#### **Thermo Fisher** S C I E N T I F I C

# Avizo Software Introductory Training

Sarawuth Wantha Product Application Specialist

The world leader in serving science

1 Proprietary & Confidential | authoremail@thermofisher.com



Avizo Software Software for CT and microscopy image data visualization and analysis

> ThermoFisher SCIENTIFIC

# A quick word about me



## **Sarawuth Wantha**

Avizo Software Product Application Specialist Thermo Fisher Scientific Bordeaux France

- Master's degree in Biomedical Engineering, RWTH Aachen University, Germany
- Doctoral degree, Ludwig-Maximilians-University Munich, Germany
- Ph.D. in Biomedical Engineering with the focus on Biomedical Imaging Modalities and Image Processing.
- Research techniques: X-Ray CT, MRI, Electron microscopy, Fluorescence Imaging
- (confocal, light-sheet, multi-photon, STED microscopy).
- Post-Doctoral research: Cardiac Electrophysiology & Stem Cell-derived Cardiomyocytes.
- Extensive experience in image processing software.



# World leader in serving science





R&D scientists/engineers



invested in R&D



# We take pride in our Mission

We enable our customers to make the world healthier, cleaner and safer

# **Avizo Software for Materials Research & Quality Control**

## Quickly and accurately obtain properties from your imaging data



**Thermo Fisher** 

Avizo Software provides unmatched imaging data analysis tools for numerous scientific and industrial applications from a single environment

# From images to knowledge





Data acquisition

From imaging data, our software tools create numerical 3D models that can be visualized and analyzed to understand the structure, properties and behaviors of live or materials samples



Thermo Fisher

General concepts

Visualization & Image Pre-Processing

3

5

2

#### Segmentation

AI Capabilities

Advanced topics





Avizo Software Software for CT and microscopy image data visualization and analysis

SCIENTIFIC

# **General concepts and tools**



# **Getting started**

Help menu access

#### Avizo start page access



# **Getting started**

Learning resources:

Tutorials

•

•

Help menu access

## 👚 Start 🛛 🛫 Project 🛭 📚 Segmentation 🛛 🌴 Meshing 🛛 🎎 Recipes 🛛 🔪 Filament 🖉 Multiplanar 🛛 📰 Animation Amira<sup>™</sup> YouTube Learning Center Welcome to Amira Software RECENT DATA RECENT PROJECTS CREATE NEW PROJECT

Amira start page access

#### User's Guide File Edit Project View Window XPand Python XScreen 😽 🕐 User's Guide 📐 --- Project 🗱 Recipes 📚 Segmentation 🕋 Start Examples **Project View** C++ Programmer's Reference 🕨 🔌 😽 Open Data... Python Programmer's Guide HELP NEWS Python Programmer's Reference Avizo ToGo Publisher Guide License Manager Go to Ed (h) Show Available Extensions System Information **Online Support** About Do no

## Getting started Tutorials Learning Center on YouTube

FOLLOW US

HELP

# Avizo start-up page

File Edit Help				
👚 Start 🧲 Project 🗱 Recipe	es 📚 Segmentation 🛛 💎 Meshi	ing 🛛 🔧 Filament 🛛 🗃 Animation		
	Avizo™ Welcome to Avizo Software			
	RECENT DATA	RECENT PROJECTS	CREATE NEW PROJECT	
	OPEN DATA	OPEN PROJECT	BLANK PROJECT	
	HELP	NEWS	FOLLOW US	
	Help Getting started	AVIZO SOFTWARE 2020.2 NOW AVAILABLE!	Corporate website	
	Tutorials Learning Center on YouTube	REDUCE YOUR LEARNING CURVE Go to Education Center	Yau Tube	
		Do not show at startup Version 2020.2		

## Thermo Fisher

# Amira start-up page

🐜 Amira - Untitled										_	×
File Edit Help											
👚 Start 🛛 🦟 Proje	ct 🛛 😤 EM Proj	ect 🛛 📚 Segmentation	💎 Meshing 💡	🎕 Recipes	🔍 Filament	🖷 Multipla	lanar	Animation			
		Amira™	и								
	N.										
	~	Welcome to Amir	a Software								
							CDEAT				
		RECENTIDATA		RECEIVER	NOJECIS		CREAT	IL NEW PROJECT			
		OPEN DATA		OF	PEN PROJECT			BLANK PROJECT			
				NEWC			50110	0.000.115			
				INEWS			FULL	00005			
		Help Cotting started			IIRA SOFTWARE 2020. W AVAILABLE!		Corpor	rate website			
	Getting started Tutorials						Yau Titte				
		Learning Center on YouTu	ıbe		to XTra Resource Libi	rary					
					Do not show at start	tup					

Version 2020.2

# **Workspace: user interface components**



	Main menu bar	Workroom bar	Viewer toolbar
Eile    Edit    Project    View    Window    XPand    Python    XScreen    He	elp 🖌 Meshing 🛰 Filament 🔛 Animat	tion	
Project View 🗆 🗙 Open Data	🕨 🌒 🕂 Q, C? C9 🖀 🕆	≝ 0 ¤ © ≪ 1 ⊶	°⊾
Project View			
Properties		Viewer Window	N
Properties Area			
auto-refresh Apply			MEMORY IKAGE
	Ready		Stop 31% 🕑

# Loading a dataset into Project Workroom – part 1





**Thermo Fisher** 

SCIENT

# Loading a dataset into the Project Workroom – part 2



# Attach a module to a dataset: e.g. Bounding Box

File Edit Project <u>V</u> ie	w Window	XPand	Python	XScreen	Help
🕋 Start 🗲 Project	🛛 🗘 Recipe	s 📚	Segment	ation 🔏	Meshi
Project View					□ ×
Open Data				) 🛞 🤫	E
Contrast Control Roi	Slice Voxel	Slice	Cylinder S	lice	
chocolate-bar.am	0			ho Slice 📎	
	—				
	Right o on da	click ata	)		
					向

### Ways to trigger module:

- Double-click
- Press Enter
- Press Create button



**Thermo Fisher** 

SCIENT

# Module properties: e.g. Bounding Box



#### **Properties:**

- Click on a module in the pool to display its properties (data module included)
- Click on "?" to access the module's documentation
- Module properties are called ports

# Navigate and interact in 3D – part 1



# Navigate and interact in 3D – part 1



# Navigate and interact in 3D – Part 2



# Navigate and interact in 3D – good practice

Most of the actions can be done in the trackball mode:

- Hold left mouse button for rotation
- Use the mouse wheel for fine zoom
- Hold left and middle mouse button for fast zoom
- Hold the middle mouse button for translation

#### When in interact mode:

- Press and hold [Alt] to switch to trackball mode
- Press [Esc] for switching between interact and trackball modes.



# Visualize a dataset: e.g. Ortho Slice



- Ortho Slice connects Automatically if Auto Display is on
- Can otherwise be created like any other module

**Thermo Fisher** 

- A dataset is displayed in the viewer only if it has a visualization module attached
- Check that visibility is turned on (e.g. workflow with multiple data)

# **Visualize a Dataset: e.g. Ortho Slice properties**



Some properties settings:

- Orientation port: choose the display plane of the slice
- Slice number: choose the slice to be displayed in the viewer - drag slider or use mouse wheel / insert value in the text box
- Adjust view: if on, the camera is reset each time a new slice orientation is selected

# **Visualize a Dataset: e.g. Ortho Slice properties examples**

#### Thermo Fisher SCIENTIFIC

#### **Orientation xy**

Adjust View off



#### **Orientation xz**





#### Orientation yz





# **Setting preferences – part 1**

<u>E</u> dit	Project	View	Window	XPand	Pytho		
	Cut			Ctrl+X			
	Сору		Ctrl+C				
l	Paste			Ctrl+V			
	Delete			Del			
	Select All			Ctrl+A			
	Preference	es	2	Ctrl+Shi	ft+P		
	Dialogs				>		

#### Preference setting e.g.:

 Set the number of recent files and projects displayed on start page

General	Layout	On exit	Molecules	LDA	Segmentation	Rendering	Performance	Network	Units	Range Partitioning	Recipes	Auto Display
Project m	odules and	l data object	ts									
🔽 Use 2-	-pass firing	algorithm										
🗹 Auto-	select new	objects										
🗹 De	select prev	iously selec	ted objects									
🗹 Draw	viewer tog <u>o</u>	gles on icons	s									
🗹 Draw	compute in	dicator										
Save Proj	ect						Maximum num	ber of recent	t documen	ts		
🗆 Incluc	de unused o	lata objects										
🗖 Inclue	de window s	sizes and po	sitions				Recent files:			7		÷
🗌 Incluc	de viewer ba	ackground s	settings									
🗹 Overv	vrite existin	ıg files in au	ito-save				Recent project	s:		7		¢
Policy:				Always ask			·····					
Language	2											
Set prog	ram langua	ae to:					English/United S	States				
		-										
Online D	ocumentati	on										
🗹 Displa	iy only avai	lable featur	es									
Preferen	ces and Set	tings										
Restore	Defaults	Load prefe	erences Sa	ive prefere	nces							
										OK Cance	el Appl	y Help

**Thermo Fisher** 

SCLEN

# **Setting preferences – part 2**



 Add compass in the 3D Viewer

General Layout On exit Molecules	LDA Segmentation Rendering	Performance Network Units Range Partitioning Recipes Auto Display
Windows		Viewer gadgets
Save window layout on exit		Camera trackball Compass
Show viewer in top-level window		
· Show "DoIt" buttons		
Enable docking "Help" panel		Show the compass
Tools buttons style: Too	l button text beside icon 🛛 🗸 🗸	
		Auto-hide the compass
		Compass position: Upper left $\checkmark$
		Project View
		Group by display/compute/data in tree view
		Show port interconnection in Project Graph View
		Show colormaps connected to objects
		Show histogram in background (colormap editor, ports)
		Glue attached display modules to data object
		Advanced mode of modules in Properties Area by default
		Preview : One Small OMedium Large
Restore default layout	Save current layout	
		OK Cancel Apply Help

**Thermo Fisher** 

SCIENT

# **Visualize a Dataset: e.g. Ortho Slice properties examples**

#### Thermo Fisher SCIENTIFIC

#### **Orientation xy**

Adjust View off



#### **Orientation xz**





#### Orientation yz





# Visualization of 2D and 3D data



# **Data visualization: Exercise 1**

Setting the orientation of the 2D view

Assemble the necessary modules to create a view like in the screenshot

Dataset available at:

 \$INSTALLDIR/data/tutorials/ chocolate-bar.am



# **Data visualization: exercise 1**



#### Solution







#### Solution:

- Connect 3 Ortho Slice modules, each having a different orientation setting
- Note: Multiple modules with different ports settings can be connected to the same data

# **Data visualization: exercise 1**











#### Tip:

 Module ports can be pinned by clicking the pin button: pinned ports are always displayed in the Properties window, even if the module is deselected

# Visualize a dataset: e.g. Ortho Slice

#### File Edit Project View Window XPand Python XScreen Help 🛛 🗲 Project 🛛 🗱 Recipes 🛛 📚 Segmentation 🛛 🏘 Meshing 🛛 🔧 Filament 🗮 Animation 🕋 Start □× ▶ ♥ + Q - C + G - M + N = N \* \* \* ■ \* \* ■ \* \* ■ \* **Project View** 🕨 🐳 😤 📑 Open Data... Roi Slice Voxel Slice Cylinder Slice Contrast Control 💶 chocolate-bar.am 🕥 🕂 匬 Properties **I** Ortho Slice Ŧ chocolate-bar.am 🗸 Data: Ŧ **Orientation:** 🔘 ху vz XZ Ŧ Slice Number: ► 147 Ŧ Mapping Type: Colormap ~ Ŧ 1000 Colormap: 1910 Ŧ 🗹 adjust view 📃 bilinear view lighting **Options:** Ŧ ✓ show width: 1 Frame: MEMORY USAGE Stop 34% Ready

 Ortho Slice connects Automatically if Auto Display is on

**Thermo Fisher** 

- Can otherwise be created like any other module
- A dataset is displayed in the viewer only if it has a visualization module attached
- Check that visibility is turned on (e.g. workflow with multiple data)

# **2D visualization with Slice**









### Slice – interpolation:

- Not necessarily axis-aligned, interpolation is necessary for reconstruction
- Interpolation can be tuned from Sample ports
  - Different sampling resolutions (fine, coarse, etc.) are available in the drop-down menu.
- Sample ports no effect if the slice is axisaligned.

# **2D visualization with Slice**









#### Slice – rotate:

- Activate virtual trackball: Press [Tab] or select "rotate" (from Options)
- Hold the left mouse button in interactive mode:
  - Click inside the white lines of a trackball axis
    => rotate along the respective axis
  - Click outside the trackball axes => rotate in all directions

# **2D visualization with Slice**

# Slice: visualize arbitrarily oriented slices in a volume







### Slice – defining a plane:

- Select "fit to points" (from Options)
- Click on 3 different points inside the object Press [Esc] to activate Interact mode.
- After clicking 3 points, "fit to points" is automatically disabled.

# **3D visualization with Isosurface**





Isosurface – visualization module that requires processing. For launching the processing:

- Press the Apply button
- Check auto-refresh (use with caution)


# **3D visualization with Isosurface**







A threshold value is necessary for computing the Iso-surface:

- set by default from data histogram
- can be manually adjusted (bottom slider)
   Top sliders zoom on the histogram

Isosurface: visualization of constant value surfaces

## **3D visualization with Isosurface**



#### **Histogram Thresholding**

- Histogram the distribution of voxel intensity values
- Can be computed by "Histogram" module
- Its shape is informative for thresholding a dataset
- In the case of chocolate bar data, the histogram has 3 lobes for:
  - background and porosities
  - for the "mousse" (inside)
  - for the chocolate and caramel (outer layers)

By setting a histogram threshold value as indicated below, The outer chocolate bar layers will be selected, as they correspond to the third histogram lobe (highest intensity values).







## **3D visualization with Isosurface**

#### Isosurface:

- For generate the surface computed by Isosurface, use "Extract Surface" module.
- For visualizing the generated surface, use "Surface View" module.





# **Planar visualization modules - clipping**

Planar visualization (orange) modules can be used for clipping. Clipping applies to all the objects that have the visibility on.





Thermo Fisher

## **Clipping:**

- Define the clipping plane by e.g. Slice
- Click on the "Clip" icon clip on one side of the object
- Click again disable clipping
- Click again clip on the other side

## **Planar visualization modules - clipping**





Thermo Fisher

#### **Setting Slice transparency:**

- Choose Binary mode (from Transparency) regions of voxel intensity values outside the colormap range are fully transparent while others are fully opaque.
- Adjust the colormap range to obtain the view in the example above.

Clipping volumes and setting transparency

Assemble the necessary modules to create a similar view:



Thermo Fisher



#### Solution – Step 1







#### Generate 2 isosurfaces for two thresholds:

- Low threshold: ~ 410
- High threshold: ~ 940



Solution – Step 2





#### Clip the 1<sup>st</sup> Isosurface with Slice:

- All visible modules are clipped => the visibility of all other modules except the 1<sup>st</sup> Isosurface should be turned off.
  - Tip: Select 1<sup>st</sup> Isosurface and press [h] => only he selected module has the visibility switched on.
- Select the clipping plane with Slice and then clip.



Solution – Step 3

#### Full solution available at:





#### Setting visibility, colors and transparency:

- Turn off the visibility of Slice
- Change the color of the 1<sup>st</sup> Isosurface: double click on the colormap to pick color
- Set a transparency to the 2<sup>nd</sup> Isosurface select "transparent" ("Draw style" port)

## **Project save**

Saving a project: File > Save Project (As) ...

- Use "Minimize project computation" when a module takes a long computation time
- Use "Minimize project size" otherwise



- Use '[pack & go]' option if you need to archive or transfer the project to a different computer.
  - This will copy the input dataset(s) inside the project folder. Otherwise, these files are only referenced via their path on the disk, and the project will not load if this path is no longer valid.

File name:	Untitled.hx
Save as type:	Avizo Project (*.hx)
	Avizo Project (*.hx)
	Avizo Project and data files [pack & go] (*.hx)
Hide Folders	

## **Project save**

Saving a project: File > Save Project (As) ...

- Use "Minimize project computation" when a module takes a long computation time
- Use "Minimize project size" otherwise



- Use '[pack & go]' option if you need to archive or transfer the project to a different computer.
  - This will copy the input dataset(s) inside the project folder. Otherwise, these files are only referenced via their path on the disk, and the project will not load if this path is no longer valid.

File name:	MyProject.hx			~
Save as type:	Amira Project (*.hx)			~
	Amira Project (*.hx)			
	Amira Project and data files [pack & go] (*.hx)			
Hide Folders	4	Save	Cancer	

# Setting a colormap: e.g. Ortho Slice

- A colormap is used to map scalar values to intensity levels or colors
- The colormap range can be modified manually in order to adjust brightness, darkness or contrast

▶ ?

• Predefined colormap settings are also proposed.

