1 What is an XTension?

XTensions are user-created plug-ins for ImarisXT. They allow you to run functions that Imaris does not have built into it already as well as create your own functionality within Imaris.

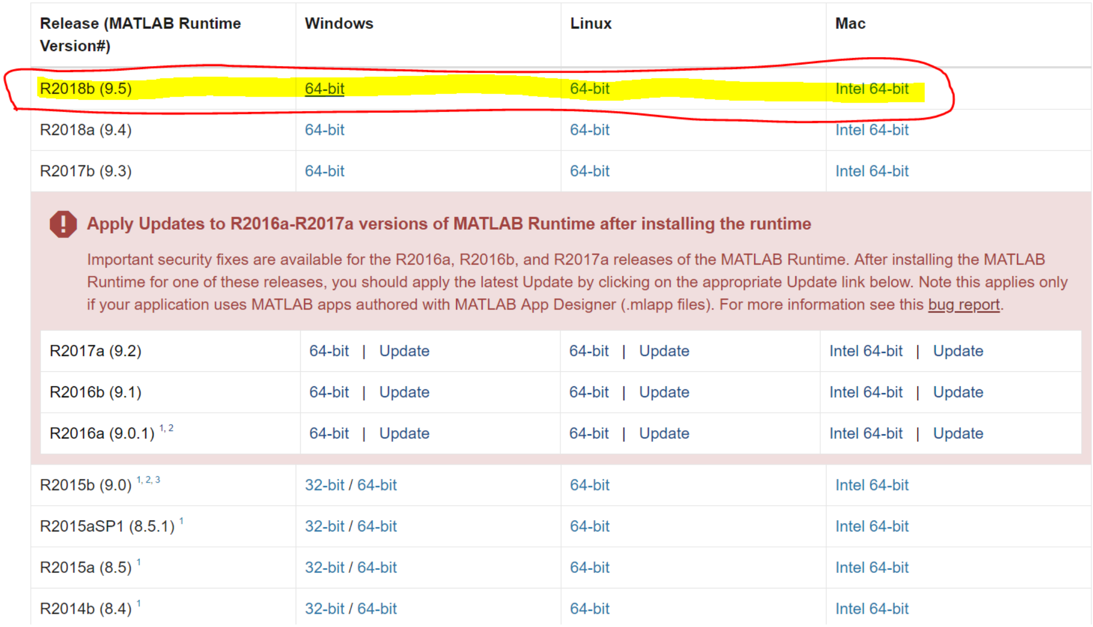
2 Running Imaris XTensions

2.1 Imaris License

In order to run Imaris XTensions, you will need a working Imaris license.

2.2 MATLAB Runtime Compiler

To run XTensions written in MATLAB (which is most of them) you will need a MATLAB Runtime Compiler. No MATLAB license is required to download or use the MATLAB Runtime Compiler. This can be downloaded from mathworks.com/products/compiler/matlab-runtime.html. Imaris XTensions require you to download and install the **R2018b (9.5)** version of the MATLAB Runtime Compiler.



Release (MATLAB Runtime Version#)	Windows	Linux	Mac
R2018b (9.5)	64-bit	64-bit	Intel 64-bit
R2018a (9.4)	64-bit	64-bit	Intel 64-bit
R2017b (9.3)	64-bit	64-bit	Intel 64-bit

