Little Objects Segmentation & Tracking Environment

What is LOBSTER?

LOBSTER is an environment to batch process multidimensional microscopy images; it can identify biological objects and measure their morphology, dynamics and intensity distribution.

The software was designed with speed and simplicity in mind and a strong emphasis on data exploration and validation; it is fit for:

➢ Cell phenotyping in large high-content screening assays
➢ Object tracking in long microscopy time-lapses
➢ Filament tracing / spot counting in massive 3D images

Why LOBSTER?

A growing number of biological images analysis software are available, but most suffer from at least one of the following shortcomings:

- Too generic: no guidance to assemble algorithms into workflows
- Too specific: 2D images only, no object tracking, etc.
- Not scalable: limited image size, slow, too sensitive to varying quality
- Not flexible: fixed workflow, difficult to customize to a specific project
- Not expressive: too much code to write, prone to errors

Highlights

✓ Handle multi-dimensional images (3D + channels + time)

✓ Virtually no image size limit

✓ Consistent set of robust, documented IA algorithms (functions)

✓ Easily assemble functions into readable IA workflows (journals)

✓ Many sample applications (images + journals)

✓ Image analysis server: queue jobs, email results

Usability

- ✓ Simple to deploy (Matlab), no programming (plumbing only)
- ✓ Streamlined flow, only 4 basic type of image masks
- ✓ Open, layered architecture, human readable data at each step
- ✓ Detailed documentation built as tutorials
- ✓ Images, results and workflows accessible from hyperlinks
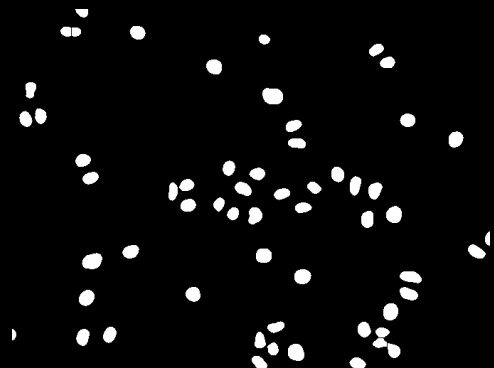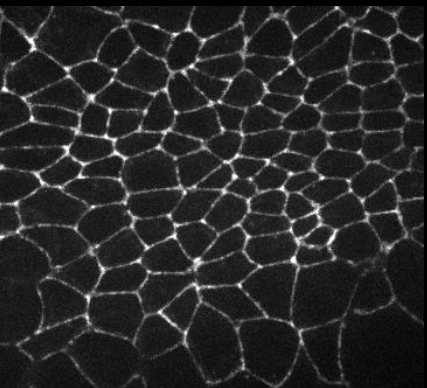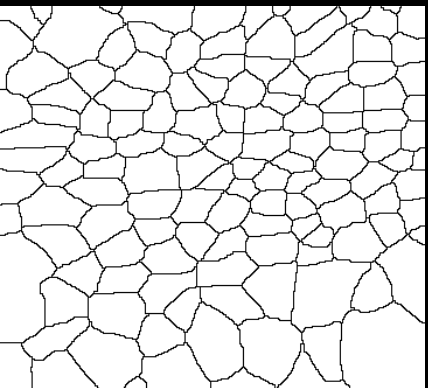
Architecture
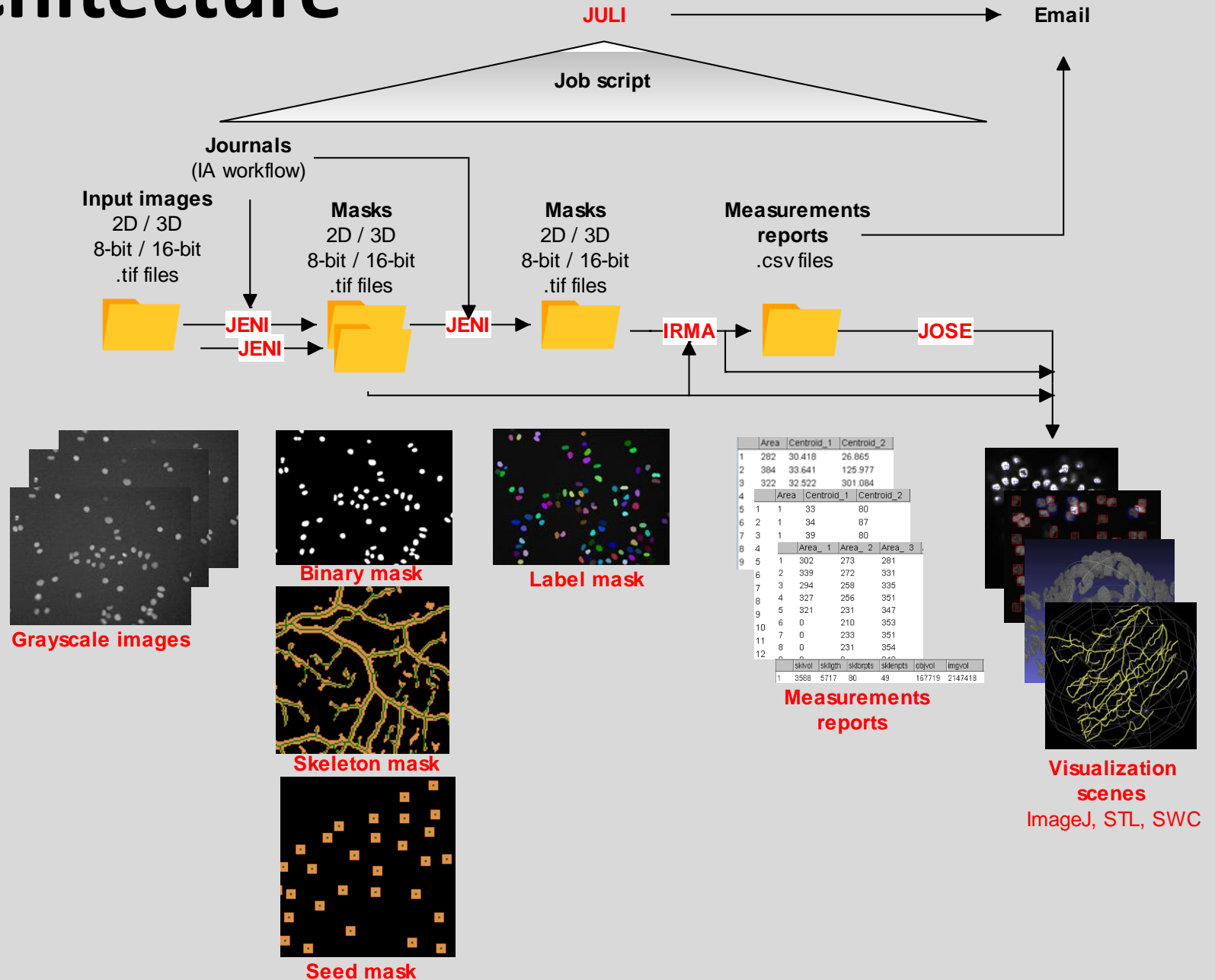
Sample Applications
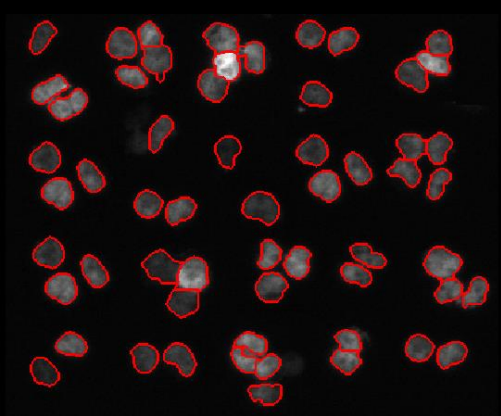
3D Object detection
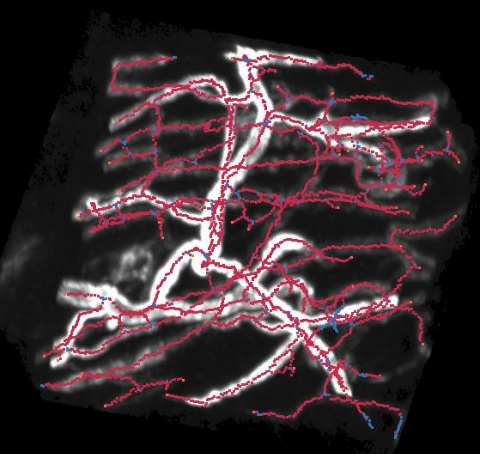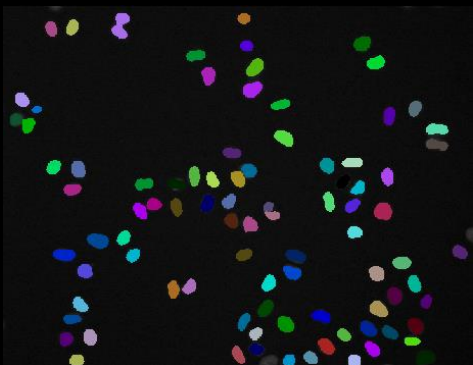
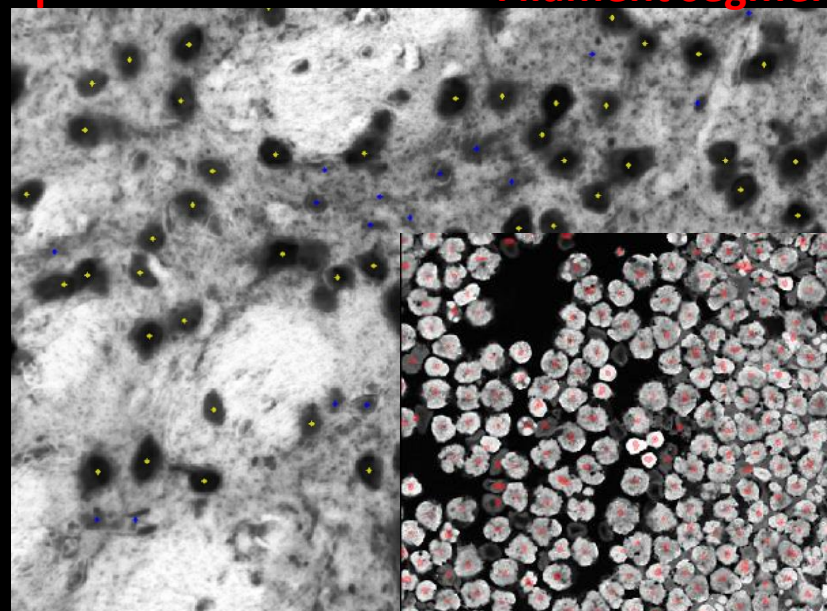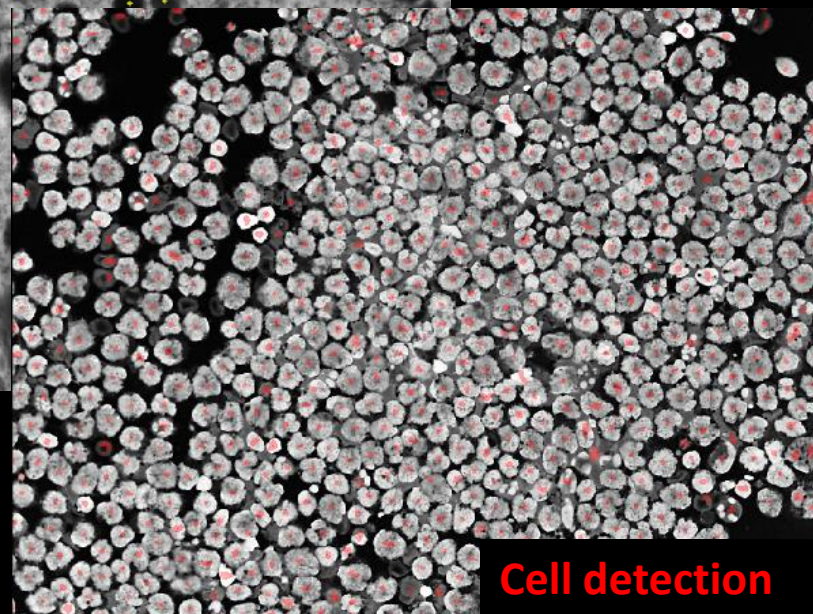**Filament / tube tracing**
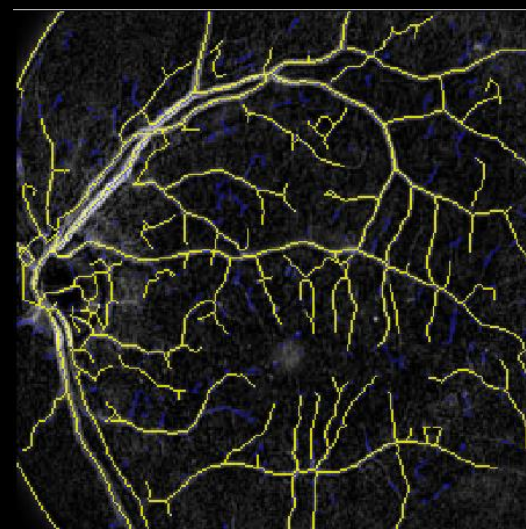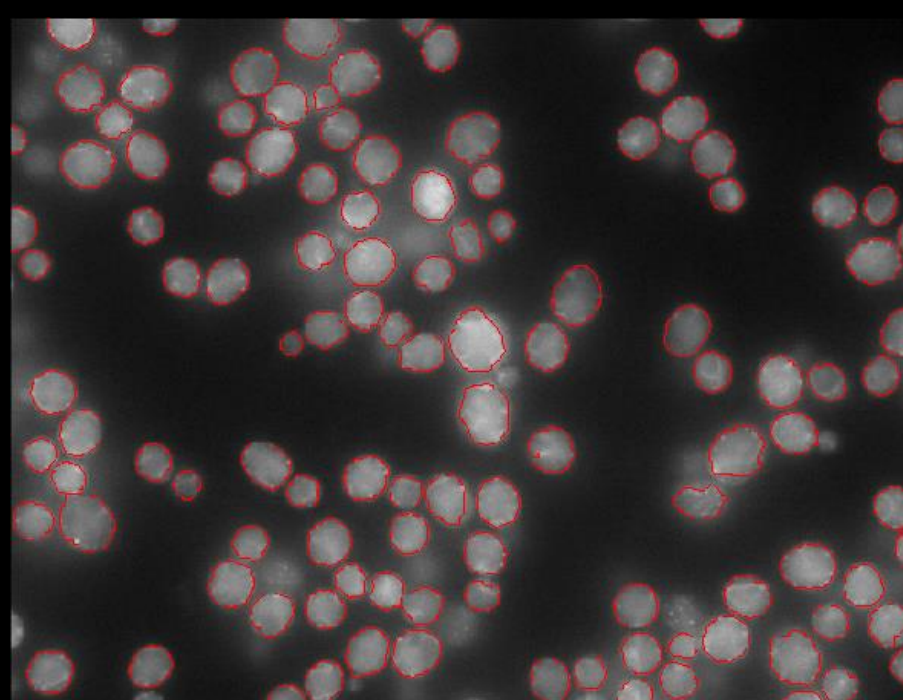
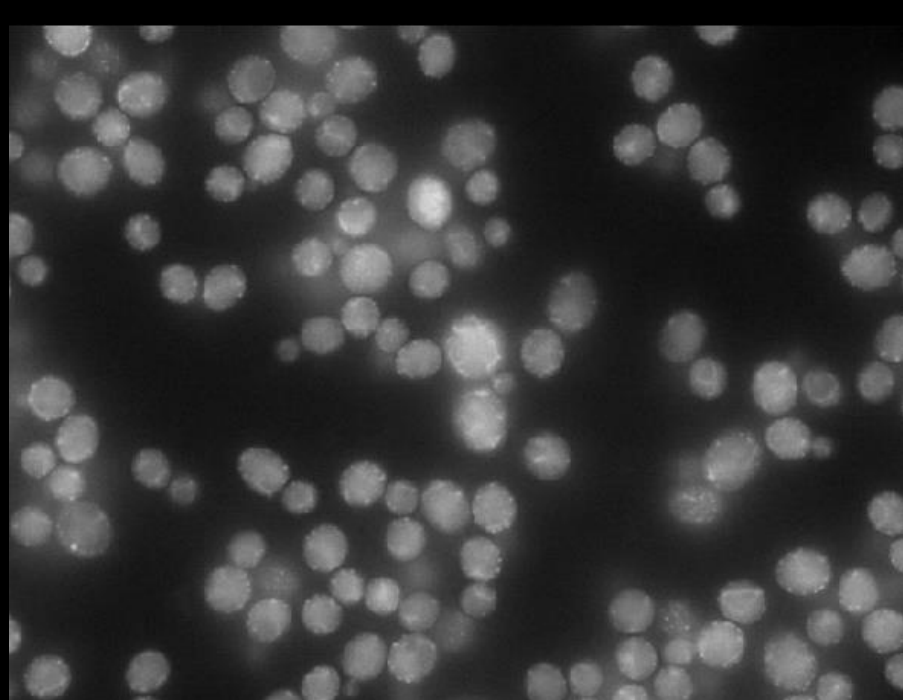**Spot detection**
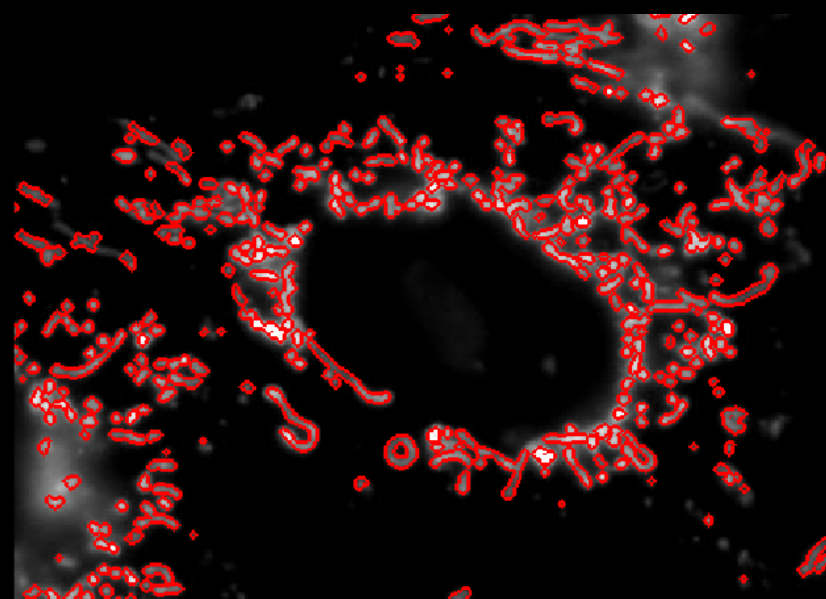
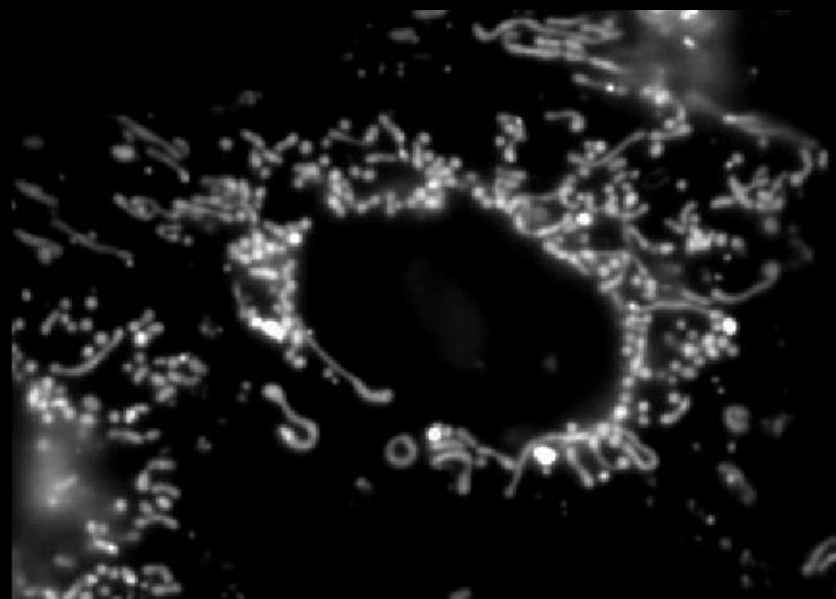**Filament segmentation**
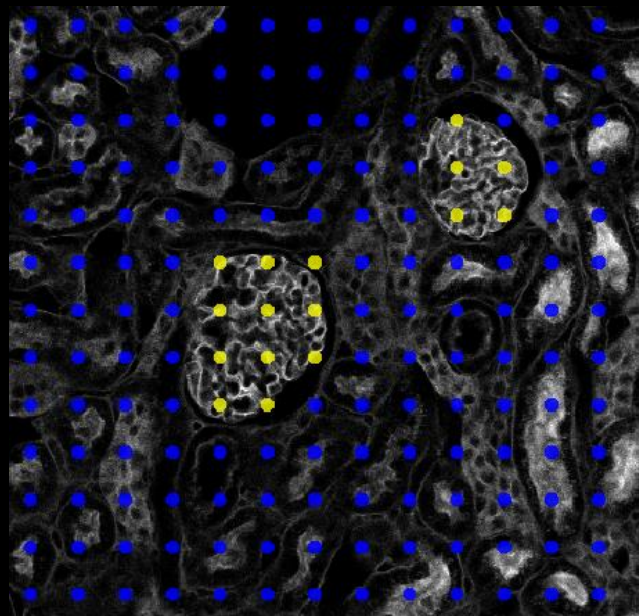
**Machine learning
texture classification**
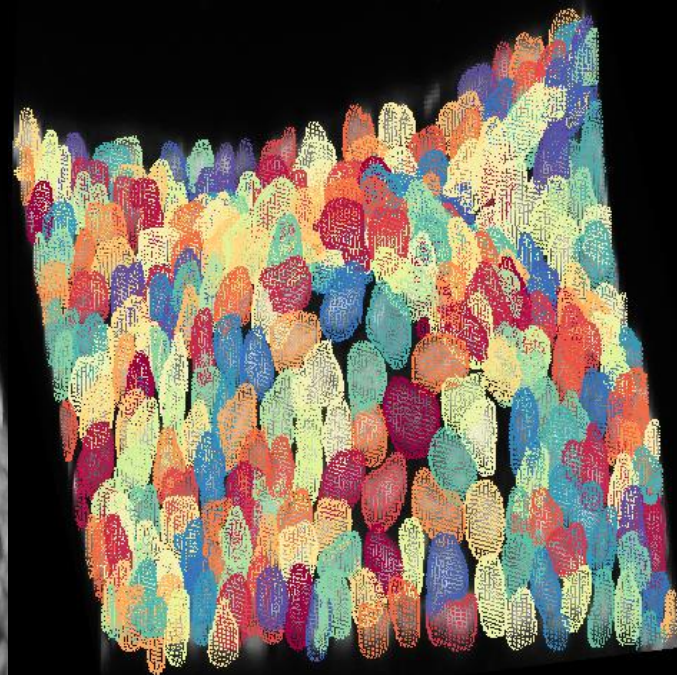
**Machine learning
nuclei detection**

