

# BITPLANE

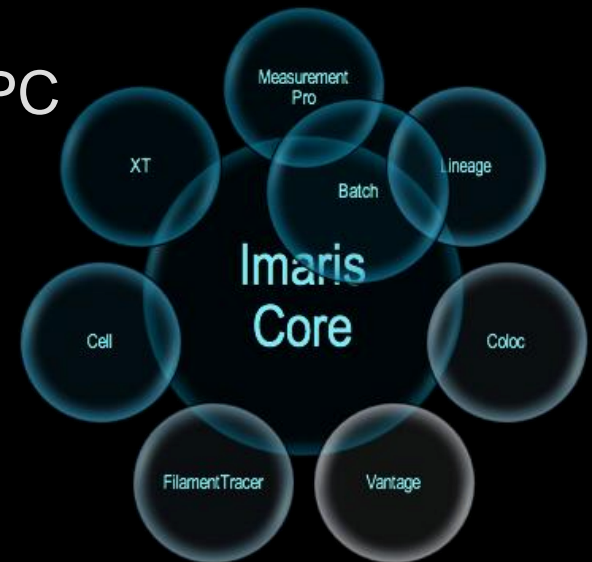
an **Oxford Instruments** company

## Imaris Introduction 2018

Xi (Nancy) NAN  
Application Support  
[nancy@bitplane.com](mailto:nancy@bitplane.com)

# What is Imaris

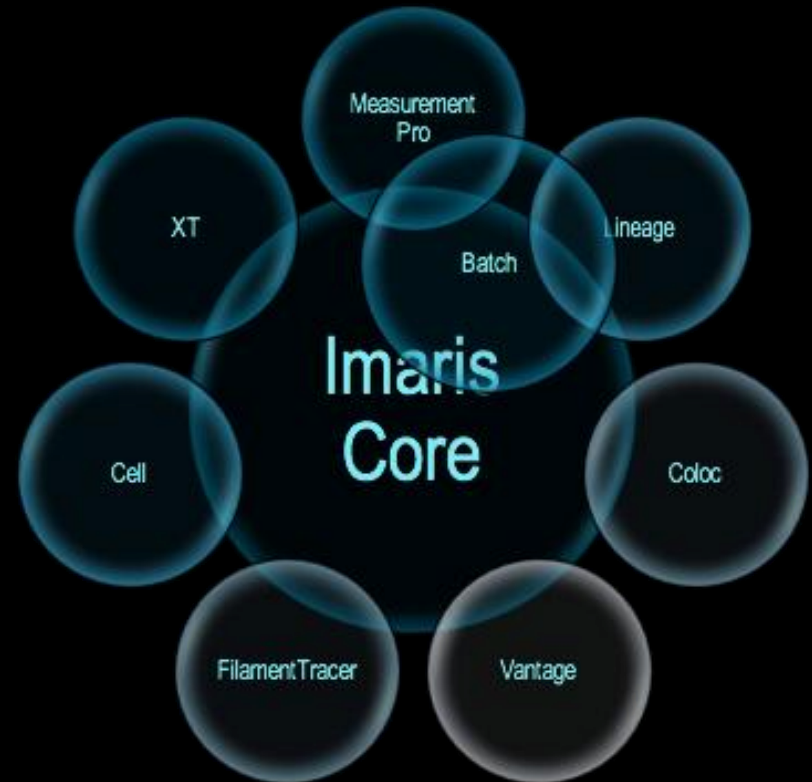
- Software for visualization, segmentation, analysis and interpretation of multi-channel 3D and 4D datasets.
- Runs on 64-bit version of both Mac and PC
- Modular based application allows customization of Imaris capabilities.
- Supports all Confocal file formats







# What is Imaris

- **Module based**

- Imaris
- Imaris MeasurementPro
- Imaris Cell
- Imaris Lineage
- Imaris Vantage
- Imaris Coloc
- Imaris FilamentTracer
- Imaris XT
- Imaris Batch



# Agenda

- **Arena View - Data Management**
- **Application - Segmentation Tools**
  - Spots/Surfaces/Cells rendering 
  - Filaments rendering 
  - Cell/Object Tracking 
  - Colocalization Analysis 





# Imaris 8 – Arena



Search field (files, tags...)

Arena tree

provides direct access to all the components of your experimental data including creation parameters, Vantage plots and (batch) results

Arena View

displays contents of the current tree location or any search results  
displays object's properties, tags and surpass objects

Image details

Parameter	Value
Channel 0	
color	0.000 1.000 0.000
colormode	BaseColor
coloropacity	1.000
colorrange	22.8358 162.388
description	(description not specified)
exposurevalue	37.500
gamma correction	1.000
lsmemissionwavelen...	583
lsmexcitationwavelen...	558
lsmphotons	1
lsmpinhole	
name	Cytoplasm
numericalaperture	1.200
objective	C-Apochromat 63x/1.20 W Korr UV VIS IR
Channel 1	

# Directly Read More than 40 Microscopy File Types

- Andor: Multi-Tiff (Series) (\*.tiff, \*.tif)
- Andor: iQ ImageDisk (\*.kinetic)
- Applied Precision DeltaVision (\*.r3d, \*.d3d, \*.dv)
- Biorad MRC 1024, 600 Series (\*.pic)
- Biovision: Ivision (\*.ipm)
- Biovision / Scanalytics: IP Lab (series) (\*.ipl)
- Bitplane: Imaris 2.7, Imaris 3, and Imaris 5.5 (\*.ims)
- Bitplane: Imaris Scene File (\*.imx)
- BMP (adjustable file series) (\*.bmp)
- Gatan Digital Micrograph (\*.dm3)
- Hamamatsu/Compix SimplePCI (\*.cxd)
- IMOD binary file (\*.imod, \*.mod), object scene file
- IMOD MRC file (\*.mrc, \*.st, \*.rec)
- 3i Slidebook (\*.sld)
- Leica Image Format LIF (\*.lif)
- Leica Vista LCS (\*.tif, \*.tiff, \*.lei, \*.raw)
- Leica Series (\*.tif, \*.tiff, \*.inf, \*.info)
- Leica TCS-NT (\*.tif, \*.tiff)
- Molecular Devices: Metamorph Stack (\*.stk)
- Molecular Devices: Metamorph ND (\*.tif, \*.nd)
- Micro-Manager Image 5D (\*.tif \*.tiff \*.txt)
- Nikon ICS – “Huygens compatible” (\*.ics, \*.ids)
- Nikon Elements ND2 (\*.ND2)

- Olympus Cell^R 1.1 (\*.tif, \*.tiff)
- Olympus FluoView (\*.tif, \*.tiff)TIFF
- Olympus OIB (\*.oib)
- Olympus OIF (\*.oif)
- Olympus VSI (\*.vsi)
- Open Microscopy Environment Tiff (\*.tiff, \*.tif)
- Open Microscopy Environment XML (\*.ome)
- Perkin Elmer: Ultraview (\*.tim, \*.zpo)
- Perkin Elmer: Volocity / OpenLab LIFF (\*.liiff)
- Perkin Elmer: Volocity / OpenLab RAW (\*.raw)
- Prairie Technologies (\*.xml, \*.cfg, \*.tif)
- Quick PALM (.quickpalm, .tif)
- TIFF (adjustable file series) (\*.tiff)
- TILL Photonics: TILLvisION (\*.rbinf)
- Zeiss Axiovision (\*.zvi)
- Zeiss LSM410, LSM310 (\*.tif, \*.tiff)
- Zeiss LSM510, LSM 710 (\*.ism)
- Zeiss CZI (Zen) (\*.czi)