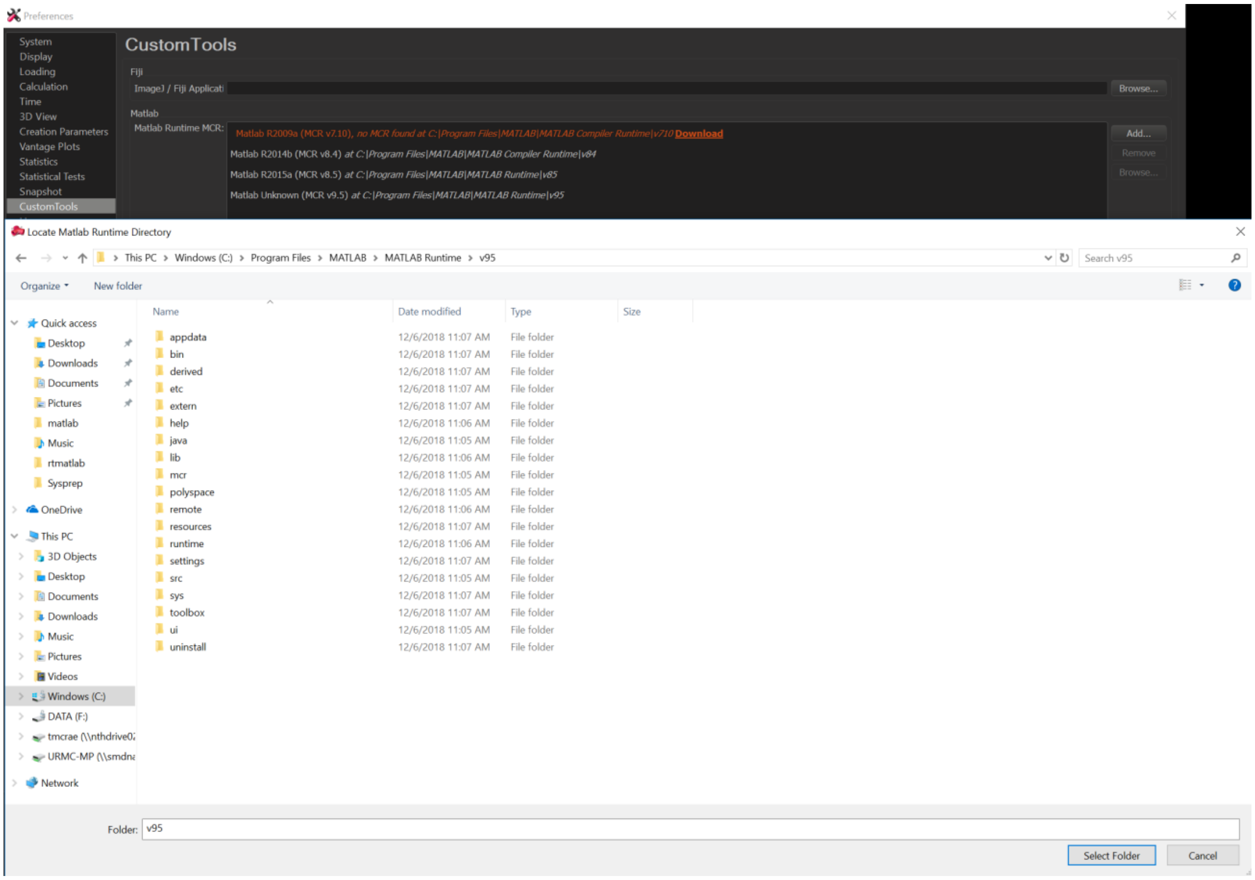
Apply Updates to R2016a-R2017a versions of MATLAB Runtime after installing the runtime

Important security fixes are available for the R2016a, R2016b, and R2017a releases of the MATLAB Runtime. After installing the MATLAB Runtime for one of these releases, you should apply the latest Update by clicking on the appropriate Update link below. Note this applies only if your application uses MATLAB apps authored with MATLAB App Designer (.mlapp files). For more information see this bug report.

Release (MATLAB Runtime Version#)	Windows	Linux	Mac
R2017a (9.2)	64-bit | Update	64-bit | Update	Intel 64-bit | Update
R2016b (9.1)	64-bit | Update	64-bit | Update	Intel 64-bit | Update
R2016a (9.0.1) [1, 2]	64-bit | Update	64-bit | Update	Intel 64-bit | Update
R2015b (9.0) [1, 2, 3]	32-bit / 64-bit	64-bit	Intel 64-bit
R2015aSP1 (8.5.1) [1]	32-bit / 64-bit	64-bit	Intel 64-bit
R2015a (8.5) [1]	32-bit / 64-bit	64-bit	Intel 64-bit
R2014b (8.4) [1]	32-bit / 64-bit	64-bit	Intel 64-bit

Once you have downloaded the MATLAB Runtime Compiler, you should run the installer with all of the default options.

After you have run the installer, you will have to point Imaris to where the runtime compiler is. To do this, in Imaris, go to *File -> Preferences -> Custom Tools.* Next to where it says “Matlab Runtime MCR” click “Add” to point to the path where you saved the MCR. When prompted, point it to *Program Files/MATLAB/MATLAB Runtime/v95* and click “Select Folder”.



CustomTools

Fiji

ImageJ / Fiji Applicati

Matlab

Matlab Runtime MCR:

Matlab R2009a (MCR v7.10), *no MCR found at C:|Program Files|MATLAB|MATLAB Compiler Runtime|v710* **Download**

Matlab R2014b (MCR v8.4) *at C:|Program Files|MATLAB|MATLAB Compiler Runtime|v84*