Properties

**□** *S* 

**Ortho Slice** 

Some examples of grayscale colormap setting for Ortho Slice module (click on the "Edit" button of "Colormap" port):





# **3D visualization with Volume Rendering**



Volume rendering – colormap settings for assigning color and transparency to each voxel value

"Opacity" port – for tuning the transparency







## **3D visualization with Volume Rendering**

### Volume rendering: Colormap Settings



For changing the default colormap:

- Click on the "Edit" button of "Colormap" port
- Pick a colormap from the drop-down list OR

Load a colormap from the disk
 Once loaded, a colormap will be added to the drop-down list

volrenGreen Colormap (default) volrenPhysics Colormap (loaded from disk)



# **3D visualization with Volume Rendering**

### **Volume rendering Settings**

■ motor.am* <b>&gt;</b>			olume Reno olume Reno	<mark>dering S</mark> dering	ettings 🤉		曲	
Properties							C	• ×
🔹 🍥 🔗 🗸 Volume Rendering Sett	ings 📃 📃				Adv	anced		?
🗄 🔷 Data:	motor.am	× •	•					
🗄 💛 Rendering:	🔘 Standard	O Pł	nysical					

## Rendering type:

- Standard
- Physical mostly used
   According to the selected rendering type,

different rendering options are proposed.

Click on the arrow on the left of the "Rendering" port in order to show more settings.



Switch on the "Advanced" ribbon for advanced Volume Rendering settings

 "Move Low Res" – low resolution mode when moving the camera, for real time rendering

# Setting a colormap: Colormap Editor

In addition to choosing a default colormap or loading one from the disk, one can also edit a colormap by means of Colormap Editor. To access Colormap Editor:

 Click the "Edit" button of the "Colormap" port of any visualization module and select Options -> Edit Colormap

Common Settings:	Volume Rendering Settings \vee 🔶	Adjust range to		
Colormap:	74 255	Options	$\rightarrow$	✓ Auto adjust range
Colormap Lookup:	🔵 alpha 🔵 luminance alpha 🥥 rgba	Zoom to		✓ Transparent
Opacity:		📲 grayScale.am		✓ Local range
opwerty.		💵 grayScaleInverted.am		Edit colormap
		🌌 temperature.icol		Edit color
		😼 physics.icol		Load colormap

Colormap Editor window will be displayed on top of the Project View window:



# Setting a colormap: Colormap Editor

Opacity curve – allows controlling the transparency of the colormap:

- Pick a default preset from the "Opacity preset" menu
- Manually adjust the curve:
  - Left-click on the curve to add a point at the respective location
  - Click and hold on a point in order to move it
  - · Right click on a point in order to remove it



Click on the arrow on the Editor's bottom-right corner for opening the Color Editor (below the histogram):



# Setting a colormap: Colormap Editor

Colormap gradient – allows modifying the colormap using color markers

- To modify the color gradient:
  - Left-click on the markers line to add a marker at the respective location
  - Click and hold a marker in order to move it
  - Right click on a marker in order to remove it
  - Double click on a marker for color settings
  - Drag the diamond shaped button to adjust the location of the inflection point
- The data points outside the colormap range will be mapped to the color defines by the extreme left and right boxes.



**Thermo Fisher** 

Setting colormaps

Use the Volume Rendering module and tune the colormap to obtain a similar view:



#### Solution

## Colormap Editor Settings:

- Add and move points on the opacity curve for setting the transparency of the colormap
- Add and tune color markers for setting the color gradient of the colormap





#### Solution

#### Volume Rendering Settings:

- Adjust the lighting effects: specular and deferred for highlighting reflections and shadows
- Choose interpolation mode: cubic for smoother result





## **Basic data manipulation**



## **Data and voxel properties**

Click on a data in the pool in order to have data and voxel properties displayed in the properties window: e.g. chocolate bar:



- Type: grayscale, label, RGBA, etc.
- Precision: number of bits used for coding the value stored by a voxel. E.g.:
  - 8-bits <-> values in [0-255]
  - 32-bits float <-> values in ~ [-1e38, 1e38], finer precision but 4x more memory required
- Minimum and Maximum voxel values give the intensity range (do not confuse with colormap range)
- Window: voxel values range outside the background

# **Converting types and re-mapping intensities**

### Changing type: Convert Image Type module



Scaling is necessary to avoid clipping. For scale tuning check:

**Thermo Fisher** 

- The intensity range of the input data
- The intensity range of the converted data

# **Converting types and re-mapping intensities**

## Changing type: Convert Image Type module



Re-mapping intensities: Different modules are available in the category: Image Processing -> Grayscale Transform

Scaling is necessary to avoid clipping. For scale tuning check:

**Thermo Fisher** 

- The intensity range of the input data
- The intensity range of the converted data

📚 chocolate-bar.to-byte 🛛 🗸	· [ ]	Ľ	Enter a search string>		
😑 Templates	>	^	Distance Maps	>	🔅 Adaptive Histogram Equaliz 🏠
🗀 Experimental	>		🗀 Edge Detection	>	🔅 Background Detection Corr
L AI	>		🗀 Enhancement Filters	>	🔅 Background Image
🗀 Animate	>		🗋 Frequency Domain	>	🔅 Beam Hardening Correction
🗀 Annotate	>		🗋 Grayscale Transforms	>	🔅 Block Face Correction
🗀 Compute	>		Morphological Operations	Cate	gory: Image Processing > Grayscale Tra
🗀 Convert	>		Propagation	>	😨 Correct Background and Fl
Correlation	>		Separating And Filling	>	🔅 Correct Z Drop
🗀 Display	>		🗋 Sharpening	>	🔅 Cylindrical Intensit
🗀 Fiber Tracing	>		Skeletonization	>	🔅 Gamma Correction
🗋 Geometry Transforms	>		Smoothing And Denoising	>	🔅 Histogram Equaliz
🗀 Image Processing	>		🔅 Filter Sandbox		🏟 Match Contrast
🗀 Image Segmentation	>		🔅 Image Stack Processing		🔅 Normalize Grayscale
🗀 Measure And Analyze	>		Image Volume Processing		🔅 Ring Artifact Removal
~~ ~ ·	χ.	$\mathbf{v}$			🏟 Shading Correction 🛛 🗡

# **Crop Editor: main functionalities**

Access Crop Editor: select a dataset in the pool and click on the Crop Editor icon in the data properties window





## Crop Editor – main functionalities:

- Crop: reduce/enlarge image frame
  - Manually
  - Automatically (by an automatically set gray-level threshold for separating the data into background and object)
- Change resolution
  - Change voxel size
  - Change bounding box size (the new voxel size is automatically computed

The number of voxels is preserved

- Modify axes:
  - Flip an axis' orientation the slices along the respective orientation will have their order reversed
  - Swap allows interchanging two axis

# **Crop Editor: good practices**

Note: Bounding Box is defined from voxel centers, i.e. : bbox\_size = voxel\_size \* (#voxels – 1) for each dimension (x, y or z) A slice is defined from voxels centers too.



### Warning: editors directly modify the data no undo available (the only way to get the original data back is by reloading/re-generating it) Good practice: duplicate data before editing:

- Keyboard shortcut: [Ctrl] + [d]
- Data object menu



## **Crop Editor: alternatives**

Extract Subvolume module can be used as an alternative to Crop Editor (for cropping only).

Extract Subvolume + ROI Box – allow cropping multiple datasets the same way:

e.g. input dataset and its segmentation result

- Extract the desired sub-volume of the input dataset (by means of Extract Subvolume)
- Connect a ROI Box to the extracted sub-volume
- Connect an Extract Subvolume module to the second dataset. Connect the ROI input of the Extract Subvolume module to the ROI Box module and then press Apply
- => The second dataset will be cropped as the first one.







## **Data resampling**

### Resampling

Allows enlarging/shrinking the regular grid on which an image is defined (interpolation is necessary):

- Change the number of voxels
   e.g. reduce => reduced data size but lowered quality
- Change voxel size

   e.g. adjust size in order to make the voxels
   isotropic

This can be done in Avizo via the Resample module.

Properties			□ <b>×</b>
<b>୍ଚ</b> ୬ ~	Resample		?
Ŧ	Data:	chocolate-bar.am 🗸 🔶	
Ŧ	Reference:	NO SOURCE -	
Ŧ	Input Resolution [px]:	235 x 175 x 295	
Ŧ	Input Voxel Size:	120000 x 120000 x 120000 [nm]	
Ŧ	Filter:	Lanczos V	
Ŧ	Memory Usage:	Resampled data size: 23.14 MB. Extra memory needed for computation: 0.00 B Available system memory: 25.46 GB	
Ŧ	Mode:	💿 dimensions 🔵 voxel size 🔵 reference	
Ŧ	Resolution [px]:	x 235 y 175 z 295	
Ŧ	Voxel Size [nm]:	x 120000 y 120000 z 120000	
auto-ref	fresh	A	oply

## **Data resampling**

### Resampling

Allows enlarging/shrinking the regular grid on which an image is defined (interpolation is necessary):

- Change the number of voxels
   e.g. reduce => reduced data size but lowered quality
- Change voxel size

   e.g. adjust size in order to make the voxels
   isotropic

This can be done in Amira via the Resample module.

Properties			□ <b>X</b>
<b>\$</b> & ~	Resample		?
Ŧ	Data:	chocolate-bar.am 🗸 🔶	
Ŧ	Reference:	NO SOURCE	
Ŧ	Input Resolution [px]:	235 x 175 x 295	
Ŧ	Input Voxel Size:	120000 x 120000 x 120000 [nm]	
Ŧ	Filter:	Lanczos 🗸	
Ŧ	Memory Usage:	Resampled data size: 23.14 MB. Extra memory needed for computation: 0.00 B Available system memory: 25.46 GB	
Ŧ	Mode:	💿 dimensions 🔵 voxel size 🔵 reference	
Ŧ	Resolution [px]:	x 235 y 175 z 295	
Ŧ	Voxel Size [nm]:	x 120000 y 120000 z 120000	
🗌 auto-re	fresh	A	pply

# Data resampling – apply transformation

### · · · · · ·

#### Resample Transformed Image module

Oftentimes, when a data is transformed in Avizo, only the visualization of the data is changed and not its representation in memory. Apply this module to implement the transformation carried by a dataset and to change the dataset representation in memory.

#### Use cases examples:

- Generate the data resulted after applying a rotation, scaling or other transformation
- Generate the resampled image after registration with a reference
- Apply the rotation necessary to a dataset after aligning the data bounding box with the object's axes.



# Data resampling – apply transformation

#### hermo Fisher CIENTIFIC

#### **Resample Transformed Image module**

Oftentimes, when a data is transformed in Amira, only the visualization of the data is changed and not its representation in memory. Apply this module to implement the transformation carried by a dataset and to change the dataset representation in memory.

#### Use cases examples:

- Generate the data resulted after applying a rotation, scaling or other transformation
- Generate the resampled image after registration with a reference
- Apply the rotation necessary to a dataset after aligning the data bounding box with the object's axes.



## **Data resampling example**

Resampling to an oblique plane

Issue: the object's axes and the bounding box's axes are not aligned Goal: align the bounding box's axes to the object's axes

**Before** alignment

## After alignment

Slice (aligned to the



## **Data resampling example**

Resampling to an oblique plane

#### Solution:

- Use "fit to points" option of Slice on an Isosurface or Volume Rendering and fit the Slice to the object's xz plane (the bottom of the chocolate bar).
- Apply a Resample Transformed Image module and connect:
  - "Data" input to the data object
  - "Reference" input to the Slice

Select "extended" option and press Apply

A new data is generated with the bounding box's axes aligned to the object's axes.





## **Export data**

The results of the different processing modules can be exported from the Avizo pool.

Exporting data on the disk:

- Right-click on the dataset you want to save
- Click on the "Export data as" icon
- Select the relevant format

□□ chocolate-ba	tam 🕥			
	📚 chocolate-bar.am 🗸 🗸	Ci 🖻	<enter a="" search="" string=""></enter>	
	💽 Favorites		kport Data As ıg Correction	$\hat{}$
	Recents	> 🕅	Bounding Box	
	Editors	> 🌣	Clear History Log	

-	Avizo binary (*.am)
	Avizo ascii (*.am)
	Avizo ZIP (*.am)
	Avizo 6 binary (*.am)
	Avizo 6 ascii (*.am)
	Avizo 6 ZIP (*.am)
	2D Tiff (*.tif)
	3D Tiff (*.tif)
	AVS Field (*.fld)
	Analyze 7.5 data (*.hdr)
	Analyze AVW data (*.avw)
	BMP (*.bmp)
	DICOM (*.dcm)
	EPS (*.eps)
	JPEG (*.jpeg *.jpg *.JPG *.JPEG)
	JPEG 2000 (*.jp2 *.jpx *.j2k *.jpc *.j2c)
	JPEG 2000 (Compatibility Avizo 9.0) (*.jp2 *.jpx *.j2k *.jpc)
	MRC Stack (*.mrc *.ali *.ALI *.MRC)
	MRC Volume (*.rec *.REC)
	Matlab mat-file (*.mat)
	Matlab v7 mat-file (*.mat)
	Nifti (*.nii)
	PDF 2D (*.pdf)
	PNG (*.png)
	PNM (*.pgm *.ppm *.pbm)
	Raw Data 2D (*.raw)
	Raw Data 3D (*.raw)
	SEGY (*.segy)
	SGI-RGB (*.rgb *.sgi *.bw)
File name:	Visilog 6 (*.im6)
ave as type:	Avizo binary (*.am)

## **Export data**

The results of the different processing modules can be exported from the Amira pool.

Exporting data on the disk:

- Right-click on the dataset you want to save
- Click on the "Export data as" icon
- Select the relevant format



File name:	chocolate-bar.am
Save as type:	Amira binary (*.am)
	Amira binary (*.am)
	Amira ascii (*.am)
a Folders	Amira ZIP (*.am)
Dete I	2D Tiff (*.tif)
Data Ir	3D lift (*.tif)
Memor	Avstreid (".fld)
incino.	Analyze 7.5 data (*.ndr) Analyze AVAV data (*.ndr)
Physica	RMP (* hmn)
	DICOM (*.dcm)
Voxel S	EPS (*.eps)
Proviou	JPEG (*.jpeg *.jpg *.JPG *.JPEG)
Fleviev	JPEG 2000 (*.jp2 *.jpx *.j2k *.jpc *.j2c)
	JPEG 2000 (Compatibility Avizo 9.0) (*.jp2 *.jpx *.j2k *.jpc)
	MRC Stack (*.mrc *.ali *.ALI *.MRC)
	MRC Volume (*.rec *.REC)
	Matlab mat-file (*.mat)
	Matlab v/ mat-file (*.mat)
	NITU (".nii) DDE 2D (* edf)
	PDF 2D (.pdi) DNG (* ppg)
Master	PNM (*.ngm *.npm *.nbm)
History	Raw Data 2D (*.raw)
Histogr	Raw Data 3D (*.raw)
Sharod	SEGY (*.segy)
Shared	SGI-RGB (*.rgb *.sgi *.bw)
	Visilog 6 (*.im6)
#### Image pre-processing: adjustments



## **Basic image data editing – Volume Edit**

Volume Edit: interactive editing with various tools

Two types of tools are available:

• A draw tool:

allows selecting the 3D projection behind a contour drawn in the viewer

• Dragger tools:

allow selecting a region by dragging, rotating, resizing a 3D shape (box, ellipsoid, cone, etc.).





#### **Basic image data editing – Volume Edit**

Volume Edit – example of creating a sub-volume with the draw tool:

#### **Draw tool selection**



#### "Cut outside" result



#### Data: MoSi2-shear-corrected.am

## **Basic image data editing – Volume Edit**

Volume Edit - example of creating a Cylindrical Mask:

- Tool port settings: TabBox, Cylinder, Z Axis
- Adjust cylinder using Orthographic Camera mode
- Set Padding value to the value of the voxels of the "exterior" (e.g. 0)
- Push buttons: Cut Outside and Edit Create Mask





- For serial sections (e.g. Light Microscopy)
- Pushing the Edit button activates the Alignment viewer and tools and the Align and Landmarks menus
- Visualization of two consecutive slices in overlay



 Align
 Landmarks
 Help

 Cravity centers
 Least-squares

 Landmarks
 Landmarks

 ✓
 Edge detection

 Align current pair
 Align all slices

 Options...
 Continue

#### Slice: 🗹 — 🍋 🕨 5 🔍 Q. 1:1 🛔 2x² 🗣 🕄 🕨 📚 📚 # 🕂 📲



#### Data: leaf image stack

- Available operations:
  - Translate
  - Rotate
  - Mirror

each slice with respect to the next one.

- Modes and options (some examples):
  - Manual
  - Land-Mark
  - Intensity-based
- Possibility to use a mask
- Resample aligned stack (by pressing the Resample button of the module).



**Thermo Fisher** 

- Rotation alignment should be disabled for FIB-SEM (Align / Options)
- No rotation while FIB-SEM data collection!
- For disabling rotation go to Align menu Options in order to access the AlignSlices Options pop-up.