**BioVision Technologies**

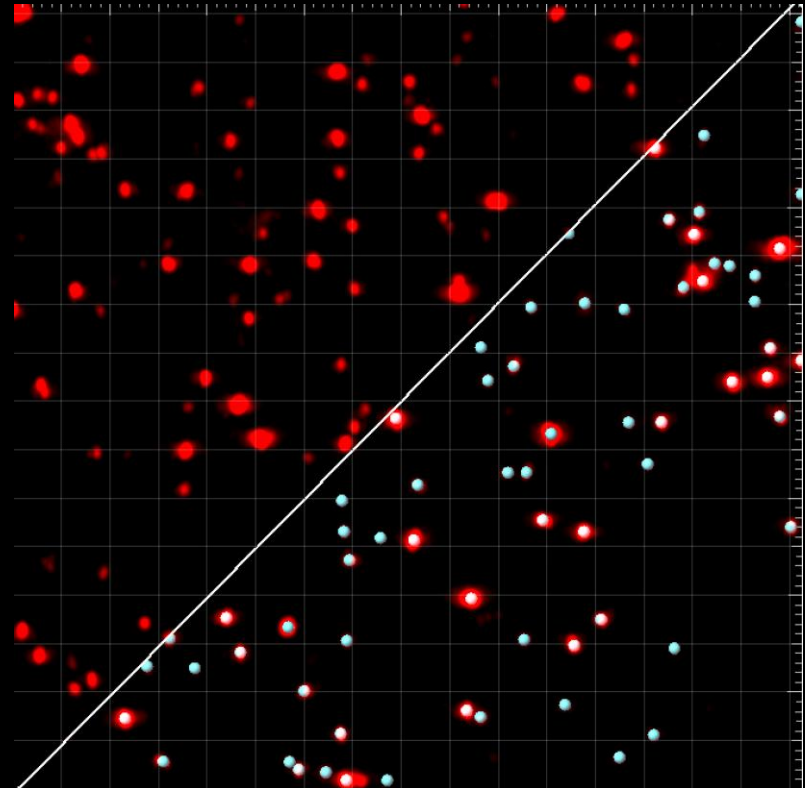
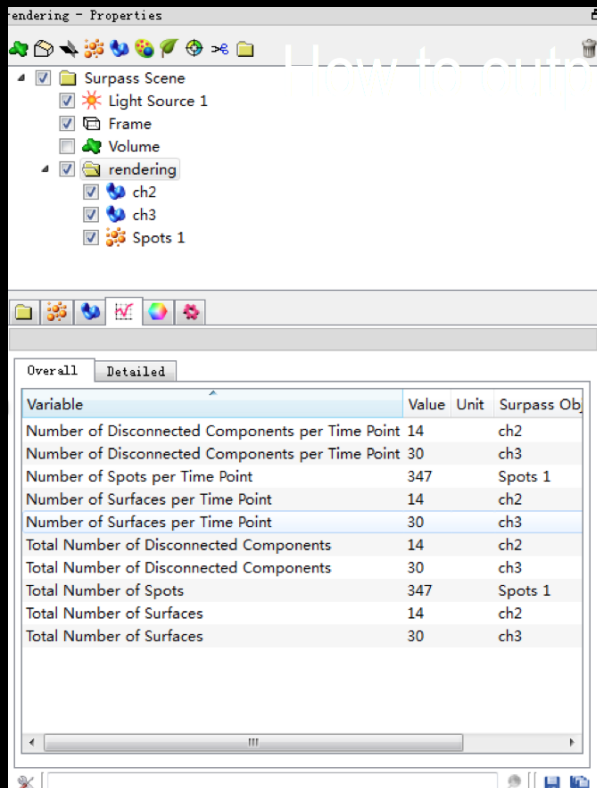
Digital Imaging Solutions...



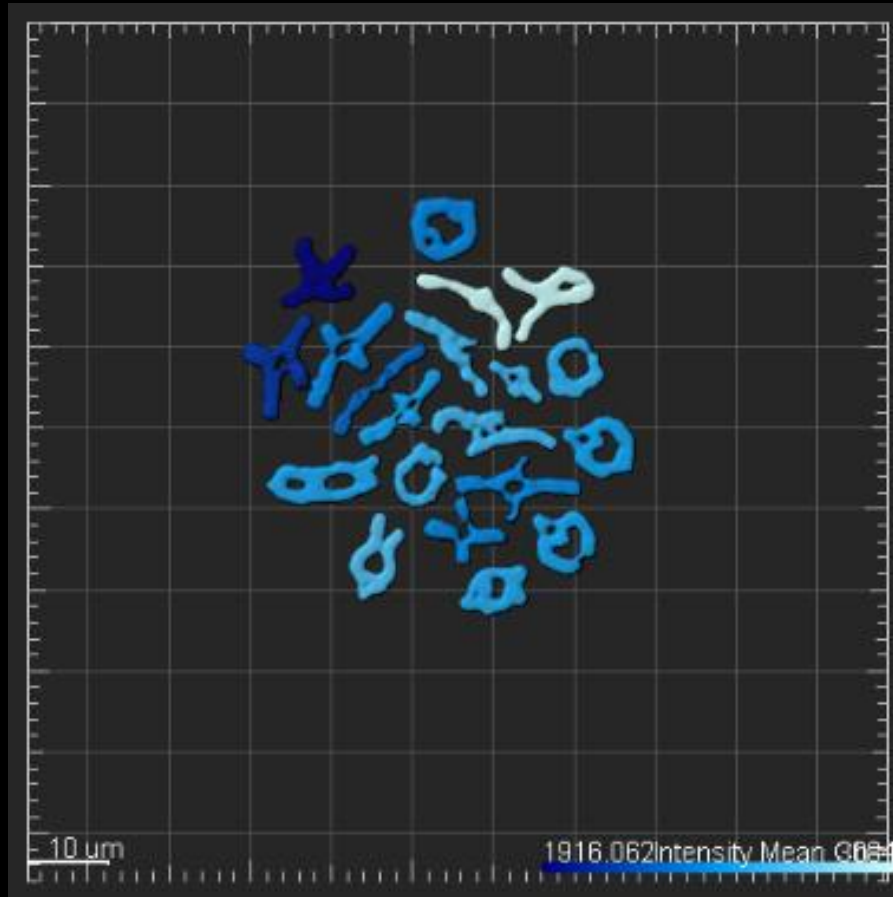
**PRAIRIE TECHNOLOGIES, INC.**

# What we do in Imaris

- What is segmentation and why we do this?



