

XTFISHInsideNucleus: Automated 3D gene position analysis

```
525 # ""BEGIN LOOP OVER ALL IMAGES""
526 # Open File and get filename
527 if wImaris is not None:
528     logfile('connected to Imaris')
529     ListOfOptions = ["Batch of images", "Just one image"], ["Segmentation & Distance"]
530     ListOfMessages = ["Do you wish to run the script on a batch of images or just on one"]
531     UserParameterList = []
532     for i in range(len(ListOfOptions)):
533         OPTIONS = ListOfOptions[i]
534         Message = ListOfMessages[i]
535         PopUpMessage(OPTIONS, Message)
536         UserParameterList = UserParameterList + [User_selection]
```

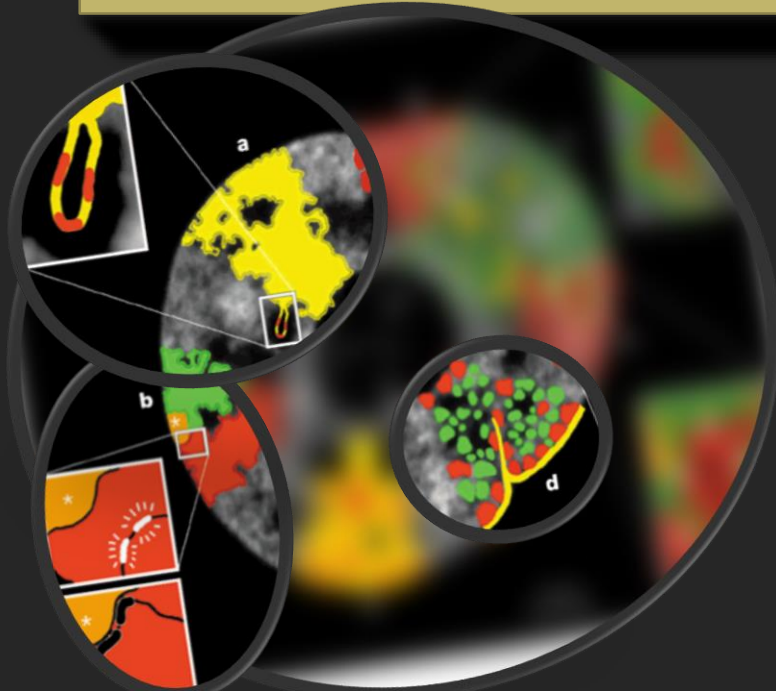
Imaris extension

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The demand

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In *Arabidopsis thaliana*:

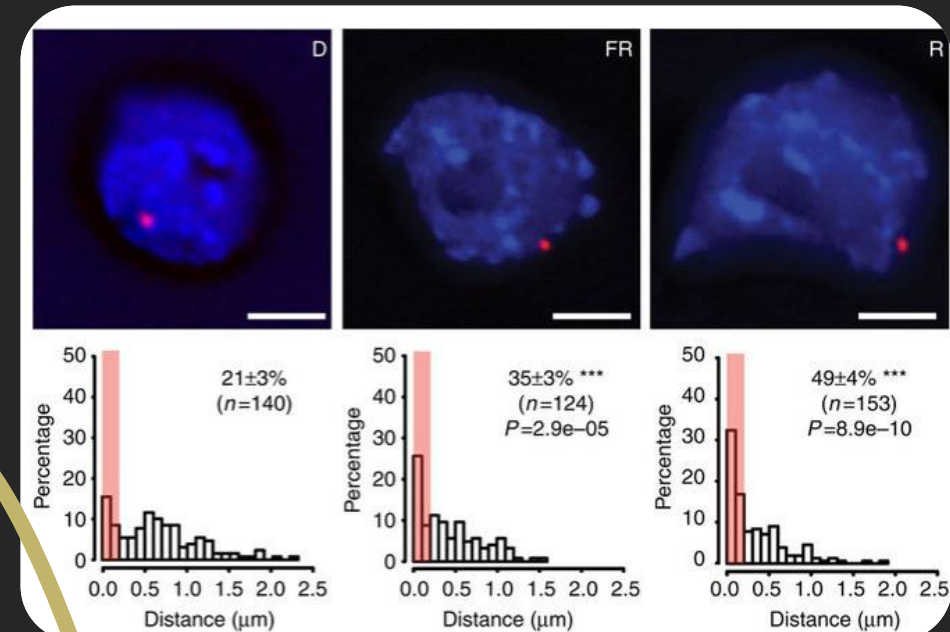
The light-inducible CAB gene (red spot, FISH) is repositioned towards the nuclear periphery upon exposure to far red (FR) or red (R) light in leaf cells.

Feng et al; 2014

In mammalian cells:

1. Several active genes are found on chromatin loops expanding from the chromosome territories (a).
2. The recruitment of active genes towards the centromeric region results in their silencing (b).
3. Gene poor chromatin are mostly positioned at the periphery of the nucleus and towards the nucleolus (d).