Matlab R2015a (MCR v8.5) *at C:|Program Files|MATLAB|MATLAB Runtime|v85*

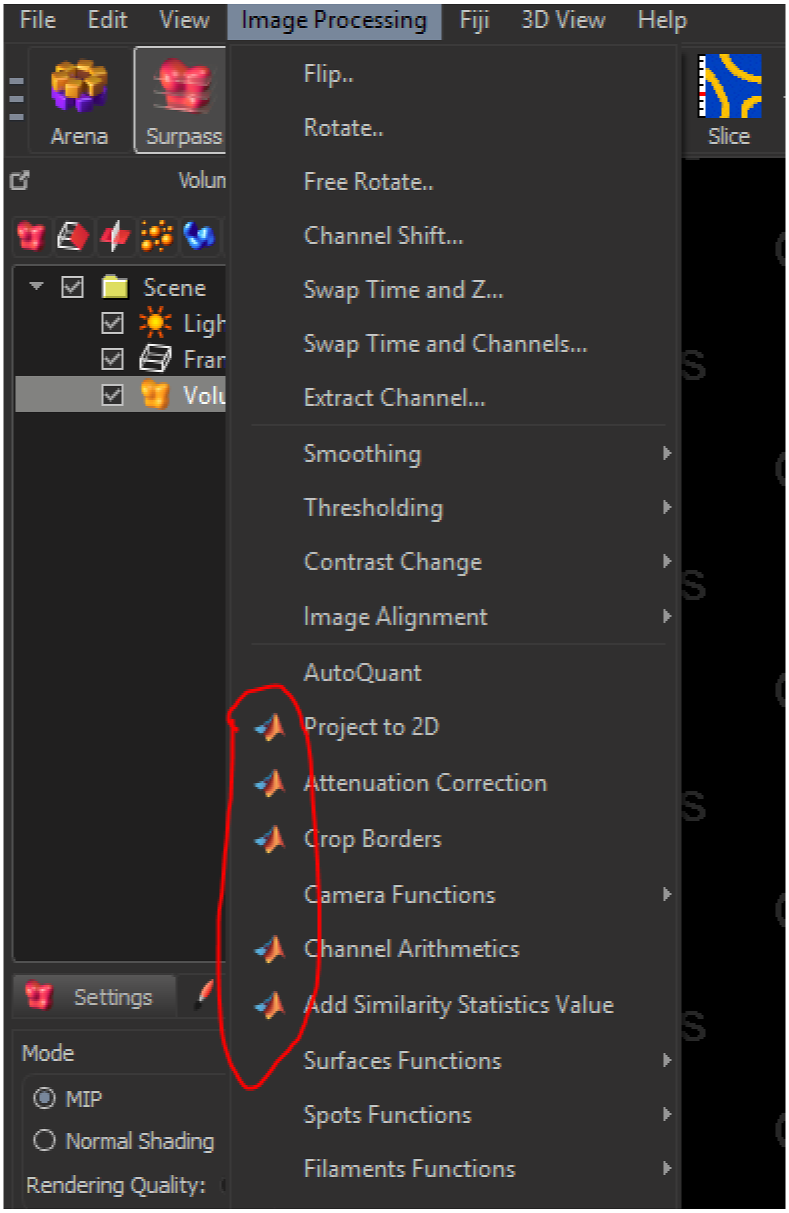
Matlab Unknown (MCR v9.5) *at C:|Program Files|MATLAB|MATLAB Runtime|v95*

Name	Date modified	Type	Size
appdata	12/6/2018 11:07 AM	File folder	
bin	12/6/2018 11:07 AM	File folder	
derived	12/6/2018 11:07 AM	File folder	
etc	12/6/2018 11:07 AM	File folder	
extern	12/6/2018 11:07 AM	File folder	
help	12/6/2018 11:06 AM	File folder	
java	12/6/2018 11:05 AM	File folder	
lib	12/6/2018 11:06 AM	File folder	
mcr	12/6/2018 11:05 AM	File folder	
polyspace	12/6/2018 11:05 AM	File folder	
remote	12/6/2018 11:06 AM	File folder	
resources	12/6/2018 11:07 AM	File folder	
runtime	12/6/2018 11:06 AM	File folder	
settings	12/6/2018 11:07 AM	File folder	
src	12/6/2018 11:05 AM	File folder	
sys	12/6/2018 11:07 AM	File folder	
toolbox	12/6/2018 11:07 AM	File folder	
ui	12/6/2018 11:05 AM	File folder	
uninstall	12/6/2018 11:07 AM	File folder	