Alignment example – comparison of raw data set (left-side) with aligned data set (right-side)

First xy slices of the volume

Last xy slices of the volume

#### ina

**ThermoFisher** SCIENTIFIC



#### Introduction to spatial domain filtering

Filtering – enhance the data quality

Most filters operate in the spatial domain – each pixel/voxel is evaluated and its filtered valued is given by applying a formula to the values of its neighbors in the input data:

- The neighborhood type needs to be defined
- The formula is filter specific.



# Image filtering: how to apply a filter in Avizo

Various filtering modules are available in Avizo.

They are mainly grouped according to their types into different sub-categories of the Image Processing category :

- Edge Detection
- Enhancement Filters
- Sharpening

. . .

Smoothing and Denoising

Right click on the data set in order to Access the module category menu.

📚 chocolate-bar.am 🛛 🗸	Ľ	1	<enter a="" search="" string=""></enter>			Ð
🗀 Animate	>	^ 🗅	Distance Maps	>	🔅 Anisotropic Diffusion	$\hat{}$
🗀 Annotate	>	C	Edge Detection	>	🔅 Bilateral Filter	
🗀 Compute	>	C	Enhancement Filters	>	🔅 Box Filter	
🗋 Convert	>	C	Frequency Domain	>	🔅 Curvature-Driven Diffusion	
Correlation	>	C	Grayscale Transforms	>	🔅 Despeckle	
🗀 Display	>		Morphological Operations	>	🔅 Edge-Preserving Smoothing	
🗅 Fiber Tracing	>	C	Propagation	>	🔅 Flow Inpainting	
🗋 Geometry Transforms	>	C	Separating And Filling	>	🔅 Gaussian Filter	
Сэ іні 🖌	>	C	Sharpening	>	🔅 Majority Filter	
🗀 Image Processing	>	C	Skeletonization		🔅 Median Filter	
🗋 Image Segmentation	>		Smoothing And Denoising	>	🔅 Nagao Filter	
🗀 Local	>	\$	Filter Sandbox		🔅 Non-Local Means Filter	
🗀 Measure And Analyze	>	\$	Image Stack Processing		🔅 Recursive Exponential Filter	
🗀 Roi	>	\$	Image Volume Processing		🔅 SNN Filter	
		$\sim$			👛 Siama Filter	$\sim$

# Image filtering: how to apply a filter in Amira

Various filtering modules are available in Amira.

They are mainly grouped according to their types into different sub-categories of the Image Processing category :

- Edge Detection
- Enhancement Filters
- Sharpening

. . .

Smoothing and Denoising

Right click on the data set in order to access the module category menu.

📚 chocolate-bar.am 🛛 🗸	Ľ	۲	Senter a search string			9
🗀 Animate	>	^	🗀 Distance Maps 🥢	>	🏟 Anisotropic Diffusion	$\hat{}$
🗋 Annotate	>		🗀 Edge Detection	>	🔅 Bilateral Filter	
🗀 Compute	>		🗀 Enhancement Filters	>	🔅 Box Filter	
🗋 Convert	>		🗀 Frequency Domain	>	🔅 Curvature-Driven Diffusion	
Correlation	>		🗀 Grayscale Transforms	>	🔅 Despeckle	
🗀 Display	>		C Morphological Operations	>	🔅 Edge-Preserving Smoothing	
🗅 Fiber Tracing	>		C Propagation	>	🔅 Flow Inpainting	
🗋 Geometry Transforms	>		🗅 Separating And Filling	>	🔅 Gaussian Filter	
Са тні 🖌	>		🗀 Sharpening 🦊	>	🔅 Majority Filter	
🗀 Image Processing	$\rightarrow$		C Skeletonization		🔅 Median Filter	
🗋 Image Segmentation	>		🗀 Smoothing And Denoising	>	🔅 Nagao Filter	
🗀 Local	>		🔅 Filter Sandbox		🔅 Non-Local Means Filter	
🗋 Measure And Analyze	>		🔅 Image Stack Processing		🔅 Recursive Exponential Filter	
🗀 Roi	>		🔅 Image Volume Processing		🔅 SNN Filter	
		$\sim$			🍅 Sioma Filter	$\sim$

# Image filtering: how to apply a filter in Avizo

Filter Sandbox module allows testing different filters in order to make the best choice for a given dataset:

- Select a filter
- Tune its parameters
- Appy result on a sub-volume of the dataset (useful for large datasets)

#### E.g. Anisotropic Diffusion







# Image filtering: how to apply a filter in Amira

Filter Sandbox module allows testing different filters in order to make the best choice for a given dataset:

- Select a filter
- Tune its parameters
- Appy result on a sub-volume of the dataset (useful for large datasets)

#### E.g. Anisotropic Diffusion







## **Image filtering: Box**

Box Filter performs the arithmetic mean of the pixel/voxel values in the neighborhood window



## **Image filtering: Median**

Median Filter outputs the median value of the pixel/voxel values in the neighborhood window



**Thermo Fisher** 

## **Image filtering: Non-Local Means**

Non-Local Means outputs the weighted average of the values in the local neighborhood. The weight of each pixel/voxel is given by how similar its neighborhood (non-local) is to the local neighborhood.



## Image filtering: adapt filter choice to dataset and problem



#### Box Smoothe

Smoothening Not edge preserving



Median Edge preserving but some details are lost



NLM Edge preserving Better preservation of details

**Thermo Fisher** 



# Image filtering: kernel type and connectivity

Kernel type – defines the neighborhood configuration, e.g. cube



Kernel connectivity in 3D (for a cube kernel)



**Thermo Fisher** 

# Image filtering: kernel size

Kernel size – refers to the half kernel. Example for a cube type kernel:



## Image filtering: denoising filters

Examples of filters for removing "salt and pepper noise" (white and black dots on the image):

- Gaussian smoothening, not effective for removing high contrast local noise
- Median fast and efficient but tends to remove small details and to blur the result



Input

Gaussian

Median

## Image filtering: denoising filters

Examples of filters for removing "salt and pepper noise" (white and black dots on the image):

- Bilateral performance in between median filter and NLM
- Non-Local Means very effective at removing noise while preserving the edges but slow



Input

Bilateral

NLM

Thermo Fi

## Image filtering: contour detection and enhancement

- Sobel and Moments (e.g. variance, kurtosis) edge detection filters •
- Unsharp Masking edge enhancement filter ٠ Best practice: employ unsharp masking after denoising





Moments



Unsharp Masking

**Thermo Fisher** 

NLM + Unsharp Masking

## **Background correction**

Basic idea: remove low frequencies in image

Can be done in Avizo by means of different modules:

- Correct Z Drop: fits an arbitrary function of z to the average intensity in each slice
- Block Face Correction: matches masked-slice average intensity to volume average intensity
- Background Image:

estimates background image, slice by slice, by fitting a 2nd order polynomial (to the masked region)

- Shading correction wizard: removes image low frequency by dividing the input image by a background image
- Background Detection Correction:

estimates the background from a B-spline model (for example) with specified grid and removes it.



## **Background correction**

Basic idea: remove low frequencies in image

Can be done in Amira by means of different modules:

- Correct Z Drop: fits an arbitrary function of z to the average intensity in each slice
- Block Face Correction: matches masked-slice average intensity to volume average intensity
- Background Image:

estimates background image, slice by slice, by fitting a 2nd order polynomial (to the masked region)

- Shading correction wizard: removes image low frequency by dividing the input image by a background image
- Background Detection Correction:

estimates the background from a B-spline model (for example) with specified grid and removes it.





## Image pre-processing: exercise 1



#### Background correction

Apply a background correction method in order to obtain a similar result:



#### Corrected



## Image pre-processing: exercise 1

#### Solution

- Example 1: Shading Correction Wizard
  - Threshold 75-255
  - Normalization 130
- Example 2: Background Detection Correction
  - Type: B-spline
  - Size: 5, 5, 5

#### Tips:

- Visualization setup for comparing images:
  - Use multiple viewers with linked camera
    - right-click on one of the images in the two viewers
    - select "Link camera to..."
    - then click on the image in the second viewer.
- Assess background
  - Use Line Probe module and increase number of samples if necessary « take average » (with increased radius / long. Width)



Thermo Fi

## Introduction to frequency domain filtering

#### **Fourier Transform**

 Filters out low frequency (small intensity variations) or high frequency (strong intensity variations e.g. edges) components in images

#### Steps

- Compute the Fourier Transform of the image
- Multiply the images Fourier Transform by a filter function (low-pass filter, high pass-filter, etc.)
- Compute the inverse Fourier Transform of the result (the result is mapped back to the spatial domain)

#### Why filter in the frequency domain ?

 Can be much faster than the spatial domain filtering (a convolution in the spatial domain is replaced by a multiplication in the frequency domain).

# Image filtering: FFT

Frequency domain filtering can be achieved in Avizo by means of FFT Filter module

#### FFT Filter has two main functioning modes:

- Spatial: removal of periodic structures or stripes
- Frequency: removal of periodic/directional structures and spots from the FFT magnitude (advanced-user mode)

#### Principal use-cases:

- Curtaining artefacts in FIB-SEM (the module's parameters are set by default for filtering vertical stripes)
- Horizontal stripes in light-sheet microscopy images







## Image pre-processing: exercise 2

#### Slice Alignment

- Load fib/MoSi2-shear-corrected.am
- Use Volume Edit to create a mask
  - Use the different transformers to rotate and position a box
  - Exclude the trench and upper surface
  - Use "Cut Outside"
- Use Align Slices on masked image
  - Translation only
  - Automatic Least Squares Mode



https://youtu.be/HKh4rCr\_blg

# **xy slice**

yz slice



#### yz slice before and after alignment



#### Image pre-processing: exercise 3

Background Correction, Frequency Domain Filtering, Denoising

- Background correction
   Try:
  - Shading Correction Wizard
  - Background Detection Correction

- Reduce curtaining artefacts
  - FFT Filter



Original

- Denoise
  - Filter Sandbox: e.g. NLM, median, bilateral

Exercise solution: <u>https://youtu.be/HKh4rCr\_blg</u>



Corrected







#### **Ring Artefact Removal**

- Adjusts the mean intensity of the pixels of concentric rings to the mean intensity of the whole image
- The rotation axis is assumed to be aligned to the Z-axis of the dataset's local coordinate system
- The center of the rings needs to be adjusted manually if: the center of the image ≠ the center of rotation during CT acquisition
- Using the settings "Lower Threshold" and "Upper Threshold", the calculation of the mean values can be restricted to a certain intensity interval. This might be necessary:
  - For objects with inhomogeneous density (large pores, multi-material, etc.)
  - For a geometry deviating from a cylinder
  - If a cylindrically shaped object was measured de-centered.
- The input data-type must be 16-bit unsigned. Use "Convert Image Type" if necessary.



#### See also

#### Ring Artefact Removal examples:



no intensity range adjustment

#### Image pre-processing: deconvolution



#### **Image deconvolution**

Iterative Maximum Likelihood Image restoration algorithm. Types of **Deconvolution**:

- Non-blind (a measured or computed PSF *Point Spread Function* is used)
- Blind (the PSF is estimated along with the data)

Theoretical PSF generation via **Generate Point Spread Function** module (Project View  $\rightarrow$  Create Object) PSF estimation via **Extract Point Spread Function** module (bead extraction).



#### Image deconvolution: measuring the PSF

Imaging beads with desired acquisition settings (dataset available at ...data/deconv/beads.am) Beads image visualization: **Image Ortho Projections + Volren** PSF Estimation:

- **Projection Cursor** (for creating Landmarks)
- Extract Point Spread Function (Adjust centers + Estimate size)

Data before deconvolution



Data after estimating the PSF and applying standard deconvolution


### Image deconvolution: implemented mathematical PSF models

For theoretical PSF computation: Generate Point Spread Function module

- Choose type of microscopy: widefield or confocal
- Set microscope parameters: numerical aperture, wavelength, refractive index







Confocal

### Image deconvolution: Blind method

Simultaneous data restoration and PSF estimation

 Can be initialized with a theoretical or measures PSF (that will only be used for the first iteration of the algorithm)

**Thermo Fisher** 



### **Image deconvolution: Standard method**

Standard deconvolution example (dataset available at: data/deconv/polytrichum.am & polytrichum-psf.am):

- Resample PSF (optional)
- Apply Deconvolution module in standard mode





### Image segmentation: Segmentation Editor

**Thermo Fisher** 

SCIEN



### **Segmentation Editor: workroom**



**Thermo Fisher** 

SCIENTIFIC

## **Segmentation Editor: workroom**

Dedicated workroom for interactive segmentation



## **Segmentation Editor: general principle**

 Generate selection using the tools available in the selection generation toolbar e.g. Brush, Lasso, Magic Wand (region growing), Superpixel



• Apply **masking** to assist the segmentation with the brush tool and magic wand.

<ul> <li>Mask</li> </ul>	king		
Range	82		255

Modify selection using the tools available in the selection modification toolbar



**Assign selection** to material using the tools available in the selection assignment toolbar



## **Segmentation Editor: Image Viewers and Navigators**

 Switch between the 1, 2 or 4 viewer modes in the Viewer toolbar. A summary of navigation shortcuts is available through the Help button, at the right of the Viewer toolbar.

• Adjust the display configuration in the display menu at the bottom right part of each viewer

Colormap				
0	255			
Adjust to:	min-max histogram			
Materials				
Draw Style: 🔵 Contour 🍥 Hatched				

Camera Mode On



Thermo Fi

## **Segmentation Editor: Selection generation tools**



### **Brush:** 2D painting

- Right click inside close contour: • flood fill in 2D
- CTRL: erase •



Eraser: 2D removing

Successive paint strokes remove voxels from • the selection.



### **Brush size**

Adjusts the size of both the **brush** and **eraser** . tools



- Generate selections in 2D and 3D by defining closed contours
- "Auto-trace" option: snap to gradient (in 2D)



### Magic Wand: 2D & 3D

Region growing within intensity range selected with the mask



### Superpixel: 2D

- Partitions the image into superpixels that share • common characteristics.
- Brush the partitions to add them to the selection
- CTRL: erase

## **Selection generation tools: Lasso**

- Draw the border of a polygonal area with the lasso tool.
- Hold left mouse button for automatically created control points
- Or click at each point individually
- Press ENTER once to edit the control points
- Press ENTER again to finalize the selection



Thermo Fisher

### **Selection generation tools: Magic Wand**

- Adjust the intensity range to mask the feature of interest.
- Click on a blue shaded area to select the connected pixels within the mask.



**Thermo Fisher** 

## **Selection generation tools: Superpixel**

- Superpixel (S): partitions the image displayed in the currently active 2D viewers into superpixels. The pixel follow the edges of the image.
- Tool parameters, colormap range, camera position and zoom level affect the size and shape of the partitions.
- Brush in the viewer to add superpixels to the voxel selection.





## **Segmentation Editor: Selection modification tools**



Interpolate: between selections from parallel slices

Shape-based interpolation



Fill holes (2D or 3D)



Shrink (2D or 3D)



Grow (2D or 3D)



**.**≉

**Invert Selection** 



圃	Clear selection
う	Undo
Ç	Redo
Ŧ	Create patches

**Thermo Fisher** 

## **Segmentation Editor: materials assignment**

### Materials assignment:

- Add selection to material (or replace / substract)
- Materials can be locked to disable any changes to the Material by clicking the lock button.

### Rules of thumb:

- Only one label per voxel
- Always keep the "Exterior" material

		3D	2D	
∨ 😑 foam.labels*		S)	۲	G
Exterior		S)	Ø)	6
Material1		Ø	٢	6
Assign Selection to Materia	1			
Material1		Active Se	lection	
+ Add	— Subtract	🕂 Rep		

### **Segmentation Editor: materials visualization**

 Click on the eye button to activate the visualization of the material in the 3D viewer



**Thermo Fisher** 

SCIENTI

## **Segmentation Editor: Selection tools**

### Tips:

Click the downward arrow on the selection tool for more options



- Right-click a material to:
  - Create a new material
  - · Select the complete material, when you want to modify the
  - Edit display color
  - Rename the material
  - Delete the material
  - Create a new group to move the material into



## **Segmentation Editor: Patch tool**

- The Patch tool is relevant for using AI Assisted Segmentation
- When a new patch set is created, it appears in the material list.

Use the Add patches tool to stamp patches on the XY image.

ନ୍ 🎢 뷲 🔓 🍓 🌞 🛄 🔍 差

 Navigate through the patch set to select individual patches. Press Delete to remove them one at a time.

匬

1

✓ Patch Set N	lavigation	
Patches	<	● > 5 / 6 🚑



### **Segmentation Editor: Automated selection tools**

### Threshold

- Select the image intensity range in the "Masking" window
- > Create a selection for the current slice or total volume
- > Add the selection to a material

Automated Segmentation Tools							
Tools	[ملم]		$\sim$	<i>‴.</i>	€.×	×	8

-	T
	•

### op-hat

- Compute a top-hat image to detect dark or bright features
- Select a threshold for the feature



#### Watershed

- Grow regions from seed points.
- Edge detection based on a computed gradient image



#### **Texture classification**

• Label regions with different textures. The classifier learns to recognize the features for the whole image.