Cremer and Cremer, 2001



1. Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Cremer T. and Cremer C. 2001, Nature Reviews Genetics 2, pp. 292-301
2. Light-regulated gene repositioning in *Arabidopsis*. Feng C., Qiu Y., Van Buskirk E. K., Yang E. J., Chen M. 2014, Nature Communication 5, p. 3027

Our goal - solution

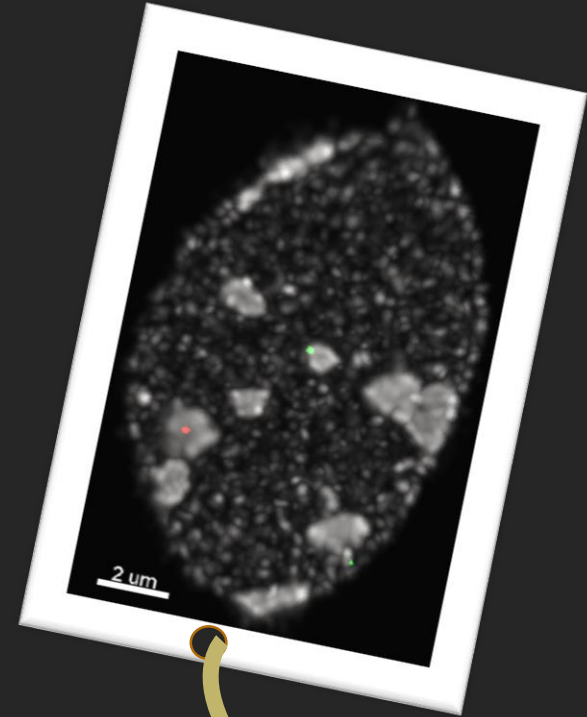
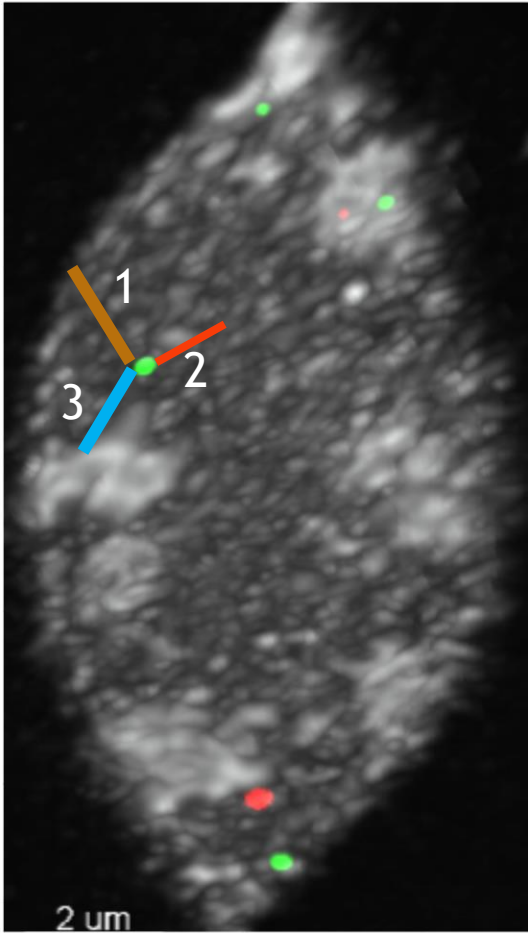
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Biological question:

3D FISH approach is a popular tool used to study the relationship between structure and function in the nucleus. The loci-of-interest are detected by specific DNA probes, recorded in distinct channels and the nucleus is generally counterstained by a general DNA dye (eg. DAPI). In dual-color FISH, a three channel image (red, green for FISH signals; grey or blue for DAPI) is recorded that can be processed for distance measurements.

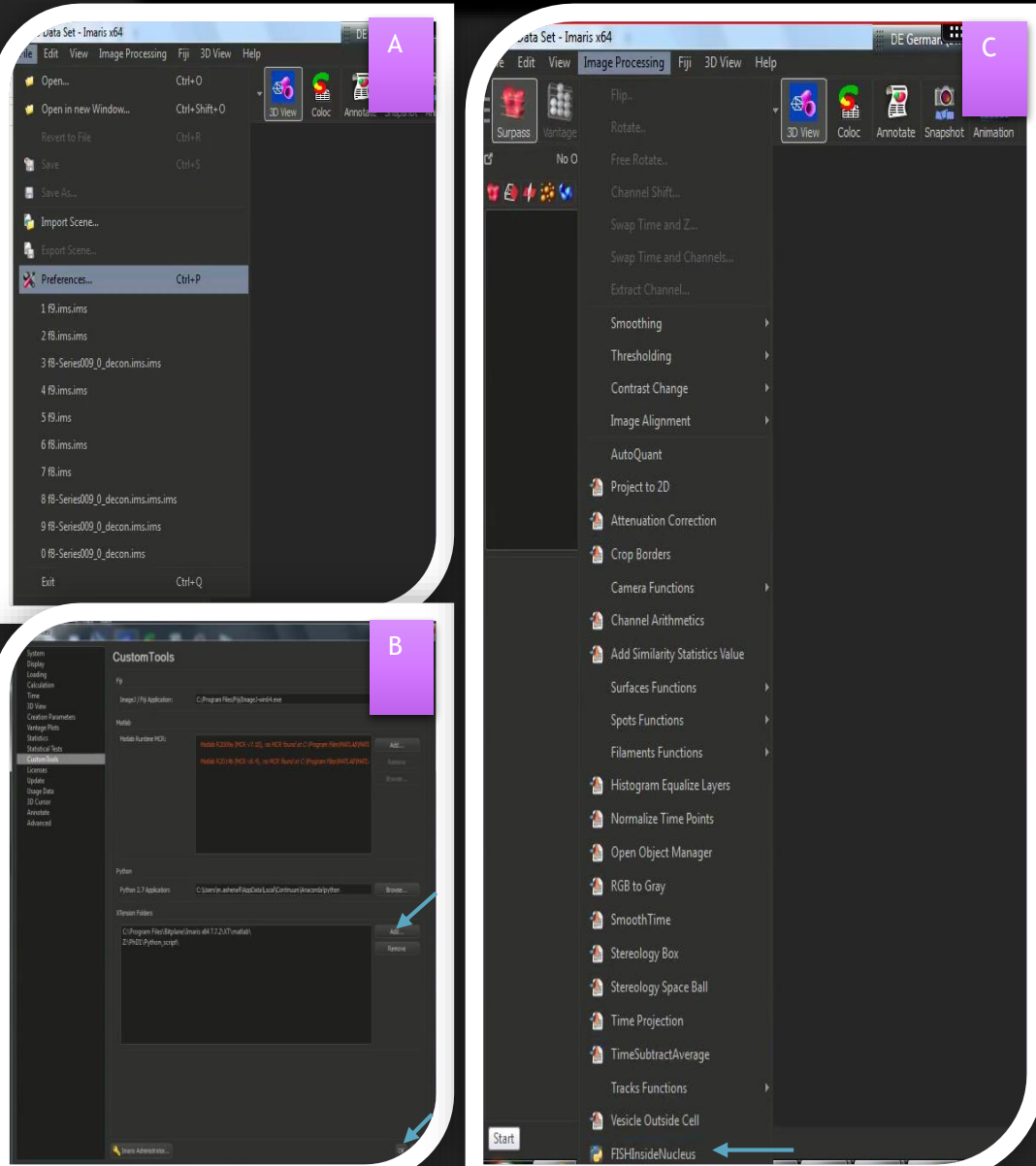
Purpose of this plugin:

- Segment DAPI channel into surfaces: nucleus, chromocenters and nucleolus
- Segment FISH signal into spots
- Calculate distances between FISH spots and 1. the nuclear periphery, 2. the nucleolus, 3. the chromocenter
- Export data
- Save image file



Running the plugin

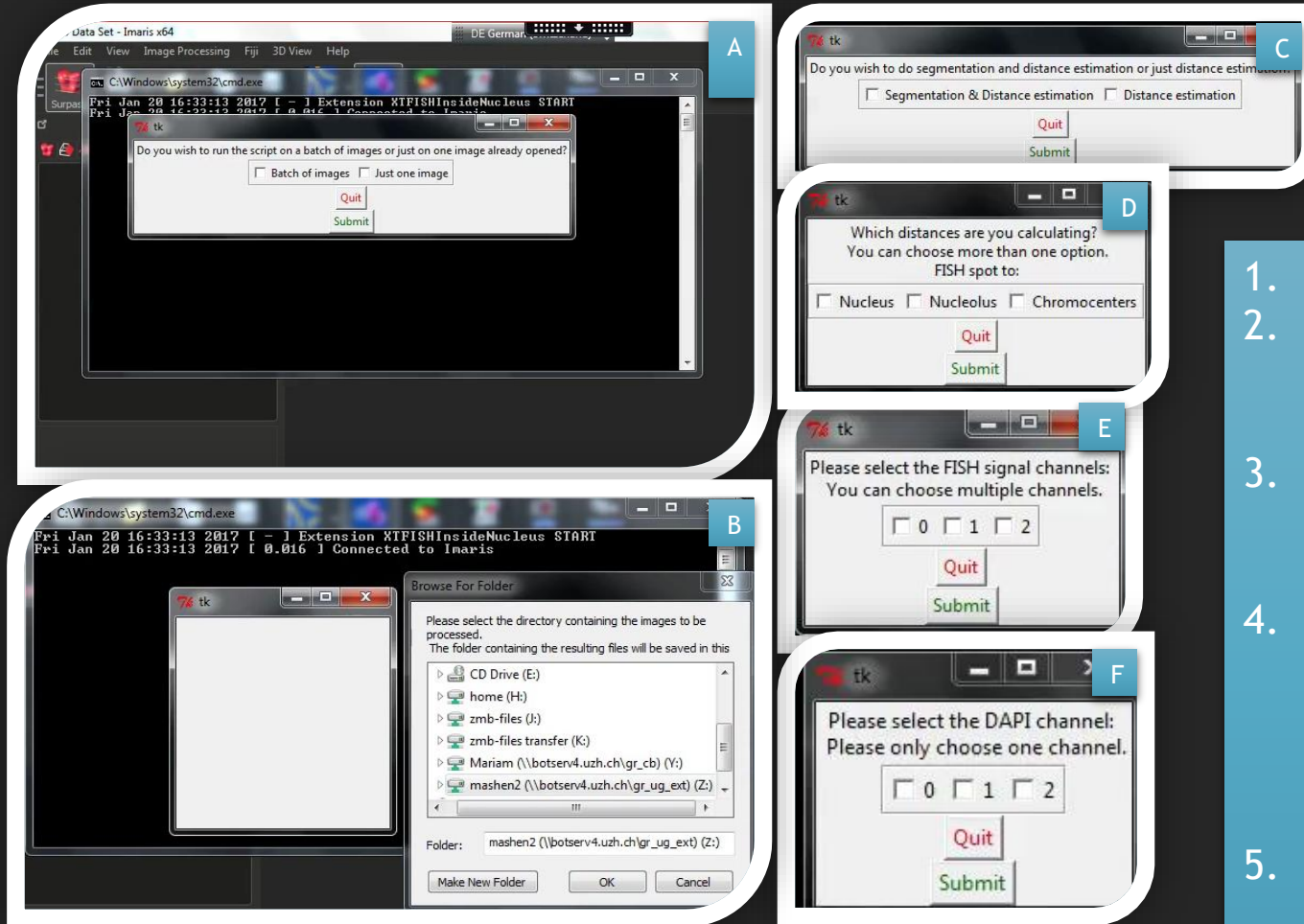
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1. Put all the .ims files in a single folder *folder1*
2. Download these two files from <http://open.bitplane.com/> : “XTFISHInsideNucleus.py” and “XTFISHInsideNucleus_Parameters.csv”. Both files are required to run the plugin
3. Put both files in a folder *folder2*
4. Open Imaris, and under File/Preferences/Calculation/ set memory limit to 4GB
5. Go to File tab and click on “Preferences” in the drop down menu . (A)
6. One the next window click on “Custom Tools” (B)
7. Locate python.exe file and select ‘Browse’ in the “Python application” field to browse for that file (B)
8. On the “XTension Folders” field, select “Add” and browse for *folder2* (B)
9. Select “Ok” on the bottom of the window (B)
10. Select Image Processing/FISHInsideNucleus (C)