Folder: v95

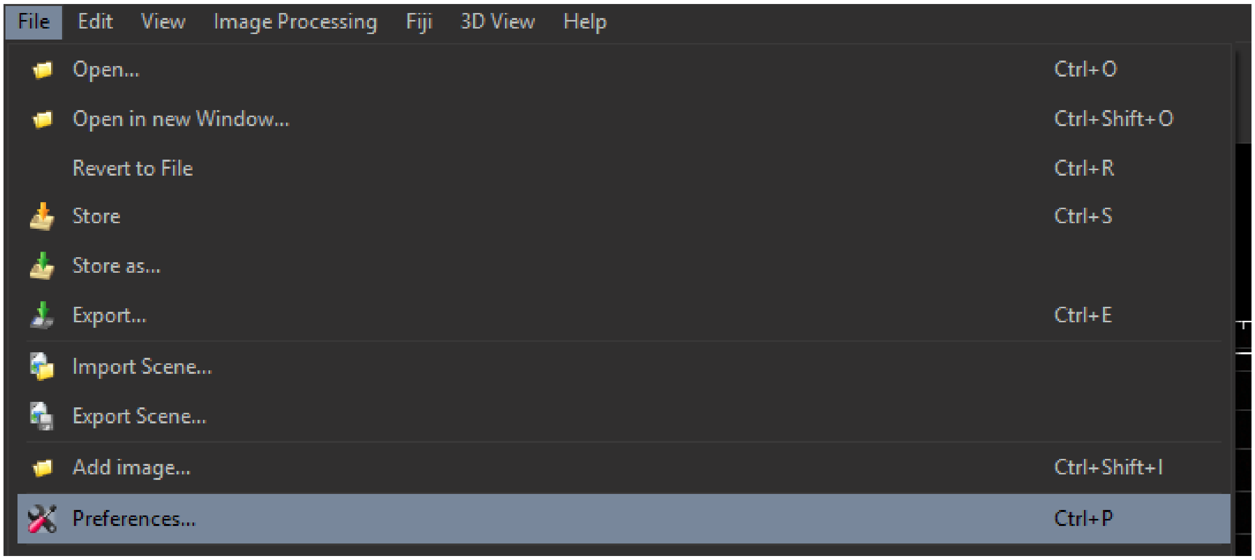
2.3 Using XTensions

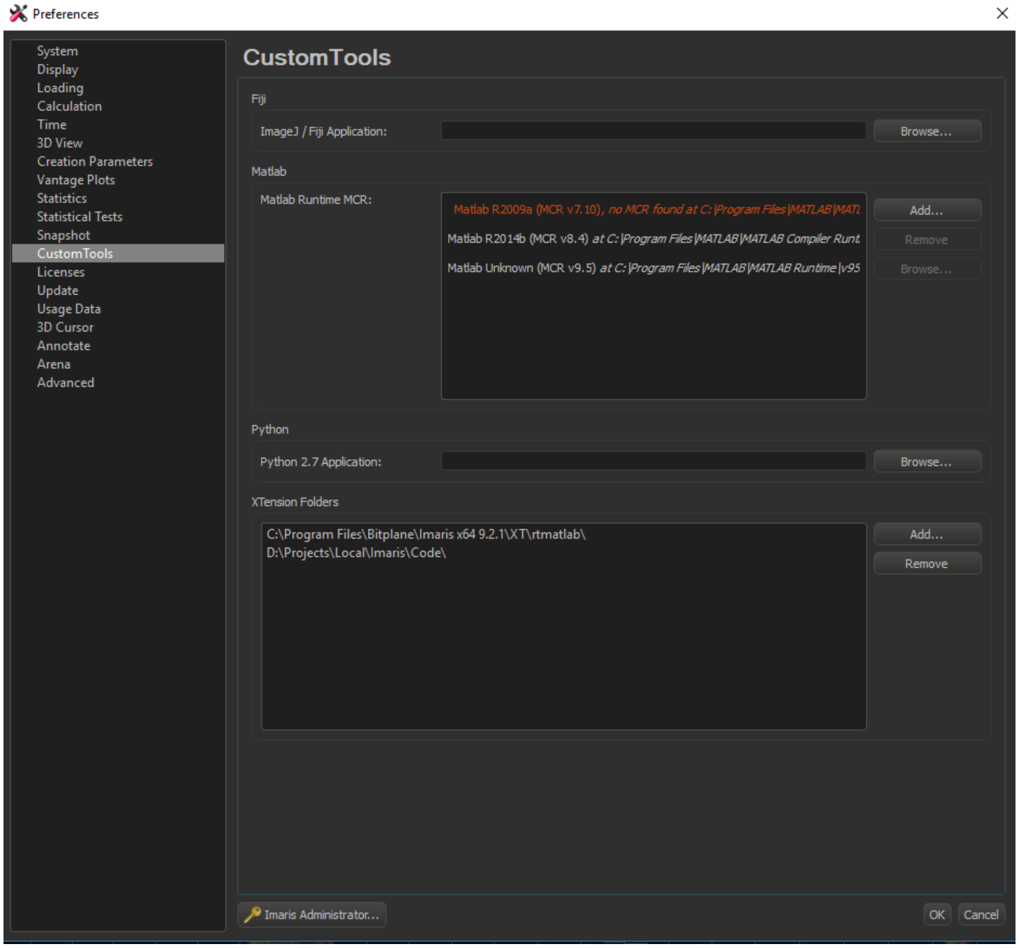
To view available XTensions in Imaris, go into Surpass and click the *Image Processing* dropdown. MATLAB XTensions will be visible with a MATLAB logo next to them. When Imaris is properly connected to MATLAB, this logo will be in color. Otherwise it will be grayed out. Imaris will come with certain XTensions already installed and available to you.



2.4 Adding Custom XTensions

When you have a MATLAB XTension in the form of a .exe file, you can add it to the XTensions Imaris can see. In order to do this, you will have to move the .m file into a folder where Imaris is looking for XTensions. To see where Imaris looks for XTensions, go to *File -> Preferences -> Custom Tools*.





