# The Ideal Solution to Interactely Analyzy Microsco y Images



#### IMARIS -- The Ideal Solution to Interactively Analyze Microscopy Images







Imaris File converter (Free)

Imaris

Imaris Viewer (Free)

### Imaris File converter

💽 Imaris File Converter				- 0 ×
Input	Output			
Drag & Drop Files, or click Button below to add Files.	Same Folder as Input File			Download ImarisConvert- Bioformats
Add Files	O Specific Folder: C:\Users\huasa\Documents\			Plugin
	Input	Output	Voxel Size	Selected Input File
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				Cancel All

Preferences... About.

### IMARIS – The Ideal Solution to Interactively Analyze Microscopy Images Imaris: a modular software







## Import -- Imaging modalities



Ref: Amat et al. 2014, Nature Methods

## Import -- Formats

Visualizing volumetric data

Rendering a 3D volume from 2D slices



## Import -- Formats

7 um

## Or Even 4D...



Time

## Background Subtraction Removes background in order to improve signal to noise



Background subtraction applies a Gaussian filter to define the background at each voxel, then performs a Baseline Subtraction of this variable background. The filter width is determined by the size of the smallest structure of interest.





2 slice view

## ClearView GPU Decon.

#### For different imaging modalities

Spinning Disk Confocal







and Brightfield, TIRF

#### Spine Morphology

#### Colocalisation

#### Golgi Segmentation

## Visualize Solutions for Correlative Microscopy



## Visualize Solutions for Correlative Microscopy



Arrange images of physical sections relative to one another and reconstruct entire specimen

Arrange images of physical sections relative to one another and reconstruct entire specimen

## Visualize Volume Rendering Modes



MIP

Normal Shading

Blend

## **Volume Rendering modes**



#### MIP Blend with step







Blend with peak

## Visualize

#### Visualization tools



Image courtoisy <u>Gaby G. Martins</u>, Advanced Imaging Unit Facility Head, Oeiras, Lisbon



#### OrthoSlicers



Clipping plane



Annotations

#### OrthoSlicer





- Orthogonal plane on which original data are projected
- Can be moved within the dataset
- "Extended section" adjusts slices thickness

🔖 Settings				
Slice Orientation	Extended Section			
YZ Plane	0,200um 🌲			
XZ Plane				
XY Plane	Show Frame			
-Slice Position				
6.200				

#### **Oblique Slicer**

👿 🔆 Light Source 1 E Frame

Ar Volume V 🥎 Oblique Slicer 1

V



- Plane on which original data are ٠ projected
- Can be freely moved and ٠ rotated within the dataset (similar to Clipping Plane)
- "Extended section" adjusts • slices thickness

Settings		
Orientation	Extended Section	
YZ Plane	0,307um	-
XZ Plane	Show Frame	
XY Plane		
Position	Camera	
Recenter	Orthogonal View	



### **Clipping Plane**

- Cuts away objects on one side of the plane
- Allows you to look inside any object
- Can be freely rotated









#### Define special Keyframes with defined settings (zoom, rotation,...)

 Imaris calculates the frames between the defined positions

### **Movie Creation: Keyframe Animation**



	Key Frame Animation				
Key Frame		Rotations	Animation	Play Back Options	
	+ Add O Modify	Custom	1600 x 1200 UXGA (4:3) - 100 Frames	☑ Camera ☑ Time Points	
	X Delete X Delete All	🛶 +360°zontal	1600 1200 Settings 🥌	<ul> <li>☑ Clipping Planes</li> <li>☑ Orthogonal Slicers</li> </ul>	
				Oblique Slicers	

#### **Setup the Animation**

- 1. Turn the image view to the starting position.
- Klick "Add" to add a keyframe
- Change the image view
- Klick "Add"...

- Choose the total number of frames of the movie
- In the settings window you can choose the frame rate of the movie
- Press the Play button to play the movie
- Press the Record button to export the movie

## Visualize

2

## Visualize Exporting data

#### Working interface: section





#### Export snapshots and videos





## Detect & Analyze





Allows for detection the boundary of structure, Object Creation and segmentation Automatic





## Adaptive Contour Tracer





Image Courtesy Tom Deerinck and Dr. Mark Ellisman, National Center for Microscopy and Imaging Research, University of California, San Diego.

## Surfaces for irregular fully stained shapes



- Source

Wei. Dai, Montell's lab, Department of Molecular, Cellular and Developmental Biology, UCSB



#### Trainable AI Image Analysis for Everyone











#### Trainable AI Image Analysis for Everyone





## Quantification





Slicer view



Count Position Volume Area Intensities <u>Morphology</u> ...