Running the plugin

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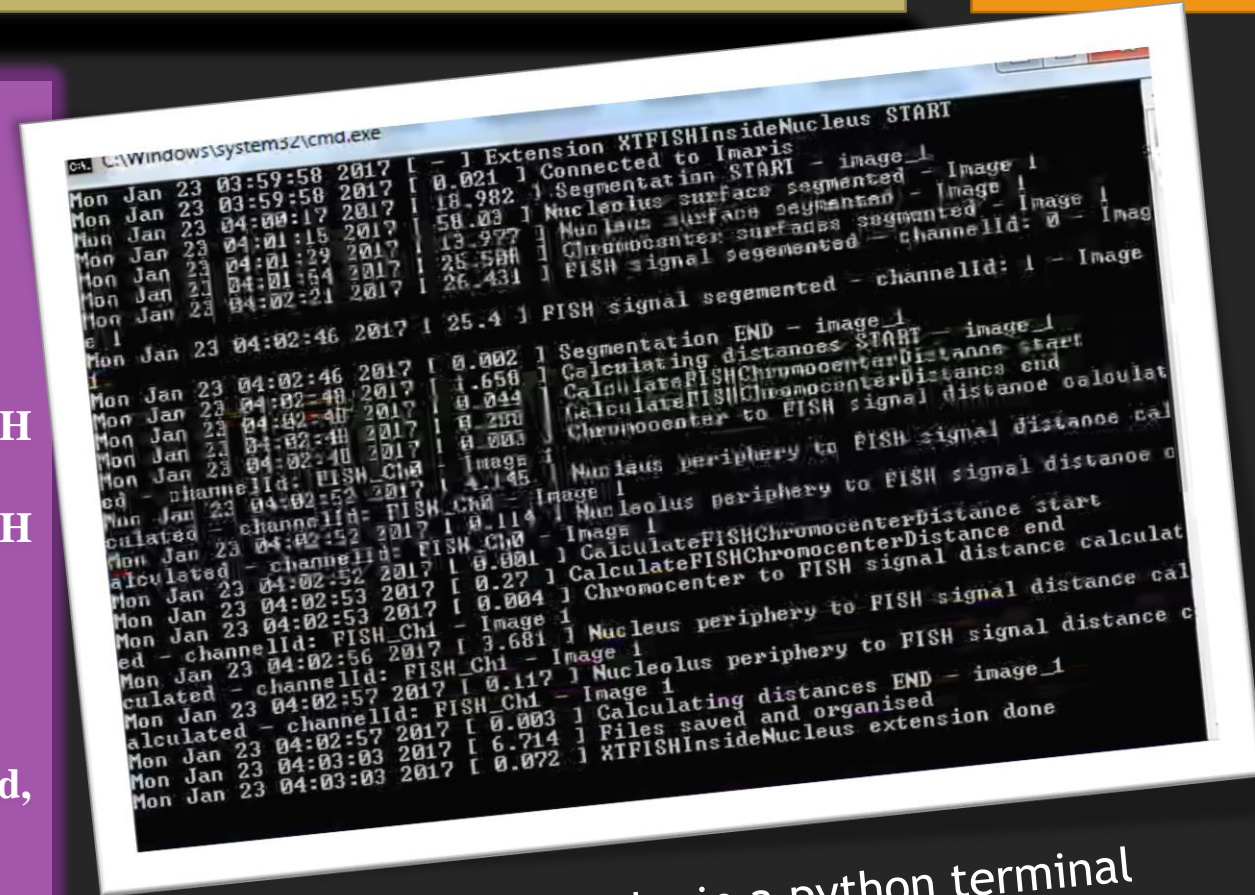
1. Select 'Batch of images' in Window1 (A)
2. Browse for *folder1* in Window2 (B), the plugin will batch process the images contained in that folder.
3. Select 'Segmentation & Distance estimation' in Window3 (C), to run a two-step program: segmentation and distance calculation
4. Select options according to the distances desired (PF, NF or CF) in Window4 (D). For PF choose: 'Nucleus', for NF: 'Nucleolus', for CF: 'Chromocenters'. More than one option can be selected.
5. Select which channels are FISH channels in Window5 (E). More than one option can be selected.
6. Select which channel is the DAPI channel in Window6 (F)

Steps of the plugin

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For each .ims file

1. Open a file
2. Segment
 - a) the nucleus
 - b) the chromocenters
 - c) the nucleolus
 - d) the FISH signal for each FISH channel
3. Calculate distance for each FISH channel
 - a) FISH to nucleus periphery
 - b) FISH to nucleolus
 - c) FISH to chromocenters
4. For each type of distance calculated, export in .csv file
5. Save file