CustomTools

Fiji

ImageJ / Fiji Application:

Matlab

Matlab Runtime MCR:

Matlab R2009a (MCR v7.10), *no MCR found at C:\Program Files\MATLAB\MATL*

Matlab R2014b (MCR v8.4) *at C:\Program Files\MATLAB\MATLAB Compiler Runt*

Matlab Unknown (MCR v9.5) *at C:\Program Files\MATLAB\MATLAB Runtime\v95*

Python

Python 2.7 Application:

XTension Folders

C:\Program Files\Bitplane\Imaris x64 9.2.1\XT\rtmatlab\
D:\Projects\Local\Imaris\Code\

Where it says “XTension Folders”, it will show the path to the folders it looks in when looking for XTensions. You can move your .m file into a folder it is already looking at. Modifying the folder it’s looking at can sometimes require administrative privileges. If this is the case and you do not have administrative privileges, you can add the folder your .m file is already in to where Imaris looks by using the “Add” button.

Once you have this path pointing to the folder with the Imaris XTension(s) you want to use, restart Imaris and the custom XTension(s) will appear in the dropdown menu with the other XTensions.

3 Spectral Unmixing XTension

3.1 XTension code

The Spectral Unmixing XTension written and maintained by the Multiphoton Research Core is available on Box here: https://rochester.app.box.com/file/363188048106 and on GitHub here: https://github.com/tristan-mcrae-rochester/Multiphoton-Image-Analysis/blob/master/Spectral%20Unmixing/Code/XTSpectralUnmixing.exe

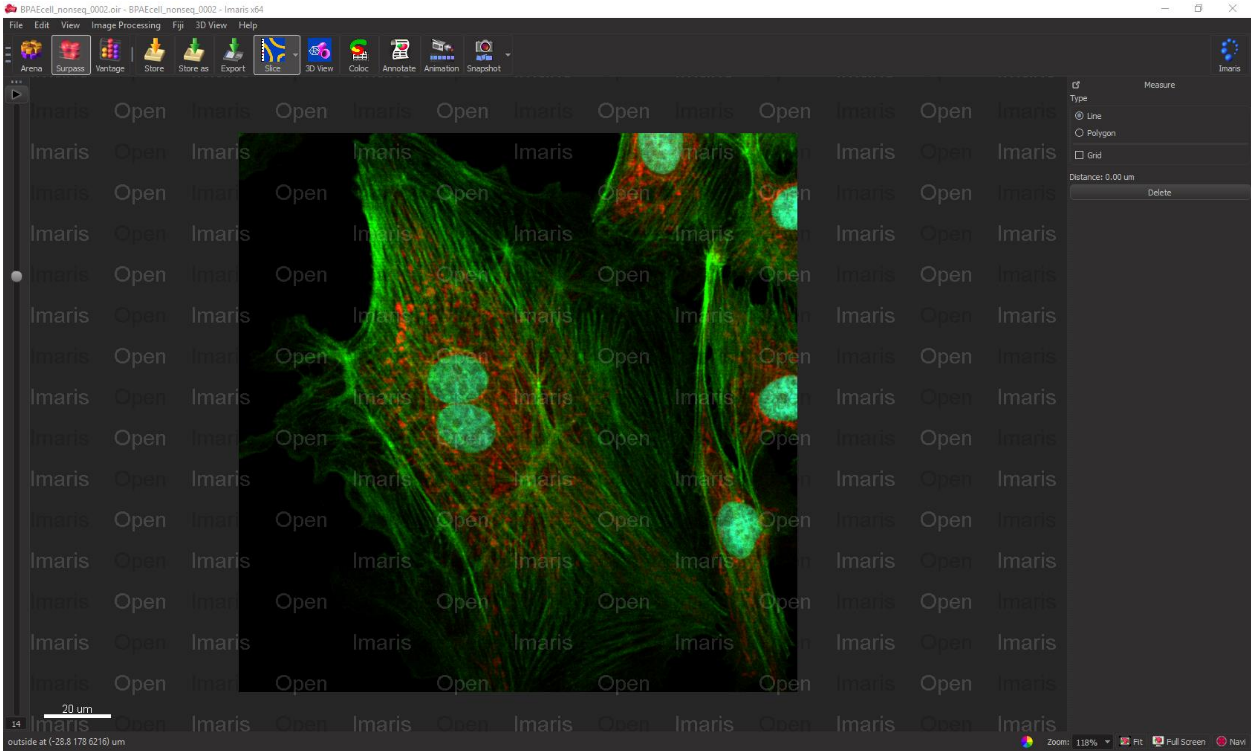
Download the XTSpectralUnmixing.exe file linked above from either source and save it to your computer.