Drosophila Egg Chamber. Image courtesy of Wei. Dai, Montell's lab, Department of Molecular, Cellular and Developmental Biology, UCSB

Volume view



#### Allows for detection the number of objects



Image courtesy of M. Belle, D. Godefroy, Dr. C. Sotelo Chédotal team, Institut de la Vision, Paris, France.









## Spots for spherical shapes



Drosophila Embryo: Embryology course at Woods Hole, Marine Biological Lab, Mass, USA.

## **Spots Statistics**



Filter / Classify objects Color code objects Count Position Diameter Area Intensities ...


## Native Distance Measurements

Easy method to understand the spatial relationship of one population of cells, organelles, or objects to another population.

## Surfaces-Spots Interactions



KunahShlama((MaKijaney Eab)and Biölmaging & Optics Platform, EPFL, Switzerland

-Source



## Searchine Learning Classification



## Measurement Pro Line and angle measurements



## **Measurement Points**

## **Intensity Profiles**





# Filament Tracer

Allows for measurements of neuronal or other branching structures

(eg. Mycorrhiza, blood vessels, mitochondria network etc.)

- Segement properties (length, diameter, etc.)
- Branch Angles
- Number of end points and branch points
- Spine classification
- Etc.

## Automatic

## Tree-like branching Structures

Network Structures



Image courtoisy <u>Dr Benjamin E.</u> <u>Deverman</u>, PhD Senior Research Scientist, California Institute of Technology.

Microglia vasculature

# Filament Tracer

### Al Powered Filament Tracer

### 1. Filament New Workflow for Neuron Tracing



Imaris 10.0 於神經追蹤模組提供最新AI功能, 全新神經追蹤分析步驟始於偵測神經體細胞 (Soma), 依照神經細絲 (Filament) 影像偵測 Seed points 。使用者可分類 Seed Points 以及所產生的Segment是"好"或"壞", 並進行訓練, 接著軟體會進行機器學習 (Machine Learning), 針對整張影像保留"好"的 Seed Points 以及 Segments 而獲得最佳結果。針對 Seed Points 以及 Segment 的分類 是Imaris10.0的最新功能, 對於更加複雜的神經影像分析, 提供更快速以及準確的分析結果。





# Filament Tracer

#### Al Powered Filament Tracer

### 2. Filament New Workflow for Network Tracing



全新網絡絲狀追蹤分析步驟,與神經追蹤分析相似,網狀追蹤分析始於偵測細絲 (Filament) 上的 Seed points。使用者可分類 Seed Points 以及所產生的 Segments 是"好"或"壞",並進行訓練,接著軟體會進行機器學習 (Machine Learning),針對整張影像保留"好" 的 Seed Points 以及 Segments 而獲得最佳結果。在細胞生物學上,可用於分析細胞內結構,如:微管(Microtubules),內質網 (Endoplasmic Reticulum), 肌動蛋白(Actin Filament)...等,也可應用於血管分析。





## Semi-automatic

Torch View





### **Filament Element Visualization**



FT

**Explore the Next Dimension** 

# Statistics

Organized into;

- Filaments
- Dendrites
- Points
- Spines

Such as length, diameter, volume, branching hierarchy, Sholl Analysis, spine density & morphology, filament tracking ...









### Automatic detection of Cells, Cell Membranes and Vesicles; analysis of data on a

"per-Cell-basis"; study of intra cellular relationships...



## **Detection modes**



Cells, Nuclei and Organelles





Cells and Nuclei





### Cells and Organelles





**Only Cells** 





# Cytoplasmic staining











# Membrane staining



Organoid, Dr Luke Boulter, Leverhulme Trust-Chancellor's Fellow, Principle Investigator, MRC Human Genetics Unit, Institute for Genetics and Molecular Medicine, University of Edinburgh.

# **Cell Statistics**

Organized into :

- Cell
- Nuclei
- Vesicles

Counts Volume, Area, Diameter Position Intensities Morphology ...





