Introduction to ImageJ
Session 4: 3D

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BIO-INSPIRED MATERIALS
NATIONAL CENTER OF COMPETENCE IN RESEARCH

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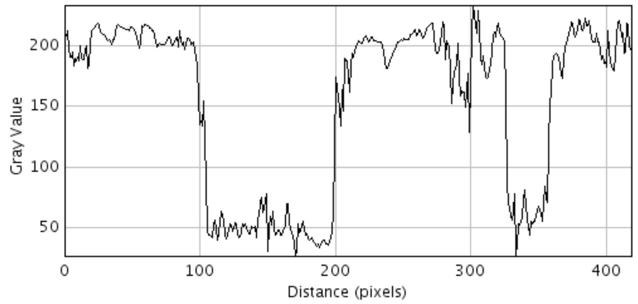
Going digital – what is a digital image?

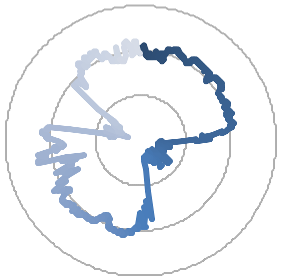
A digital image is an ordered rectangular array (or grid) of **integers (numbers: 0,1,2,3…)**.
Each element (=number) in the grid is also known as a picture element or 'Pixel'

Spectrum

1 dimensional array

208
209
213
198
191
191
195
184
190
188
191
188
200

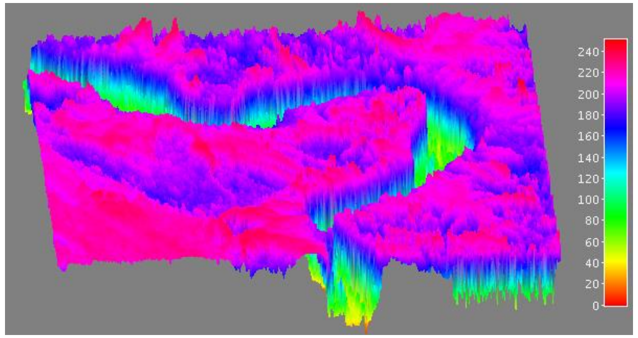




Image

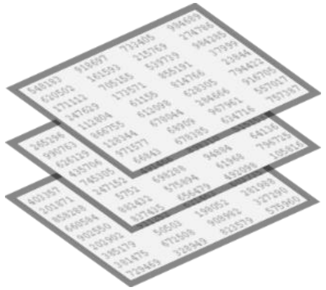
2 dimensional array

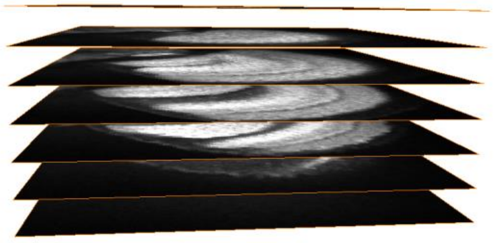
82	72	78	86	65	41
157	144	167	188	201	19
185	191	195	188	188	19
193	195	195	191	189	17
173	170	181	192	194	19
210	214	206	202	203	20
237	224	221	230	232	22
183	180	190	188	192	18
178	170	159	187	195	18
167	164	170	186	192	18
159	162	164	184	170	16
180	172	165	172	185	17
193	180	196	195	185	17
167	184	182	183	180	17
195	191	182	189	195	18
183	188	184	183	174	16
191	196	185	179	188	19



Stacks

3D array
(= volume stack or video/Timelapse





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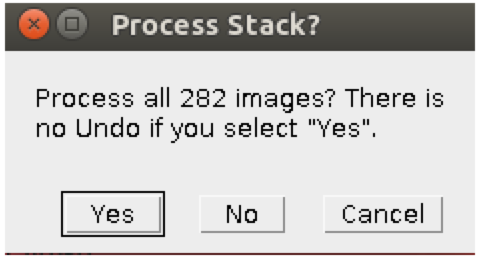
Types of 3D data

Filters, point operators, … and stacks

Upon running a function over a stack, you will often get a question:



Hence, all
- Filters
- Bandpass filters
- Point operators
- Binary functions
- etc...

Are also valid for stacks

Sobel filter on RGB Lena

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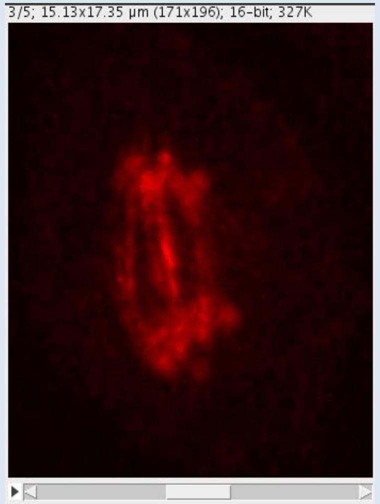
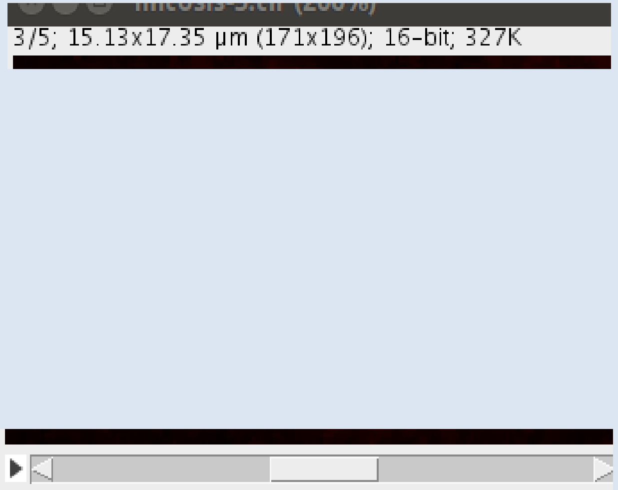
Stacks

Prerequisites

- All the slices in a stack must be the same size (X,Y) and bit depth.
- The slice thickness is considered constant (Z)





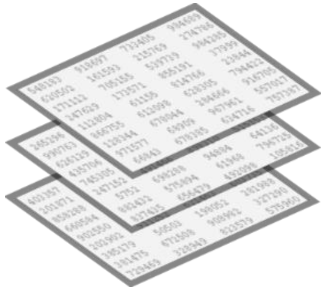
Type of stacks

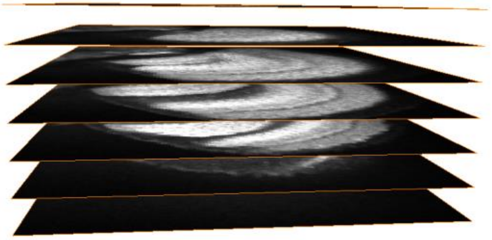
1. 2D images with **encoded Z information** (e.g. AFM)
2. **Channels (or layers)** are multiple images stored within one file. Typically, they contain different color absorption functions of the same object
3. **Z layers** are images recorded at different depth positions through the object
4. **Time lapse** are images recorded at a different time point
5. **Hyperstacks** (n>3)

Stacks

3D array

(= volume stack or video





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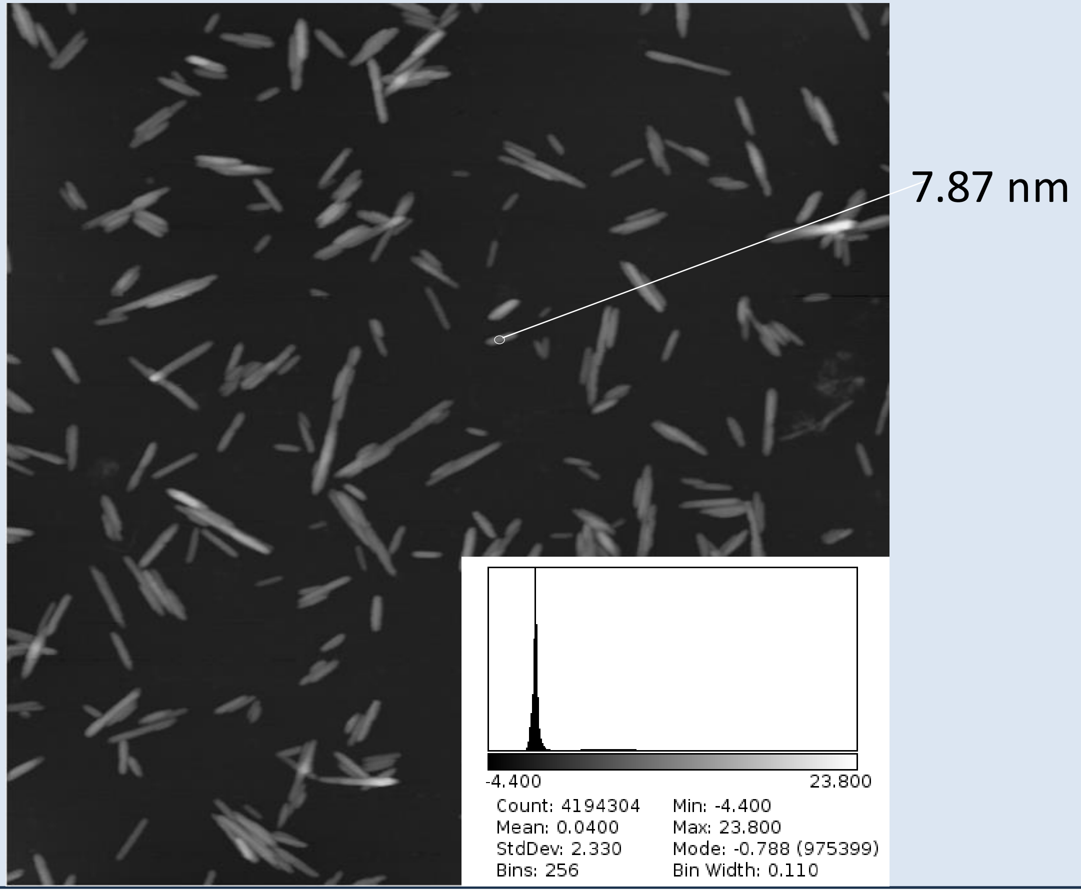
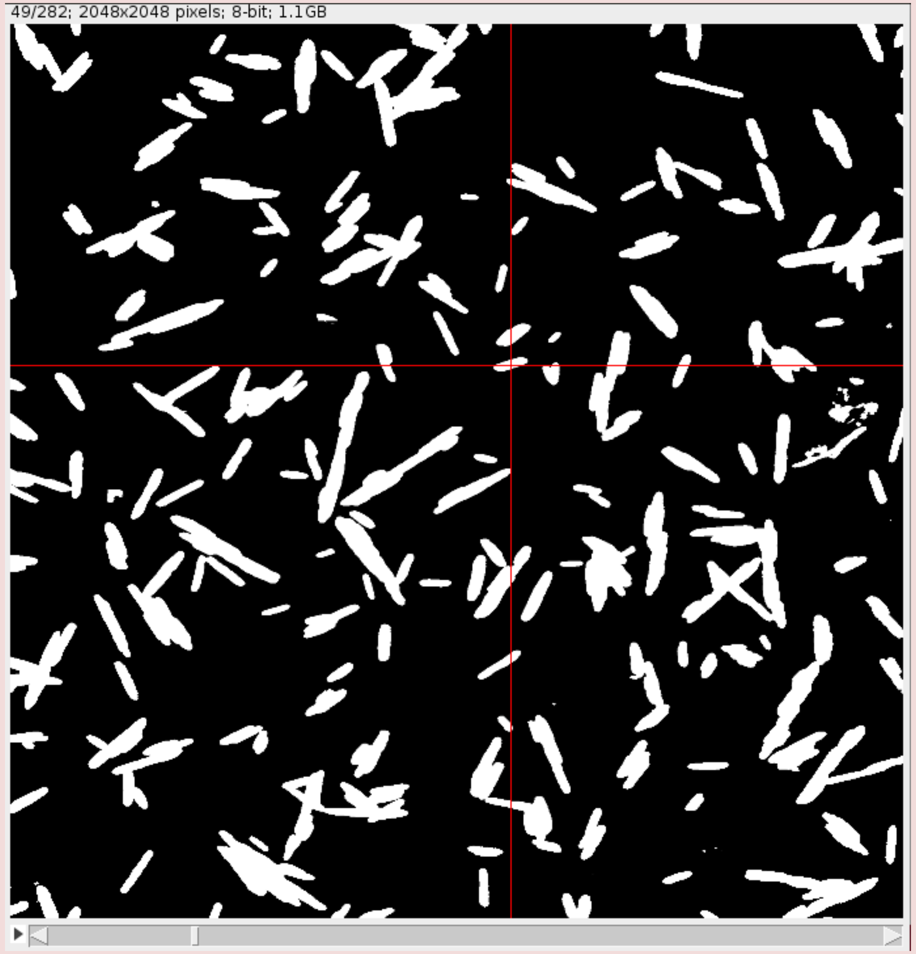
1. Height maps

Gwyddion
Open SPM (AFM, SNOM/NSOM, STM, MFM, …) data analysis software

e.g. AFM height maps
- 2D image
- Pixel value = height = height map



3D Map (XY view, transformed height map)

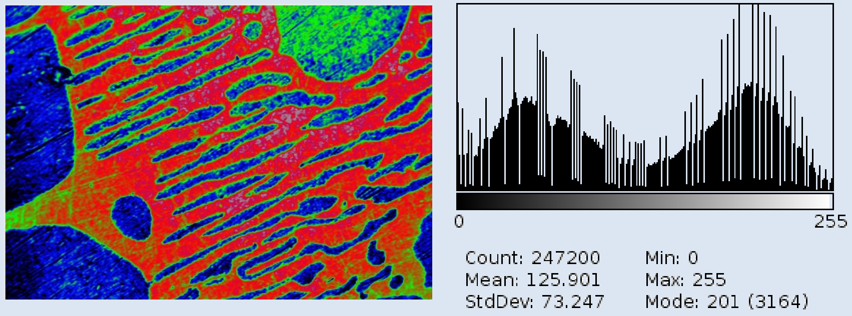


XZ view (10X)

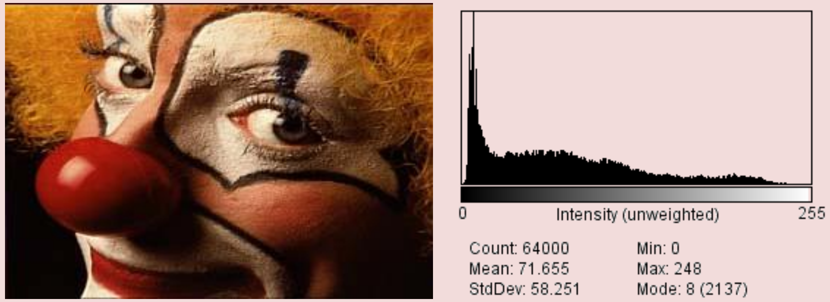
Data by Gwendolyn Delepierre

2. Channels

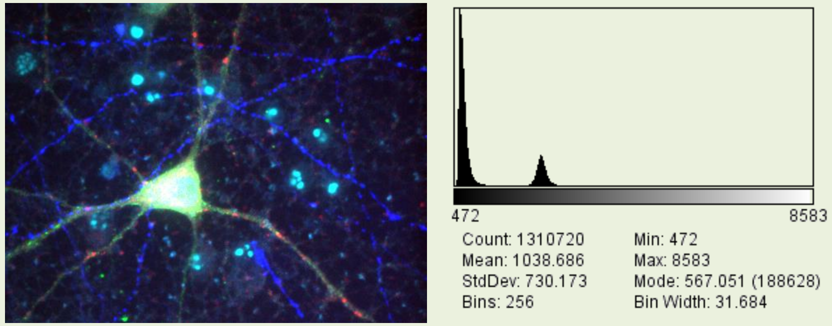
Pseudo-color
= a single channel (grayscale) equipped with a LUT



RGB images (24 bit=3x8bit)
3 layers, reflecting the natural red, green and blue colors
(or HSL, CMYK, HSV, …)



Composite images (flexible: e.g. 5x16bit)
n layers, separated. For example LSM multi channel data



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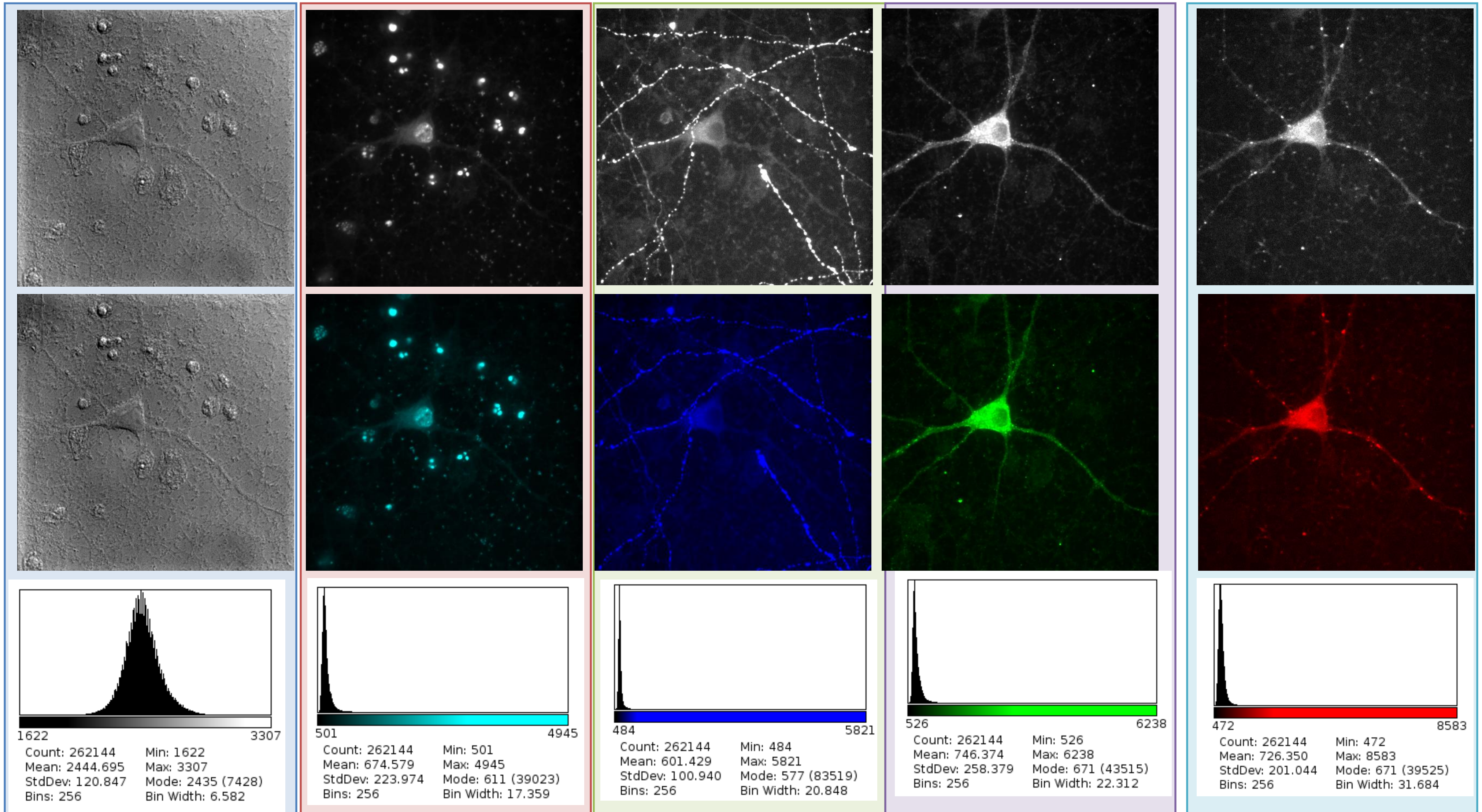
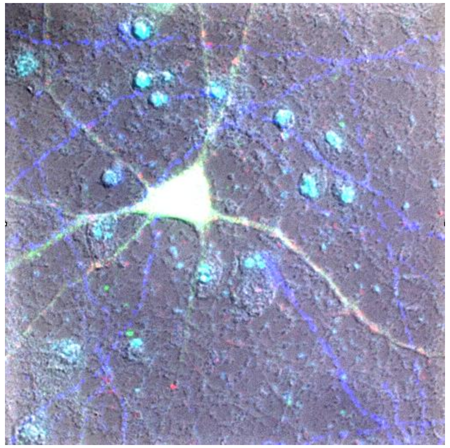
Channels: composite images



Brightfield | Astrocyte marker | Synaptic vesicles | Syntaxin | Actin



Composite image

Channels tool

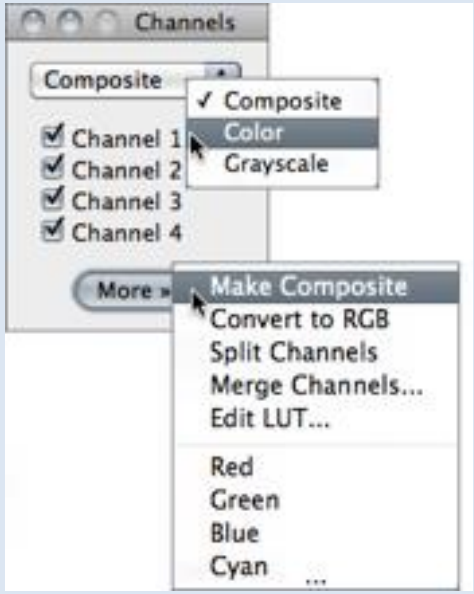


Image > Color > Channels tool

Composite: overlaying the layers of choice (also for RGB images)
Color: showing only one layer, with LUT. Change the LUT of the selected layer
Grayscale: showing only one layer, in grayscale LUT
(Clicking on the channel selector = use the channel scrollbar below the image)

Make composite: splits the color image in its layers
Convert to RGB: joins the layers into a 2D RGB image (you will end up with 1 window)
Split channels: makes n windows of each channel
Merge channels: Tool to put n single channels together into a composite stack

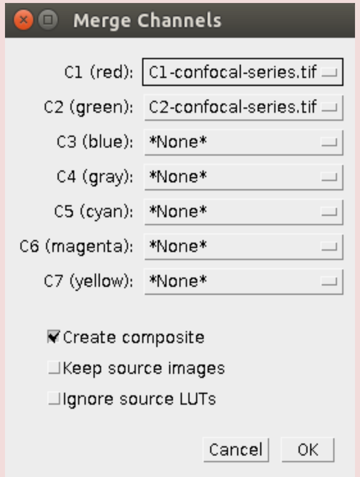


Image > Color > Merge Channels

Combines n images into a composite image

- Prerequisite: all images have the same size (width, heigth and bitdepth)
- Choose the LUT (color)
- Once merged: check the "Arrange" menu entry (Image > color > Arrange...)

Channels: split, arrange, and merge

EXERCISE
Open Example 1

Convert to Composite
Convert a color image to a composite image (Image > color > channels tool: More > make composite)

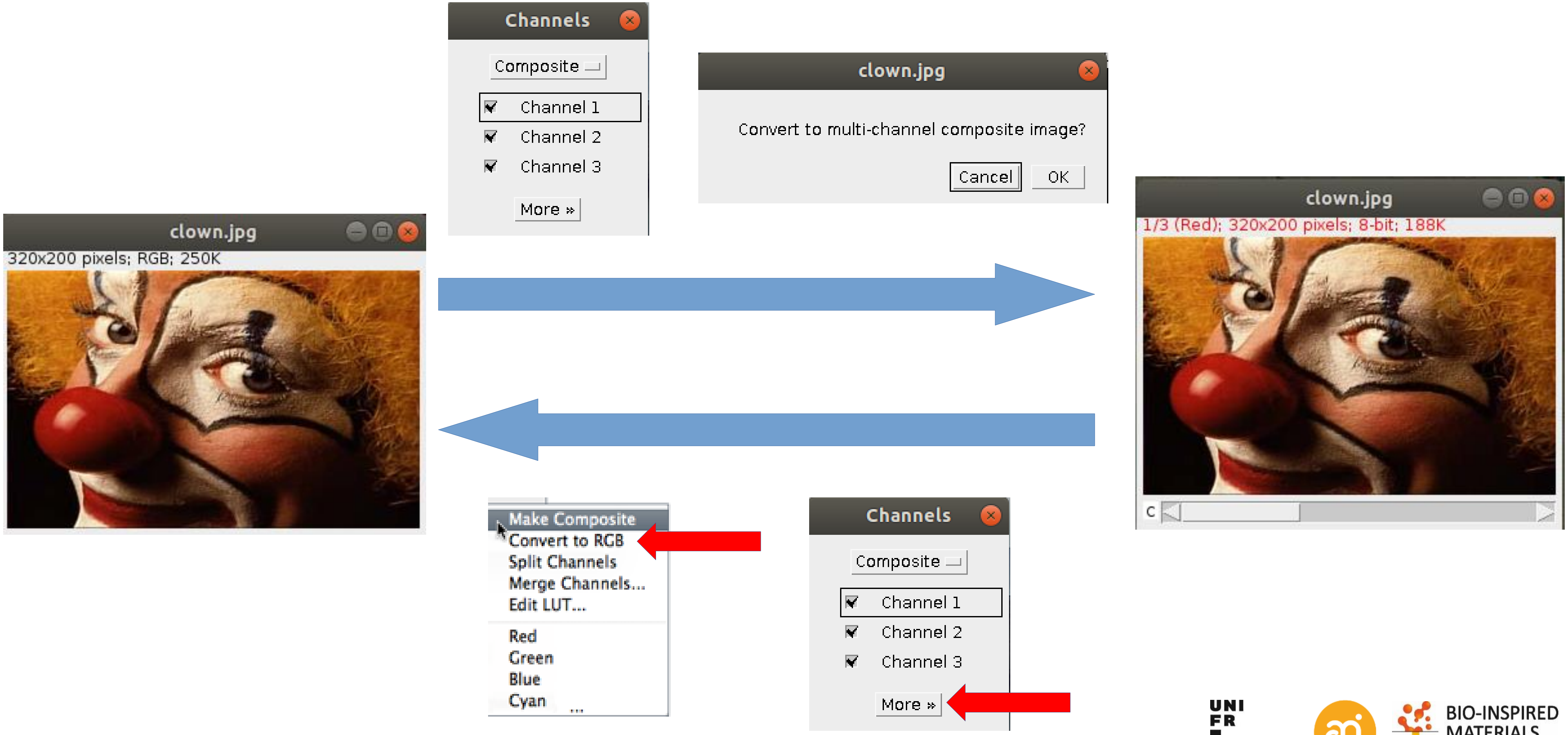
Split a composite dataset in its grayscale components
Split the three channels (Channels tool: More > split channels)

Optional: change the LUT of each of the grayscale components
Change LUTs if required (Image Lookup tables)

Merge channels
Merge the channels again to an RGB image (Image > color > Merge channels OR Channels tool: more > merge channels)

Change the order of the grayscale channels in the composite dataset
Arrange: Change the order of the layers in the stack (Image > color > Arrange Channels...)