# What we do in Imaris



Overall Detailed Selection

Specific Values

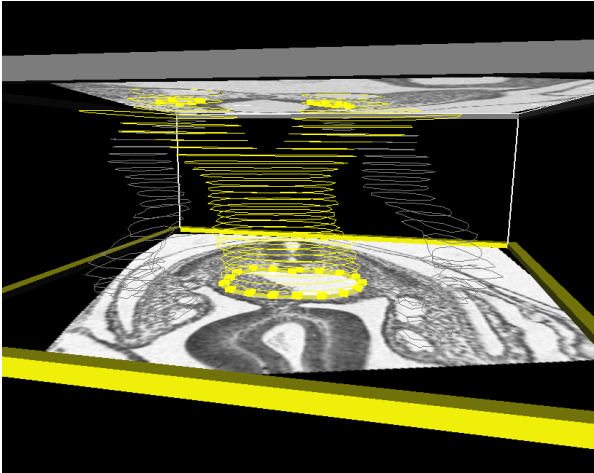
Volume

Value	Unit	Category	ID
✓ 1653	um <sup>3</sup>	Surface	0
✓ 797	um <sup>3</sup>	Surface	1
✓ 747	um <sup>3</sup>	Surface	2
✓ 1343	um <sup>3</sup>	Surface	3
✓ 912	um <sup>3</sup>	Surface	4
✓ 1345	um <sup>3</sup>	Surface	5
✓ 1776	um <sup>3</sup>	Surface	6
✓ 305	um <sup>3</sup>	Surface	7
✓ 1019	um <sup>3</sup>	Surface	8
✓ 2369	um <sup>3</sup>	Surface	9



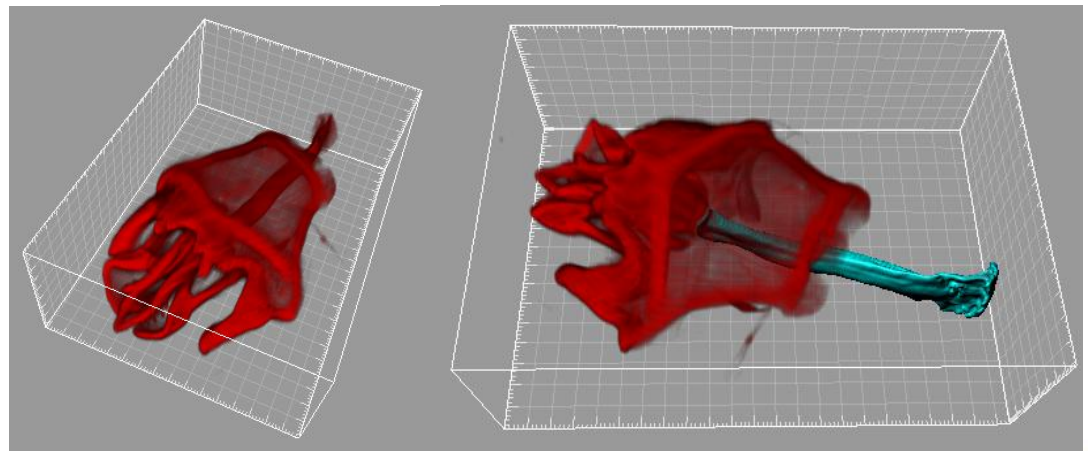
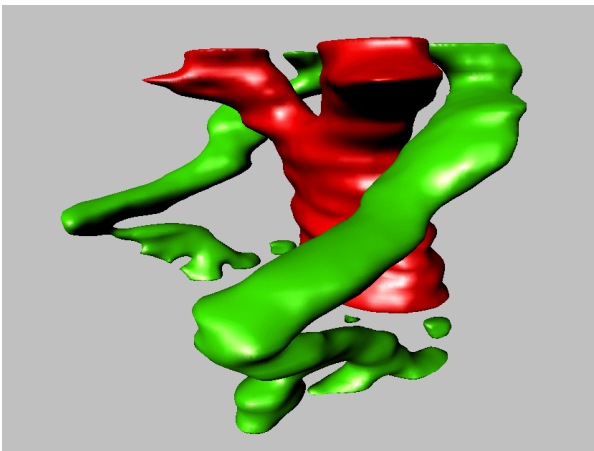


# Creating Surface manually via Contour tracing



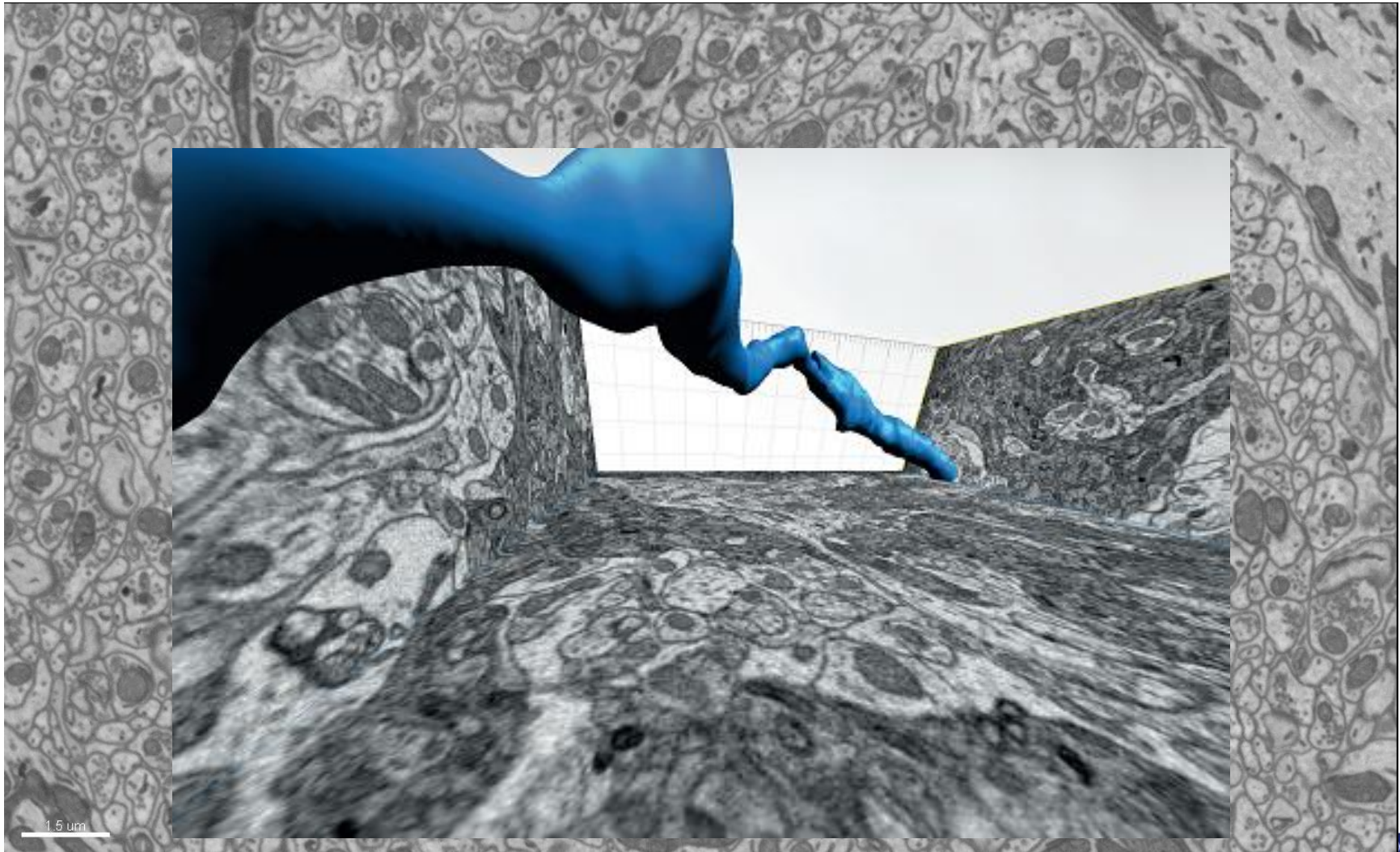
Visualization & Measurement of structures which cannot be easily segmented from background or neighboring structures