#### **Remove islands**

• Merge small islands with the surrounding material.



#### Al Assisted segmentation

Train and apply a shallow neural network on patches containing ground truth.

#### **Thermo Fisher** S C I E N T I F I C

# **Automated Segmentation Tools: Threshold**

Segment the pores in *foam.am* using the Automated Thresholding tool

- 1. Set the desired intensity range in the Masking window
- 2. Select if you want to apply it on the current slice only or the full volume
- 3. Click Create Selection
- 4. Add the selection to the material





The Top-Hat segmentation extracts small elements and details from given images. It detects the dark or the white area, corresponding to the valleys or the narrow peaks.

Top-Hat computation perform (1) Grayscale Closing  $(C{f})$  of Original Grayscale followed by (2) Subtract Grayscale Closing by Original Grayscale  $(C{f} - f)$  to yield a Top-Hat image and (3) Thresholding of the Top-Hat image

There exist two types of Top-Hat transform:

- The Black Top-Hat: it is defined as the difference between the cube closing with a given size kernel and the input image. A threshold allows selecting the darker elements of the Top-Hat result.
- The White Top-Hat: it is defined as the difference between the input image and its opening cube. The threshold allows selecting the brighter elements of the Top-Hat result.













**Thermo Fisher** 

SCIENTIFIC



**Thermo Fisher** 

SCIENTIFIC



**Thermo Fisher** s c | e N T | F | C



**Thermo Fisher** 

SCIENTIFIC

#### Avizo 3D - Untitled File Window Help 📚 Segmentation 🌼 Recipes 🛛 🛸 Image Recipe Designer 🛛 🛰 Filament Start - Project The Meshing Animation Multiplanar 🖌 🥙 Size 👥 🔍 👂 🧨 🎠 🐁 🤙 🖷 🕲 🕲 🌲 🌐 🕤 で Segmentation Camera Mode On chocolate-bar.am Grayscale Image 147 / 294 Label Image Create New Label chocolate-bar.labels\* Patch Set NO SOURCE Create New Patch Set Create New Material 3D 2D $\odot$ G Material1 Assign Selection to Material Material1 No Selection Add **Replace** Th Automated Segmentation Tools Tools $\overline{\mathcal{D}}$ Top-hat 2/2: Use the Top-hat Range to change black areas depth Type Black White Size 3px Kernel Faster 🔘 Precise Selection Settings Select only in material Filter Top-hat Range 1239 Avizo Avizo Apply to Current slice All slices Volume 3D~ MEMORY USAGE Stop

**Thermo Fisher** 

SCIENTIFIC





Texture supervised classification aims at performing image segmentation based on local textural features, when typical intensity-based methods are not appropriate.

A texture classification workflow is composed of 3 main steps:

- Selection of texture features considered in the model
- Learning of a texture model on representative annotated training data
- Application of the texture model to segment a new image

During the learning step, all the selected features are computed on a local neighborhood of pixels belonging to labels defined in a training image. A subset of discriminant features is retained and only these features are computed during the segmentation step.





**Thermo Fisher** S C I E N T I F I C









### **Automated Segmentation Tools: Remove Islands**

Merge small areas to the surrounding material.

- Define the island max size.
- Click Compute highlight to get a preview of which islands will be removed.
- Select if you want to overwrite the current label or create a new label.
- Click *Compute Label* to apply the action.





Thermo Físher

### **Segmentation Editor: AI-Assisted Segmentation**




**Thermo Fisher** 

SCIENTI



**Thermo Fisher** 

SCIENTI



**Thermo Fisher** 



### **ThermoFisher** SCIENTIFIC



Create New Label

3

90

**Active Selection** 

• > 6/6 🚣

2 Replace

Volume

Create New Patch Set

All slices

Create New Material

File Window Help

Grayscale Image CryoTomo.am

😂 CryoTomo.labels\*

Exterior

Material1

Assign Selection to Material No material selected in the list

Patch Set Navigation

Automated Segmentation Tools

[.....]

Current slice

Patches

Masking

Range 55551

Tools

CryoTomo.patchSet\*

Add

CryoTomo.labels\*

CryoTomo.patchSet\*

Segmentation

Label Image

Patch Set

### **Thermo Fisher** SCIENTIFIC 🎓 Start 🛛 🛫 Project 🛛 📚 Segmentation 🛛 🐨 Meshing 🥨 Recipes 😪 Image Recipe Designer 🛸 Filament 🚟 Animation 🖉 Multiplanar $\Box \mathbf{X}$ Size 🔵 ?,",★ ि d, ♠ 🗮 🛛 ₺ 🗇 ♡ 🕒 🕨 Interaction Mode On 38 3D 2D G 0 C. 3 ۲ C.







#### **Thermo Fisher** SCIENTIFIC





File Window Help

# **Thermo Fisher** SCIENTIFIC 🕨 Interaction Mode On



Label Image

Patch Set

Patches

Tools

Patch Size

Epochs

Material

Apply to

#### File Window Help 🔺 Start 🖃 Project 📚 Segmentation 🔷 Meshing 🗱 Recipes 😪 Image Recipe Designer 🛰 Filament 📰 Animation 🔚 Multiplanar $\Box \mathbf{X}$ Segmentation 🖌 🥖 Size 🔵 ₽, ℤ, ╊, ि 🤙 👾 🗱 🔍 ≵, 🗇 ᠑ ᢗ' 🛓 🛤 Camera Mode On 🛛 🔳 📰 😭 Grayscale Image 38 / 80 Create New Label CryoTomo.labels\* CryoTomo.patchSet\* Create New Patch Set **Create New Material** 3D 2D G 🚔 CryoTomo.labels\* 3 (D) (D) C Exterior Assign Selection to Material Material1 No Selection + Add **C**Replace Yeatch Set Navigation > 6 / 20 🚣 Range 55551 **Automated Segmentation Tools** [.1.] **AI Assisted Segmentation** 96 x 96 px 20 Elastic Deformation Strength 3.00 Material1 O Current slice All slices Volume Create Selection

**Thermo Fisher** 

#### File Window Help 🛷 Meshing 🛛 🎎 Recipes 🛛 🗞 Image Recipe Designer 🛛 😪 Filament 🛛 🚟 Animation 🖉 Multiplanar Segmentation 🖌 🍠 🛛 Size 🌒 ₽,ℤ,≒,ि,⊴,♠,ሺ0,₺,๗ つெ ± || = | = = = | 🥝 Camera Mode On Grayscale Image CryoTomo.am 38 / 80 Create New Label Label Image CryoTomo.labels\* Patch Set CryoTomo.patchSet\* Create New Patch Set **Create New Material** 3D 2D C 0 Exterior 3 3 Assign Selection to Material Material1 Active Selection +Add **∂** Replace -Subtract Year Patch Set Navigation > 6 / 20 🚣 Patches Range 55551 Automated Segmentation Tools [عالم] 11. Tools AI Assisted Segmentation Patch Size 96 x 96 px 20 Epochs Elastic Deformation Strength 3.00 Material Material1 Apply to Current slice All slices Volume Create Selection

**Thermo Fisher** 

File Window Help

# **Thermo Fisher** SCIENTIFIC



File Window Help

Grayscale Image CryoTomo.am

Material1

+Add

Patch Set Navigation

**Automated Segmentation Tools** 

Elastic Deformation Strength 3.00

∿ ℤ

96 x 96 px

Material1

Current slice All slices

20

Material1

Patches

Range 55551

Tools

Patch Size Epochs

Material

Apply to

CryoTomo.patchSet\* Assign Selection to Material

CryoTomo.labels\* CryoTomo.patchSet\*

🕋 Start 👘

Segmentation

Label Image

Patch Set

### 🖂 Project 🛭 📚 Segmentation 🛛 🌴 Meshing 🛛 🎇 Recipes 🛛 🗞 Image Recipe Designer 🔌 Filament 🛛 📇 Animation 🖉 Multiplanar $\Box \mathbf{X}$ 🖌 🖉 size 🌒 🚽 🥐 🎢 🎝 🖓 🏞 🔓 🤙 🌺 🛄 🕘 🖓 🛓 💽 Camera Mode On 🛛 🗖 📰 📰 🧣 38 / 80 Create New Label Create New Patch Set Create New Material 3D 2D O C. ۲ No Selection **∂** Replace > 6 / 20 🚣 8 **AI Assisted Segmentation**

Volume

**Thermo Fisher** 



**Thermo Fisher** S C I E N T I F I C



#### File Window Help 👘 🐨 Meshing 🛛 🗱 Recipes 🛛 🗞 Image Recipe Designer 🔧 Filament 🛛 🚟 Animation 🖉 Multiplanar 👚 Start 🛛 ---- Project 🛛 🕪 Segmentation $\Box \mathbf{X}$ 🖌 🍠 Size ———— 👂 🎢 🏝 🔓 🤙 🌞 🛍 🕤 🔿 Segmentation Camera Mode On Grayscale Image Image 92 / 184 Image.labels ~ **Create New Label** Label Image **Create New Patch Set** Patch Set NO SOURCE 🗸 **Create New Material** 3D 2D Image.labels\* 3 0 C C 93 Exterior 30 ſ. Material1 Assign Selection to Material Material1 No Selection + Add **C**Replace 255 Range 12 **Automated Segmentation Tools** ₩ 🗠 🗸 📈 🖾 🖗 Tools O Current slice All slices Ø Volume XY

**Thermo Fisher** 

### **ThermoFisher** SCIENTIFIC







#### File Window Help segmentation 🛛 💎 Meshing 🛛 🌼 Recipes 🐟 Image Recipe Designer 🛛 🕊 Filament 🔤 Animation 🖉 Multiplanar 🕋 Start 🛛 🛁 Project $\Box \times$ Segmentation 🕨 Interaction Mode On 🔳 💷 📰 💡 Grayscale Image Image 92 / 184 Label Image Image.labels ~ Create New Label **Create New Patch Set** Patch Set Image.patch! ~ **Create New Material** 3D 2D Material1 📕 Image.patchSet Assign Selection to Material No material selected in the list No Selection Add 2 Replace Yeatch Set Navigation > 0 / 0 🚣 < • Patches 255 Range 12 Automated Segmentation Tools 🗠 🔨 깼 📖 🛤 [**.**]. Tools O Current slice All slices Volume

**ThermoFisher** 

#### **ThermoFisher** SCIENTIFIC



File Window Help

Grayscale Image Image

Material1

Image.patchSet\*

Assign Selection to Material No material selected in the list

Patch Set Navigation

Automated Segmentation Tools

Patches

Range 12

Tools

+Add

< 0

Segmentation

Label Image

Patch Set

### 🆀 Start 🛛 🚽 Project 🛛 🕪 Segmentation 🛛 💎 Meshing 🗱 Recipes 😪 Image Recipe Designer 🔌 Filament 💥 Animation 🖷 Multiplanar 🖌 🍠 Size 🌒 🕨 Interaction Mode On 🔳 💷 📰 💡 92 Image.labels ~ Create New Label Image.patch! <</td>Create New Patch Set Create New Material 3D 2D 3 ۲ G 0 Active Selection 2 Replace > 1 / 17 🚣 ^ ∥ 🖾 🕺 [ala] Current slice All slices Ø Volume

**Thermo Fisher** 

### Thermo Fisher SCIENTIFIC



File Window Help 🐨 Meshing 🗱 Recipes 🛭 😪 Image Recipe Designer 🛛 🛰 Filament 🛛 🚟 Animation 🖉 Multiplanar Segmentation Segmentation 🗾 🖉 Size 🥥 ९१६ ि े ् छ 0 ₺ @ २० ₺ 🕨 Interaction Mode On 🔳 💷 📰 🧧 Grayscale Image Image 92 Create New Label Label Image Image.labels ~ **Create New Patch Set** Patch Set Image.patch! ~ **Create New Material** 3D 2D Material1 📕 Image.pa ۲ Assign Selection o Material Material1 tive Selection +Add **∂** Replace - Subtract Patch Set Navigation > 1 / 17 🚣 < • Patches Masking Range 12 Automated Segmentation Tools щ 🗠 🗸 🔣 🛤 Tools O Current slice All slices Volume 1 to

**Thermo Fisher** 

File Window Help 📚 Segmentation 🛛 🏘 Meshing 🐗 Recipes 🛭 🗞 Image Recipe Designer 🛛 🕊 Filament 🔤 Animation 🖷 Multiplanar Segmentation 🖌 🥖 Size 🔷 🕨 Interaction Mode On 🔳 💷 📰 💡 ?,",★ ि 4, ∰ 📓 🔍 ≵ 🗇 つ ெ 🛓 Grayscale Image Image 92 Create New Label Label Image Image.labels ~ **Create New Patch Set** Patch Set Image.patch: ~ **Create New Material** 3D 2D Material1 Image.patchSet\* Assign Selection to Material Material1 No Selection +Add Re Patch Set Navigation > 1 / 17 < • Patches Masking Range 12 **Automated Segmentation Tools** V ∭ 🖾 🕅 [علم] Tools Current slice All slices Ø Volume th

Thermo Fisher



#### **Thermo Fisher** s c | e N T | F | C



### Thermo Fisher



### Thermo Fisher





**Thermo Fisher** 



### **Image segmentation: Segmentation Editor**

**Thermo Fisher** 

SCIEN



## **Segmentation Editor: workroom**

Dedicated workroom for interactive segmentation



## **Segmentation Editor: workroom**

Dedicated workroom for interactive segmentation



**Thermo Fisher** 

SCIENTI

### **Segmentation Editor: 3D Viewer Position**

The default **3D Viewer Position** in Amira is "Bottom Right". You can switch it to "Upper Left" from: Edit -> Preferences ->Segmentation

General Layout On exit Molecules LDA Segmentation Rendering Performance Network Units Range Partitioning Recipes Auto Display   3D Draw Syle   Transparent Contour Hatched Dotted Light Dots   Color	Amira 🗛	Preferences												×
B) Draw Style Points 1 Points 2 Solid Selection Draw Style Transparent Contour Hatched Dotted Light Dots Determined Dotted Light Dots Viewer Position: Upper Left Bottom Right Undo Memory Limit (MB): 32 \$	General	Layout	On exit	Molecules	LDA		Rendering	Performance	Network	Units	Range Partitioning	Recipes	Auto Displa	y
Points 1 Points 2 Solid   Selection Draw Style  Transparent Contour Hatched Dotted Light Dots Opacity: 9 Golor:  Color:  Labels Draw Style Contour Hatched Dotted Light Dots Viewer Position:  Upper Left Bottom Right Undo Memory Limit (MB): 32 Memory Limit (MB): 32	-3D Dra	w Style												
Selection Draw Style Transparent Contour Hatched Dotted Light Dots Opacity: Color: Labels Draw Style Contour Hatched Dotted Light Dots Viewer Layout 3D Viewer Position: Upper Left Bottom Right Undo Memory Limit (MB): 32 OK Cancel Apply Help	Points 1 O Points 2 Solid													
Tansparent Contour Hatched Lobted   Opacity: 96   Color: 96   Lables Draw Style Contour   Contour Hatched Dotted   Light Dots   Viewer Position: Upper Left Bottom Right Undo Memory Limit (MB): 22	Selecti	on Draw Styl	e											
Opacity: 96     Color:     Labels Draw Style        Contour     Hatched           Viewer Layout   3D Viewer Position:   Upper Left   Bottom Right        Undo   Memory Limit (MB):   32     Memory Limit (MB):     32     OK     Cancel   Apply	💿 Tra	ansparent (	Contour	Hatched	O Dotte	d 🔵 Light Dots								
Color:  Labels Draw Style Contour Hatched Dotted Light Dots Viewer Layout 3D Viewer Position: Upper Left Bottom Right Undo Memory Limit (MB): 22	Opacit	у:	•		96	¢								
Labels Draw Style • Contour • Hatched • Dotted • Light Dots Viewer Layout 3D Viewer Position: • Upper Left • Bottom Right Undo Memory Limit (MB): 32 •	Color:													
Contour Hatched   Dotted Light Dots     Viewer Layout   3D Viewer Position:   Upper Left   Bottom Right   Undo   Memory Limit (MB):   32     OK   Cancel   Apply   Help	Labels	Draw Style												
Viewer Position: Upper Left Bottom Right Undo Memory Limit (MB): 32	🔘 Co	ntour 🔵 H	latched 🔘	Dotted 🔵 L	ight Dots									
3D Viewer Position:  Upper Left Bottom Right Undo Memory Limit (MB): 32 C K Cancel Apply Help	Viewer	Layout												
Undo Memory Limit (MB): 32	3D Vie	wer Position	: 💿 Upper	Left 🔵 Botto	om Right									
Memory Limit (MB): 32	Undo													
OK Cancel Apply Help	Memo	ry Limit (MB)	: 32 🛟											
OK Cancel Apply Help														
OK Cancel Apply Help														
OK Cancel Apply Help														
OK Cancel Apply Help														
OK Cancel Apply Help														
OK Cancel Apply Help														
OK Cancel Apply Help														
OK Cancel Apply Help														
										ОК	Cancel	Apply	Help	