Channels tool: example RGB image



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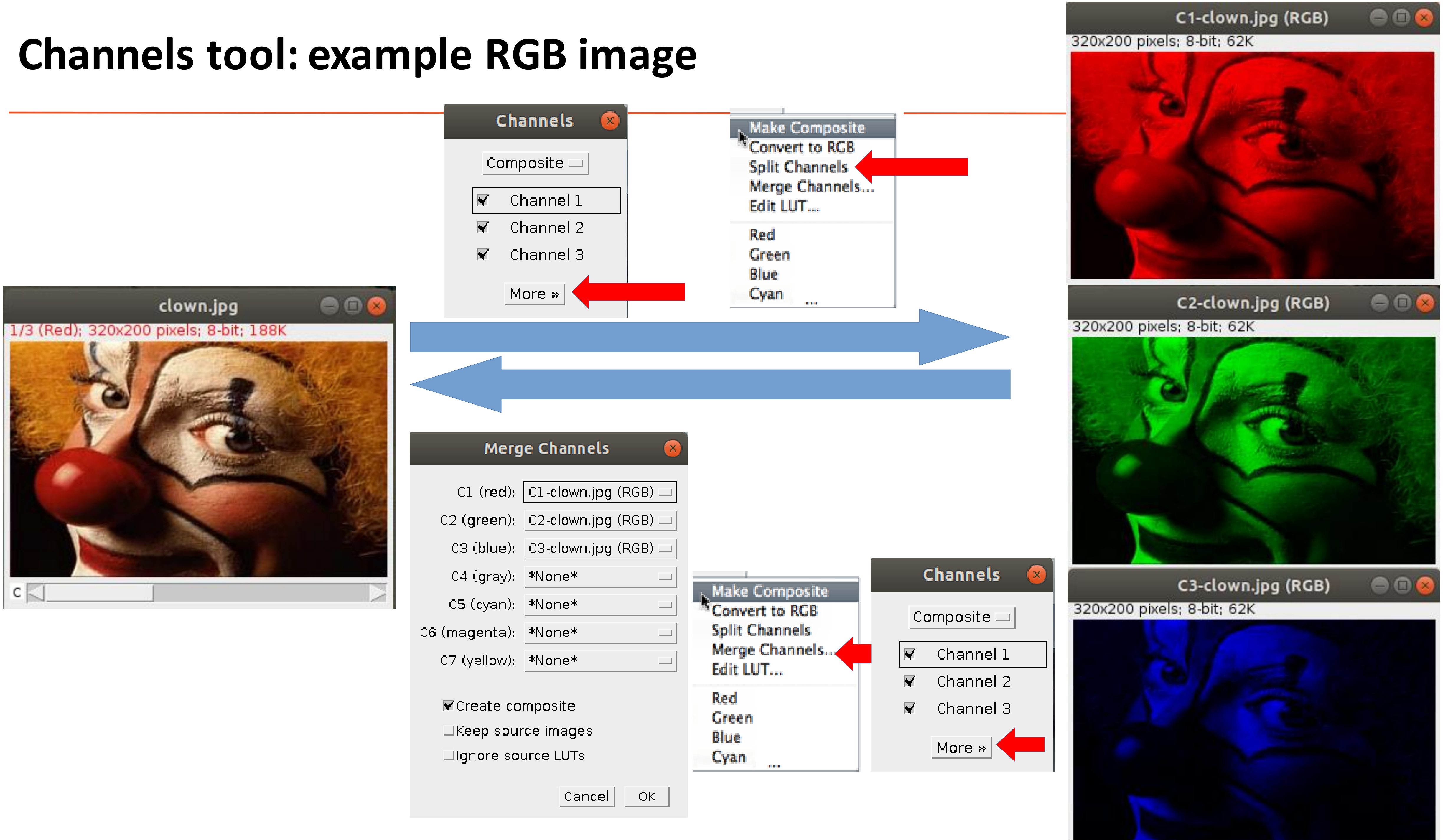
Channels tool: example RGB image

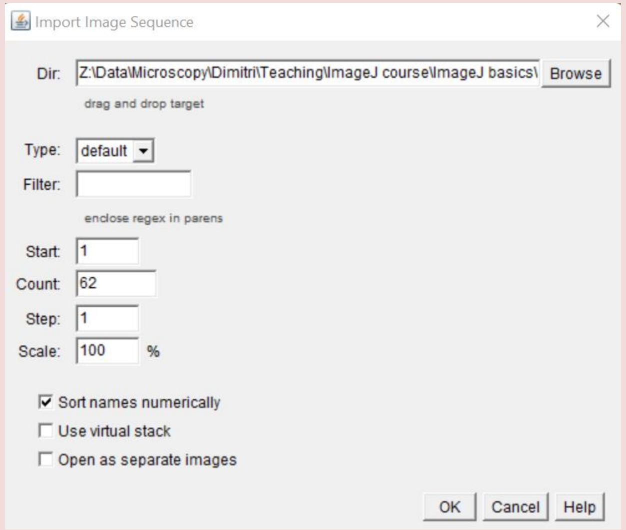


3. Z-stacks

1. File format including the entire Z-stack

Native	TIFF	
Non-Native	lsm (Zeiss)	Use LSM toolbox
	lif (Leica)	Use Bio-Formats plugin

2. Sequence = a number of 2D images (same XY size, same bitdepth) in a single folder



File > Import > Image Sequence

Enter or browse the folder path

Filter: regex patterning, e.g. 'tif' will only select images that have 'tif' in their filename

Possibility to reduce the stack

Import options

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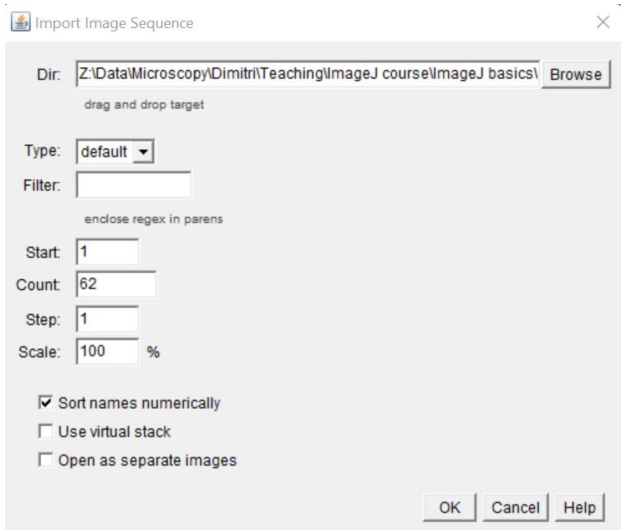
Opening sequences

EXERCISE
Open Example 2 (the folder) and import the sequence



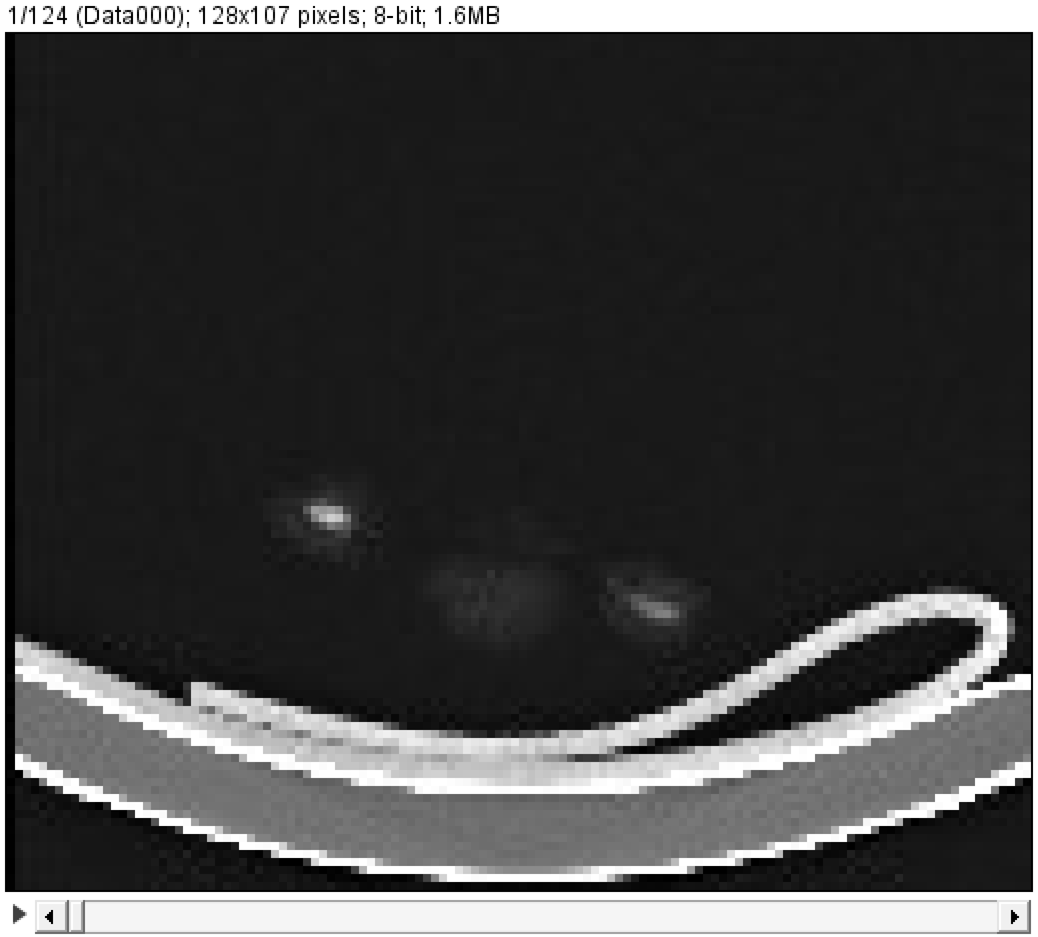
- **File > Import > Image Sequence**
- Locate the folder
- (you do not see the actual files in the folder)
- Filter: allows filename filtering (e.g. tif will only include files that have tif in the filename)

All images must have the same size! (X, Y and bitdepth!)
Watch out for **OS generated thumbnail files**

Possibility to open as virtual stack

Opening sequences

EXERCISE
Open Example 2 (the folder) and import the sequence



Stack of 124 Slices, now looking at slice 1
X = 128
Y = 107
Z = 124

Works exactly the same if you would have opened a multi-image file (eg. Tiff)

What is the difference between TIF and TIFF?

Move through the stack

Operations on Z-stacks

Image > stacks Add Slice, Delete Slice, Next Slice, Previous Slice, Set Slice…

Image > Stacks > Make montage — Produces a single grid-image containing the individual images that compose stacks and hyperstacks

Image > stacks > Stacks to Images — Releases the n images in the one stack window into n windows (watch out with large stacks!)

Image > stacks > Images to Stacks — Takes all open Images and puts them into 1 stack. Regex filter possible.

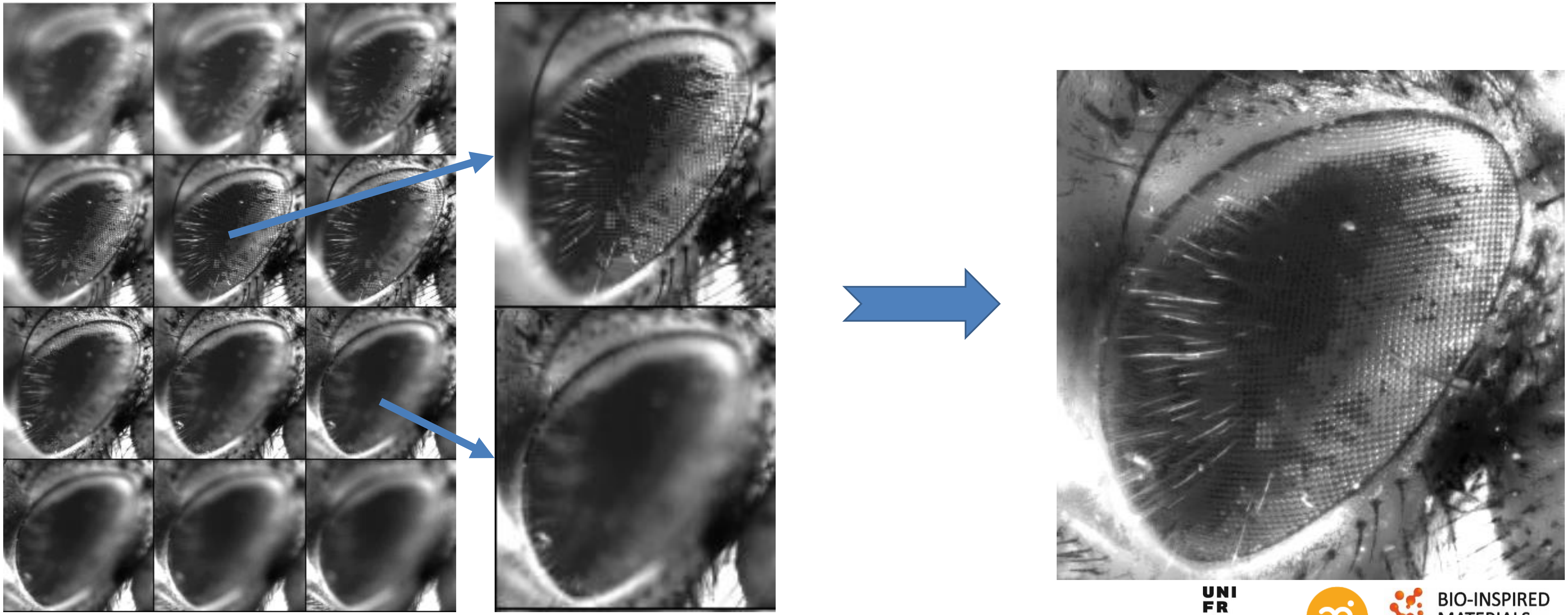
Image > Stacks > Z project — Projects the entire stack onto a 2D image
Image > Stacks > 3D project — Maximum intensity projection of the stack

Image > Stack > Tools — Combine, Reduce, Make substack, …
Image > Stacks >Tools > Grouped Z project…Output: a new stack, but with each slice the average/max/sum or each group

Z-Stacks: Extended depth of field

Image > Plugins > Extended depth of field (EPFL: bigwww.epfl.ch/demo/edf)

Projects a brightfield image of a large object in focus based on a focal series



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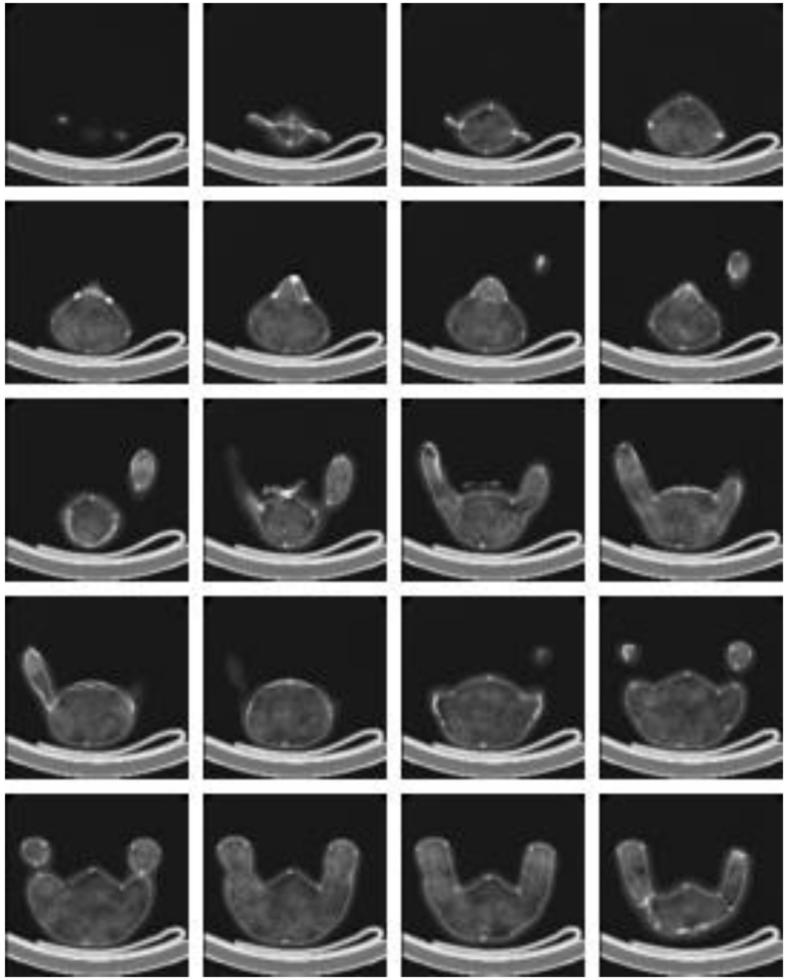
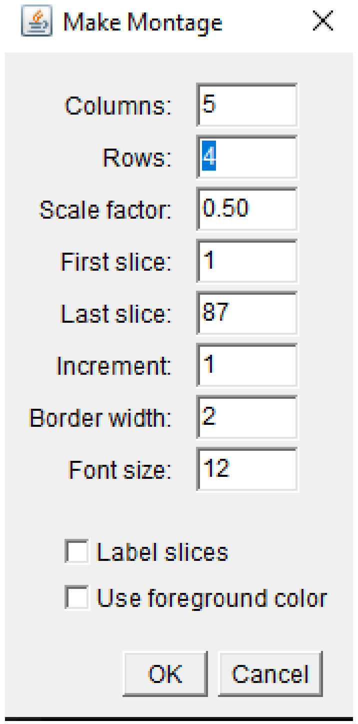
Montage tool

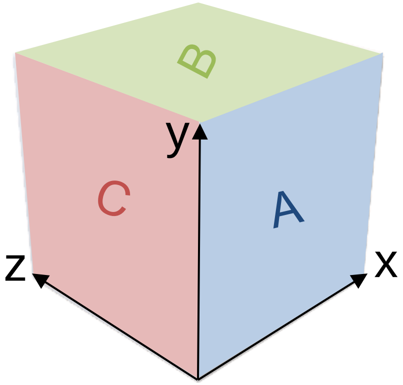
EXERCISE
Try out the tools in the Images > Stack menu and with Example 2

Open a stack, then:
Image > stack > Make montage...

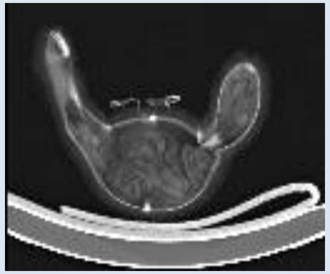




Z-Stacks: Reslice (orthogonal rotation)



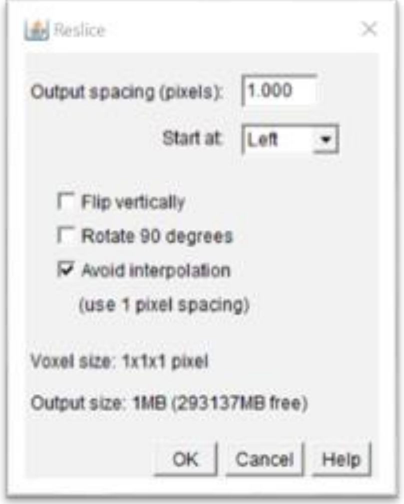
(Plane A) XY view

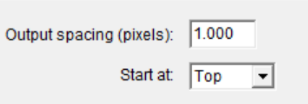


X=128
Y=107
Z=124

Bold: on-screen depicted dimensions

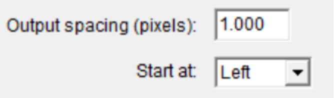
Image > stack > Reslice...





Reslice from top/bottom
(Plane B) XZ view

X=128
Y=124
Z=107



Reslice from left/right
(Plane C): YZ view

X=107
Y=124
Z=128

Other tools:

Radial reslice
orthogonal recon-structions of a stack by rotating a line ROI around one end of its center. Useful for data with rotational symmetry

Dynamic reslice
Creates an arbitrary cross section along a user-defined line

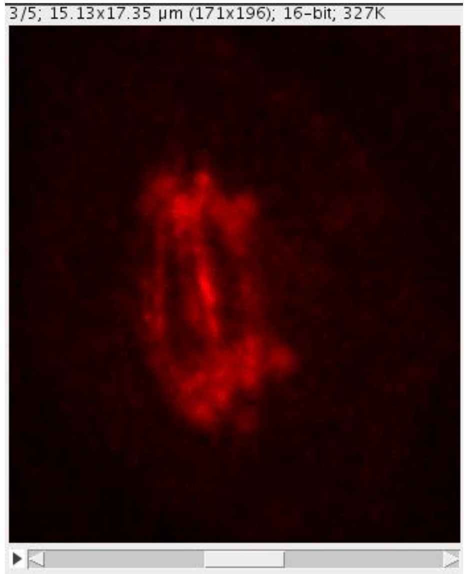
4. Hyperstacks

Hyperstacks are multidimensional images, extending image stacks to four (4D) or five (5D) dimensions:

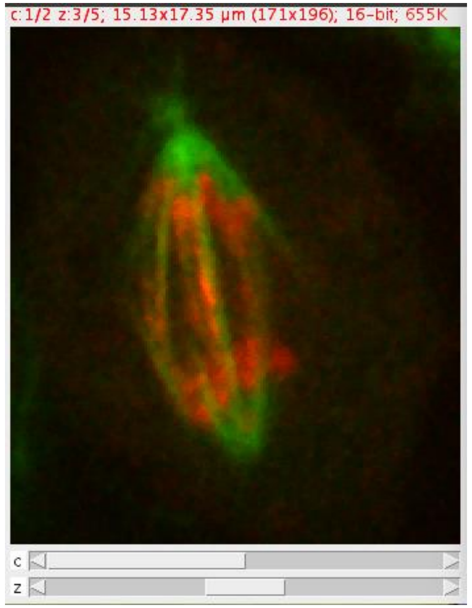
x (width),
y (height),
z (slices),
c (channels or wavelengths)
t (time frames)

Hyperstacks are displayed in a window with 2 or 3 labelled scrollbars. Similarly to the scrollbar in stacks, including a play/pause icon.

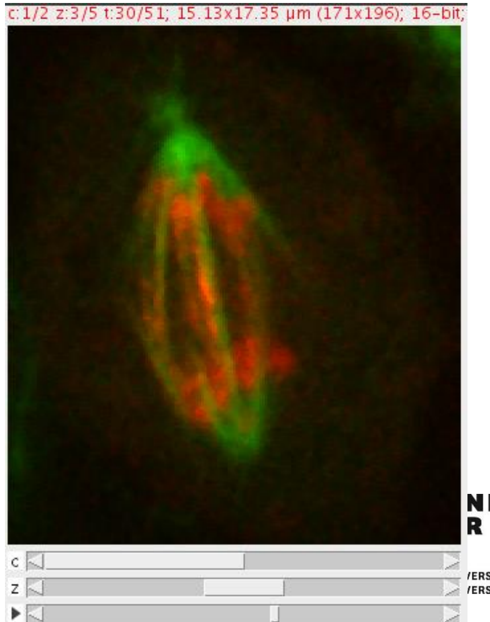
3D stack (z=5)



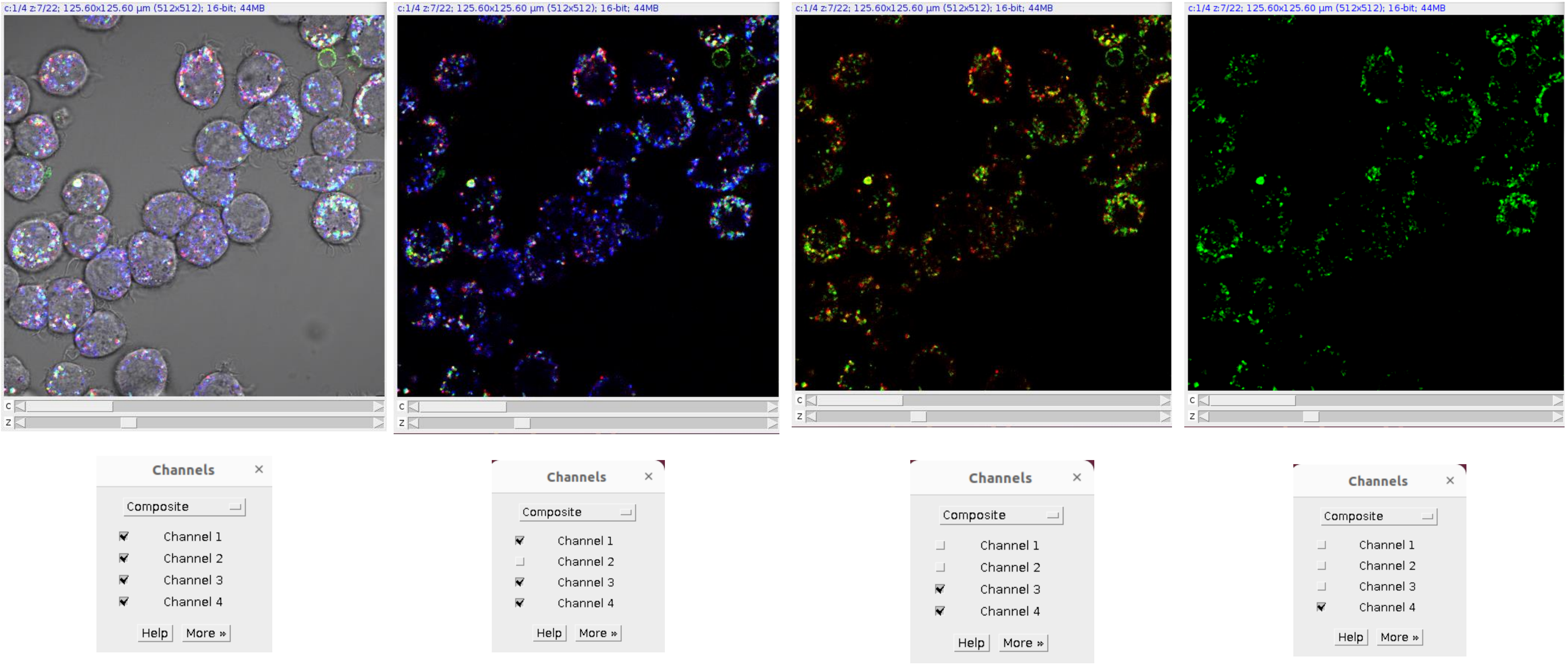
4D stack (z=5, C=2)



5D stack (z=5, C=2, t=51)



Hyperstacks

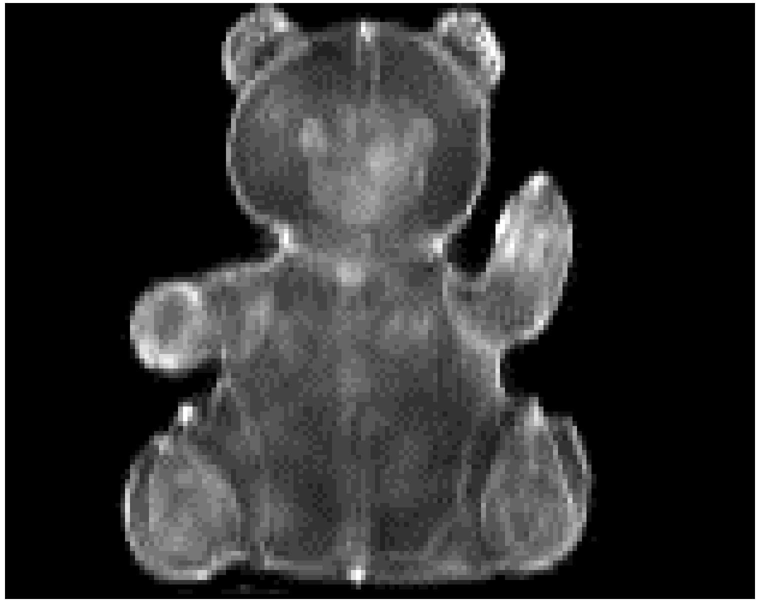


Videos/timelapse

Out of the box, ImageJ has limited support (no codecs, no audio). However, it can open/close uncompressed AVI formats.

