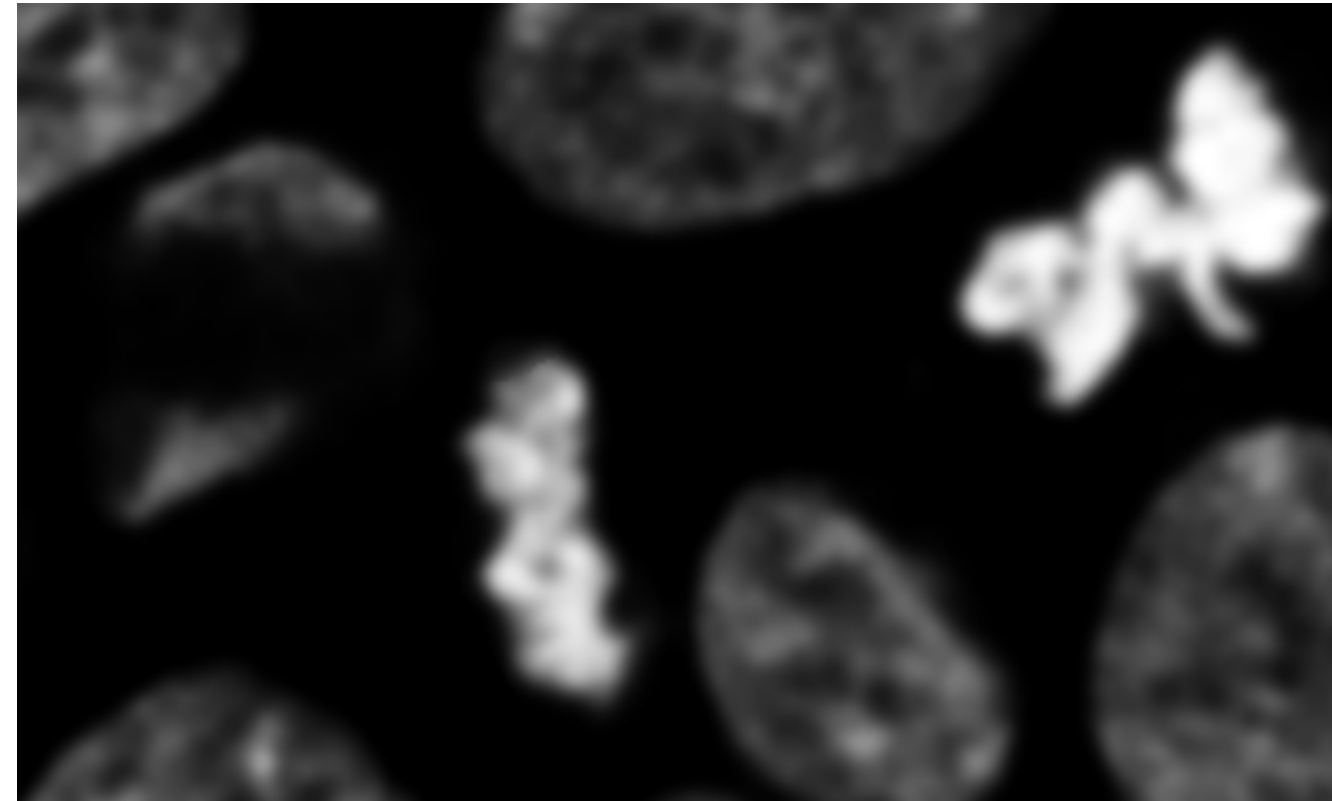
Bit conversion

what are we losing?
can we still quantify?



Quantization **Graylevel**

How many
bit-depth?



256



Human vision

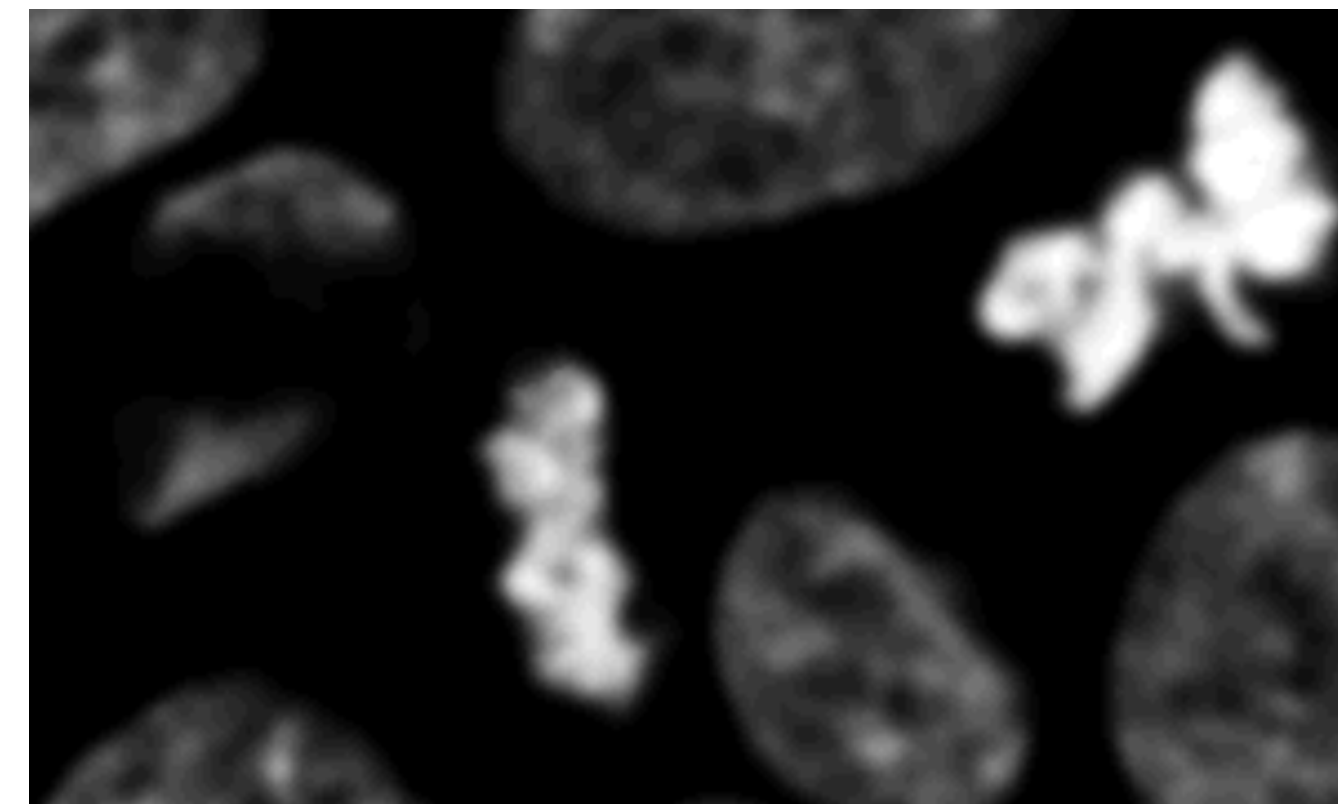
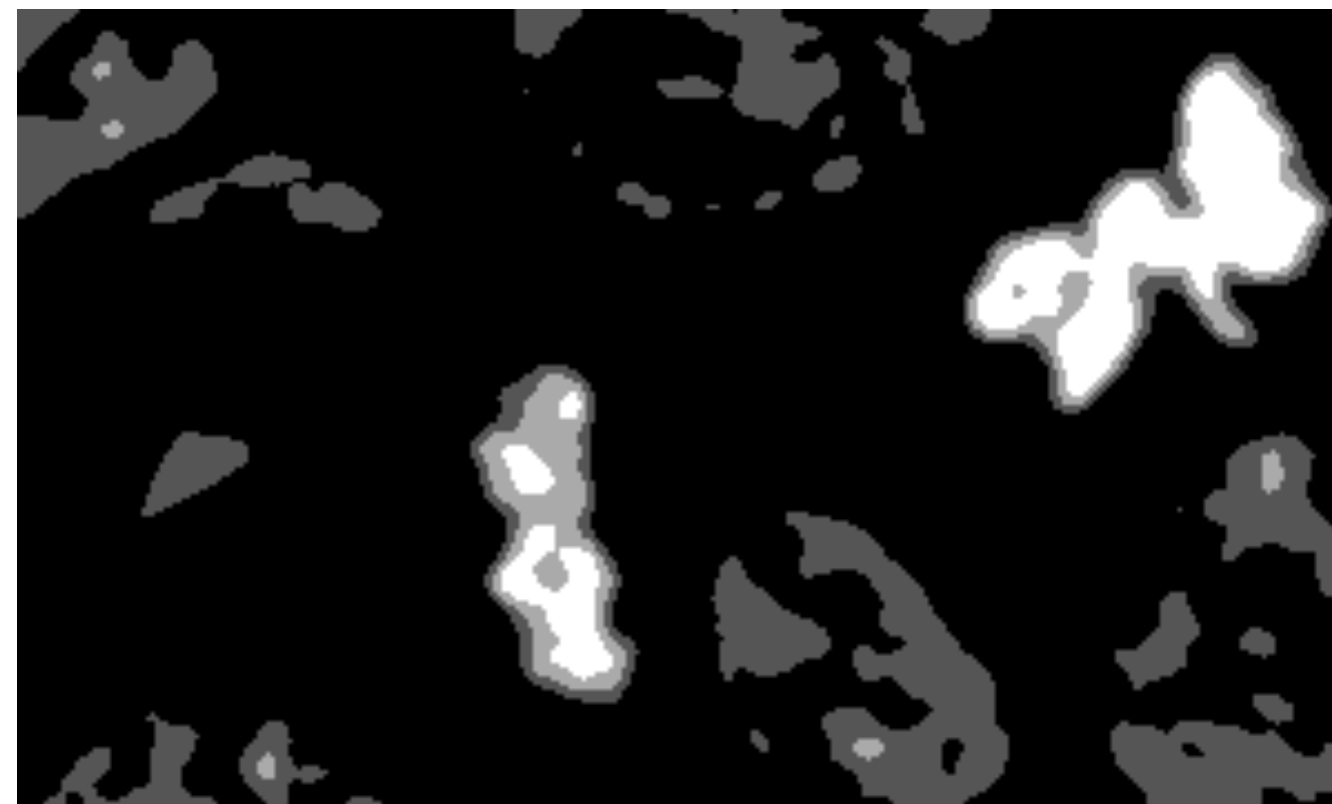
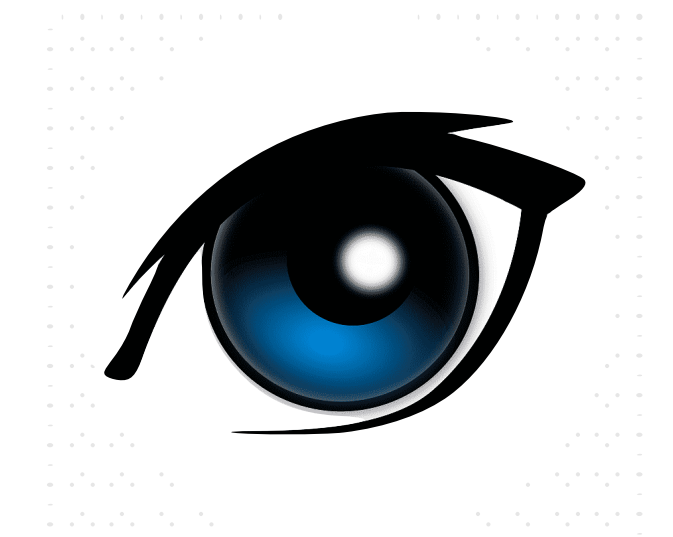




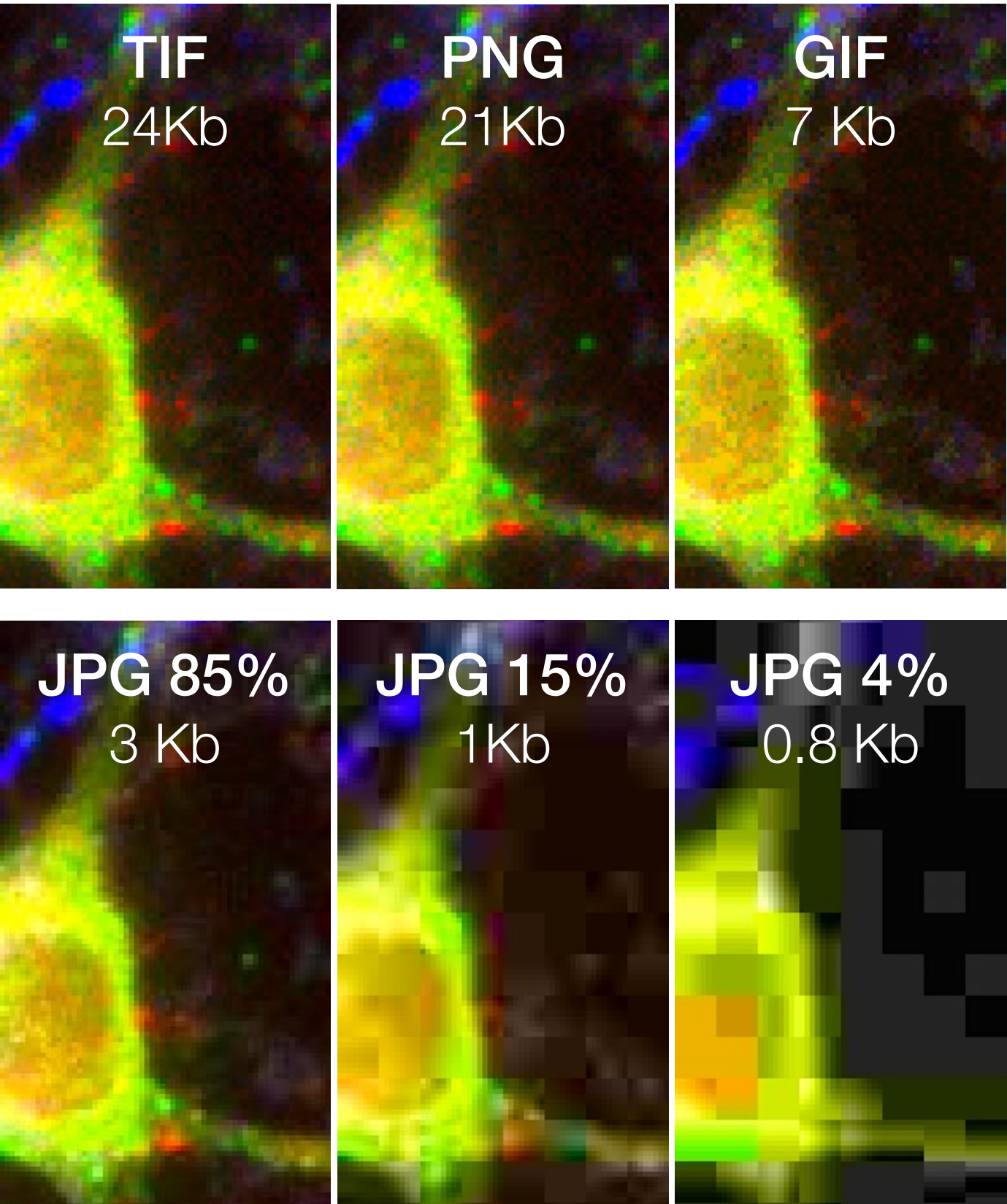
Image File Format

Raster
array of pixel values

Format	Compression	Metadata	Transp.	Open	3D	Time	Software	Common Use
JPEG	✗ Lossy	✗	✗	✓	✗	✗		Web, photography
PNG	✓ Lossless	✗	✓	✓	✗	✗		Graphics, web images
BMP	None	✗	✓	✓	✗	✗		Windows-based
TIFF	✓ Lossless	✓	✓	✓	✓	✓		Medical, scientific imaging
DICOM	✓ Lossless	✓	✗	✓	✓	✓		Medical (CT, MRI, X-ray)
HEIC	✗ Lossy	✗	✓	✗	✗	✓		Mobile photography
NPY	None	✗	✗	✓	✓	✓		Python geeks
RAW	None	✗	✗	⚠	✓	✗		Unprocessed, raw data
SVG	None		✓	✓	✗	✗		Logos, diagrams
PDF	✗ Lossy		⚠	✓	✗	✗		Documents, print graphics
AI	None		✓	✗	✗	✗		Adobe Illustrator graphics
AVI	✗ Lossy		⚠	✗	✗	✓		Videos
MP4	✗ Lossy		✓	✓	✗	✓		Videos
GIF	⚠ Lossy		✓	✓	✗	✓		Simple animations

Vector
objects

Movie



- Lossless**
- GIF (indexed color)
 - ZIP or LZW
 - RLE (Run-length encoding)
 - **Lossy**
 - JPEG (on 8x8 block)
 - JPEG2000

Image File Format

Domain

❖ DICOM

Digital pathology

❖ WSI

Microscopy (proprietary)

❖ Nikon ND2

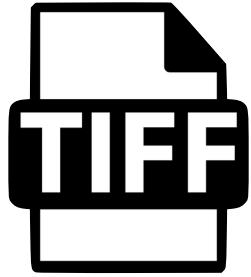
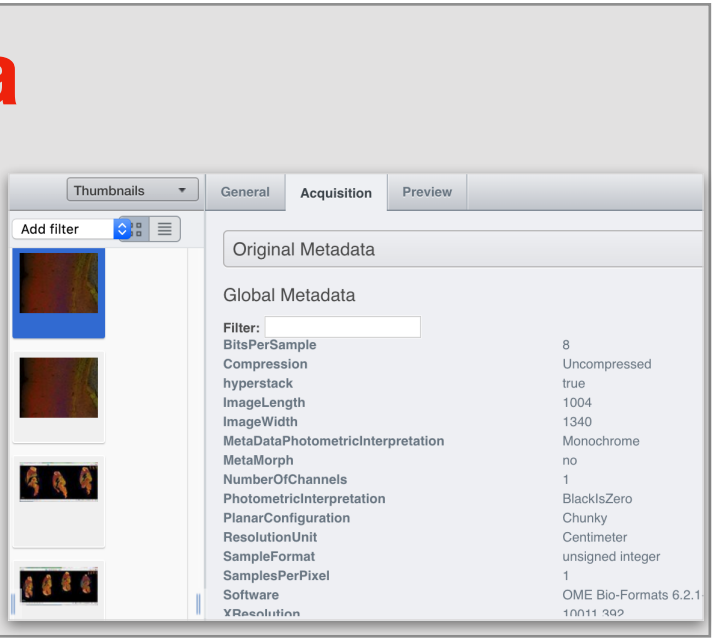
❖ Leica LIF

❖ Zeiss ZVI, LSM

❖ Imaris IMS

Metadata

pixelsize
display
reporter
dimension
scale



Tagged Image File Format

The most common format in science



- Compatible with all software, 3D -> 5D
- Some metadata, 8-bits -> 64-bits
- Limited size: 4GB

Format for Science

❖ TIFF / OME-TIF

❖ HDF5

❖ ZARR / NGFF

❖ BIDS

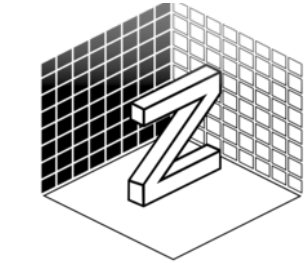


Hierarchical Data

Diverse data types and structures

- For datasets too large for in-memory
- Random access to large blocks of data.

BIO-FORMATS										
Format	Extensions	Pixels	Metadata	Openess	Presence	Utility	Export	BSD	Multiple Images	Pyramid
TIFF (Tagged Image File Format)	.tiff, .tif, .tf2, .tf8, .btf	▲	▲	▲	▲	▼	✓	✓	✓	✓
DICOM	.dcm, .dicom	▲	▲	▲	▲	▲	✓	✓	✓	✓



Zarr

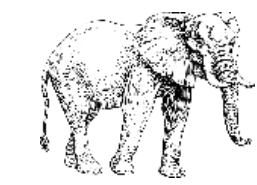
Chunk storage of array

nD data for cloud-based or distributed environments and parallelized processing

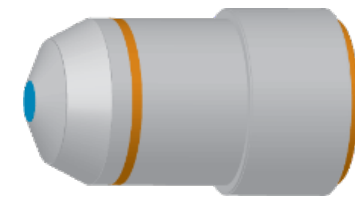
- Ideal for: very large-scale, highly nD data
- Python-based

Image Data Display

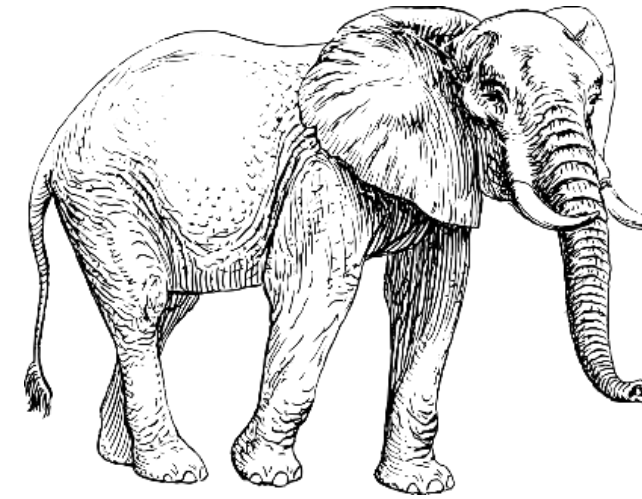
How a Digital Image Reaches your Eyes ?



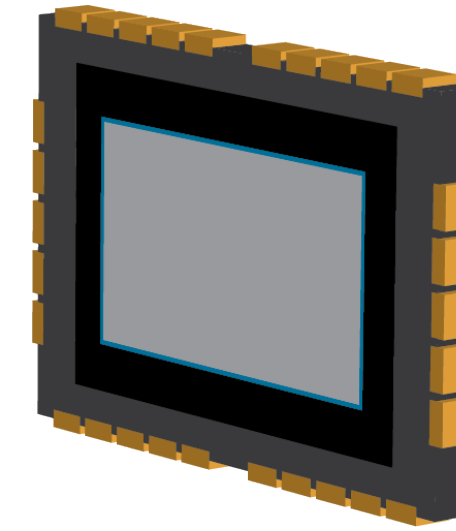
Object



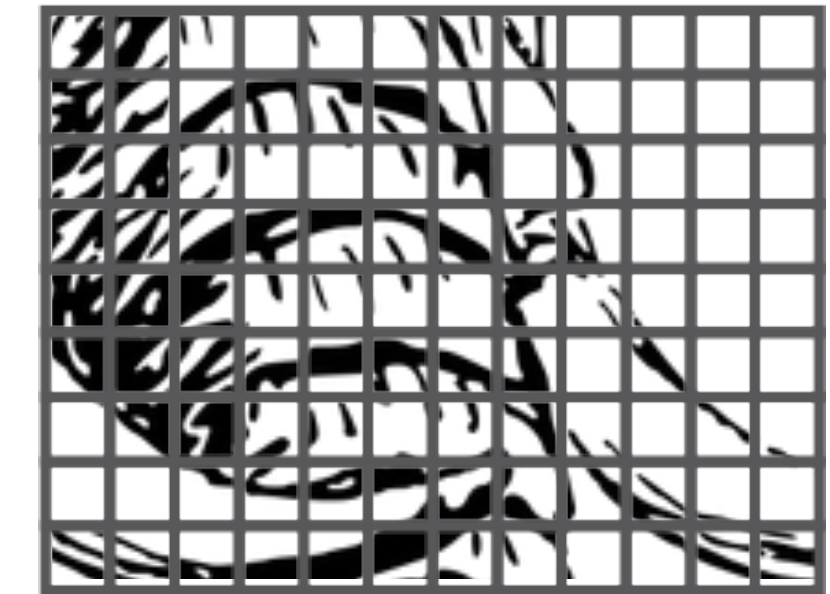
Imaging System



Image

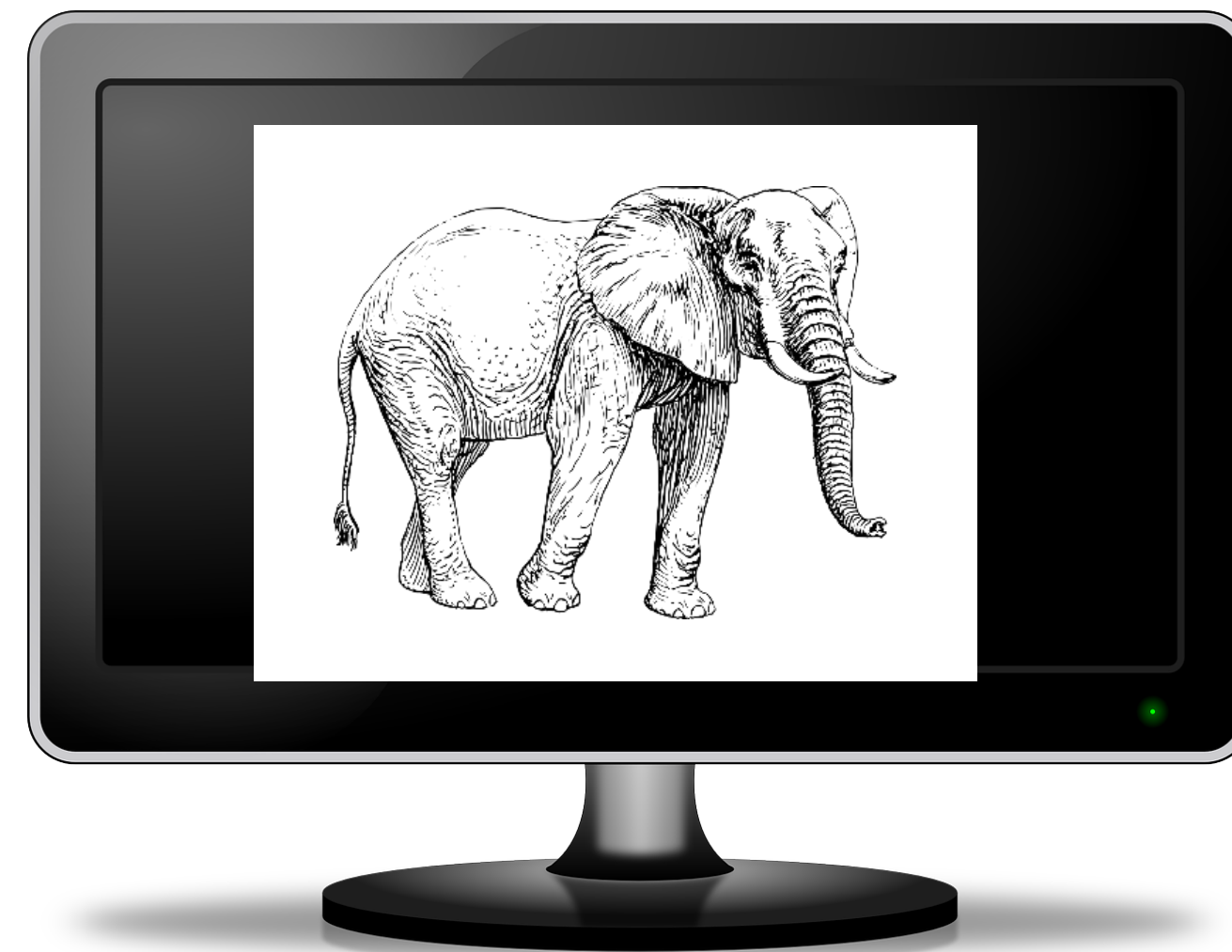


Detection System



Digital Image (Raster)

Pixel Intensity
values

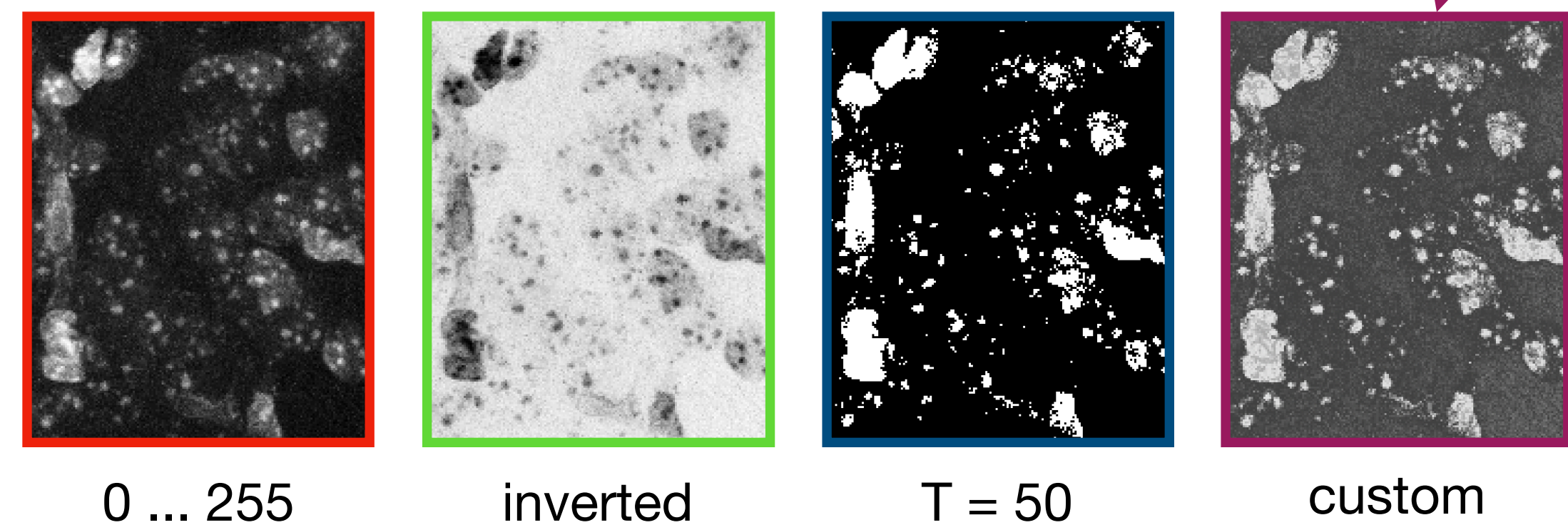
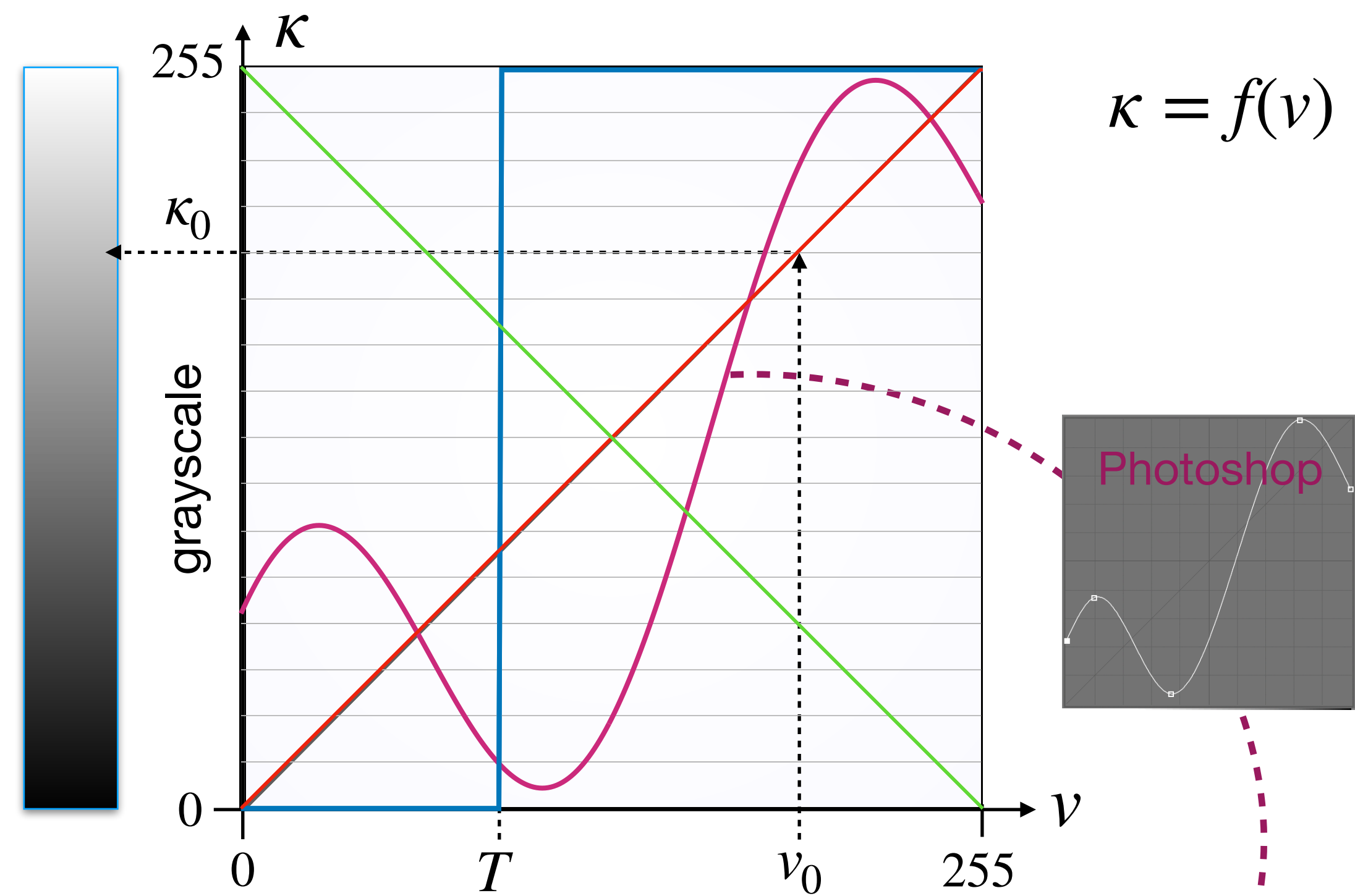


Screen
luminosity
value

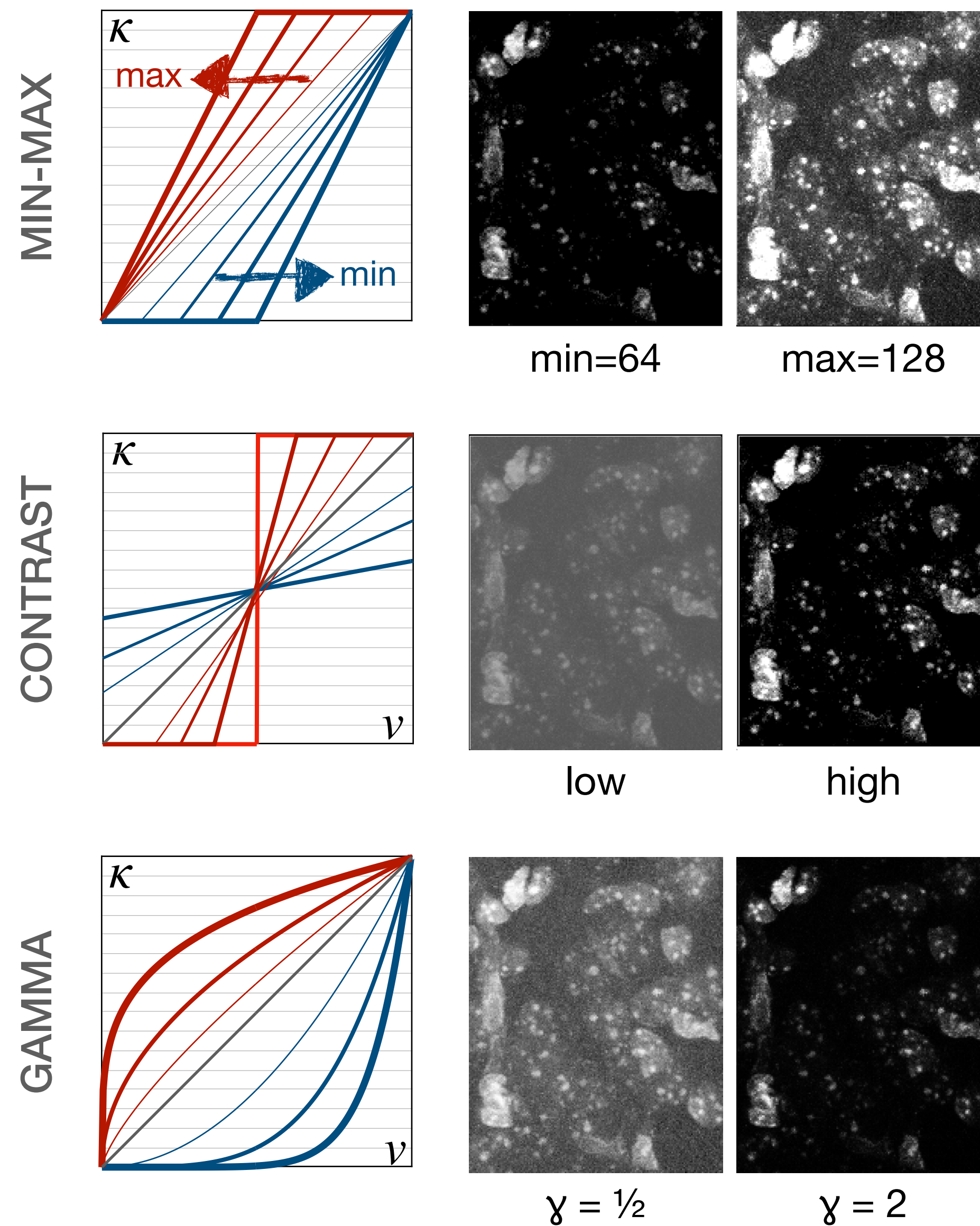
MAPPING PIXEL VALUES
TO GRAYSCALE COLOR

MAPPING PIXEL VALUES
TO GRAYSCALE COLOR

Color Mapping **Transfer Function**

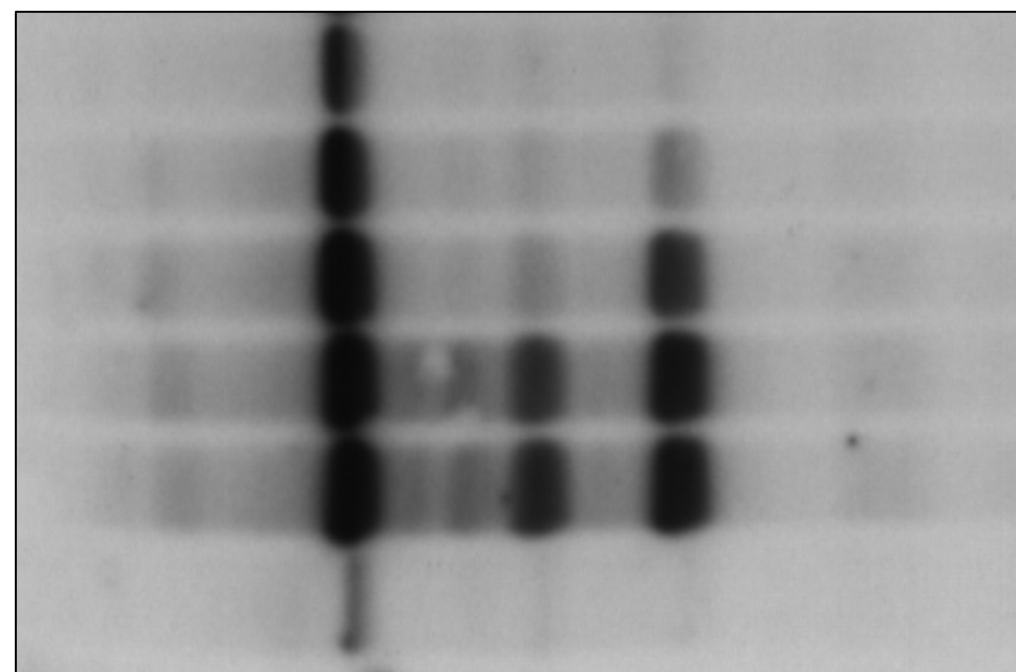


Which one is a linear operation ?