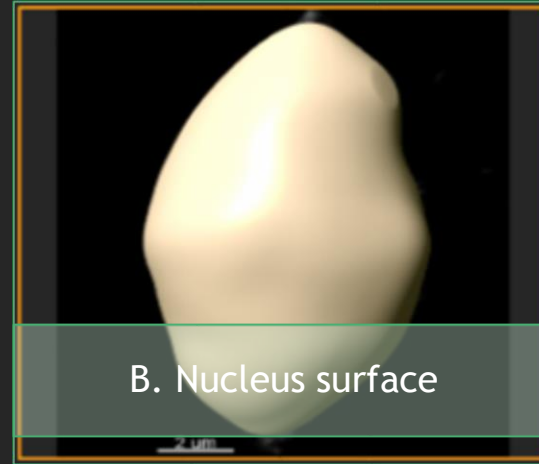
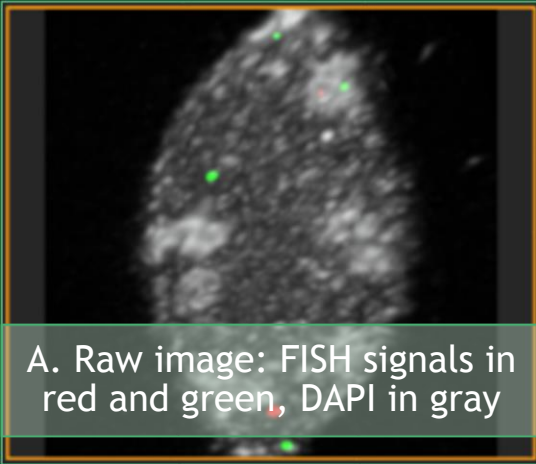


```
Mon Jan 23 03:59:58 2017 [ - ] Extension XTFISHInsideNucleus START
Mon Jan 23 03:59:58 2017 [ 0.021 ] Connected to Imapis
Mon Jan 23 04:00:17 2017 [ 18.982 ] Segmentation START - image_1
Mon Jan 23 04:01:15 2017 [ 58.03 ] Nucleolus surface segmented - Image 1
Mon Jan 23 04:01:29 2017 [ 13.977 ] Nucleolus surface segmented - Image 1
Mon Jan 23 04:01:54 2017 [ 25.584 ] Chromocenter surfaces segmented - channelId: 0 - Image 1
Mon Jan 23 04:02:21 2017 [ 26.431 ] FISH signal segmented - channelId: 1 - Image 1
Mon Jan 23 04:02:46 2017 [ 25.4 ] FISH signal segmented - channelId: 1 - Image 1
Mon Jan 23 04:02:46 2017 [ 0.002 ] Segmentation END - image_1
Mon Jan 23 04:02:46 2017 [ 1.658 ] Calculating distances START - image_1
Mon Jan 23 04:02:46 2017 [ 0.044 ] CalculateFISHChromocenterDistance start
Mon Jan 23 04:02:46 2017 [ 0.250 ] CalculateFISHChromocenterDistance end
Mon Jan 23 04:02:46 2017 [ 0.003 ] Chromocenter to FISH signal distance calculated
Mon Jan 23 04:02:46 2017 [ 4.145 ] Nucleus periphery to FISH signal distance calculated - channelId: FISH_Ch0 - Image 1
Mon Jan 23 04:02:52 2017 [ 0.114 ] Nucleolus periphery to FISH signal distance calculated - channelId: FISH_Ch0 - Image 1
Mon Jan 23 04:02:52 2017 [ 0.001 ] CalculateFISHChromocenterDistance start
Mon Jan 23 04:02:52 2017 [ 0.27 ] CalculateFISHChromocenterDistance end
Mon Jan 23 04:02:53 2017 [ 0.004 ] Chromocenter to FISH signal distance calculated - channelId: FISH_Ch1 - Image 1
Mon Jan 23 04:02:53 2017 [ 3.681 ] Nucleus periphery to FISH signal distance calculated - channelId: FISH_Ch1 - Image 1
Mon Jan 23 04:02:56 2017 [ 0.117 ] Nucleolus periphery to FISH signal distance calculated - channelId: FISH_Ch1 - Image 1
Mon Jan 23 04:02:57 2017 [ 0.003 ] Calculating distances END - image_1
Mon Jan 23 04:03:03 2017 [ 6.714 ] Files saved and organised
Mon Jan 23 04:03:03 2017 [ 0.072 ] XTFISHInsideNucleus extension done
```

After starting the plugin a python terminal displays logs of completed tasks throughout the application.