Videos/timelapse

Can be understood as a 3D stack where the third dimension is not spatial but temporal



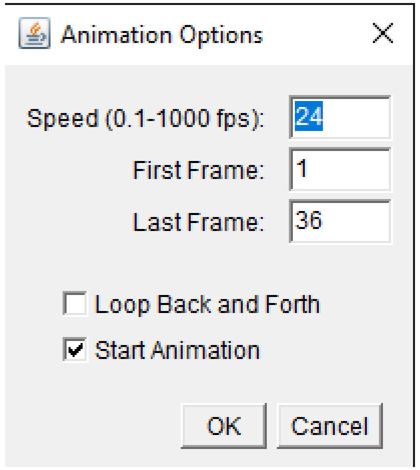
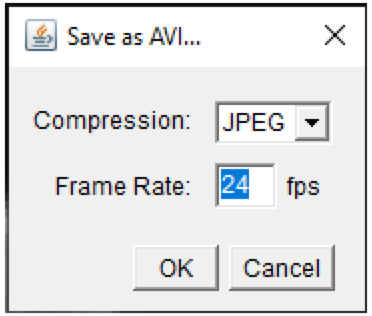


Image > Stacks > Animation > Animation options



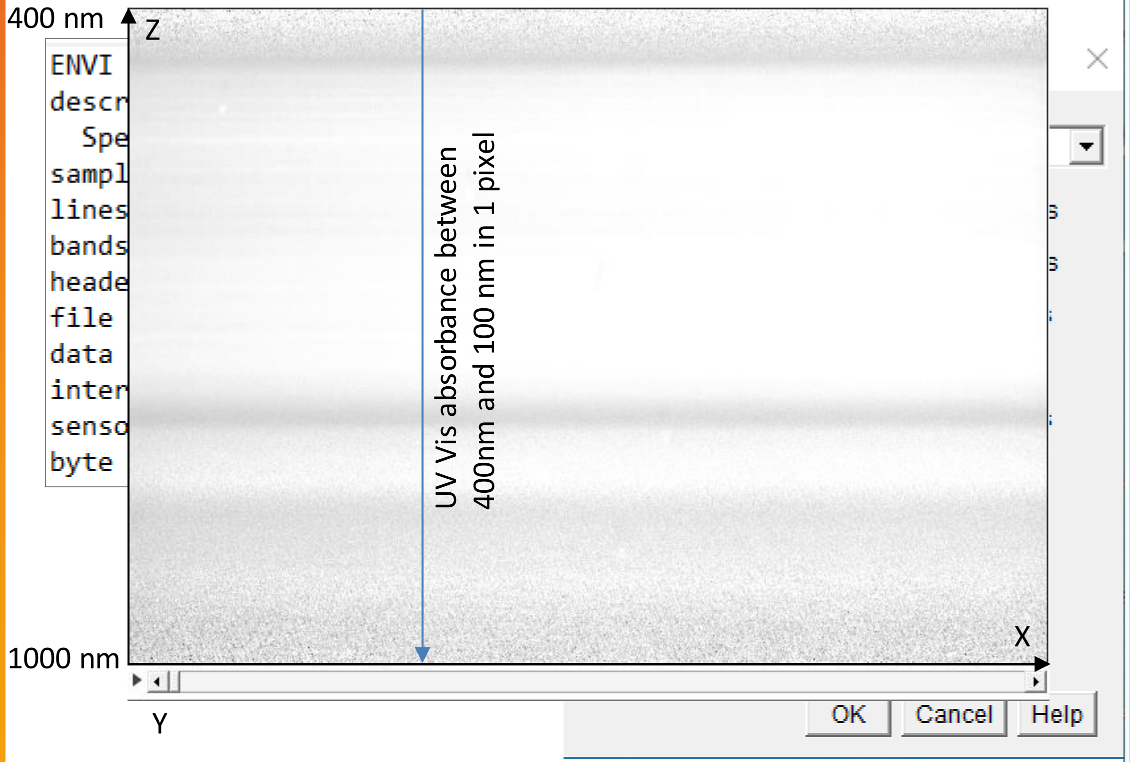
File > Save as... > Avi...

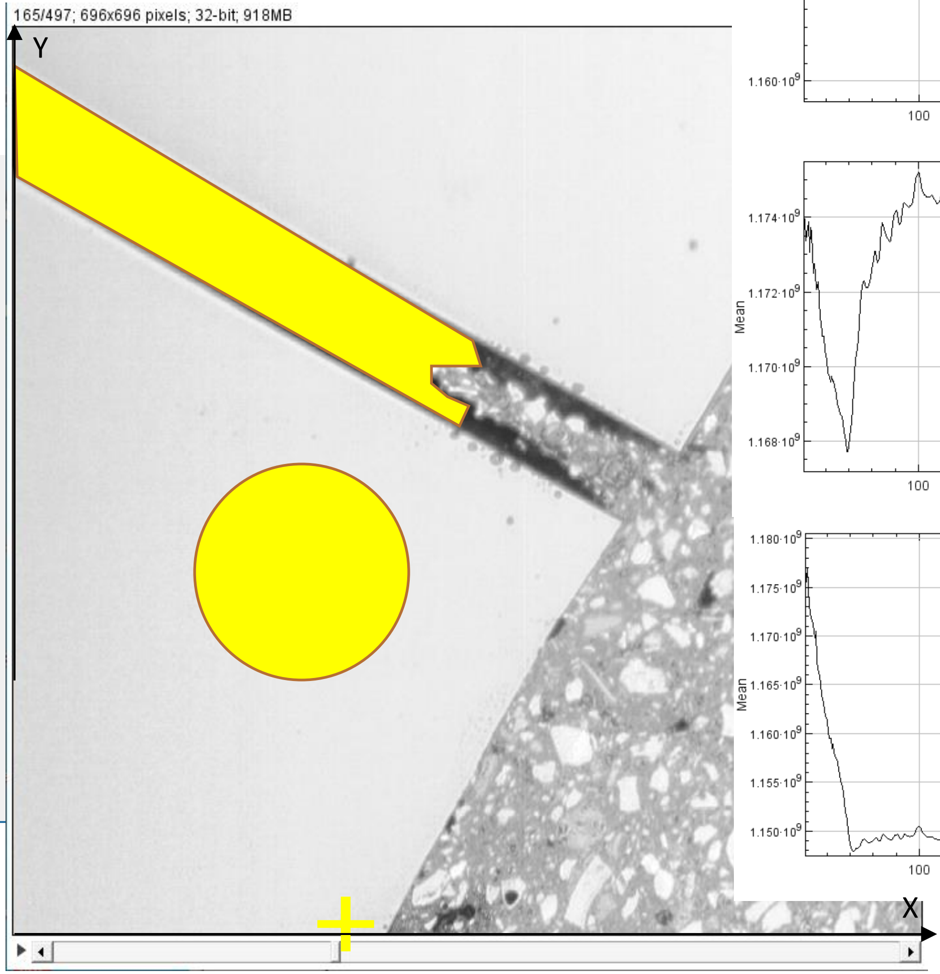
5. Custom multi-dimensional datasets

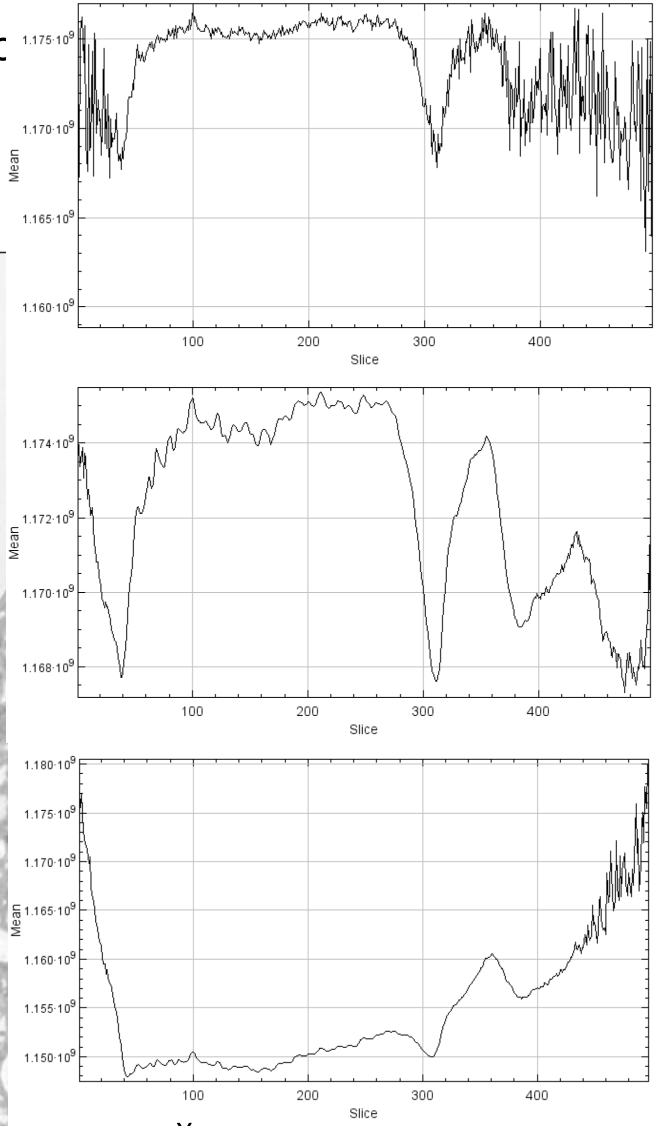
FIJI does not interprete your data, just reads it (remember Lecture 1) according to a model yo

Image > stack > Plot Z-axis profile…

Example: Cytoviva dataset







XY: image dimension
Z: spectral dimension

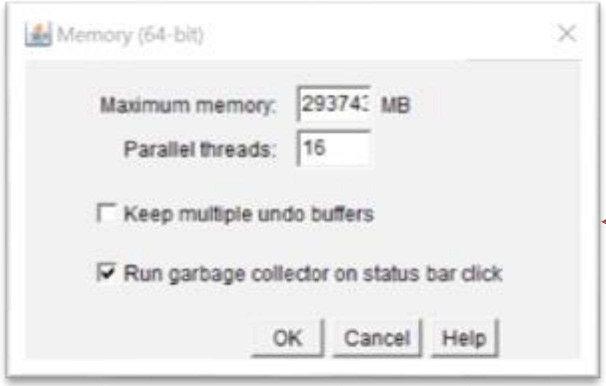
6. Virtual stacks

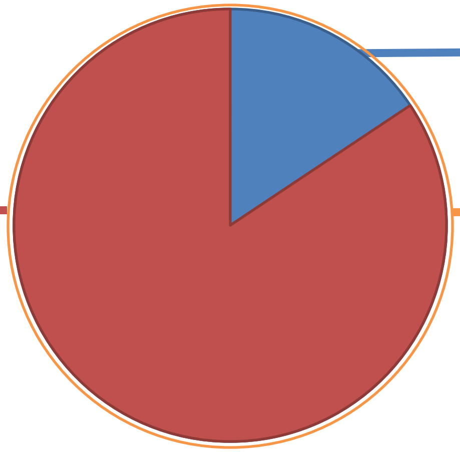
- Virtual stacks are disk resident (as opposed to RAM resident) datasets
- The only way to load image sequences that do not fit in your RAM.

1. Virtual stacks are read-only, so changes made to the pixel data are not saved when you switch to a different slice

2. Commands like Crop [X] may create a RAM issue since any stack generated from commands that do not generate virtual stacks will be RAM resident.

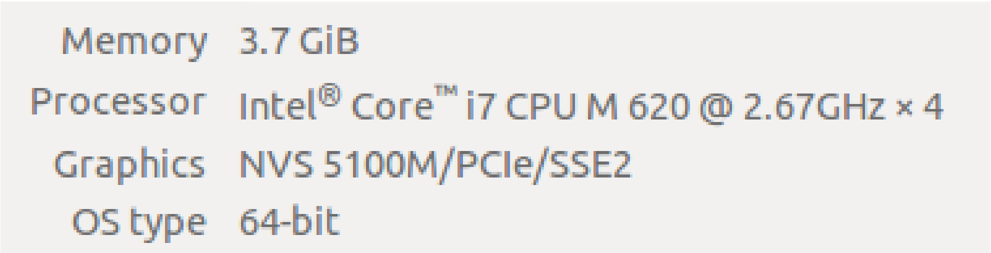
Edit > options >memory & threads will allow you to change the RAM allocated



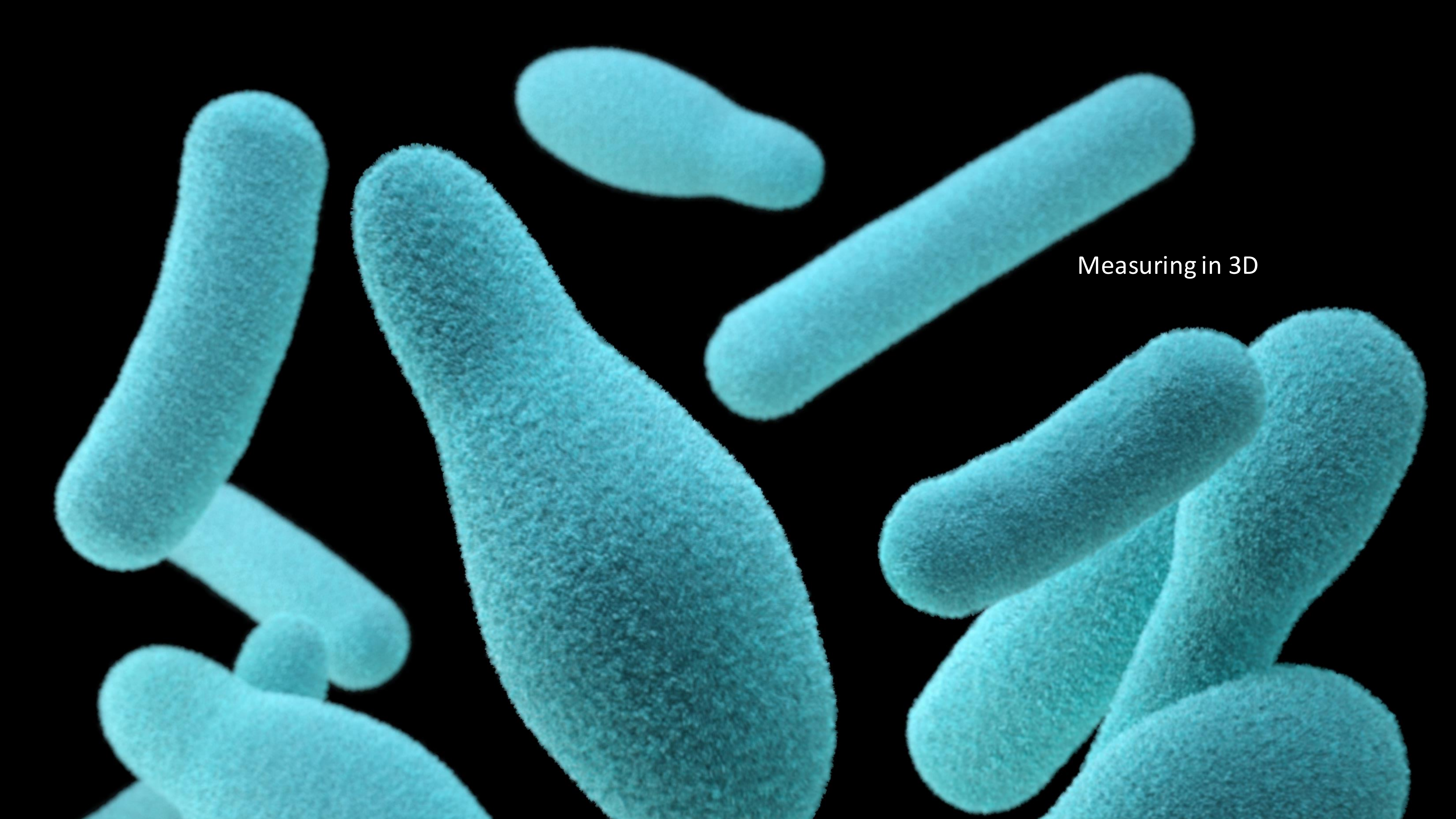


RAM for all other software

Total RAM of PC

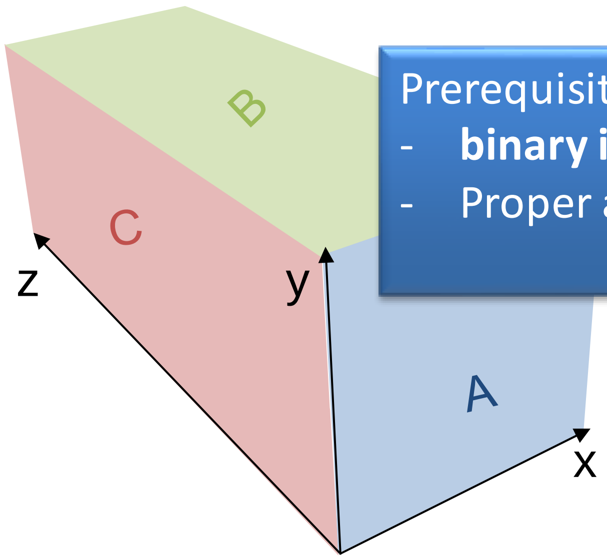


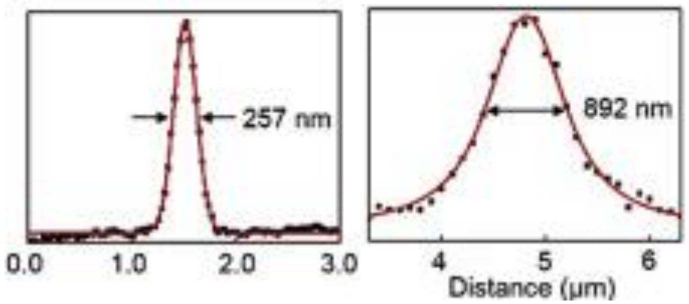
RAM for ImageJ


Measuring in 3D

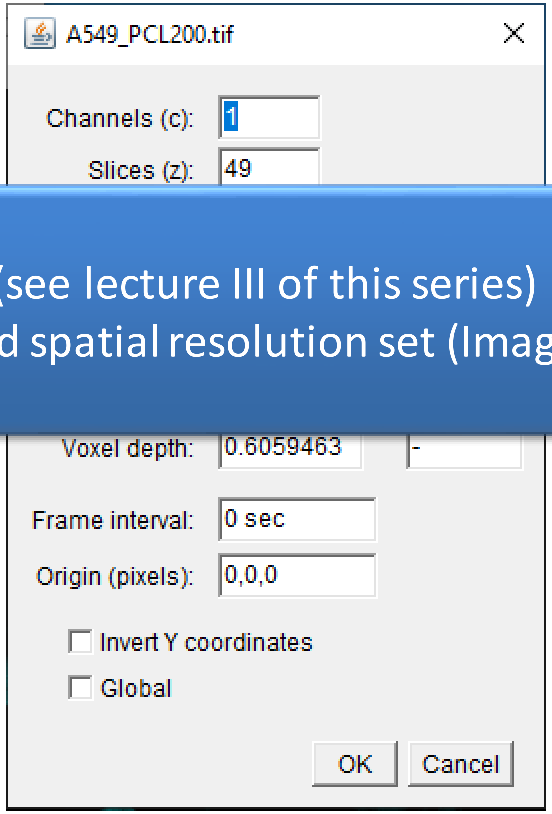
Note on non-isometric data (LSM, FIB, …)

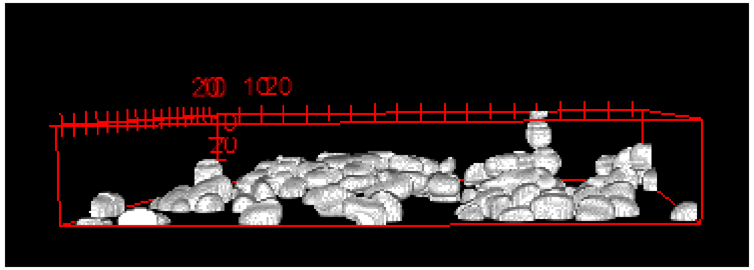
When the axial resolution (in Z) is not the same as the spatial resolution (in XY):
Image > Properties



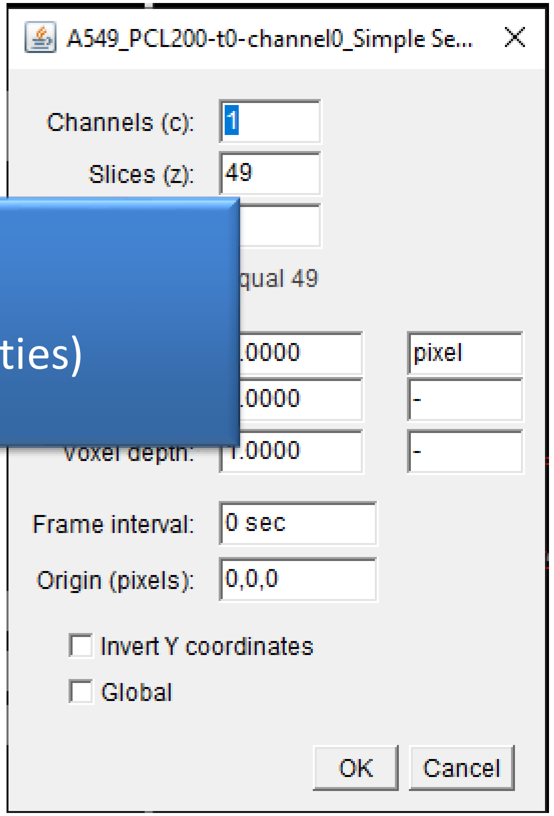


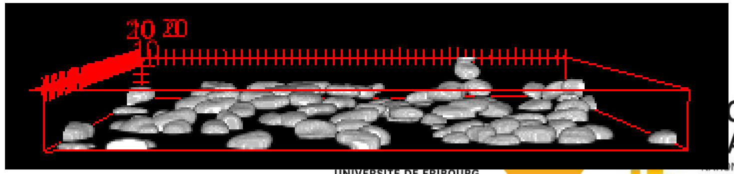
Original data





After segmentation (from iLastik)





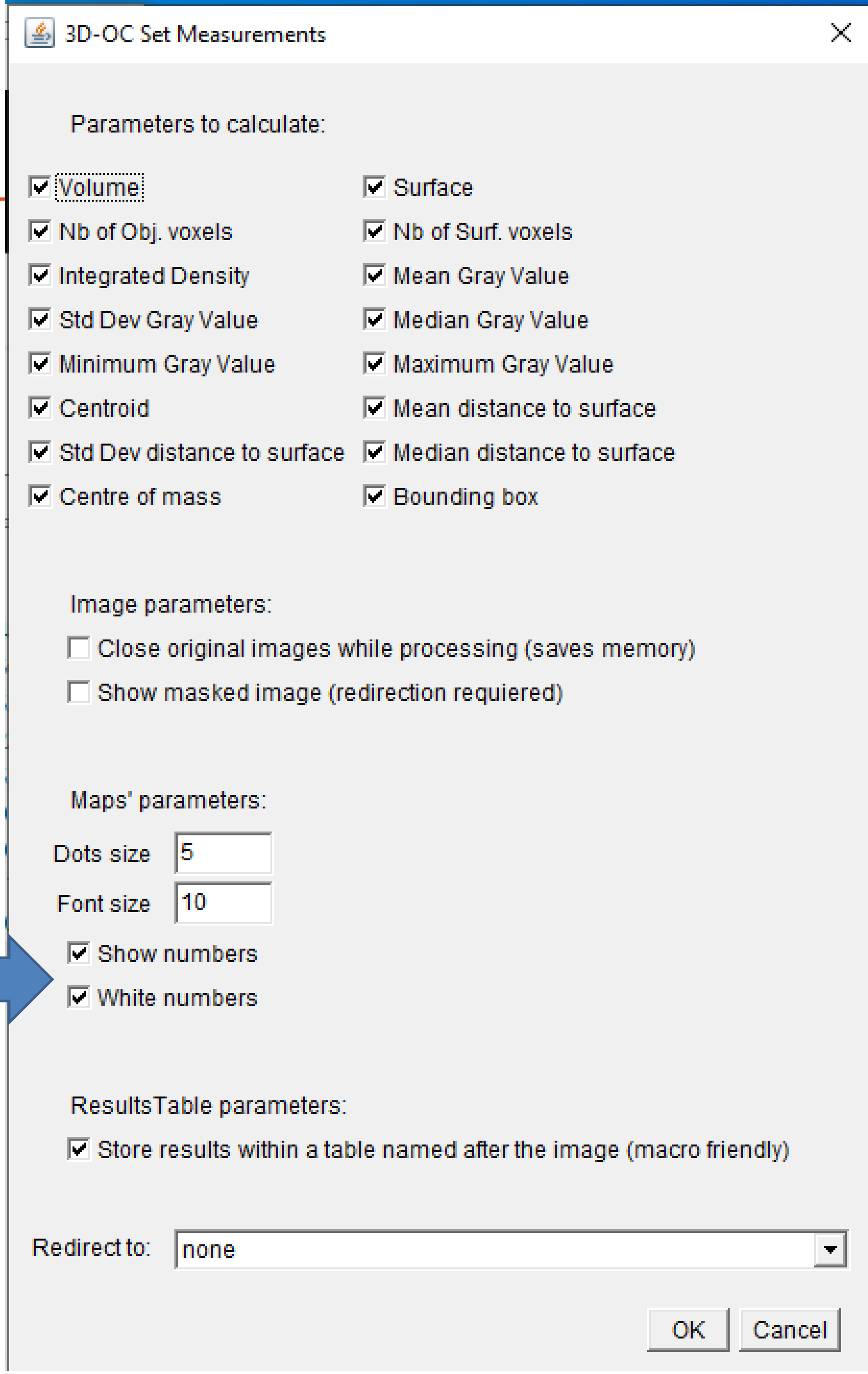
Prerequisites:
- **binary images** (see lecture III of this series)
- Proper axial and spatial resolution set (Image > Properties)

3D Objects counter

Analyze > 3D OC options

Allows to set the Measurements that will be performed

Turn off



3D Objects counter

Analyze > 3D Objects Counter
Similar to ‘Measure particles’, but: Threshold can be set