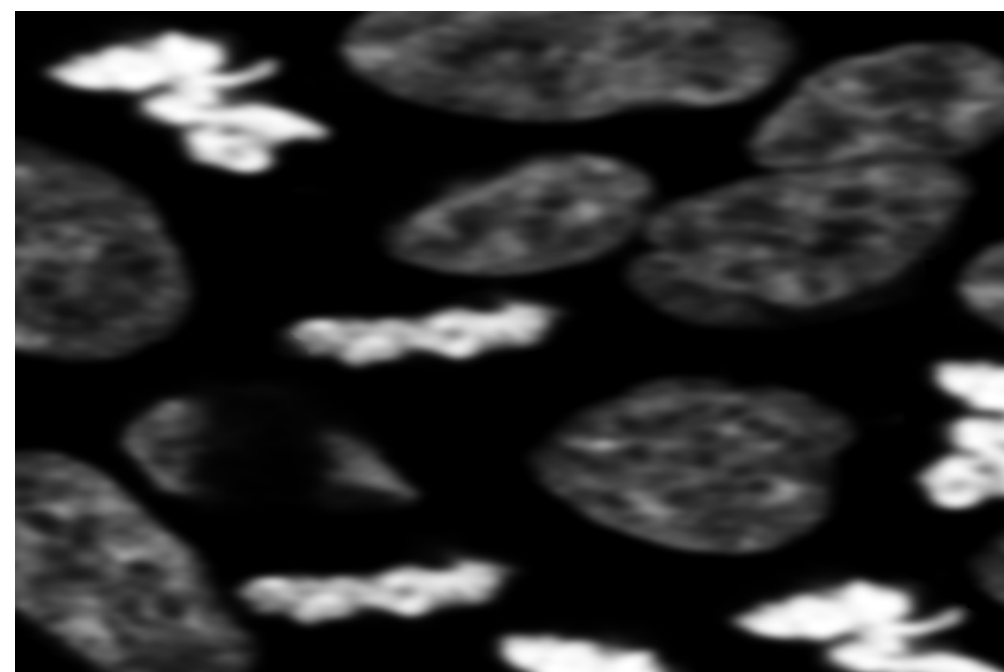


Histogram and Statistics

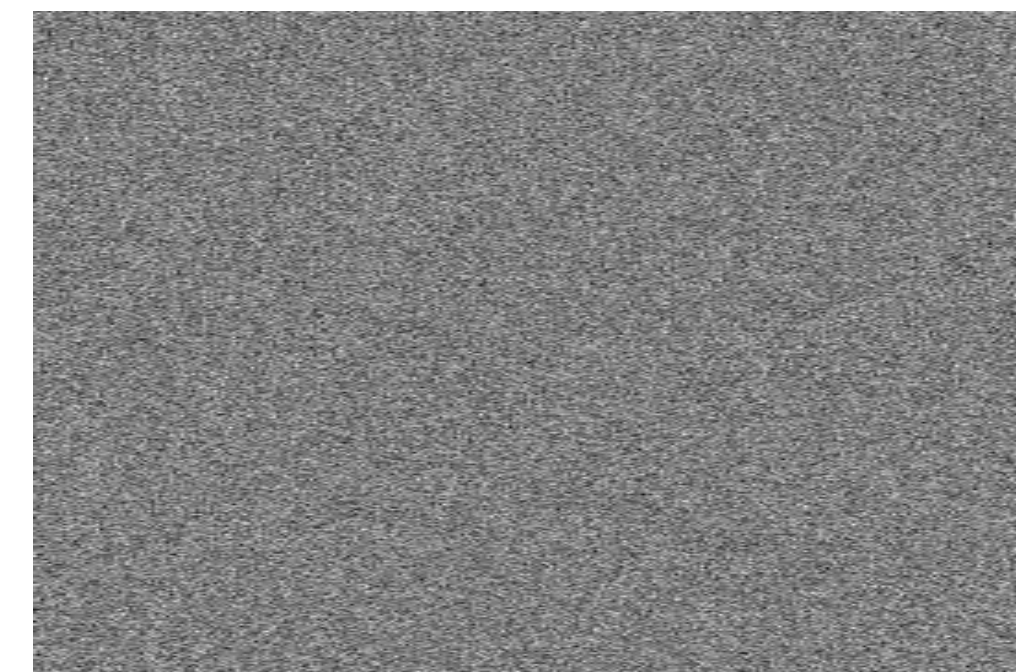
8 BITS



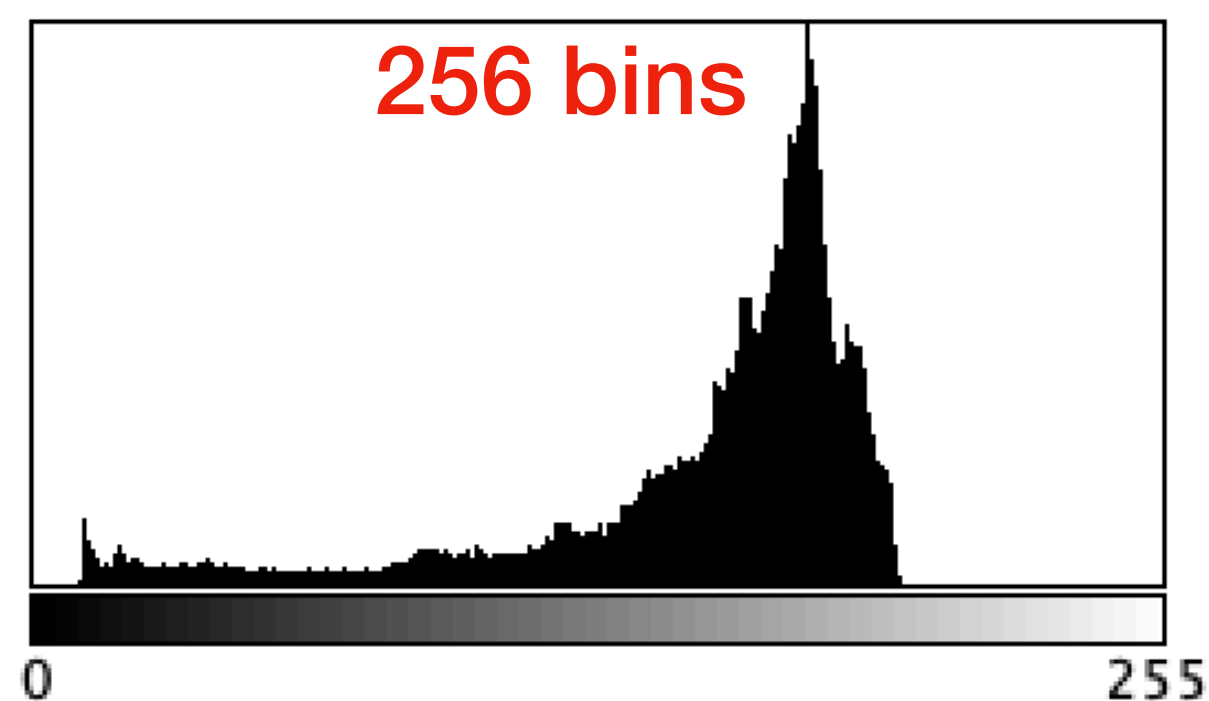
16 BITS



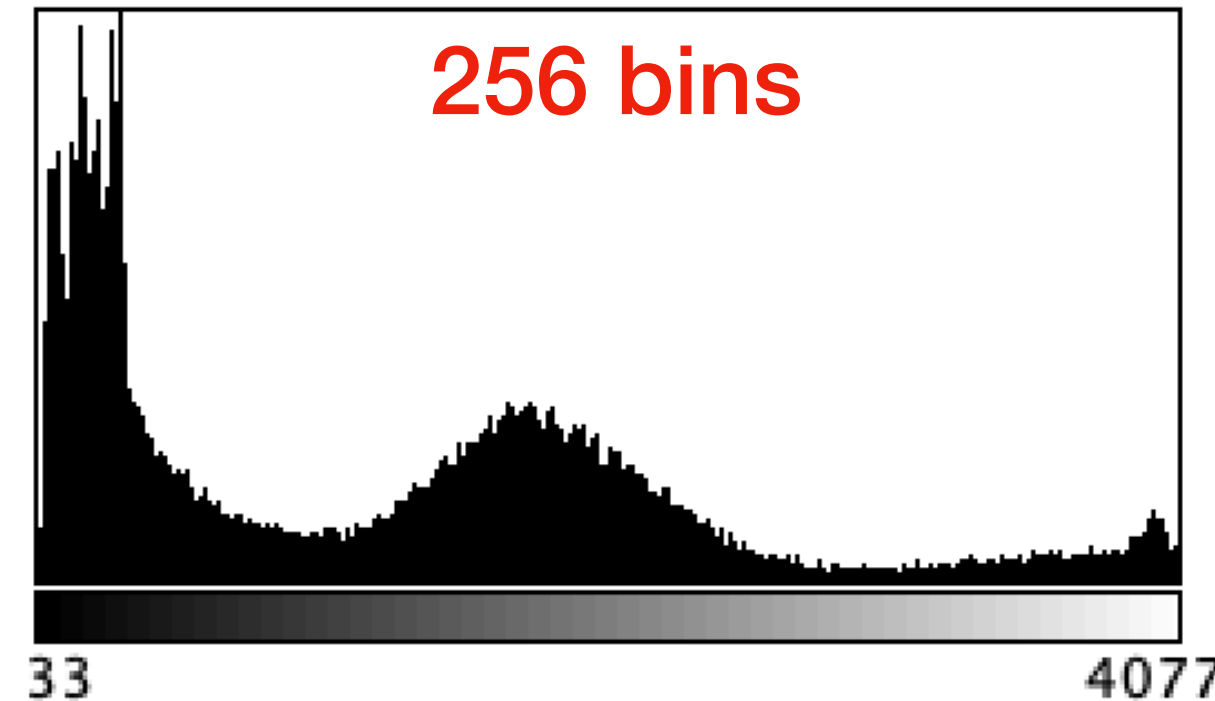
32 BITS



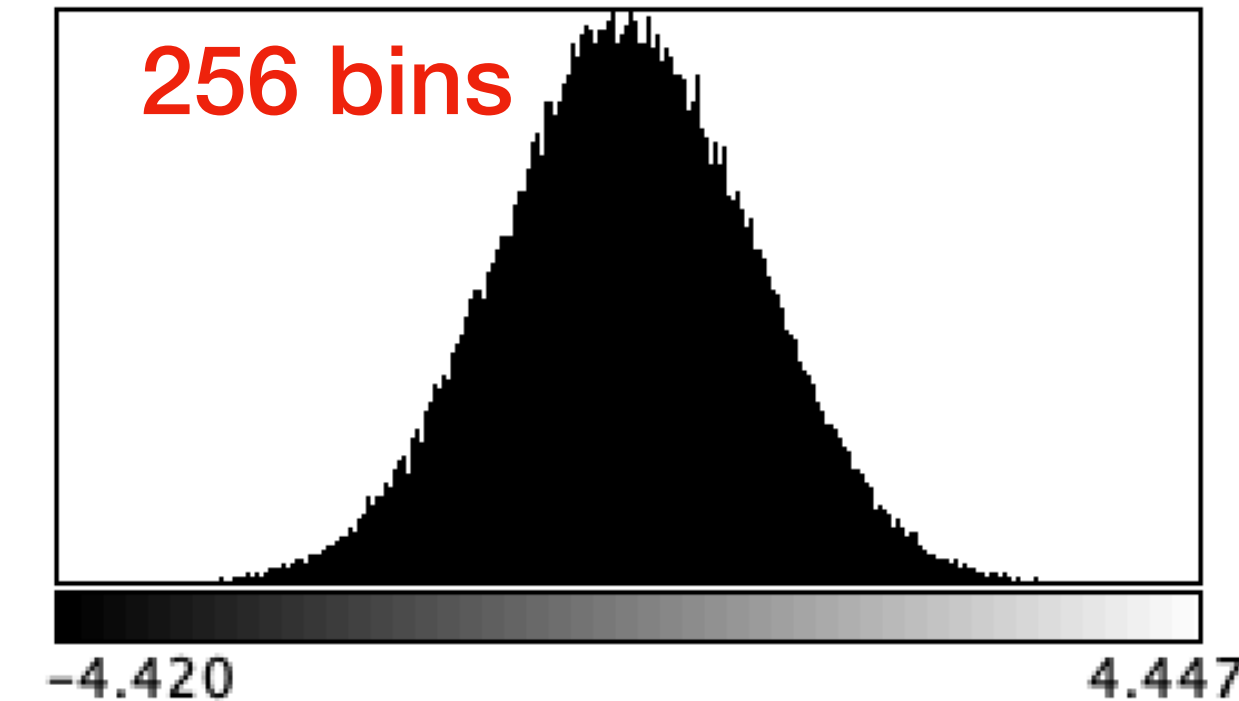
256 bins



256 bins



256 bins



0

255

33

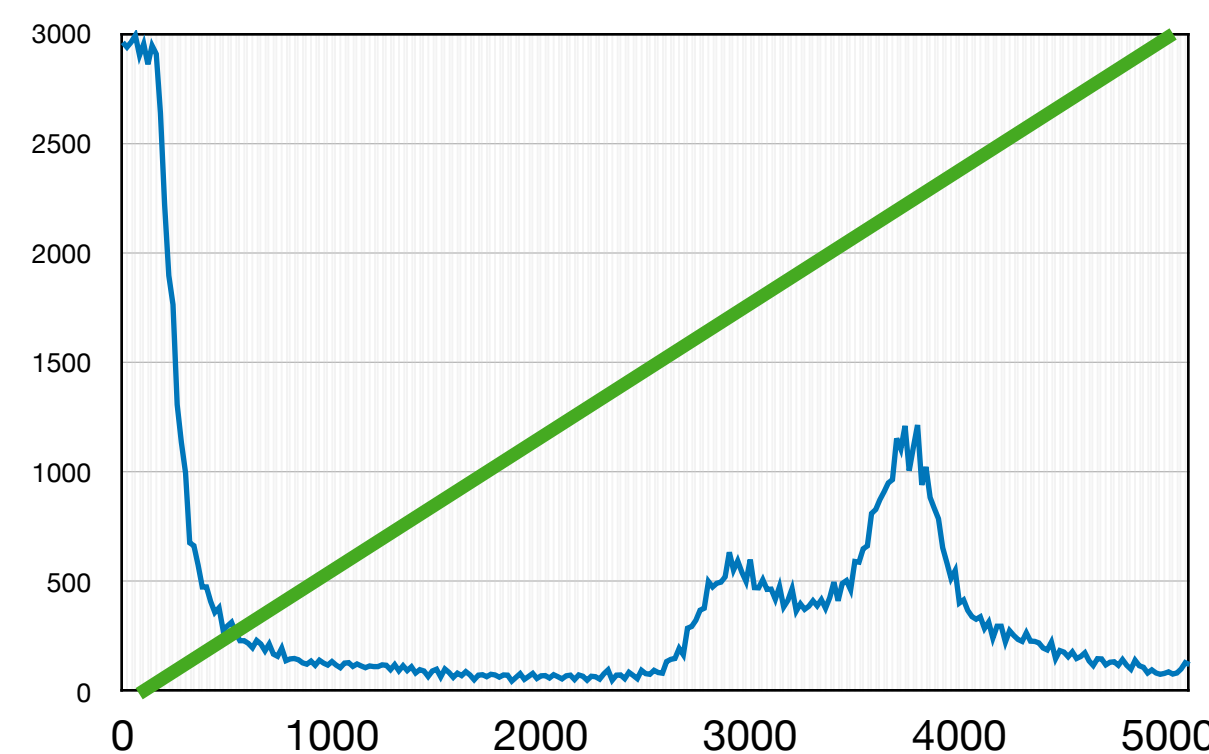
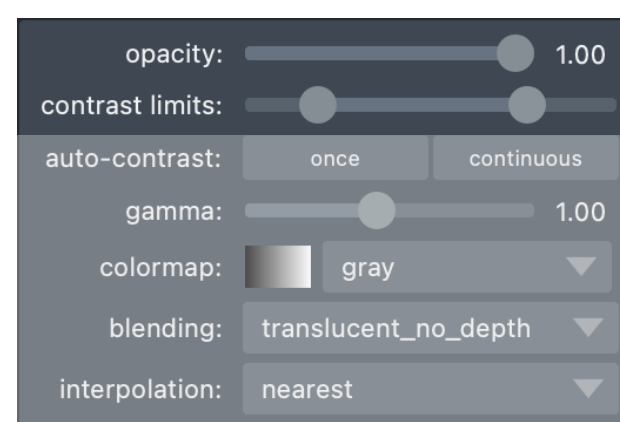
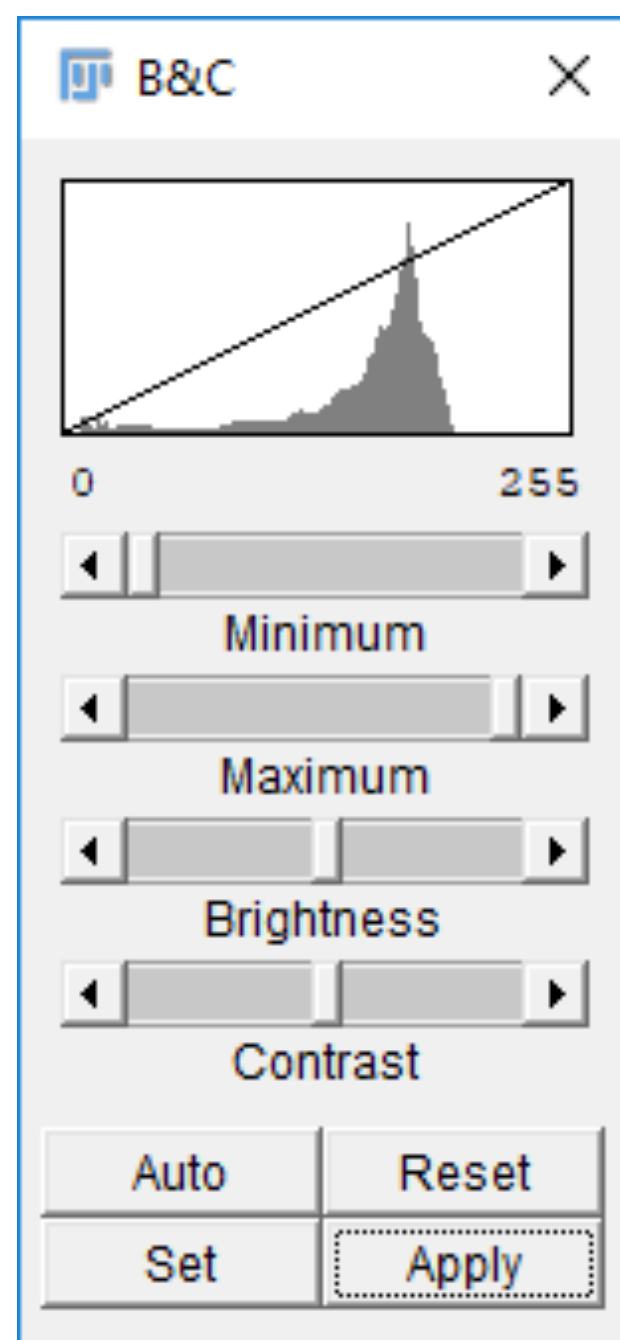
4077

-4.42

-4.45



Brightness & Contrast



m

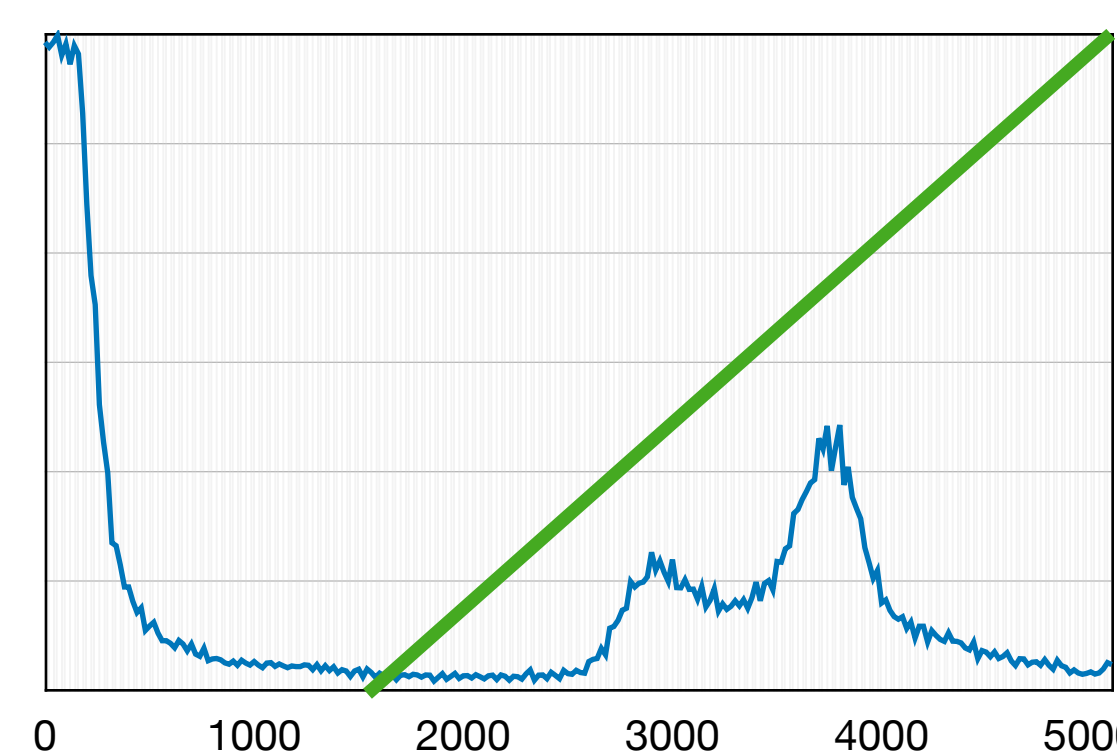
M

0

5000

$f_{min}(\mathbf{x})$

$f_{max}(\mathbf{x})$

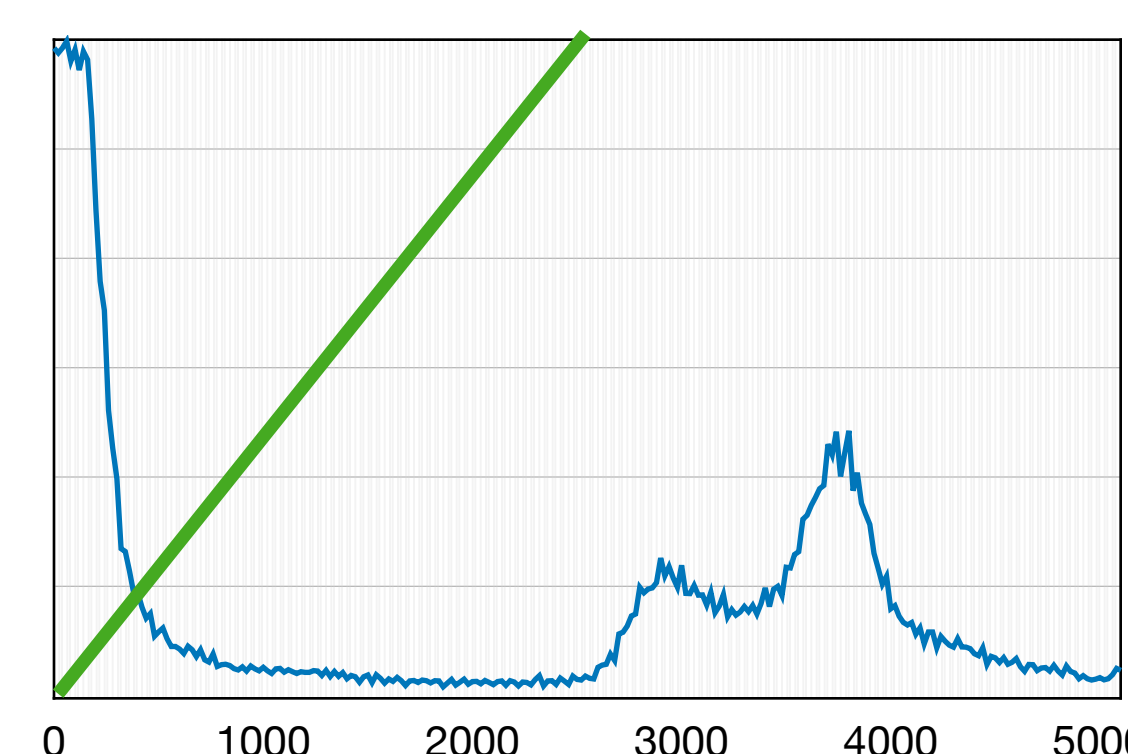


m

M

1500

5000



m

M

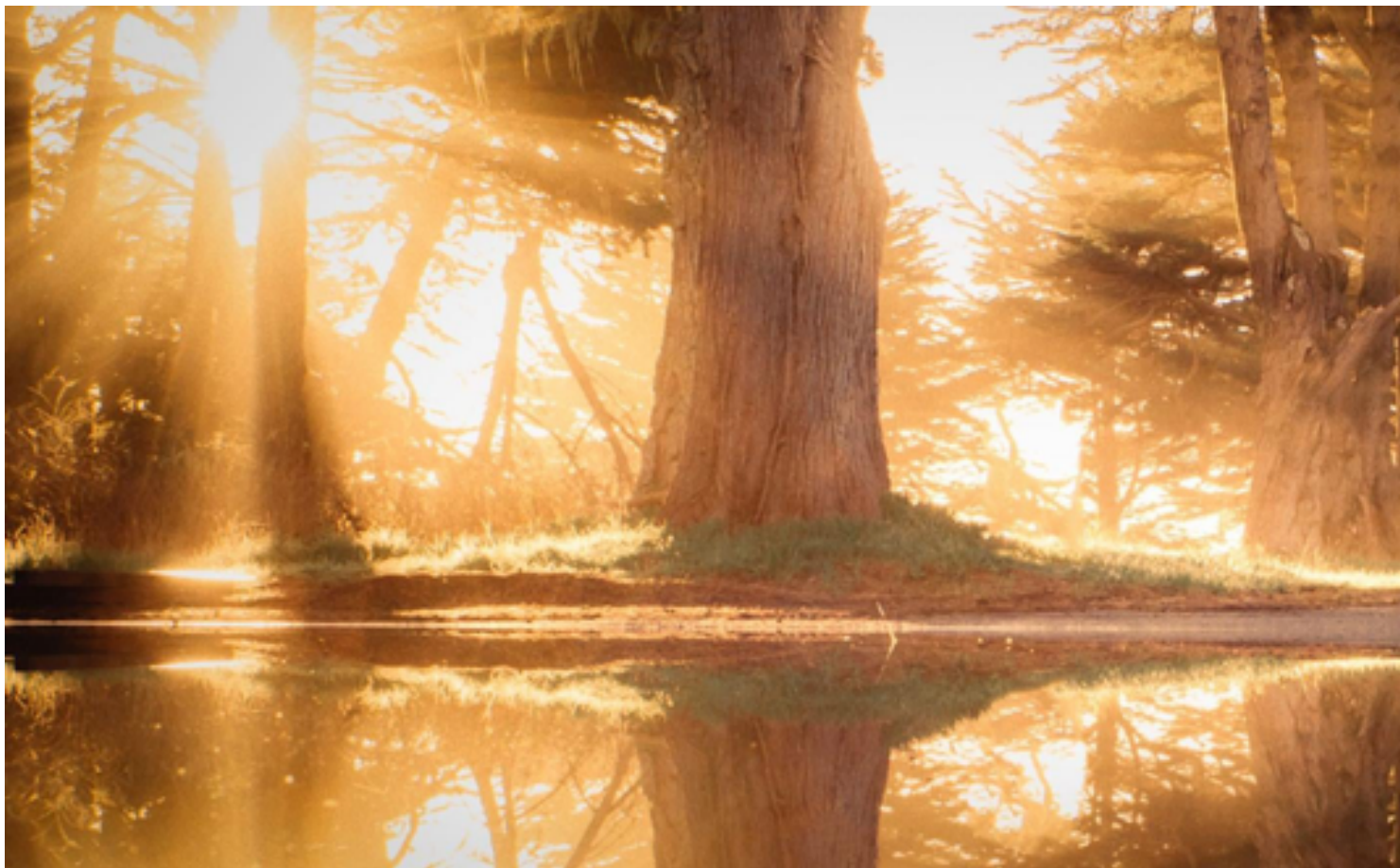
2500



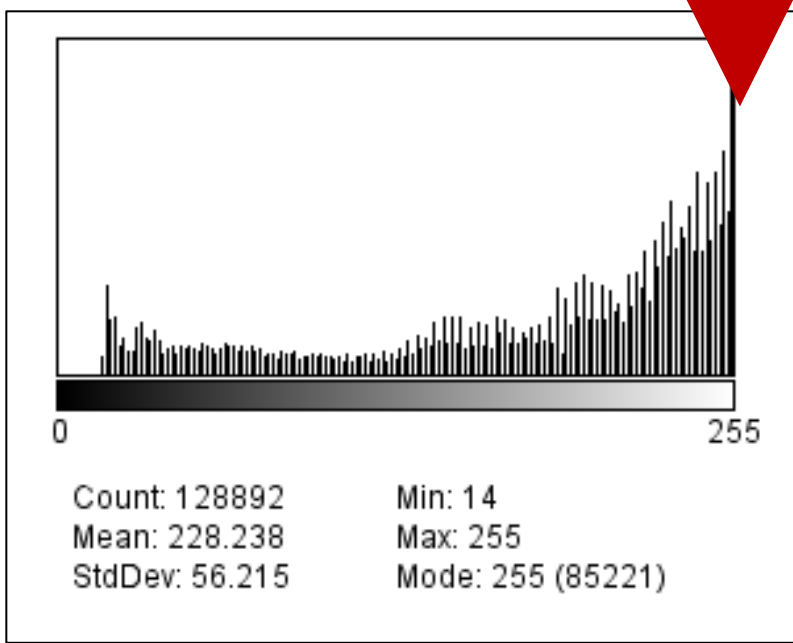
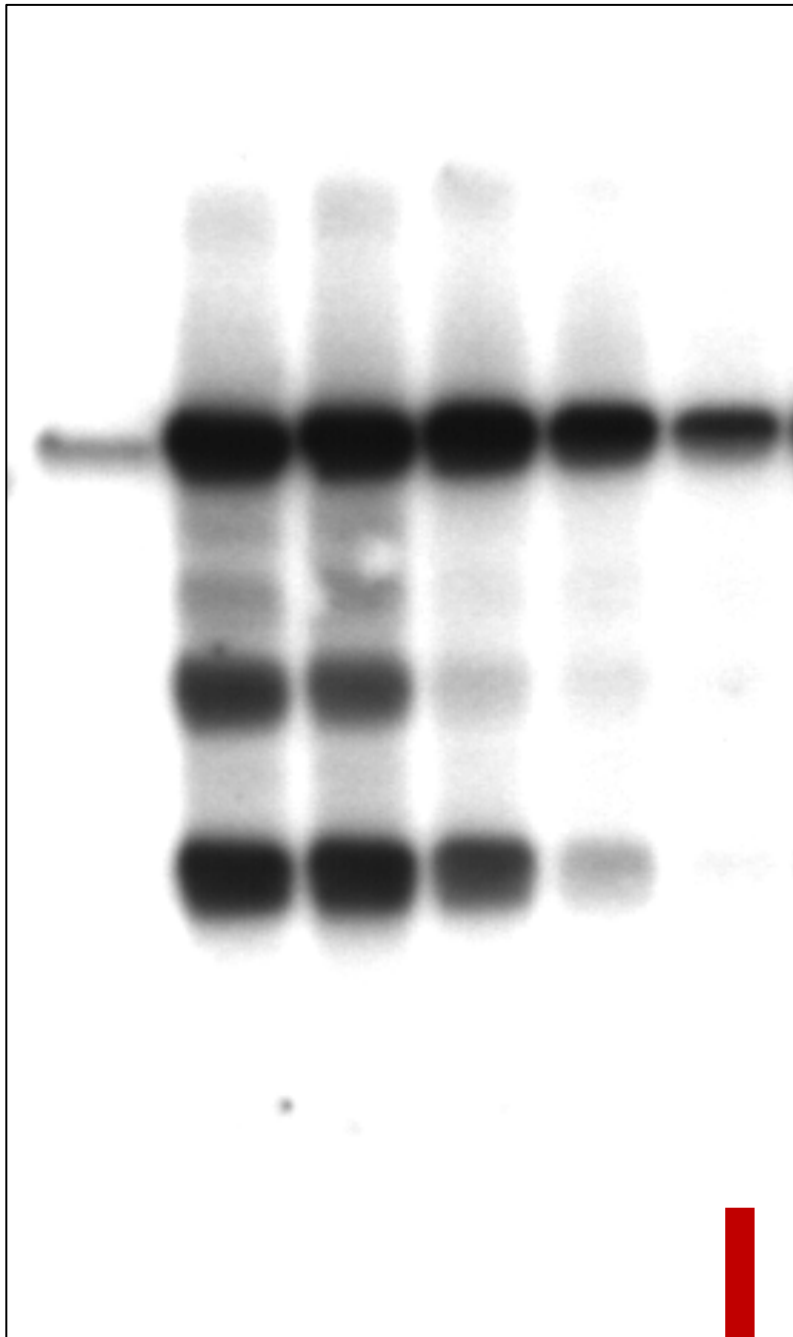
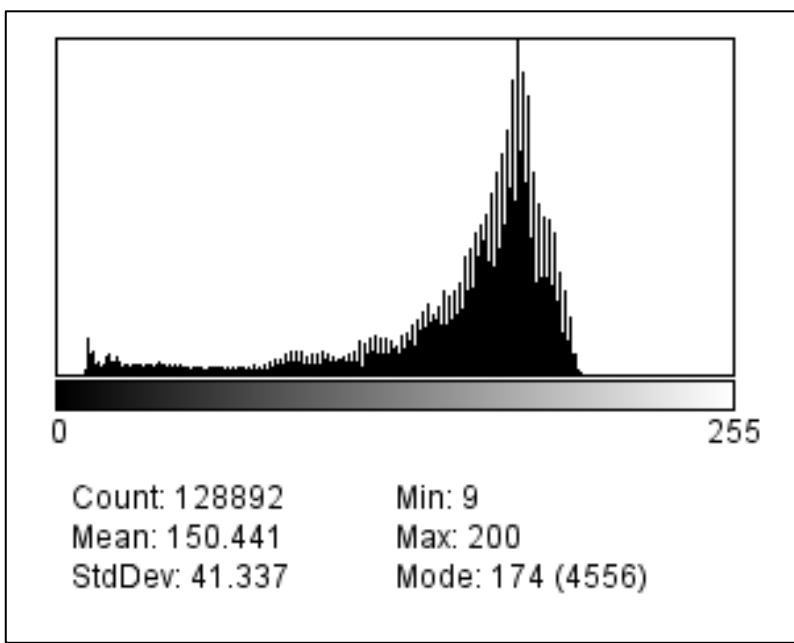
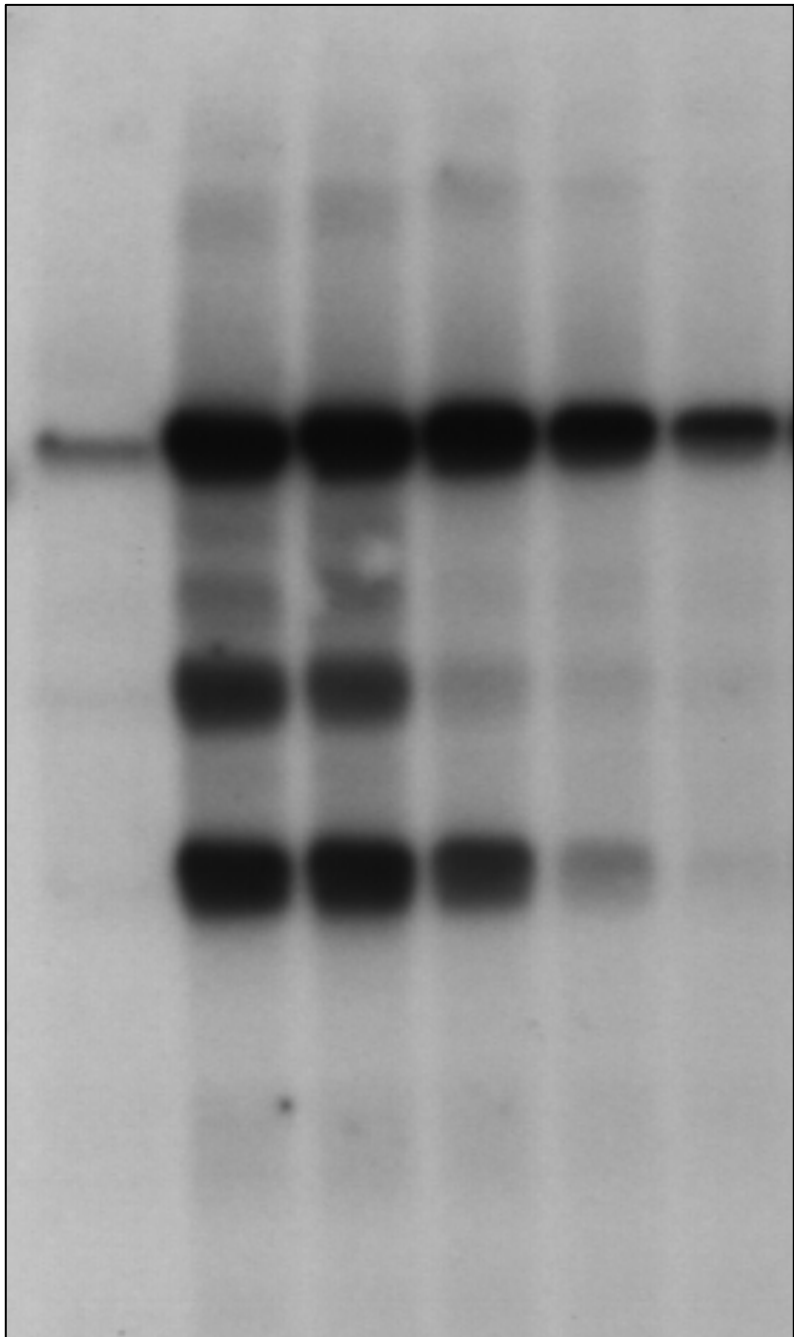
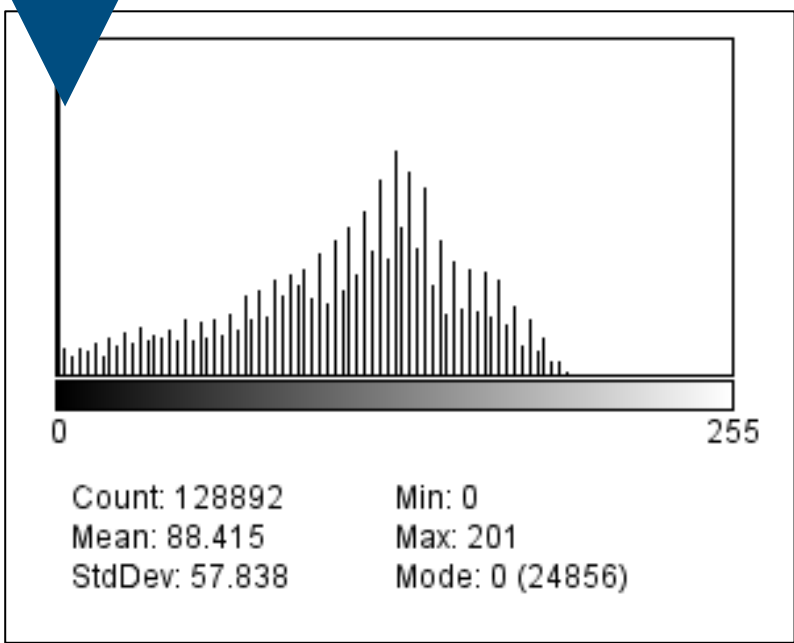
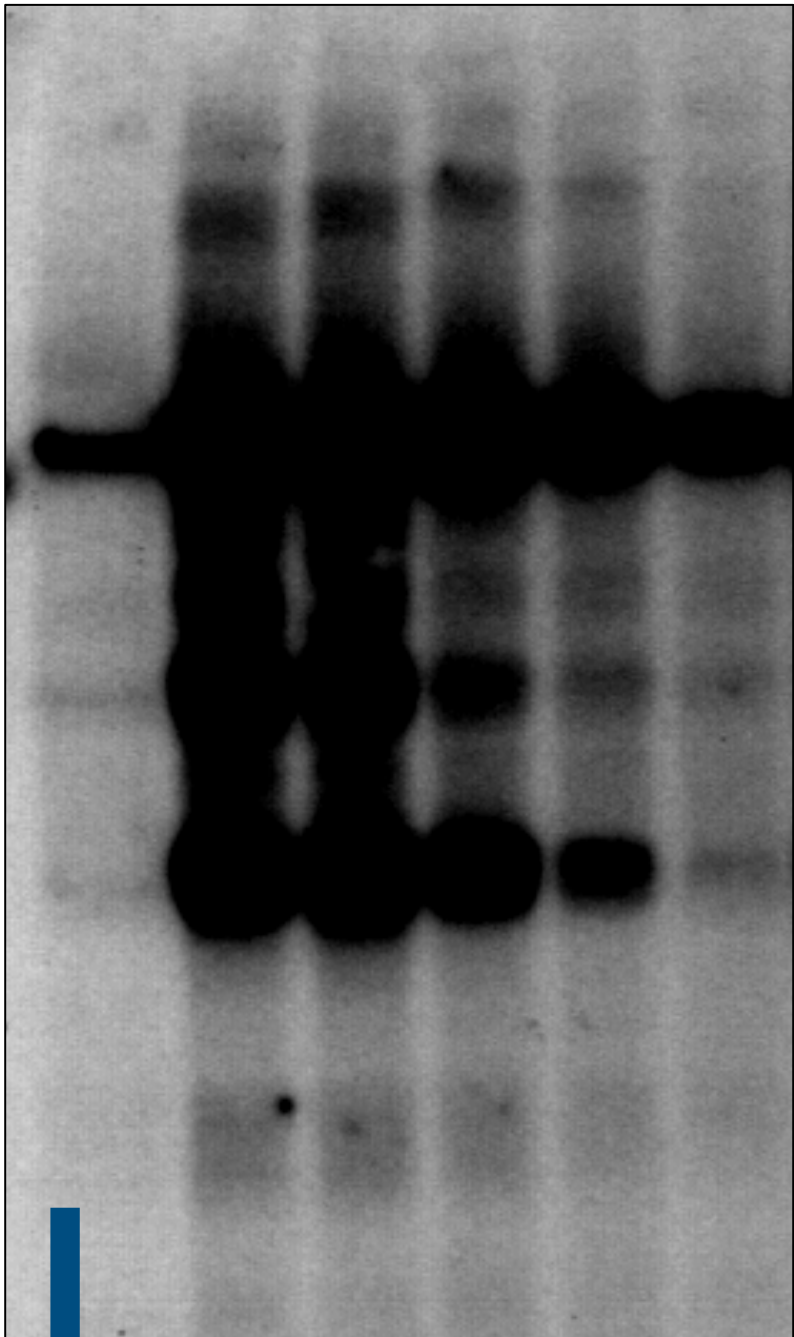
Image Saturation