# **Segmentation Editor: general principle**

• **Generate selection** using the tools available in the selection generation toolbar e.g. Brush, Lasso, Magic Wound (region growing), Threshold, Blow



• Modify selection using the tools available in the:

ĥ

Selection menu

 $\odot$ 

- With keys (shift/ctrl)
- Selection modification toolbar

Current slice 🔽 Show in 3D



• Assign selection to material using the tools available in the selection assignment toolbar

Active selection

### Rules of thumb:

SELECTION

2

- Only one label per voxel
- Always keep the "Exterior" material



# **Segmentation Editor: selection tools**

- Brush: 2D painting
- Right click inside close contour: flood fill in 2D
- CTRL: erase



- Lasso: 2D & 3D closed contours
- Generate selections in 2D and 3D by defining closed contours
- "Auto-trace" option: snap to gradient (in 2D)



### Pick & Move: 2D & 3D

- Pick and move (translate/rotate) selection
- Can be applied to all slices or current slice only

- Threshold: 2D & 3D masking
  - Select all voxels in intensity range



### Magic Wand:

- Region growing within intensity range
- CTRL: add new seeds
- Draw 2D barriers ("Draw limit line" option)



### **Blow:**

Blow a 2D balloon that stick to edges
## **Segmentation Editor: selection tools**

Tips:

• "Same Material Only" option available for: Brush and Magic Wand tools



• "All Slices" mode available for: Threshold, Magic Wand and Pick & Move tools



• "Masking" can be enabled: selection only within specified intensity range



## **Segmentation Editor: selection modification**

#### **Selection modification**

- Grow / Shrink (2D or 3D)
- Fill Holes (2D or 3D)
- Smooth (2D only)
- Invert Selection
- Snake: propagate a 2D selection to the next/previous slice, following grayscale intensities
- Interpolate: between selections from parallel slices
  - Shape-based interpolation
- Wrap: between selections from orthogonal slices
  - Shape-based RBF interpolation





## Segmentation Editor: materials assignment and modification tools

#### Materials assignment:

- Add selection to material (or replace / substract)
- Materials can be locked

#### Materials Menu:

- Fill holes (slices only)
  - For 3D rather use Selection > Fill Holes
- Smooth Label:
  - 2D or 3D smoothing of the label map (shape only)
- Remove Islands:
  - Select small connected components
  - Relabel them according to the dominant neighboring label



SELECTION

ર

Ð

Θ

Current slice 🔽 Show in 3D



3D

2D

 $\mathbf{\nabla}$ 

Colorize Lock

Select

Select

Select

## **Segmentation Editor: exercise 1**

Bubble segmentation

Use "Thresholding" and "Fill Selection" to segment chocolate bar and bubbles:



## **Segmentation Editor: exercise 2**

Caramel and biscuit segmentation

- Use "Brush" or "Blow" tool and "Interpolation" to segment the chocolate mousse.
- Use "Lasso" tool and "Interpolation" to segment the caramel.



#### Tips:

- Segment the **denoised** image rather than the noisy one
- "Blow" tool and "Lasso" (Auto trace) are sensitive to the visualization range. Adjust the contrast accordingly.

## **Segmentation Editor: solution to exercises**

#### Tutorial: <a href="https://youtu.be/IQsKXRr9Njs">https://youtu.be/IQsKXRr9Njs</a>

File Edit Project View Window XPand Python XScreen Seg	mentation Selection Help		
🕋 Start – 🤆 Project 🕫 Recipes 📚 Segmentation 💎	Meshing 🔌 Filament 🖼 Animation		
Segmentation Editor	- ×	↓ ▶ ♥ ♥ ₽ ₽ ₽ ₽ ▲ ▲   ₽ ™ ™ * *	≠ ~ @ ! ■ ■ = [=]
Image: chocolate-bar.filtered 🗸	¢?		
Label field: chocolate-bar.labels	New Rename Delete		
MATERIALS			
Color Name Exterior (Not Assigned) Chocolate_Bar Bubbles Mousse Caramel	3D 2D Colorize Lock Select Select Select Select Select Select Select Select		
> DISPLAY CONTROL	Add Delete Locate 2D Crosshairs		49 7 294
Select TON Volume Current slice Show in 3D Volume Current slice	<ul> <li>C C &gt; </li> <li>C → </li> <li>Hidden selection</li> <li>Ø</li> <li>41</li> </ul>	7Z	YZ
Masking 462 Preview 2 2D 3D Pos: Material:	928 Edit V V Enable Index: Intensity:		
Reauy			Stop 31% 📐

## **Segmentation Editor: solution to exercises**

#### Tutorial: <a href="https://youtu.be/lQsKXRr9Njs">https://youtu.be/lQsKXRr9Njs</a>

For setting the Viewer position as in the illustration, go to Edit-> Preferences->Segmentation-> ViewerLayout-3D Viewer Position: Upper Left

File Edit Project View Window XPand Python XScreen Segmentation Selection H	lelp		
🖀 Start – 🗄 Project 🌼 Recipes 📚 Segmentation 🐨 Meshing 🛰 Filamen	t 📟 Animation		
Segmentation Editor	□ ×	│ ▶ 🔍 ⊕ Q ଫ ଫ @ @ "@ "@ "@ "@ ≪   ⊨ % ,	≠ ∽ ◙ ⊨ ■ ■ ■
Image: chocolate-bar.filtered V	• ?		
Label field: chocolate-bar.labels	New Rename Delete		
MATERIALS			and the second
Color Name Exterior (Not Assigned) Chocolate, Bar Bubbles Mousse Caramel	3D 2D Colorize Lock Select Select Select Select Select Select Select Select Select Select		
> DISPLAY CONTROL	Add Delete Locate 2D Crosshairs		
SELECTION			
<ul> <li>Z O O</li> <li>Volume O Current slice I Show in 3D</li> </ul>	🖆 🗈 < ≽ 🎂	22	YZ
Auto hide cursor   Select only current material Square brush   Masking   462 Preview 2D 3D	41 928 Edit ∨ <b>V</b> Enable		
Pos: Index:		83 / 174	117 / 234
Material: Intensity:			
кеаду			Stop 31% 🕑

## **Visualization of segmentation maps**



## Visualization of segmentation maps: main modules

- Voxelized Rendering: displays the boundaries of voxels in a 3D volume,
- Create Label Colormap: Generates a label colormap with the colors defined in the Segmentation Editor
- Colorwash: details on next slide.
- Volren: displays 3d scalar fields volumes.
  - Connect a greyscale volume to 'Data' port, optionally a labeled image to port 'Labels'
  - Tune Transfer functions and materials.



Thermo Fi

## **Visualization of segmentation maps: Color Wash**

- Configure an Orthoslice to visualize the greyscale image
- Attach the Colorwash to Orthoslice
- Connect a label image to the port 'Data' of Colorwash
  - Adjust label transparency.





Thermo Fi

• Colorwash can also be connected to a grayscale image, various fusion rules are available

## Visualization of segmentation maps: side by side viewers

- Attach Ortho Slices on 2 datasets
- Set 2 viewers and visibility of Ortho Slices on each viewer Tip: pin "Data" port of one Ortho Slice to have it visible on both Ortho Slices
- Link cameras: right click in a viewer, link camera to... left click on 2<sup>nd</sup> viewer

**Tip**: For independent viewers, make sure to switch off "Link object visibility"





# Image: Image



## Visualization of segmentation maps: linking ports

#### Linking ports – e.g. "Slice Number" port:

- Activate "Connection Editor" for "Ortho Slice 2"
- Click on the connection icon next to the port and drag over the "Ortho Slice" in the Project View





• Slice number ports are now linked, changing one will change the other simultaneously.

#### **Before linking pots**



#### After linking pots



## Image segmentation: general principles



## Segmentation in image processing workflows

#### Step 1. Optimise image acquisition:

- Lower noise
- Improve contrast
- Remove artefacts

#### Step 2. Image pre-processing:

- Noise reduction filters
- Background correction
- Deconvolution

#### Step 3. Segmentation:

- Thresholding
- Mathematical morphology
- Watershed

#### Step 4. Post-processing:

- Separate objects
- Clean segmentation maps

## **Image segmentation: concepts**

#### **Thresholding:**

Binarization: separating the dataset pixels/voxels into object and background. •



Multi-thresholding: separating the dataset pixels/voxels into several groups. •



Filtered input data

#### Multi-thresholding result



## Image segmentation: thresholding methods

#### Main modules for performing thresholding:

- Interactive modules:
  - Interactive Thresholding
    - Used for binarization, allows setting the threshold value interactively
  - Multi-Thresholding
    - Up to five different regions separated by four different thresholds can be extracted
    - All thresholds are set interactively
- Automatic modules:
  - Auto Thresholding
    - Binary or 3-phase segmentation
    - The threshold or (thresholds for 3-phase mode) are computed automatically
    - 4 methods available for threshold computation
- Local thresholding modules:
  - Local Thresholding
  - Adaptive Thresholding



Thermo

## Image segmentation: thresholding methods example

- Use Multi-Thresholding module:
  - For setting segmentation regions: write a name for each segmentation region
  - For setting threshold values: study the histogram place thresholds between histogram lobes
  - The intensity range between two threshold values defines a region
  - Push Histo button to generate the histogram



## Image segmentation: thresholding methods example

#### **Multi-thresholding:**



Histogram

#### Multi-thresholding result



## Image segmentation: thresholding advanced

#### Hysteresis thresholding

- Starts from regions selected with high threshold
- Propagates into voxels with intermediate intensities up to a given length



Thermo

Tip: Use probes, or an interactive thresholding module to help set both threshold values

## Image segmentation: thresholding methods example

#### Local thresholding:

- Use Local Thresholding module:
  - For foreground object detection
  - For datasets presenting small background variations
- Three methods are available

Example on FoamPoro.am dataset:

Input slice







#### Local-thresholding\_result



## Image segmentation: thresholding methods example

#### Adaptive-thresholding:

- Use Adaptive-Thresholding module:
  - For thresholding problems that require to adapt the threshold locally e.g. in the case of intensity variation along the data

#### Example on ??? dataset:

Properties			×
<b>ଚ</b> ୬ ~	Adaptive Thresholding		?
Ŧ	Input Image:	foam.am 🗸 🔶	
Ŧ	Interpretation:	🔵 3D 🥥 XY planes	
Ŧ	Window Size X [px]:	30	
Ŧ	Window Size Y [px]:	30	
Ŧ	Threshold:	1	
Ŧ	Comparison Criterion:	greater-or-equal 🗸	
Ŧ	Threshold Mode:	Multiplicative mode 🛛 🗸	

#### **Global-thresholding result**

#### Local-thresholding result







## Image segmentation: thresholding methods limitations

#### Thresholding limitations (e.g. on Multi-thresholding):

• Segmentation artefacts at the boundary between regions (alternative: watershed)



Thermo



### Image segmentation: advanced concepts Part 1: mathematical morphology

204 Proprietary & Confidential | authoremail@thermofisher.com

## Image segmentation: erosion and dilation - binary

#### Mathematical morphology (mm):

- Structuring element neighborhood of voxels, defined by:
  - Size
  - Shape (cube, line, disk, ball)
  - Connectivity type reminder for a cube neighborhood of size 1:



- Basic mm operations:
  - Erosion shrinks the object
    - If any voxel in the neighborhood is 0, the voxel is set to 0 in the eroded image, else to 1
  - Dilation grows the object
    - If any voxel in the neighborhood is 1, the voxel is set to 0 in the dilated image, else to 1

## Image segmentation: erosion and dilation – binary

#### **Binary Erosion and Dilation exemple:**



**Erosion** result

**Dilation** result

**Thermo Fisher** 



## Image segmentation: erosion and dilation – grayscale

#### **Erosion and Dilation on grayscale data:**

- Erosion
  - Replace voxel value by the minimum intensity value in neighborhood
  - Shrinks bright objects
- Dilation
  - Replace voxel value by the maximum intensity value in neighborhood
  - Expands bright objects









input image



dilated image



**Erosion** result

**Dilation** result



## Image segmentation: erosion and dilation – grayscale

#### **Erosion and Dilation on grayscale data:**

- Erosion
  - Replace voxel value by the minimum intensity value in neighborhood
  - Shrinks bright objects
- Dilation
  - Replace voxel value by the maximum intensity value in neighborhood
  - Expands bright objects





eroded image



input image



dilated image

**Erosion** result



**Dilation** result



## Image segmentation: opening and closing - binary

#### Mathematical morphology operations derived from Erosion and Dilation:

- Opening:
  - Erosion + Dilation
    - (using the same structuring element SE)
  - All detection objects smaller than the size of the structuring element are removed
- Closing:
  - Dilation + Erosion (using the same SE)
  - All void regions (label=0) smaller than the size of the structuring element are filled

## Image segmentation: closing example - binary

porosities.



Closing: fills small holes (e.g. porosities) and connects detection objects that are close to each other.

## **Image segmentation: opening example - binary**



**Thermo Fisher** 

Opening: removes small structures (clean segmentation results – remove artefacts of small size).

## Image segmentation: opening example- binary



**Thermo Fisher** 

SCIENTIFIC

Opening: removes links (connections) of small size (e.g. separate detection object).

## Image segmentation: opening and closing – grayscale

#### **Opening and Closing on grayscale data:**

- Opening
  - Removes small bright structures.
- Closing
  - Removes small dark structures.





opened image



input image



closing



#### **Opening** result

**Closing result** 



## Image segmentation: opening and closing – grayscale

#### **Opening and Closing on grayscale data:**

- Opening
  - Removes small bright structures.
- Closing
  - Removes small dark structures.





opened image



input image



closing



**Thermo Fisher** 



**Closing result** 



## Image segmentation: when thresholding does not work





**Thermo Fisher** 

## **Image segmentation: Top-Hat Transform**

#### **R**: Apply Top-Hat transform and then thresholding



**Thermo Fisher** S C I E N T I F I C

## **Image segmentation: Top-Hat Transform**

#### Top-Hat (TH) Transform:

- Derived from Opening and Closing
- Highlights small size structures
- Two types of TH transform:
  - White TH:
    - Highlights bright structures
    - Mathematical expression: Input Data Opening result
  - Black TH:
    - Highlights dark structures
    - Mathematical expression: Closing result input data
- Good practice: apply before thresholding in order to corrects non-uniform lighting
# Image segmentation: Top-Hat Transform example

**Q**: How does one get from A to B

Α



Thermo Fisher

Β

# Image segmentation: Top-Hat Transform example



**Thermo Fisher** 



Getting from A to B:

- Apply Closing to the Input
- Subtract Input from Closing Result  $\rightarrow$  TH transform image
- Apply thresholding on the TH transform image
- Overlay result on Input (Colorwash)

# Image segmentation: Top-Hat in Avizo

#### Ways of applying (TH) Transform in Avizo:

- Dedicated module
  - Interactive Top-Hat



- Use the modules that correspond to the operations composing the TH transform
  - Opening, Closing, Arithmetic/Subtract Image
  - Advantage more flexibility in the choice of the:
    - Structuring element
    - Thresholding method
- Segmentation Editor:
  - Top-hat selection tool
    - TH transform image computation
    - Thresholding on the TH transform image



# **Image segmentation: Top-Hat in Amira**

#### Ways of applying (TH) Transform in Amira:

- Dedicated module
  - Interactive Top-Hat



- Use the modules that correspond to the decomposed TH transform
  - Opening, Closing, Arithmetic/Subtract
  - Advantage more flexibility in the choice of the:
    - Structuring element
    - Thresholding method
- Segmentation Editor:
  - Top-hat selection tool
    - TH transform image computation
    - Thresholding on the TH transform image



# Image segmentation: Top-Hat exercise

Bubble detection in chocolate bar

Apply the necessary module(s) and ports parametrization to create a similar view:



**Thermo Fisher** 

# Image segmentation: Top-Hat exercise

**TH** transform

image histogram

#### Solution

- e.g. Interactive Top-Hat module 2 steps:
- (1/2): Computation of the Black TH transform
- (2/2): Thresholding of the TH transform image





#### Image segmentation: advanced concepts Part 2: watershed segmentation

# **Introduction to Watershed**

Watershed is the area of land that drains into catchment basins



**Thermo Fisher** S C I E N T I F I C

# Watershed Divide Watershed Divide Headwaters Riparian Wetlands Rainwater JULIE LABOR COLOR Water table Groundwater flow

# **Watershed Transformation**

Any grayscale image can be considered as a topographic surface.







# **Watershed Transformation**

If we flood this surface from its minima and, if we prevent the merging of the waters coming from different sources, we partition the image into two different sets: the catchment basins and the watershed lines.



If we apply this transformation to the image gradient, the catchment basins should theoretically correspond to the homogeneous grey level regions of this image.