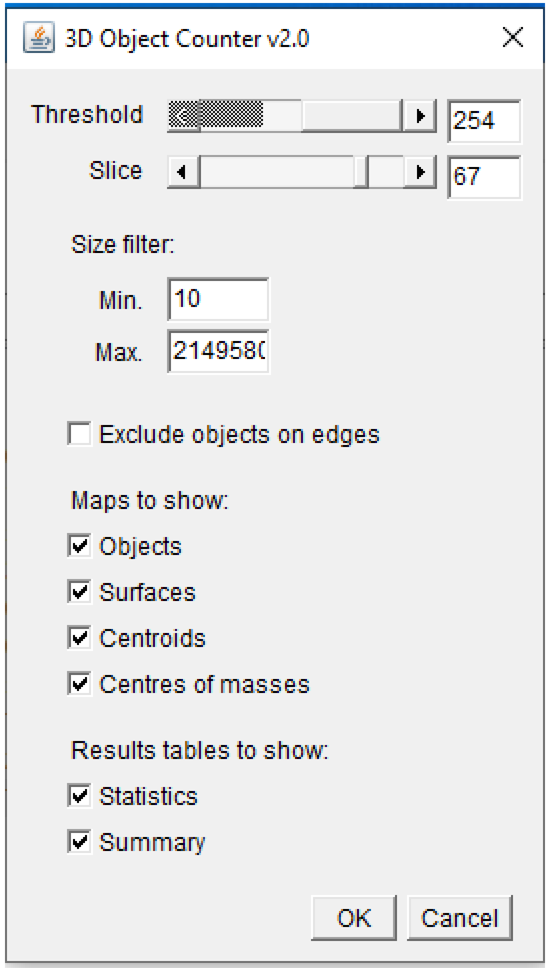
(Poor) thresholding attempt

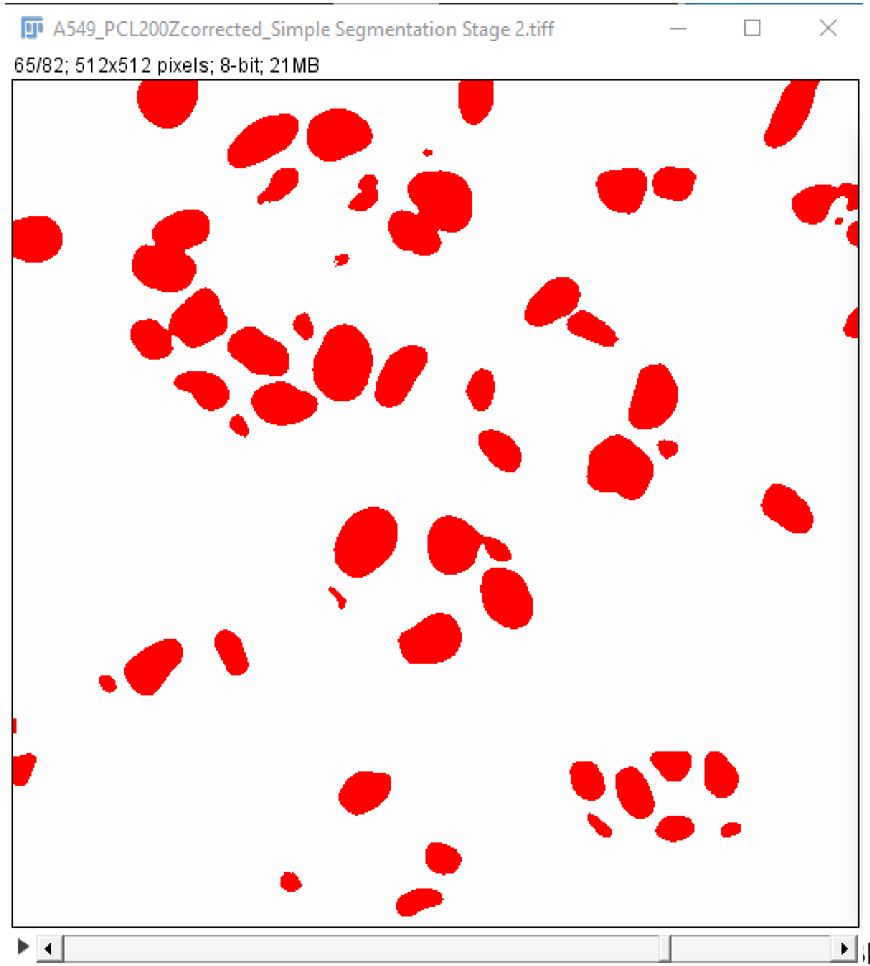
Move through stack

Eliminate noise or large clumps

Eliminate partial objects

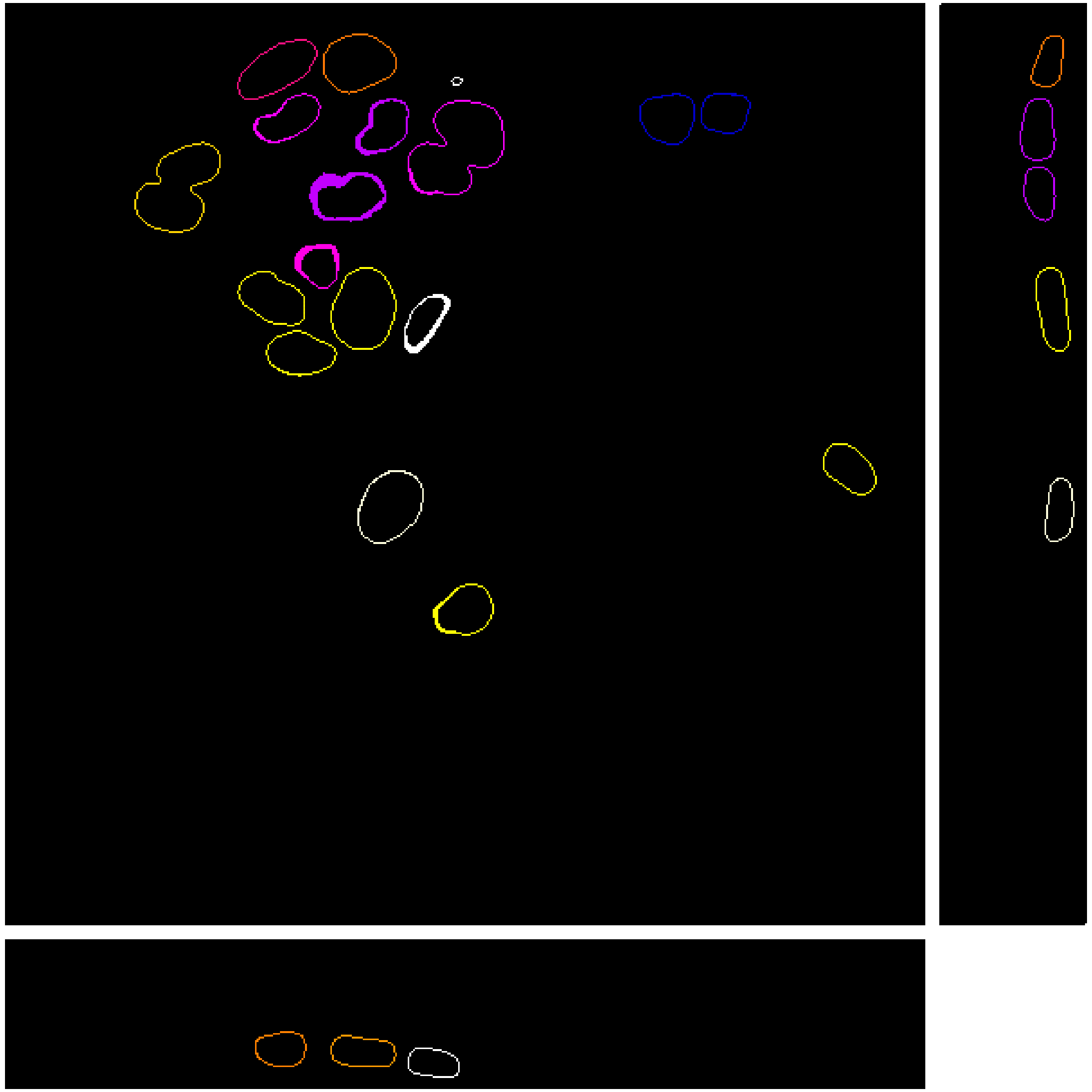
Output

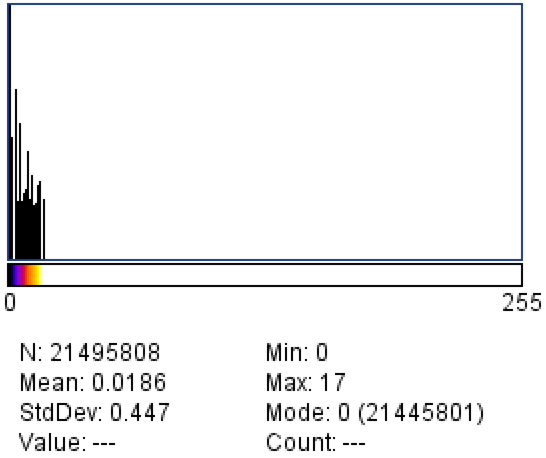




3D Objects counter: Output

Volume
Surfaces





Check the Look up table

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3D Objects counter: Output

Statistics for A549_PCL200-t0-channel0_Simple Segmentation Stage 2-1.tiff

File Edit Font

Volume (micron^3)	Surface (micron^2)	Nb of obj. voxels	Nb of surf. voxels	IntDen	Mean	StdDev	Median	Min	Max	X	Y	Z	Mean dist. to surf. (micron)	SD
1408.099	1201.742	17891	3974	4562205	255	0	255	255	255	382.541	60.942	35.786	7.534	2.
2118.878	1497.572	26922	6070	6865110	255	0	255	255	255	197.877	89.038	33.429	9.103	3.
647.894	585.138	8232	2013	2099160	255	0	255	255	255	155.839	63.630	34.102	5.357	1.
643.565	558.641	8177	2092	2085135	255	0	255	255	255	172.481	142.474	33.814	5.120	0.
697.950	649.447	8868	2294	2261340	255	0	255	255	255	151.261	37.186	36.641	5.656	1.
1707.412	1195.269	21694	4806	5531970	255	0	255	255	255	250.222	80.860	36.413	7.609	2.
747.534	633.551	9498	2437	2421990	255	0	255	255	255	195.318	34.086	36.335	5.452	1.
1255.649	993.930	15954	3658	4068270	255	0	255	255	255	96.251	102.124	37.521	6.826	2.
682.367	581.667	8670	2050	2210850	255	0	255	255	255	148.327	164.184	36.652	5.336	1.
1001.277	758.804	12722	3025	3244110	255	0	255	255	255	198.978	170.228	37.802	6.027	1.
598.783	537.068	7608	1945	1940040	255	0	255	255	255	162.643	195.234	39.588	5.080	1.
644.431	582.112	8188	2171	2087940	255	0	255	255	255	234.675	177.968	41.452	5.394	1.

3D Objects counter

EXERCISE
Open Example 7 and calculate the volume of the objects using the 3D object counter.

1. Check calibration	Image > Properties… (for 3D spatial and axial settings)
2. Do the analysis	Analysis > 3D object counter
3. Change the settings and repeat	Analysis > 3D OC settings

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3D suite (plugin)

Help > Update … > ☑ 3D ImageJ Suite https://sites.imagej.net/Tboudier/

Plugins > 3D suite

- Analysis ▸
- Binary ▸
- Filters ▸
- Relationship ▸
- Segmentation ▸
- 3D Manager V4 (testing)
- 3D Manager V4 Macros
- 3D Manager
- 3D Manager Options
- Spatial ▸
- Tools ▸

See next slide

(Morphological) filters in 3D

Local Linear filters in 3D

Measuring distances (e.g. border to border)

Binary segmentation tools (e.g. 3D watershed)

(experimental)

(scripting)

ROI 3D manager (see next)

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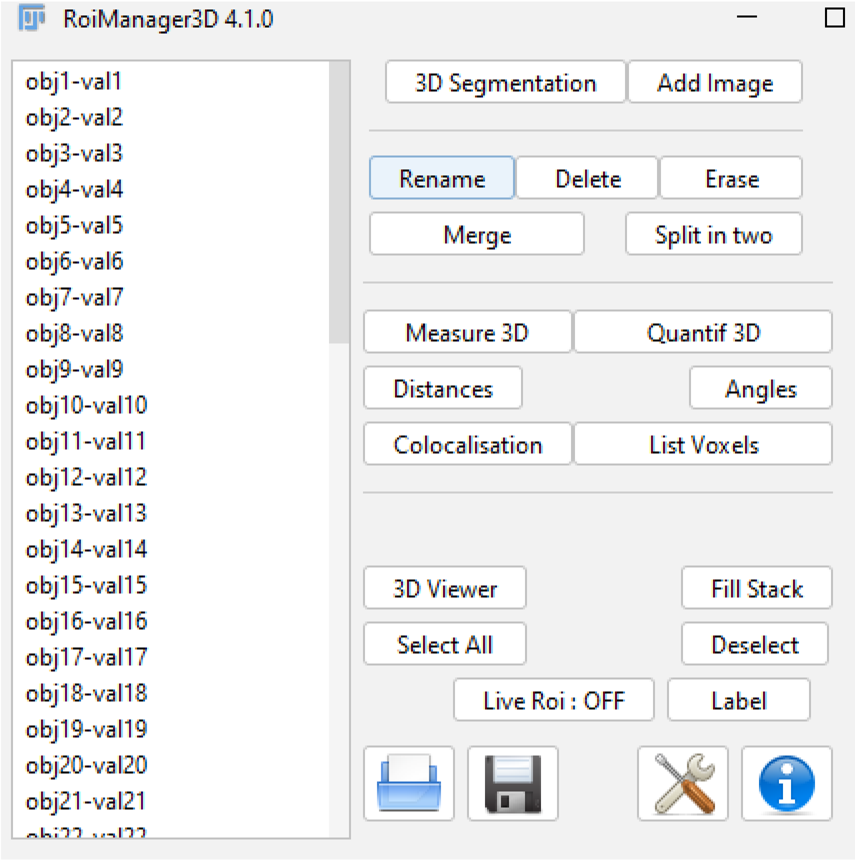
3D suite (plugin)

	Input	Output
3D Intensity Measure	Raw data and binary mask	Intensity stats of each object
3D Centroid	Binary mask	Position of centroid of each object (X,Y,Z)
3D Volume	Binary mask	Volume of each object
3D Surface	Binary mask	Surface of each object
3D Distance Contour	Binary mask	Distance stats between center and shell
3D Feret	Binary mask	Caliper distances in 3D and ortho-planes
3D Compactness	Binary mask	Sphericity and 3D compactness
3D Ellipsoid measure	Binary mask	Goodness of fit measurements
3D RDAR	Binary mask	Ellipsoid: how much is sticking out
3D Mesh Measure (slow)	Binary mask	
3D Ellipsoid Fitting	Binary mask	Fitting measures to elliposoid
3D Convex Hull (slow)	Binary mask	3D convex hull

3D suite (plugin)

ROI3D manager

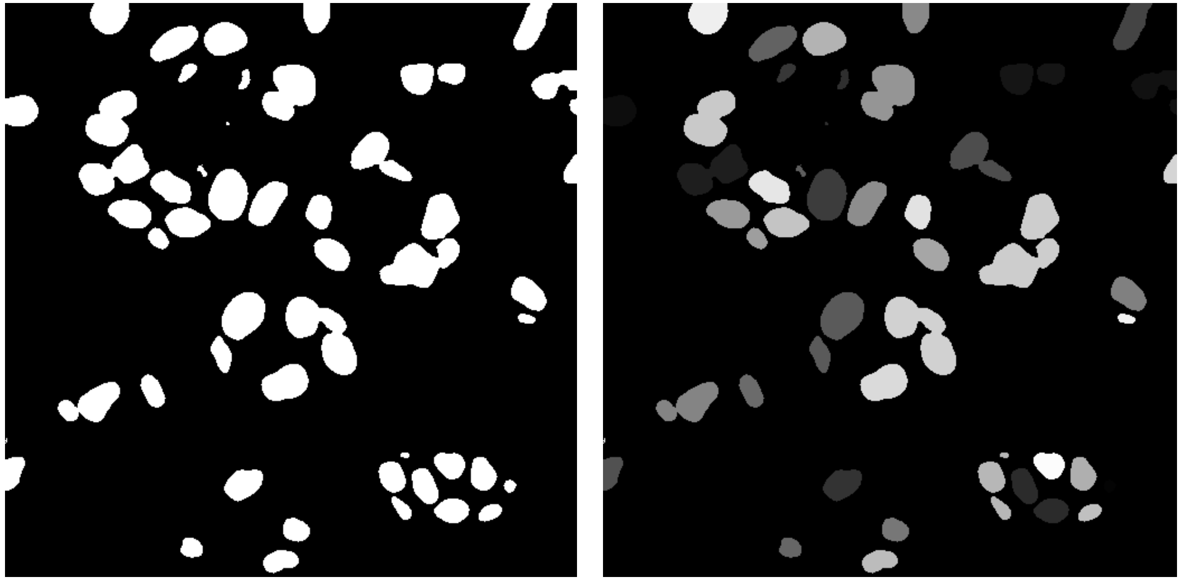


1. **3D segment (use binary Data!!)**

you get a new window with your objects in different shades

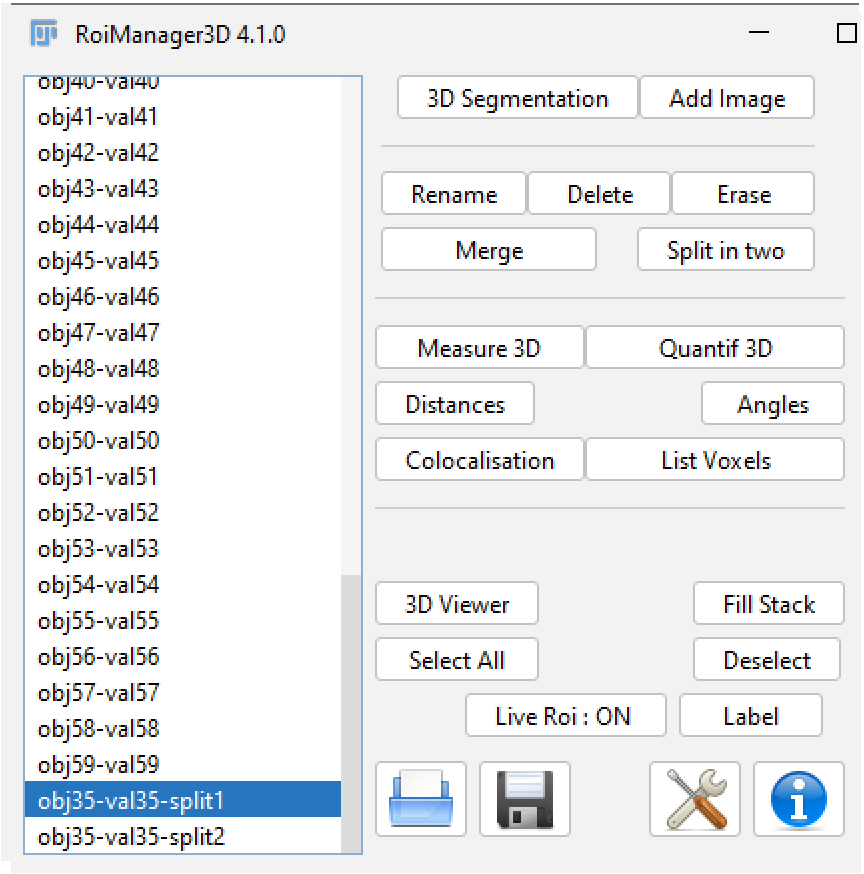
2. **Add an image**

this adds the objects

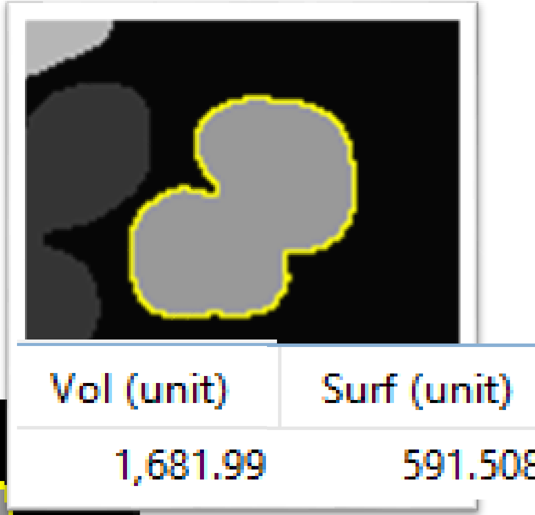


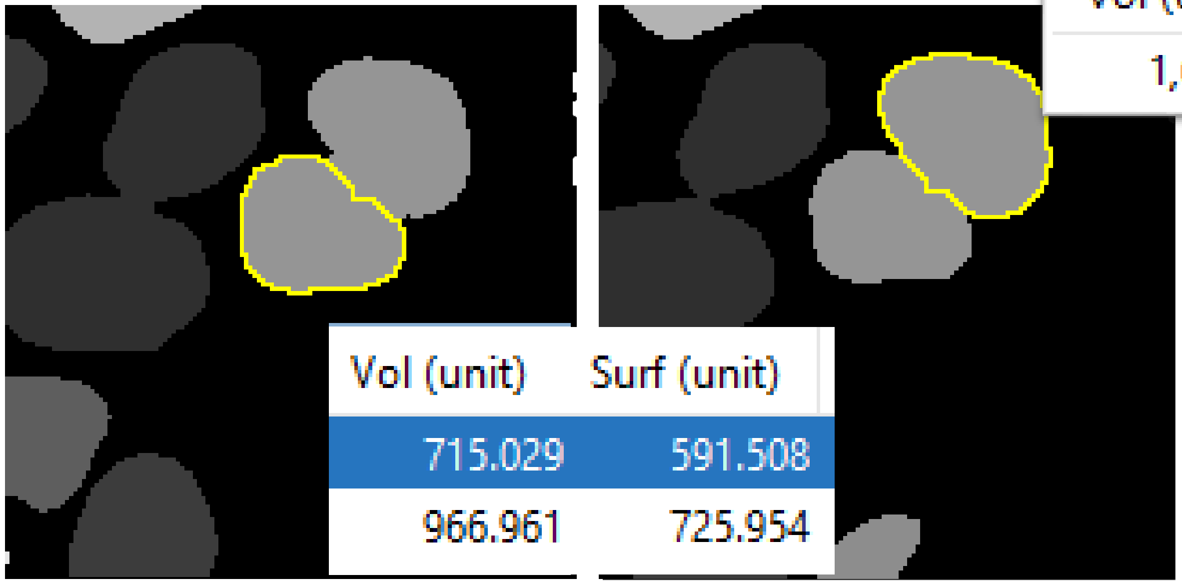
3D suite (plugin)

ROI3D manager



1. 3D segment (use binary Data!!)
2. Add an image
3. Click “Live ROI: OFF” (makes it “ON”)
4. From the list, select obj35-val35
5. Then click “split in two”





3D suite (plugin)

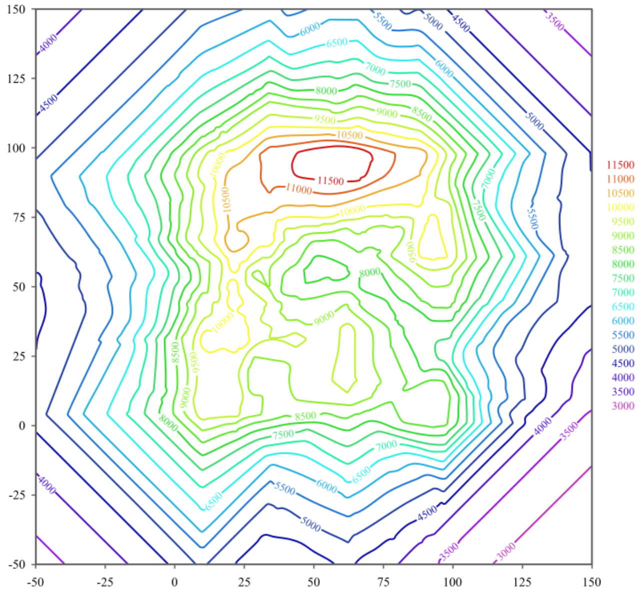
EXERCISE
Open Example 7 and calculate the volume of the objects using the 3D manager of 3D suite.
Try to split some objects in the 3D suite

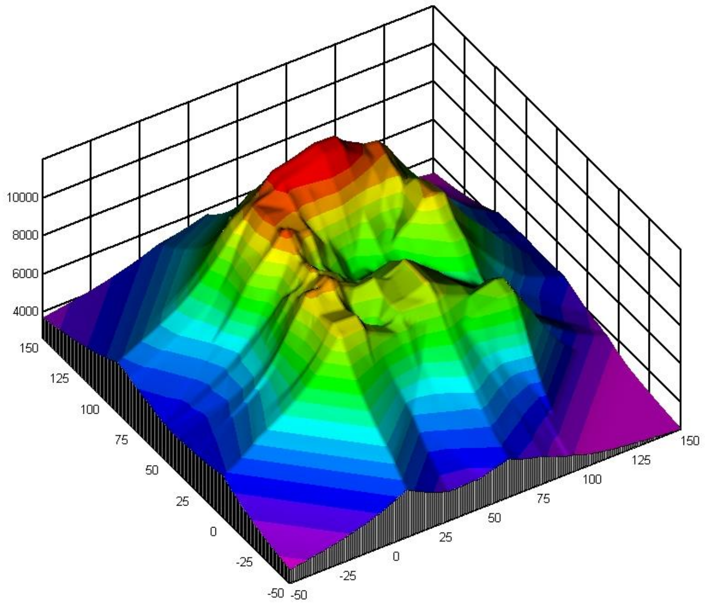
Image > Properties… (for 3D spatial and axial settings)
Analysis > 3D object counter (and 3D OC settings)
Plugins > 3D suite > 3D manager
- Segmentation
- Add image

Visualization of 3D data

Visualizing 3D data

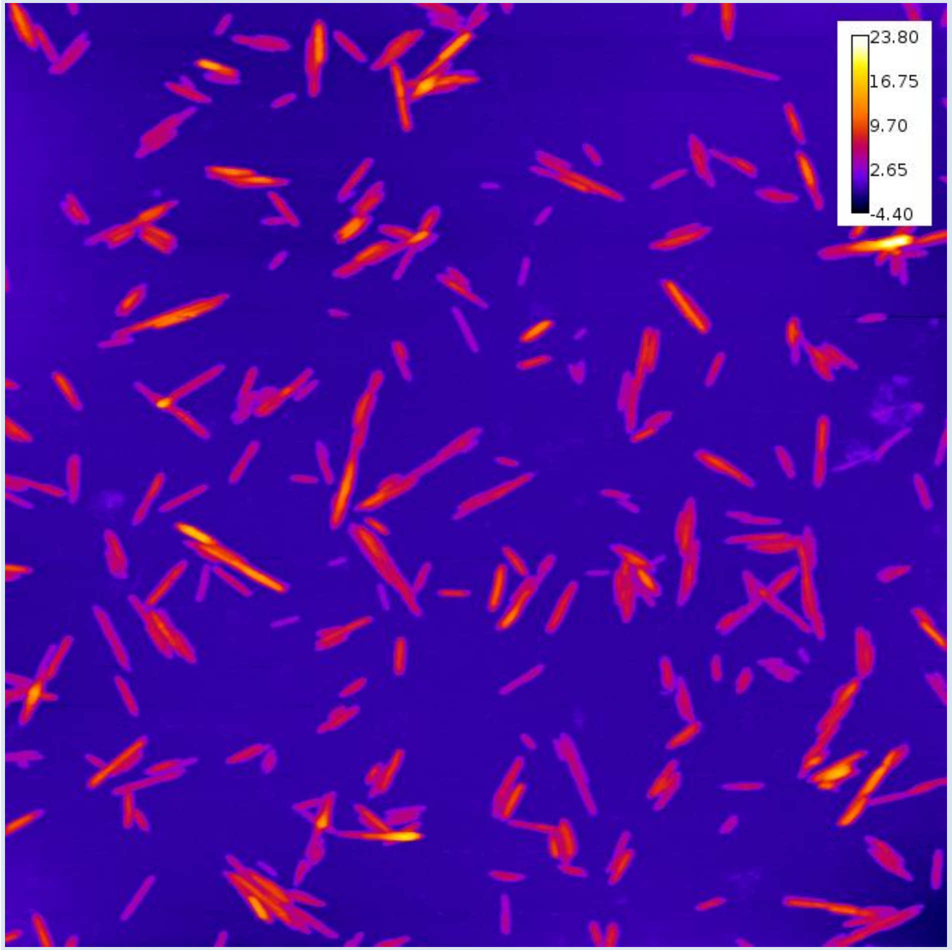
1. 2D depictions
2. Renderings
    1. Surface rendering
    2. Volume rendering