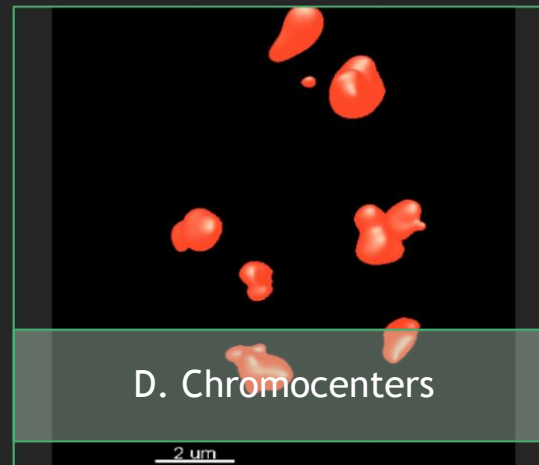
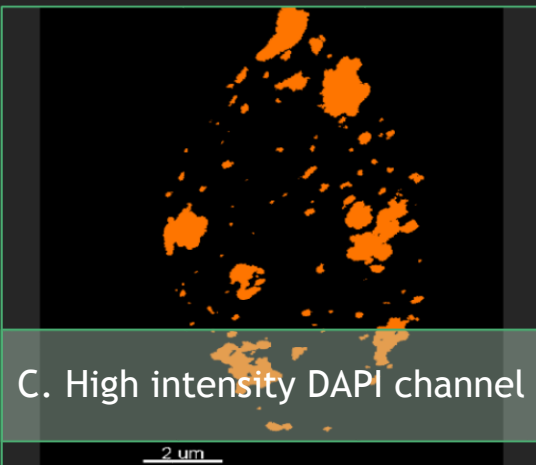
Segmentation - nucleus and chromocenter surface

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Step 2.a) Segment the nucleus:

The only input parameter used here is the Surface grain size used for smoothing prior to segmentation.



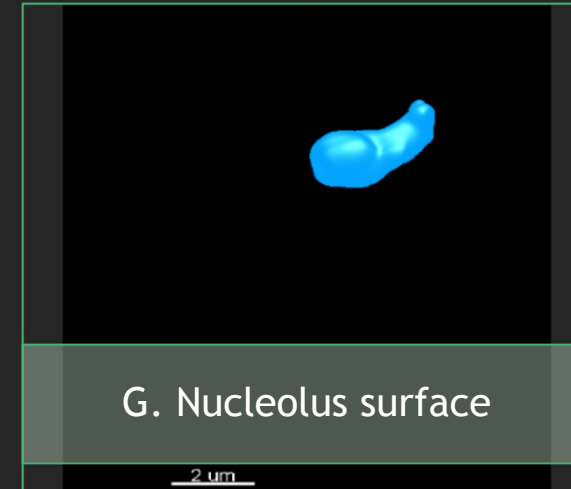
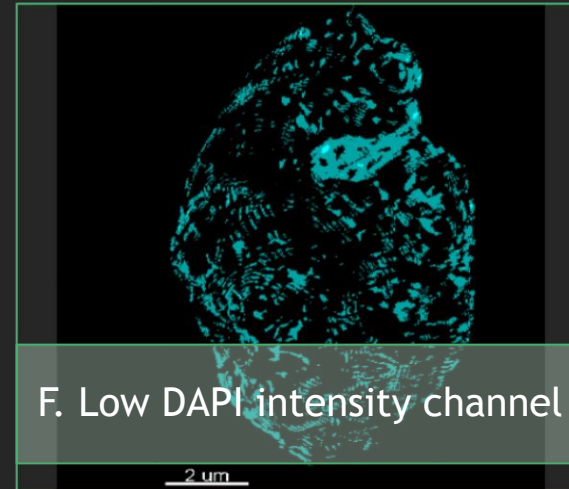
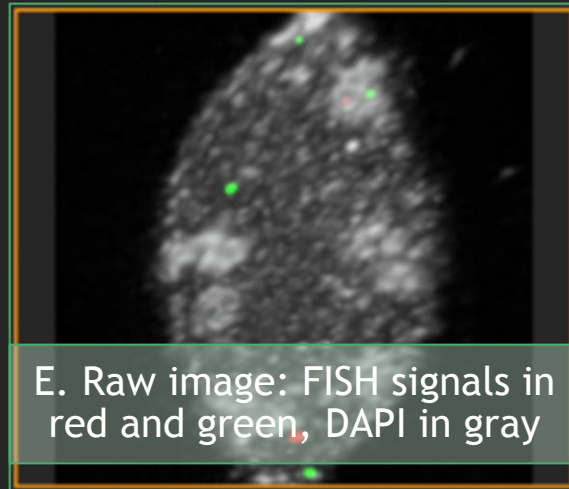
Step 2.b) Segment the chromocenters:

A “High intensity DAPI channel” is created by selecting only voxels with above average DAPI intensity

Parameters used for segmentation are:
Surface grain size and Diameter Of Largest Sphere

Segmentation - nucleolus surface

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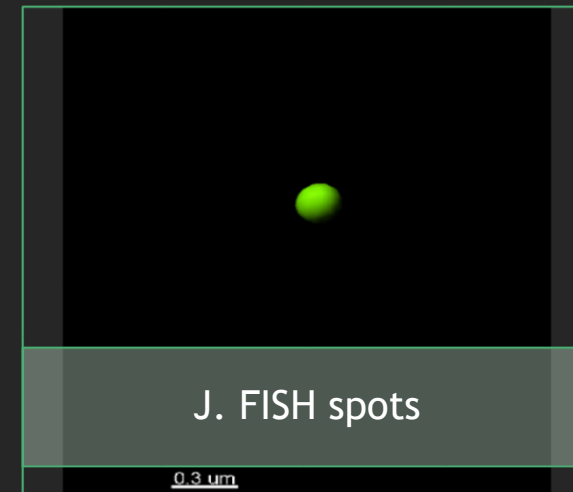


Step 2.c) Segment the nucleolus:

The parameters used here are:
Surface grain sizes used for the detailed and rough nucleus segmentation, and that used the nucleolus segmentation, and the Diameter Of Largest Sphere.