Cell detection

Out of focus resilient object segmentation
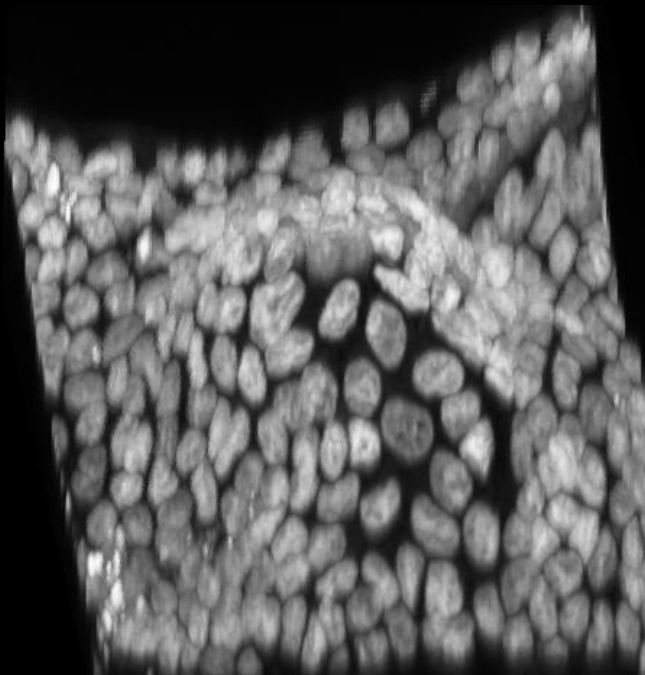
Clustered 3D object segmentation
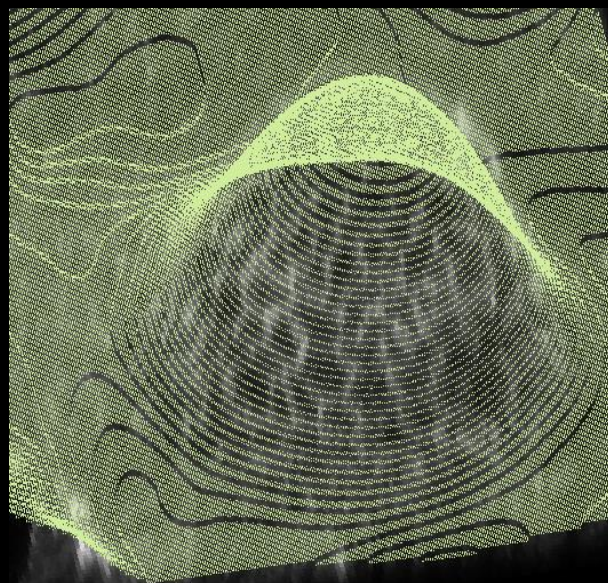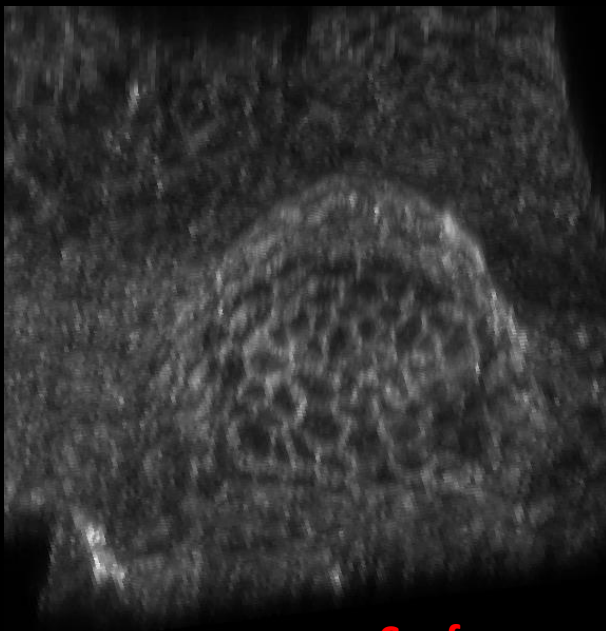
Surface segmentation
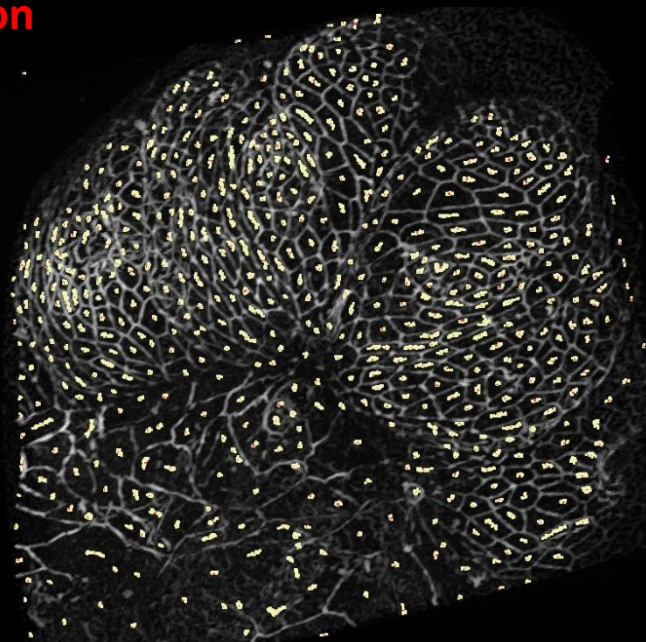
Cell detection over 3D mesh
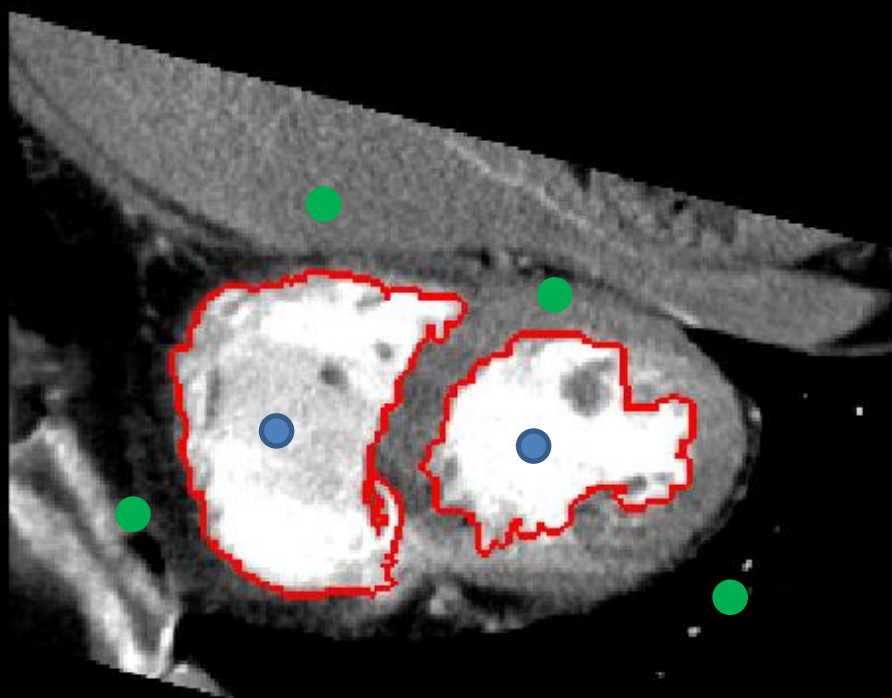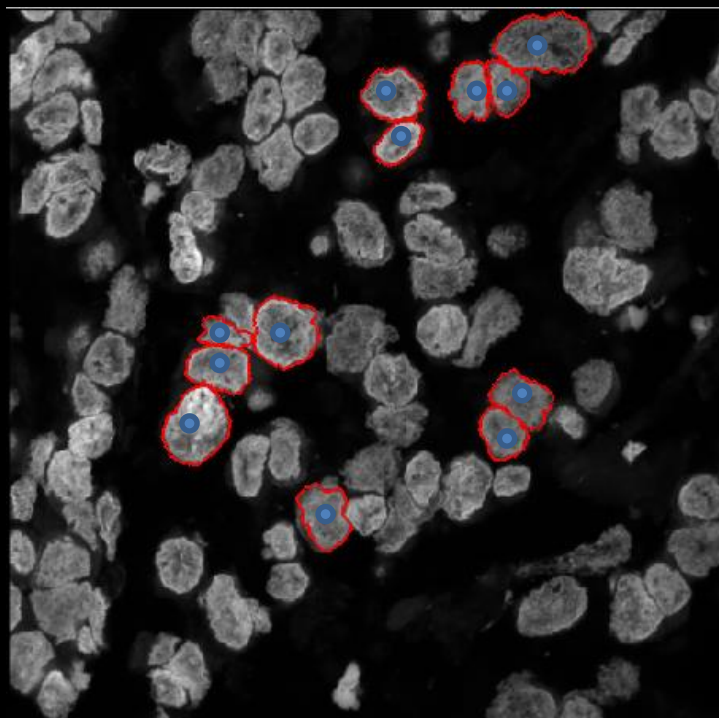
Objects extraction from seeds
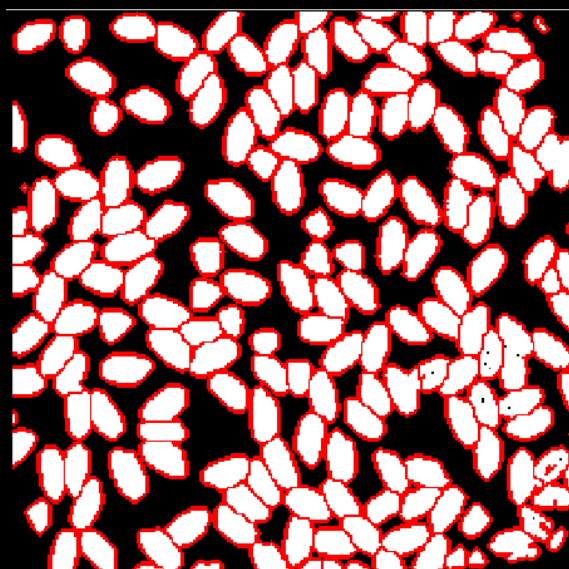
Robust convex particles splitting