Saturation

Is an irreversible loss of information?



Cannot be fixed by post-acquisition

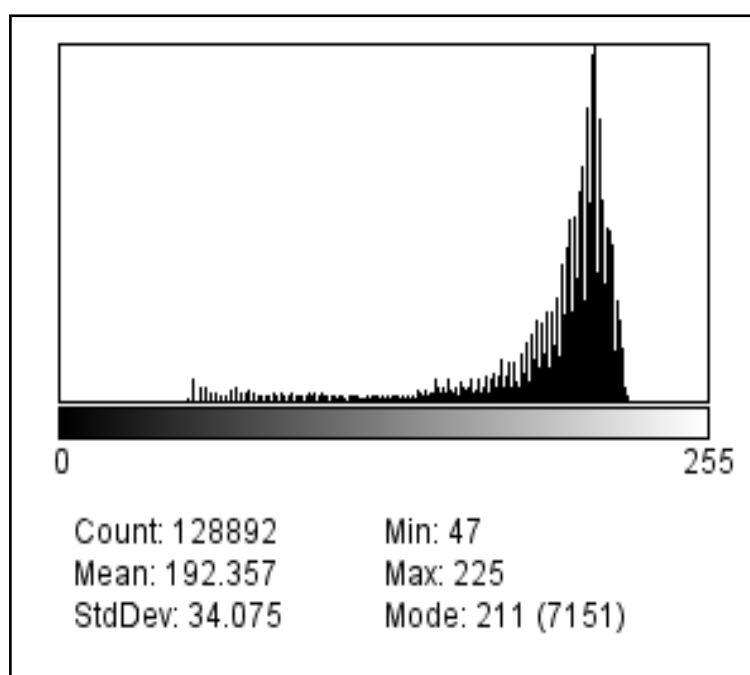
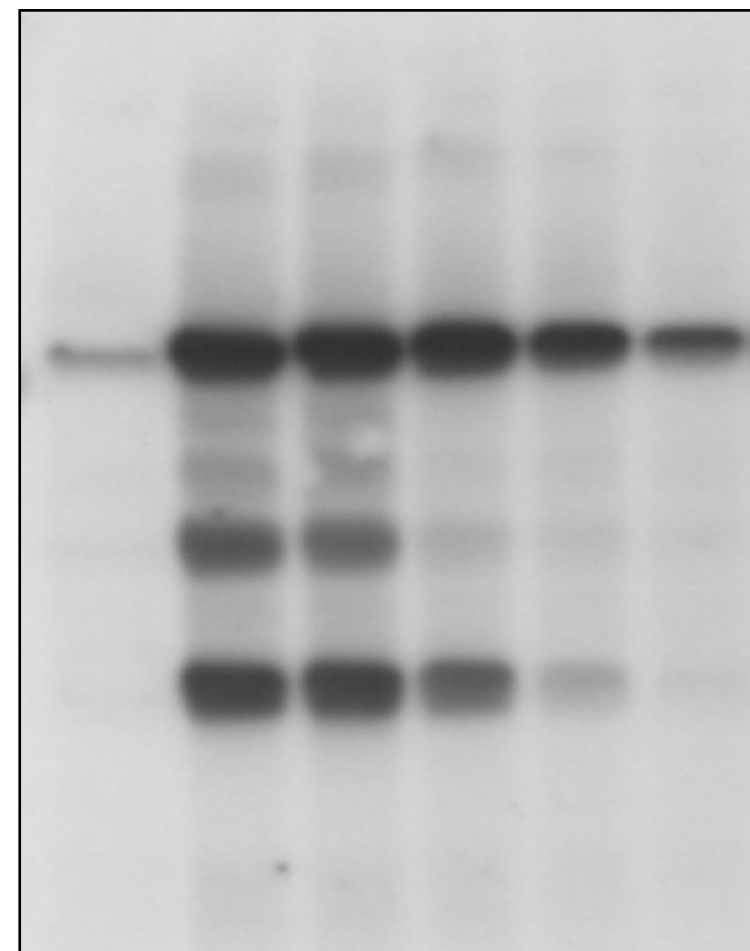




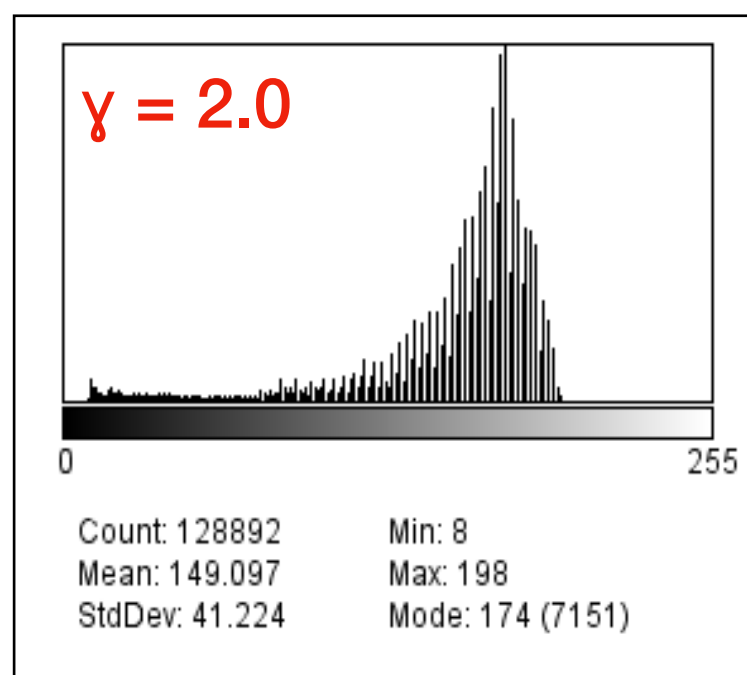
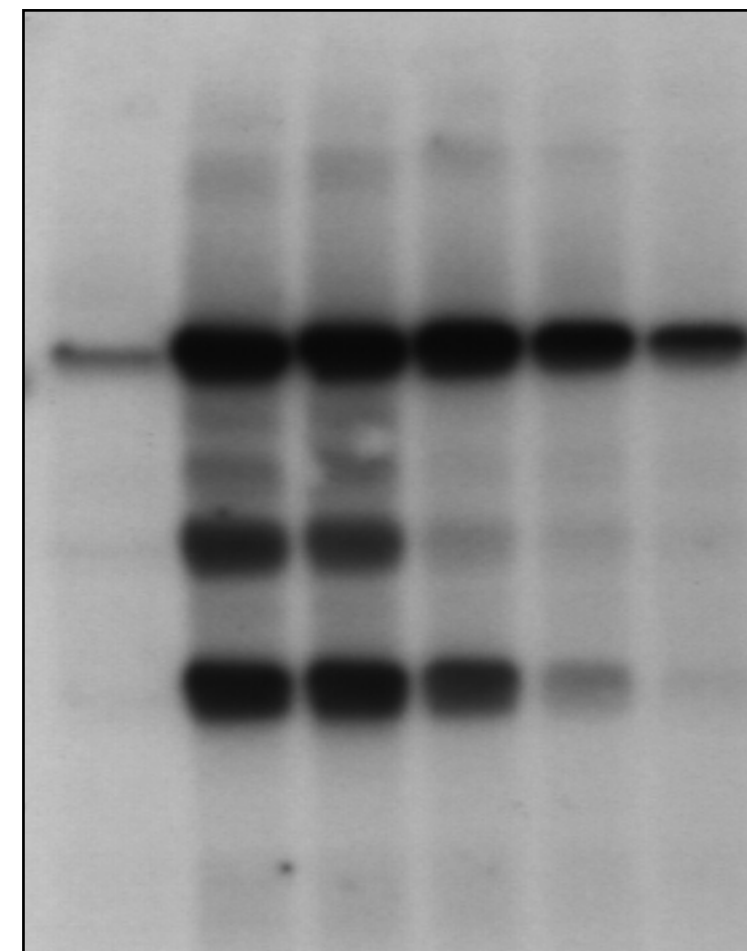
Contrast Enhancement

$$g(x, y) = (f_{max} - f_{min}) \times \left[\frac{f(\mathbf{x}) - f_{min}}{f_{max} - f_{min}} \right]^\gamma$$

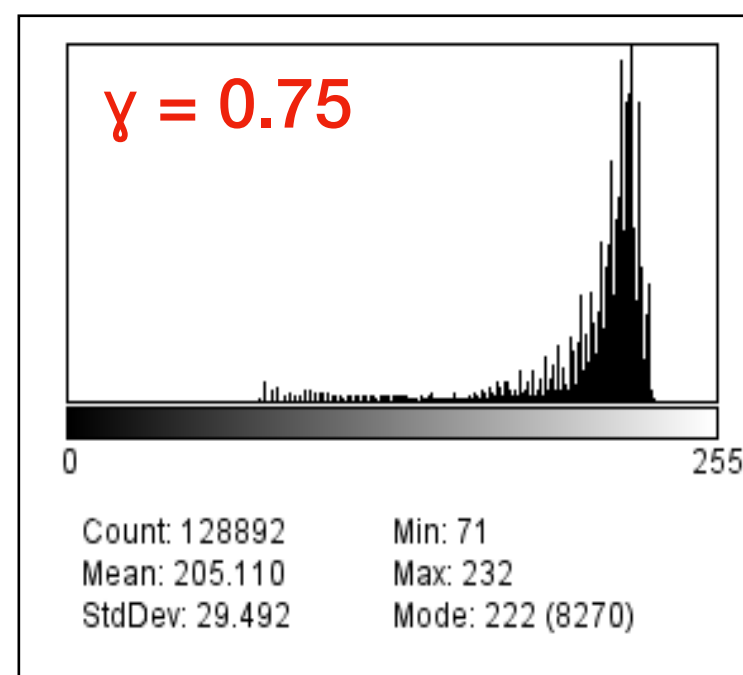
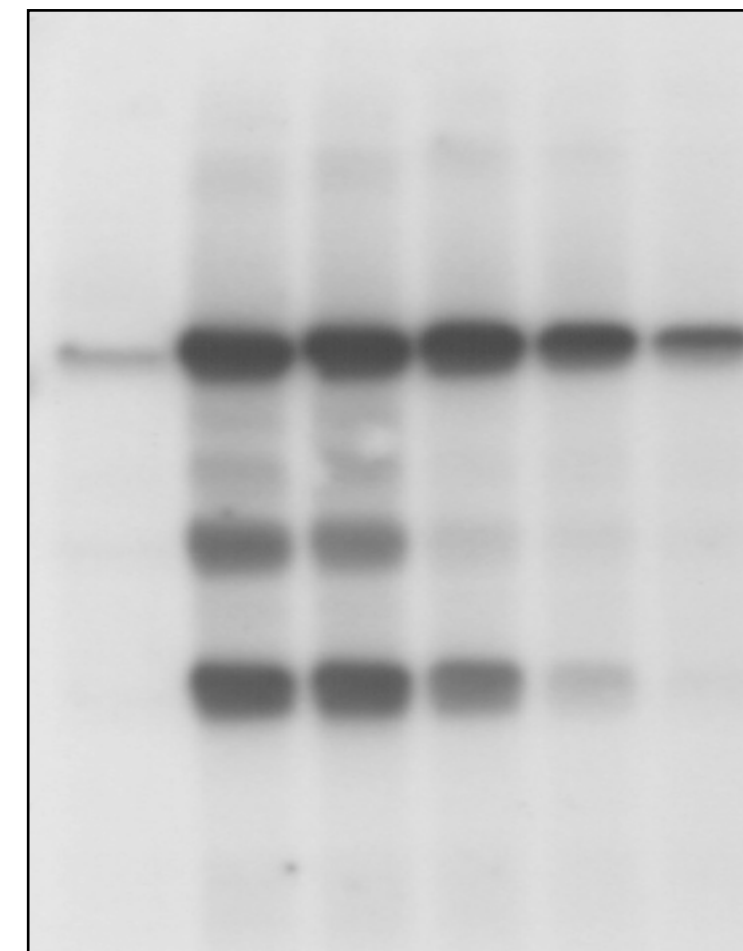
Original
Image



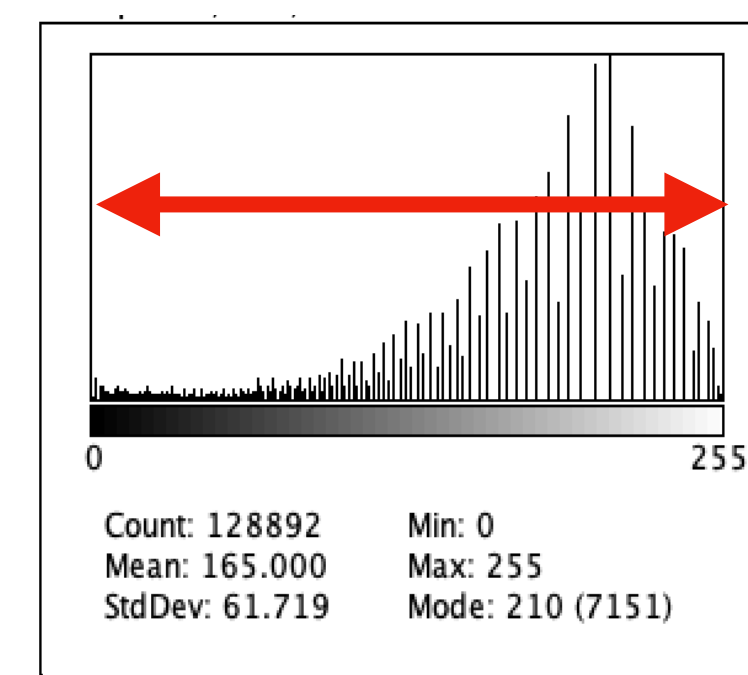
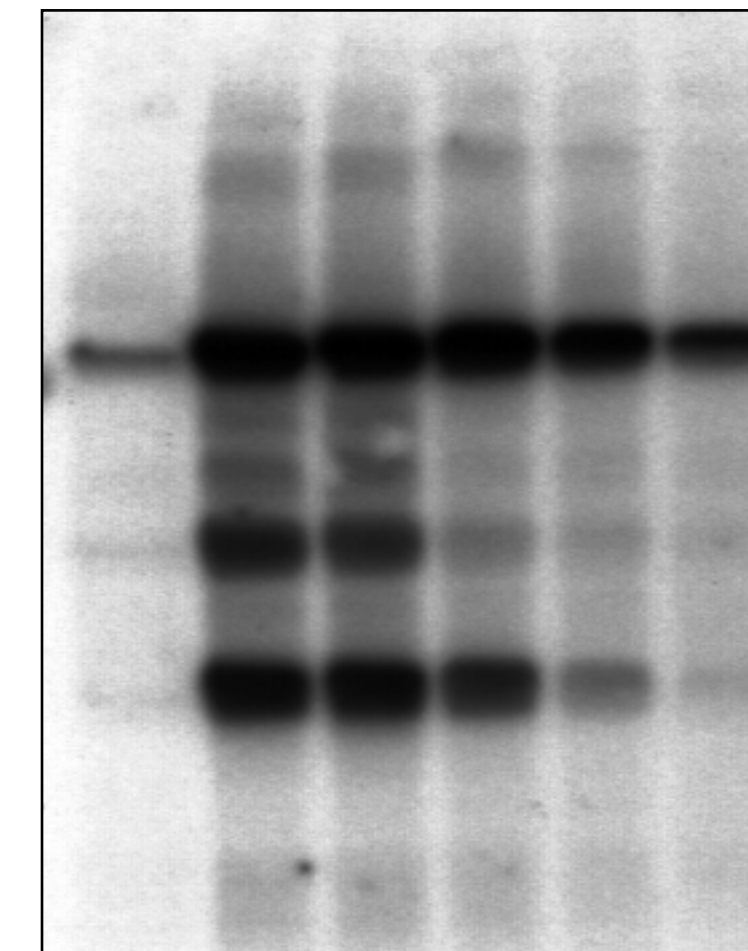
Gamma
Correction



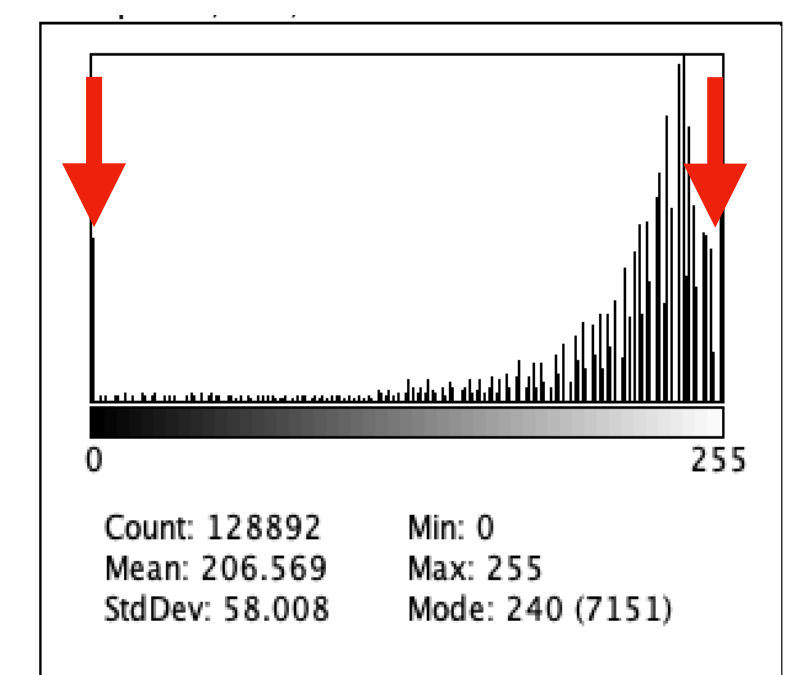
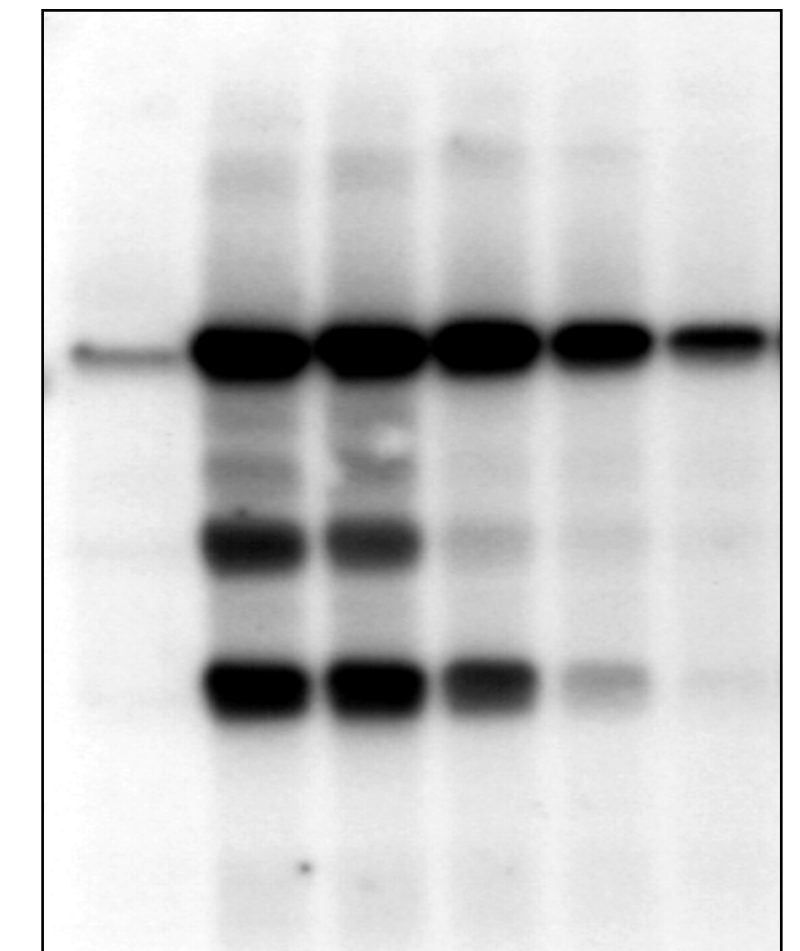
Gamma
Correction



Equalize
Histogram



Normalize
saturated pixels=0.5%





Dataset Normalization

Similar Data Distribution in Machine Learning

Mandatory preprocessing to ensure that the dataset have a similar dynamic range



Normalization

- Divide by max (e.g. 256, 65536, ...)

- Min-Max

$$g(\mathbf{x}) = \frac{f(\mathbf{x}) - f_{min}}{f_{max} - f_{min}}$$

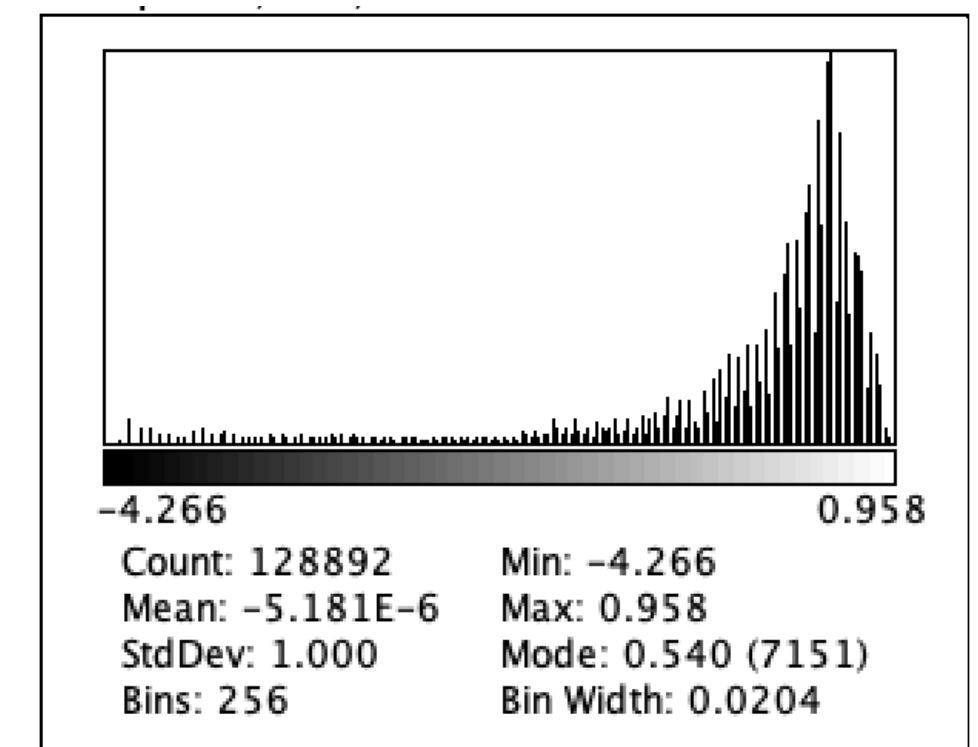
- Quantile normalization
- Zero-centering

Standardization

- ≡ Statistical normalization
- ≡ Z-score normalization
- ≡ Zero-mean and unit-variance

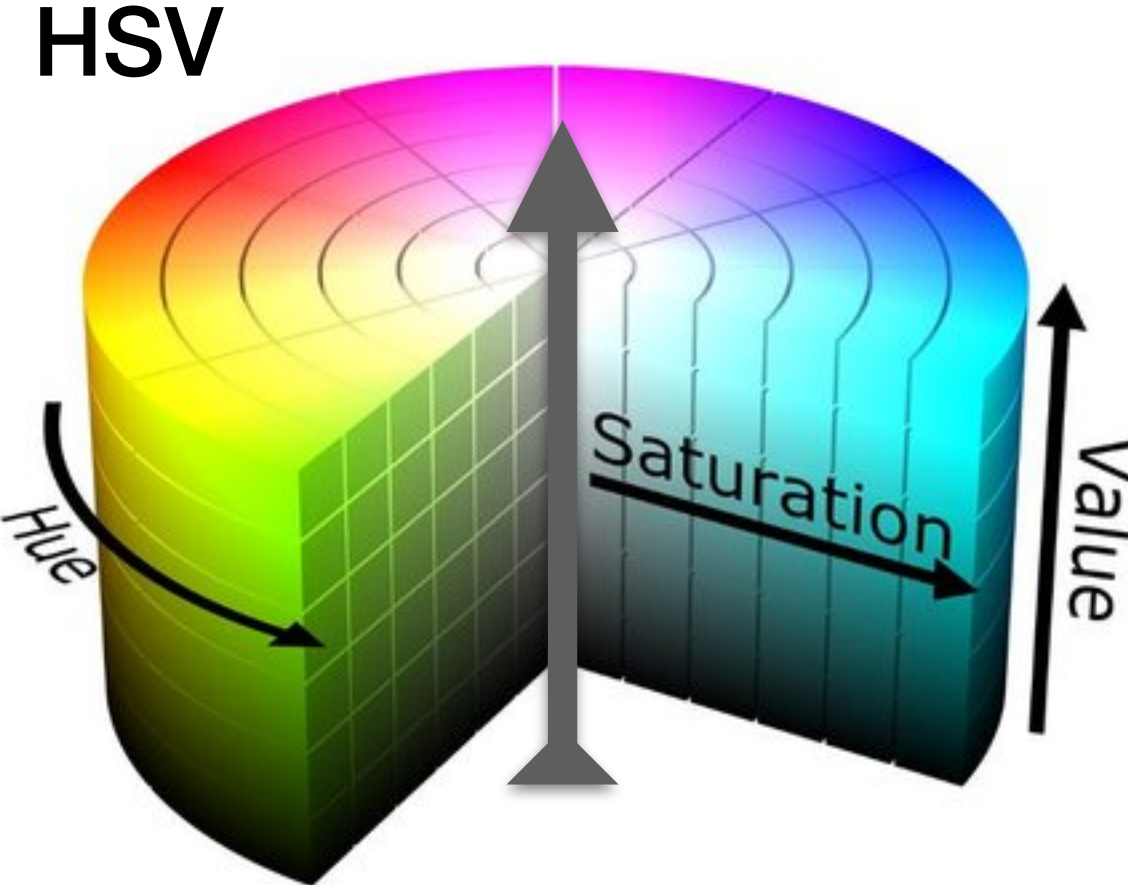
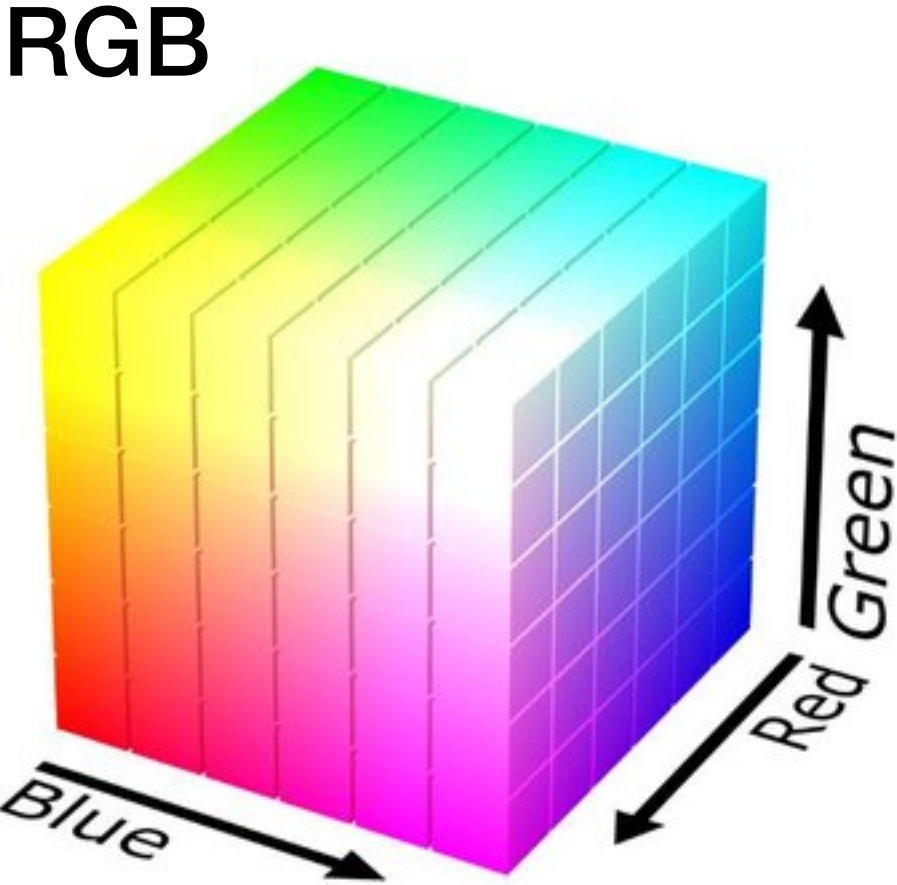
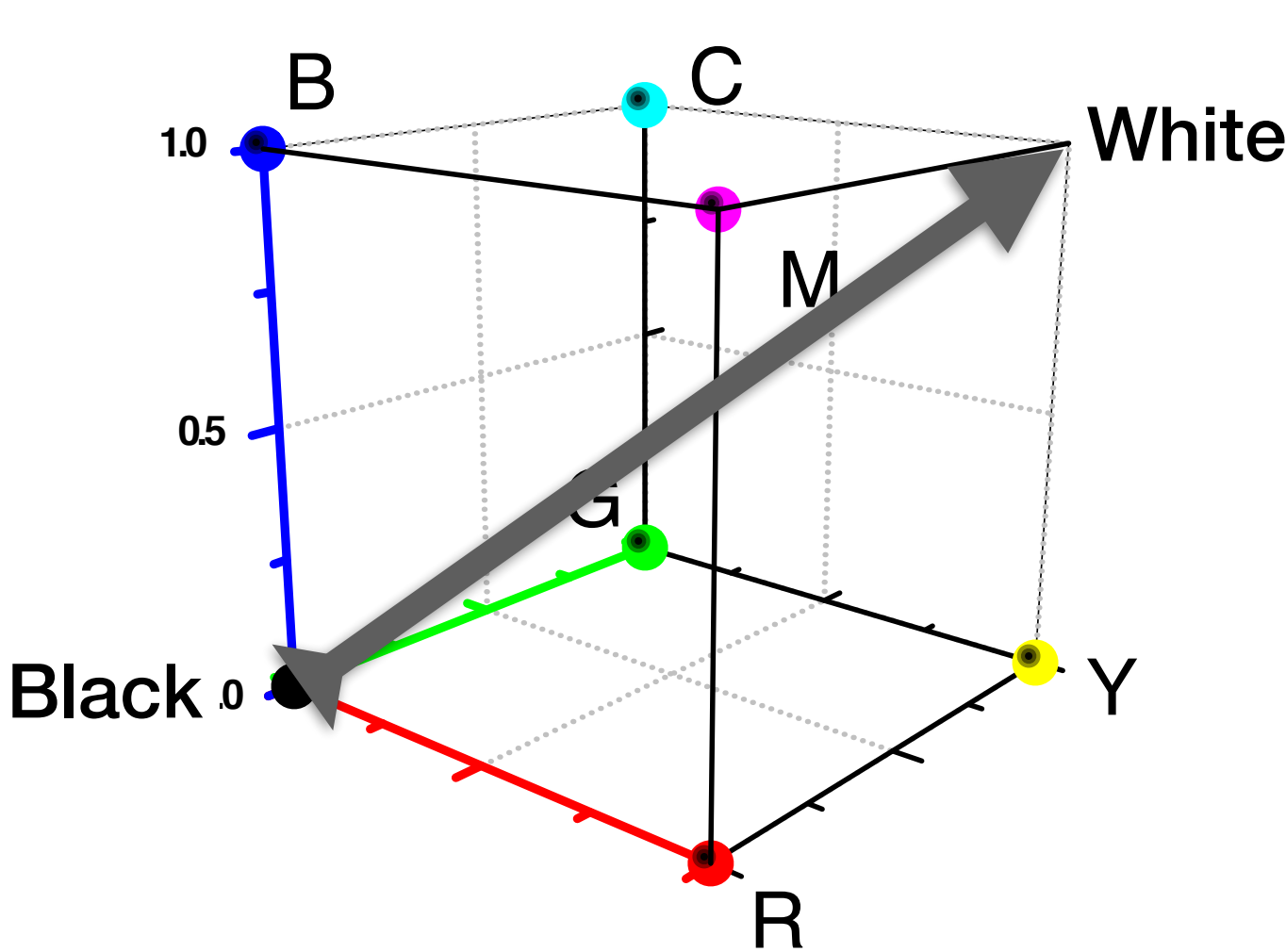
$$g(\mathbf{x}) = \frac{f(\mathbf{x}) - \bar{f}}{\sigma_f}$$

Which normalization
was applied ?