Draw 3D Regions of Interest to get Statistics for those regions or for masking part of one or more channels



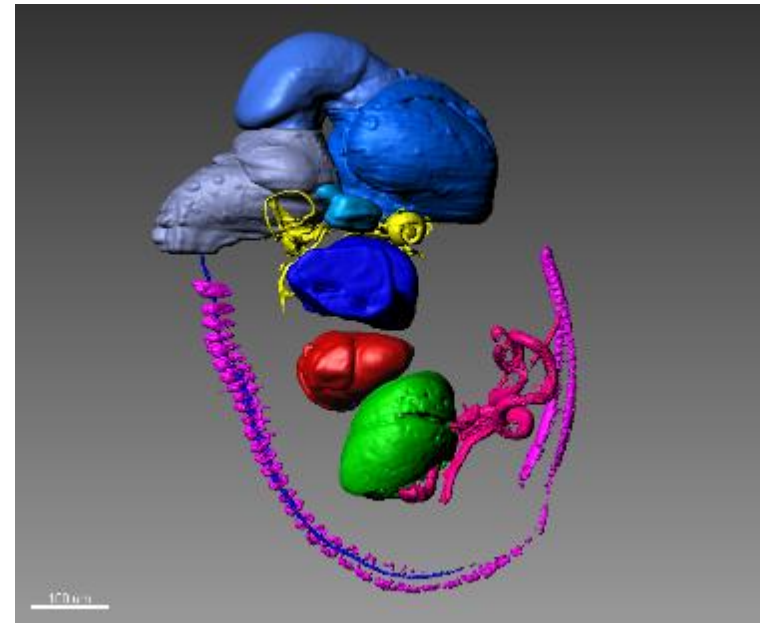
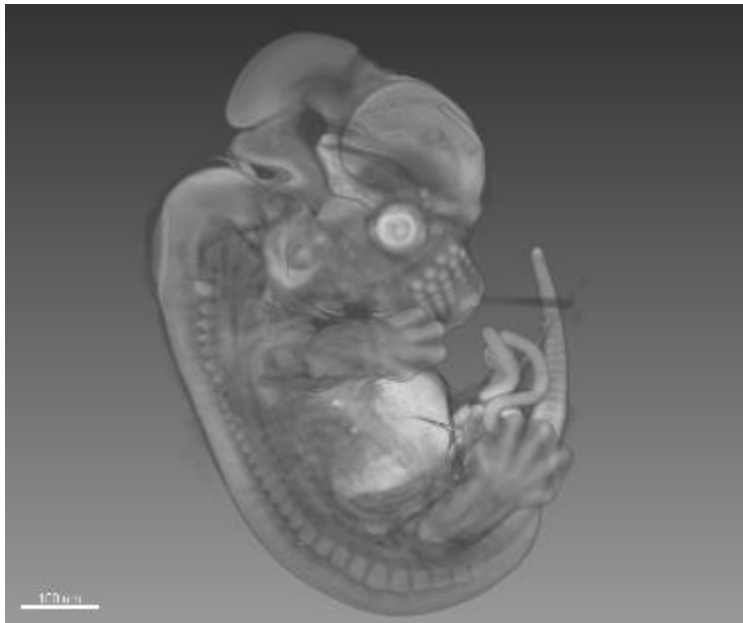


# Creating Surface manually via Contour tracing



# Creating Surface manually via Contour tracing

Optical projection tomography "Haeckaliens"





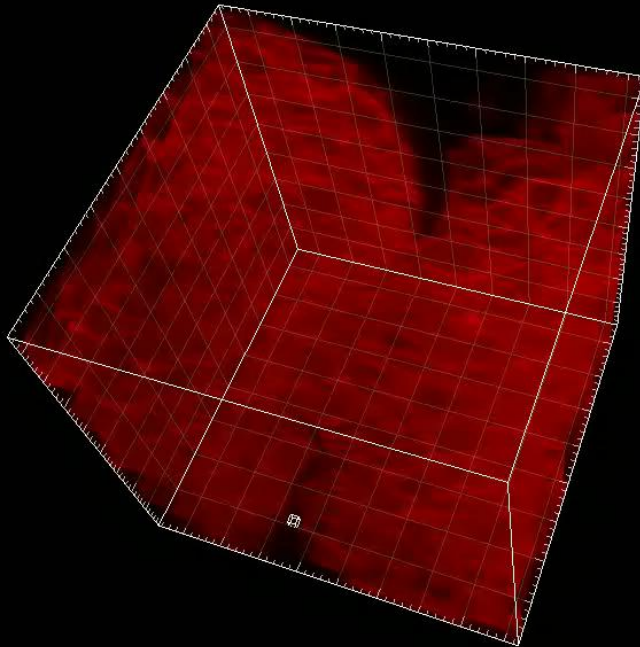
# Cell/Object Detection



20 um

- Qualitatively and Quantitatively examine micro relationships that exists between cells and between the cell and its subcellular components
- Analysis is done in 2D, 3D and 4D
- Calculate Statistical parameters such:
  - Distance to membrane
  - Distance to nucleus
  - Number of vesicles per cell
  - Nucleus to Cytoplasm volume ratio
  - Relative Tracking of subcellular objects

# Cell/Membrane Detection



Qualitatively and Quantitatively  
examine micro relationships that exists  
between cells and between the cell  
and its subcellular components  
Analysis is done in 2D, 3D and 4D

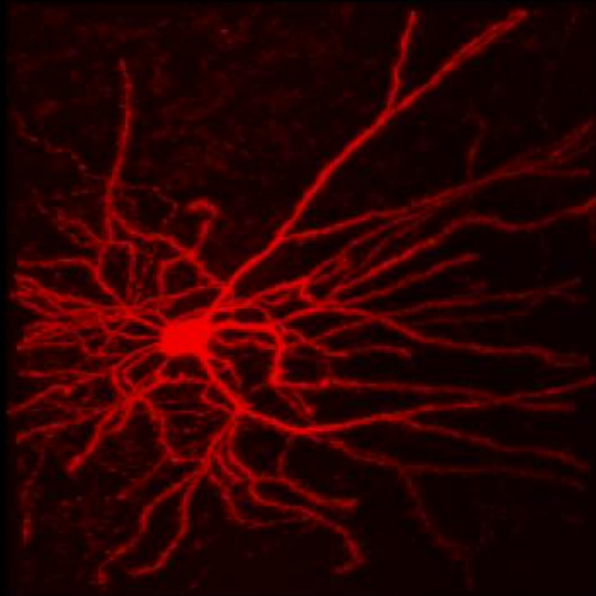
Calculate Statistical parameters such:

- Distance to membrane
- Distance to nucleus
- Number of vesicles per cell
- Nucleus to Cytoplasm  
volume ratio
- Relative Tracking of  
subcellular  
objects

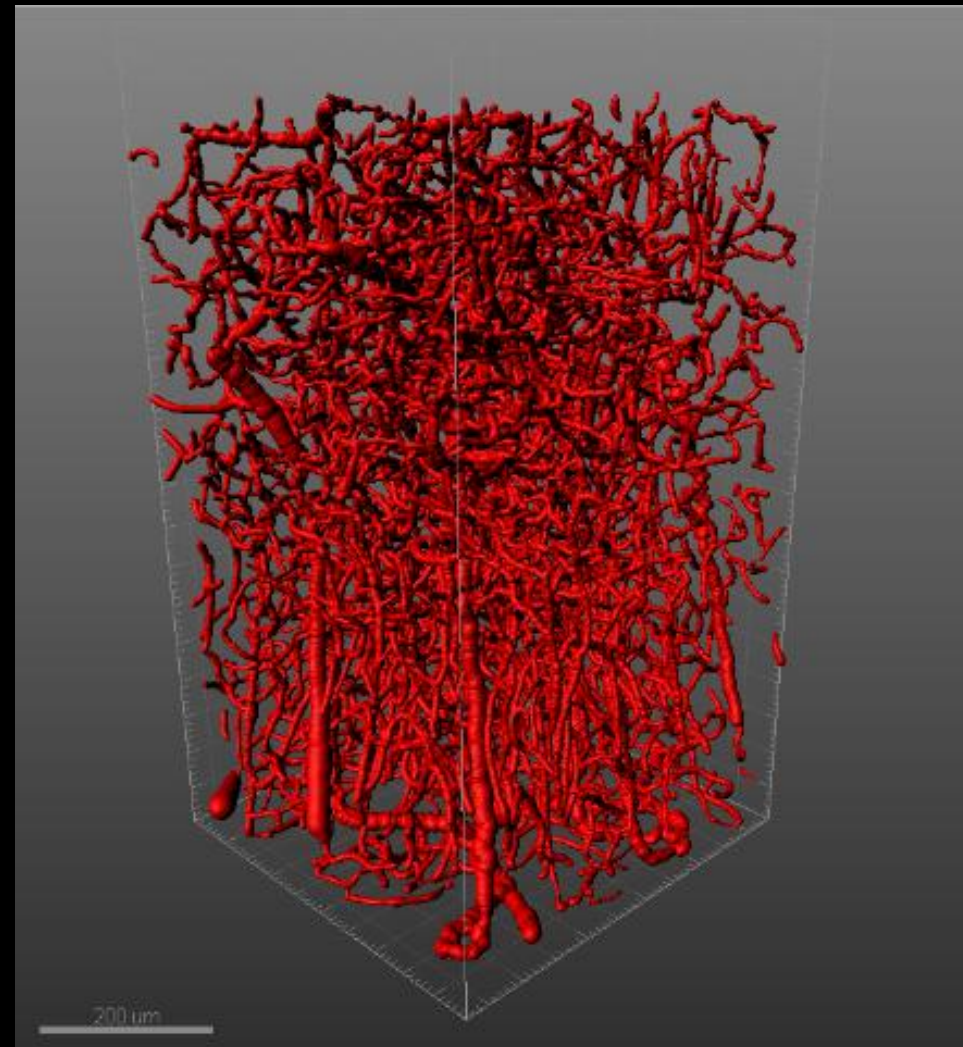
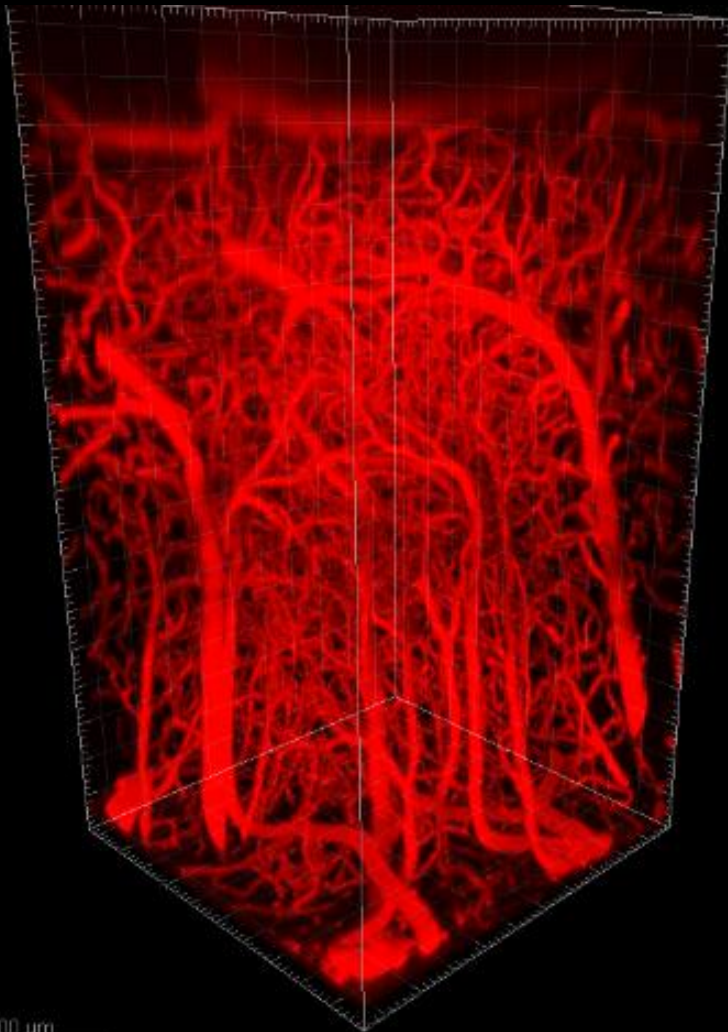
10  $\mu$ m