Advanced denoising
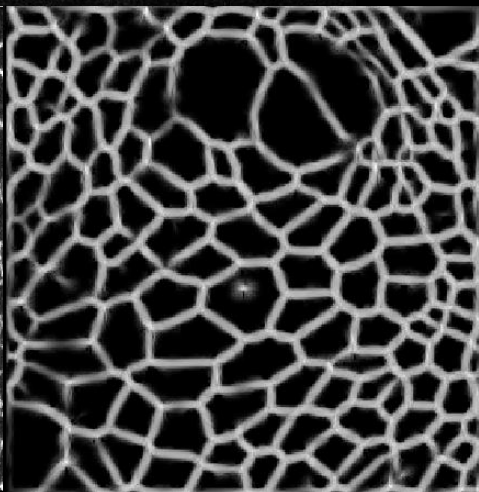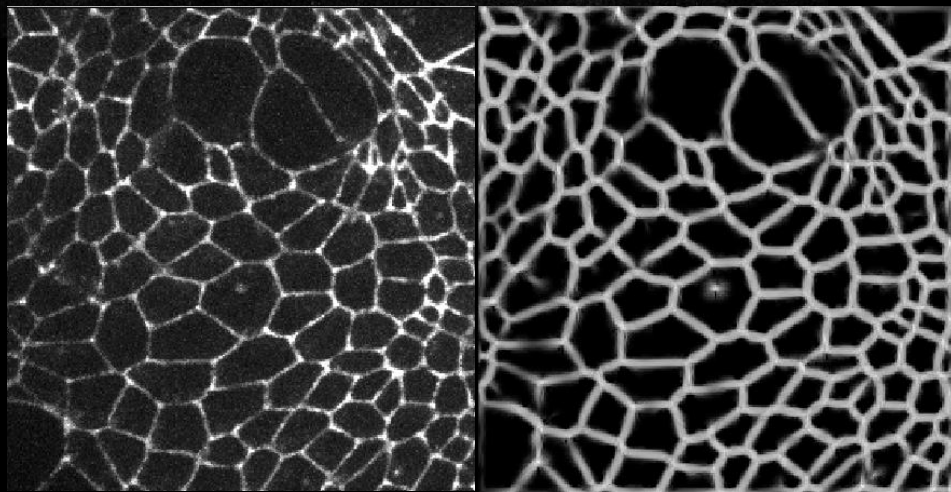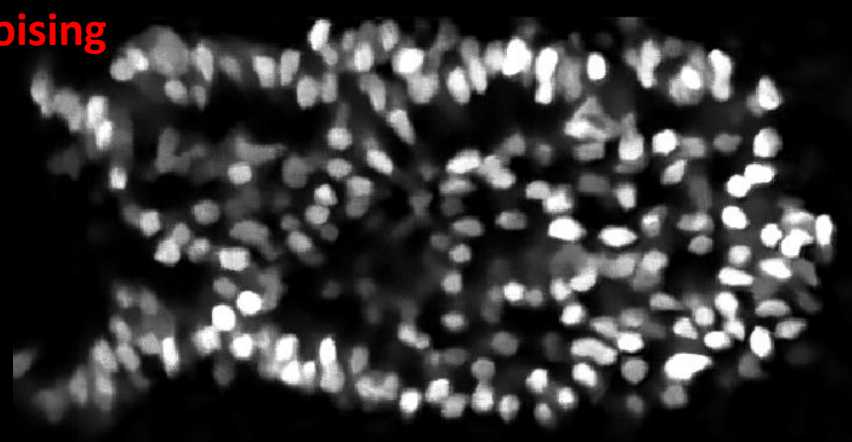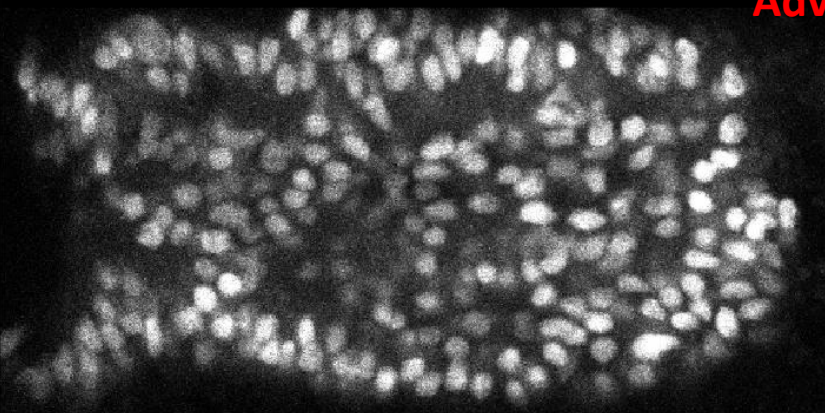
Membrane restoration
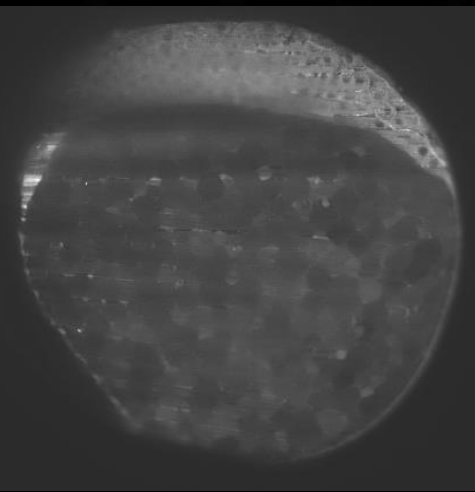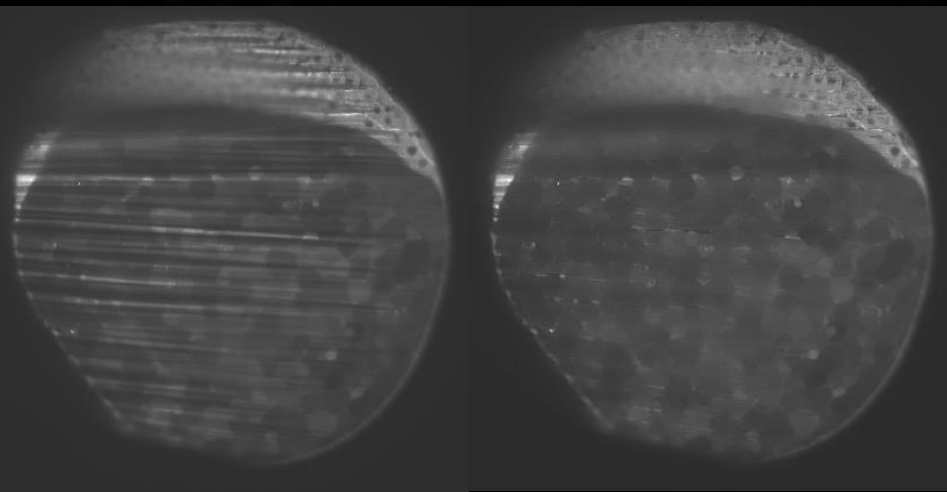
Destriping

Restoration
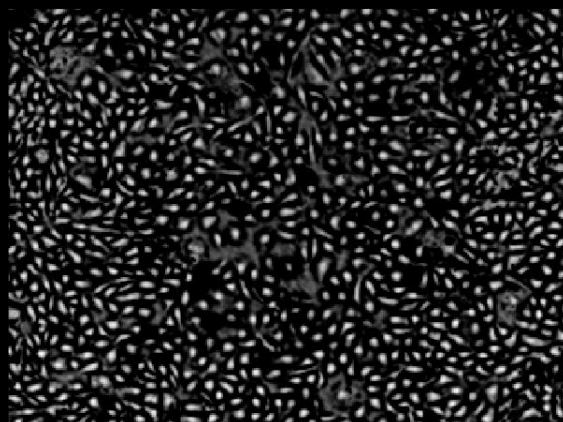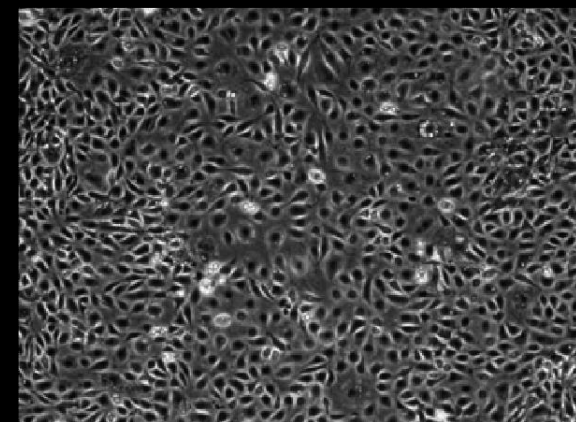
Phase contrast restoration