A 2D Image can be viewed as a Height Map. The gradient of an image, computed in each point as the first order spatial derivatives along the x and y directions (dx and dy) gives an estimation of the slope of the equivalent landscape that the image might represent.

#### Grayscale Image



#### Gradient = First Order Derivatives



The gradient magnitude – computed as the squared root of the sum of the squared spatial derivatives allows an estimation of the slope steepness.





Gradient Magnitude = local steepness ~= contours





- Transform grayscale image into gradient image (topographic surface)
- Typically: use the gradient magnitude as landscape image
- Simulate flooding of water (markers) in the landscape image
- Start from low level landscape (local minima)
- Fill the watershed into the basins with respective markers until reach the watershed line (local maxima)



Markers
B,C: Local minima in the landscape image
D,E: Ridges (local maxima) in the landscape image

- Transform grayscale image into gradient image (topographic surface)
- Typically: use the gradient magnitude as landscape image





- Simulate flooding of water (markers: Green, Blue, Yellow) in the landscape image
- Start from low level landscape (local minima)





Thermo Fi

• Fill the watershed into the basins with respective markers (Green, Blue, Yellow) until reach the watershed line (local maxima) where two marker sources meet





**ThermoFis** 

# **Marker-based Watershed: Segmentation Editor**

- Start with user-defined markers (e.g., brush tool)
- Compute the watersheds (basins) separating the markers (seeds)
- Create a landscape image (Gradient Image) once
- Grow the markers in each Watershed basin







# **Marker-based Watershed: Segmentation Workroom**

#### **ThermoFisher** SCIENTIFIC



# **Segmentation Editor: 3D Viewer position**

The default **3D Viewer position** in Avizo is "Upper Left". You can switch it to "Bottom Right" from: Edit -> Preferences ->Segmentation

Thermo

Nizo Preferences						×
General Layout On exit Molecules LDA Segm	nentation Rendering	Performance	Network U	nits Range Partitioning	Recipes	Auto Display
3D Draw Style						
🔿 Points 1 💿 Points 2 🔵 Solid						
Selection Draw Style						
💿 Transparent 🔿 Contour 🔿 Hatched 🔿 Dotted 🔵	Light Dots					
Opacity: Opacity:						
Color: 📕						
Labels Draw Style						
Contour S Hatched Dotted Light Dots						
Viewer Layout						
3D Viewer Position: 🔿 Upper Left 💿 Bottom Right						
Undo						
Memory Limit (MB): 32						
	[	•				
				OK Cancel	Apply	Help

File Edit Project View Window XPand Python XScreen Segmentation Selection Help

New

2D

30

∧ ⊕ ..... ∿ 11/2

Rename

Lock

1.0 6

£

C.

Ð

Colorize

Segmentation Editor

chocolate-bar.am

chocolate-bar.labels

Crosshairs Slices Volume rendering

Current slice 🛛 🗸 Show in 3D 1

100

Image:

Label field:

MATERIALS Color

2D -24

**3D** 478

SELECTION

1 2

O Volume

All slices Select all

Name

✓ DISPLAY CONTROL

 $\odot$ 

Material\_1

Material\_2

Material\_3

#### - | 🗇 🤴 🧐 🔇 🐇 | 🖬 😘 🏕 < [µm] < 🔯 | 🔳 🔳 🚍 📰 🕨 💐 🖶 Q 😳 🤁 🍵 💣 ? ÷. **€** 2:1 **€** Select Select Select Select 2D Crosshairs 117 / 234 147 / 294 n n < > 🔅 No selection



**Thermo Fisher** 

SCIENTLELC

87 / 174







#### Thermo Fisher SCIENTIFIC











File Edit Project View Window XPand Python XScreen Segmentation Selection Help 🕋 Start 🛛 – 🗧 Project 📚 Segmentation 🛛 💎 Meshing 🛛 🛱 Recipes 🛸 Filament 🖉 Multiplanar 🖉 Animation Segmentation Editor  $\Box \mathbf{X}$ | 🐼 🌤 🏷 🏷 «< | 🖬 💊 🏕 ~ [µm] ~ 🔯 | 🔳 🔳 🚍 📰 🕨 💐 🕂 Q ଫ 🕀 🍙 💣 •? chocolate-bar.am Image: ⊕ 2:1 Q Rename Label field: chocolate-bar.labels New MATERIALS Color Name Colorize Lock Select R Exterior (Not Assigned) Ē Select C Material 1 Select C Material 2 Select V Material 3 🗧 Select Delete ✓ DISPLAY CONTROL 2D Crosshairs **NAME** 2D 0 Edit 🗸 3D 478 117 / 234 147 / 294 Crosshairs Slices Volume rendering SELECTION / € ⊕ Θ G G < > 🔿 💿 Volume Current slice 🔽 Show in 3D No selection 🎢 🔨 🕀 📶 🗠 🕥 🕅 🎯 ଚ . . Auto hide cursor Select only current material Square brush Masking 478 1910 Edit 🗸 🗌 Enable Preview 🔽 2D 📃 3D 87 / 174 Pos: Index: Material Intensity





#### ThermoFisher scientific







**ThermoFisher** SCIENTIFIC



# Image segmentation: Watershed Segmentation Wizard





- Define markers for phases via thresholding
- Mask out regions with high gradient magnitude
- Expand markers with watershed







**Thermo Fisher** 

# Image segmentation: Watershed Segmentation wizard

r	Chocolate-bar.am
{	Colorwash 3




















Apply

Step 7 of 7: Compute Watershed

Back

Properties

Ŧ

Ŧ

Ŧ

Ŧ

Ŧ

Ŧ

Info:

Action:

**.** 9







**Thermo Fisher** 

SCIENTI

#### Thermo Fisher SCIENTIFIC

#### Watershed in Segmentation Editor: exercise

Multi-phase segmentation of chocolate bar



#### Watershed in Segmentation Editor: exercise

🕋 Start → – – – – Project	Segmentation 🔷 Meshi	ing 🕫 Recipes 🛰 Filament	🖷 Multiplanar	🖽 Anima	tion			
Segmentation Editor			□ <b>X</b> :	<b>x x</b>	4 Q &	0) 🍙 💣	·	% ≪ ⊑⊔ °⊾ ⊅
Image: chocolate-ba	ar.am	New Rename	Delete	Y				
MATERIALS							-	
Color Name Exterior (Not As: Chocolate Caramel	signed)	3D 2D Colorize Lock S	select Marker Select V Select V Select V				5	
	ור	Add Delete	Locate					estentionen.
J     C     O       O     Volume     Current       ▶     ✓     P	slice 🔽 Show in 3D	6 6 <	> ় ় X ● No selection	Z				
Landscape image:	chocolate-bar.am	<ul> <li>Create a new gradient image</li> </ul>	ge		- 63			
Markers:	Selected materials $\checkmark$ Select a	ll Deselect all			. Reve			THE REAL PROPERTY OF
Output catchment basins:	separated 💿 side-by-side						a state of	
Apply and create a new l	abel field					•	-	

# Watershed in Segmentation Editor: exercise





# Image segmentation: post-processing



# Segmentation post-processing: morphological filtering

- Binary mathematical morphology
  - Interactive Shrink & Grow in Segmentation Editor
  - Opening and Closing
- Fill Holes
- Dilate + Fill Holes + Erode: may close more open cavities/pores
- Remove Small Spots
- Border Kill: removes objects touching image bounding box

# Segmentation post-processing: object separation

- Separate Objects
  - Smaller 'Extent' value means more separation
  - Criterion relates to convexity of the particles
  - See tutorial "Separating, Measuring and Reconstructing -> Separation using Watershed step by step"



**Thermo Fisher** 

# **Segmentation post-processing: exercise**

Separate objects after sand-pack segmentation

- Data to use is Data/Sandpack/SandPack128.am
- Follow steps given in tutorial "Separating, Measuring and Reconstructing -> Separation using Watershed step by step"



Thermo

#### **Surface generation**



### **Surface reconstruction**

Open the label image chocolate-bar-labels (data>tutorials) then attach Generate Surface module to the label image. In the properties window, using the default parameters:

	Properties			<b>×</b>
🗖 🗖 chocolate-bar-labels.am 📀	🌩 Ø 🗸	Generate Surface		?
🕞 🖂 🖂 Generate Surface 📀	Ŧ	Data:	chocolate-bar-labels.am 🗸 🔶	
chocolate-bar-labels surf*	Ŧ	Options:	Compactify Minimum edge length: 0	
	Ŧ	Algorithm Mode:	repeatable 🗸	
	Ŧ	∨ Border:		
	Ŧ	Settings:	🔽 Adjust Coords 📄 Extra Material 📄 Create All Patches	
	Ŧ	$\sim$ Smoothing:	Unconstrained Smoothing $\sim$	
	Ŧ	Smoothing Extent:	5	
	Ŧ	Smooth Material:	None V	

- Data: chocolate-bar-labels.am (label image)
- Border Settings: Adjust Coords
- Algorithm Mode : repeatable
- Smoothing: Unconstrained Smoothing (use None/Constrained smoothing to preserve thin structures)
- Smoothing Extent: 5

#### Surface data export

The result surface chocolate-bar-labels.surf can be exported by right click at the surface (or left click and then go to > File) and select Export Data As then select the format (e.g. .stl or .obj) to export.



Open Inventor binary compressed (\*.iv) Wavefront (\*.obj) ABAQUS Input (\*.inp) ANSYS Input (\*.ans) AVS UCD ascii (\*.inp) AVS UCD binary (\*.inp) CGNS (\*.cgns) COMSOL ascii (\*.mphtxt) COMSOL binary (\*.mphbin) DXF (\*.dxf) Ensight Gold binary (\*.case) FLUENT/UNS (\*.cas) Hypermesh ascii (\*.hmascii \*.hm) MSC/NASTRAN Bulk Data (\*.bdf) Matlab m-file (\*.m) SDRC/IDEAS Universal (\*.unv) STL ascii (\*.stl) STL binary Big Endian (\*.stl) STL binary Little Endian (\*.stl) Stanford PLY (\*.ply) Tecplot 10 binary (\*.plt) Avizo Binary Surface (\*.am)

#### **Surface view**

#### Attach Surface View to the chocolate-bar-labels.surf to visualize the surface.



- Data: chocolate-bar-labels.surf
- Draw Style: Transparent
- Colors: patch
- Base Trans: 0.5

Surface View usage, tips & tricks: <u>https://youtu.be/zXq3A4bKcFg</u>

# **Surface simplification**



**Thermo Fisher** 

#### **Simplification Editor**

- Simplify: faces 18000 (0 max & min dist)
- Action: Simplify now

# **Surface simplification example**







180000 faces

18000 faces

# **Surface remeshing**

After reconstruction, the surface can be coarse: for refining, remeshing is necessary. Attach Remesh Surface module to the chocolate-bar-labels.surf.

Proper	ties			
۰	<i>ତ</i> ~	Remesh Surface		?
Ŧ		> Data:	chocolate-bar-labels.surf	
Ŧ		Objective:	Best isotropic vertex placement 🛛 🗸	
Ŧ		Desired Size:	#vert. 216991 #tris 433982 % 50	
Ŧ		Error Thresholds:	smoothness 0 distance 0	
Ŧ		Interpolate Orig. Surface:	smoothly on none	
Ŧ		Contour Options:	📕 fix contours 🛛 🔽 contract boundary edges	
Ŧ		Modify Result:	Apply	
Ŧ		> Zone		
Ŧ		> Advanced Options		

- Data: chocolate-bar-labels.surf
- Objective: Best isotropic vertex placement
- Desired Size: #vertex = 216991, #tris = 433982, % = 50
- Interpolate Original Surface: none
- Contour Options: contract boundary edges

# **Surface remeshing**

Dataset: Chocolate-bar-labels.surf

Thermo Fisher



Original surface

After simplification

After remeshing

# Surface view: exercise

Tuning Surface View module

Load *motor.labels* (data->tutorials) then use Generate Surface and Surface View to obtain a similar view.



# **Data visualization: exercise 3**

#### Solution

- 1. Generate Surface:
  - Smoothing: Existing Weights
- 2. Simplify Surface:
  - 18000 faces
- 3. Surface View:
  - Draw Style: Outline
  - Colors: Patch
  - Buffer: Remove Material3



# Surface view: exercise

Tuning Surface View module

Load lobus.labels (data>tutorials) then use Generate Surface and Surface View to obtain a similar view.



# **Data visualization: exercise 3**

#### Solution

- 1. Generate Surface:
  - Smoothing: Existing Weights
- 2. Simplify Surface:
  - 18000 faces
- 3. Surface View:
  - Draw Style: Outline
  - Colors: normal
  - Buffer: Remove Medulla



**Thermo Fisher** 

#### Quantification



## **Quantification on segmentation results**

**Q**: How can one identify segmentation objects and extract measurements and statistics ?



**Thermo Fis** 

#### Label segmentation objects and extract measures

- A: Label Analysis module. It
- Generates a label image: a unique label is assigned to each connected component (if input is binary)
- Allows extracting individual measures for each label object
- Allows extracting global statistics
- Intensity input (optional): allows extracting gray level statistics (e.g. mean, min, max)



#### **Label Analysis**

A: Label Analysis module. Default port initialization:



- "basic" Measures group of pre-defined measures:
  - Volume3d
  - Area3d
  - BaryCenterX
  - BaryCenterY
  - BaryCenterZ
  - Mean

#### **Label Analysis**

Label Analysis results for default ports initialization.



#### **Label Analysis**

Different tools are available for manipulating the spreadsheet measures:

• E.g. "Sort descending" sorts the values of a column in descending order.



# Label Analysis: pre-defined measures list

Some measures are pre-defined and ready to use by "Label Analysis" module. For checking the list of pre-defined measures:

- Go to "Label Analysis" Help page
- Click on "list of available measures"





**Thermo Fi** 

#### Label Analysis: pre-defined measures list

Help page with the list of pre-defined individual label measures.



# Label Analysis: custom measures list, custom measures

#### A custom measures list with pre-defined measures can be created:



- Click on a measure on the left side to add it to the group
- Select a measure added to the group (right side) and click on "Deselect the measure form the measure group" to remove it from the group.

Selection of measure groups		×	Selection of measure groups			×
Choose a measure group: basic			Choose a measure group: NewGro	oup 🗸	2 🖬 🖿 I 🚨	
Custom measures: 🚨	Create a new measure group.	e group: This list can not be modified.	Custom measures: 🚨		Measures selected in the group:	
Name	Formula Name	Formula Native Native Native	Name	Formula	Name Anisotropy	Formula Native
	Please enter a new name: NewGroup	Native Native Native Native	Native measures: Name	 Formula		
Native measures: Name > Cooccurrence > Feret > Geometry > Histogram > Inertia	OK Cancel		HistoStddev HistoVariance V Inertia Anisotropy BinMom2x BinMom2y BinMom2z	Native     Native     Native     Native     Native     Native     Native     Native     Native     Native	Deselect the measure from the measure	group.
		OK Câncel Help			ОК	Cancel Help

## Label Analysis: custom measures list

- Click on "Create a new measure" icon
- Type the name of the custom measure
- Type the measure in the "Measure Editor" (it's green if valid, red if not).

Selection of measu	ire groups		×	Measure Editor			
Choose a measure gro	NewGroup ~			Choose a measure: sphericity	~ I		
Custom measures: 🔓		Measures selected in th	ne group:				
Name	Create a new measure. mula	Name	Formula	Output unit dimension:			
		Anisotropy	Native			/3))/Area3d	
	New label measure	×					
Nativo moscuroci				Type the measure formula here.			
auve measures:	Please enter a new name:			- arithmetic: +, -, *, /, **			
HistoStddov	r. sphericity			- brackets: (), []			
HistoVariance				- logical: !, &&,   , and, or			
<ul> <li>Inertia</li> </ul>				- constants: pi, e			
Anisotropy BinMom2x	ОК	Cancel					
BinMom2y	HULIYE	15					
BinMom2z	Native 🗸				Variables Description	Functions	Measures
			OK Cancel Help		cx width of voxel (.	abs(a)	> Cooccurrenc
					cy height of voxel .	sqrt(a)	> Feret
				You can also <b>double-click</b> in the	cz depth of voxel (	log(a)	> Geometry
				following lists to quickly insert elements in the formula or directly	gx width of input i.	exp(a)	> Histogram
				drag and drop them.	gy neight of input.	cos(a)	> Intensity
						sin(a)	> Intensity
					NDFeret Num angul	acos(a)	> Intercept
						acio(a)	i -
### **Quantification: removing unwanted detection**

**Q:** how can one remove parasite detection from a label image and its corresponding measures in the measures spreadsheet ?

E.g.: for the segmentation example on chocolate bar, remove the label corresponding to background and keep only the porosity labels and measures



#### Unwanted detection to be removed

### **Quantification: removing unwanted detection**

#### A: Analysis Filter module

- Filters out from the measures spreadsheet, labels that do not fulfill a filtering criterion
- Same behavior on the label image label image (when provided as input optional)
- Filtering criterion: choose one (or more) measures that allow to discriminate the parasite detection and write filtering formula.