-46.268 Cell Position Z -2.145

# Filament/Neuron Detection



# Filament/Blood Vessels Detection





# Imaris TrackLineage - Object Label

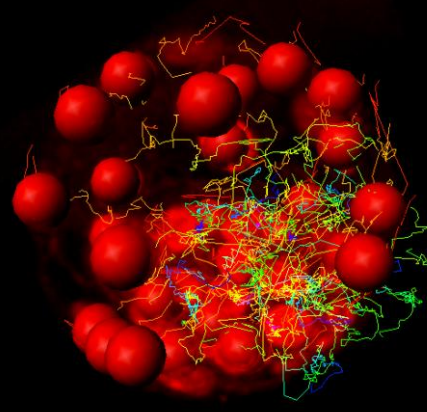
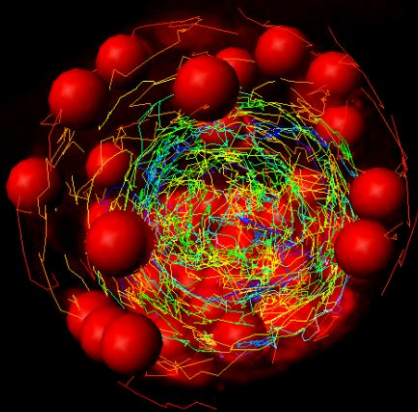
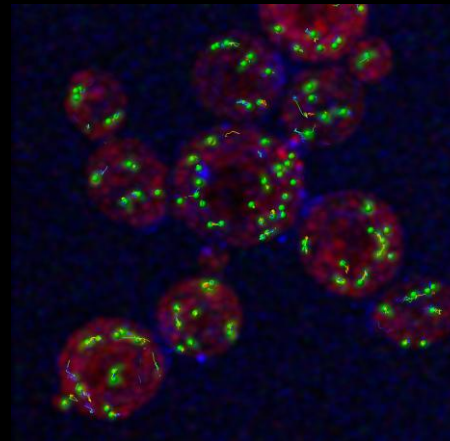
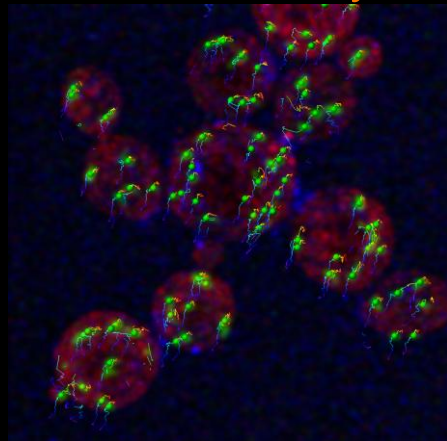
The screenshot displays the Imaparis software interface for analyzing cell lineage data. The main 3D view shows a complex, multi-colored structure representing a cell lineage tree, with spheres of various colors (green, yellow, red, blue, purple) and connecting lines. A scale bar indicates 10  $\mu\text{m}$ . A color scale for 'Track Number Of Generations' ranges from 1,000 to 10,000. A 'Time' scale is also visible, ranging from 1:55:20.497 to 1:55:20.497.

The interface includes several panels and controls:

- Top Menu:** Arena, Surpass, Vantage, Store, Store as, Export, Slice, 3D View, Color, Annotate, ADP, Snapshot, Animation.
- Scene Properties:** Spots 3 - Properties. Objects include Light Source 1, Frame, Volume, and Spots 3.
- Mouse Selects:** Object, Segment, Branch, Pivot, Track. Actions include Connect, Disconnect, Move Left, Move Right, Flip, Reorganize, Invert Axis, and Correct Drift...
- Track Lineage Graph:** A large graph showing the lineage of the selected object over time, with different colors representing different lineages.
- Camera / Labels Panel:** Camera Type (Orthogonal, Perspective 45°), InMotion (Center to Selection, Fit to Selection, Set Center...), and Labels of Selection (No labels found).
- Add a Label Panel:** NumberOfSplits (One, Three, Two).
- Bottom Panel:** Show Objects, Frame 116, and a timeline from 0 to 6928.

# Reference Frame / Coordinate System

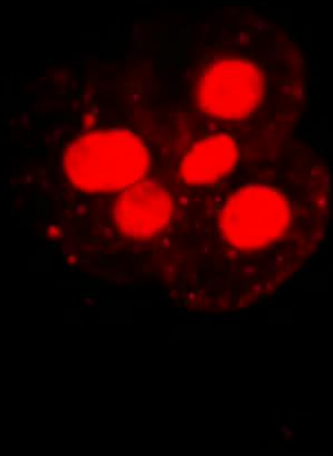
## - Correct Drift by RF



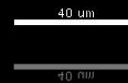
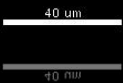
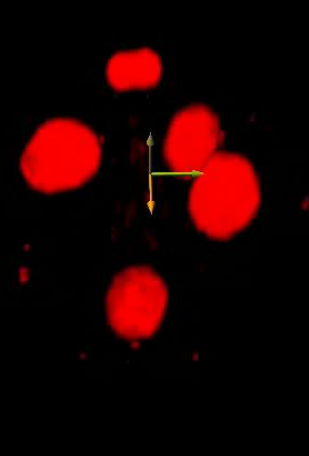
- ✎ Track Position
- ✎ Track Position Reference Frame
- ✎ Track Position Start
- ✎ Track Position Start Reference Frame
- ✎ Track Speed Max
- ✎ Track Speed Max Reference Frame
- ✎ Track Speed Mean
- ✎ Track Speed Mean Reference Frame
- ✎ Track Speed Min
- ✎ Track Speed Min Reference Frame
- ✎ Track Speed StdDev
- ✎ Track Speed StdDev Reference Frame
- ✎ Track Speed Variation
- ✎ Track Speed Variation Reference Frame
- ✎ Track Straightness
- ✎ Track Straightness Reference Frame
- ✎ Track Volume Mean
- ✎ Velocity Angle
- ✎ Velocity Angle Reference Frame