Deconvolution
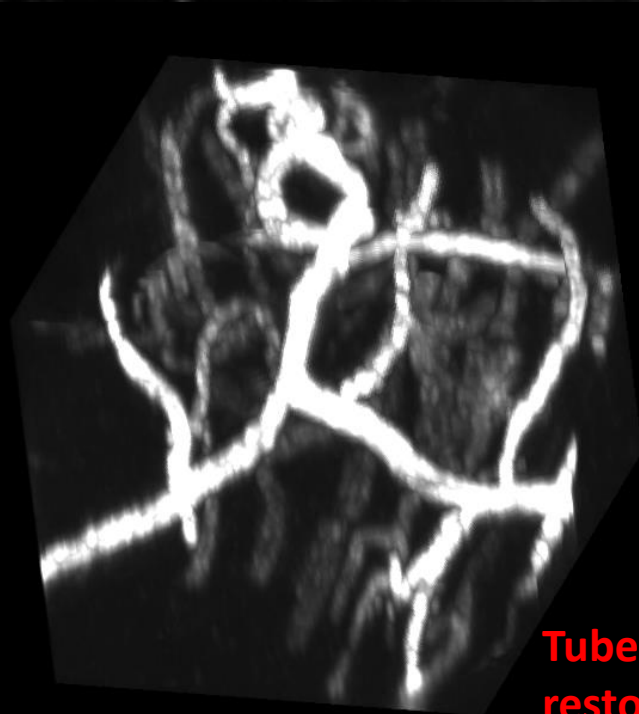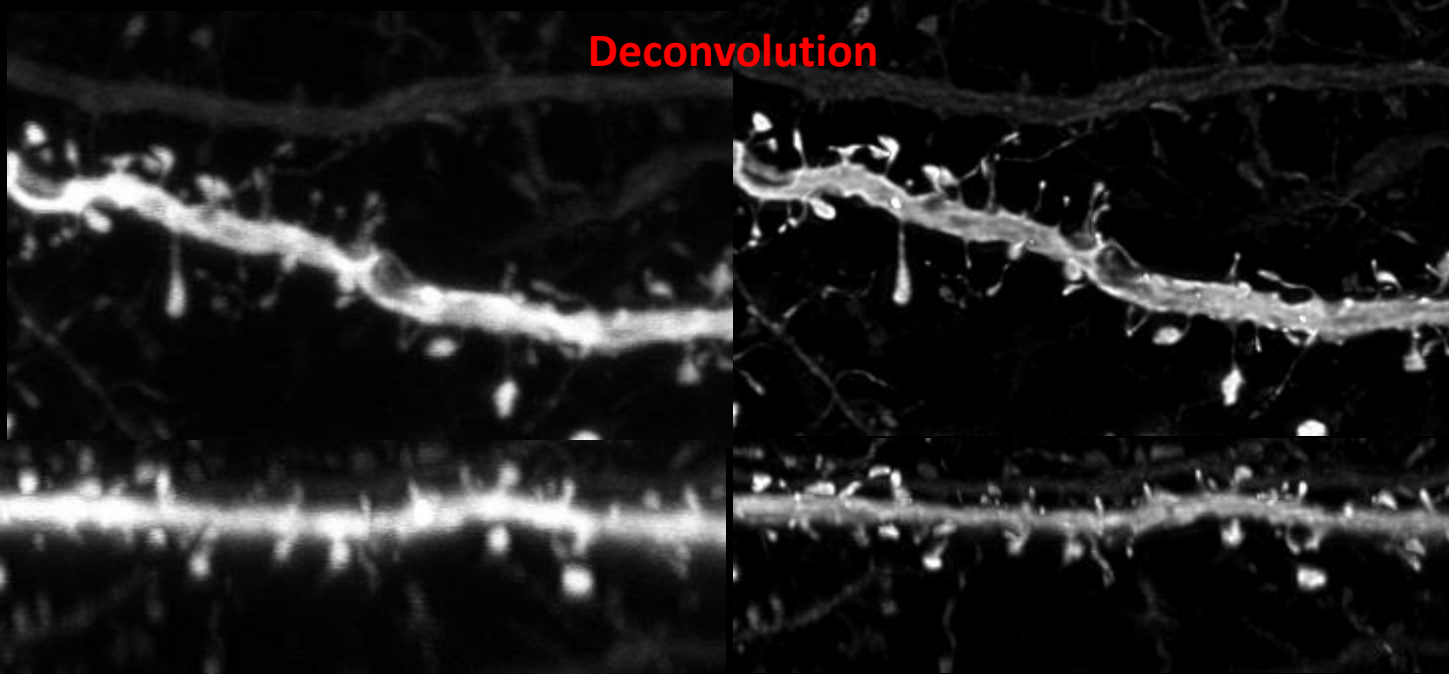
Tube to filament restoration
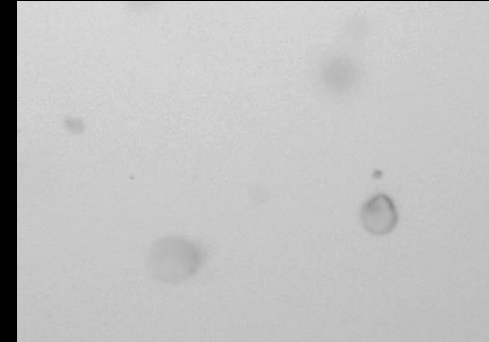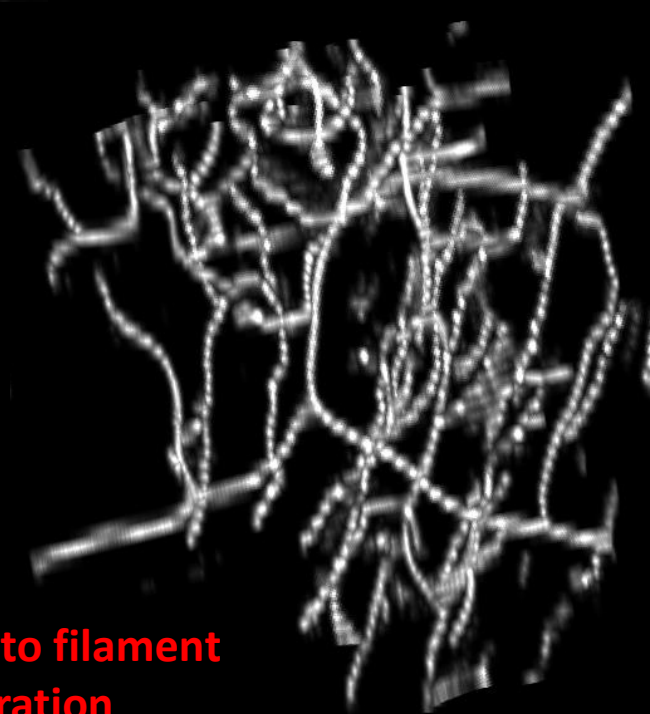
Extended depth of view
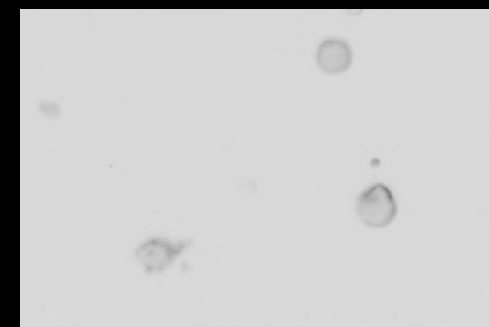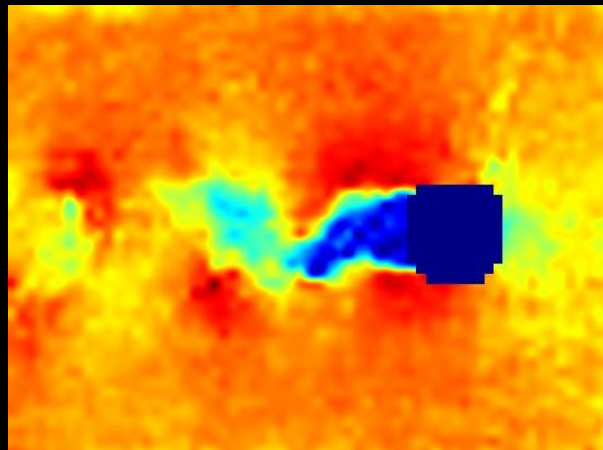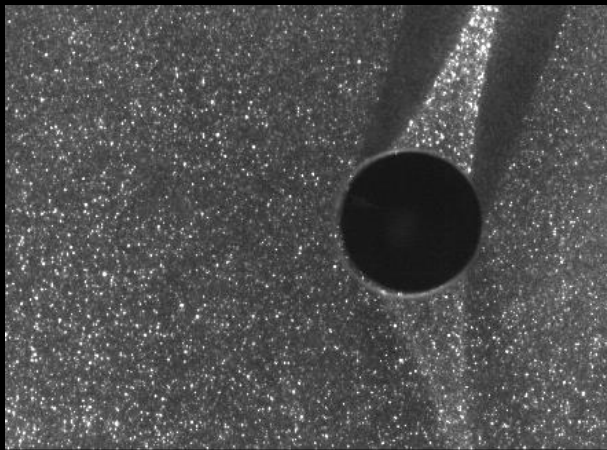
Speed measurement (PIV)
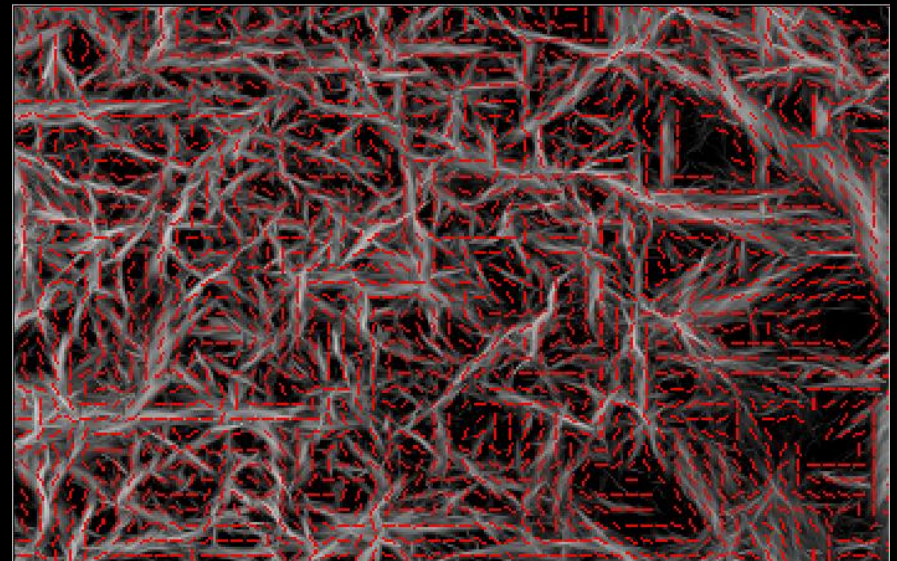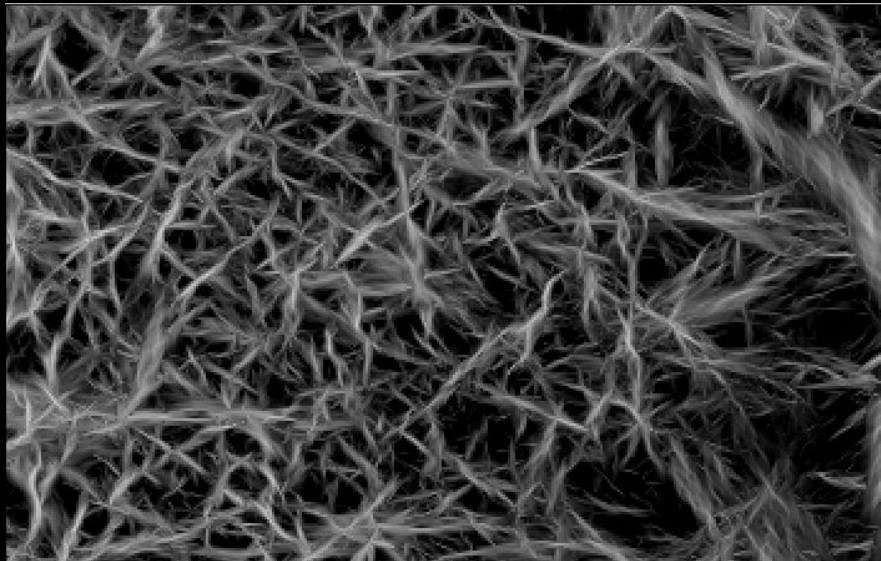
Orientation measurement
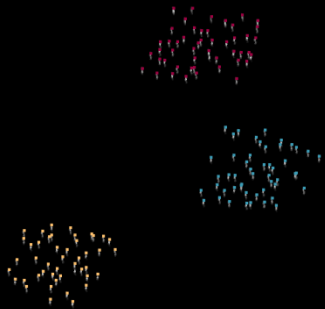
Points clustering