Thermo Fis



### **Quantification: removing unwanted detection**

Analysis Filter result for the filtering formula "Volume3d < 1e-7":



**Thermo Fisher** 

#### Example: porosities analysis in FoamPoro.am

#### Step 1:

Do a binary segmentation of the porosities



#### Segmentation workflow

#### Porosities before and after object separation



### Example: porosities analysis in *FoamPoro.am*

#### Step 2:

- Apply Label Analysis with "Standard Shape Analysis"
- Measures for shape analysis:
  - Eigenvectors
  - Eigenvalues



Selection of measure groups				×
Choose a measure group: Standa	ard Shape Analysis		I	
Custom measures: 🚨		Measures selected in the grou	p: This list can not be mod	
Namo	Formu	Name	Formula	^
Name	Formu	BaryCenterZ	Native	
		Anisotropy	Native	
		Elongation	Native	
		Flatness	Native	
		EigenVal1	Native	
		EigenVal2	Native	
		EigenVal3	Native	
		EigenVec1X	Native	
Native measures:	•	EigenVec1Y	Native	
		EigenVec1Z	Native	
Name	For	EigenVec2X	Native	
> Cooccurrence		EigenVec2Y	Native	
> Feret		EigenVec2Z	Native	
> Geometry		EigenVec3X	Native	
> Histogram		EigenVec3Y	Native	
> Inertia		EigenVec3Z	Native	
> Intensity	×	ExtentMin1	Native	~
<		<		>
		ОК	Cancel Help	

**Thermo Fisher** 

#### Example: porosities analysis in FoamPoro.am

#### Step 3:

- Generate Bounding Box and Ellipsoid representation for individual labels via "Spreadsheet to Point Cloud" module
  - Check "Bounding Boxes" Output
  - Check "Point Cloud" Output and "Fill Bounding Boxes" for ellipsoid representation



Proper	rties							□ <b>×</b>
ø	<i>ତ</i> √	Spreadsheet to Point Cloud						?
Ŧ		Data:	FoamPoro.Label-An	alysi	∕ →			
Ŧ		Output:	Bounding Boxes	<b>V</b>	Point Cloud			
Ŧ		Tensor :	🔵 None 💿 Fill Bo	ound	ing Boxes 🔵 Distril	outio	n	
Ŧ		Table:	FoamPoro.Label-An	alysi	s ~			
Ŧ		Value:	EigenVal1	~				
Ŧ		Coordinates:	BaryCenterX	~	BaryCenterY	~	BaryCenterZ	-
Ŧ		First Direction:	EigenVec1X	~	EigenVec1Y	~	EigenVec1Z	-
Ŧ		Second Direction:	EigenVec2X	~	EigenVec2Y	~	EigenVec2Z	-
Ŧ		Third Direction:	EigenVec3X	~	EigenVec3Y	~	EigenVec3Z	-
Ŧ		Extent Min:	ExtentMin1	~	ExtentMin2	~	ExtentMin3	-
Ŧ		Extent Max:	ExtentMax1	~	ExtentMax2	~	ExtentMax3	-

#### Example: porosities analysis in *FoamPoro.am*

#### Step 4:

- Visualization:
  - Line Set View for Bounding Boxes
  - Tensor View for the ellipsoids (click on "Apply" for generating the visualization)



#### 

#### **Binarisation** representation

#### Bounding Boxes and Ellipsoid representation



### **Surface measurements and statistics**

Other Surface measurements and statistics modules are available in the "Measure and Analyze" object category (for access: right click on the surface object in the pool).

Thermo Fisher



## **Extract skeletons and graphs**

For filamentous data, automatic extraction of centerlines with local thickness can be done via Auto Skeleton module. It generates a spatial graph data:

Thermol

- Spreadsheet with information on nodes, points (thickness info available too), and segments
- Can be visualized with:

Example: neuron.am and Neuron-SpatialGraph.am data in ...\data\tutotials\neuron

🗖 🗖 Neuron-SpatialGraph.am\* 📀 🛏



🚽 🗖 Spatial Graph View 📀

### **Measurements and annotations**



- Units are necessary to interpret numbers as physical values.
- Two "types" of units must be distinguished:
  - Working units:
    - All calculations are done in those units.
    - Can only be changed before loading the first data-set in the project.
  - Display units:
    - Used to display numerical values.
    - Can be changed anytime, independently of working units

Note: Display Units do not change data.

Use adequate working units because of the impact on numerical precision!

Apply

- Units management is implemented for spatial size only (coordinate and angle units).
- Units management settings can be accessed in preferences:

File       Edit       Help	General Layout On Units management O None	exit Molecules	LDA Segmentation	Rendering Performan	e Network formation only	Units	Range Partitioning	Recipes	Auto Display
<ul> <li>Default is:</li> <li>enabled <ul> <li>("Spatial information only")</li> </ul> </li> <li>default unit: nm</li> </ul>	Options       Display units         Automatically determine         Lock display units on weater         When the coordinate units         Show Units Editor ditale         Use       nm         Use       nm	Working units ne working units rorking units its are unknown at d alog efault coordinate uni	ata loading ts						

### Loading data

- When loading data, either spatial information is given in the same unit (default coordinate units), or the units of each data-set must be set correctly independently.
  - Default setting
  - Recommended:

Set to your most likely unit, as this will be pre-selected in the **Units Editor dialog**.

Use nm  $\vee$  as default coordinate units



If you are always using data with the same unit, set the appropriate unit here:



#### Beware:

- If you save a project, the information about working units is saved with it
- If you load a project having working units set differently from your current ones, the settings from the project are loaded and applied permanently, until you explicitly change it back!

#### **Display units:**

May be changed anytime, either via the preferences, or via the Viewer Window settings.

																				Z
General	Layout	On exit	Molecules	LDA	Segmentation	Rendering	Performance	Network	Units	Range Partitioning	Recipes	Auto Display	(	••••	° <b>⊳</b>	u ~ <i>6</i> *	~ [nm	) 🗸 💿 🕴		
Units ma	nagement																n	anometer [	nm]	
🔘 None							Spatial infor	mation only									n	nicrometer	[µm]	
																	n	nillimeter [r	nm]	
Options	Display	vunits V	Vorking units															ontimotor [	cml	
Spatial	informatio	n																		
Coordi	nates:						nanometer [nm	]												
Angle:							degree [deg]													

• Affect all measures with units management, e.g.:



#### Measurement tool:

- invoke with
  - Measure button in viewer toolbar (shortcut "M")
  - Via "Create object ... → Annotations → Measurement"
- Different measurements types available
- works on visualization modules in
  - 2D, e.g. Ortho Slice, Slice
  - 3D, e.g. Surface Rendering, Volume Rendering, Voxelized Rendering



#### Measurement tool:

• is active when Measurement module is selected

■ chocolate-bar.am ④	Ortho Slice	,	ň
Properties	ش 	Line: 24.95 mm	Note _ Caramel phase 🗘 .::
	rate		
<ul> <li>All</li> <li>Line 24.95 mm</li> <li>■ In 20</li> <li>■ Note</li> <li>■ In 20</li> </ul>	<ul> <li>Snapping</li> <li>Width: </li> <li>30</li> <li>Snap: chocolate-bar.am </li> <li>max gradient </li> <li>Trigger: Snap-it</li> <li>Text</li> <li>Title: Line</li> <li>Note:</li> <li>Callout Properties</li> <li>Shape Properties</li> </ul>		
auto-refresh	Apply		

#### Measurement module:

- snapping possible click on a point
  - within search window (semi-transparent square)
  - to min, max, or gradient (min or max)

#### Snapping example





#### Measurement module:

- editable properties:
  - snapping
  - text (title and note)
  - callout and shape properties
    - measure points (colors, font, etc.)

Select point to modify (#1 or #2)



Edit x, y, z coordinated of the selected point

#### **Quick Probe**

- show value of data at mouse position (interactive mode should be on) •
- works with e.g. Slice, Ortho Slice, and Volume Rendering ٠
- 2 modes: •
  - Continuous update ٠
  - Click for update (Shift + Click) ٠
  - → prefer "Click for update"
- value is shown in status bar •





chocolate-bar.am - Coord [mm]: 12.273, 11.905, 17.640, Value: 815

Thermo Fi

### **Point Probe**

- get callout with position and value
- select Point Probe and click with middle mouse button to pick a location, or move the handles
- works with e.g. Ortho Slice, Slice, Voxelized Rendering
- editable callout settings
- local averaging
- hide dragger:





#### Line Probe (2D and 3D)

- evaluate the data-values along a straight line
- select point to modify (#1 or #2) to change the coordinates in the text boxes or
- click with middle mouse button to pick new location or use handles to position the points
- for arbitrary orientation de-select "orthogonal"



#### **Display line-profile in plot window:**

- adjust number of sample-points
- possibility for local averaging



### **Spline Probe**

- similar to Line Probe, but:
  - arbitrary number of control points
  - sampling along smooth, curved Spline



#### Histogram

- distribution of values in the data-set
- optionally limited to ROI or mask

- adjust settings:
  - range
  - number of bins
  - absolute/relative counting
  - Iinear/logarithmic Y-axis



#### Histogram

- further settings:
  - axis control for X and Y:
    - range
    - number format
    - tick-marks
    - linear/log
    - labels

• ...





#### **Thermo Fisher** S C I E N T I F I C

# **Spreadsheet visualization**

- Plot Spreadsheet
- Histogram
- Spreadsheet To Point Cloud + Point Cloud View for the display
  - e. g. create bounding-box information and orientation tensors

Spreadsheet to Point Cloud	
Data:	cellbodies.labels.Label-Analysis \vee 🔶
Output:	🗹 Bounding Boxes 🛛 Point Cloud
Tensor :	🔵 None 🥥 Fill Bounding Boxes 🔵 Distribution



# **Spreadsheet visualization**

- Spreadsheet To Point Cloud + Point Cloud View for the display
  - e. g. point cloud as sphere visualization





# **Displaying plots in the main viewer**

Normally, all kinds of plots are displayed in a separate viewer window. With **Plot In Viewer**, plots can be displayed in the main viewer window(s).

- can be attached to:
  - Histogram
  - Point Probe
  - Line Probe
  - Spline Probe
  - Plot Spreadsheet
- options:
  - position
  - size
  - transparency
  - frame



## **Annotation: Scale Bar and 2D Scale Bar**

There are two types of scale-bars available:

### 2D Scale Bar

- attached to a slice object
- options: length, position, color, ...

### Scale Bar

- located in the 3D viewer
- invoked via click on background and
   "Create object → Annotations → Scalebars"
- only meaningful in orthographic ( 
   , parallel) view!
   (because of perspective shortening)
- options: length, position, color, label, font, ticks, ...







#### Thermo Fisher SCIENTIFIC

### **Annotation: Colorbar**

Attach Colormap Legend to the display module.

- options:
  - size
  - vertical
  - background
  - font
  - title
  - custom text
  - Histogram

Tip: set alpha=0 to hide the histogram



### **Annotation: Colorbar**

If you want to get rid of the checkerboard-pattern:

- in the display-module's (e.g. Color Wash) colormap port: select "Options → Edit colormap"
- in the Colormap Editor: set transparency to "Opaque"



Thermo

# **Annotation: Axes, Caption**

#### Axes

- visualization of global coordinate system:
  - no data-set connected
  - invoked via click on background and "Create object → Annotations → Axes"
- visualization of local coordinate system:
  - · connected to a data object
  - invoked via the object's context menu ("Annotate → Axes")
- default coloring convention: X: red, Y: green, Z: blue

### Caption

- any text in the viewing plane of the viewer
- invoked via click on background and
   "Create object → Annotations → Caption"
- options: position, text, color, font





### Data registration and alignment



### Alignment to an oblique plane: example

Load chocolate-bar.am then attach Volume Rendering, Bounding Box and Slice to the dataset (oblique view).

### Alignment to an oblique plane: example

Rotate Slice using rotate mode in Slice properties port (activate trackball) to rotate to the desired tilt angle.



## Alignment to an oblique plane: example

Attach Resample Transformed Image to chocolate-bar.am and set reference to Slice.





**Thermo Fisher** 

SCLEN
## Alignment to an oblique plane: example

At Resample Transformed Image properties select Interpolation: Standard, Mode: extended then click Apply.

**Thermo Fisher** 

SCIEN



#### Alignment to an oblique plane: example

Attach another Bounding Box to chocolate-bar.transformed and visualize with Volume Rendering. The transformed result is now aligned with Slice (in green).



# **Data registration: introduction**

- General concepts
  - All datasets are positioned in 'physical' space
  - This position is control by a 'Transform'
- Registration
  - · Optimization of the 'alignment' with respect to the degrees of freedom given by the 'transform'
  - Difficult mathematical problem, sensitive to the initialization
- Avizo can register:
  - Volume to volume (grayscale or label images)
  - Surface to surface
  - Using Linear transform: translation, rotation, optionally scaling and shearing

# **Data registration: introduction**

- General concepts
  - All datasets are positioned in 'physical' space
  - This position is control by a 'Transform'
- Registration
  - · Optimization of the 'alignment' with respect to the degrees of freedom given by the 'transform'
  - Difficult mathematical problem, sensitive to the initialization
- Amira can register:
  - Volume to volume (grayscale or label images)
  - Surface to surface
  - Using Linear transform: translation, rotation, optionally scaling and shearing

Open motor.part1-reference.am and motor.part2-model.am (data -> registration) then attach Volume Rendering to each dataset.



Properties				$\square \mathbf{X}$
s & ~ moto	r.part2-model.am		ば 🖦 🚄 🖂 😂	?
₹ Latt	ice Info:	310 x 156 x 178, uniform coordinates		
ቿ Data	a Info:	grayscale, 8-bit unsigned, min-max: 0255, window: 74255	5, intensity ranges: 2	
± Mer	nory Size:	8.2 MB		
₹ Phys	sical Size:	0.680764, 0.479531, 0.60844 [mm] from 0, 0.00309375, 0.7 [m	m]	
E Voxe	el Size:	0.00220312 x 0.00309375 x 0.00343751 [mm]		
E Prev	view:			



Thermo

Move the handle box and bring motor.part2-model.am (in yellow) to overlap with motor.part1-reference.am (in green) as much as possible.





**ThermoFi** 

# **Data registration: Register Images module**

#### Attach Register Images to motor.part2-model.am and set the reference to motor.part1-reference.am.



Properties	□ <b>×</b>
🏟 🔗 🗸 Register Images	Advanced ?
₹ ∨ Model:	motor.part2-model.am 🗸 🔶
E Reference:	motor.part1-reference.am \vee 🔶
E Reference2:	NO SOURCE ~ ->
E Reference3:	NO SOURCE ~ 🔶
🗄 Transform:	🗸 Rigid 📄 Iso-Scale 📄 Aniso-Scale 📄 Shear
E Disable Rotation:	
E Register:	🔵 2D 💿 3D
E Prealign:	Align centers Align principal axes
🗄 Metric:	Normalized Mutual Information $$
E Localizers:	

- Transform: Rigid
- Register: 3D
- Prealign: Align centers & Align principal axes
- Metric: Normalized Mutual Information

# **Data registration: Resample Transformed Image module**

Attach Resample Transformed Image to motor.part2-model.am to apply the transformation (otherwise the transformation will be available for visualization only).



# **Image fusion & stitching**

Attach Merge module to motor.part2-model.transformed and set motor.part1-reference.am as a reference. Merge using standard interpolation with blend option.

Propert	ies		
•	𝔗 ∨ Merge		
Ŧ	∨ Data:	motor.part2-model.transformed	<ul> <li></li> </ul>
Ŧ	Lattice1:	motor.part1-reference.am	<ul> <li>+</li> </ul>
Ŧ	Lattice2:	NO SOURCE	∕ →
Ŧ	Lattice3:	NO SOURCE	∕ →
Ŧ	Lattice4:	NO SOURCE	∕ →
Ŧ	Lattice5:	NO SOURCE	∕ →
Ŧ	Lattice6:	NO SOURCE	∕ →
Ŧ	Lattice7:	NO SOURCE	∕ →
Ŧ	Lattice8:	NO SOURCE	∕ →
Ŧ	Lattice9:	NO SOURCE	<ul> <li>+</li> </ul>
Ŧ	Lattice10:	NO SOURCE	<ul> <li>→</li> </ul>
Ŧ	Interpolation:	Standard 🗸	
Ŧ	Padding Value:	0	
Ŧ	Options:	Jend use existing result	



#### **Image fusion & stitching**



Open chocolate-bar.part1-reference.am and chocolate-bar.part2-model.am (data > registration) then attach Volume Rendering to each dataset.



In the properties window of chocolate-bar.part2-model.am, activate Transform Editor, the transform box will appear.





Move the handle box and bring chocolate-bar.part2-model.am (in yellow) to overlap with chocolate-bar.part1-reference.am (in green) as much as possible.