# Reference Frame / Coordinate System

- raw data

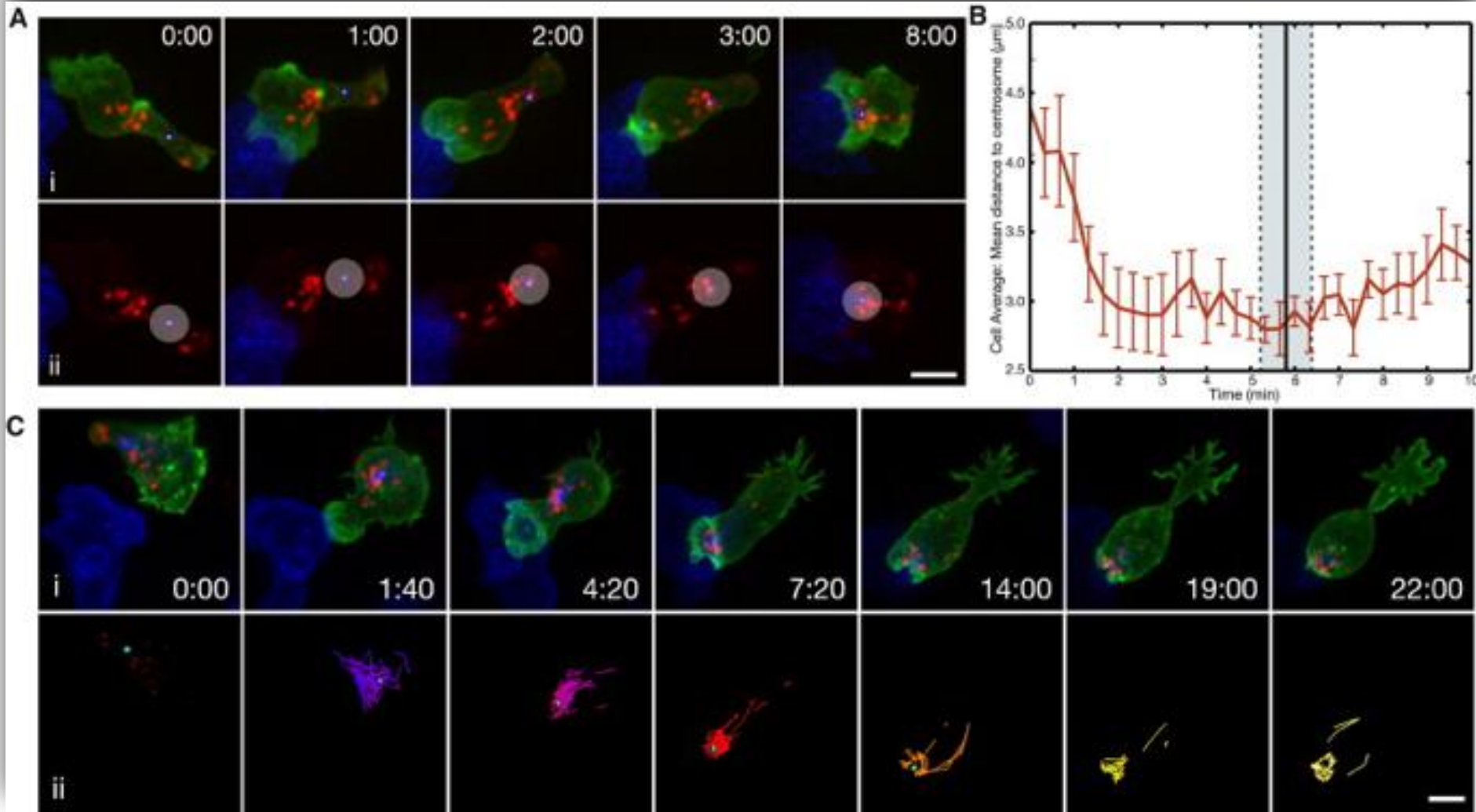


rf aligned





# Killing Cancer - Cytotoxic T-Cells on Patrol

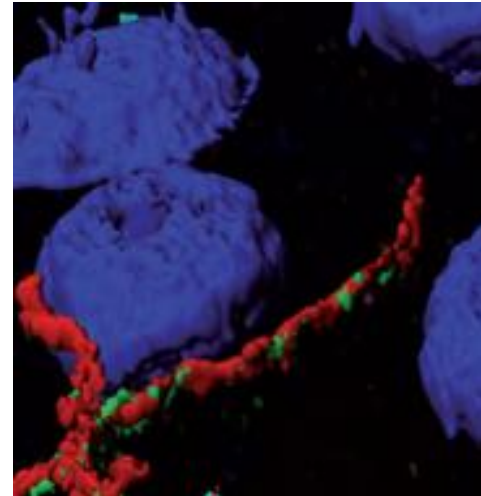
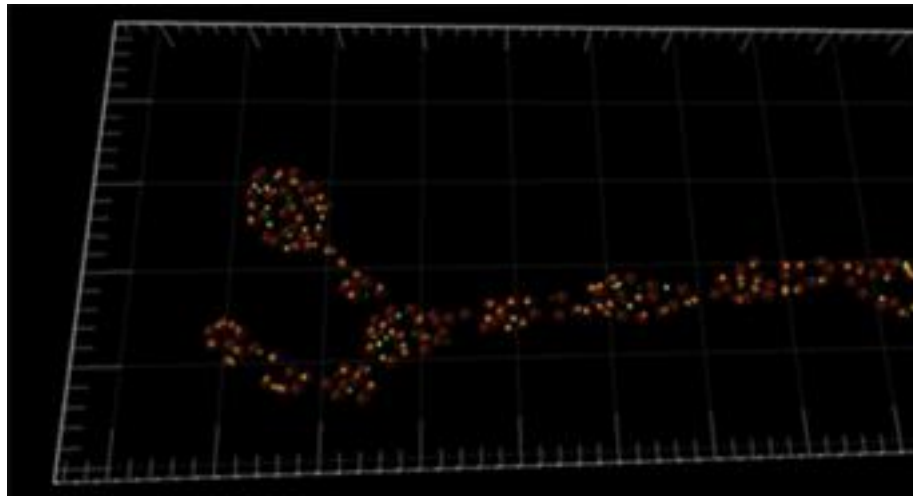
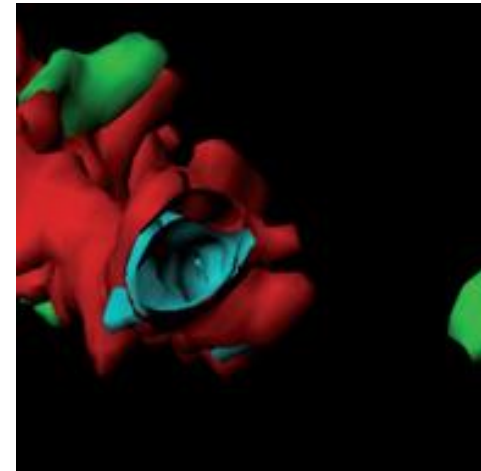
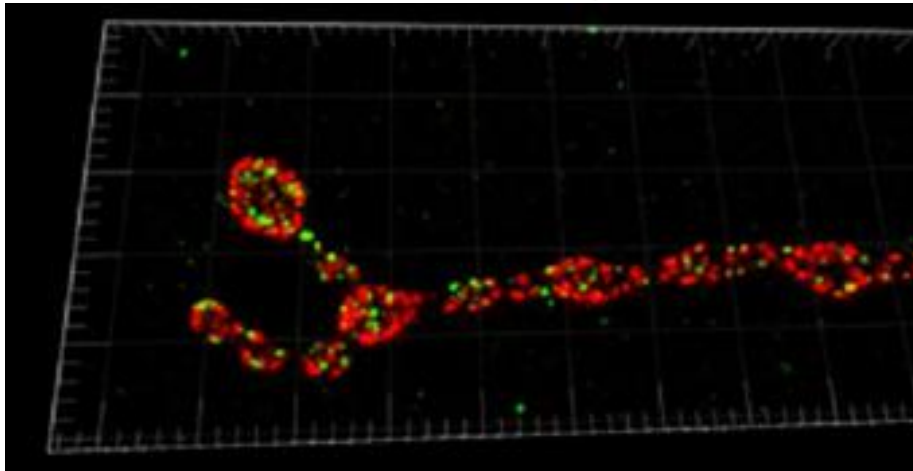