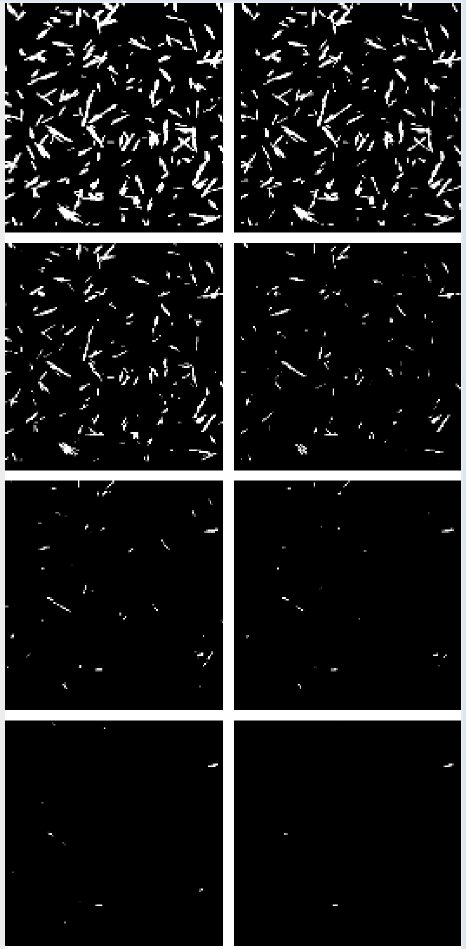
Visualizing 3D data: Depth encoded

AFM image

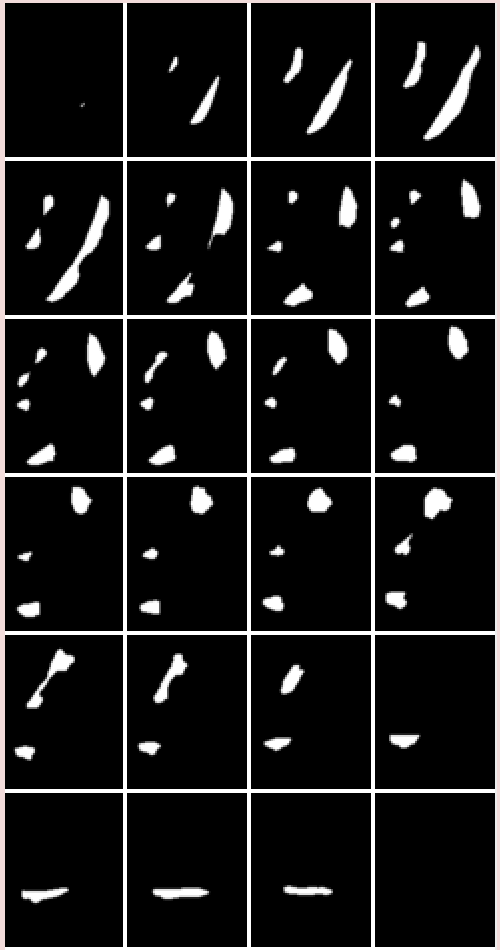


(= depth coded, Fire LUT)

AFM as 3D stack



3D stack

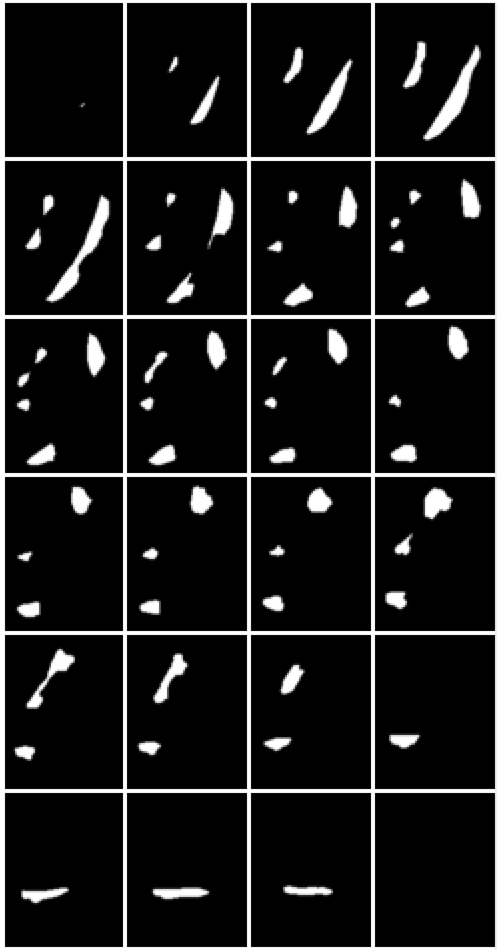


Depth coded 3D stack

Image > Hyperstacks > temporal color-coded

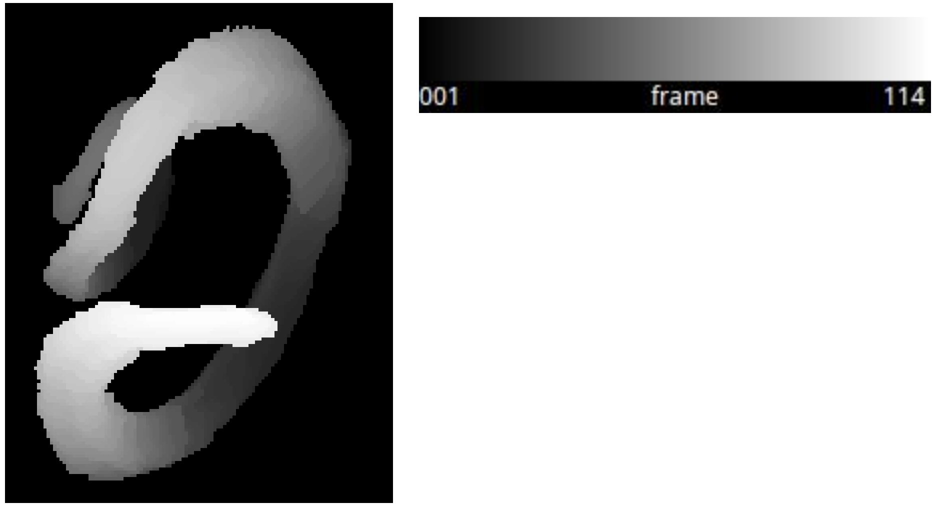
Visualizing 3D data: Orthogonal views and depth coding

EXERCISE
Open Example 3.

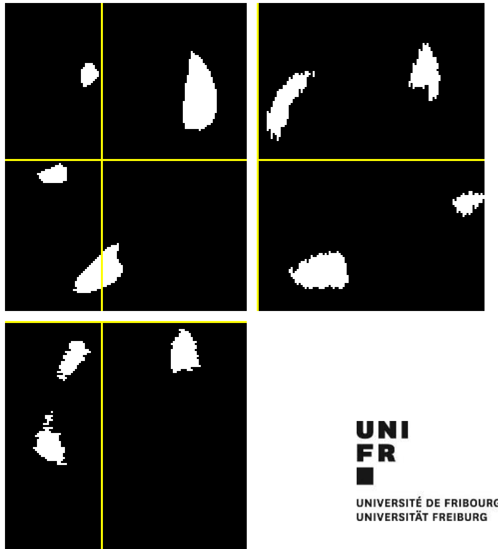


Try both:

Depth-encoded Color
Image > Hyperstacks > Temporal color-code
/ choose a LUT (e.g. Grays)



Orthogonal views
Image > stack > orthogonal views



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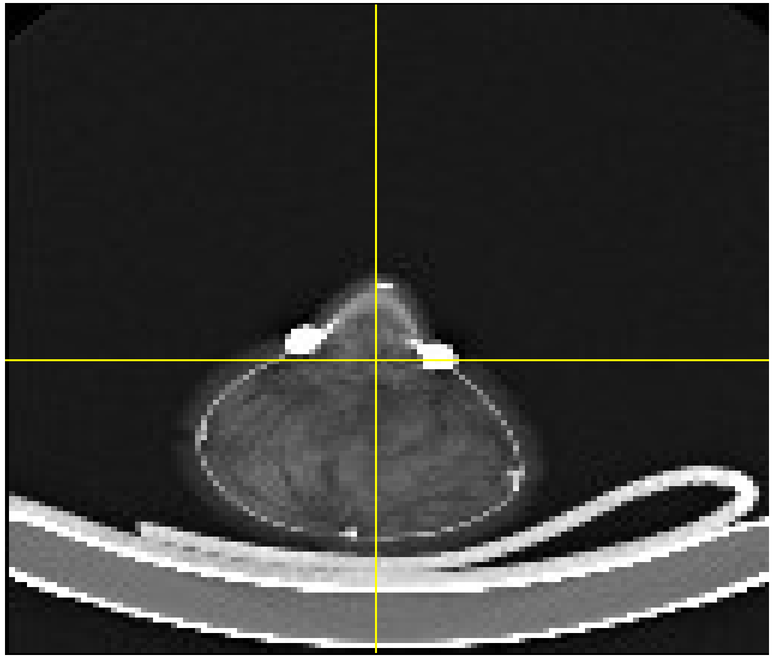
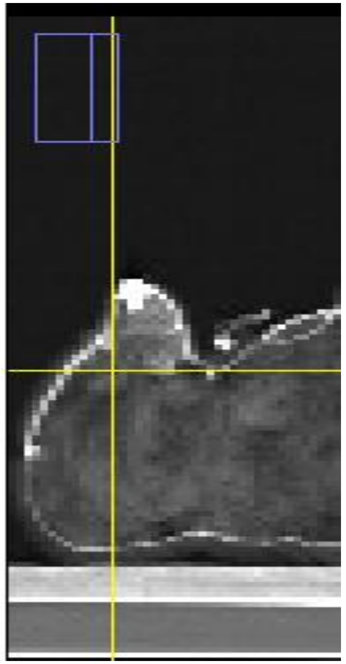
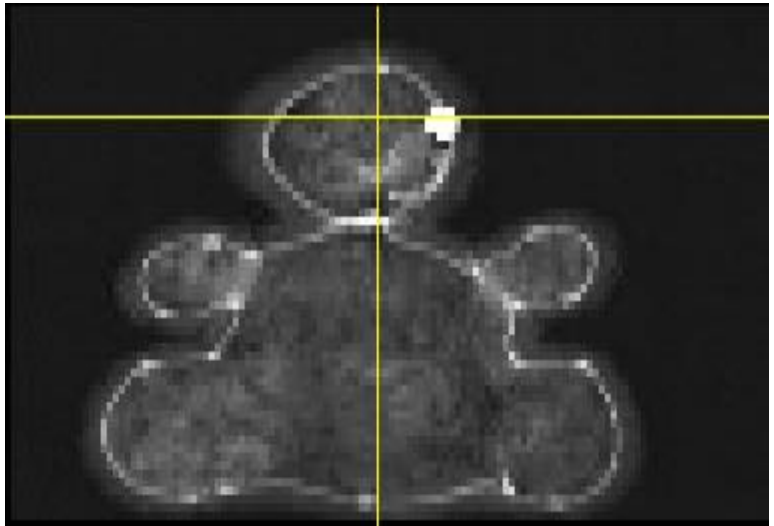
Visualizing 3D data: Orthogonal views







Orthogonal views

The intersection point of the three views follows the location of the mouse click and can be controlled by clicking and dragging in either the XY, XZ or YZ view.

XY and XZ coordinates are displayed in the title of the projection panels. The mouse wheel changes the screen plane in all three views.

How to get rid of the marker lines?
Image > Overlay > hide overlay (or remove overlay)

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3D rendering

Note: renderings require **interpretation** by the user. Hence, they are the convolution of the raw scientific data and the feature the user would like to see.

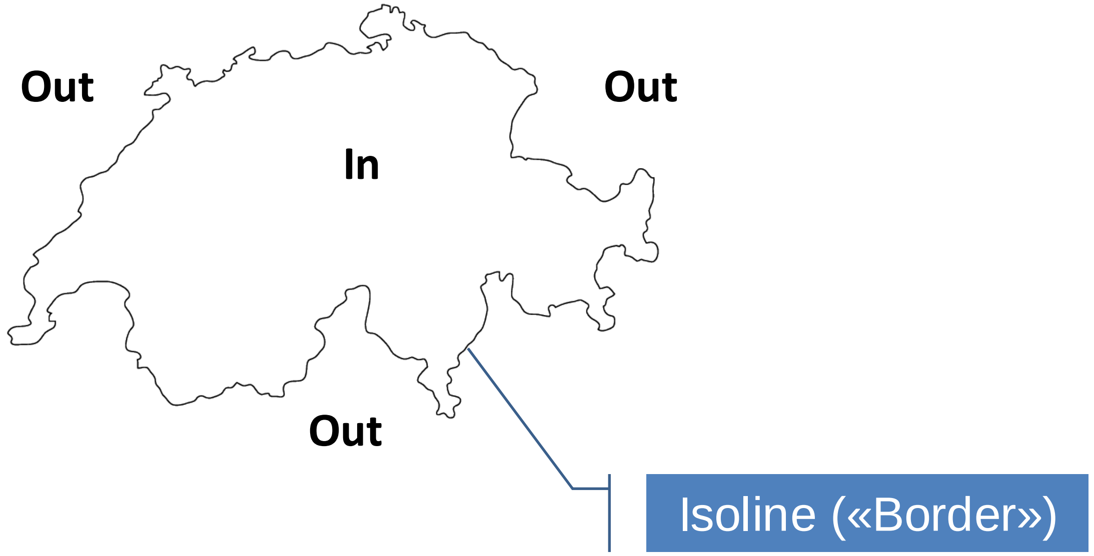
1. Surface rendering
= binary threshold-based

2. Volume rendering
= percentage threshold-based

Never publish only renderings. Always provide the raw data (i.e. orthogonal views)

Surface rendering: Isosurfaces

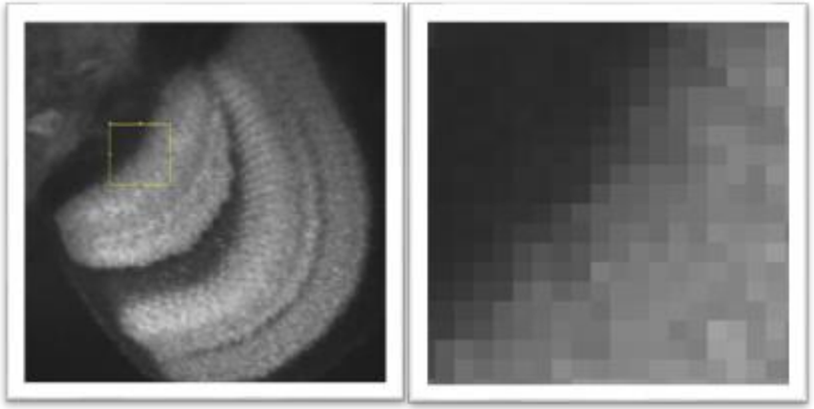
Isosurface
A three-dimensional analogue of an isoline. It is a surface that represents points of a constant value within a volume.

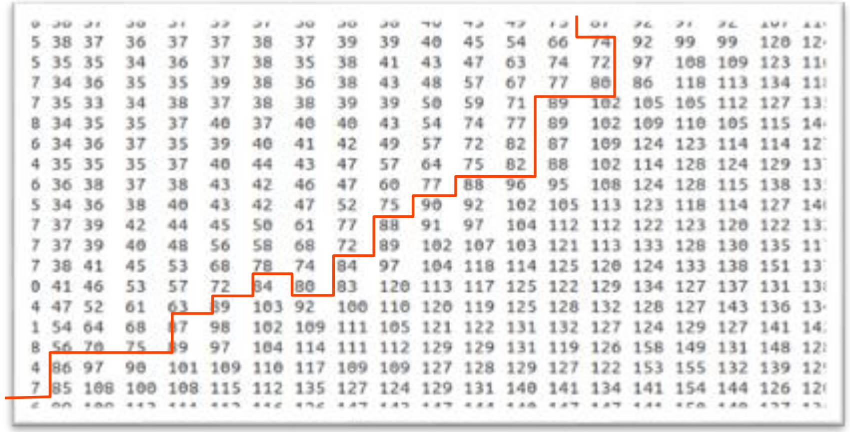


Step 1: Creating an isoline by thresholding
Step 2: voxels to mesh by marching cubes
Step 3: Mesh to rendering through shaders

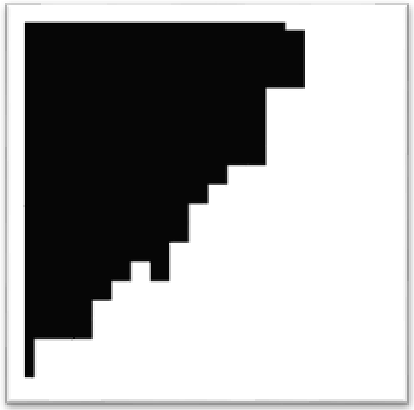
Isosurfaces: Step 1: Thresholding the voxels

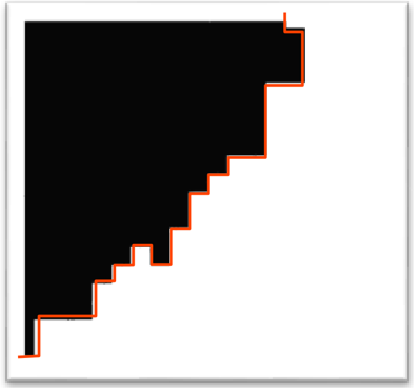
Original





Binary





Threshold = 83

Edge only = isoline

A threshold is calculated

- Pixel value > threshold, the voxel is considered to contain the signal (=object).
- Pixel value < threshold, the voxel is considered not to contain the signal (=background).
- This classification system is binary; it defines each voxel as containing either 100% or 0% of the signal
- Once classified, a surface is defined as the boundary between the pixels (=isoline)

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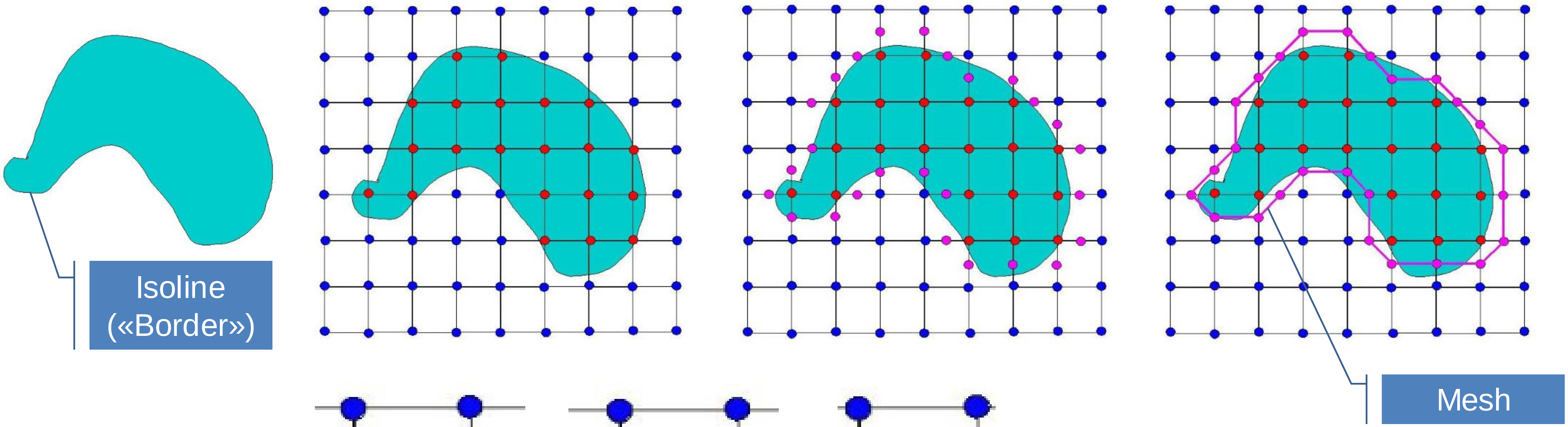
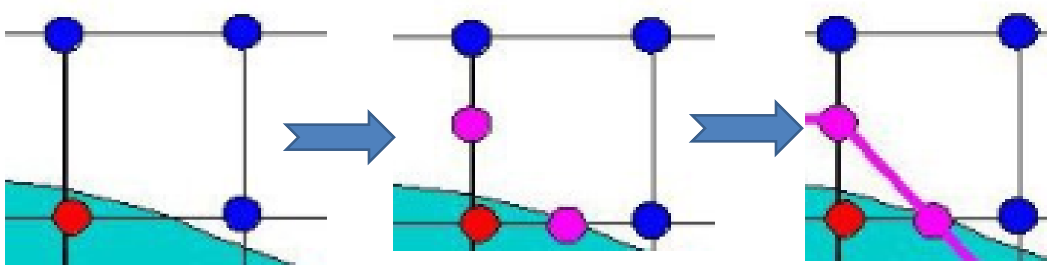
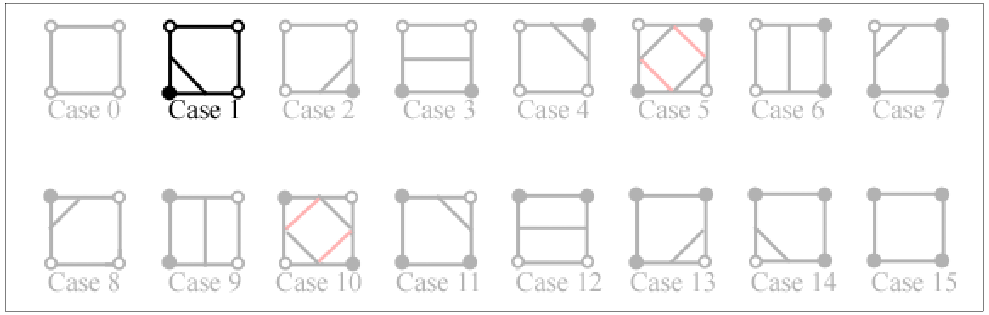
Isosurfaces: Step 2: **Isoline/Voxel to mesh conversion**



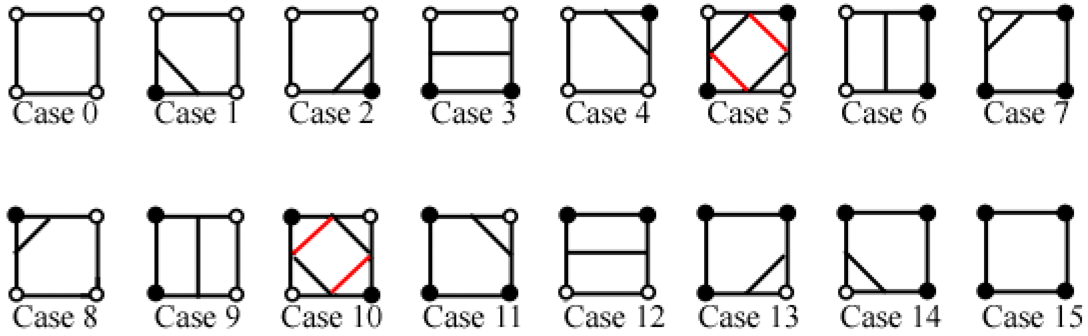


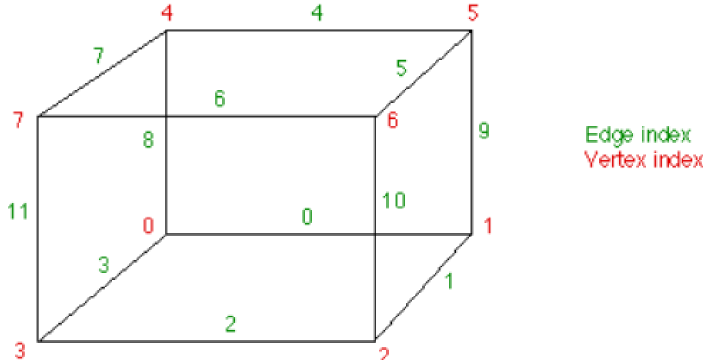


Marching squares

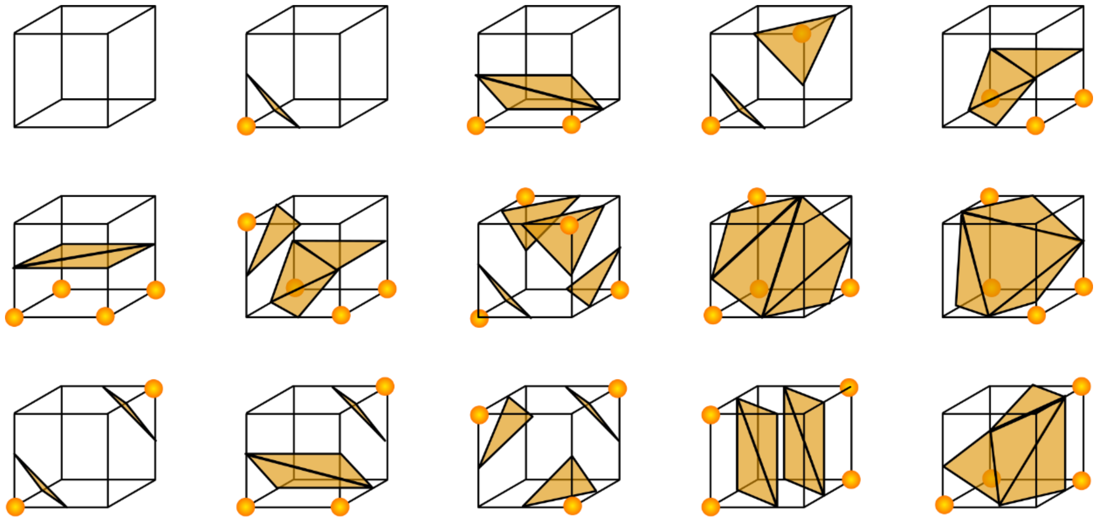
Isosurfaces: Step 2: **Voxel to mesh conversion**