Image Features Measurements

Calling Journals: JENI
(Journal ENgine Interface)

>> [InputFolder MaskFolder] = **JENI** ('NucleiCytoo_GradWaterTilesMerge.jl');

Input and output image folders (MATLAB variables)

Journal to call

```
Journal: E:\LOBSTER/Journals/jl/NucleiCytoo_GradWaterTilesMerge.jl-->Launch
Input Folder: ./Images/NucleiCytoo/
Output Folder: ./Results/Images/NucleiCytoo/
Warning: Output folder not empty!!
> In JENI_Images (line 53)
  In JENI (line 95)
-------------------------------------------------------------
Found 2 image(s) / group(s)
Rescale: 1
MinLocalFocus: 0
ExportDist: 0
-------------------------------------------------------------
processing... 100%    [..........]
Time spent inside function fxg_mGradWaterTiles : 0.11 s
Time spent inside function fxm_lTilesMerge : 0.39 s
Total processing time: 0.51 s
```

Open journal

Rerun journal

Parsed files + configuration

Execution time

Open folder

Open folder

Open file

Name	Date
NucleiCytoo1_C00.tif	06/05/2015 13:24
NucleiCytoo1_C01.tif	06/08/2017 21:46
NucleiCytoo1_C02.tif	04/12/2017 18:30
NucleiCytoo3_C00.tif	31/01/2017 18:22
NucleiCytoo3_C01 .tif	06/08/2017 21:46
NucleiCytoo3_C02 .tif	04/12/2017 18:31

Name	Date
NucleiCytoo1_C00_L2.tif	26/02/2018 14:24
NucleiCytoo3_C00_L2.tif	26/02/2018 14:24

Open file
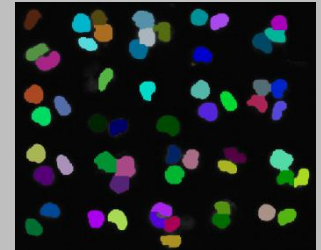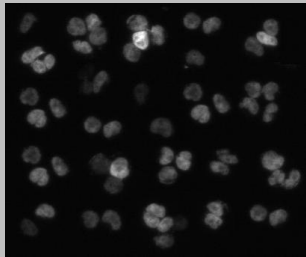
Measurements: IRMA

(Image Regions Measurements & Analysis)

Coming from JENI

```
>> ReportFolder = IRMA (MaskFolder, '.', 'Objs', 2, 1, InputFolder , '*C01*.tif');
```

Report folder

- Mask images to measure
- File filter

- Measurement type
- 2D
- Zratio = 1

Intensity channels (optional)

```
Intensity measurements in 1 channels
Intensity channels folder: ./Images/NucleiCytoo/
Found 2 datasets in input folder
------------------------------------------------

------------------------------------------------
Processing dataset 1 of 2
Number of objs:        58    Sum area    : 24568
Mean intensity:    103.71    Non null pix: 24568
------------------------------------------------
Processing dataset 2 of 2
Number of objs:         2    Sum area    : 781
Mean intensity:     75.00    Non null pix: 781
------------------------------------------------
Masks folder: ./Results/Images/NucleiCytoo/
Chan folder: ./Images/NucleiCytoo/
Reports folder: ./Results/Reports/NucleiCytoo/
```

Parsed files

Measurements summary

	Area	Centroid_1	Centroid_2	BoundingBox_1	BoundingBox_2	BoundingBox_3	BoundingBox_4	MeanInt	NonNullPix
1	375	30.50	26.87	20.50	14.50	22	26	50	375
2	485	33.56	126.00	20.50	112.50	25	26	50	485
3	410	32.57	301.09	21.50	289.50	23	23	50	410
4	423	36.46	69.86	22.50	58.50	30	23	50	423
5	485	36.56	204.00	23.50	190.50	25	26	50	485
6	492	43.76	154.51	30.50	142.50	25	25	50	492
7	492	46.76	232.51	33.50	220.50	25	25	50	492
8	512	52.65	82.52	35.50	68.50	32	26	50	512

Open folder

Name	Date modified
NucleiCytoo1_C00_L2.csv	01/03/2018 16:37
NucleiCytoo3_C00_L2.csv	01/03/2018 16:37

Open file

Exportation: JOSE
(**JO**b **S**cene **E**xporter)

Coming from JENI

Coming from IRMA

>>JOSE(InputFolder,'*_C00*',ReportFolder,'Objs',ReportFolder, 'IJ',);

Link C00 from
original images

Link Objects
bounding boxes

Export scene to this folder
for IJ (SceneViewer macro)

```
0000: E:\LOBSTER\Results\Reports\BloodVessels3DSkl/zzz_Jobscene.sce
0001: E:\LOBSTER\Results\Reports\FISH_nuc/zzz_Jobscene.sce
0002: E:\LOBSTER\Results\Reports\HeLaMCF10AMovieOvlLbl/Movie1/zzz_Jobscene.sce
0003: E:\LOBSTER\Results\Reports\HeLaMCF10AMovieOvlLbl/Movie2/zzz_Jobscene.sce
0004: E:\LOBSTER\Results\Reports\LymphGland3D/zzz_Jobscene.sce
0005: E:\LOBSTER\Results\Reports\LymphGland3D_folders/zzz_Jobscene.sce
0006: E:\LOBSTER\Results\Reports\NucleiCytoo/zzz_Jobscene.sce
0007: E:\LOBSTER\Results\Reports\TissuePilar3DNucDet/zzz_Jobscene.sce
```
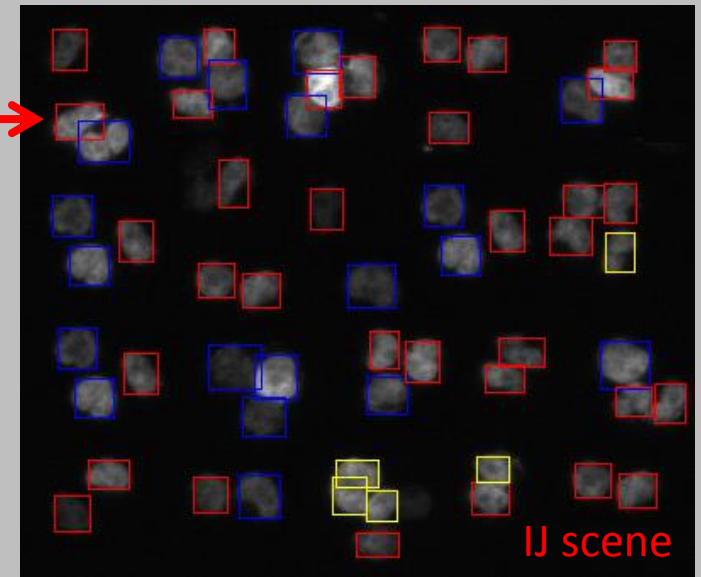
.sce files parsed by IJ
SceneViewer macro
from report folder

Scene index

OK   Cancel

IJ scene
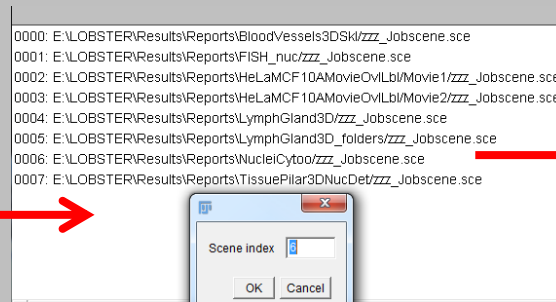
3 steps together: job file (*.m)

```
[InputFolder MaskFolder] = GENI('NucleiCytoo_GradWaterTilesMerge.jl');
ReportFolder = IRMA(MaskFolder,'.','Objs',2);
JOSE(InputFolder,'*_C00*',ReportFolder,'Objs',ReportFolder, 'IJ');
```

Job 'NucleiCytoo_scene.m'

Journals


```
InputFolder = './Images/NucleiCytoo/';
OutputFolder = './Results/Images/NucleiCytoo/';
Fill = 1;     display filled masks
Lbl = 1;      display labeled masks (colors)


@iA = '*C00*.tif';     image filter (InputFolder)


@fxg_mGradWaterTiles [iA] > [L];      first function
params.GaussianRadInt = 2;            + parameters
params.ExtendedMinThr = 2;
/endf


@fxm_lTilesMerge [L, iA] > [L2];
params.GaussianRad = 2;
params.MinObjArea = 175;              second function
params.MinSal = -0.5;                 + parameters
params.MaxValleyness = 1.075;
params.ConcavityThresh = 0.4;
/endf


/show iA > L2;     display iA + L2 overlay (image viewer)
/keep L2 > tif;    export L2 to tif (OutputFolder)
```
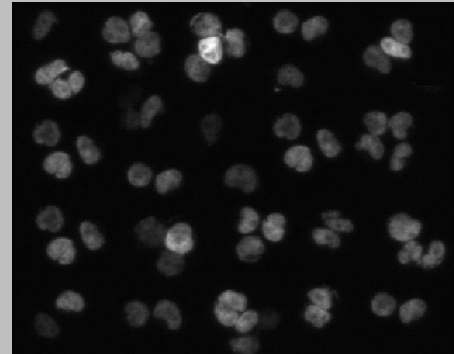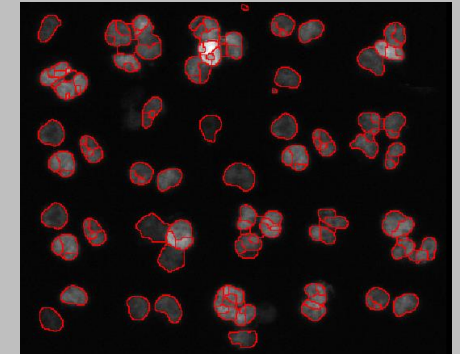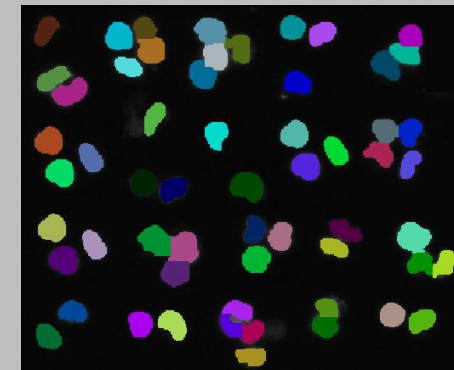
iA

L

L2

Journal 'NucleiCytoo_GradWaterTilesMerge.jl'

Documented Functions

>> help fxm_lTileMerge

Merge/split particles in binary mask to reconstruct loosely convex bright objects.
The algorithm also requires the original image to use intensity information.