The spectral unmixing application also requires a matching XML file to be saved in the same folder. The XML file for XTSpectralUnmixing is available for download in the box here: https://rochester.app.box.com/file/363271106451 and on github here: https://github.com/tristan-mcrae-rochester/Multiphoton-Image-Analysis/blob/master/Spectral%20Unmixing/Code/XTSpectralUnmixing.XML

Using the instructions from section 2.4, point Imaris to the location where you saved the XTension.

3.2 Running the XTension

Open the image you wish to unmix in Imaris



BPAEcell_nonseq_0002.oir - BPAEcell_nonseq_0002 - Imaris x64

Measure

Type

● Line

○ Polygon

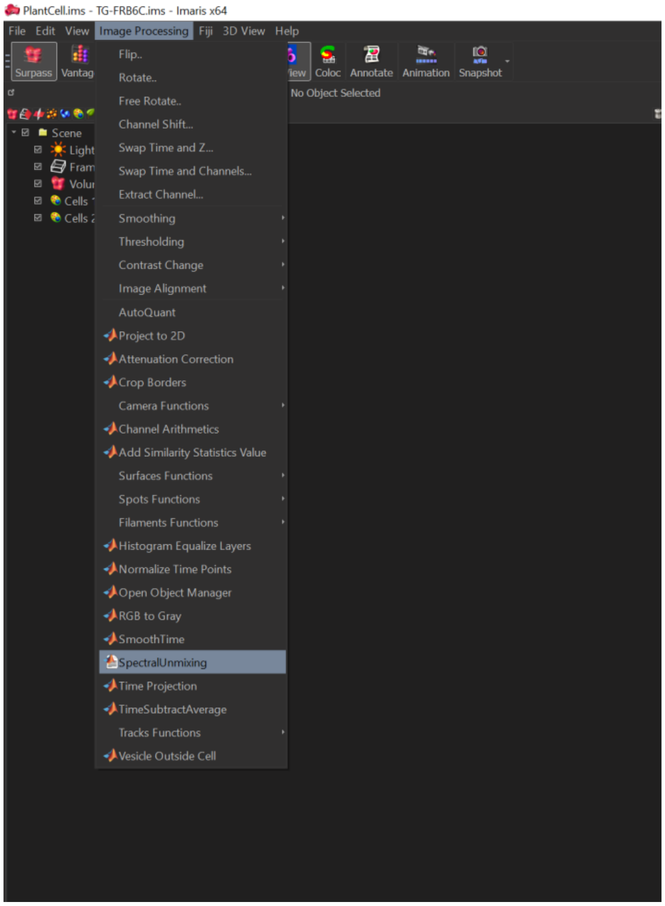
□ Grid

Distance: 0.00 um

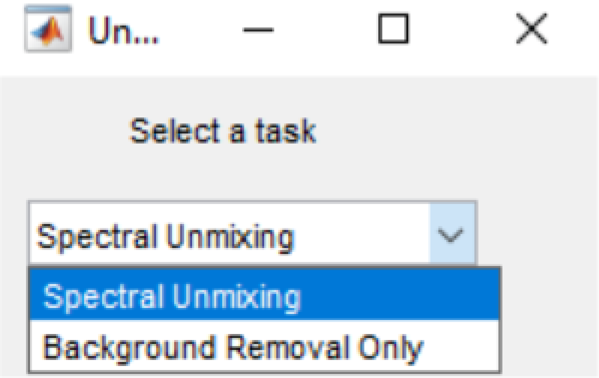
outside at (-28.8 178 6216) um

Zoom: 118%

The Spectral Unmixing XTension can be found under *Image Processing -> Spectral Unmixing*



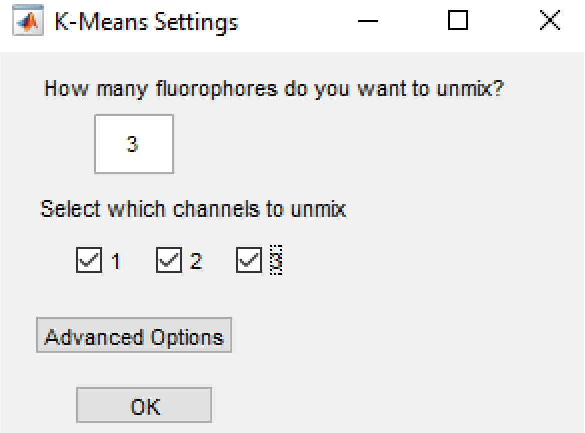
After you select Spectral Unmixing, you will be prompted to choose your task. You have the choice between *Spectral Unmixing* and *Background Removal Only*. *Spectral Unmixing* will separate each fluorophore into a single channel while at the same time separating the background of the image into its own channel. *Background Removal Only* will remove the background from an image while leaving the rest of the image untouched.



3.3 Spectral Unmixing

The *Spectral Unmixing* option uses *k*-means to cluster each pixel into a channel corresponding to either a fluorophore or the background.

After selecting *Spectral Unmixing*, you will be asked about your image:



K-Means Settings

How many fluorophores do you want to unmix?