# **Data registration: Register Images module**

Attach Register Images to chocolate-bar.part2-model.am and set the reference to chocolate-bar.part1reference.am.



- Transform: Rigid
- Register: 3D
- Prealign: Align centers & Align principal axes
- Metric: Normalized Mutual Information

## **Data registration: Resample Transformed Image module**

Attach Resample Transformed Image to chocolate-bar.part2-model.am to apply the transformation (otherwise the transformation will be available for visualization only).



# **Image fusion & stitching**

Attach Merge module to chocolate-bar.part2-model.transformed and set chocolate-bar.part1-reference.am as a reference. Merge using standard interpolation with blend option.

Prope	rties			
٠	𝔗 ∨ Merge			
Ŧ	∽ Data:	chocolate-bar.part2-model.transformed	~	<b>→</b>
Ŧ	Lattice1:	chocolate-bar.part1-reference.am	$\sim$	+
Ŧ	Lattice2:	NO SOURCE	~	<b>→</b>
Ŧ	Lattice3:	NO SOURCE	$\sim$	<b>→</b>
Ŧ	Lattice4:	NO SOURCE	$\sim$	<b>→</b>
Ŧ	Lattice5:	NO SOURCE	$\sim$	<b>→</b>
Ŧ	Lattice6:	NO SOURCE	$\sim$	<b>→</b>
Ŧ	Lattice7:	NO SOURCE	$\sim$	<b>→</b>
Ŧ	Lattice8:	NO SOURCE	$\sim$	<b>→</b>
Ŧ	Lattice9:	NO SOURCE	$\sim$	<b>→</b>
Ŧ	Lattice10:	NO SOURCE	$\sim$	<b>→</b>
Ŧ	Interpolation:	Standard 🗸		
Ŧ	Padding Value:	0		
Ŧ	Options:	Jend use existing result		



#### **Image fusion & stitching**



## **Data registration: exercise**

Register and merge parts of motor data

Register and merge the motor parts (...data/registration/motor.part1-reference.am & motor.part2-model.am)

<ul> <li>Volume Rendering Settings</li> <li>Volume Rendering</li> <li>Volume Rendering</li> <li>Volume Rendering 2</li> <li>Volume Rendering 2</li> <li>Notor.part1-reference.am</li> </ul>	<image/>
---	----------

#### Thermo Fisher

#### **Data registration: exercise**

Register and merge parts of chocolate bar data

Register and merge the chocolate bar parts

(...data/registration/chocolate-bar.part1-reference.am chocolate-bar.part2-model.am)

<ul> <li>Volume Rendering Settings </li> <li>Volume Rendering </li> <li>Colume Rendering </li> <li>Colume Rendering </li> <li>Colume Rendering </li> </ul>	
<ul> <li>chocolate-bar.part2-model.am</li> <li>Volume Rendering Settings 2</li> <li>Volume Rendering 2</li> </ul>	

## **Nominal-Actual comparison**

Open Pump-bracket-CAD-model.surf and Pump-bracket-CT-scan.am (...data/pump-bracket) then generate a binary image (thresholding) and then generate surface from Pump-bracket-CT-scan.am and attach Surface View to both surfaces.

Pump-bracket-CAD-model.surf	<ul> <li>Pump-bracket-CT-scan.am</li> <li>Pump-bracket-CT-scan.surf*</li> <li>Surface View 2</li> </ul>		
-----------------------------	---	--	--

In the properties window of *Pump-bracket-CT-scan.surf*, activate Transform Editor, the transform box will appear.

Dropo	rtion							
riope	nues							- ~
-	<i>&amp;</i> ~	Pump-bracket-CT-scan.surf					• 🗵 🖾 🗉	�?
Ŧ		Physical Size:	93.5704, 127.068	, 97.3715 [mm] fr	om 3.77167, 11.1	1576, 6.56884 [m	m]	
Ŧ		Surface:	572788 points, 1	145596 faces, 1 p	atches, 0 contou	rs, 0 edges		
Ŧ		Master:	NO SOURCE		~ →			
Ŧ		$\sim$ Transform Editor						
Ŧ		Manipulator:	Transformer	<ul> <li>✓ Dialog</li> </ul>				
Ŧ		Reset:	All	Translation	Rotation	Scale		
Ŧ		Action:	Undo	Redo	Сору	Paste	Apply transform	



Thermo

Move the handle box and bring *Pump-bracket-CT-scan.surf* to overlap with *Pump-bracket-CAD-model.surf* as much as possible.



#### Attach Align Surfaces to Pump-bracket-CAD-model.surf and set the reference to Pump-bracket-CT-scan.surf.



- Options: iterate
- Transformation: rigid
- Stop: relative RMS = 0.001, max iter = 15
- Align: Surfaces



# Nominal-Actual comparison: e.g. Surface Distance

Attached Surface Distance module to Pump-bracket-CAD-model.surf (surface 1) and Pump-bracket-CT-scan.surf (surface 2).



- Direction: Surface 1->2
- Output: Distance
- Maximal Distance: 1
- Above Threshold: 0.5

## Nominal-Actual comparison: e.g. Surface Distance

#### Visualize distance with Surface View, set color map to Physics



**Thermo Fisher** 

SCIENTIE

- Landmarks are useful for registration and alignment of multiple 3D images.
- It allows you to store multiple sets of corresponding marker positions.
- The data type can also be used to represent a simple list of 3D points.

Open chocolate-bar.part1-reference.am and chocolate-bar.part2-model.am (data -> registration) then attach Volume Rendering to each dataset.



In the properties window of chocolate-bar.part2-model.am, activate Transform Editor, the transform box will appear. Move the handle box and bring chocolate-bar.part2-model.am (in yellow) to align with chocolate-bar.part1-reference.am (in green) as much as possible.

Once satisfied apply Resample transform Image to save the transformed dataset.

In Project View, right-click and "Create Object", select Landmarks (2 sets) (Points And Lines).

land		 0
Objects (3)		
CombineLandmarks (Compute)		
📔 Landmarks (2 sets) (Points And Line	es)	
Landmarks (Points And Lines)	Landmarks (2 sets)	
C Search "land" within documentation		

Activate Landmark Editor in the properties port of Landmarks-2-sets, Landmark View will appear.



In Landmark-View select to show lines. Then go back to Landmarks-2-sets to start adding points by click on chocolate-bar.part1-reference.am (yellow dots) and connect to chocolate-bar.part2-model.am (light-blue dots). Line sets between the two volumes will be shown. Rotate the volumes and add more points and lines to connect common landmarks between the two volumes. Do not make crossing lines.





Open Tcl console, then come back to "Project View" and click on "Landmarks-2-sets". Next go back to Tcl Console and then press "Tab" to activate- > "Landmarks-2-sets" and input "computeLinearTransform 1 0 0":

**Thermo Fisher** 

• "Landmarks-2-sets" computeLinearTransform 1 0 0

Press enter.



Go back to Project View and click on chocolate-bar.part2-model\_transformed.am. Then go back to Tcl Console and then press "Tab" to activate -> "chocolate-bar.part2-model-transformed.am" and input setTransform and paste the values from previous step:

"chocolate-bar.part1-reference.am" setTransform 0.999631 -0.0174218 -0.020835 0 0.0176621 0.999779 0.0114049 0 0.0206318 -0.0117689 0.999718 0 -0.375237 0.593657 -18.5194 1
 Press enter.

Consoles						□ ×
Tcl Console	Main Python Console					
Reading labels Reading labels Amira 2020.2 (a Type 'help' for Reading choco Reading choco LandmarkEdito >"Landmarks-2	Binary.am 256.am arch-Win64VC12-Optimize) getting started. late-bar.part1-reference.a late-bar.part2-model.am or Object 312 created 2-sets" computeLinearTran	n sform 1 0 0				Î
0.999631 -0.01/ >"chocolate-ba 0.999718 0 -0.3 1 >	'4218 -0.020835 0 0.017662 r.part2-model.transforme 75237 0.593657 -18.5194 1	1 0.999779 0.0114049 0 0.0 d" setTransform 0.999631	206318 -0.0117689 0.99 -0.0174218 -0.020835 0	9718 0 -0.375237 0.593657 0.0176621 0.999779 0.0114	-18.5194 1 1049 0 0.0206318 -0.0	117689
				Sto		32%

**Registration result:** 





# **Animation generation**










#### Attach Camera Path

Right-Click anywhere in the project view

- Create Object



**Attach Camera Path** 

Right-Click anywhere in the project view

- Create Object
- Camera Path

■ ■ ch	ocolate-bar.am 🕥	-{	-	i Volu I Volu	ume Rendering Setting ume Rendering	IS 🕑		
	<enter a="" search="" string=""></enter>	_	_	_				•
	😧 Favorites	>	$\hat{}$	٥	Calculus MATLAB			
	Recents				Camera Orbit			
	😑 Templates	>			Camera Path			
	🗀 Experimental	>		т	Caption	Creates a p	th for the camera	
	🗋 Animate	>		• •	Clipping Plane			
	Animations And Scripts	>			Recipe Player	HxKeyframe	CameraPath	
	Annotations	>		Ţ	Scalebars			
	🗀 Compute	>			Time			
	🗀 EM Toolbox	>						
	Tmane Processinn	>	$\sim$					

**Camera Path** 

Click on the Camera-Path module

- Properties



**Camera Path** 

Click Camera Path Editor icon (top-right) to start adding key frames.

A small camera path view will also appear.

🌆 Viewer 5



**Camera Path** 

Click Add to start adding key frames

For each key frame;

- Turn, rotate, zoom data in or out
- The path of camera rotation will be shown in the small Camera Path Viewer



Click the small viewer to close Camera Path Editor when finish with key frames (or click at the Camera Editor icon one more time).



#### **Camera Path**

Click Play button to preview the animation



Making movie

Right click at Camera-Path module to attach Movie Maker module

Camera-Pat	n* 💿			
	📋 Camera-Path 🗸	🗋 蔖 <	ing>	9
	😧 Favorites	🔪 🔅 Clear History	y Log	
	Recents	📕 Movie Make	r	
	Dittors	>	Creates a movie as single images or MPEG	
	Experimental	<u>&gt;</u>	HxMovieMaker	
Properties	🗋 Animate	>		
Camera-Pat	🗋 Annotate	>		
I Camora Da	Compute	>		
I Calliera Pa	Display	>		
⊥ nme:				638
🛨 Camera Or	ientation: Smooth			
				201

#### **Movie Maker**

Set the parameters (**Filename** field is **mandatory**) then click Apply to create a movie file.

	[		 ₩
	Came		
Propertie	25		□ ×
<b>≣</b> d	တ္ 🗸 Movie Maker		Advanced ?
Ŧ	Time:	Camera-Path 🗸 🔶	
Ŧ	Viewer:	Viewer 0 🗸	
Ŧ	Antialiasing Quality:	•	
Ŧ	✓ Format Options		
Ŧ	Info:	Frames: 200 - Total time: 8.3 s - Frame rate: 24 (fixe	d)
Ŧ	Filename:	uth.wantha/Desktop/Animation.mpg	
Ŧ	File Format:	MPEG movie 🗸	
Ŧ	$\sim$ Resolution Options		
Ŧ	Size:	💿 Viewer 🔵 360p 🔵 480p 🔵 720p 🔵 10	180p 🔵 Custom
Ŧ	Resolution [px]:	X 1056 Y 880	
aut 🗌	to-refresh		Apply

#### Animation: Camera Path (rotation) movie example



**Thermo Fisher** S C I E N T I F I C



**Thermo Fisher** S C I E N T I F I C



#### **Animation Director**

Attach Clipping Plane to the dataset and "clip"

#### **Animation Director**

Then activate the Animation workroom. The Animation Director will appear.

File E	lit Pro	ject View Window XF	and Python XScreen H	ielp														
🎓 Star	t 🖂	Project 🕫 Recipes	Segmentation 👘	🏹 Meshing 🛛 😪	Filament 📰 Ar	nimation												
Project V	iew					X		• a	<b>२ ⊕</b>		Ø 🖏	<u>ة</u> (8	« 🖬	° <b></b> ~	Ø ∨ [µm] ∨	ōō	- 🔳 🚥	= =
Open D	ata					* 3 8												
Cylinde	Slice	Dual Ortho Slices Inter	nsity Ranges Contours Iso	contour Slice														
ſ	= chor	olate-bar am		🗕 🗖 🗖 Volume Rend	dering Settings 💿					_	ALCONT CO		-					
	a choc			🗖 🗖 Volume Rend	dering 💿						well have	termen	de.					
												12						
		Camera-Path* 📀		Clipping Pla	ane 📀							V		1				
													*					
													(All and a second se					
												M						
						団												
<b>0</b> ť						- <b>- -</b>												
Propertie	÷)		Ĩ.			~	Animation	Director										
<u>ا</u> ا	₽ Ō. \	Clipping Plane				▶ ?	6				0.2	00.0	+					
Ŧ	ō	> Data:	chocolate-bar.am 🗸	<b>→</b>							0.0	00:0	0.000	00:00	00:08	00:16	00:24	00:32
Ŧ	ō,	Frame:	🗹 show width: 1				Current A	nimation: Ne	wAnimation $$	· 🗘 🛍	🕂 Add sp	ecial event	🛛 🛢 🌩					
Ŧ	Ō,	Orientation:																
Ŧ	Ō,	Options:	🔲 adjust view 🔲 ro	otate 💟 immediate	e 📄 fit to points				G	ET START	ED!							
2	0	Translate [%]:		50														
t	Ø	Plane Definition:					To anir	The curr mate a compo	ent Animation E nent (object <u>, po</u>	Director does rt, viewer), (	not contain a click on the Ō	y animation button l <u>oca</u>	ted near the					
									comp	onent to be a	nimated.							

1. Start with the Timeline at 00:00:000

2. In Clipping Plane Properties translate to 0%.Pay attention to the clipping direction: full volume should be visible at 0% (Translate).



## Translation Animation

1. Start with the Timeline at 00:00:000

2. In Clipping PlaneProperties. Translateto 0%

3. Hide the Clipping Plane bounding box.



**Thermo Fisher** 

SCIENTIFIC

# Translation Animation

4. Click the Stopwatch button next to Translate (%) in Clipping Plane properties to start adding the first key frame (Translation) to the timeline at 00:00:000.



#### Translation Animation

5. Move the timeline bar to 00:03:000

6. Translate to 50 %



Translation Animation

Repeat the steps

8.Move the timeline bar to 00:06:000

9. Translate to 80 %



Translation Animation

Repeat the steps

11.Move the timeline bar to 00:09:000

12. Translate to 0 %



14. When done, bring timeline bar to 00:00:000

15. Set the limit of the animation at 00:09:000

16. Click Play button in Animation Director to preview the animation.



**Thermo Fisher** 

#### Translation + Rotation Animation

17. At time 00:00:000

18.Click on Camera-Path module (with previously created rotation key frames).

19. Make sure to have Time 0 in Camera Path properties port before clicking on Stopwatch button next to Time.

Select Time value to add to Animation Workroom.



Translation + Rotation Animation

17. At time 00:00:000

18.Click at Camera-Path module (with previously created rotation key frames).

19. Make sure to have Time 0 in Camera Path properties port before clicking on Stopwatch button next to Time.

Select Time value to add to Animation Workroom.



Translation + Rotation Animation

20. Move the timeline bar to 00:03:000

21. Move Camera Path Key frame to time 30



Translation + Rotation Animation

Repeat the steps

23. Move the timeline bar to 00:06:000

24. Move Camera Path Key frame to time 60



Translation + Rotation Animation

Repeat the steps

26. Move the timeline bar to 00:09:000

27. Move Camera Path Key frame to time 90



**Translation + Rotation** 

#### Animation

29. When done, bring timeline bar to 00:00:000

30. Click Play button in Animation Director to preview the animation.

Please note that the animation limit was already set at 00:09:000



**Thermo Fisher** 

## **Animation director: movie generator**

#### **Movie Creation**

Activate Movie Creation in Animation Director and input all parameters as needed.

Then click Create Movie.

Animation Director	
Current Animation: NewAnimation 🗸 🧐 🗇 🕂 Add special event 🗸 🧮 🏟	
Movie Creation	
Info: Frames: 225 - Total time: 9.0 s - Frame rate: 25 (fixed)	
Viewer: Viewer 0 🗸	
Antialiasing Quality: O	
File Format: MPEG movie 🗸	
Filename: ific Avizo 2020.2/data/NewAnimation	
Frames: < > 225	
Frame Rate: 25 V	
Quality: 0.8	
Type: monoscopic ~	
Format: 💿 RGB 🔵 RGBA	
Tiles: X 1 Y 1	
Size: 💿 Viewer 🜑 360p 🜑 480p 🜑 720p 🜑 1080p 🜑 Custom	
Resolution [px]: X 1344 Y 528	
? Create Movie	

#### **Animation Workroom: Animation Director movie example**

**Thermo Fisher** 



# Thank you!

Find out more at thermofisher.com/avizo

392 Proprietary & Confidential | authoremail@thermofisher.com