# Two primary kinds of colocalization

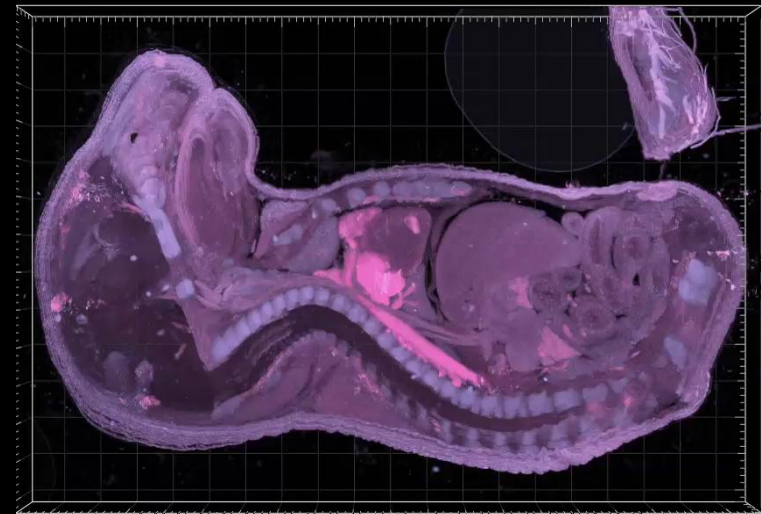
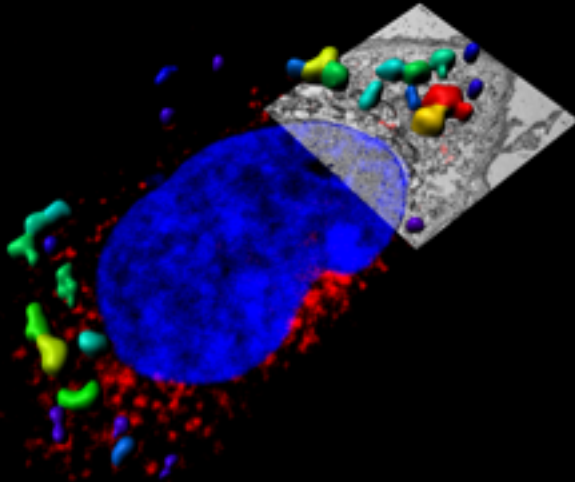
Neuron 66, 724–738, June 10, 2010, DOI 10.1016/j.neuron.2010.05.020

Mensa L, Crespo G, Gastinger M, et al. Hepatology, 2011 (53), pp 1436 - 1445



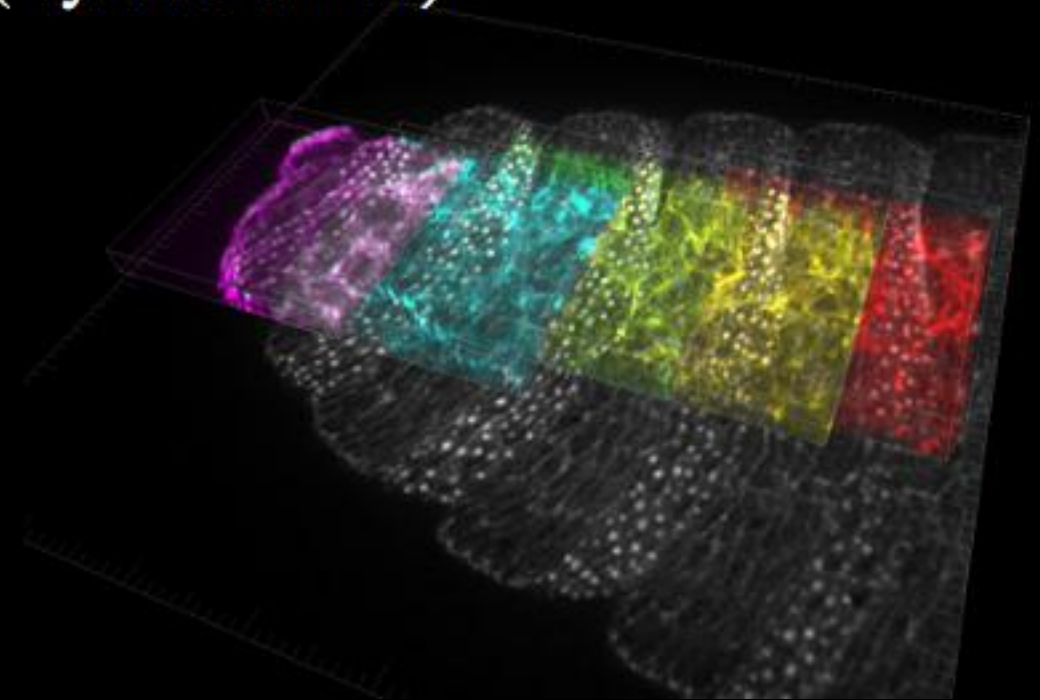
# Simultaneous Visualization of Multiple Images

- Correlative Microscopy
  - Confocal &:
    - EM
    - TIRF
    - DIC, Phase, etc.
- High-mag/Low-mag Overlay
  - Staging
  - Tissue identification



# Simultaneous Visualization of Multiple Images

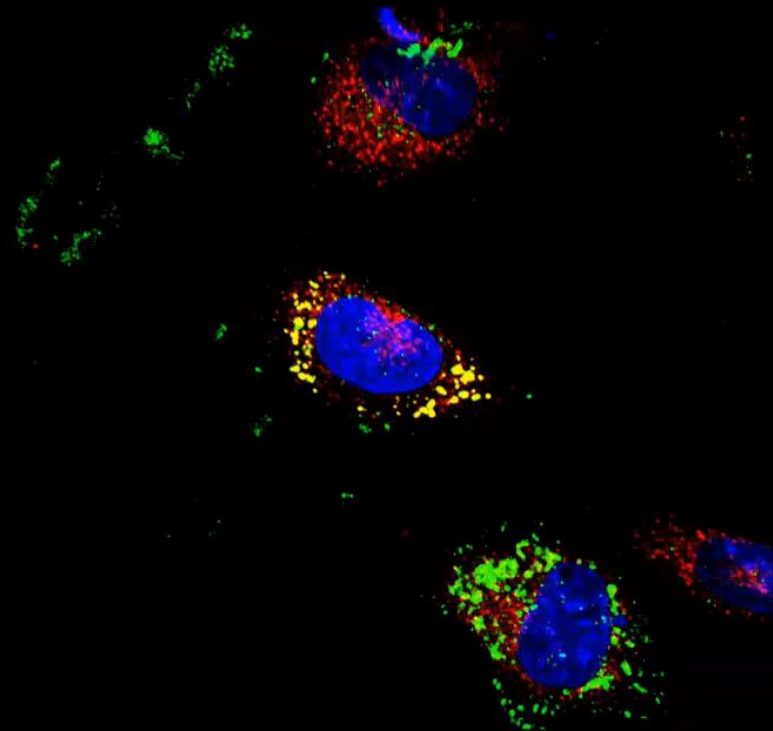
- Flexibility
  - Overlay many images
  - Of differing dimensions (x/y/z/t/channels)
  - Of differing resolutions



# Simultaneous Visualization of Multiple Images

Physical section

- Working distance
- Antibody cost



20  $\mu$ m



# Any Questions?



Thank you !





# BITPLANE

an **Oxford Instruments** company