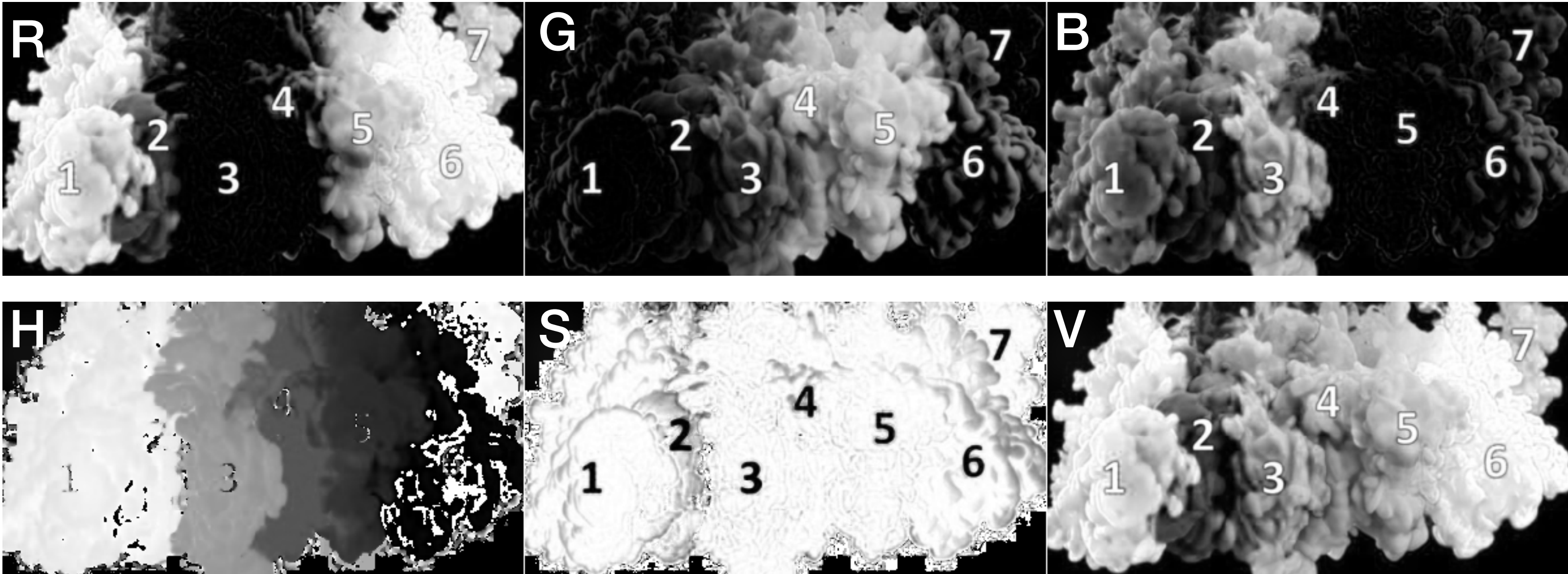




Color Representation



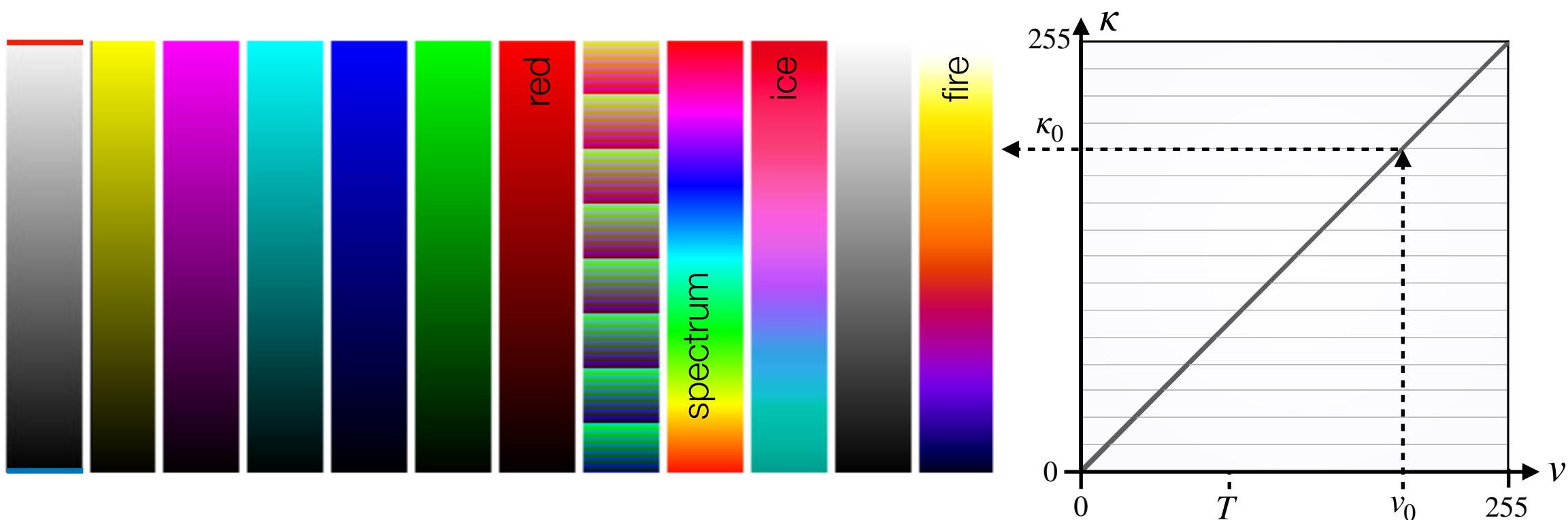
Hue
Saturation
Value = brightness



Hue spans on a circular scale



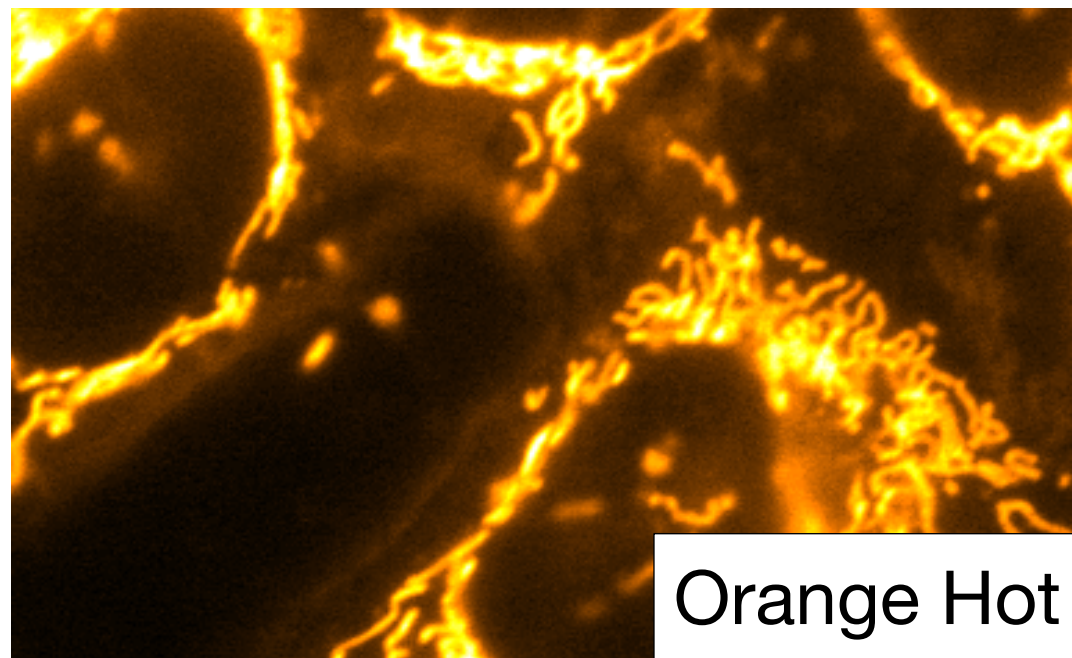
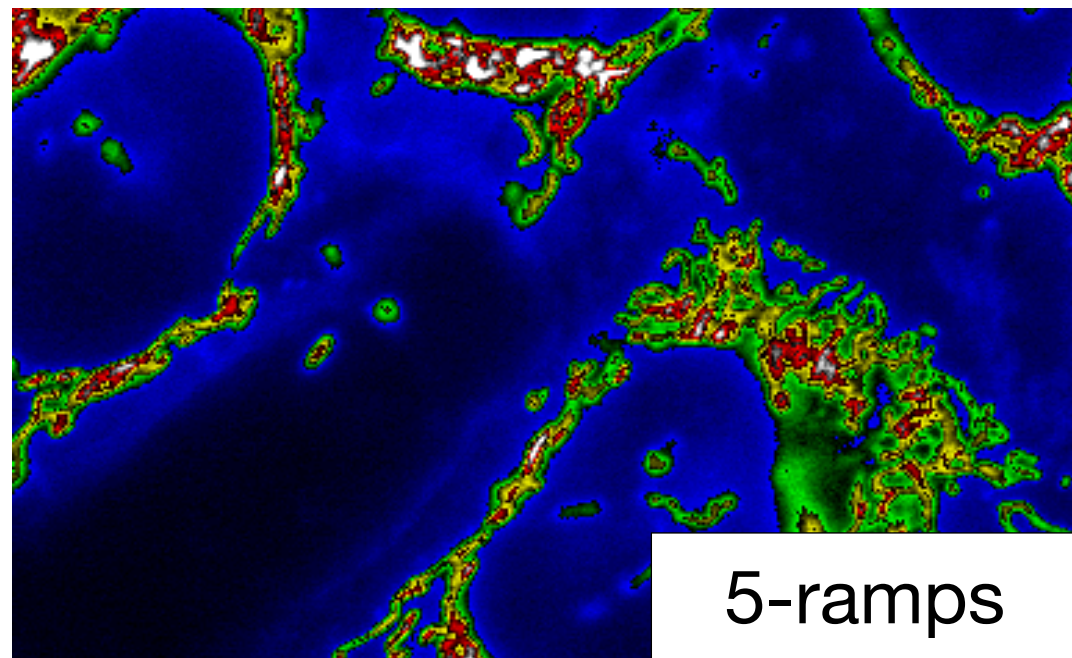
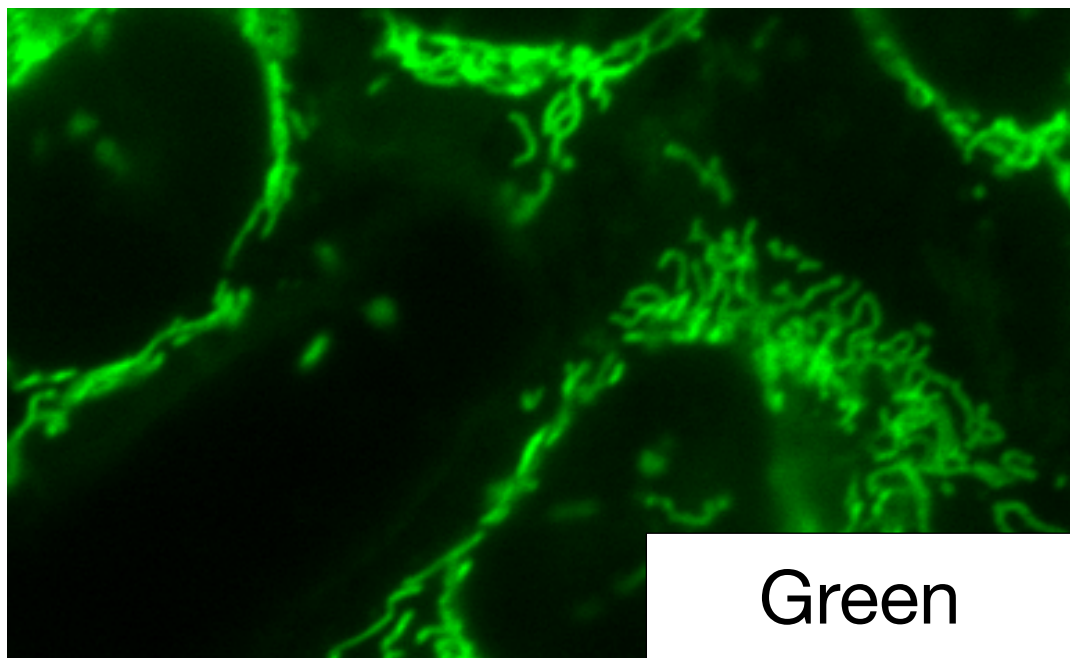
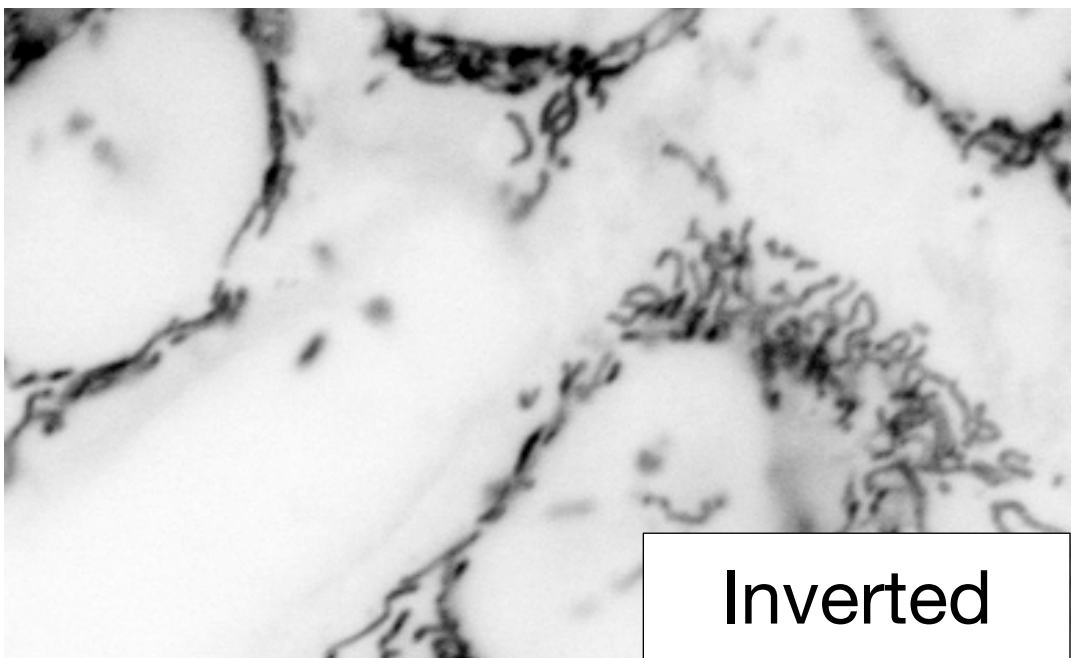
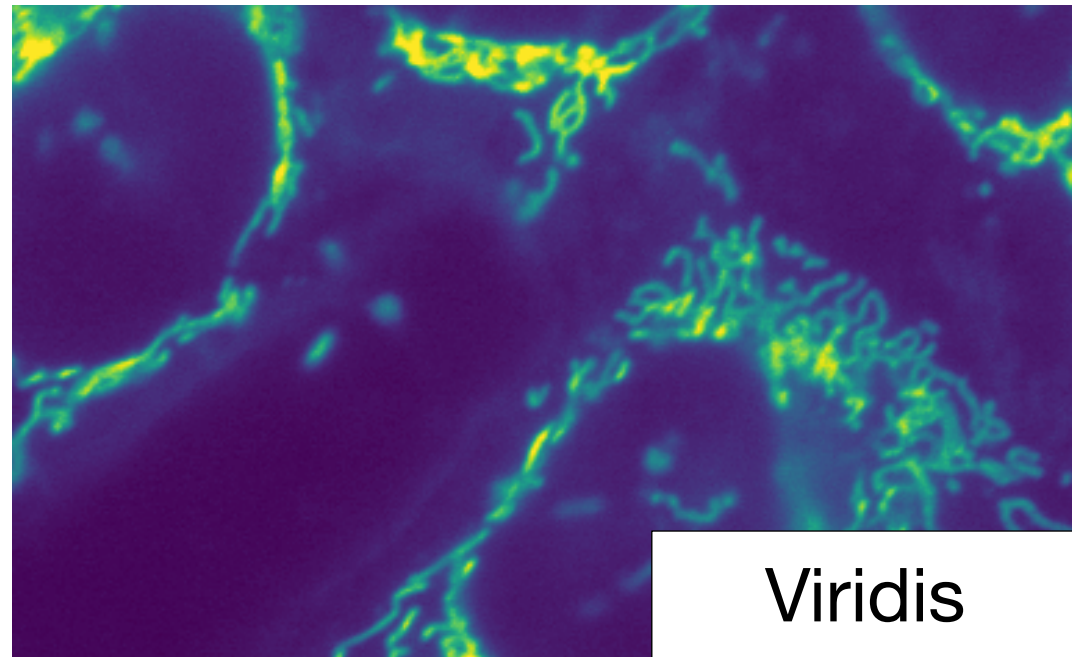
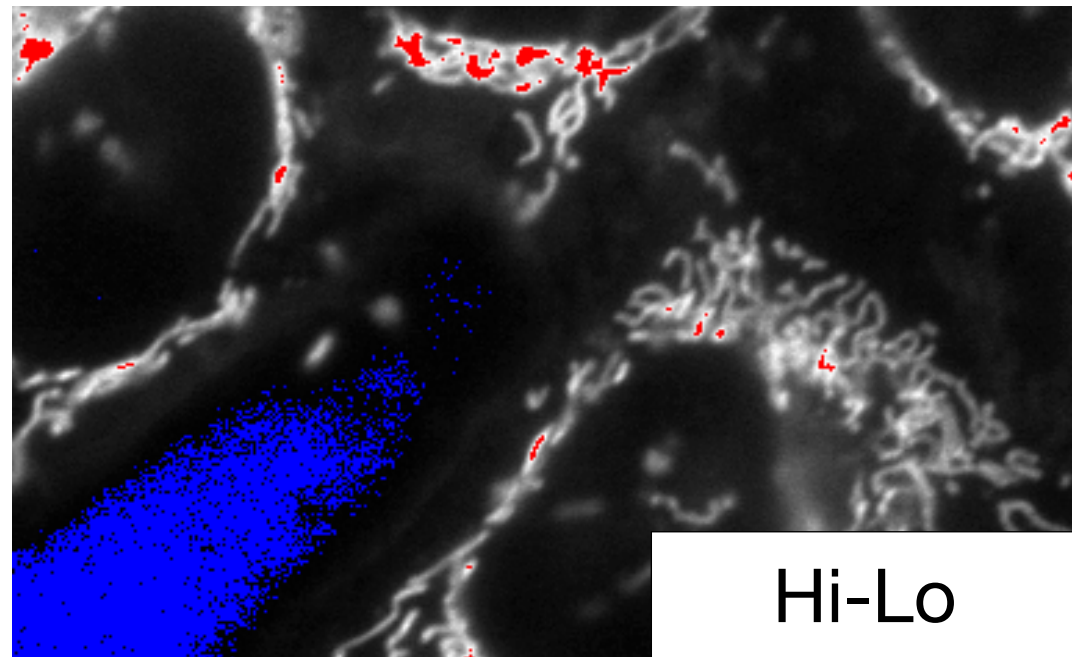
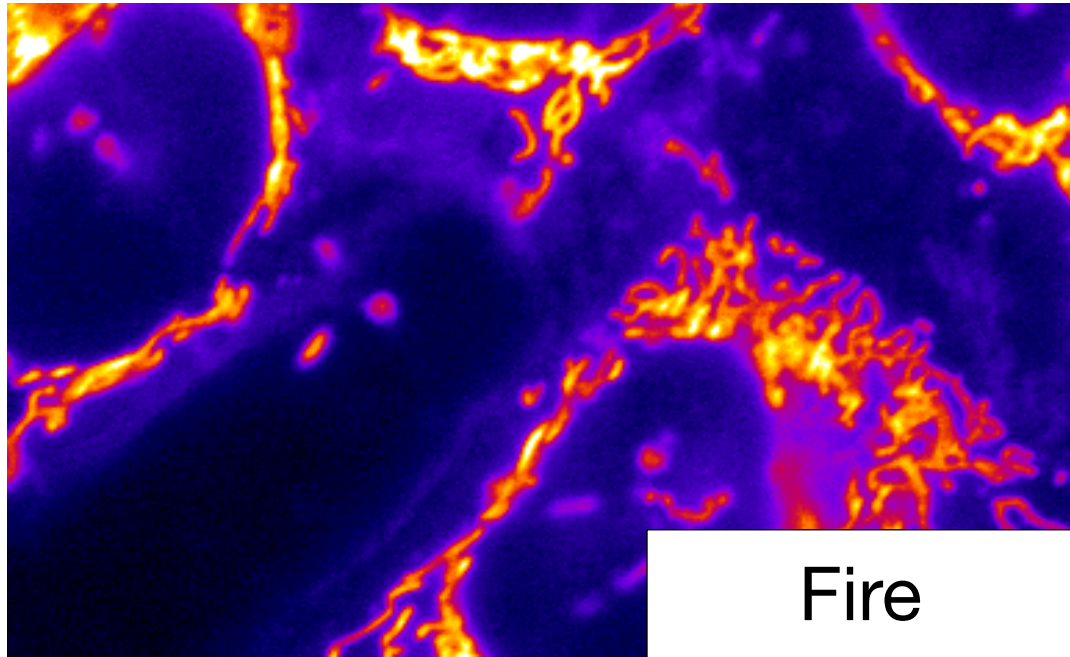
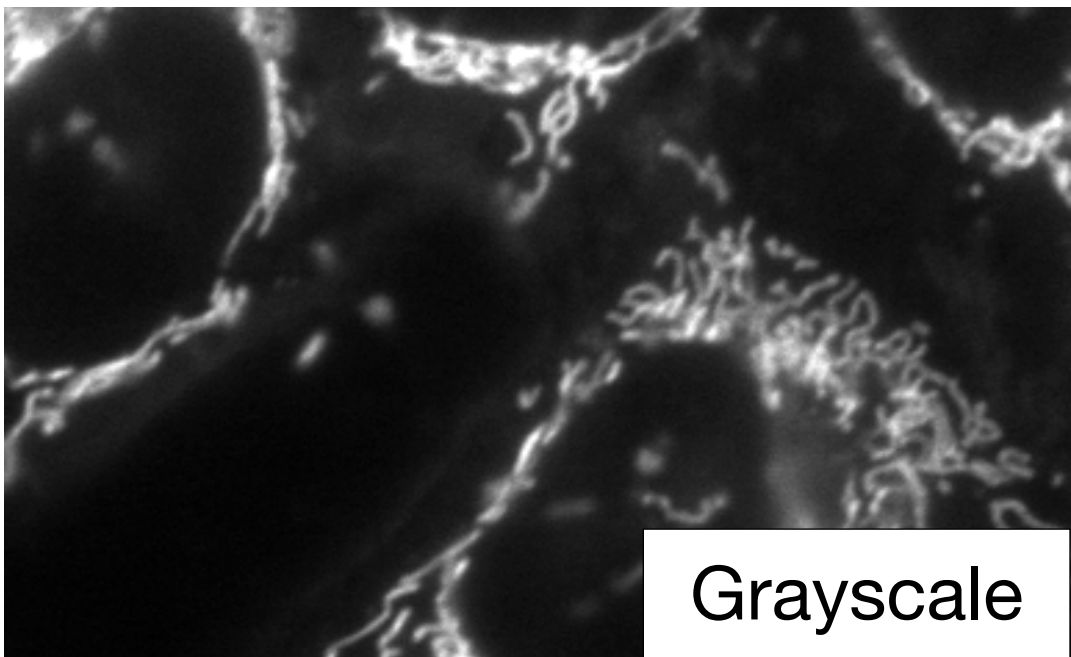
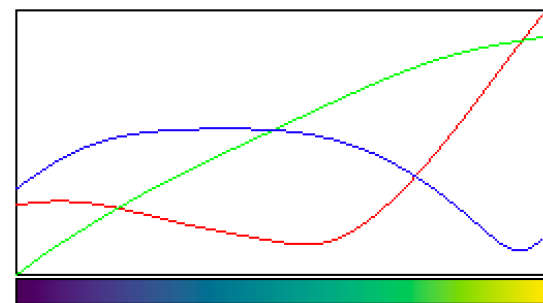
Color Mapping



LUT DO NOT CHANGE PIXEL VALUES

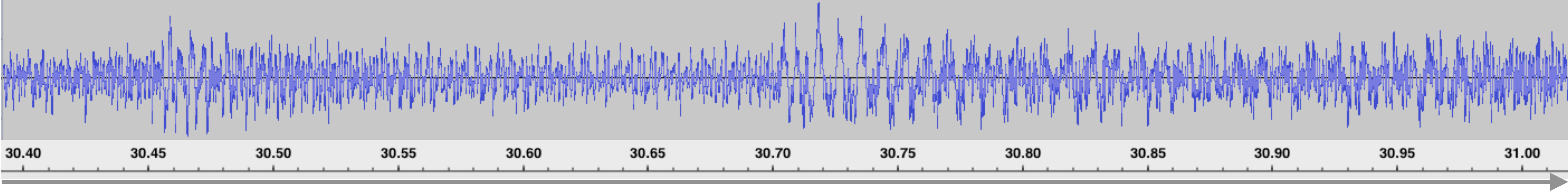
LUT
Pseudo Color

Continuity in human perception





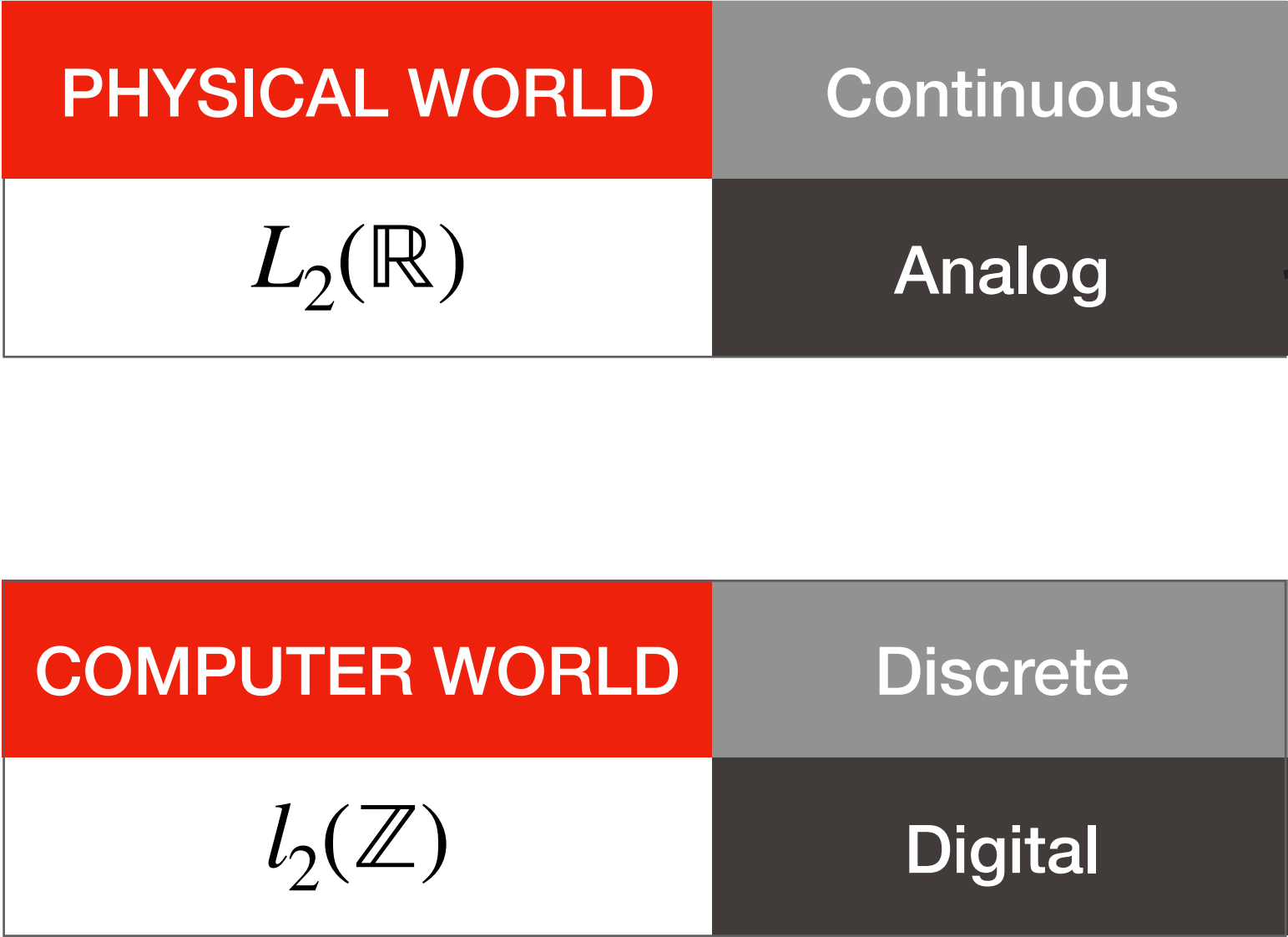
Notion of Signal



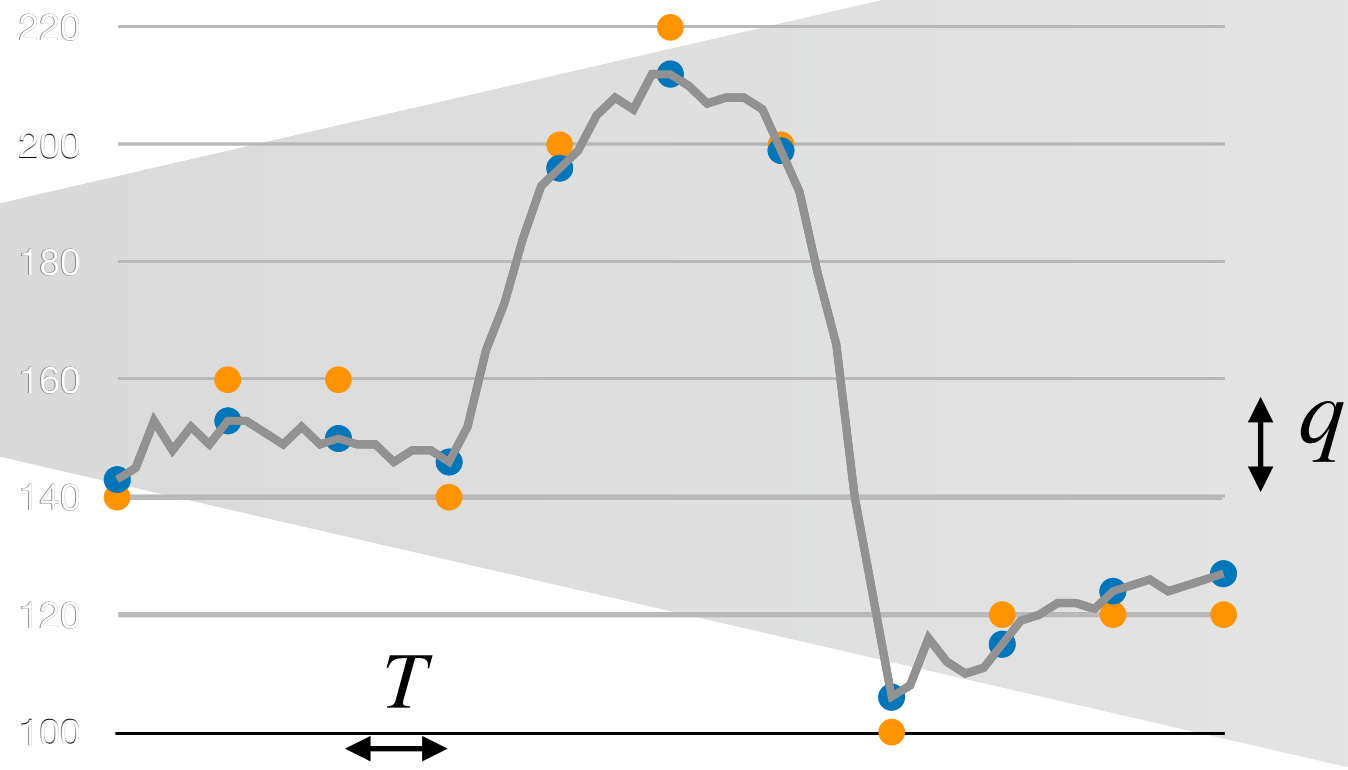
Bob Dylan, 2016
*Knocking on the
heaven's door*



$$f : \Omega \longrightarrow [0, f_{max}]$$
$$t \longmapsto f(t)$$



ADC



Analog-to-discrete

Sampling

- Sampling rate = $1/T$

Quantization

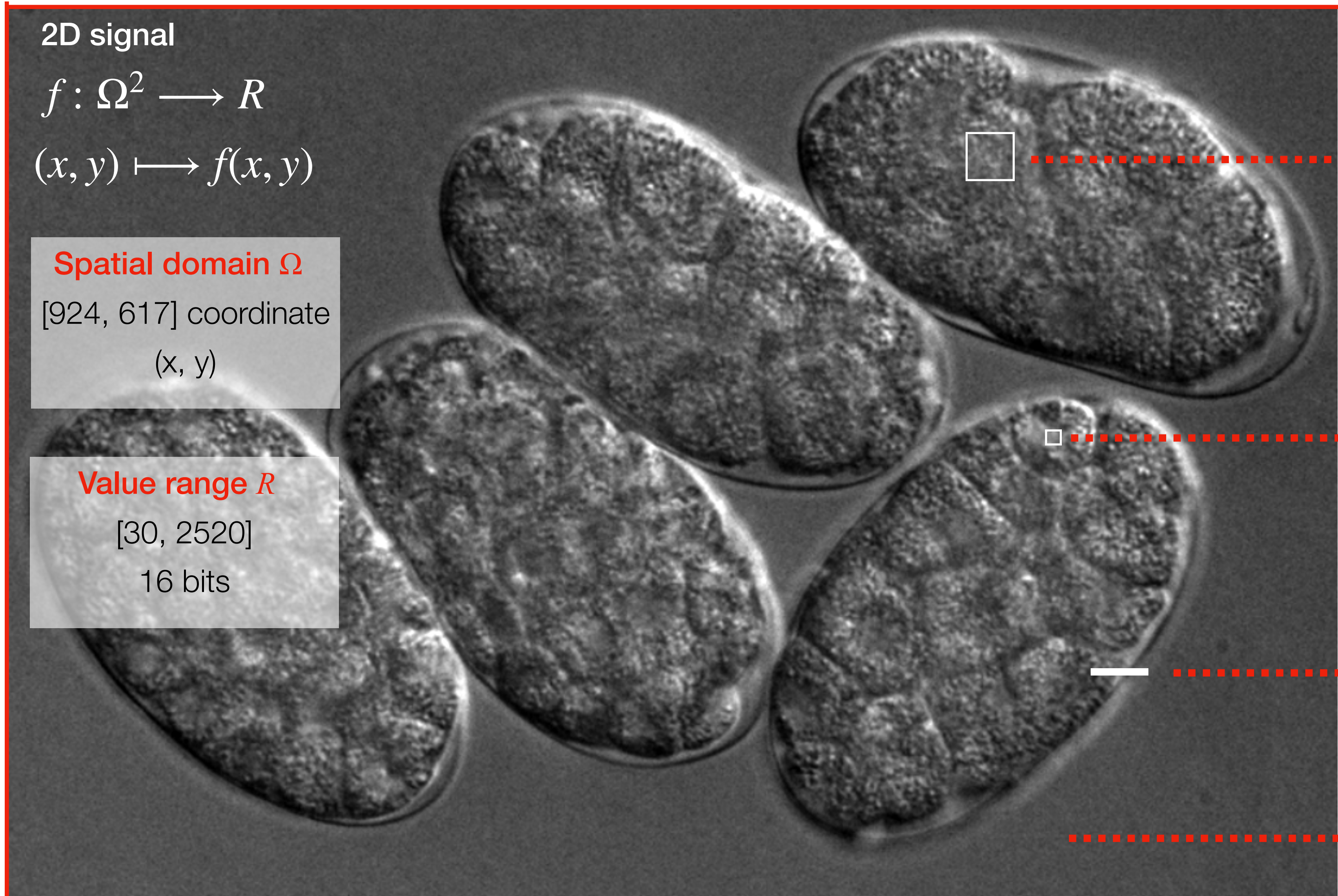
- Bit-depth (q: step)

Nyquist-Shannon theorem



Lateral Plane 2D

Lateral XY



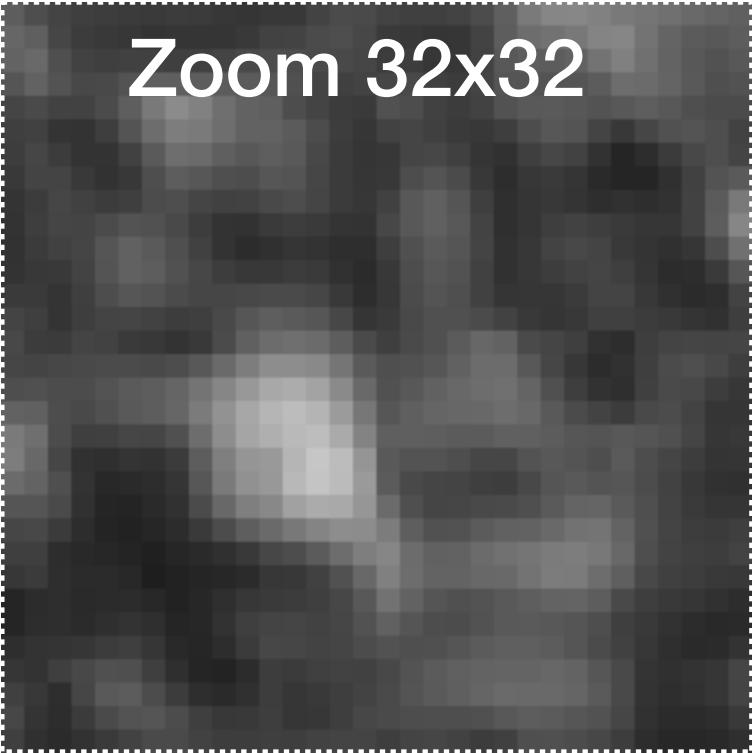
2D signal

$$f: \Omega^2 \longrightarrow R$$

$$(x, y) \longmapsto f(x, y)$$

Spatial domain Ω
[924, 617] coordinate
(x, y)