Vesicles colored according to their distance to the cell membrane

# Lineage/Tracking

Lineage enables the study of cell lineages, including cell division detection Tracking is the solution for 3D and 4D object tracking



# **Different Algorithms**



- Autoregressive
- Autoregressive Expert
- Connected Components
- Brownian Motion
- Lineage (standard, cells with space between them)
- Lineage (Maximum overlap)(Cell)

Image courtesy Willy Supatto, Laboratory for Optics and Biosciences, Ecole Polytechnique, Palaiseau

# Autoregressive



## Connected Component





## Lineage Tracking - Standard





## Lineage Tracking – Maximum Overlap



# Manual

## Edit





# Statistics

- Track object properties over time (morphology, intensity, volume, position)
- Track length, duration, straightness
- Speed, acceleration
- Cell cycle / division (generation, time between divisions, etc...)
- Generation of Cell Lineage Trees





# Colocalisation

- Co-localization in 2-D, 3-D and 4-D images
- Possible of >2 channels

- Histogram
- Intensity scaling

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<u>File E</u> dit <u>V</u> iew Image Processing <u>S</u> urpass <u>H</u> elp	
Den Save as Load Scene Save Scene Snapshot InFress Gallery Surpass Animation InMotion Coloc	Bitplane
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## Colocalisation



• Calculate statistical parameters such:

Pearson Coefficient Manders Coefficient % of Colocalization

XLS	XML	CSV
$\Box$		

## **Customisation (XTension)**

Incorporate your own Xtensions

Ø	×	1	1	Ŧ	W	•	4	
		Add 9	Similar	ity St	atistics	s Value	2	
			Ang	les Sta	atistics			
			Bran	ch Hie	rarchy	/		
Convex Hull								
			Crea	ate Ch	nannel			
		Fi	lamen	nts Poi	nts Tra	ack		
	Split Into Branches							
Find Spots Close to Filaments								

Filament Analysis Classify Spines

# Add Similarity Statistics Value Distance Transformation Compute Distance between Spots And Surfaces Find Spots Close To Surface

Find Spots Close To Surface Merge Surfaces Split Connect Tracks Plot Angles of selected Track Plot Distance Between Tracks Plot Length of selected Track Split Tracks Translate Tracks dilate Surface Center of Mass to Spots Surface-Surface Contact Area Surface-Surface coloc

### 🐲 🔨 🖍 👔 🗛

Add Similarity Statistics Value Distance Transformation Intensity Profile Compute Distance between Spots And Surfaces Spots to Spots Closest Distance Find Spots Close to Filaments Find Spots Close To Surface Colocalize Spots Create Channel Split Spots Split Into Surface Objects Super Resolution Localization Data To Image Super Resolution Localization Data To Spots Connect Tracks Plot Angles of selected Track Plot Distance Between Tracks Plot Length of selected Track Split Tracks Translate Tracks Vesicle Outside Cell

### Custom your analysis in:









## **Xtensions : example Colocalize Spots**

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Distance Transformation					
Intensity Profile					
Compute Distance between Spots And Surfaces					
Find Spots Close to Filament					
Find Spots Close To Surface					
Colocalize Spots					
Create Channel					
Find Spots On Ends Of Lines					
Split Spots					
Split Into Surface Objects					
Connect Tracks					
Plot Angles of selected Track					
Plot Distance Between Tracks					
Plot Length of selected Track					
Translate Tracks					
Split Tracks					



## Share XTensions

- Offers source code of numerous XTensions to Imaris and allows researchers to share ...
- More than 40 XTensions already coded and fully editable



## https://imaris.oxinst.com/open/



## Interpret(Vantage)

Allows users to interpret their results using interactive multi-dimensional plots



- t-test, f-test
- Wilcoxon test
- Kolmogorov-Smirnov test

# Hypothesis Testing







	continuous distributions with different medians				
	Report				
		faces 1 Set 1 Clas	faces 1 Se		
	Cardinality	120	80.0		
	Rank Sums	7264	1.28e4		
	Confidence Level Alpha: 0.0500				
	Test Type: Two Tails T	est			
	P-Value: 6.08e-5				
	Accept H1: The two samples come from normal distributions with unequal means				
	Report				
		faces 1 Set 1 Clas	faces 1		
	Mean Value	2594	3953		
	Variance	9.01e5	7.75e6		
	•				
	Confidence Level Alpha: 0.0500 Test Type: Two Tails Test				
	P-Value: 7.81e-25				
	Accept: H1: The two samples comes from normal distributions with different variances				
	Report				
		faces 1 Set 1 Clas	faces 1		
	Mean Value	2594	3953		
	Variance	9.01e5	7.75e6		

# Data Plotting



### Microscopy Image Browser - ARENAXFORD | IMARIS



## Batch processing in Arena

File Arena View Help						
Arena Surpass Vantage Observe Folder	New Folder New Batch New Plot					Imaris
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## Thank you!