Sample journal: NucleiCytoo_GradWaterTilesMerge.jl ← Hyperlink to sample journal
demoing the use of this function

Input: 2D binary mask, 2D original image
Output: 2D label mask
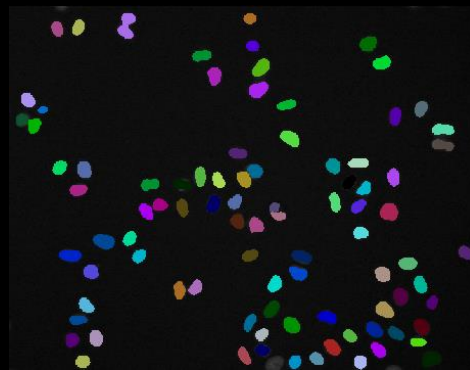
Parameters:
GaussianRad: Gaussian blur pre-filter radius (pix)
MinObjArea: Minimum object area (pix)
MinSal: Minimum tiles saliency (low -0.5 -> 0.5 high)
MaxValleyness: Maximum tiles valleyness (rescue more 0.75 -> 1.25 rescue less)
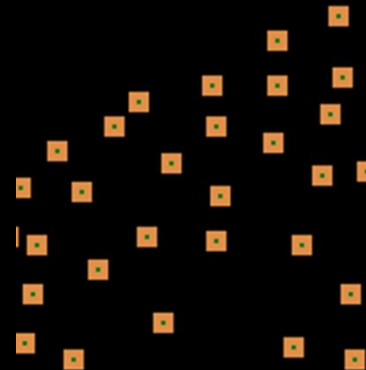ConcavityThresh: Concavity threshold (sensitive 0.25 -> 0.5 coarse)

Basic Measurements

Object mask (binary)
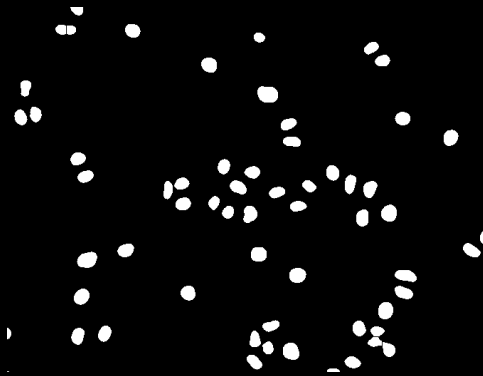
Object label mask

Seed mask

Skeleton mask

Objects
Area
Centroids (X,Y,Z)
Bounding boxes (6 coords)
Mean intensity

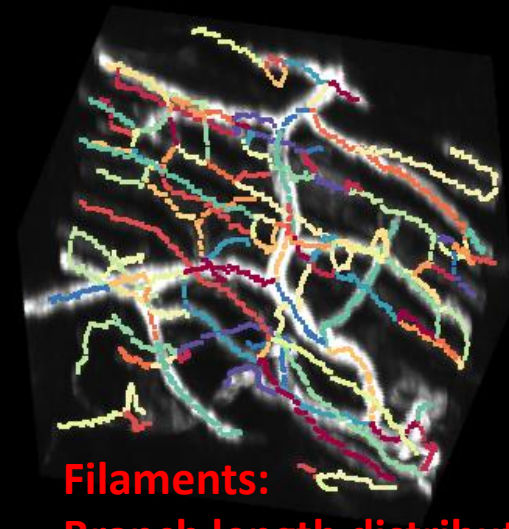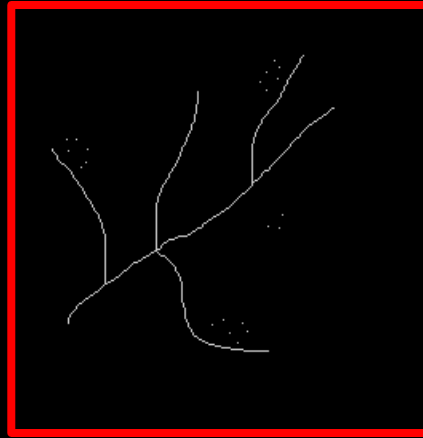**Objects**
Area
Centroids (X,Y,Z)
Mean intensity

Seeds
Centroids (X,Y,Z)
Mean intensity

Skeletons
Mask volume
Length
Nb brch/end points
Object volume
Image volume
Mean intensity

Advanced Measurements

Secondary objects count

Objects distance distribution
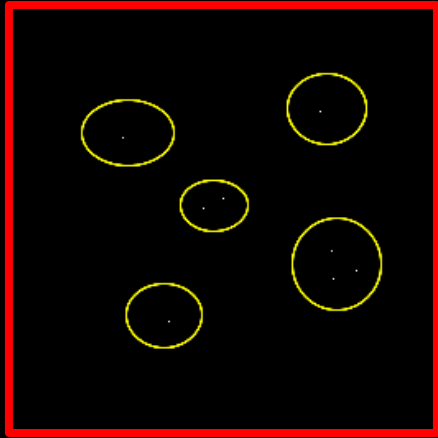
Filaments:
**Branch length distribution**
Width distribution
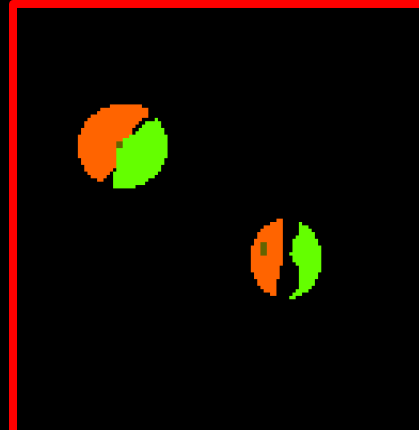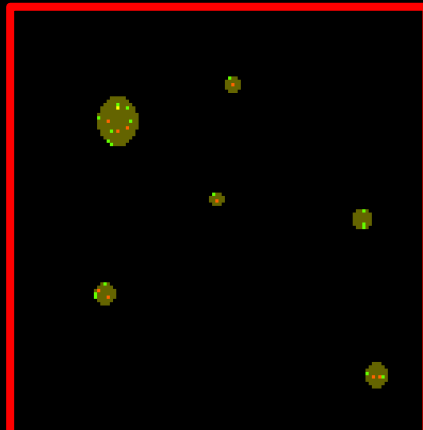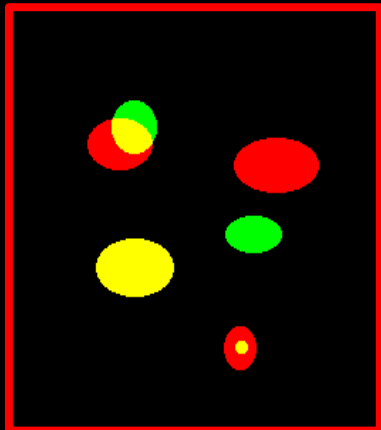
Objects colocalization

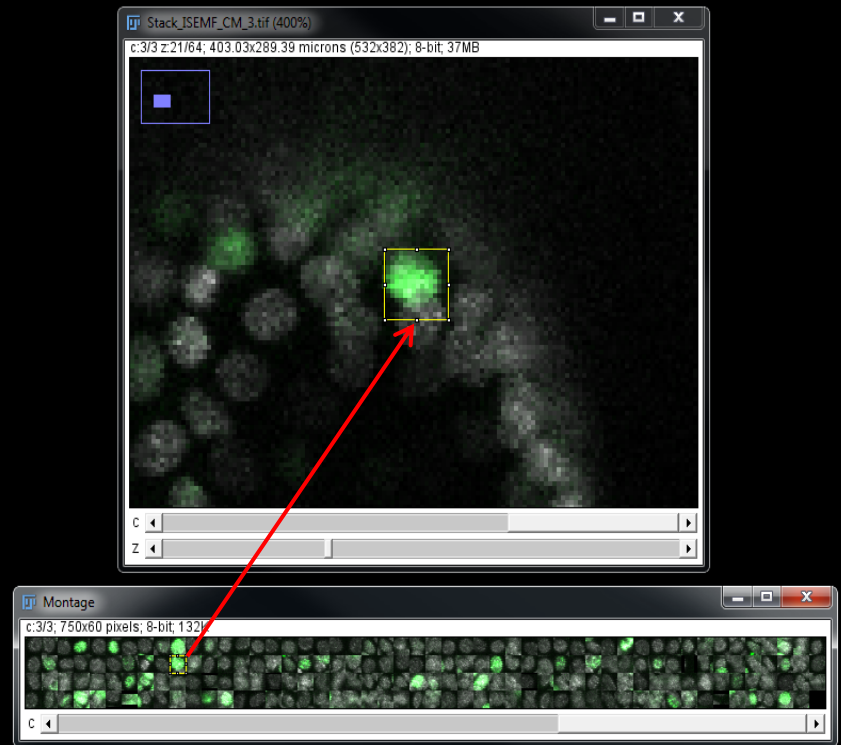**Statistical colocalization**

Clustering

- **Normalized objects overlap**
- **Seeds FP / FN / TP rates**
- **Predicted ++ probability**
(assuming random seeds)
- **Actual ++ observation prob.**
(assuming random seeds)
- **Colocalization evidence**
P(r+ | g+) / P(r+ | g-)
- **Exclusion evidence**
P(r- | g+) / P(r- | g-)
- **Clustering factors (r and g)**