Value range R
[30, 2520]
16 bits



Array of pixels

1681	1742	1692	1591
1308	1237	1344	1230
884	891	937	1022
579	520	742	821

Line profile

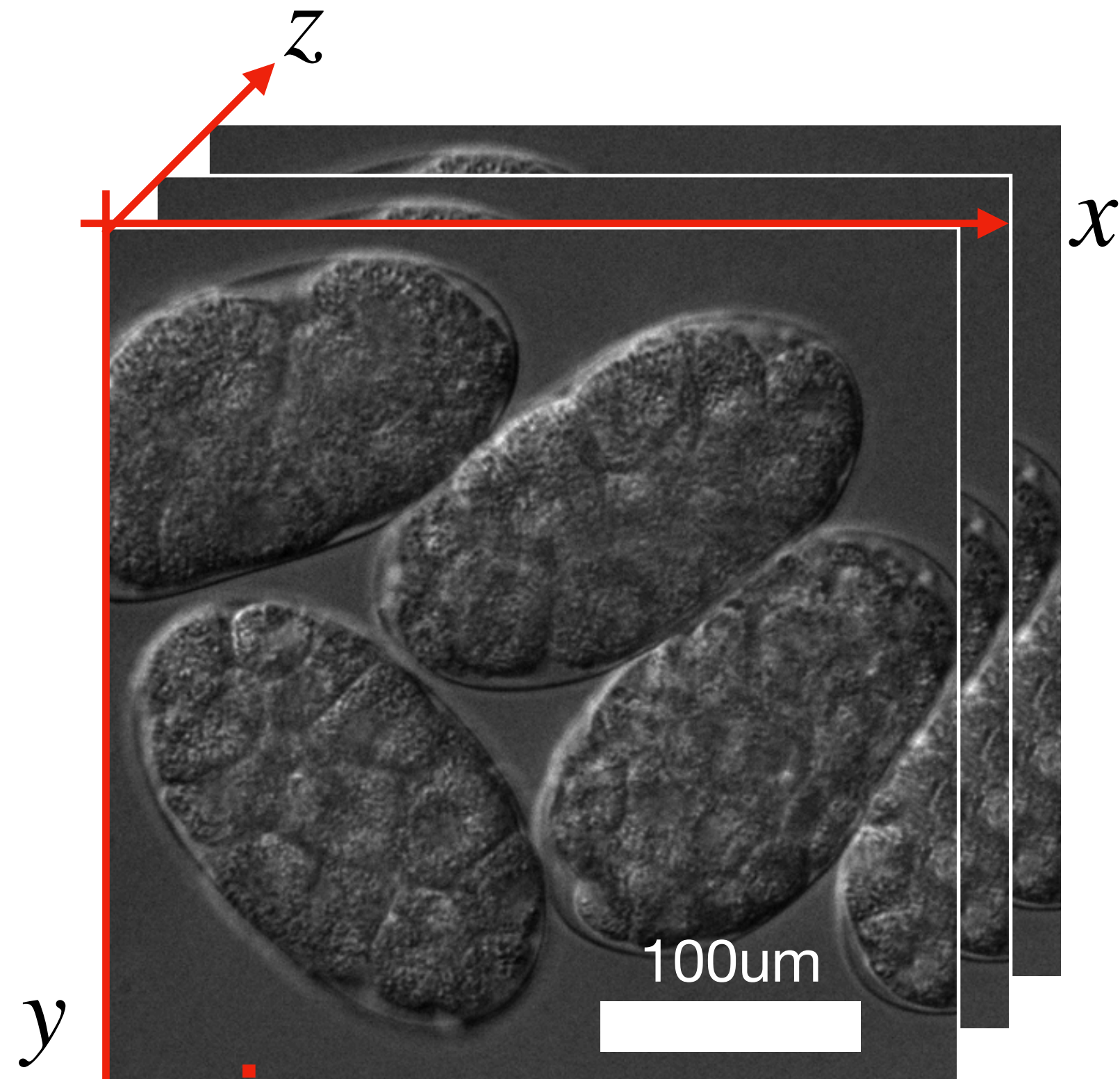


$$f(802,571) = 1141$$

C. elegans embryo. DIC microscopy. Source: Pierre Gönczy and Radek Jankele, EPFL

👁 Axial 3D

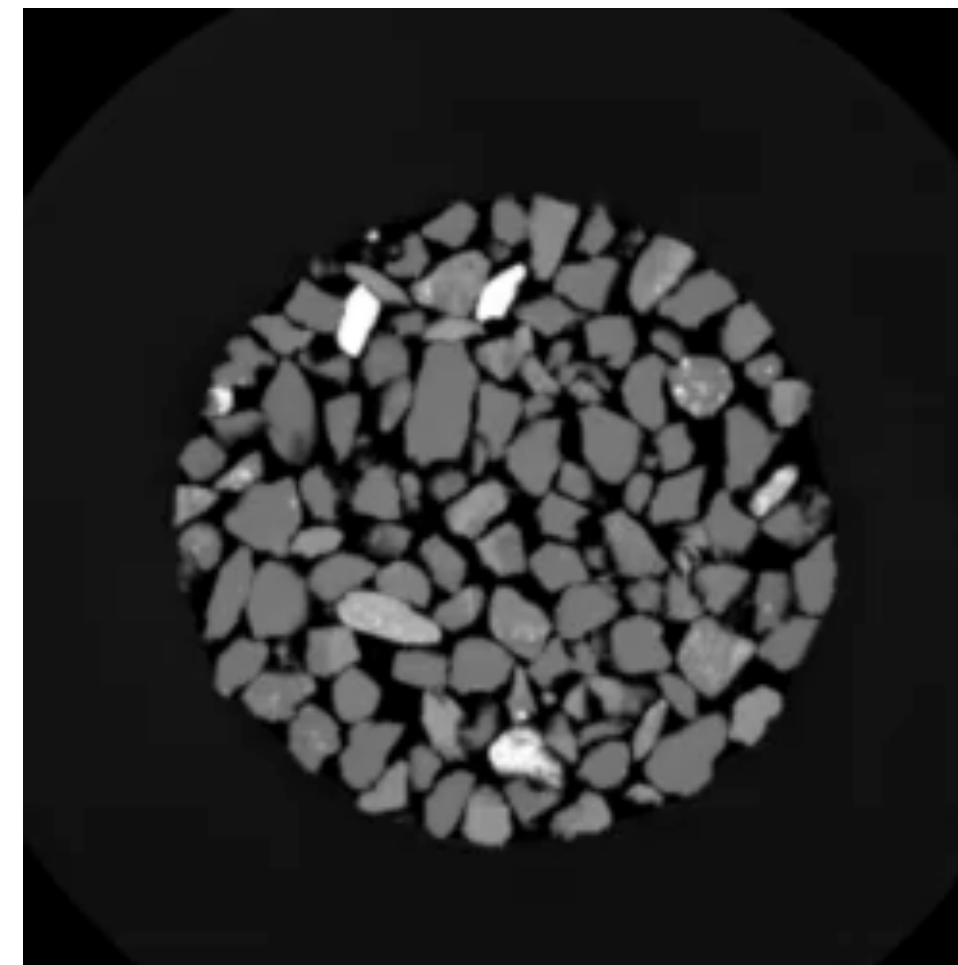
Slice Z



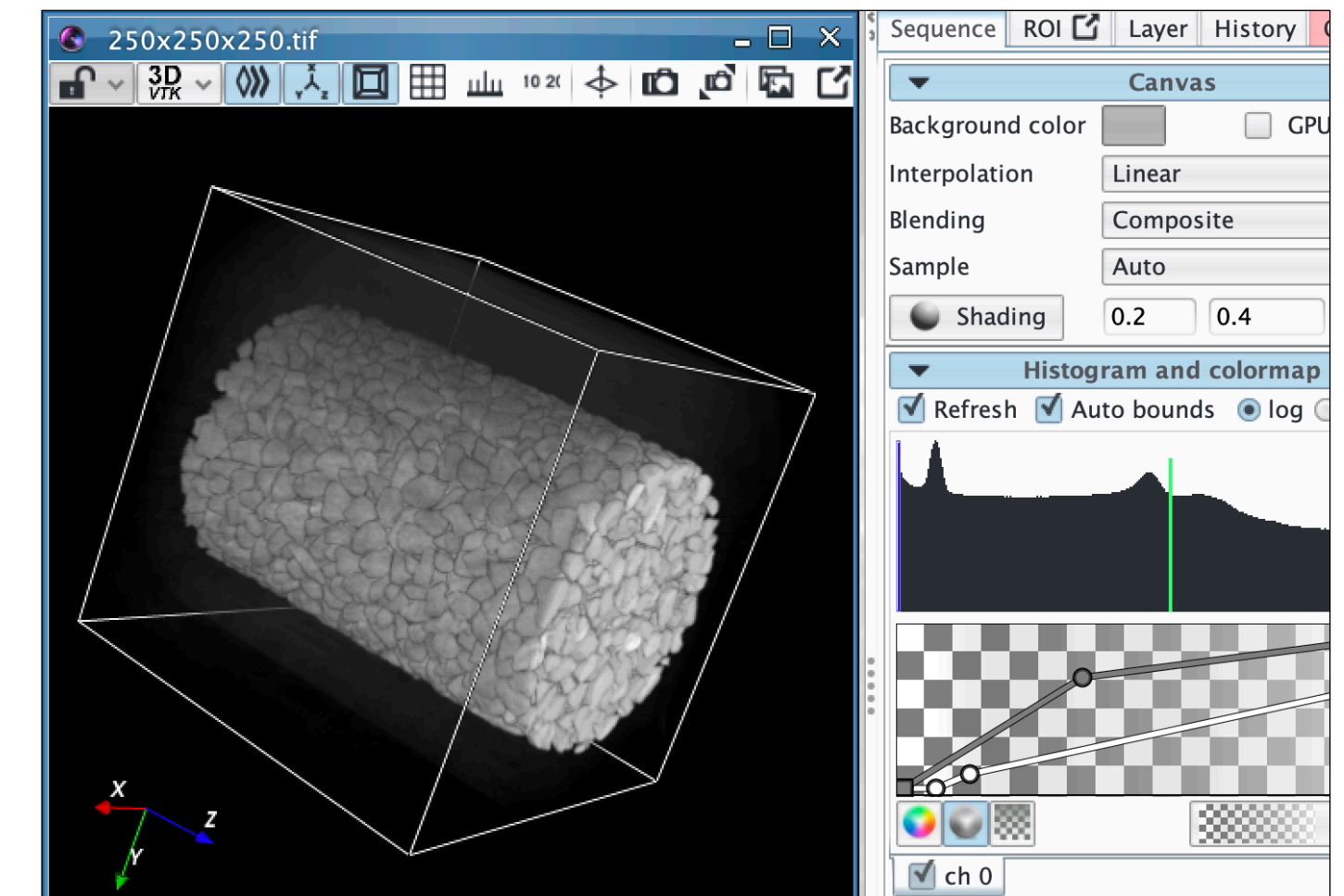
Volume, stack, or z-stack

Spatial calibration: **voxel** size

- Lateral resolution **pixelsize** (often square pixel)
- Axial resolution **z-step** ($>$ pixelsize)

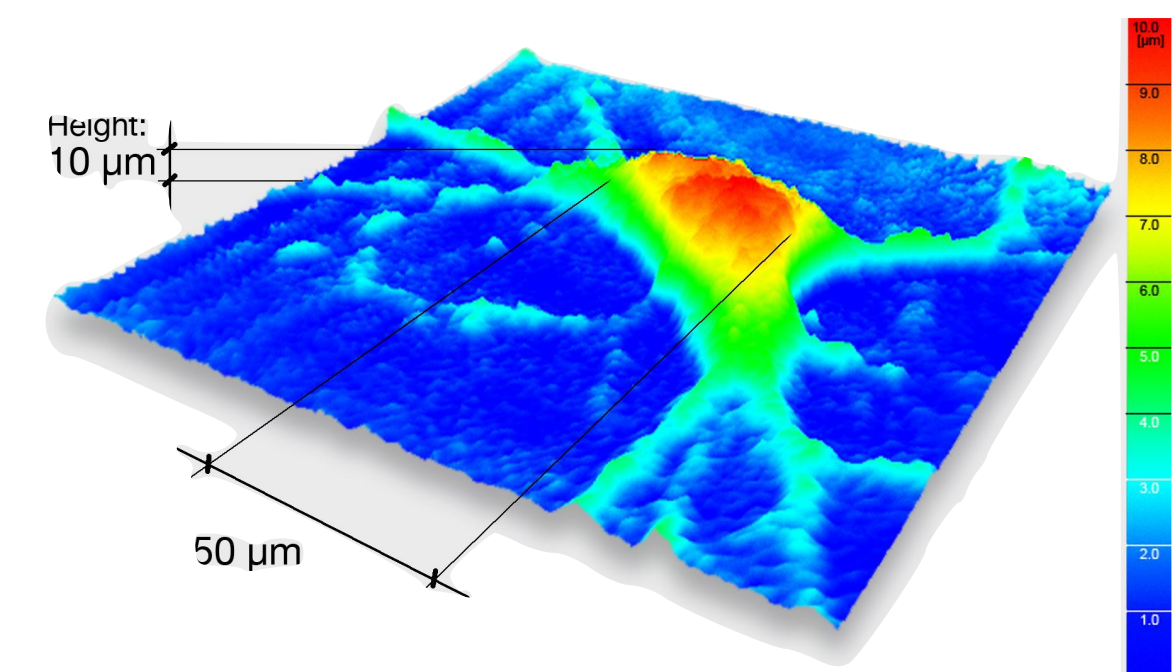


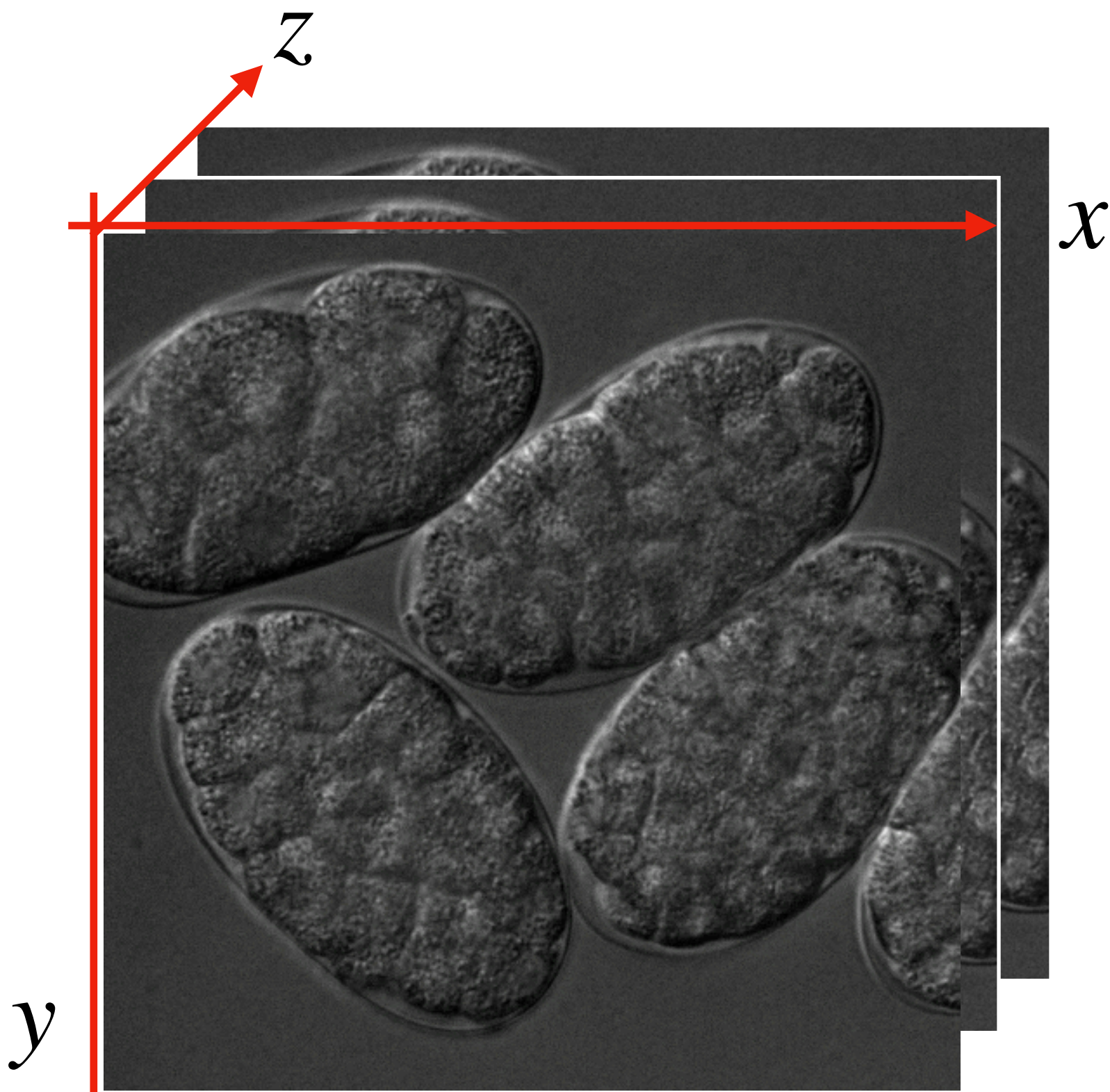
As a movie
Z as time



Volume rendering

3D SURFACE IS NOT VOLUMETRIC DATA





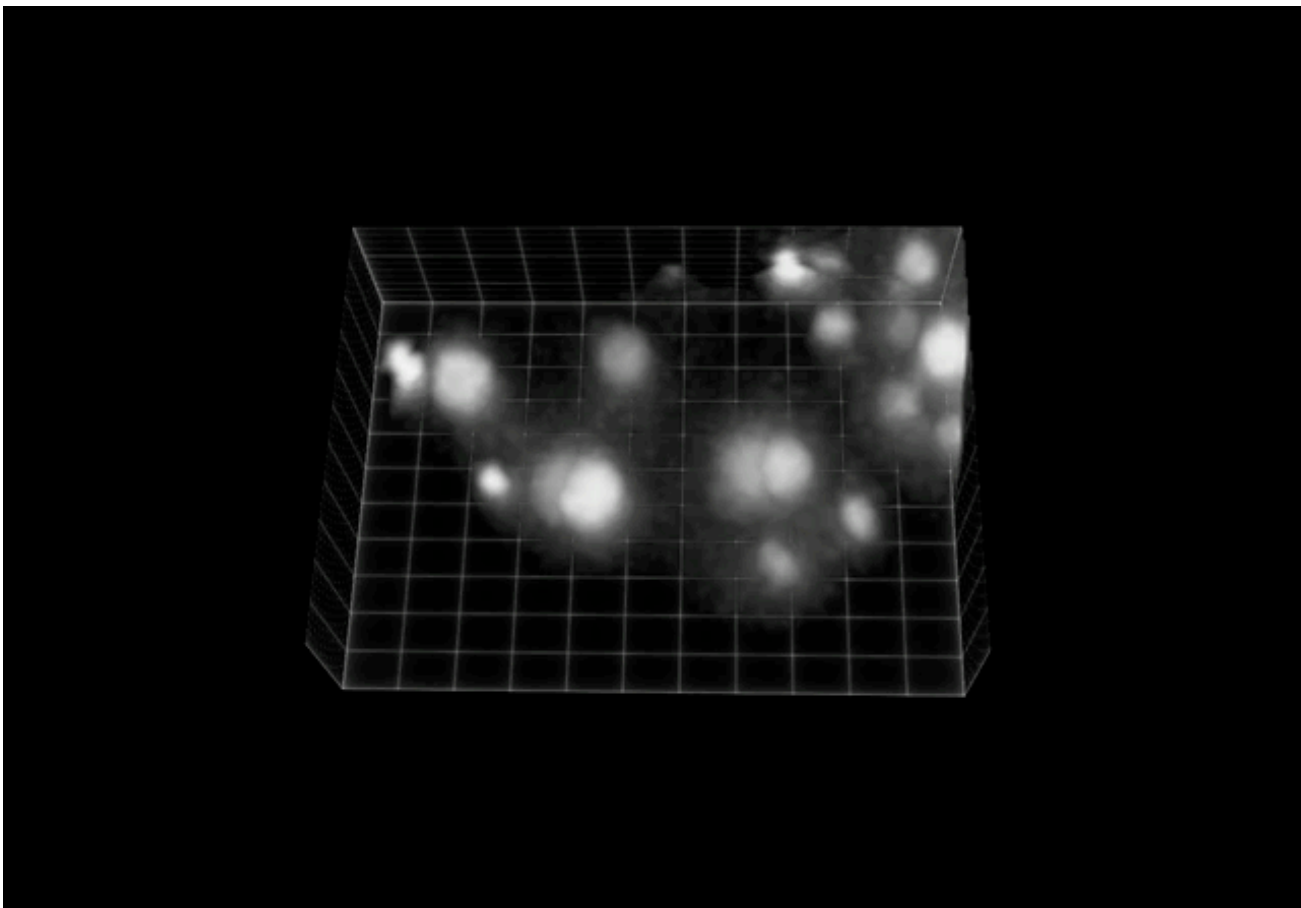
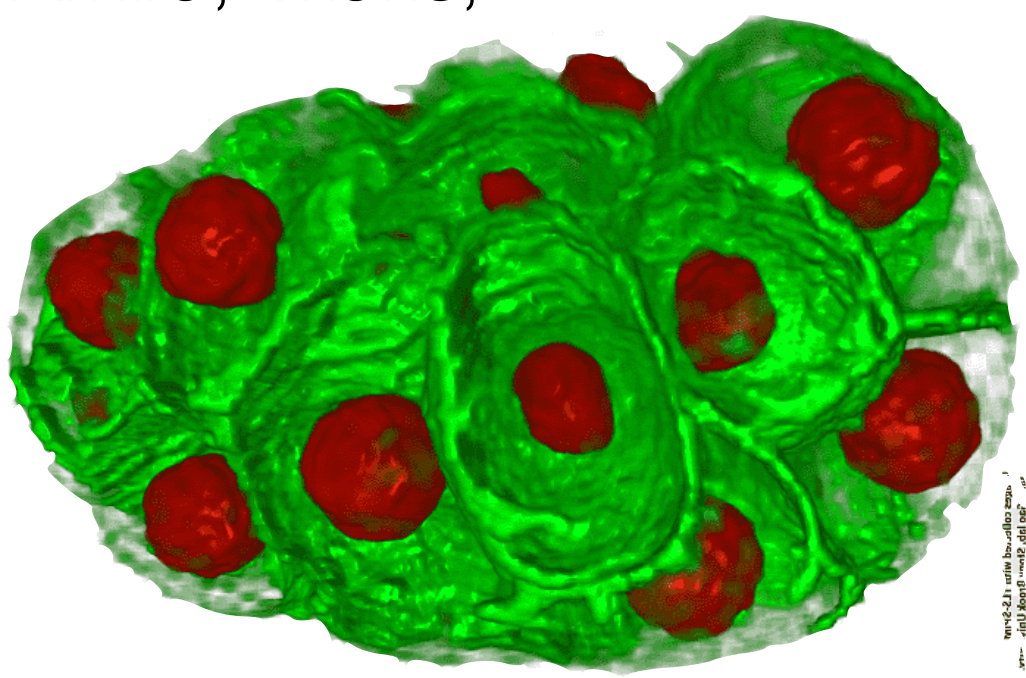
Volume, stack, or z-stack

Spatio-temporal calibration

- Volumetric information in nm
- Time-lapse: Frame rate in Hz or frame / seconds

Commercial Software

- Osirix, Amira, Imaris, ...



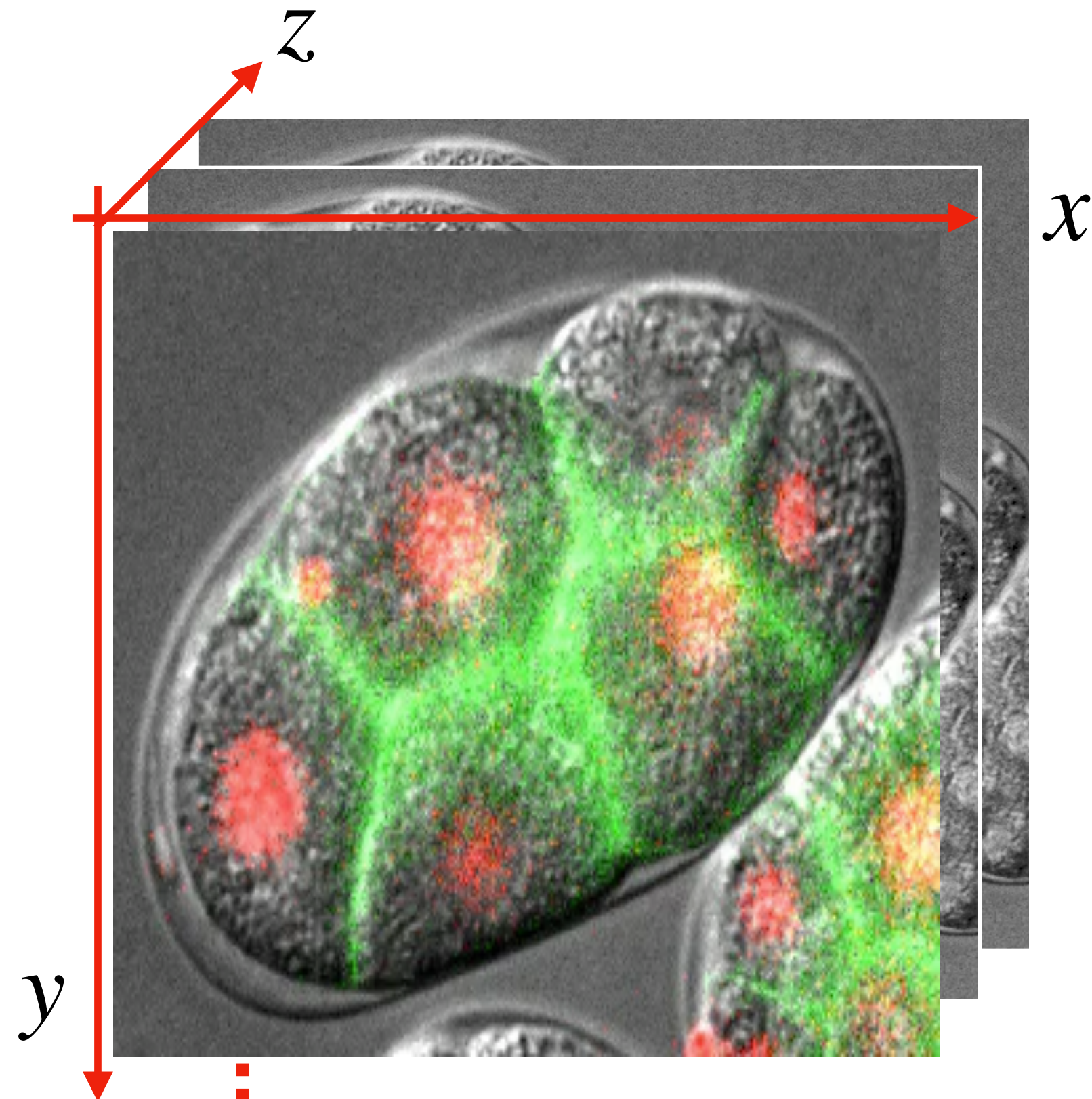
Free Software

	Free	Multiple OS	GUI Control	One-click Install	Intuitive use	Time	Multiple Channel
Imaris Free Viewer	✓	✓	✓	✓	✓	✓	✓
ClearVolume	✓	⚠	✓	✓	✓	✓	✓
BigDataViewer IJ	✓	✓	✓	✓	✗	✓	✓
Volume Viewer IJ	✓	✓	✓	✓	✓	✗	✓
3D Viewer ImageJ	✓	✓	✗	✓	✓	✗	✓
Paraview	✓	✓	✓	✓	✗	✗	✗
Napari	✓	✓	✓	✗	✓	✓	✓
Icy	✓	✓	✓	⚠	✓	✓	✓
Slicer	✓	✓	✗	✓	✗	✗	✓



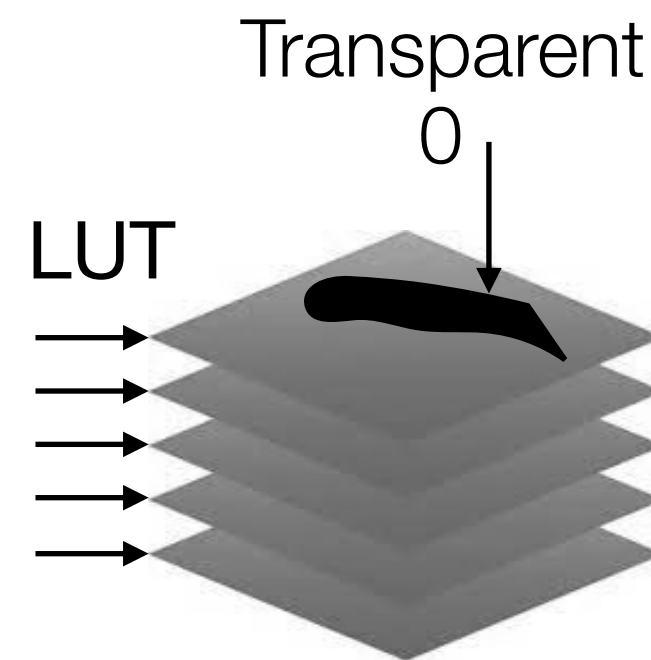
Multichannels 5D

Channel C

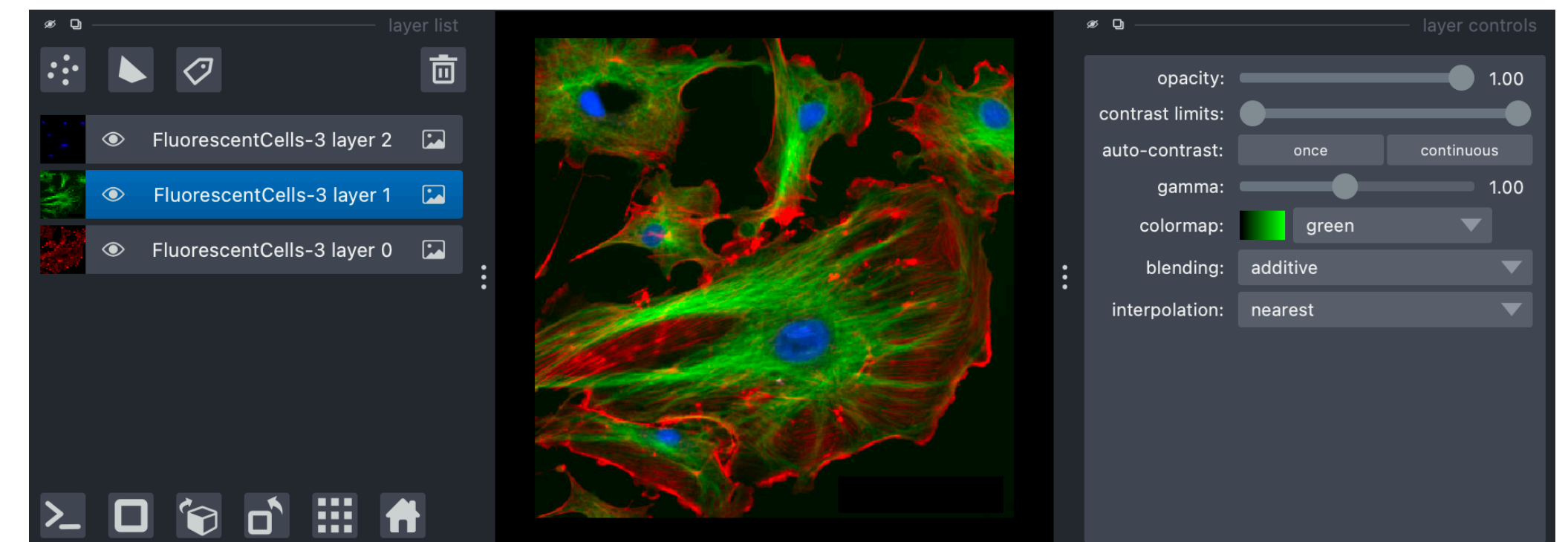


Multiple channels

Use on color to display the channel

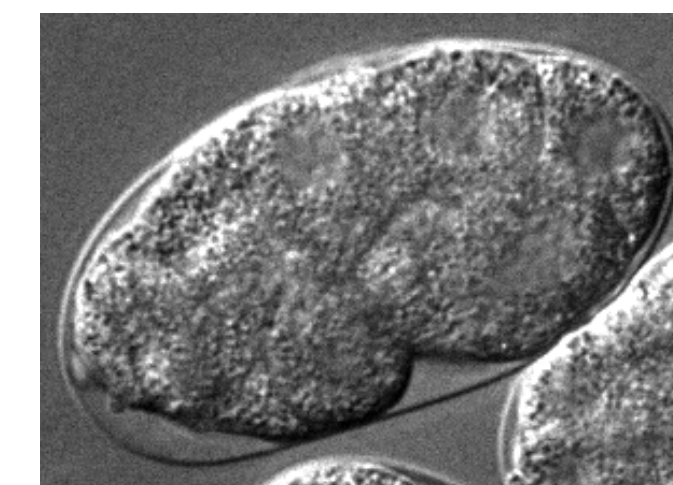
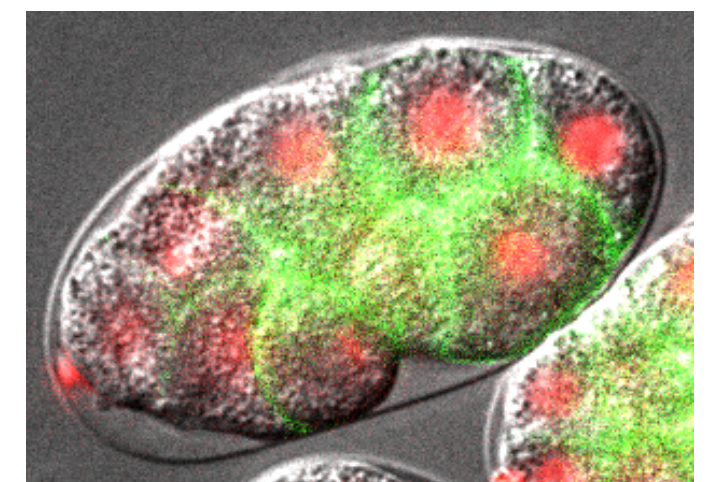