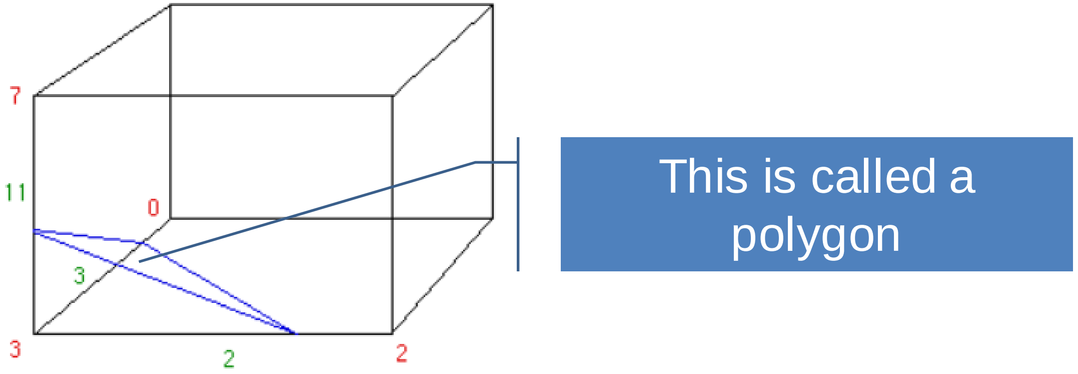
Marching squares





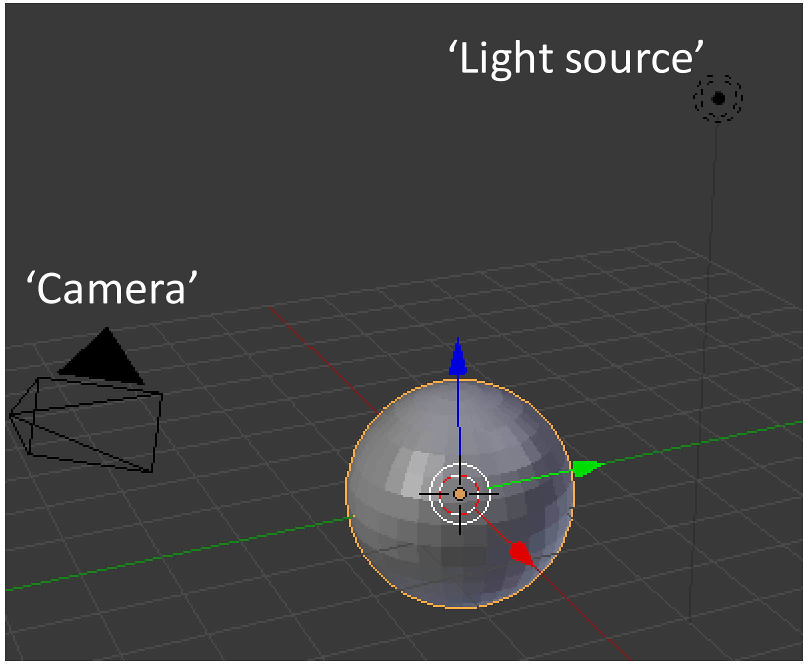
Marching cubes

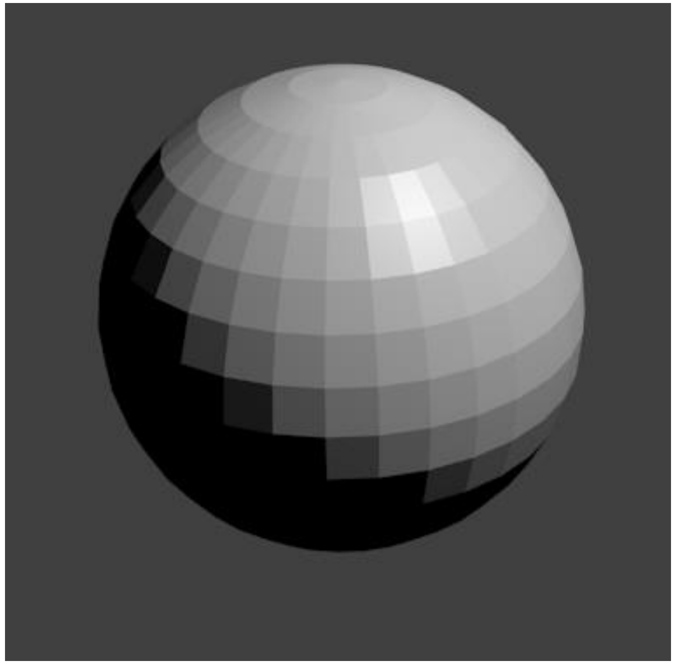




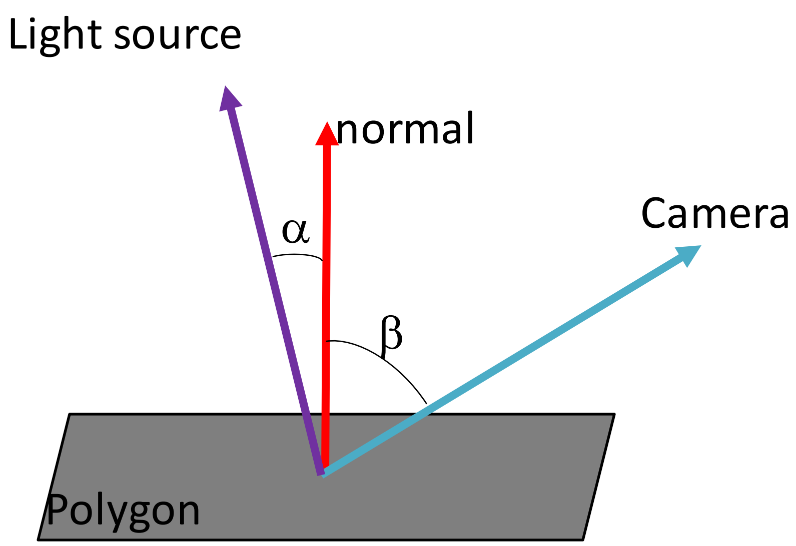
Intensities -> Binary -> 64 predefined values / marching cubes

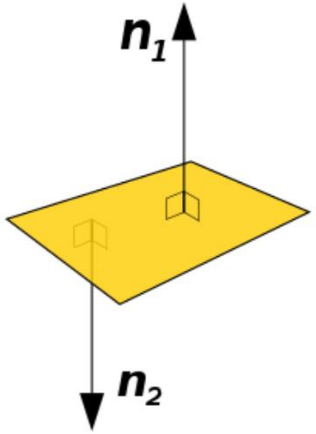
Isosurfaces: Step 3: **Reflection and intensity**





Polygons rendered without shader (flat)





Normal: vector perpendicular to the polygon

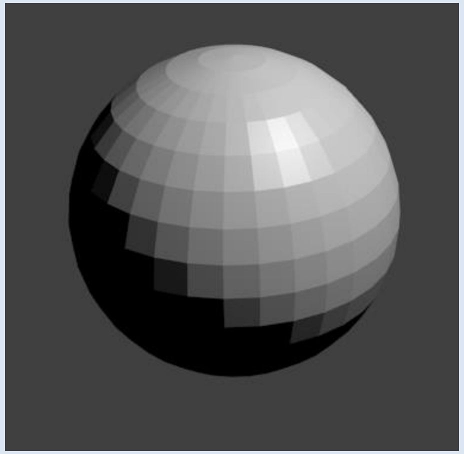
The normal defines the color (or shade) of the polygon

α = angle between light and normal
β = angle between camera and normal

The smaller the difference between and ,
The brighter the polygon

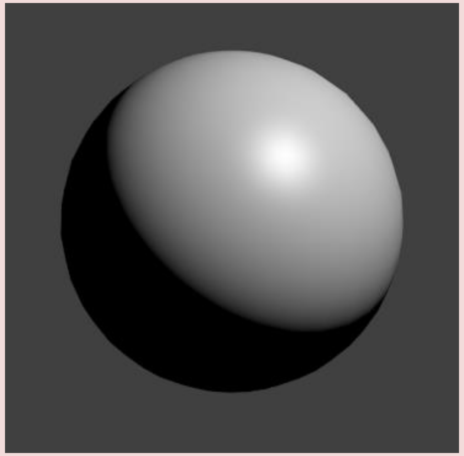
Isosurfaces: Step 3: **Illumination**

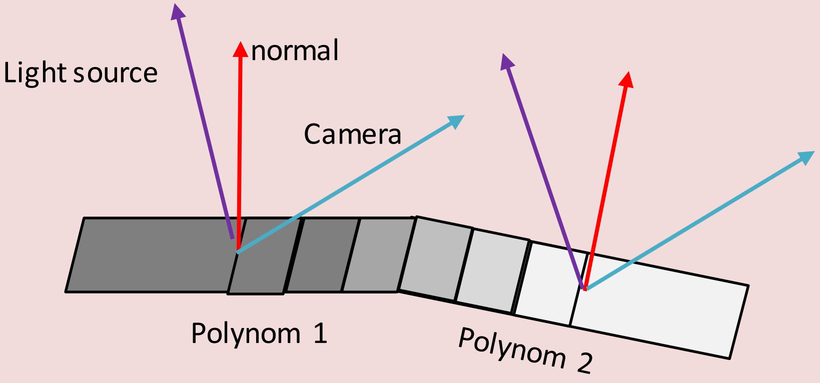
No shader



Gouraud shading

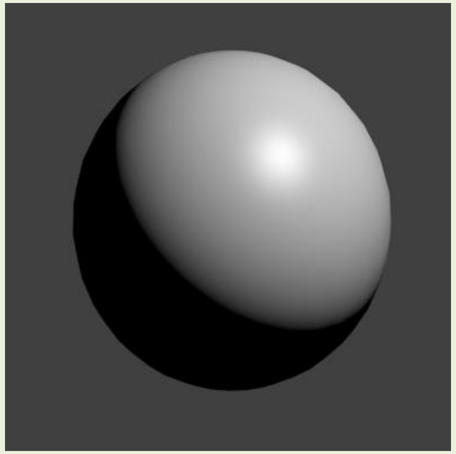
Bilinear interpolation of the intensities (color) between two normals

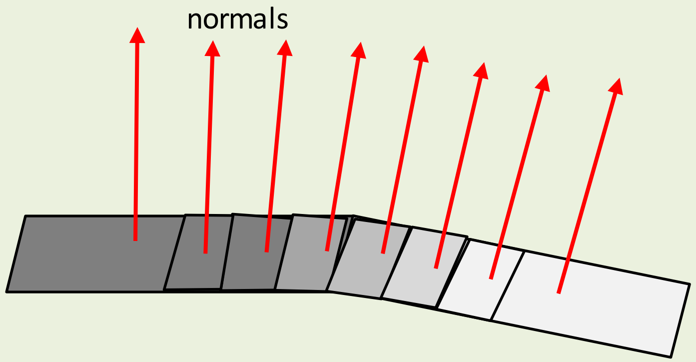




Phong shading

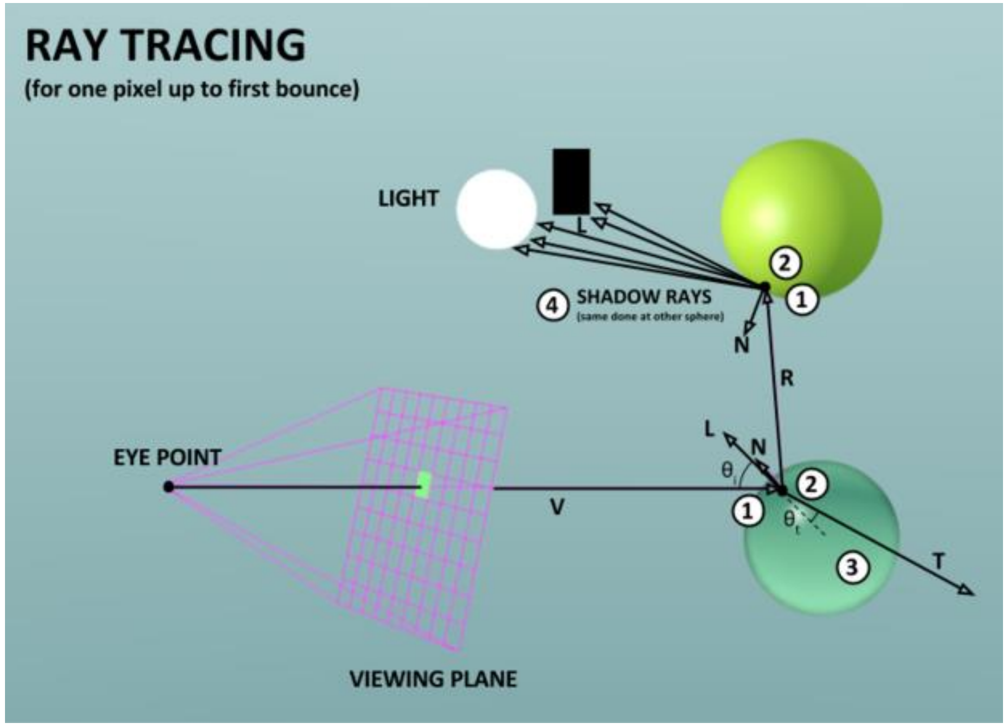
Barycentric interpolation of the normals themself





Modern hardware: use Phong (better than Gouraud, but a bit more intensive computing)

Isosurface: towards ray tracing



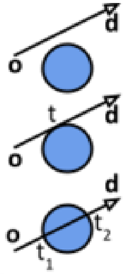
1. Sphere equation: $(\vec{p}-\vec{c})\cdot(\vec{p}-\vec{c})=r^2$

   Ray equation: $\vec{r}(t)=\vec{o}+t\vec{d}$

   Intersection:

   $$\left(\vec{o}+t\vec{d}-\vec{c}\right)\cdot\left(\vec{o}+t\vec{d}-\vec{c}\right)=r^2$$

   $$t^2\left(\vec{d}\cdot\vec{d}\right)+2\left(\vec{o}-\vec{c}\right)t\vec{d}+\left(\vec{o}-\vec{c}\right)\cdot\left(\vec{o}-\vec{c}\right)-r^2=0$$



2. **Illuminiation Equation (Blinn–Phong) with recursive Transmitted and Reflected Intensity:**

   $$I=k_aI_a+I_i\left(k_d\left(\vec{L}\cdot\vec{N}\right)+k_s\left(\vec{V}\cdot\vec{R}\right)^n\right)+\underbrace{k_tI_t+k_rI_r}_{recursion}$$

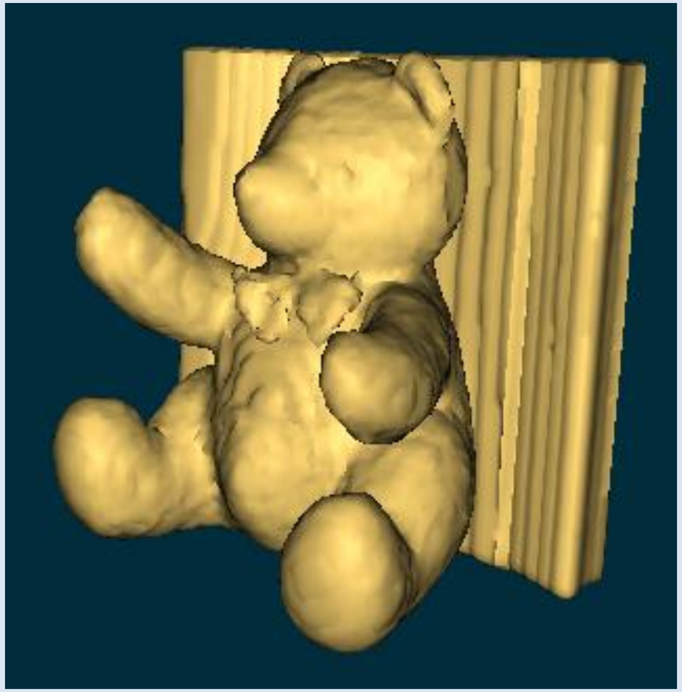
3. Snell's law: $\frac{\sin\theta_1}{\sin\theta_2}=\frac{v_1}{v_2}=\frac{n_2}{n_1}$ $\quad n_{air}\sin\theta_i=n_{glass}\sin\theta_t$

   refraction coefficients:
   n_{air} = 1, n_{glass} = 1.5

4. **Area Light Simulation:** $I_{light}\frac{\#\,(\text{visible shadow rays})}{\#\,(\text{all shadow rays})}$

The more bounces, the more realistic the image becomes

Isosurface: Example

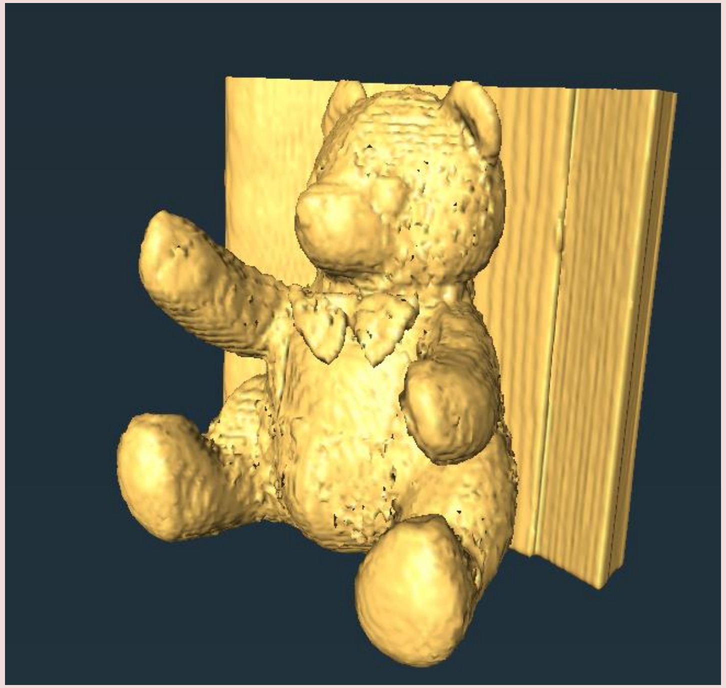


ImageJ 3D viewer

Isosurface and (very basic) volume renderer
Good quality, but limited
Buggy (in my view)

But:
export as STL, wavefront ♦☐ 3D printer

And volume calculation



Commercial renderer

Avizo/Amira/Imaris
Very flexible, commercial software
Good quality, extensive renderer

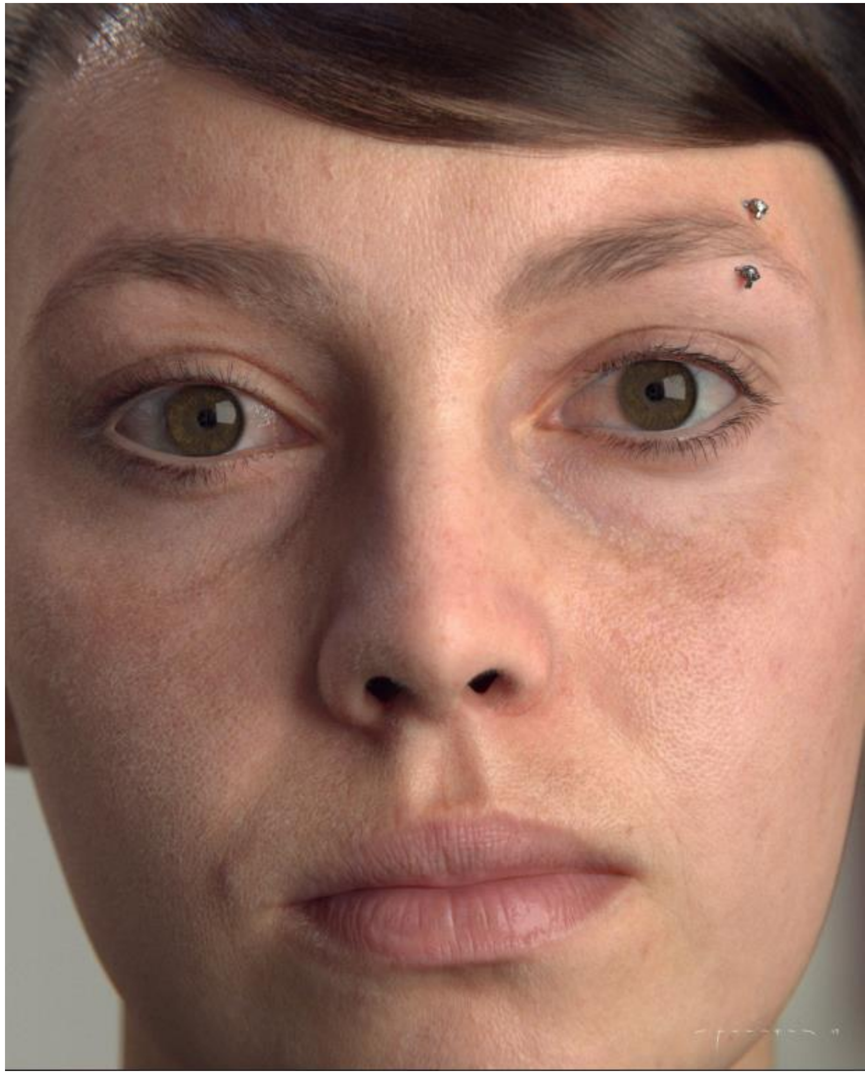
Available through SciIT
(BioNano workstation)

Open source ray-tracer

Blender 2.82 cycles renderer
Realistic rendering possible
Slow

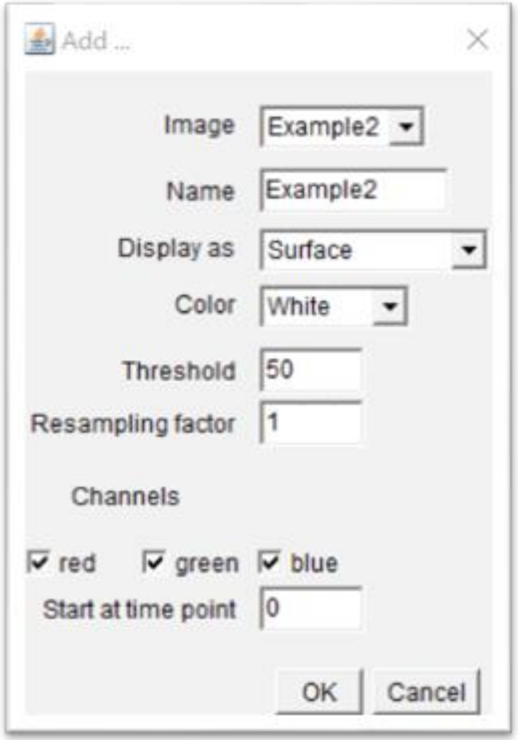
Free to download

Isosurface: Ray-tracing and GANs



Isosurface: surface rendering

EXERCISE
Open Example 2 and try out the 3D viewer.

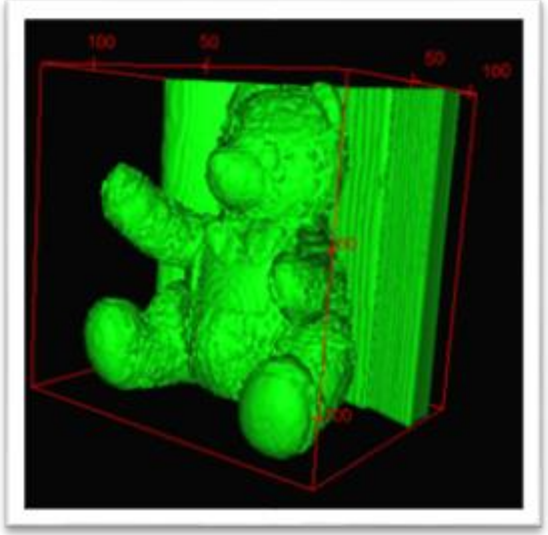


Select surface
Select a color

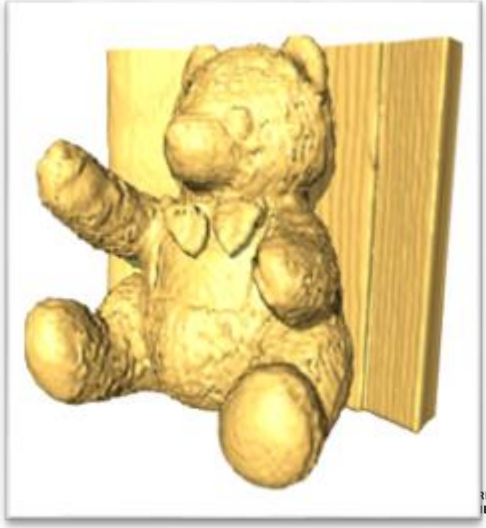
Set threshold
Do not downsample (value =1)

1. Plugins > 3D viewer
2. Select Display as surface, color (your choice) and resampling factor of 1)
3. Change the threshold (Edit > Adjust threshold). Set it to 50

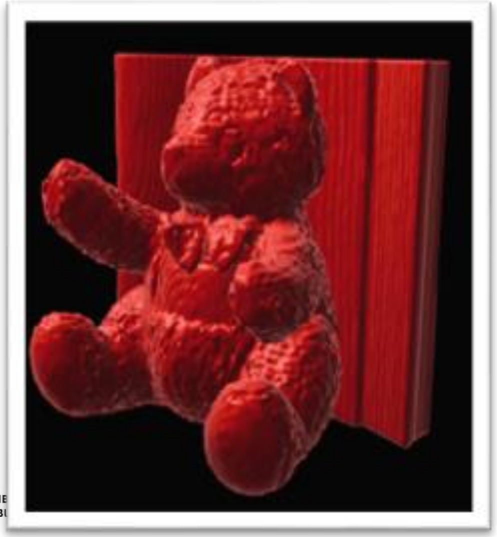
FIJI



Avizo



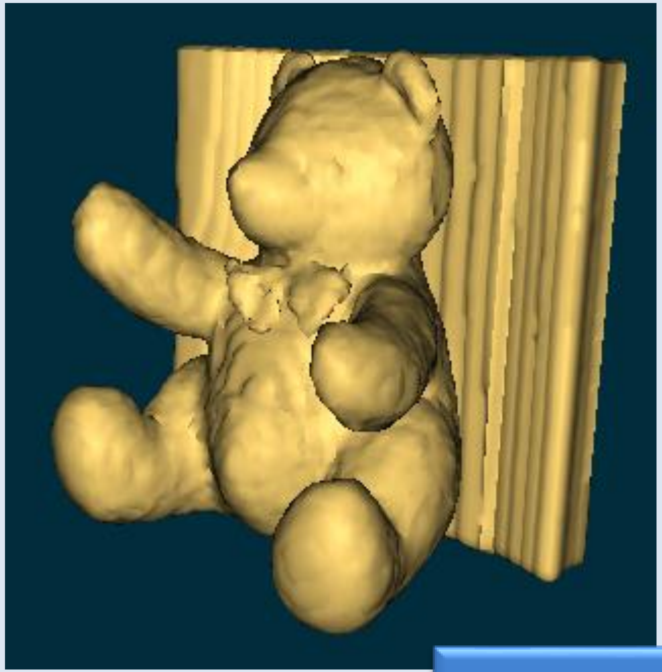
Imaris



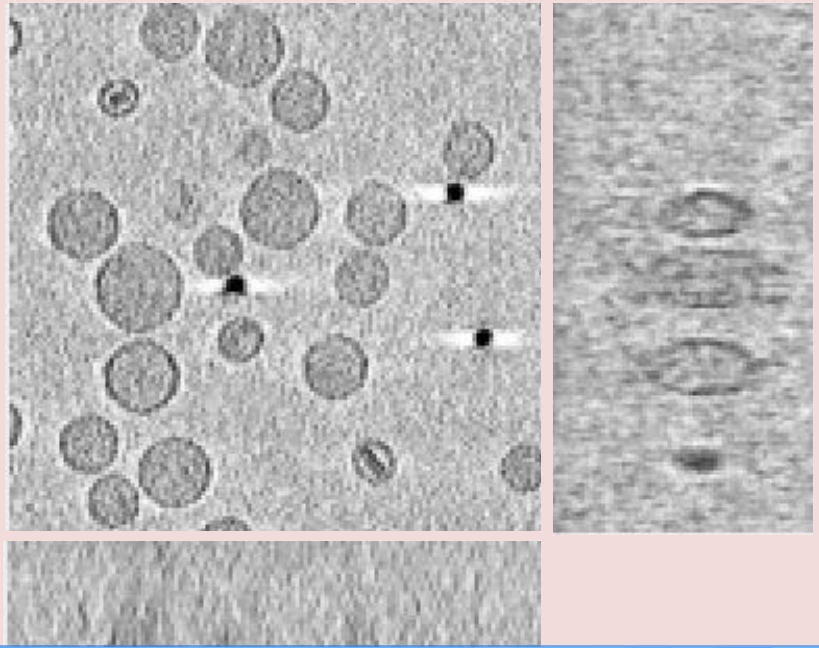
File > Export surfaces (Wavefront, STL, ...)
♦□ 3D printer

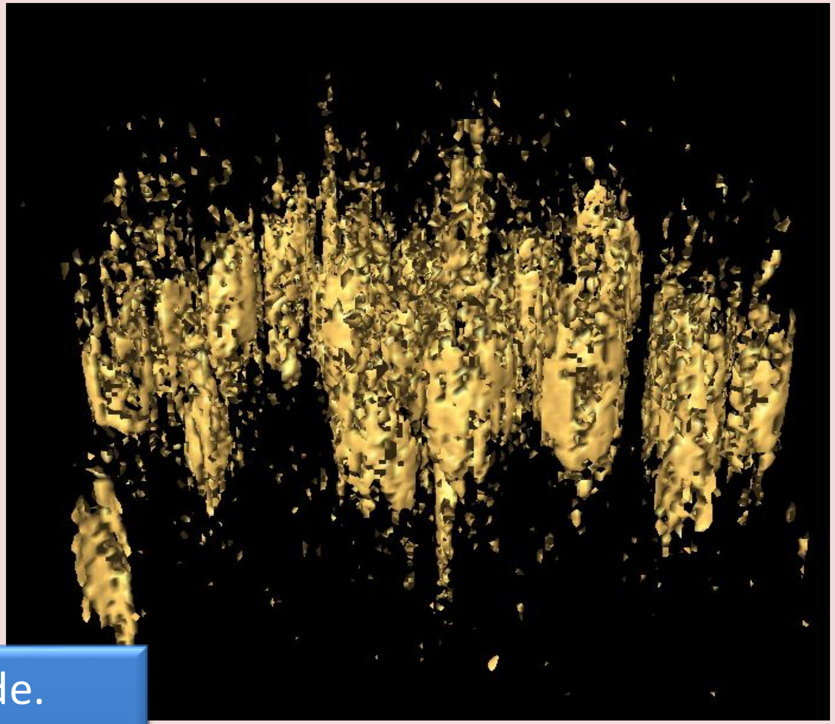
Isosurfaces: example

Advantages:
- Computationally fast
- Good 3D interpretation



Disadvantages:
- Noise effects only one signal (e.g. LSM channel, segmented/thresholded)
- Hence: not suitable for noisy data (e.g. electron tomography)
- Preferably: thresholded/segmented (binary) data





Main disadvantage: A decision for every voxel must be made. This can produce:
- false positives (spurious surfaces)
- false negatives (erroneous holes in surfaces)

3D rendering

Never publish only renderings.
Always provide the raw data.