Exportation & validation
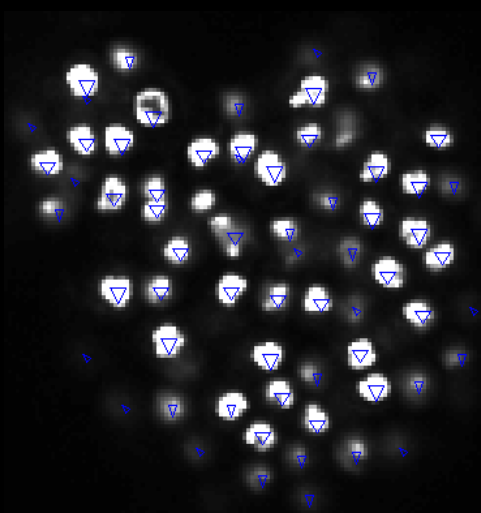
… in massive images
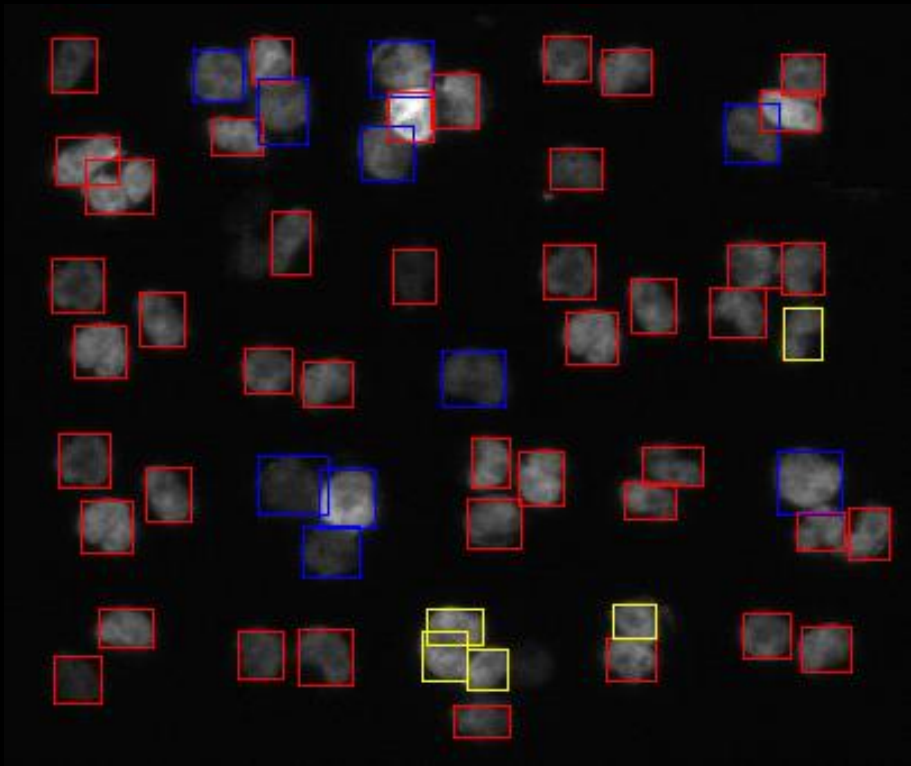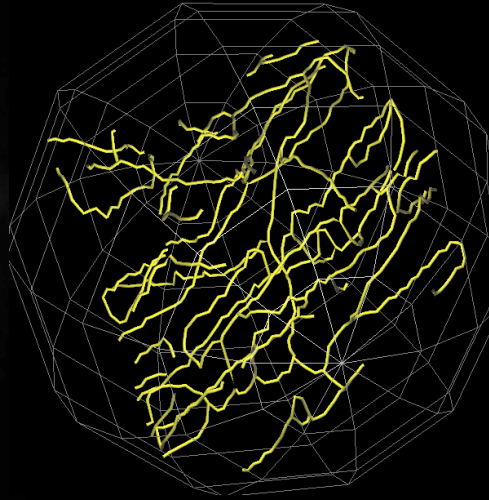

Display object bounding Box + color-code (IJ)

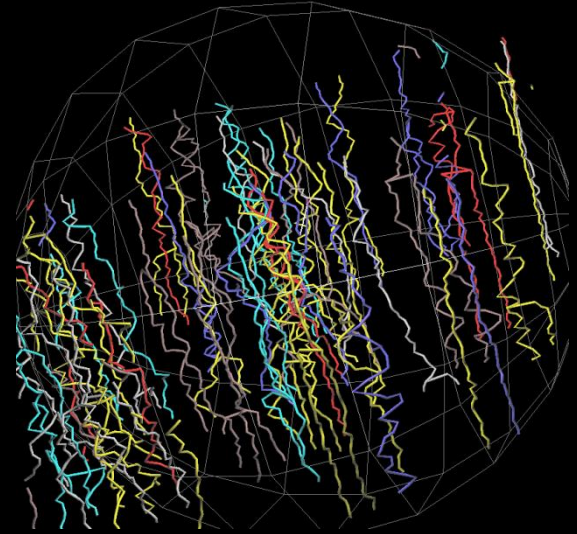**Montager IJ macro: Auto-montage objects + link to their locations**
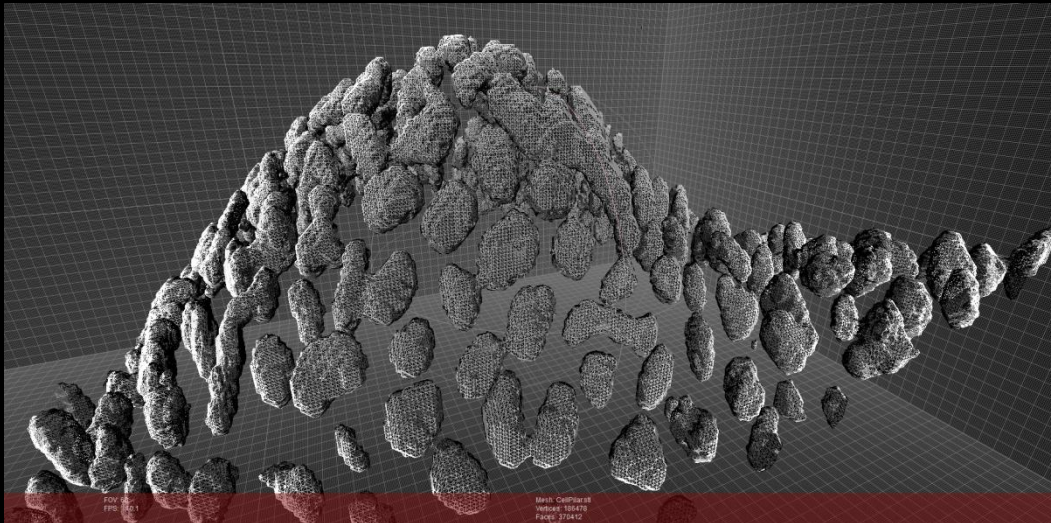
3D models exportation

Seeds (xls)
CellInsight (IJ)

Filaments (SWC)
Neuromantics

Tracks (SWC)
Neuromantics
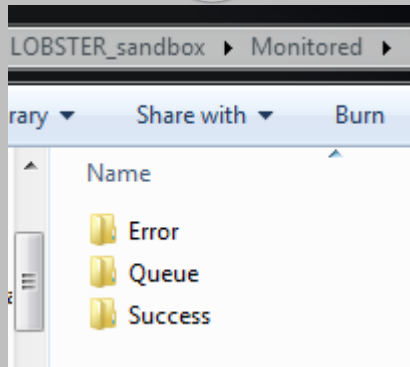
Objects (STL)
MeshLab

Image Analysis server: JULI
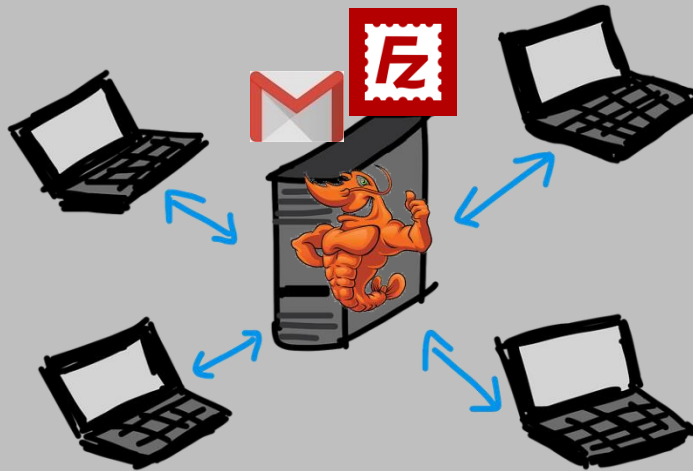## (**Job UpLInk**)

JULI enabled job

```
>> JULI (PathToMonitoredFolder, PathToLogFile, 'user@gmail.com', 'password');
```
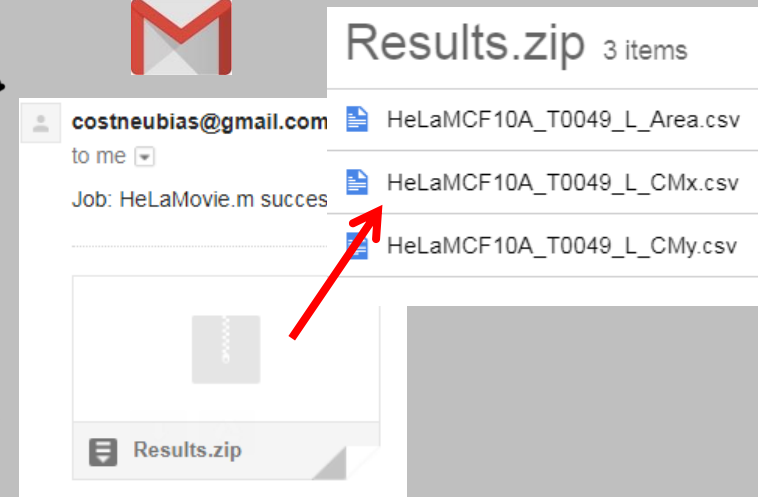
```
InputFolder = 'E:/LOBSTER_sandbox/Images/FISH/';
Dstmail = youremail@youremailprovider.com';
[InputFolder MaskFolder1] = GENI('FISH_nucseg.jl',InputFolder,1);
[InputFolder MaskFolder2] = GENI('FISH_sptdet.jl',InputFolder,2);
AttachmentFolder = IRMA(MaskFolder1,1,'Objs',2,1, MaskFolder2);
```
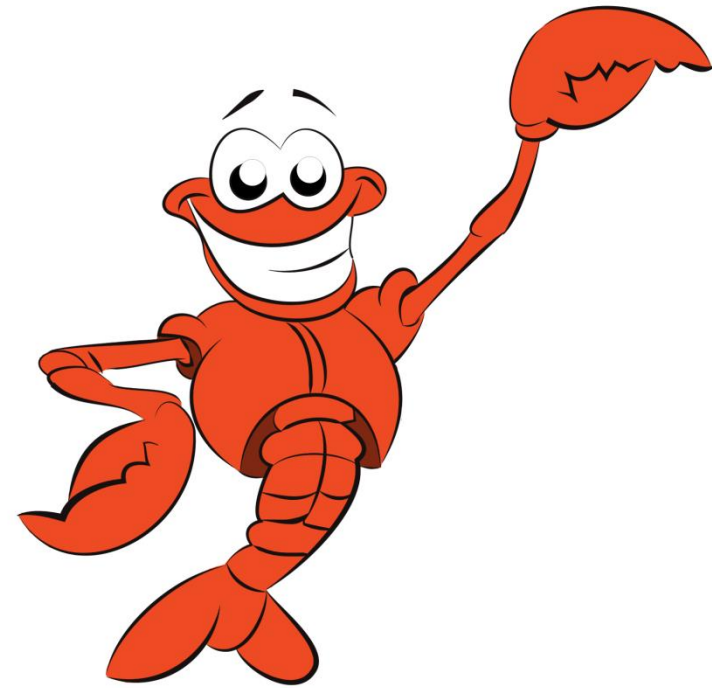

Monitored Folder (FTP)

LOBSTER + FTP + mail server (MATLAB + Filezilla server)

Results.zip 3 items

costneubias@gmail.com
to me
Job: HeLaMovie.m succes

HeLaMCF10A_T0049_L_Area.csv
HeLaMCF10A_T0049_L_CMx.csv
HeLaMCF10A_T0049_L_CMy.csv

Results.zip

Attachment Folder (csv, scenes, models) zipped and sent to Dstmail

OUTLOOK

- 3D annotations edition tool (partly done)
- Deep learning all rounder module: images -> masks (in works)
- Job builder: graphical wizard
- Whatever else you find useful...

                    --> sebastien.tosi@irbbarcelona.org