Napari Control Layer / Opacity / LUT

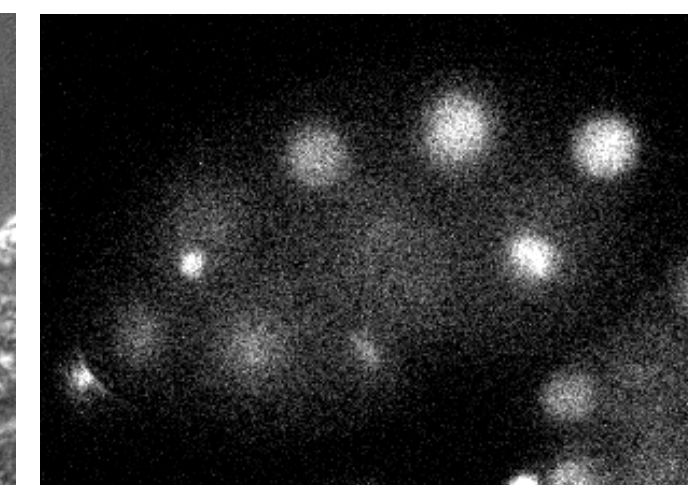


Fiji / ImageJ

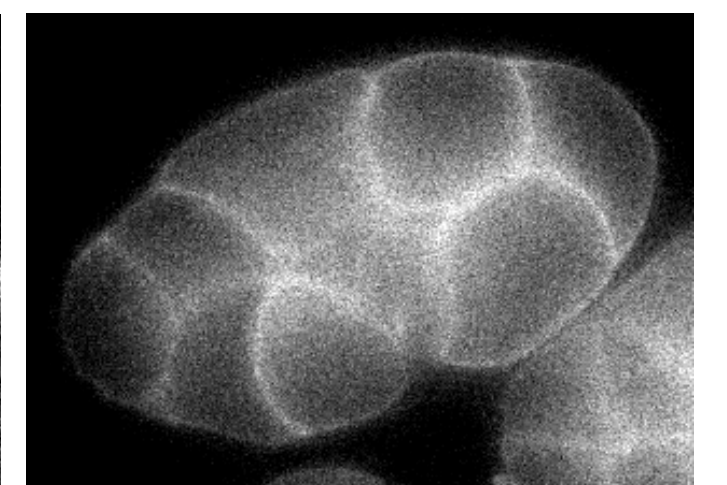
Composite mode / Channel Tool / LUT



DIC Microscopy



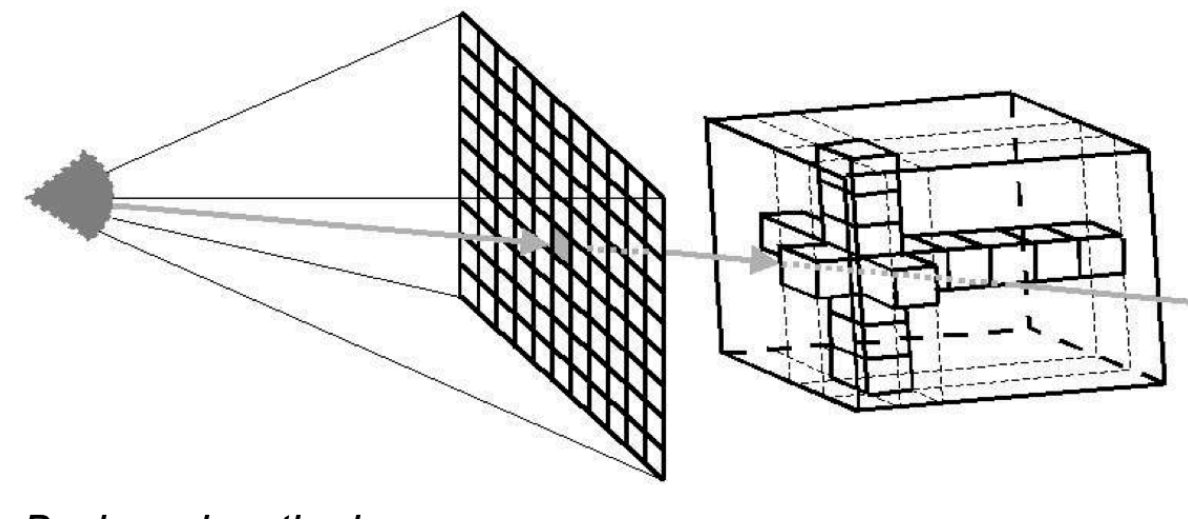
DAPI Fluorescence



GFP Fluorescence

Volume Rendering Techniques

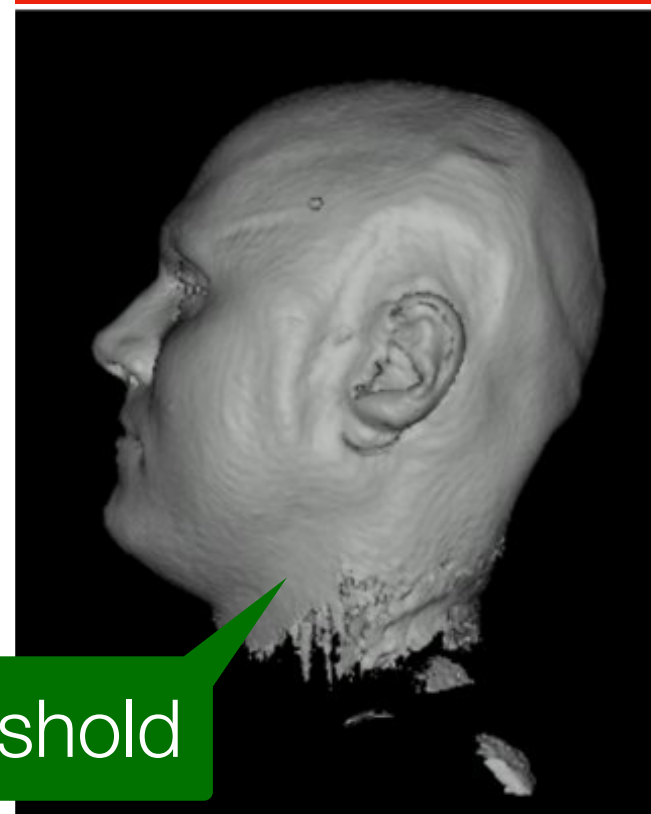
3D



Computer graphics

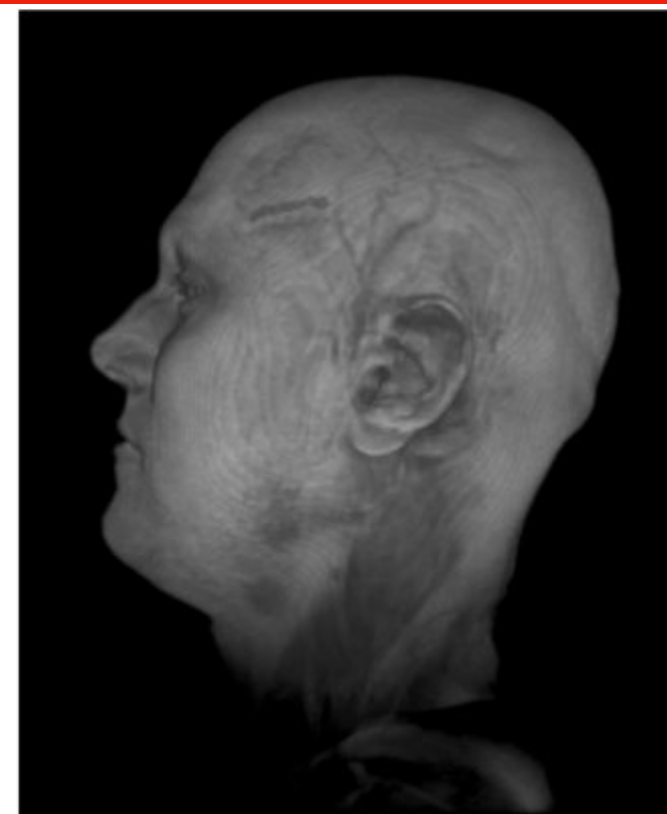
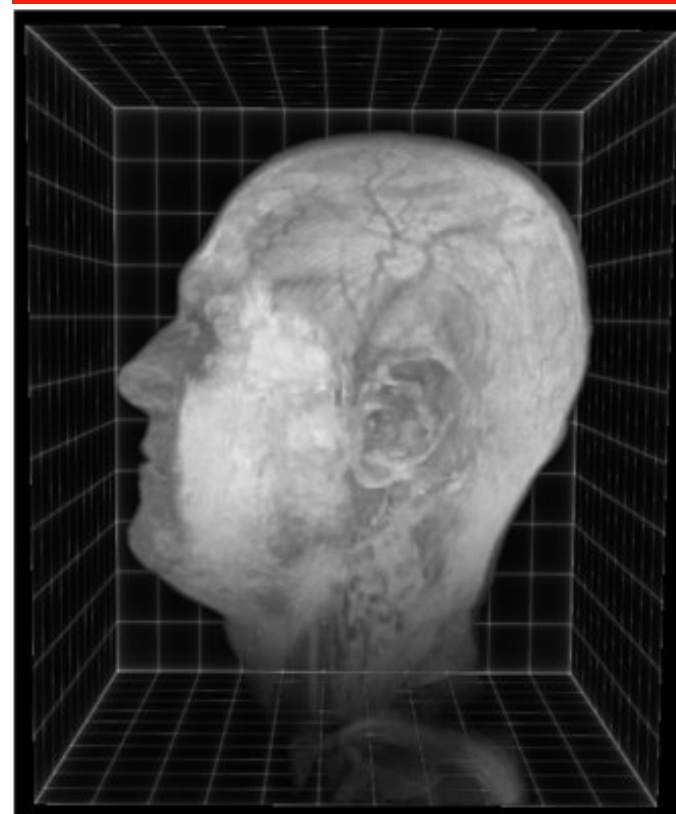
- Transparency
- Shadows
- Ray casting
- Lightning

Surface



Threshold

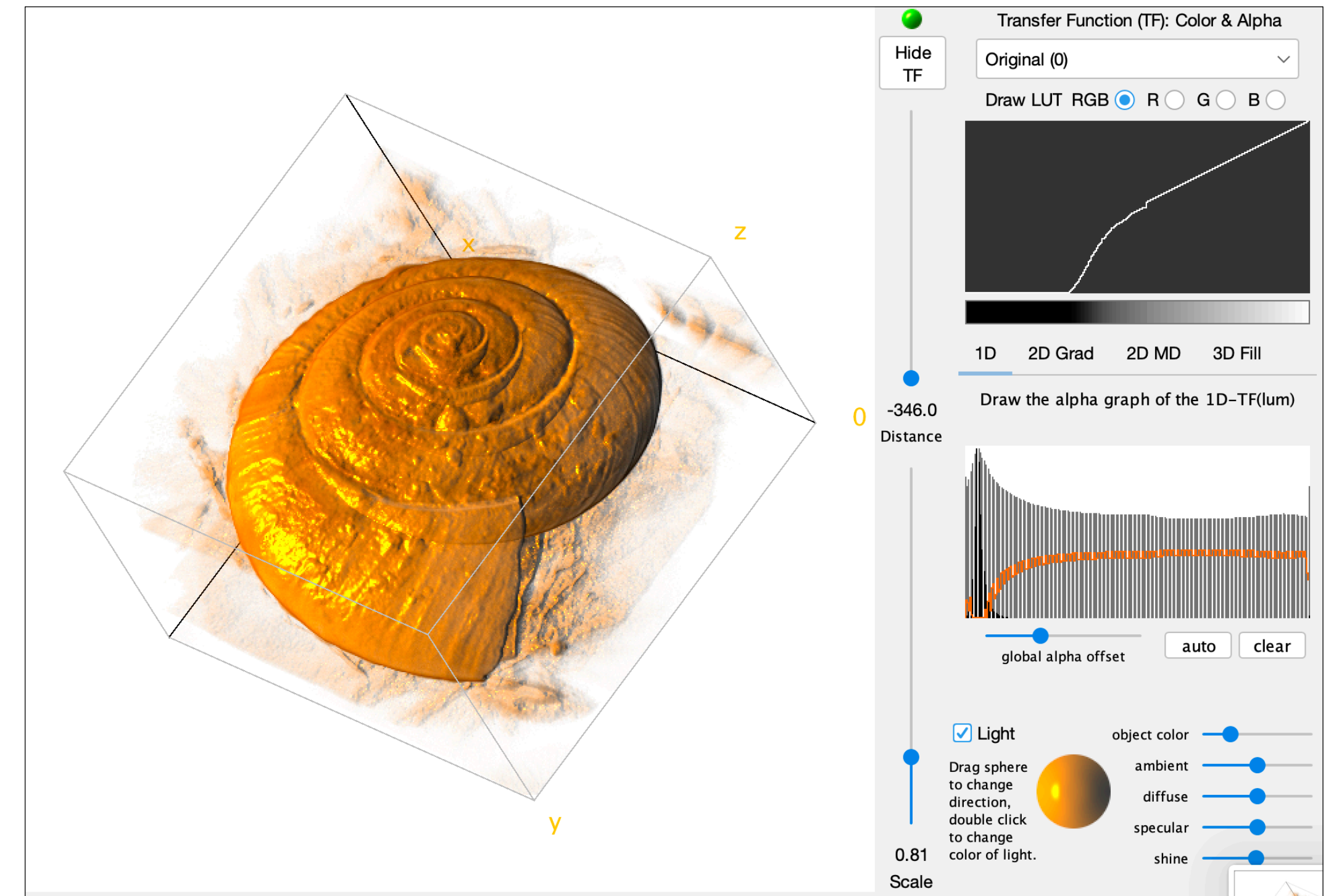
Volume rendering



Isosurface

Maximum

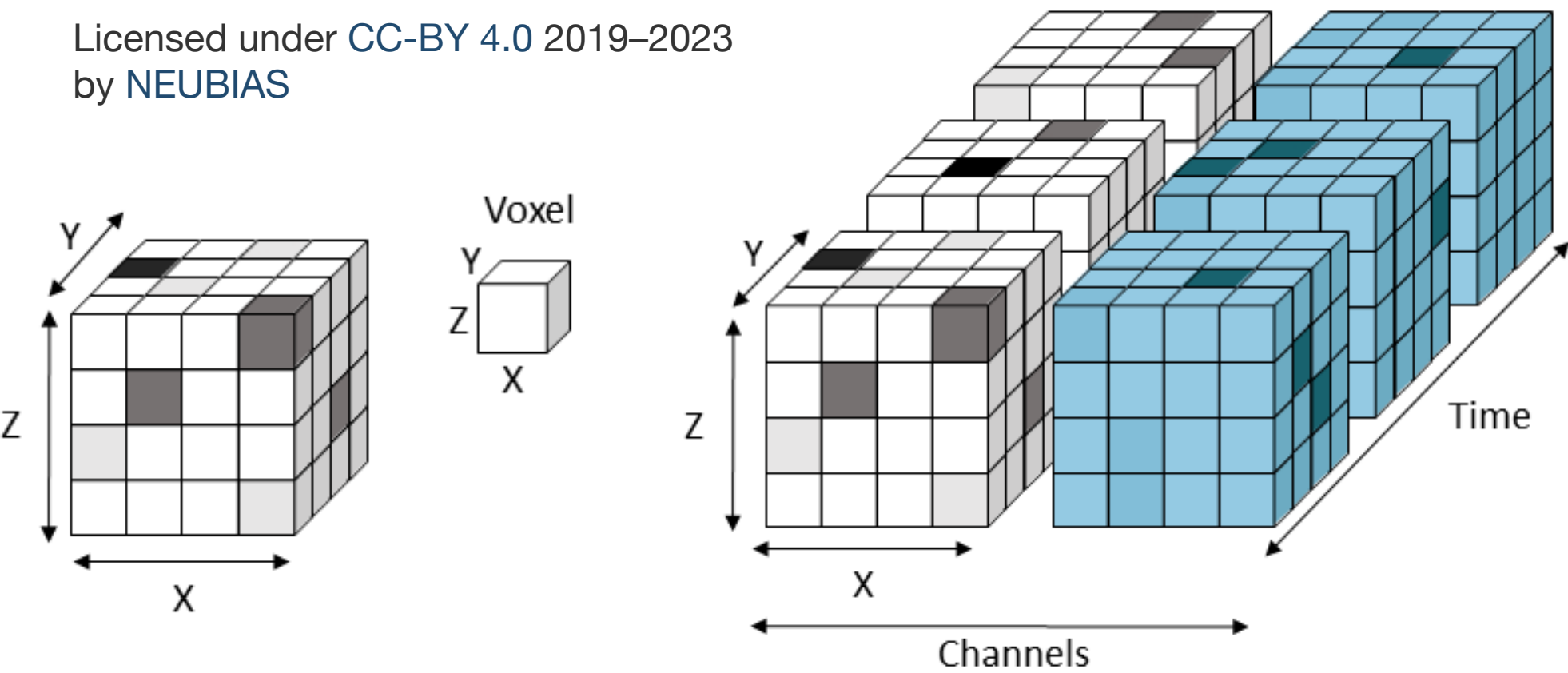
Translucent





Slicing nD Data

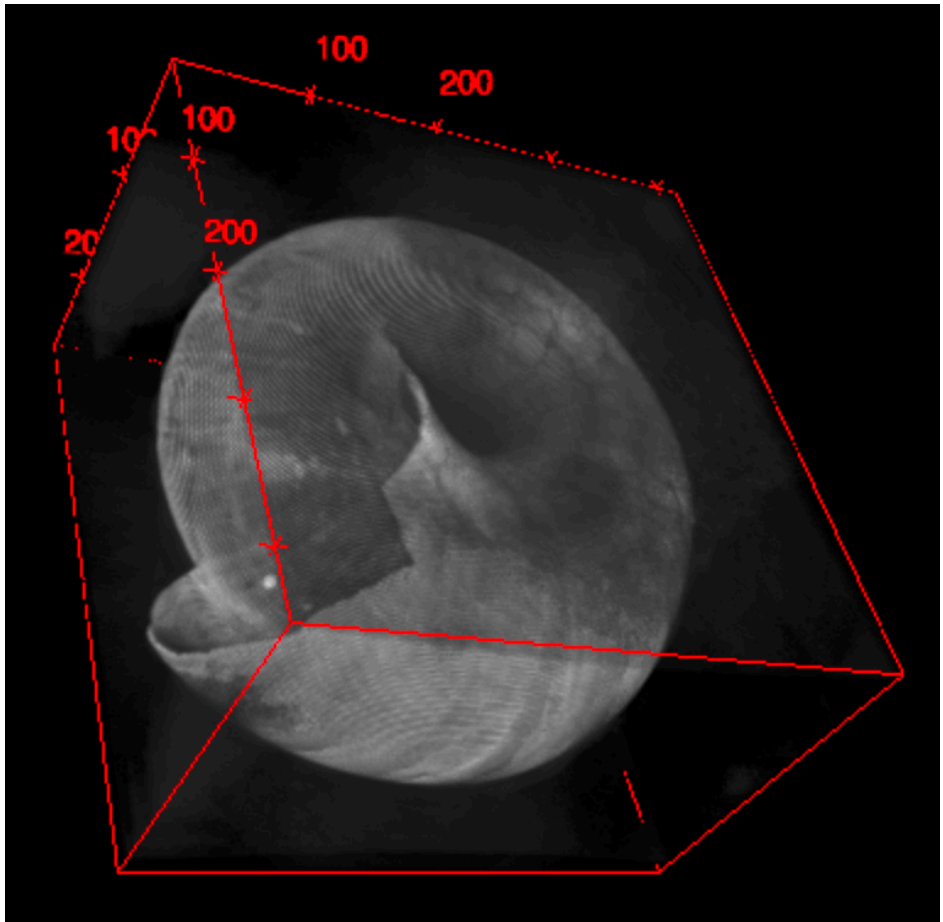
Licensed under CC-BY 4.0 2019–2023
by NEUBIAS



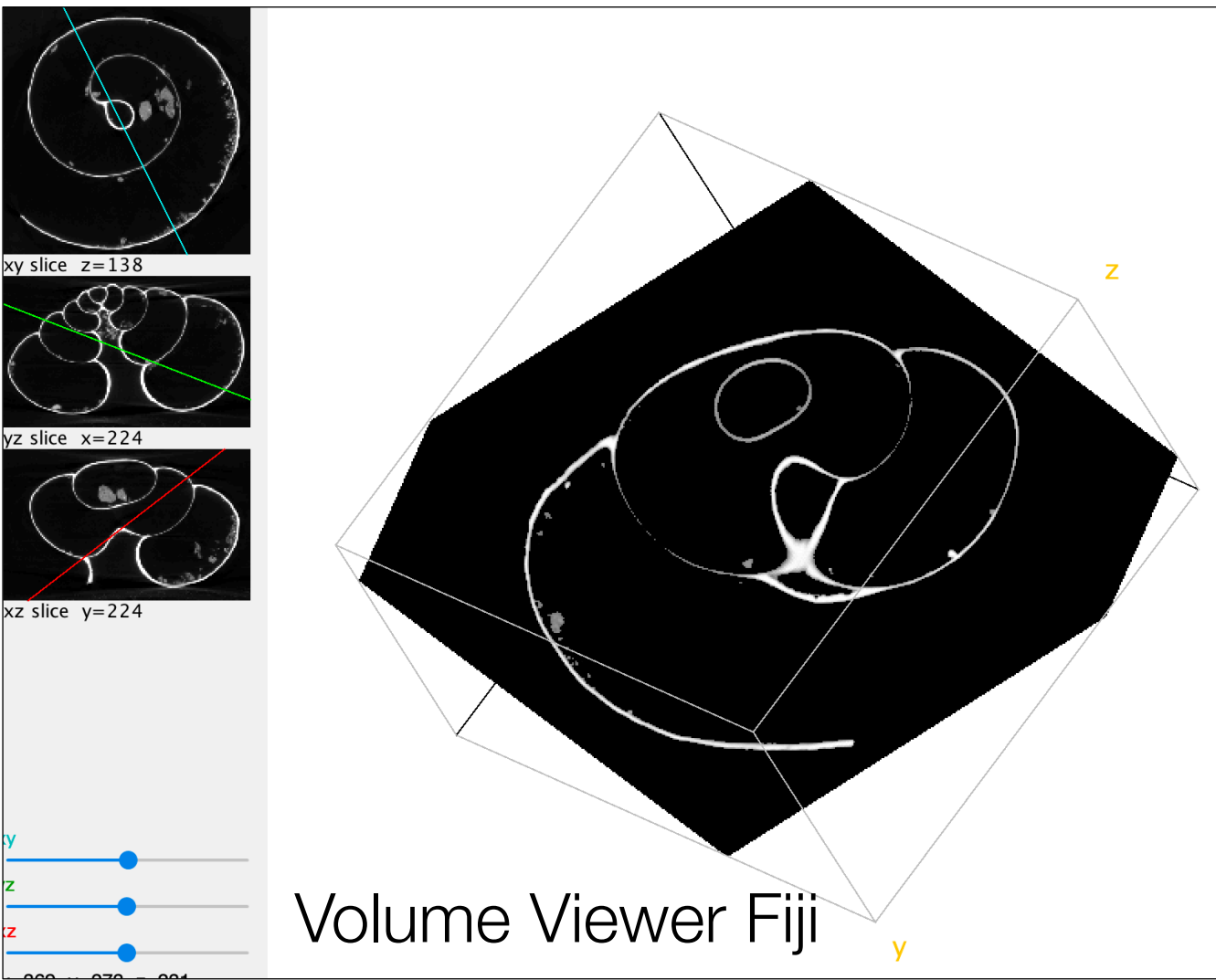
Orthogonal reslicing



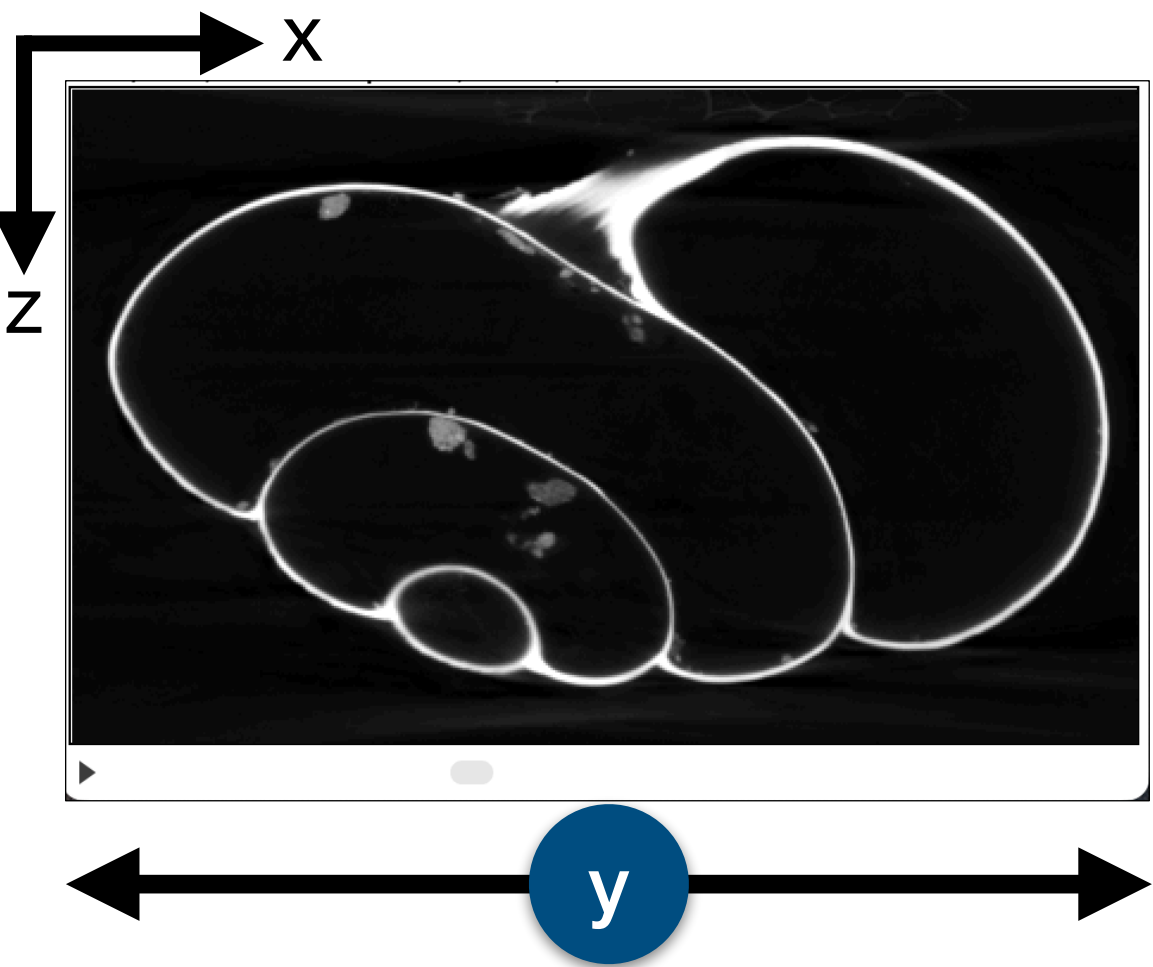
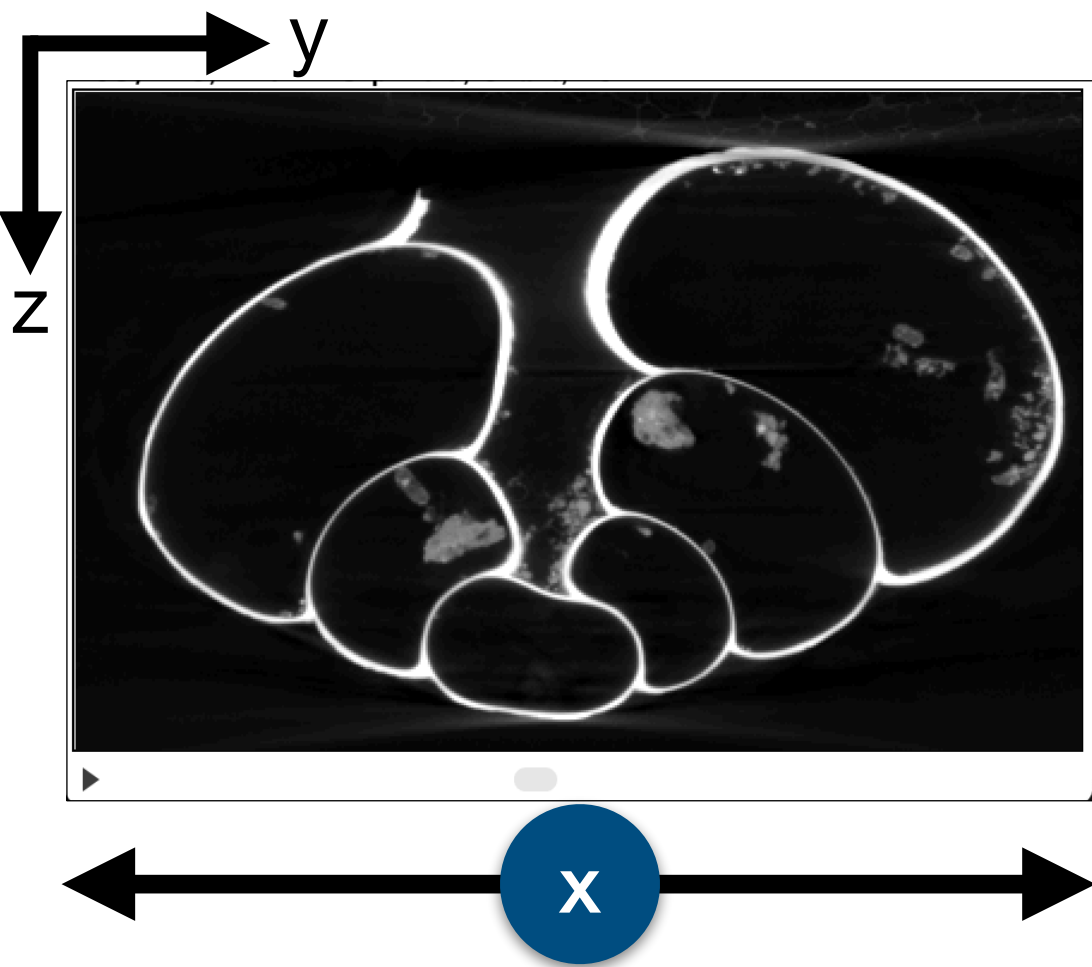
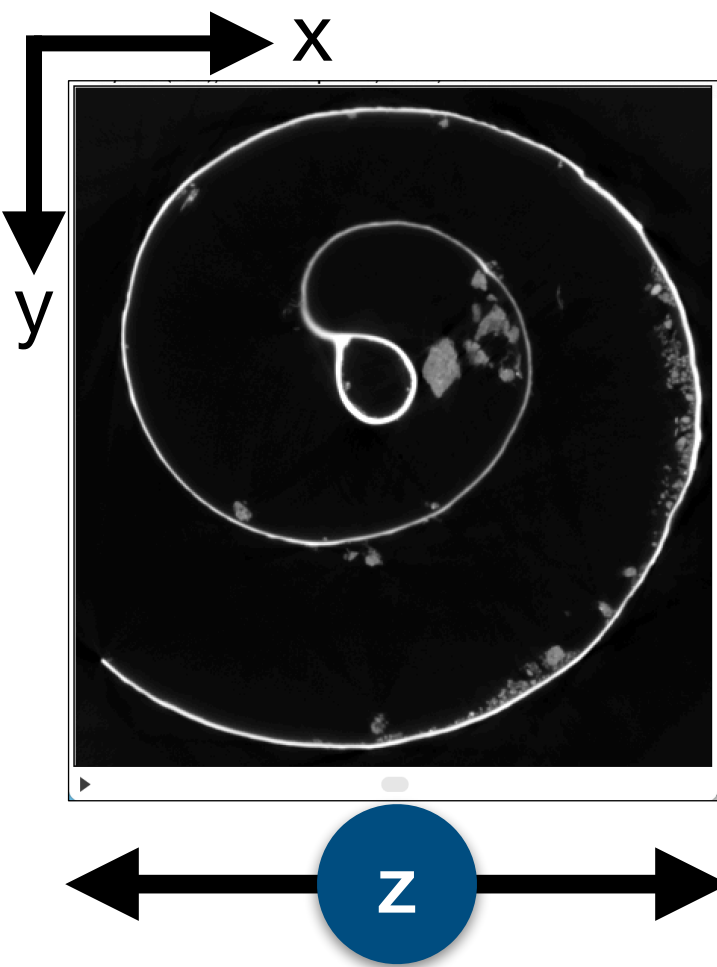
Image → Stacks
→ Reslice



Oblique reslicing

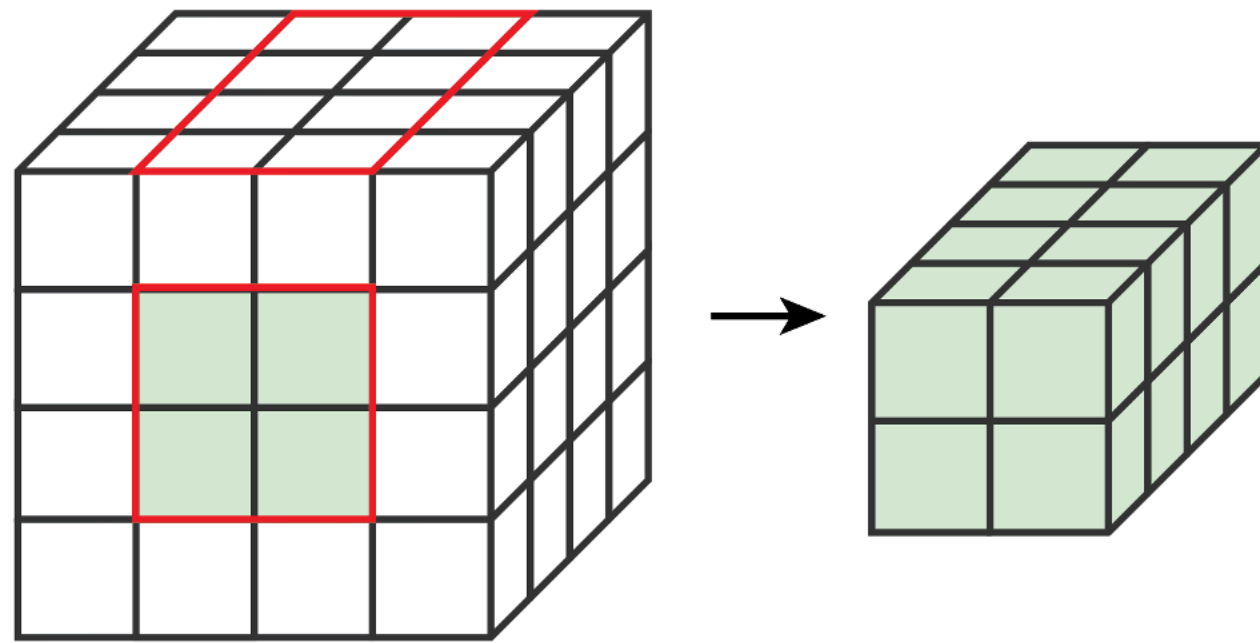


Permutation of axis

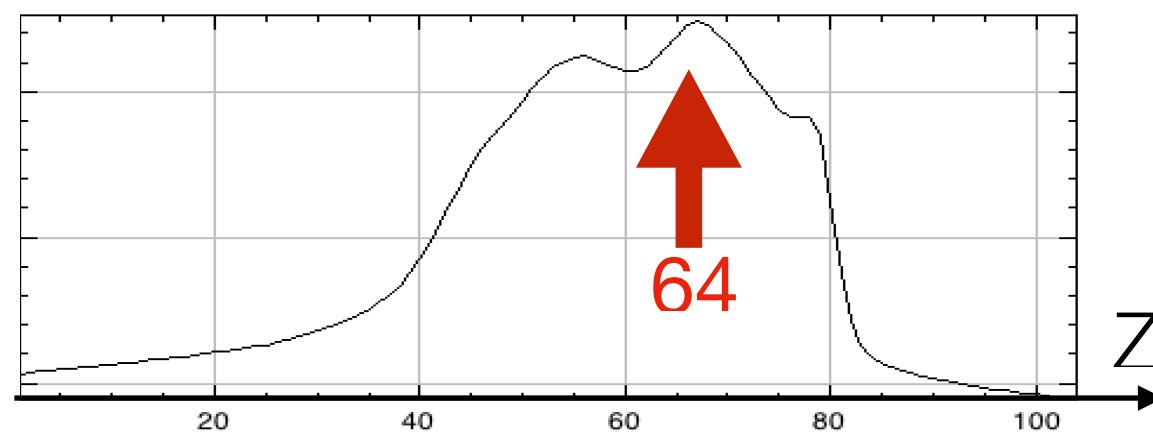




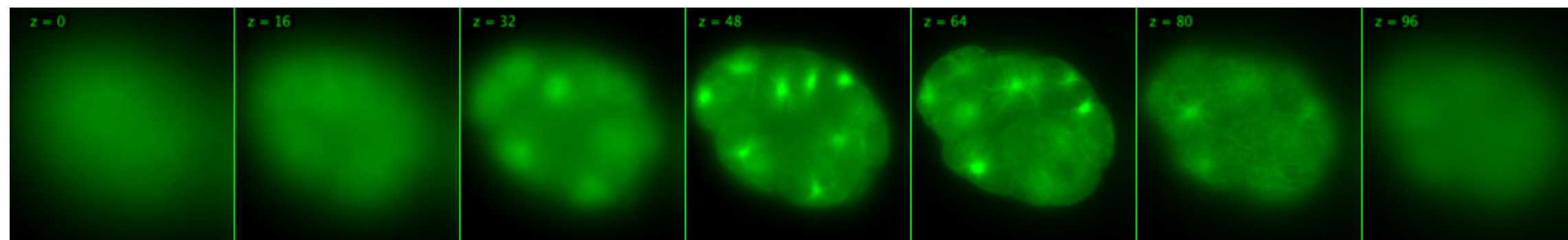
Subsetting nD Data



Automatic selection



Example: Local
variance per slice



Manual selection



Subhyperstack Maker

Enter a range (e.g. 2-14), a range with increment (e.g. 1-100-2) or a list (e.g. 7,9,25,27)

Channels:

Slices:

Frames:

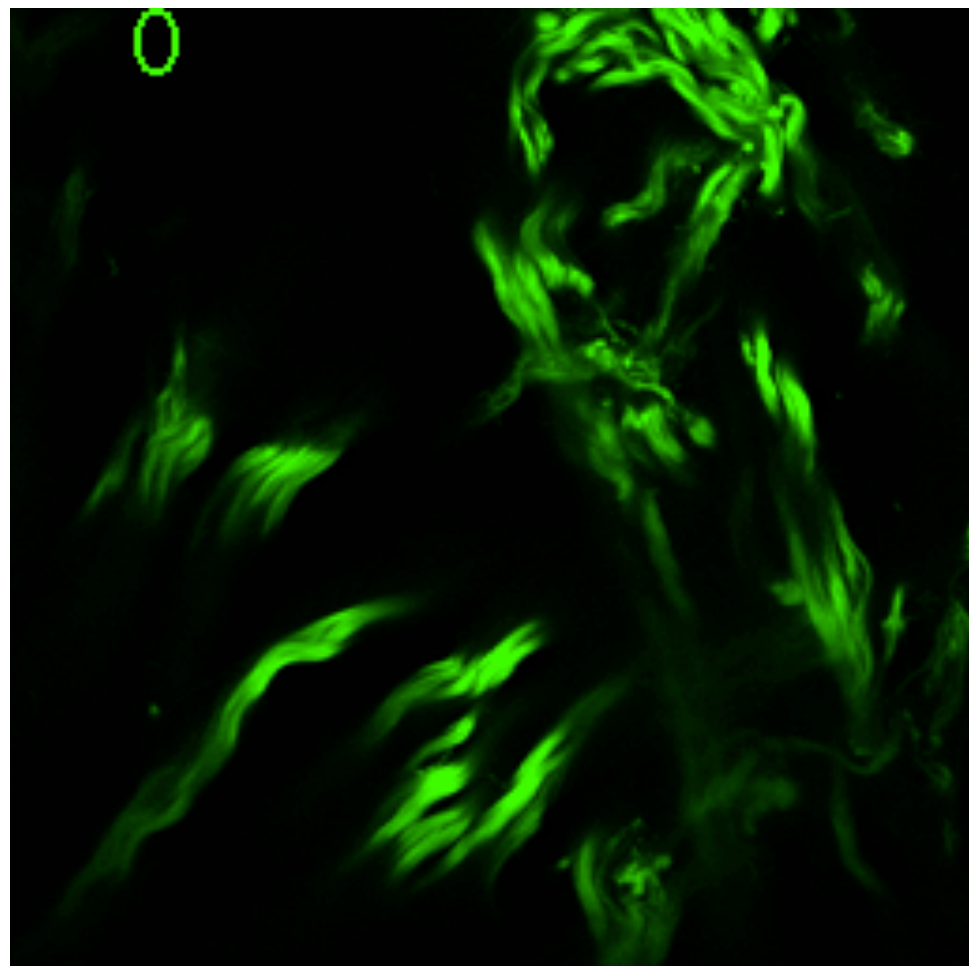


Select subarray
numpy array

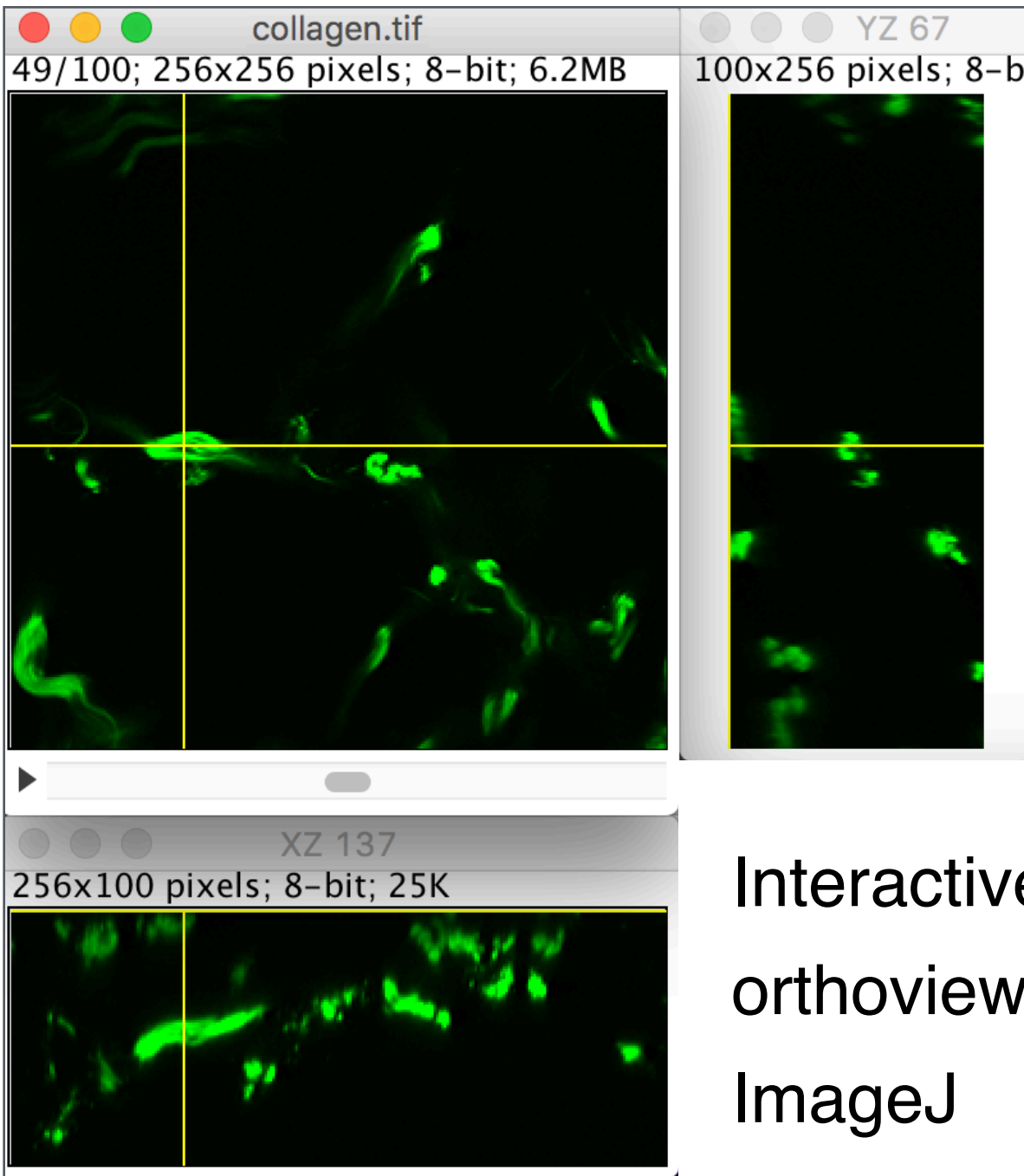
```
import numpy as np
im = viewer.layers['img'].data
print(im.shape)
crop = im[:, 200:300, :100]
viewer.add_image(crop)
```