Segmentation - FISH spots

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Step 2.d) Segment the FISH signals:

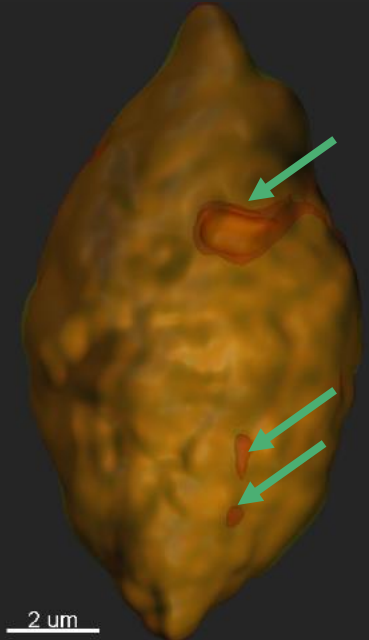
A “High intensity FISH channel” is created for every FISH channel, by selecting only voxels with above average intensities

Parameters used for segmentation are: Estimated XY Diameter and Estimated Z Diameter

Imported parameters used to run the plugin

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Parameters	Default values	Unit
Surface grain size for nucleus segmentation	0.8	μm
Surface grain size for Chromocenters segmentation	0.2	μm
Diameter Of Largest Sphere for Chromocenters segmentation	1.6	μm
Surface grain size for nucleolus segmentation	0.2	μm
Diameter Of Largest Sphere for nucleolus segmentation	1.6	μm
Surface grain size for detailed segmentation of the nucleus	0.2	μm
Surface grain size for rough segmentation of the nucleus	0.3	μm
Estimated XY Diameter for FISH signal segmentation	0.26	μm
Estimated Z Diameter for FISH signal segmentation	0.3	μm

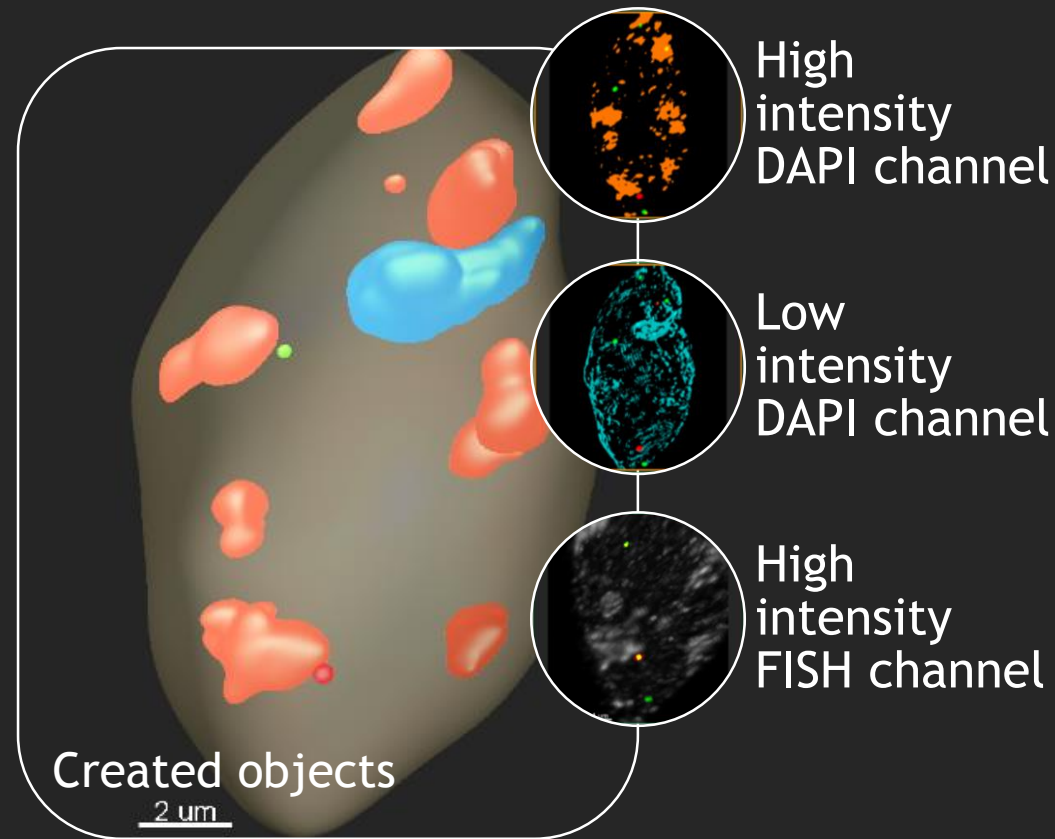


For nucleolus segmentation a “Low DAPI intensity channel” is created, by doing a rough nucleus segmentation and a detailed one to select voxels with below average DAPI intensity, that are exclusively outside the detailed nucleus segmentation and inside the rough nucleus surface. These voxels are situated inside the surfaces indicated by the arrows in the figure on the left.

These parameters are found in the `XTFISHInsideNucleus_Parameters.csv` file

Plugin result - processed image

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Four objects (nucleus - gray, nucleolus - light blue, chromocenters - orange, FISH spots - green & red) and three new channels

Plugin result - Exported data

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Exported tables saved in the
XTFISHInsideNucleus_Result folder.
(.CSV format)

Number of column = number of image analysed
Number of row = number of FISH spots segmented