3

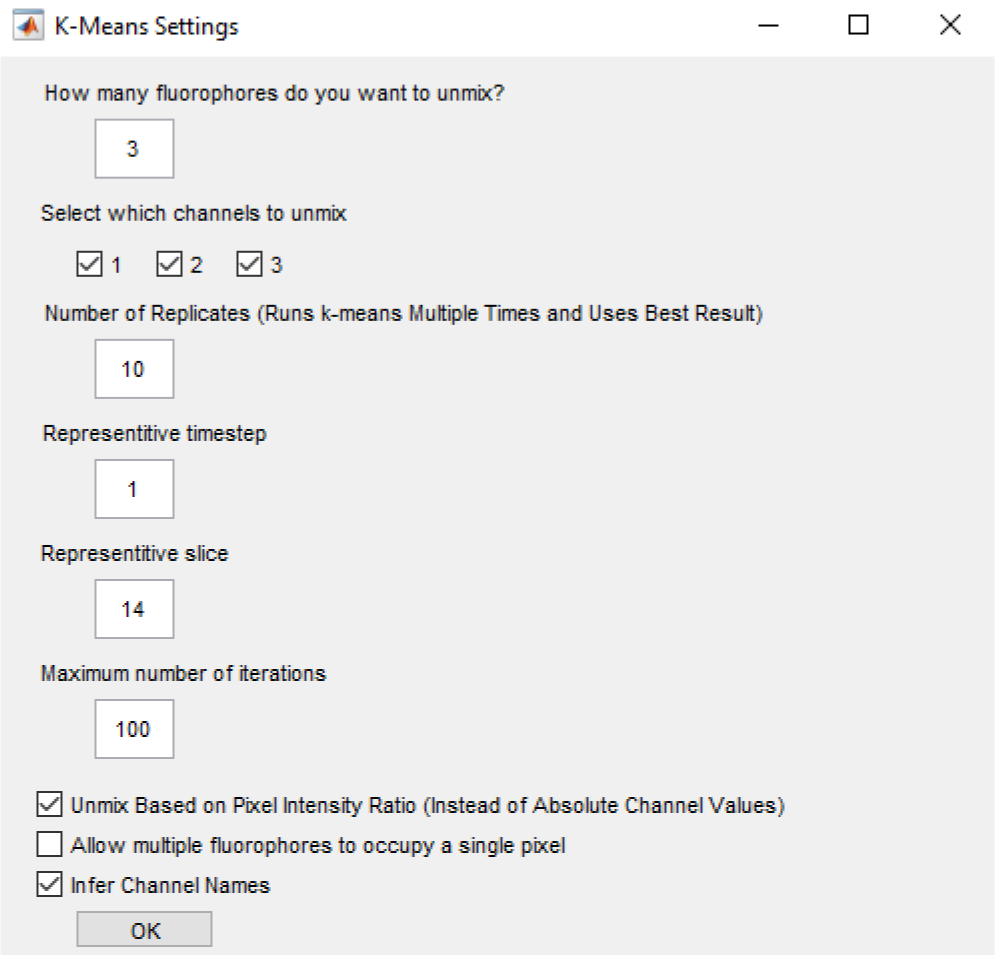
Select which channels to unmix

☑ 1 ☑ 2 ☑ 3

How many fluorophores you want to unmix – The number of channels the algorithm will create for your fluorophores. An additional channel is added behind the scenes. This is the "*k*" in *k*-means. A good starting point is to select every channel for unmixing and ask it to unmix however many fluorophores were present during imaging. If there are some channels that you already know only include one fluorophore, you can reduce the number of fluorophores you want to unmix by one. If, after unmixing, it seems like there are still multiple fluorophores contained in some of the output channels, you can increase the number of fluorophores you ask it to unmix. This will have the effect of adding more clusters to k-means. If successful, this will result in two or more clusters that represent the same fluorophore but no clusters that represent multiple fluorophores.

Select which channels to unmix - Any channel whose box you check will be included in the unmixing and any unchecked channel will simply be preserved in the final image. If there are some channels that you know already only include one fluorophore, you can uncheck their box.

Advanced Options – Selecting this will give you a new pop-up with more choices. If this is not selected, default values will be used for all advanced options.



How many fluorophores do you want to unmix?

3

Select which channels to unmix

☑ 1 ☑ 2 ☑ 3

Number of Replicates (Runs k-means Multiple Times and Uses Best Result)

10

Representitive timestep

1

Representitive slice

14

Maximum number of iterations

100

☑ Unmix Based on Pixel Intensity Ratio (Instead of Absolute Channel Values)

☐ Allow multiple fluorophores to occupy a single pixel

☑ Infer Channel Names

Number of replicates – Runs k-means multiple times and uses the best result. More replicates will improve clustering quality at the expense of taking longer. There tends to be diminishing returns with number of replicates. Default is 10.