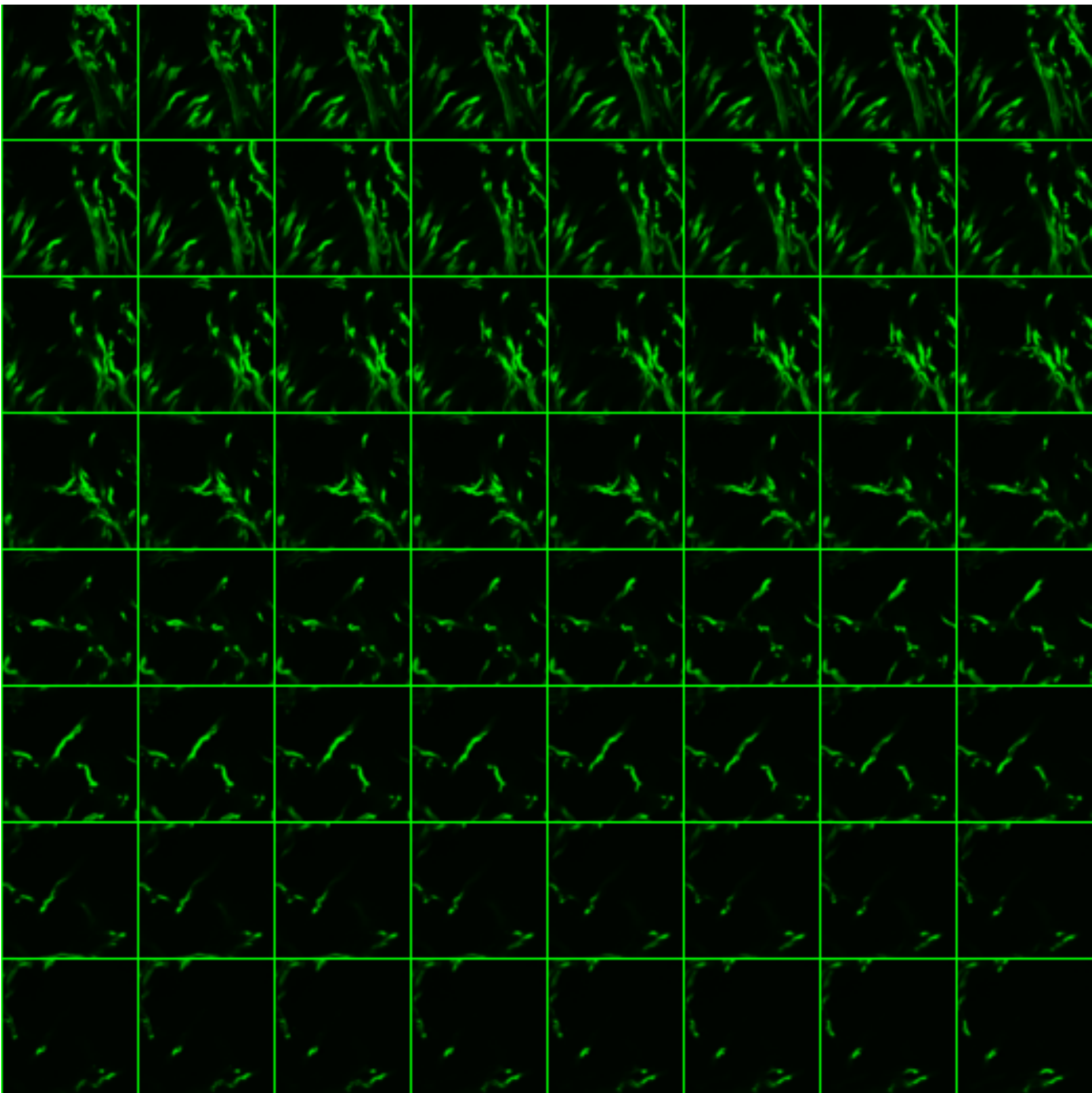

Orthogonal Section



Pancreatic islet vasculature
Blood vessels, intravital images of tumor. *Marta Alabrudzinska, Karolinska Institute*



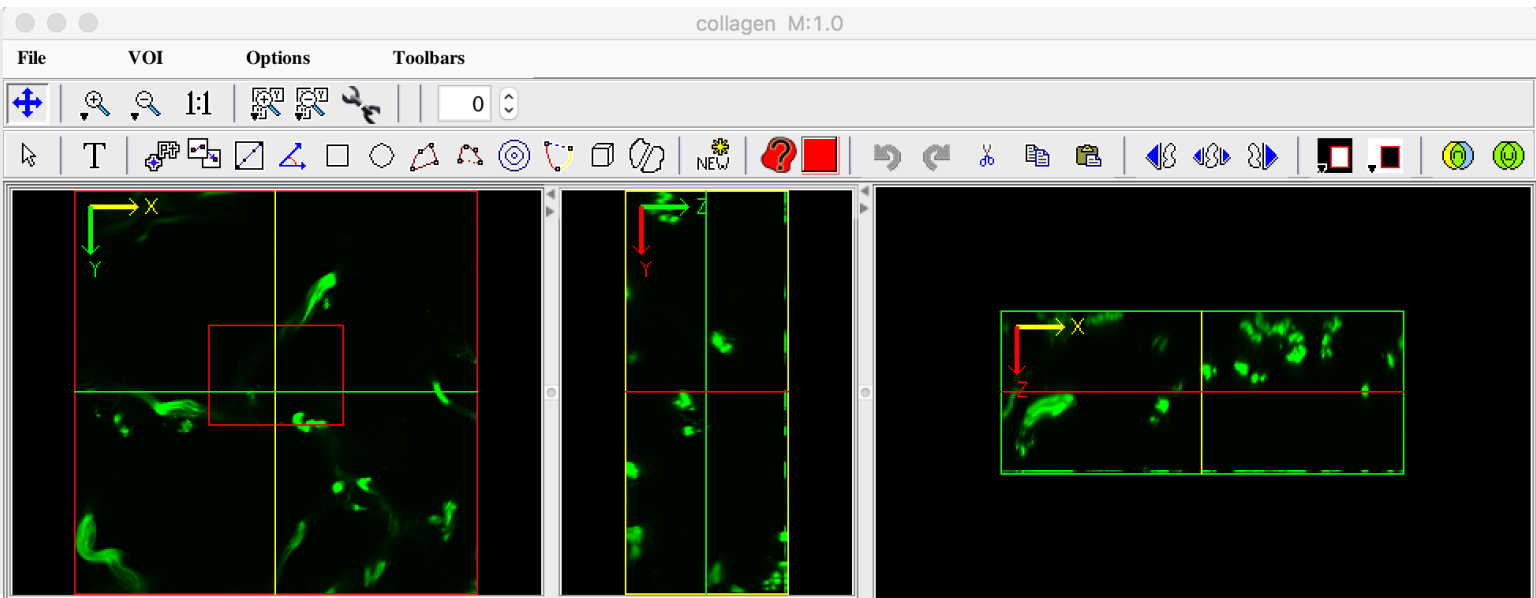
Interactive
orthoview
ImageJ



Montage of ImageJ

Radiology

- Transversal (XY)
- Frontal
- Sagittal





Spatial Projection

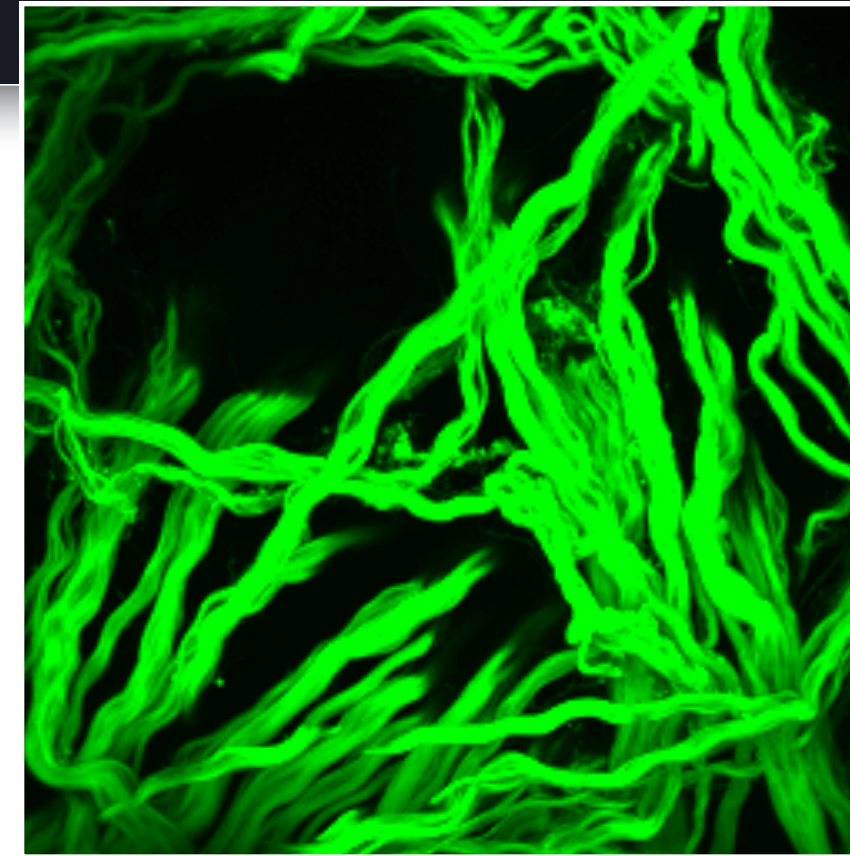
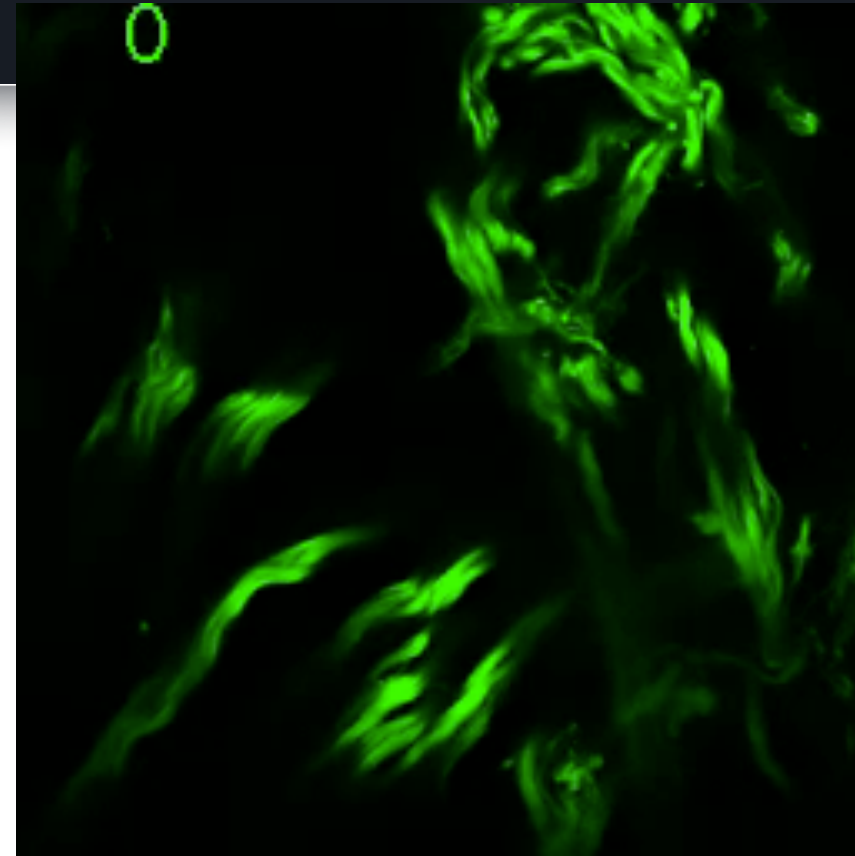
Global

Global

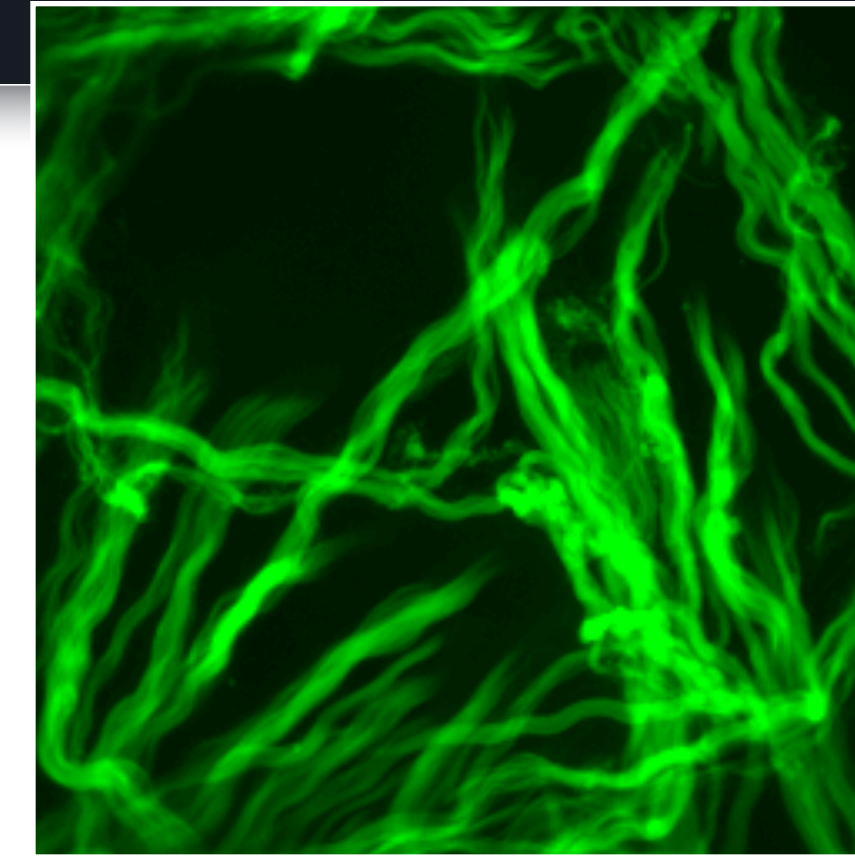
Pancreatic islet vasculature

Blood vessels

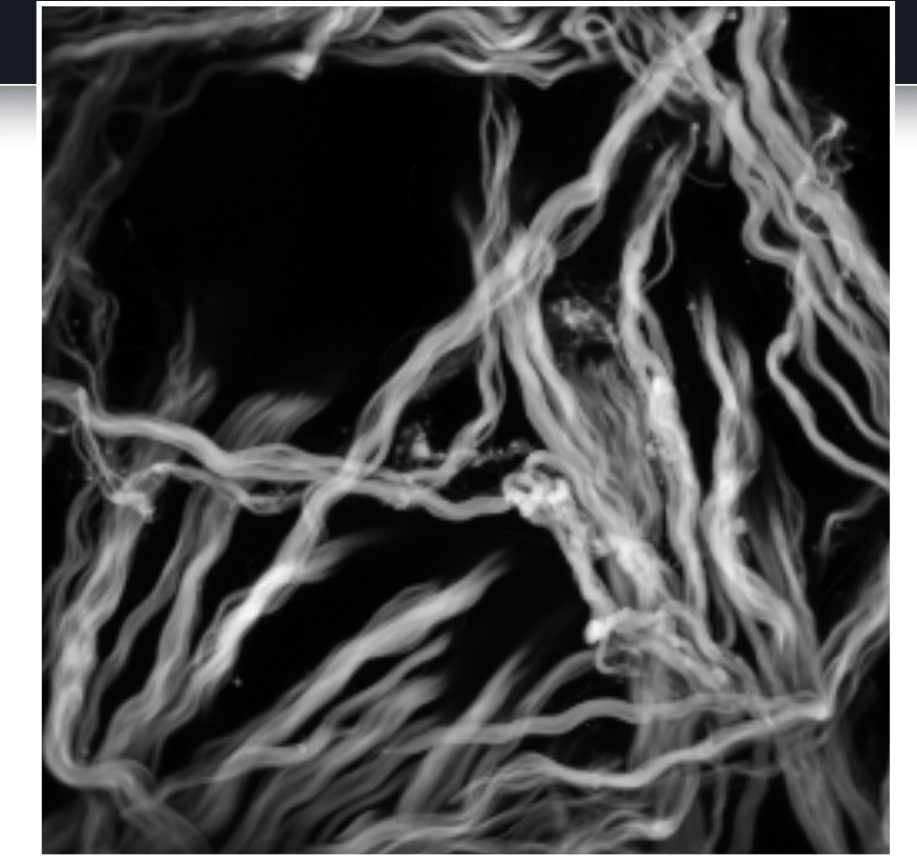
Marta Alabrudzinska, Karolinska Institute



Maximum projection



Average projection



Stdev projection

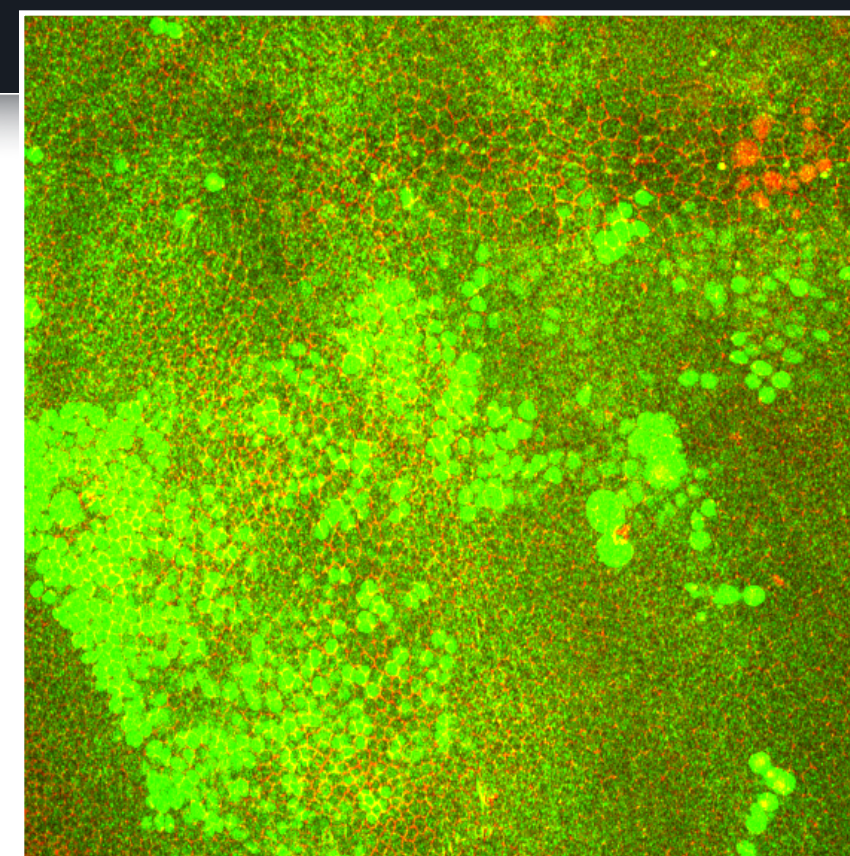
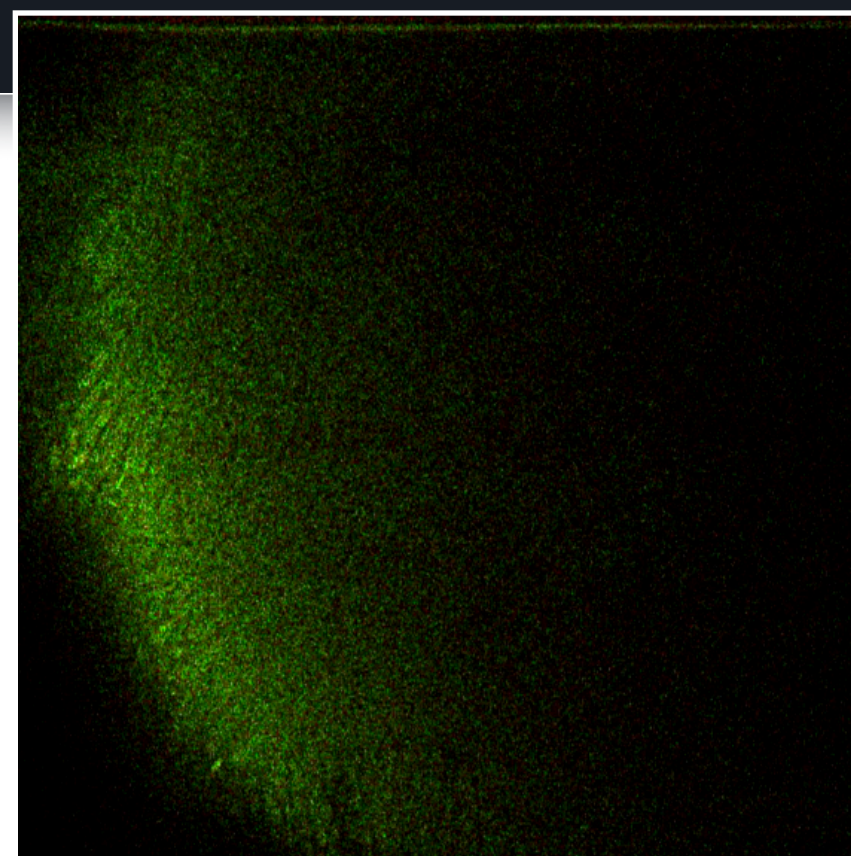
Smart

Smart

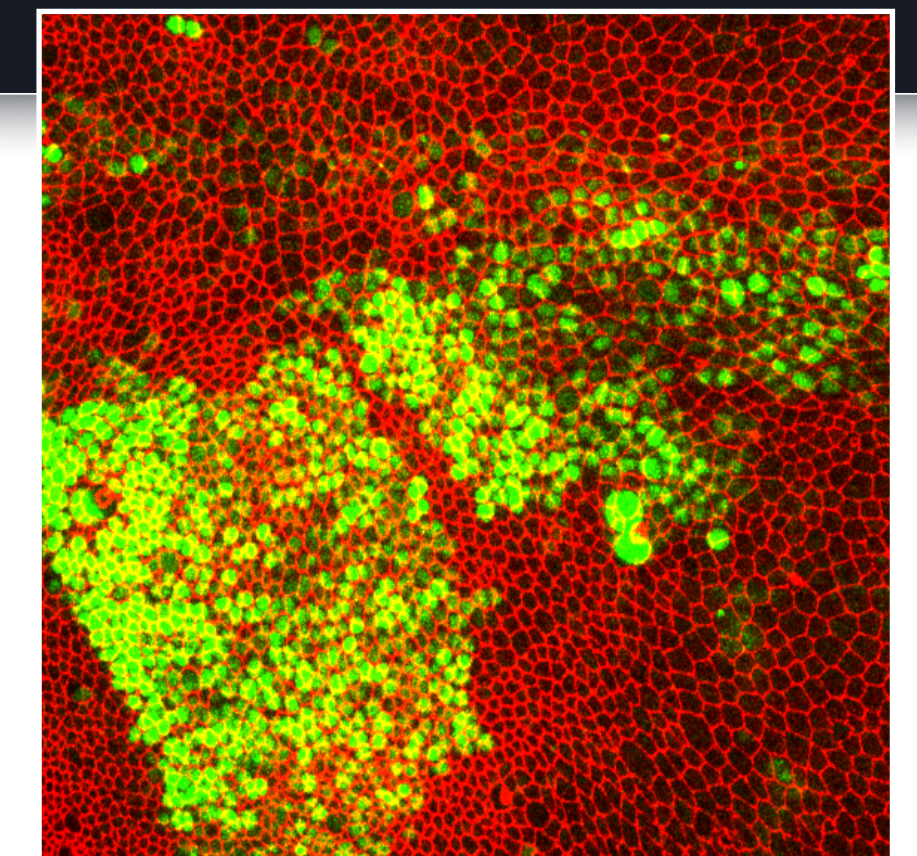
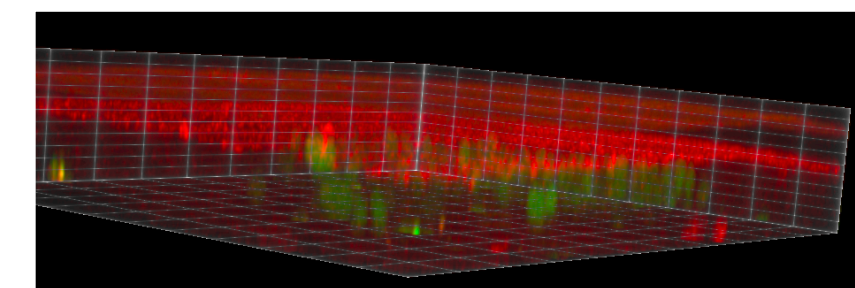
Drosophila pupa notum

LSM 880, Zeiss, 26 slices

Jean-Yves Tinevez, Institut Pasteur



Maximum projection

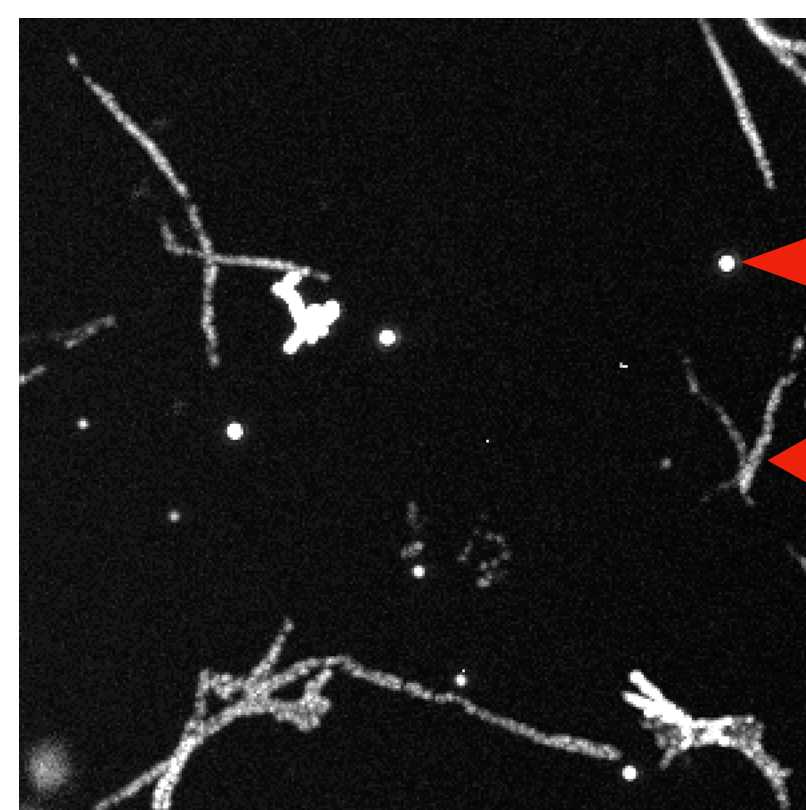


Local Z Projection



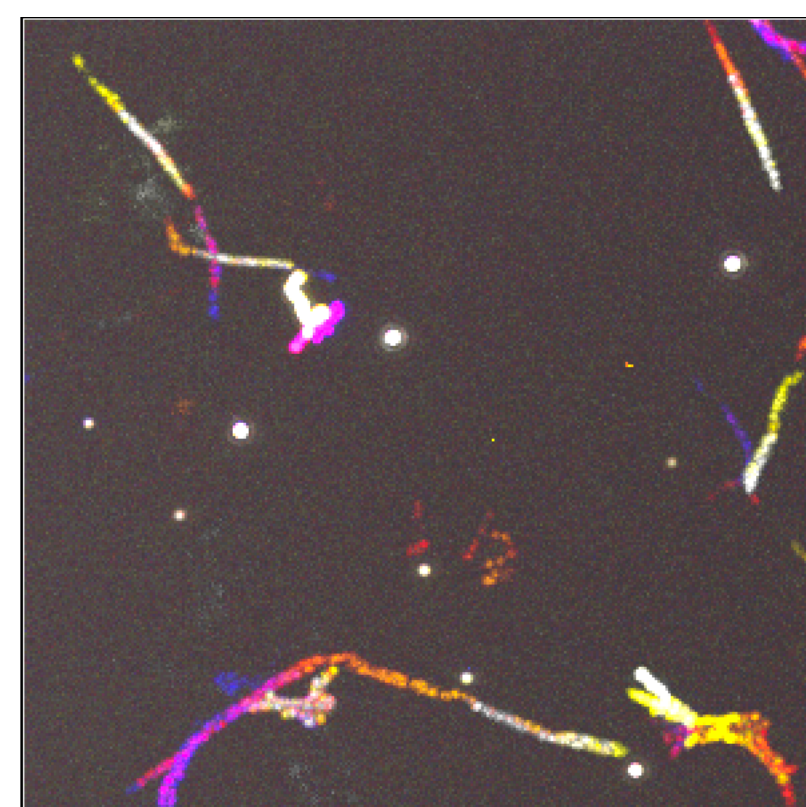
Temporal Projection

Time projection



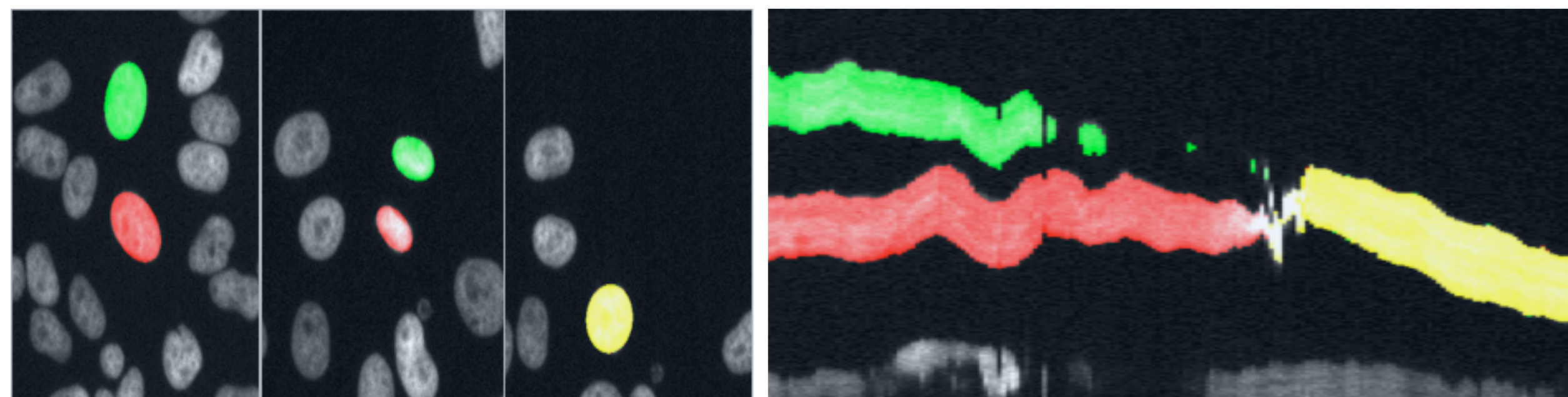
stationary particles

moving particles

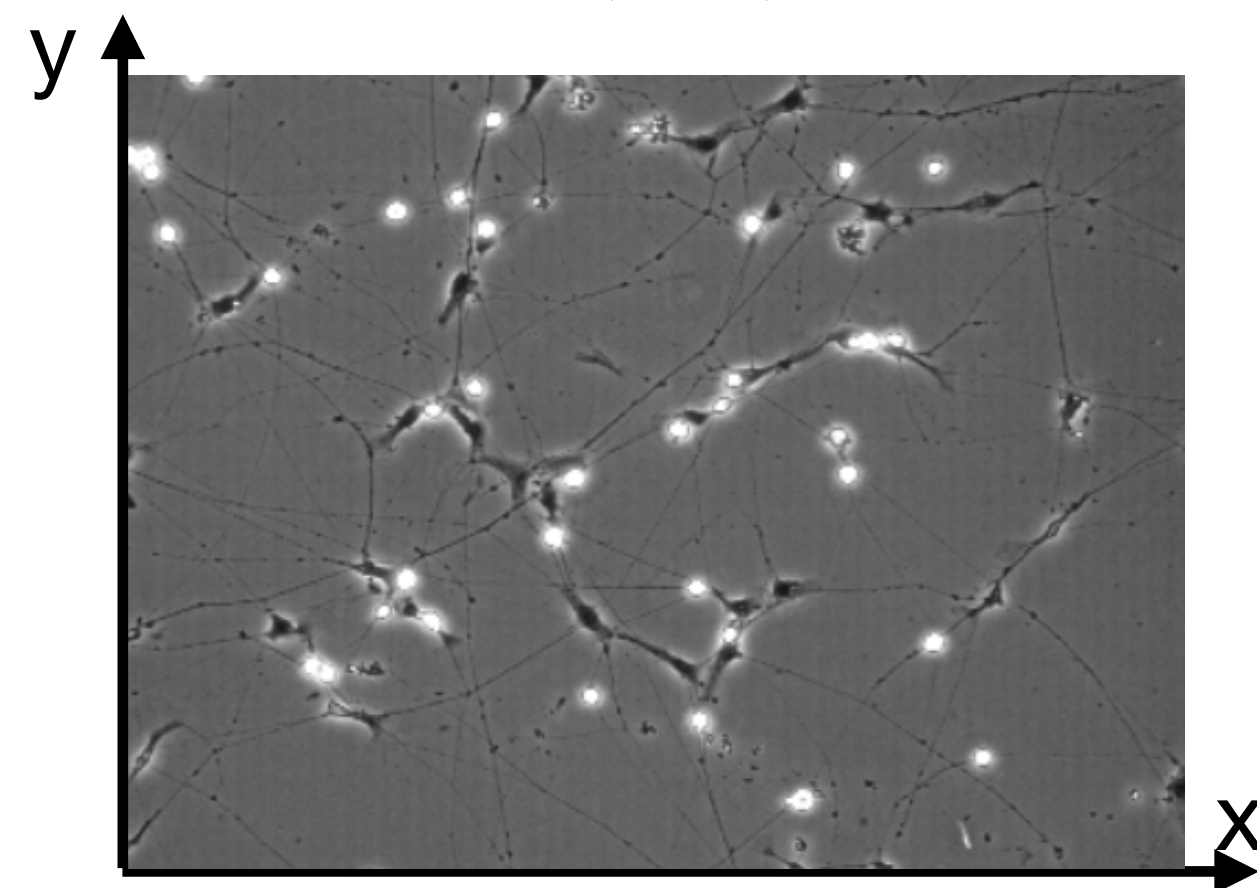


temporal-color coded
projection

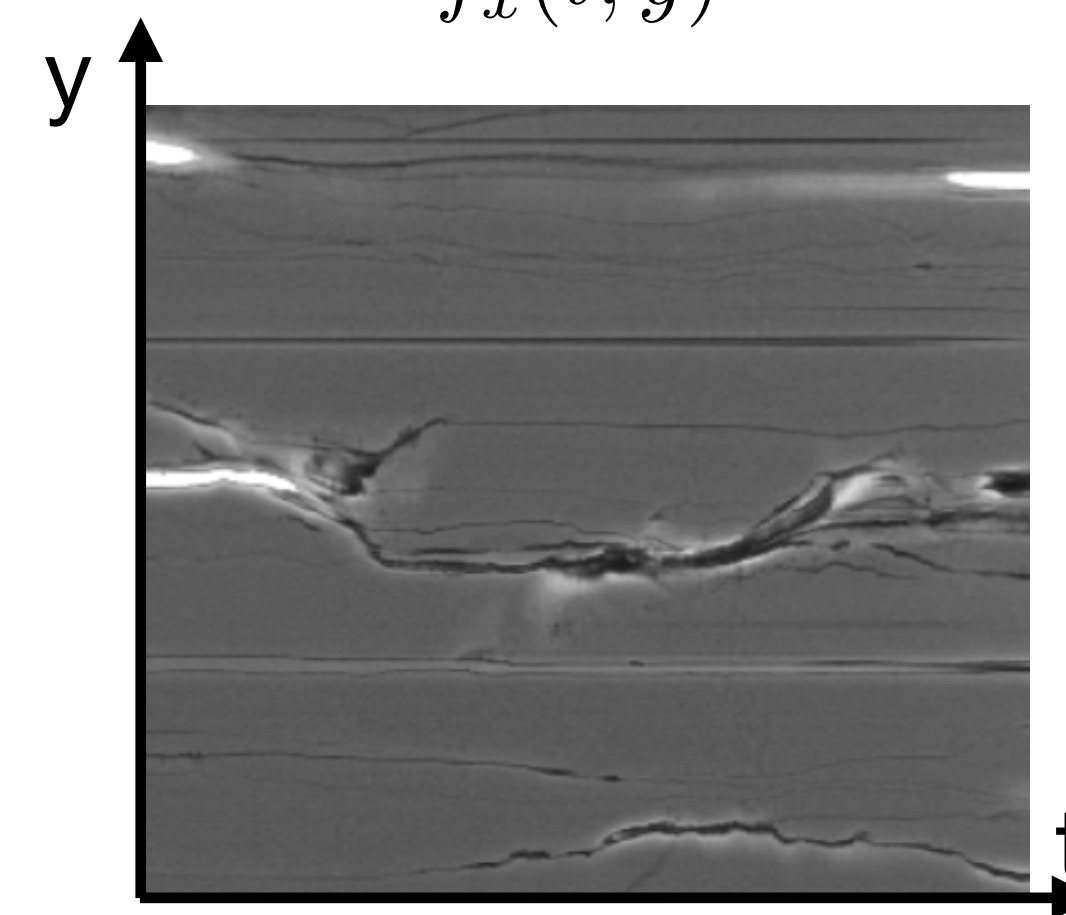
Permutation of axis



$$f_t(x, y)$$



$$f_x(t, y)$$



RECAP

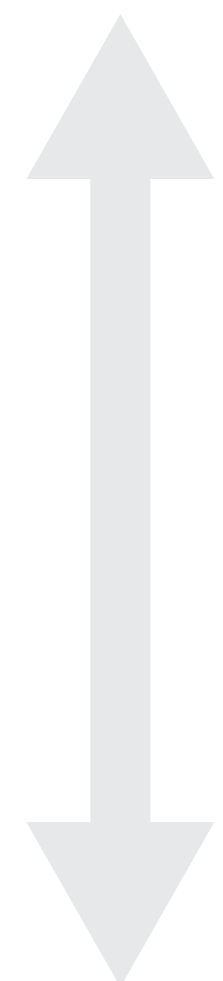
- DATA SOURCE
- DATA TYPE, BIT DEPTH
- MAPPING PIXEL VALUE TO COLOR
- CONTRAST, BRIGHTNESS, SATURATION, GAMMA
- HISTOGRAM, STATS
- IMAGE NORMALISATION
- LUT VS. COLOR VS. COMPOSITE IMAGE
- 5D IMAGES: SLICES, FRAMES, CHANNELS
- VOLUME RENDERING
- PROJECTION OF ND DATA

Extra Material

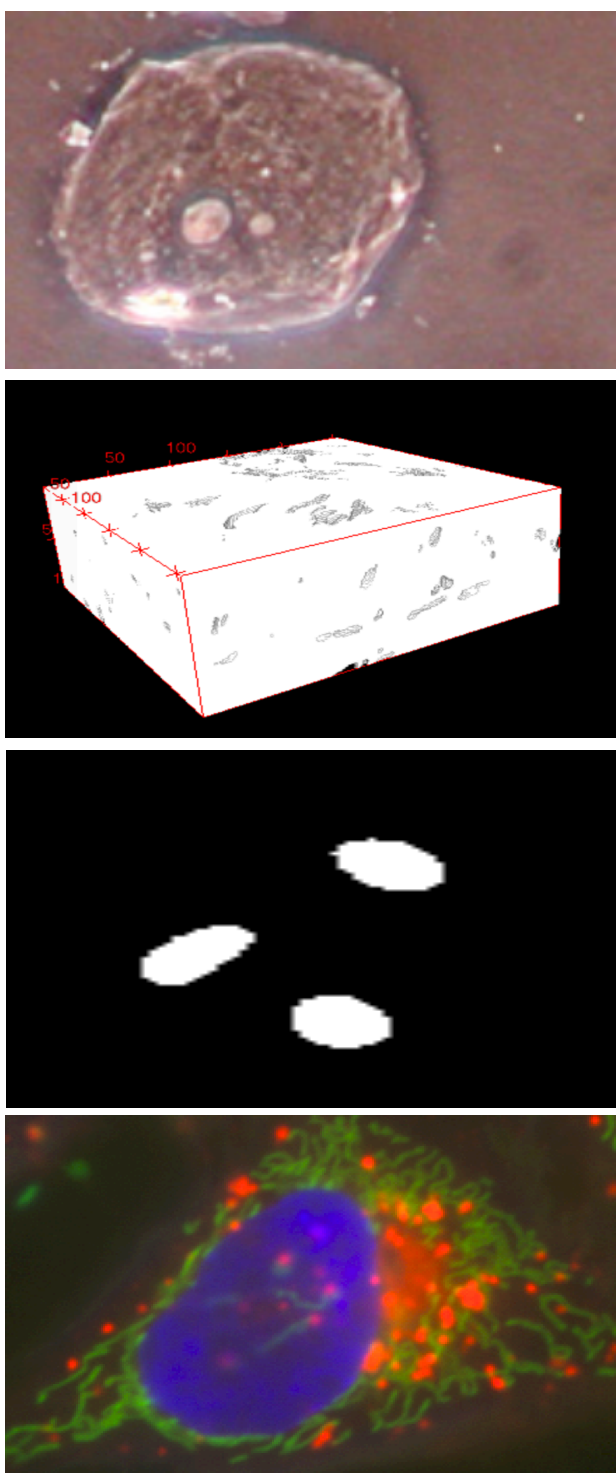
Digital Images □ Applications nD



Why Does Multidimensional Matter?