Note: renderings require **interpretation** by the user. Hence, they are the convolution of the raw scientific data and the feature the user would like to see.

1. Surface rendering
   = binary threshold-based

2. Volume rendering
   = percentage threshold-based

Direct volume rendering methods generate images of a 3D volumetric data set without explicitly extracting geometric surfaces from the data (Levoy 1988).

Volume rendering offers the possibility for displaying weak or fuzzy surfaces. This frees one from the requirement to make a decision whether a surface is present or not.

Every voxel should contribute to the image

How does it work?

1. **VOLUME RAY-CASTING (or ray marching)**: Cast imaginary rays through the entire 3D stack
2. DEFINE TRANSFER FUNCTION: setup rules for color and alpha (opacity)
3. DEFINE EDGES AND LIGHT SOURCE: shading
4. ACCUMULATE THE DATA

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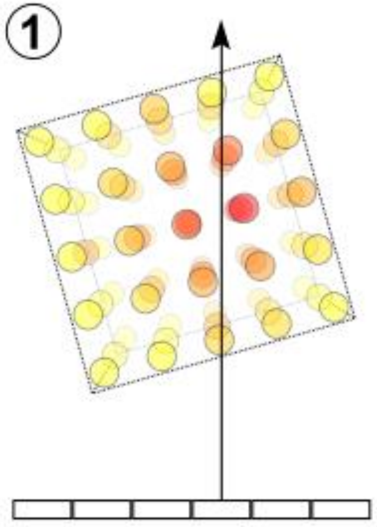
Volume rendering: 1. Ray casting & interpolation

For each pixel of the final image, a ray of sight is shot ("cast") through the volume.
At non-orthogonal angles, **interpolation** is needed



Ray casting

Volume rendering: Example - Maximum intensity projection

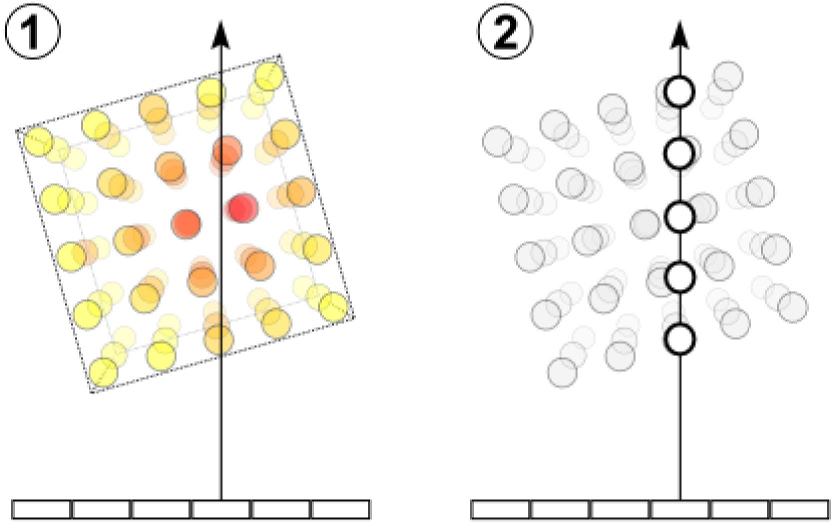
projects in the **visualization plane** the voxels with maximum intensity that fall in the way of **parallel rays** traced from the **viewpoint** to the plane of projection

110	48	38	37	38	37	39	37	38	38	38	40	43	49	75	87	92	97	92	107	110
124	45	38	37	36	37	37	38	37	39	39	40	45	54	66	74	92	99	99	120	124
123	35	35	35	34	36	37	38	35	38	41	43	47	63	74	72	97	108	109	123	116
134	37	34	36	35	35	39	38	36	38	43	48	57	67	77	80	86	118	113	134	118
135	37	35	33	34	38	37	38	38	39	39	50	59	71	89	102	105	105	112	127	135
144	38	34	35	35	37	40	37	40	40	43	54	74	77	89	102	109	110	105	115	144
127	36	34	36	37	35	39	40	41	42	49	57	72	82	87	109	124	123	114	114	127
137	34	35	35	35	37	40	44	43	47	57	64	75	82	88	102	114	128	124	129	137
138	36	36	38	37	38	43	42	46	47	60	77	88	96	95	108	124	128	115	138	135
140	35	34	36	38	40	43	42	47	52	75	90	92	102	105	113	123	118	114	127	140
132	37	37	39	42	44	45	50	61	77	88	91	97	104	112	112	122	123	120	122	132
135	37	37	39	40	48	56	58	68	72	89	102	107	103	121	113	133	128	130	135	117
151	37	38	41	45	53	68	78	74	84	97	104	118	114	125	120	124	133	138	151	137
138	40	41	46	53	57	72	84	80	83	120	113	117	125	122	129	134	127	137	131	138
143	44	47	52	61	63	89	103	92	100	110	120	119	125	128	132	128	127	143	136	134
142	51	54	64	68	87	98	102	109	111	105	121	122	131	132	127	124	129	127	141	142
158	58	56	70	75	89	97	104	114	111	112	129	129	131	119	126	158	149	131	148	128
155	64	86	97	90	101	109	110	117	109	109	127	128	129	127	122	153	155	132	139	129
154	67	85	108	100	108	115	112	135	127	124	129	131	140	141	134	141	154	144	126	120
150	96	89	108	113	111	112	116	126	147	143	147	144	140	147	147	141	150	140	137	136

For each sampling point: RGBA is computed (Red, Green, Blue and Alpha)

Volume rendering: step 2: Sampling and interpolation

For each pixel of the final image, a ray of sight is shot ("cast") through the volume.
At non-orthogonal angles, **interpolation** is needed

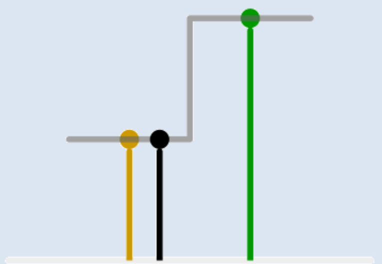


Ray casting Interpolation

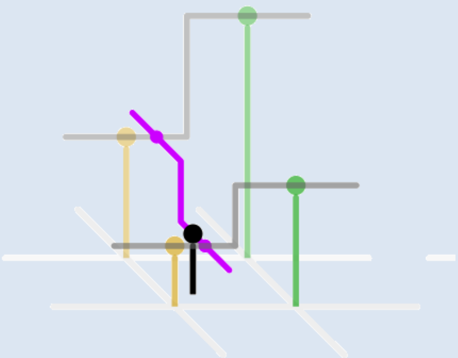
Volume rendering: step 2: Sampling and interpolation

Nearest Neighbour
= unweighted
➔ Take the value of the closest voxel

1D NN: closest of two points

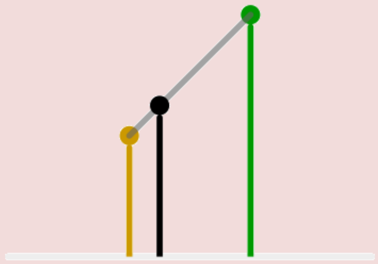


2D NN: closest pixel of four corners of a square

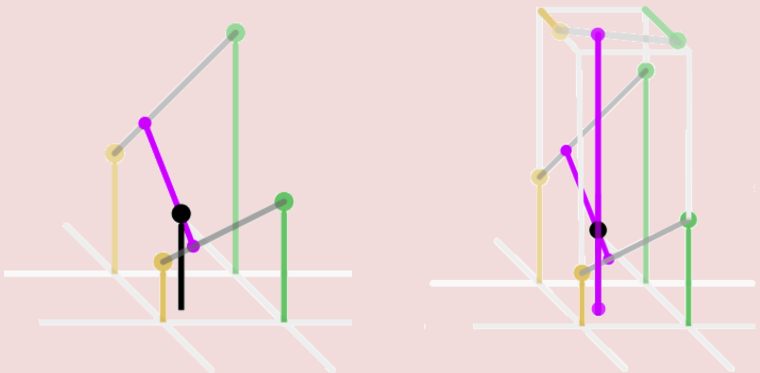


Linear
= Center of mass
➔ Take the linear average of the two pixels the ray is intersecting

1D Linear: Center of mass of two points

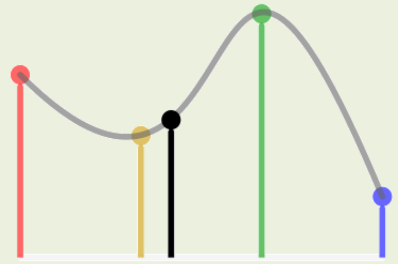


Bilinear: Center of mass of square corners
Trilinear: Center of mass of cube lattice points

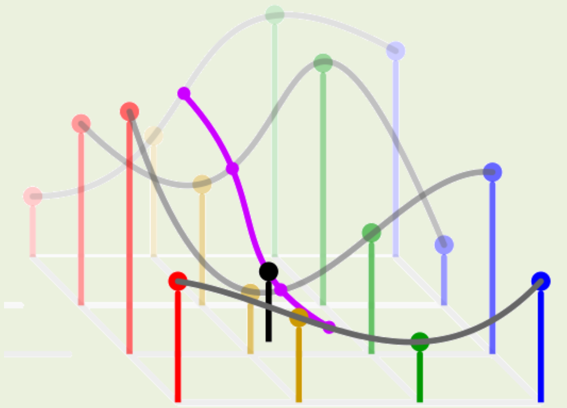


Cubic
➔ Center of mass
= Lagrange polynomials, cubic splines or cubic convolution

1D Cubic: Center of mass of 3^{th} degree polynomial



Bicubic: Center of mass of 16 pixels
Tricubic: Center of mass of 64 pixels

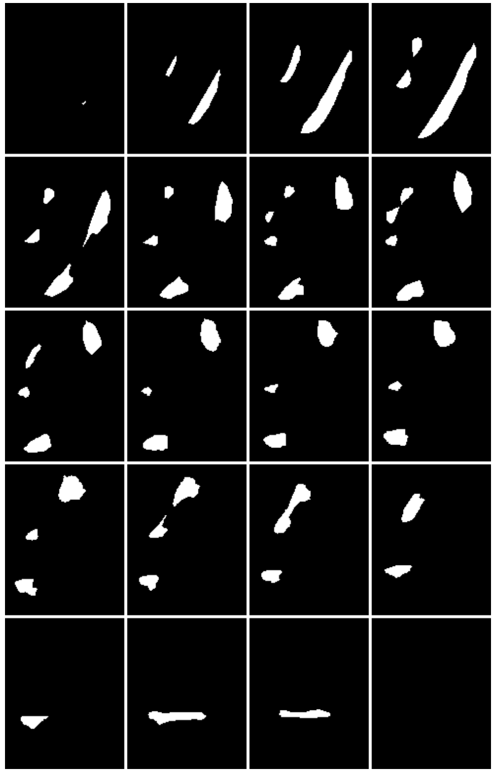


Volume rendering: Example - Maximum intensity projection

projects in the **visualization plane** the voxels with maximum intensity that fall in the way of **parallel rays** traced from the **viewpoint** to the plane of projection

Image > Stack > 3D Project...

Original stack



Maximum intensity (brightest point)

Advantages
computationally fast

Disadvantages
May not provide a good sense of depth of the original data.
Two MIP renderings from opposite viewpoints are symmetrical images
No difference between left or right, front or back.

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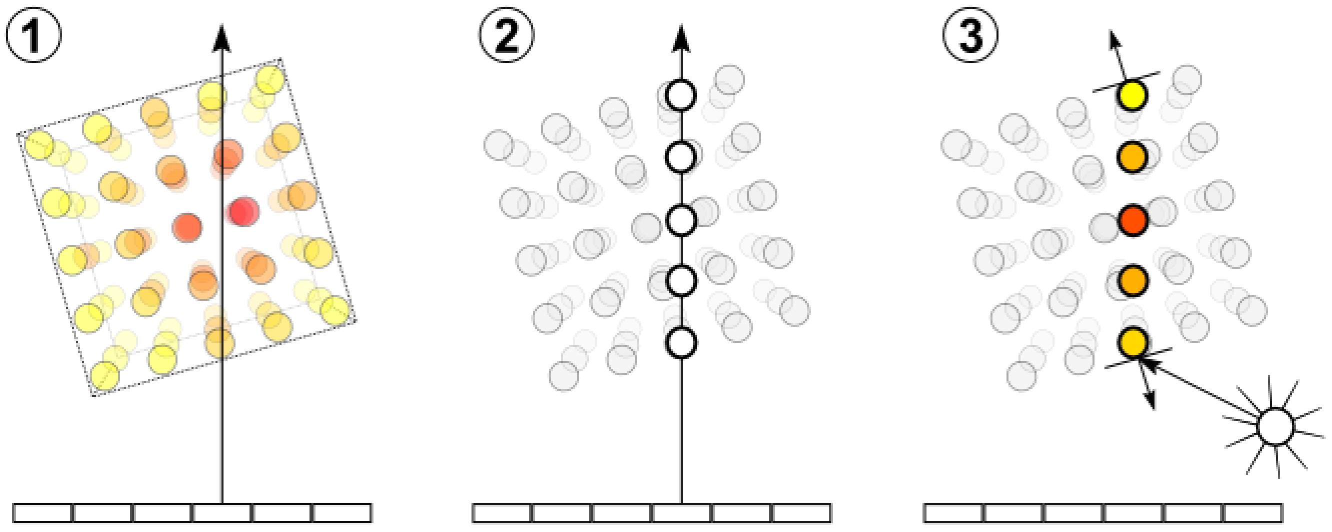
Volume rendering: step 3: shading



Imaris

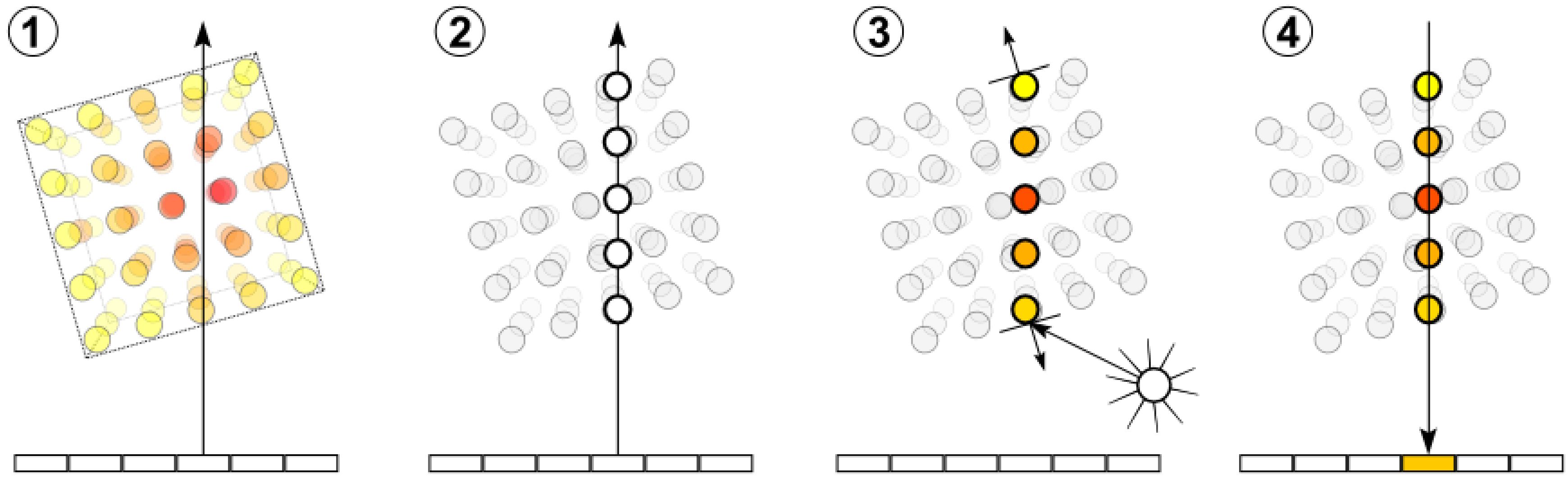
Avizo

Shading
For each sampling point, a gradient of illumination values is computed. These represent the orientation of local surfaces within the volume. The samples are then *shaded* (i.e. coloured and lit) according to their surface orientation (normal) and the location of the light source in the scene.

Each sampling point is shaded according to its normal

Note: thresholding needed!

Volume rendering: step 4: compositing



Compositing

After all sampling points have been shaded, they are composited along the ray of sight, resulting in the final colour value for the pixel that is currently being processed.

$$L_o(\mathbf{x}, \omega_o, \lambda, t) = L_e(\mathbf{x}, \omega_o, \lambda, t) + \int_\Omega f_r(\mathbf{x}, \omega_i, \omega_o, \lambda, t)\, L_i(\mathbf{x}, \omega_i, \lambda, t)\, (\omega_i \cdot \mathbf{n})\, \mathrm{d}\,\omega_i$$

The total spectral radiance
x = position
ω_0 = direction (angle)
λ = wavelength
T = time point

The emitted spectral radiance

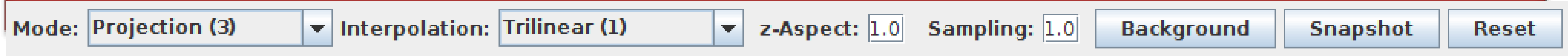
The bidirectional reflectance distribution function

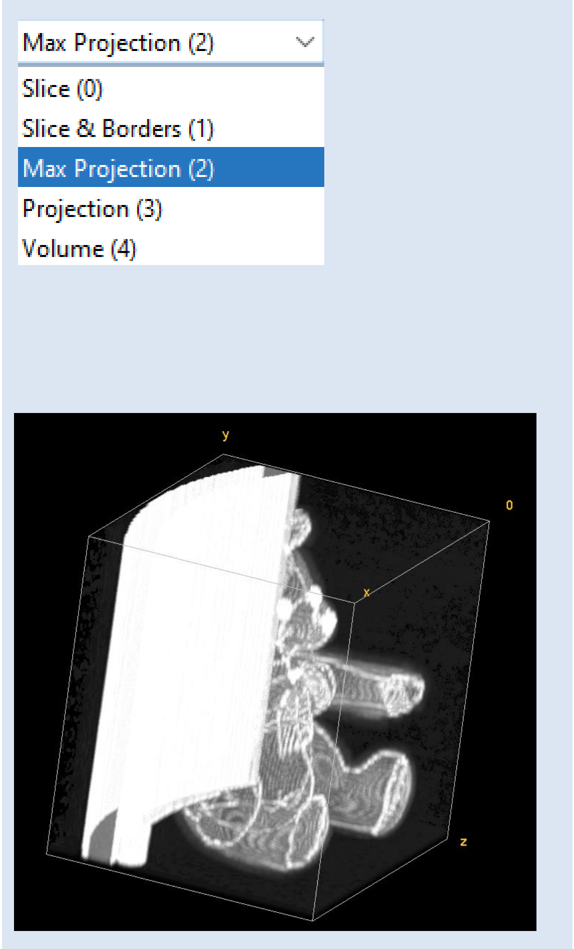
The spectral radiance

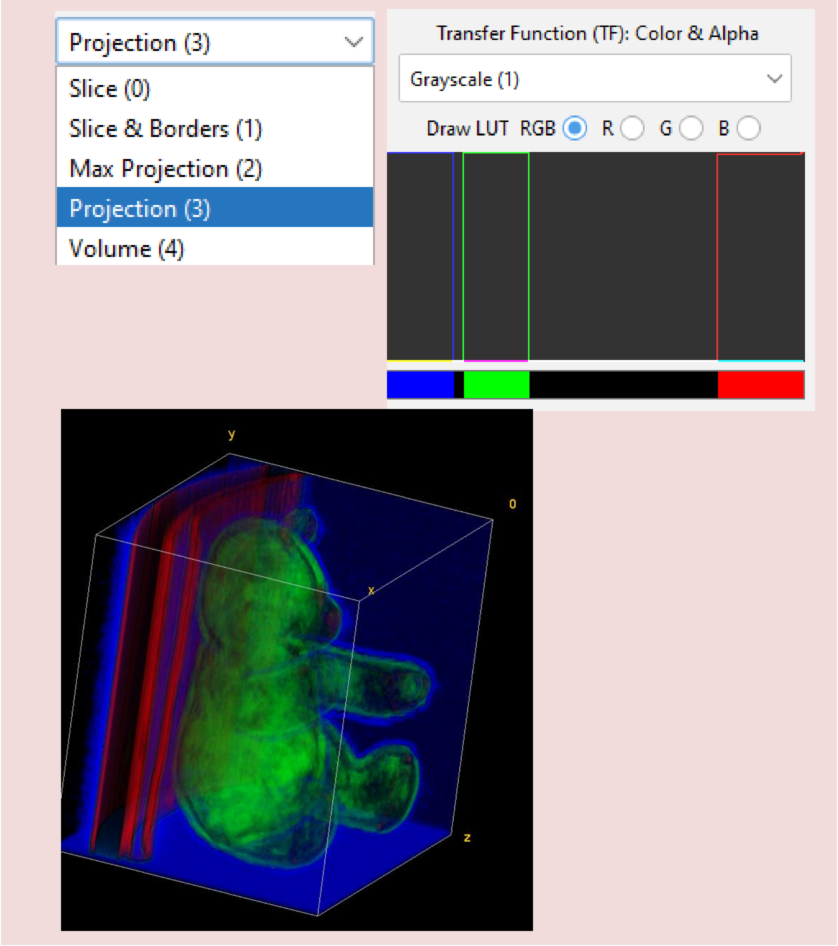
Volume rendering: Maximum intensity projection

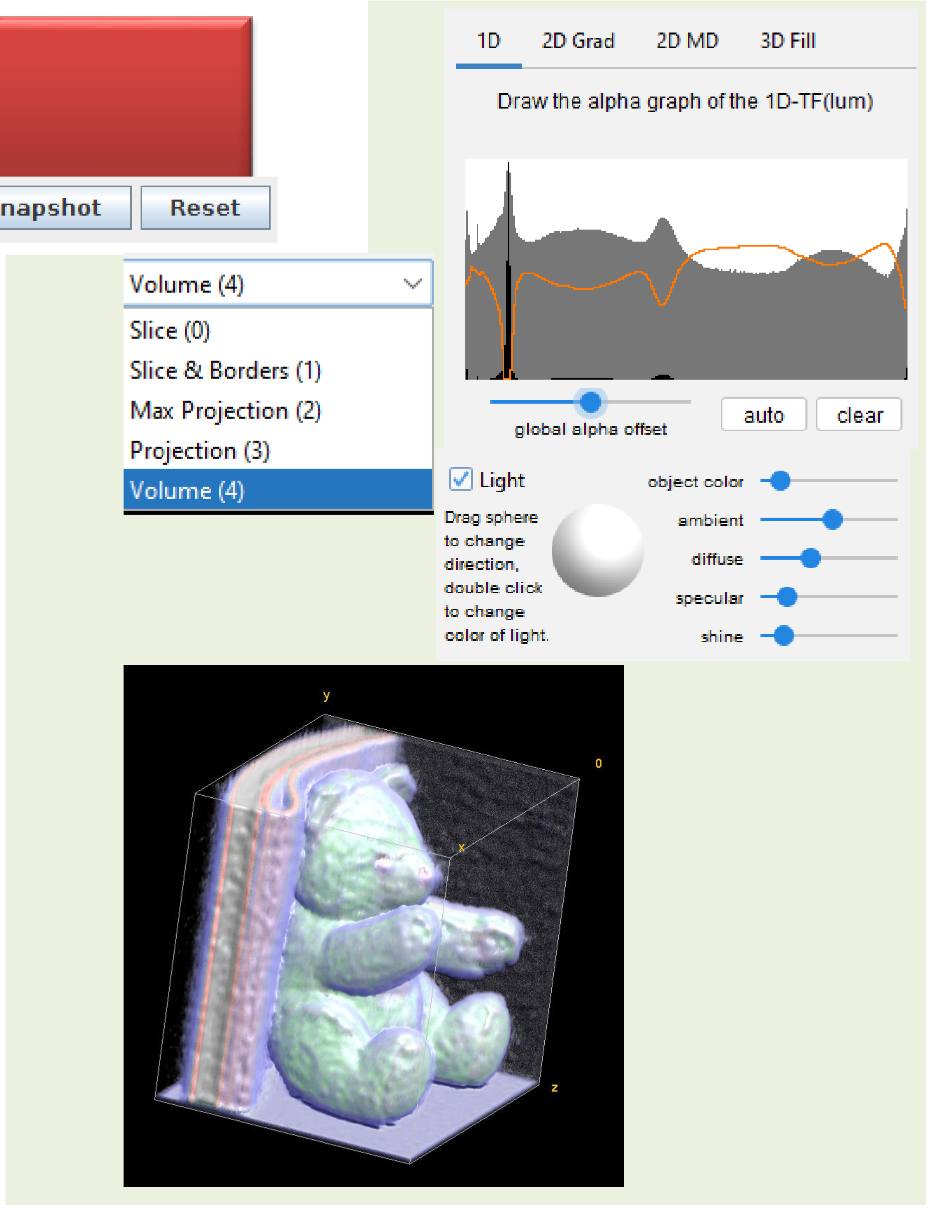
EXERCISE

Open Example 2 and try out the Volume viewer (plugins > volume viewer)





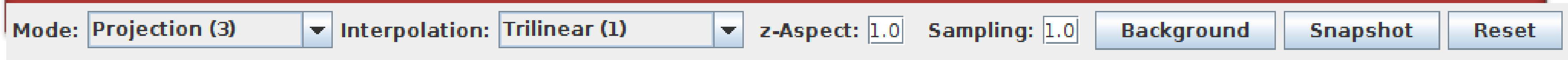




Volume rendering: Projection

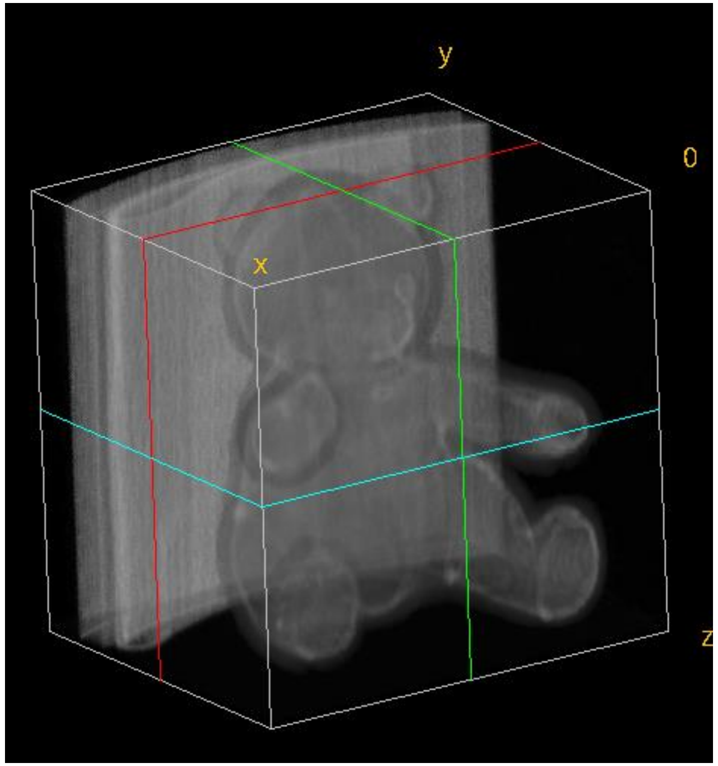
EXERCISE

Open Example 2 and try out the Volume viewer (plugins > volume viewer)