Name	Date modified	Type	Size
f8.ims	23.01.2017 04:03	Imaris File	125'693 KB
FISHChromocenterDistanceTable_FISH_Ch0	23.01.2017 04:03	Microsoft Excel C...	1 KB
FISHChromocenterDistanceTable_FISH_Ch1	23.01.2017 04:03	Microsoft Excel C...	1 KB
FISHNucleolusDistanceTable_FISH_Ch0	23.01.2017 04:03	Microsoft Excel C...	1 KB
FISHNucleolusDistanceTable_FISH_Ch1	23.01.2017 04:03	Microsoft Excel C...	1 KB
FISHPeripheryDistanceTable_FISH_Ch0	23.01.2017 04:03	Microsoft Excel C...	1 KB
FISHPeripheryDistanceTable_FISH_Ch1	23.01.2017 04:03	Microsoft Excel C...	1 KB
FISHPositionTable_FISH_Ch0	23.01.2017 04:03	Microsoft Excel C...	1 KB
FISHPositionTable_FISH_Ch1	23.01.2017 04:03	Microsoft Excel C...	1 KB

FISH X,Y,Z coordinates:
FISHPositionTable_ChX.csv

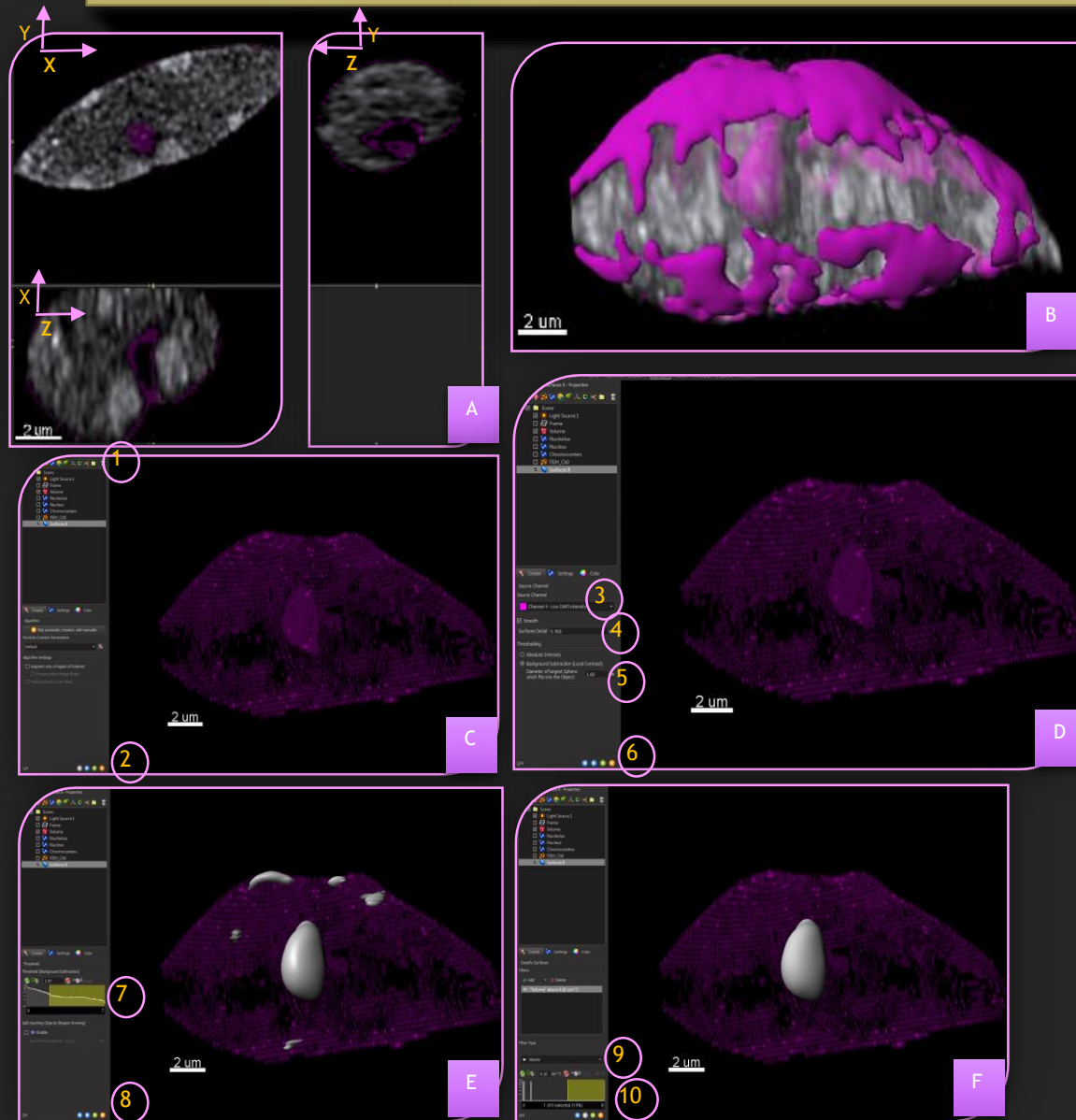
FISH spot distance to nucleus periphery:
FISHPeripheryDistanceTable_ChX.csv

FISH spot distance to nucleolus:
FISHNucleolusDistanceTable_ChX.csv

FISH spot distance to chromocenters:
FISHChromocenterDistanceTable_ChX.csv

Troubleshoot a segmentation error

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In case of. erroneous nucleolus segmentation (B).

1. Create a new surface (C).
2. Set the channel index (D. 3), the smooth surface detail (D 4) and diameter of the largest sphere which fits into object (D 5) Set the lowest intensity threshold (E7).
3. Select Volume and set a threshold of minimum value (F 9)
4. Run the plugin by selection the "Distance estimation" option in the first pop-up window

Option 2: If this segmentation error occurs systematically, user can run the plugin several times for a gradient of values for the input parameters described on page 7

Acknowledgments

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