2D	X Y	Lateral plane	Shape, flat structure, counting	Resolution Sensitivity	volumetric info. live info.
3D	Z	Optical axis	3D structure, volume	Z-step Depth	Out-of-focus Toxicity
4D	T	Time	Dynamics, motility, diffusion, tracking	Video recording Time-lapse Frame-rate	Stabilization Bleaching
5D	C	Multiple channels (modalities)	Color, specific, colocalization	Fluorescence Brightfield PC, DIV	Cost Toxicity



ImageJ
CZT

Think multidimensional to design robust algorithm



Extended Depth-of-Field

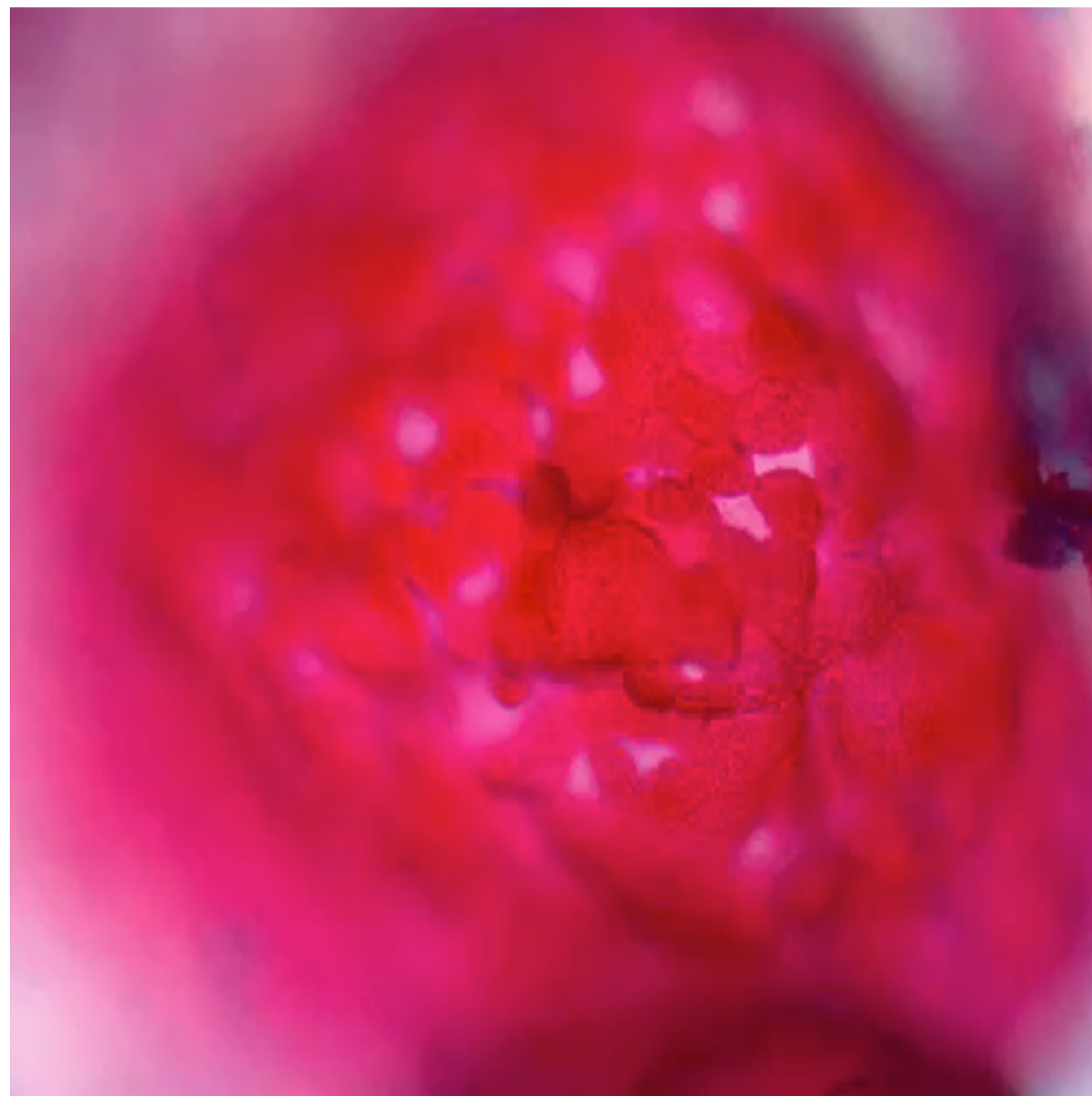
EDF ImageJ plugin

- Merge focal planes
- Height-map
- 3D surface plot



<http://bigwww.epfl.ch/demo/edf/>

Series of focal planes



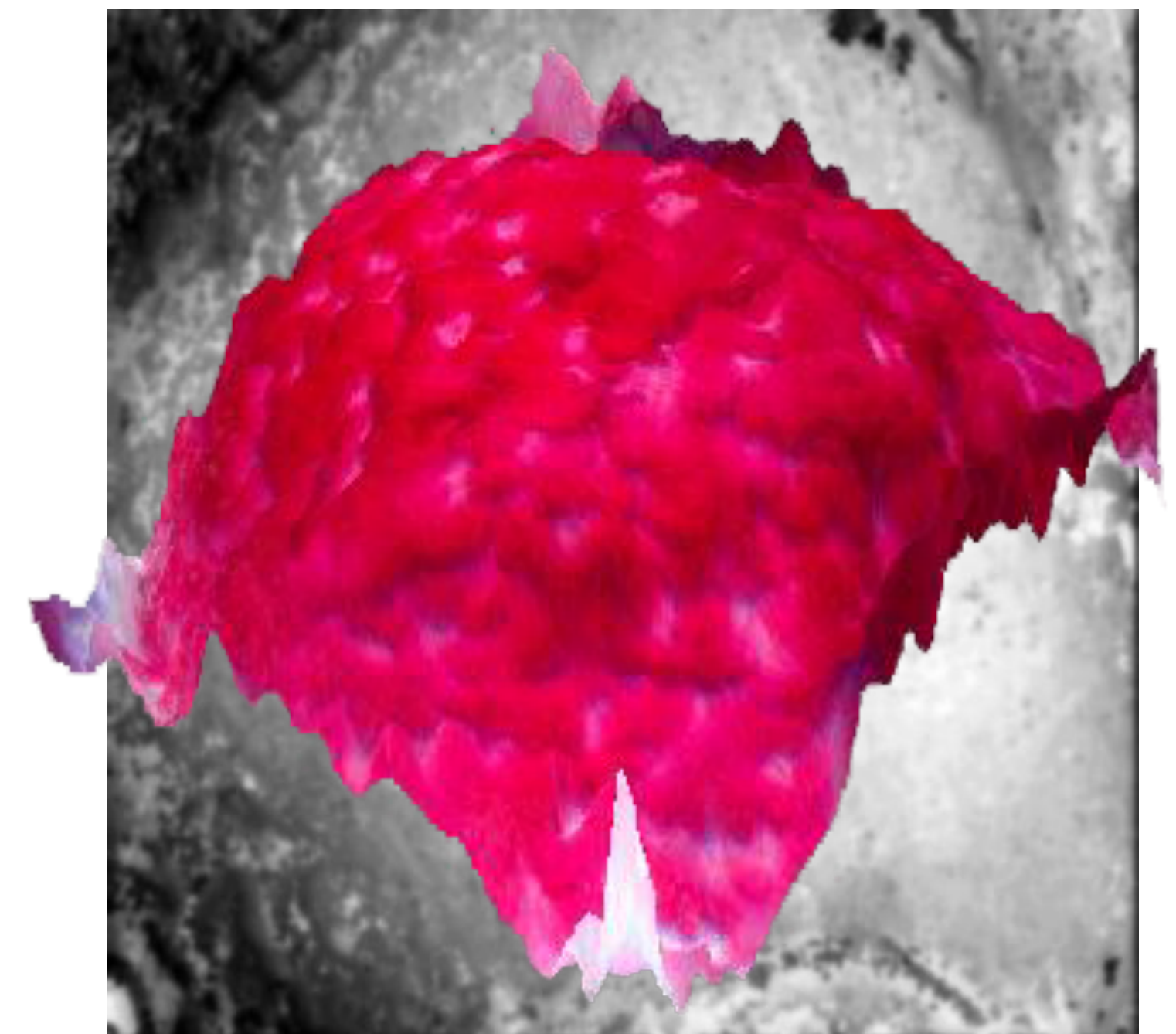
Peyer patch in mouse intestine
Stack of 20 images.

Source: Nathalie Garin, ISREC, EPFL

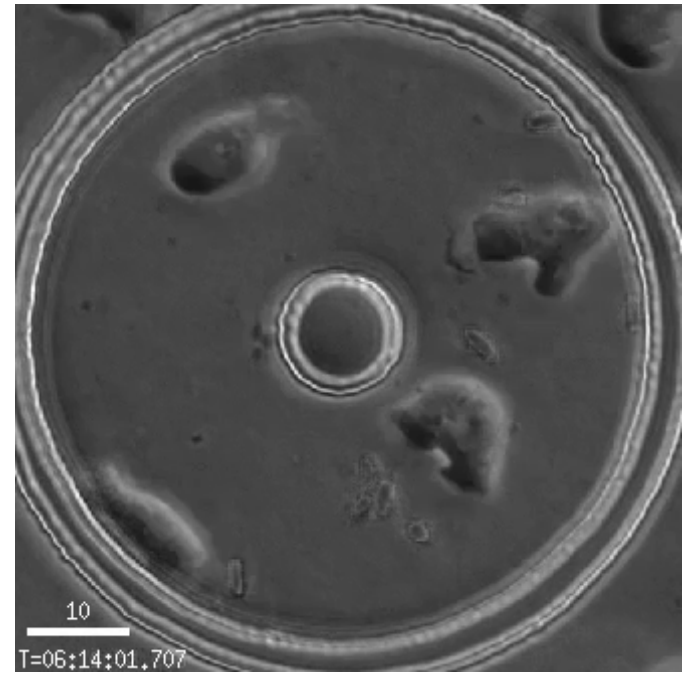
In-focus image composite



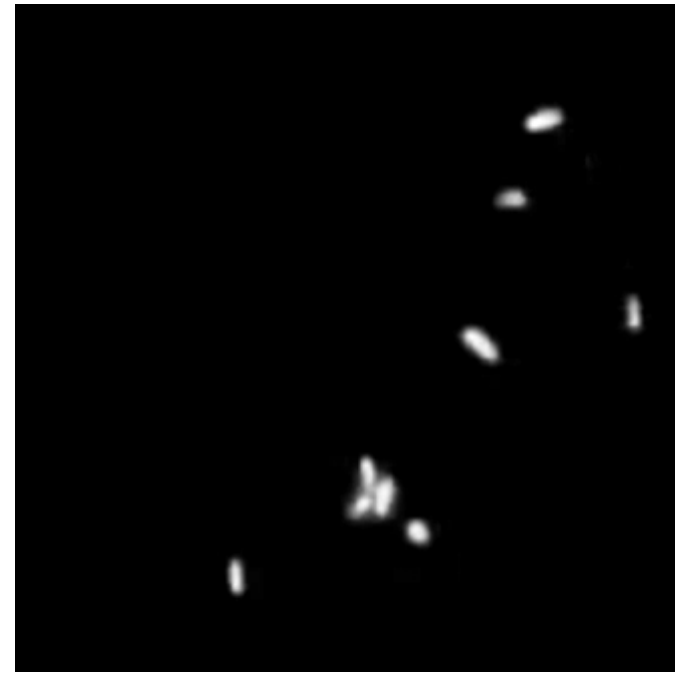
Height-map



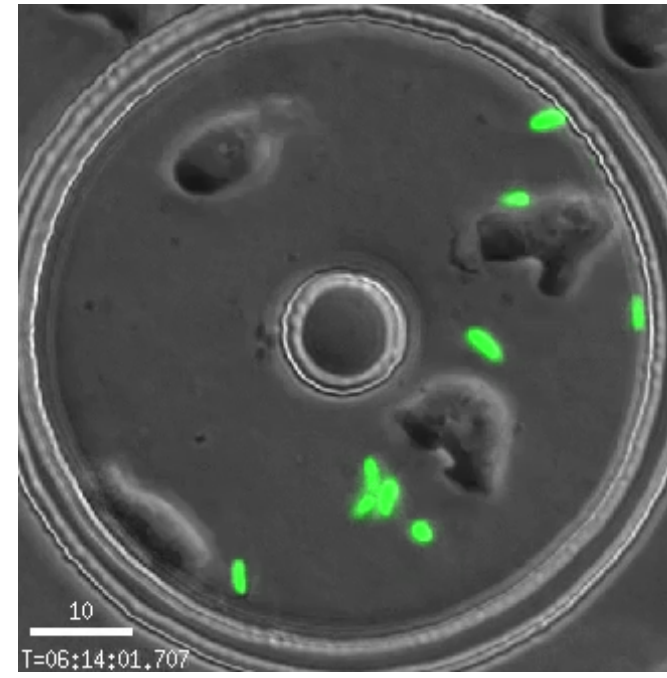
Multiple Channel Quantification



Phase contrast



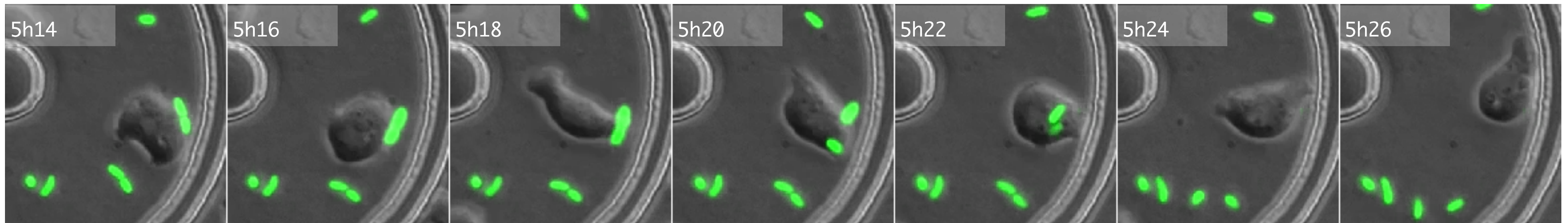
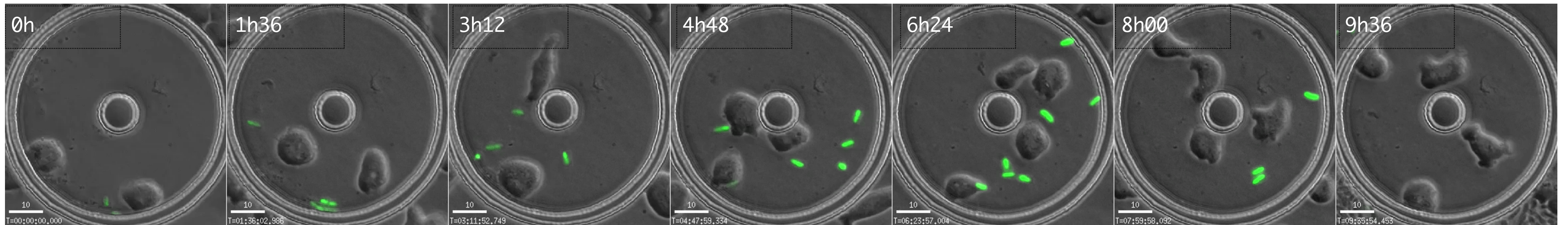
GFP



Composite

Cell bacteria interaction

source: Matthieu Delincé and Chiara Toniolo, McKinney Lab, EPFL





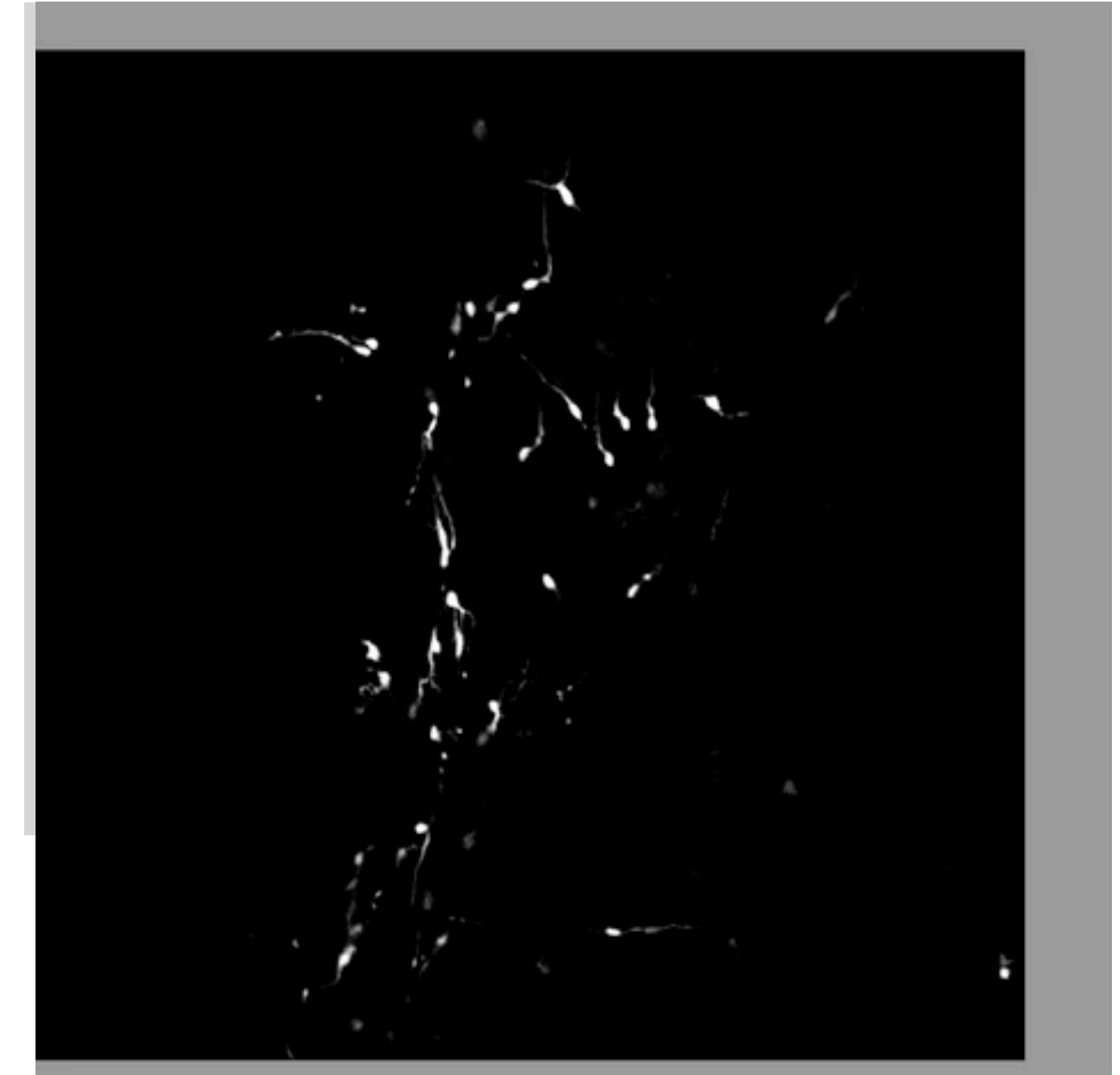
Time Processing

Drift correction

- Pixel-based image registration
- Landmarks registration

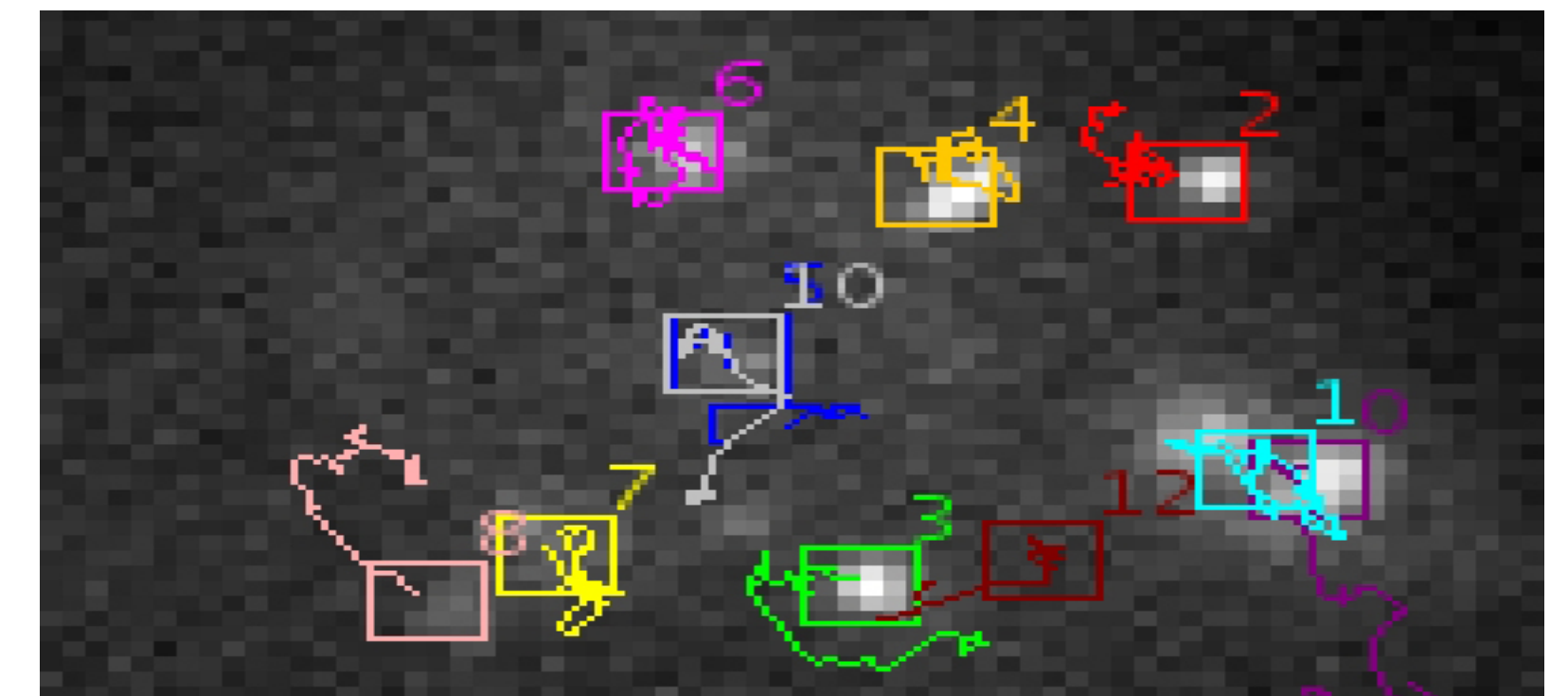
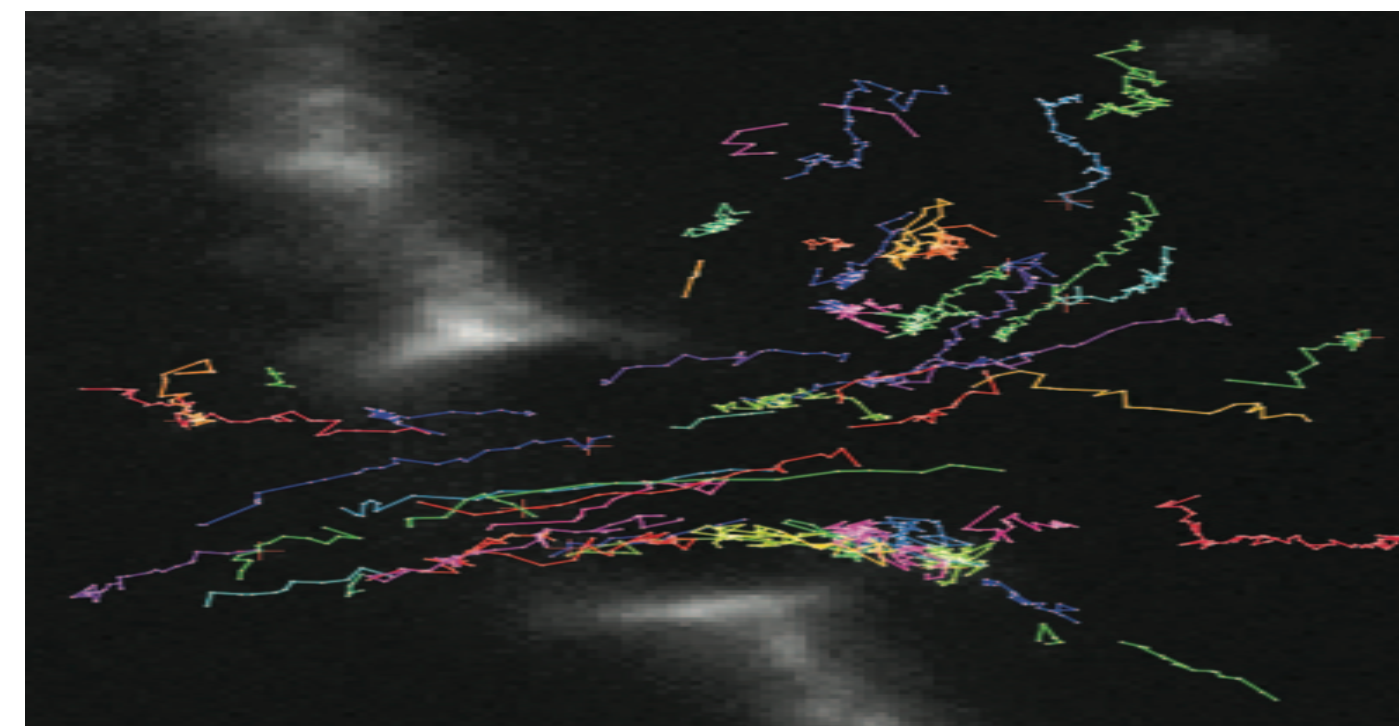


ImageJ plugin: StackReg

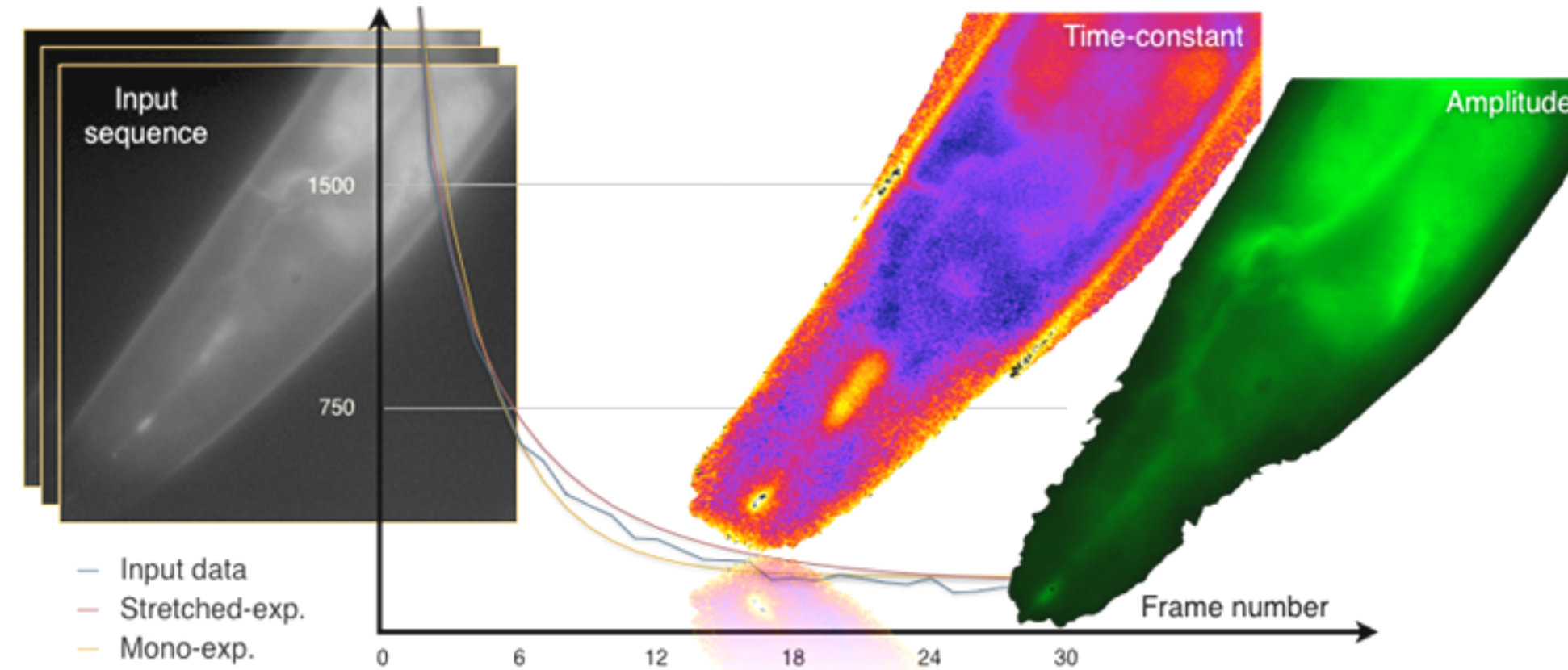


Tracking particles

- Detection
- Temporal association



👁 Finding the Photobleaching Rate



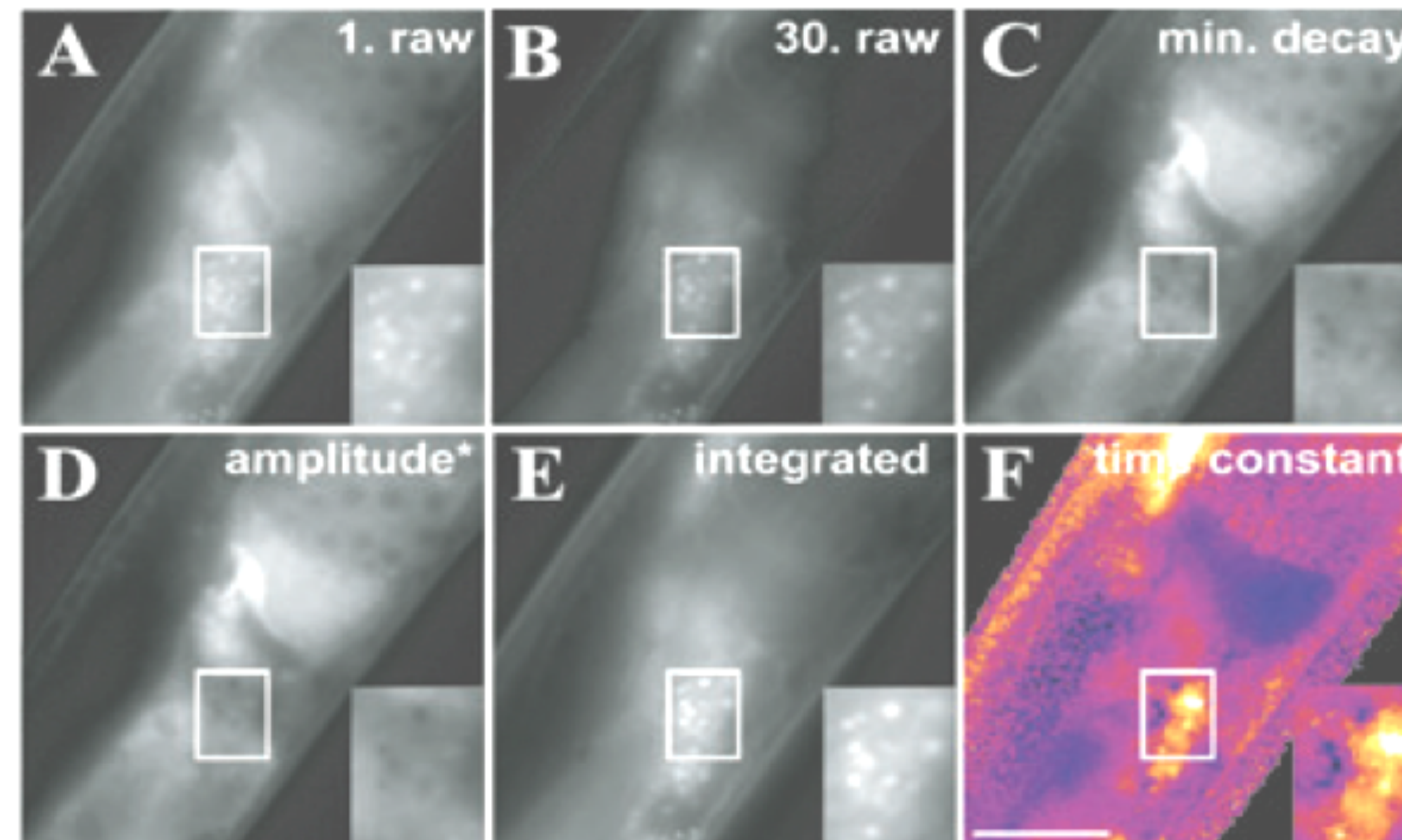
stretched-exponential time model
h: heterogeneity factor

$$I(t) = e^{\left(-\frac{t}{\tau}\right)^{\frac{1}{h}}}$$

PixBleach ImageJ plugin

<http://bigwww.epfl.ch/algorithms/pixbleach/>

Application
Detection of dehydroergosterol
DHE in adult nematodes based on
its bleaching rate



A: first image
B: last image
C: minimal decay map
D: calculated amplitude
E: integrated intensity
F: result of time-constant map