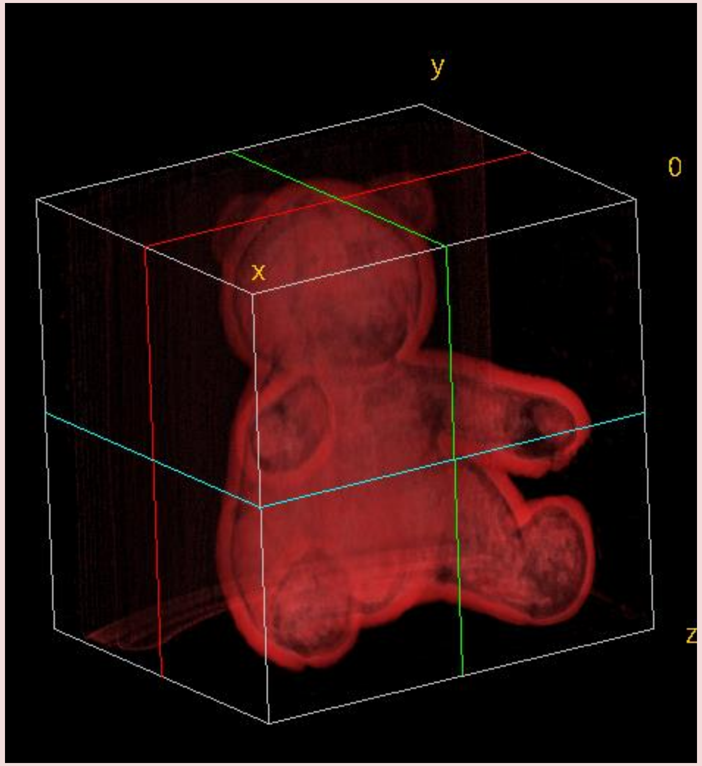
Projection

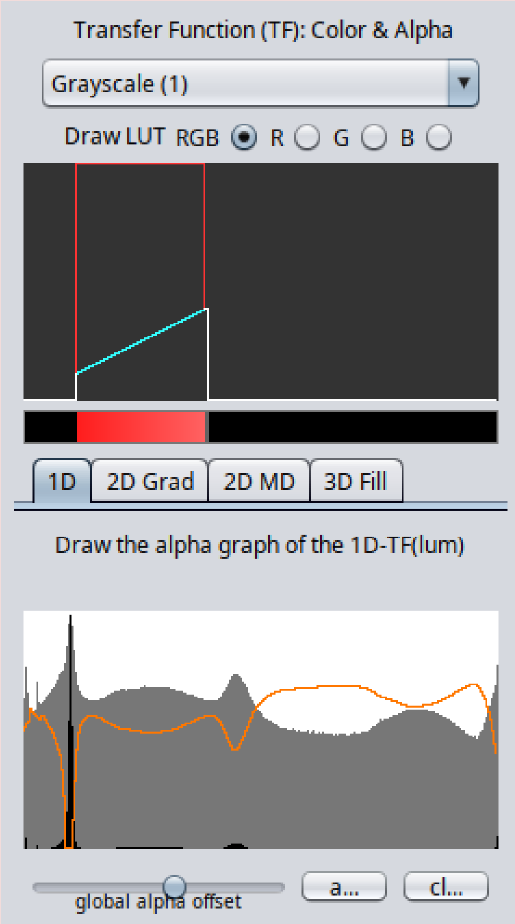
Alpha without transfer function adjustments



Projection

Alpha with 1D transfer function adjustments





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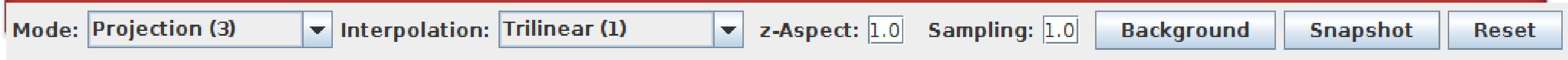
Volume rendering: Projection

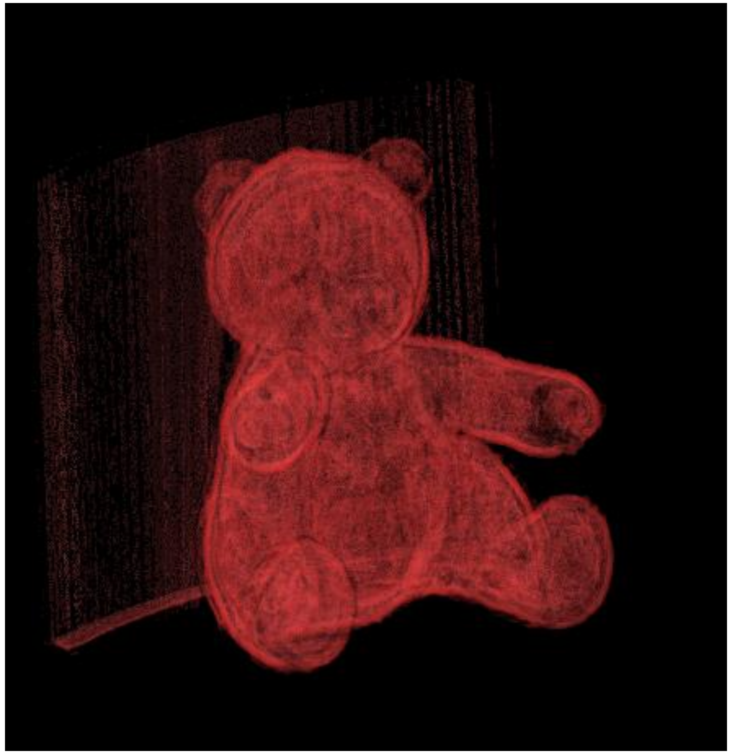
EXERCISE

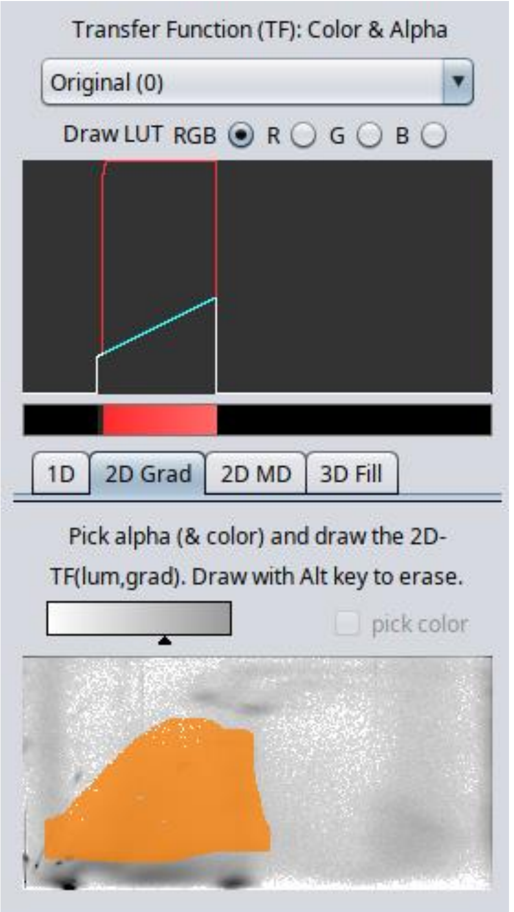
Open Example 2 and try out the Volume viewer (plugins > volume viewer)



Projection

Alpha with 2D transfer function adjustments





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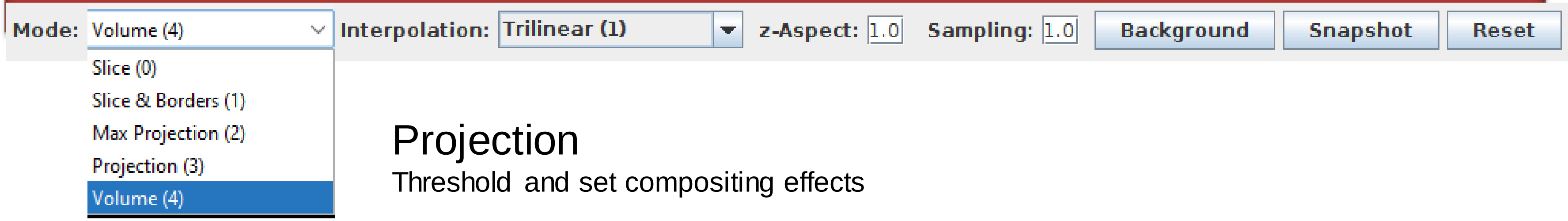
Volume rendering: Volume

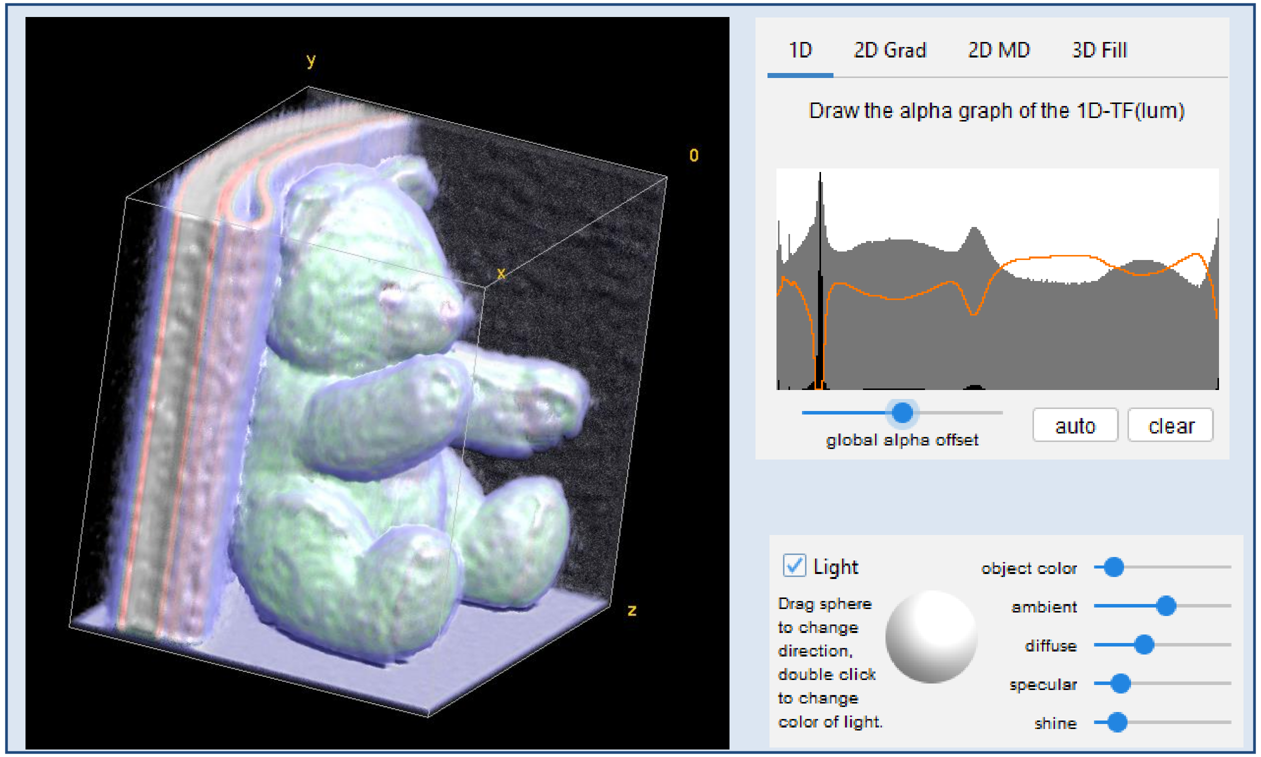
EXERCISE

Open Example 2 and try out the Volume viewer (plugins > volume viewer)



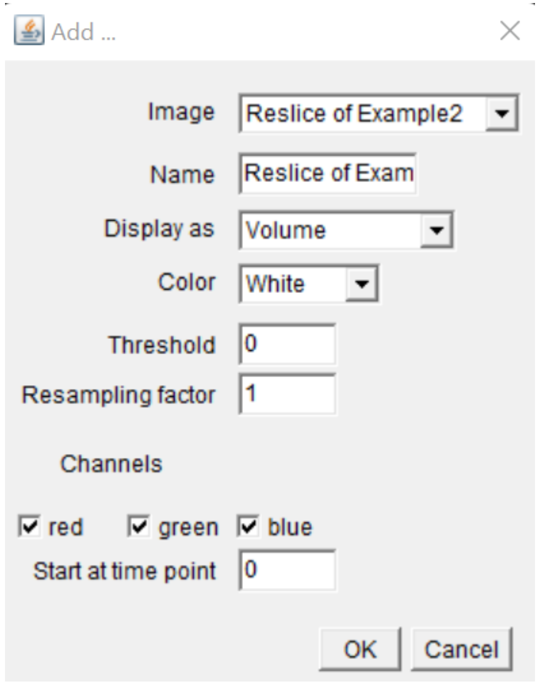
Projection

Threshold and set compositing effects



Volume rendering

EXERCISE
Open Example 2

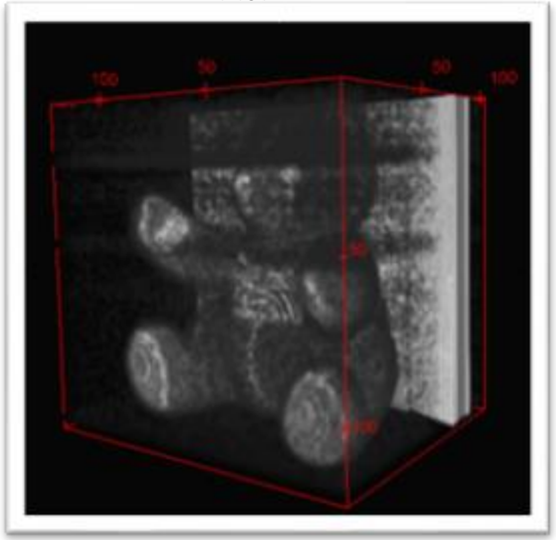


Select Volume
Select a color

NO threshold
Do not downsample (value =1)

1. Plugins > 3D viewer
2. Select Display as volume, color (your choice) and resampling factor of 1)
3. No need to set a threshold

FIJI



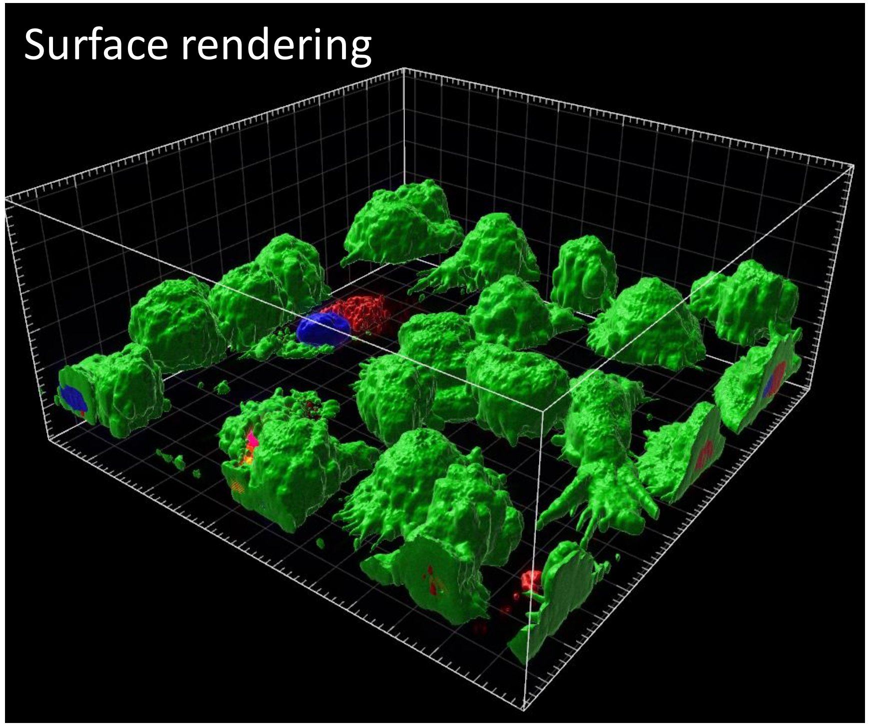
Avizo

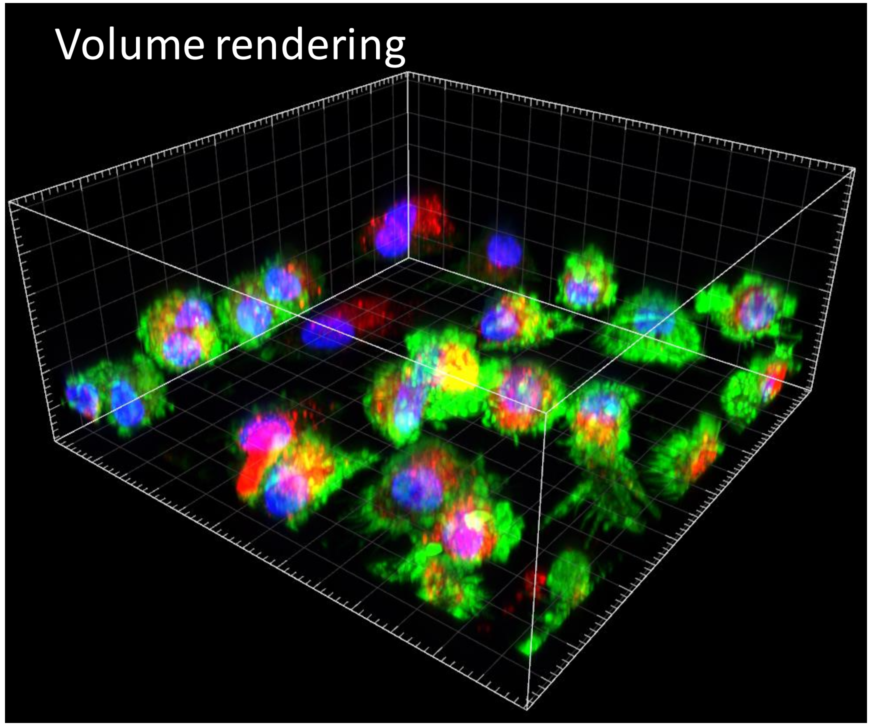
Imaris

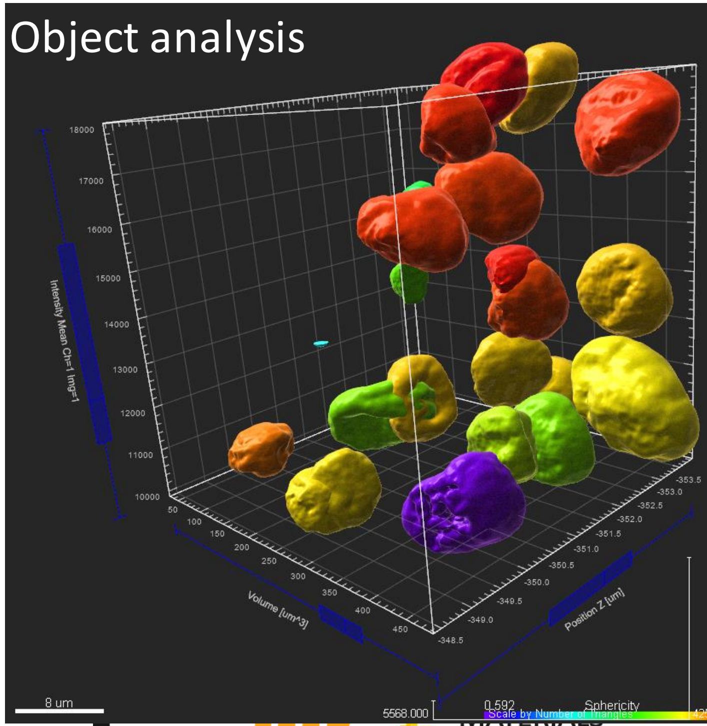
Volume rendering: Imaris

BioNano has a workstation dedicated to Image rendering (amipc22.unifr.ch)
Soft Matter physics has also a workstation
More number cruncher available at Biology, Medicine, (physics?)

Imaris: dedicated to 3D LSM data



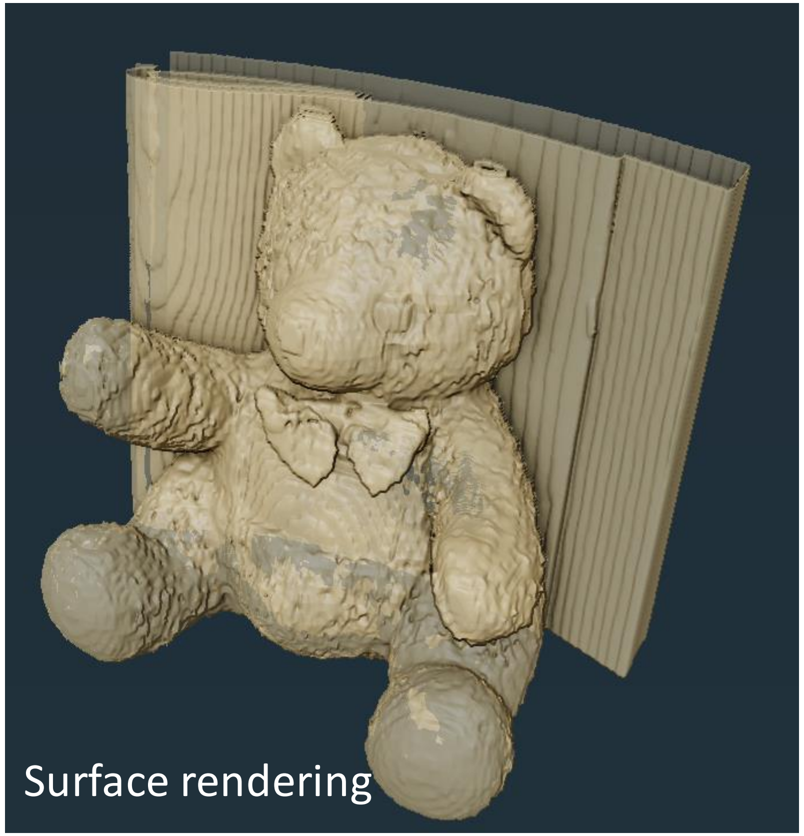


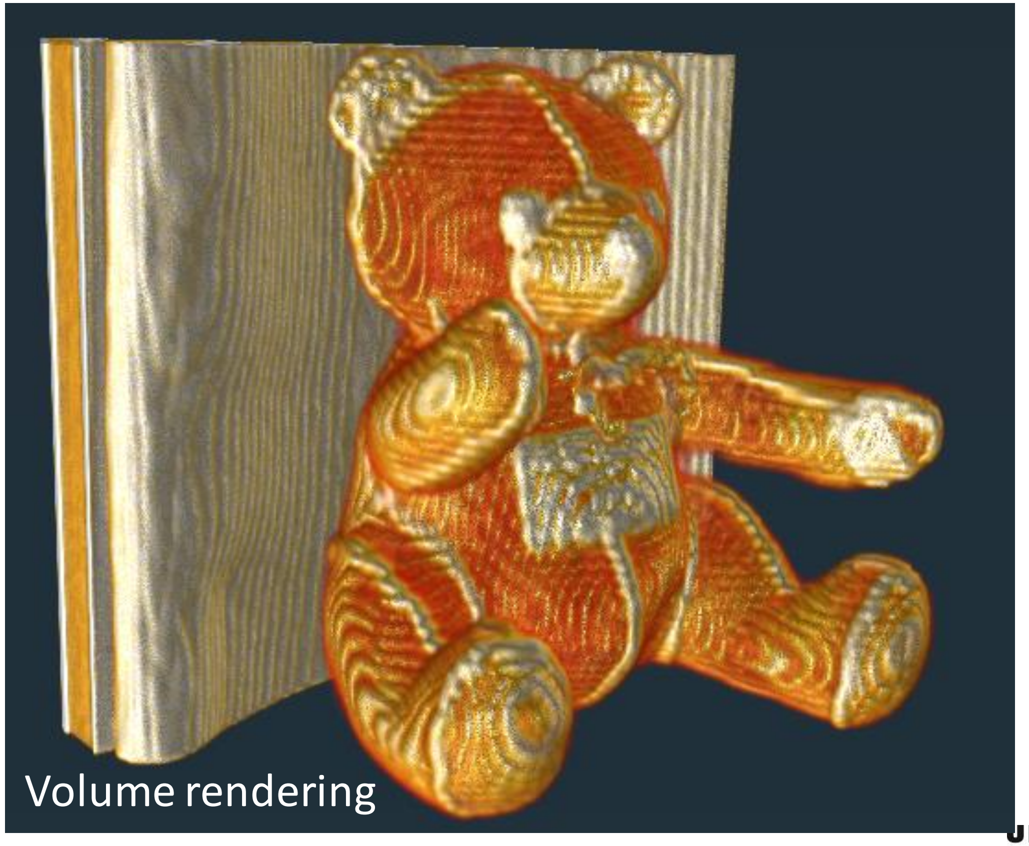


Volume rendering: Aviso

BioNano has a workstation dedicated to Image rendering (amipc22.unifr.ch)

Aviso: dedicated to 3D non-fluorescent 3D and 4D data





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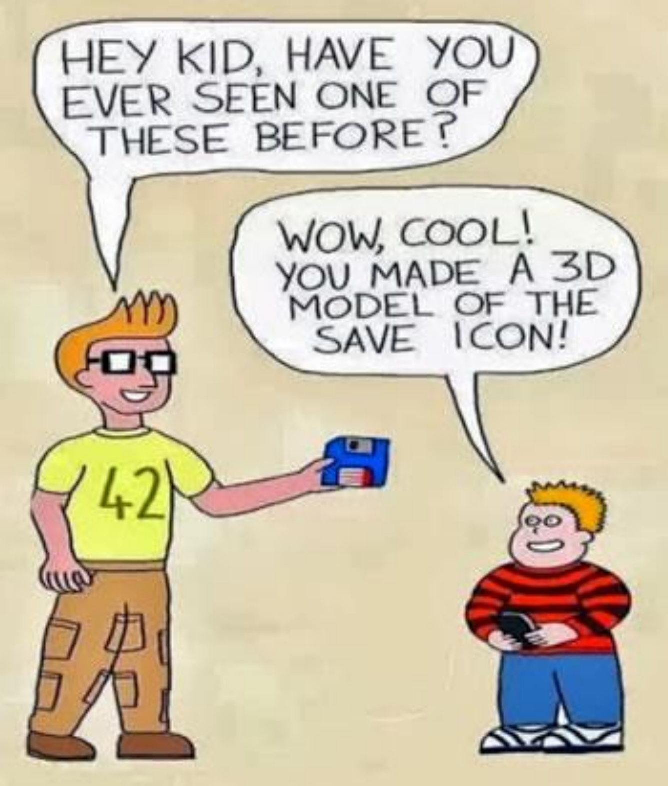
Z-Stacks

✓ Congratulations,
You finished Part IV, 3D



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