Representative timestep– In order to speed up clustering, clusters are defined based one 2D slice of your data and then applied to the rest of your data afterwards. Choose a timestep where you can see all of the different structures and colors. This number indexes from 1. If you only have one timestep, put 1 here. Default is the middle timestep.

Representative slice– Same as representative timestep but for an axial slice. Default is the middle slice.

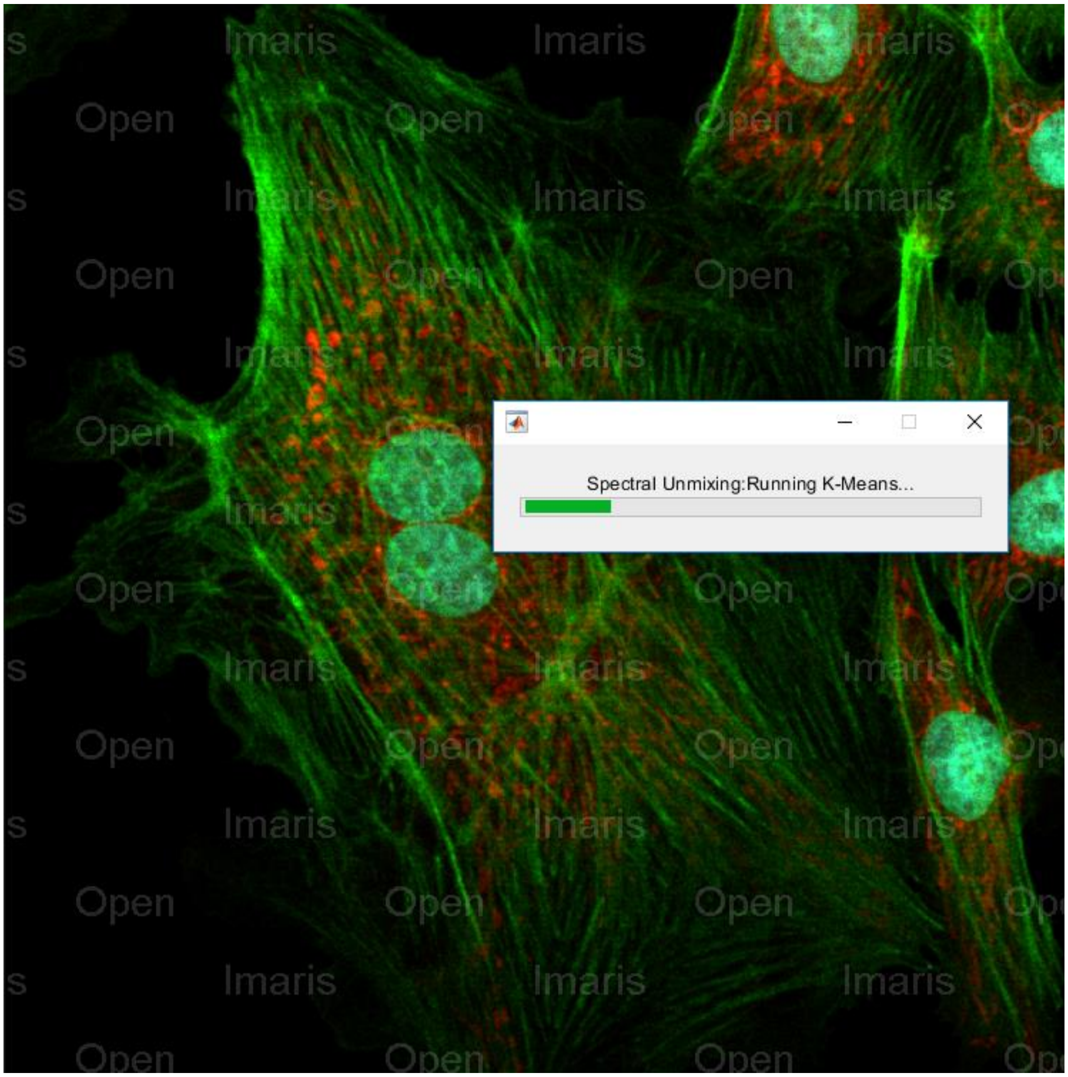
Maximum Number of Iterations – K-means is an iterative algorithm and this option can cap the number of iterations in each replicate of k-means. A lower number cuts off the algorithm earlier which could potentially speed the algorithm up at the expense of quality. Setting this to -1 tells the algorithm to run until it converges. Default is 100.

Unmix Based on Pixel Intensity Ratios – If checked, the algorithm will treat two pixels with the same ratio of channel intensities as being similar to one another. This is useful when overall intensity drops off with depth but the color stays the same. If pixels of different absolute intensity can represent different things in the image, leave this box unchecked. Default is true.

Allow multiple fluorophores per pixel – Select if you want each pixel to be able to belong to multiple output clusters. Implements fuzzy *c*-means instead of k-means. Default is false.

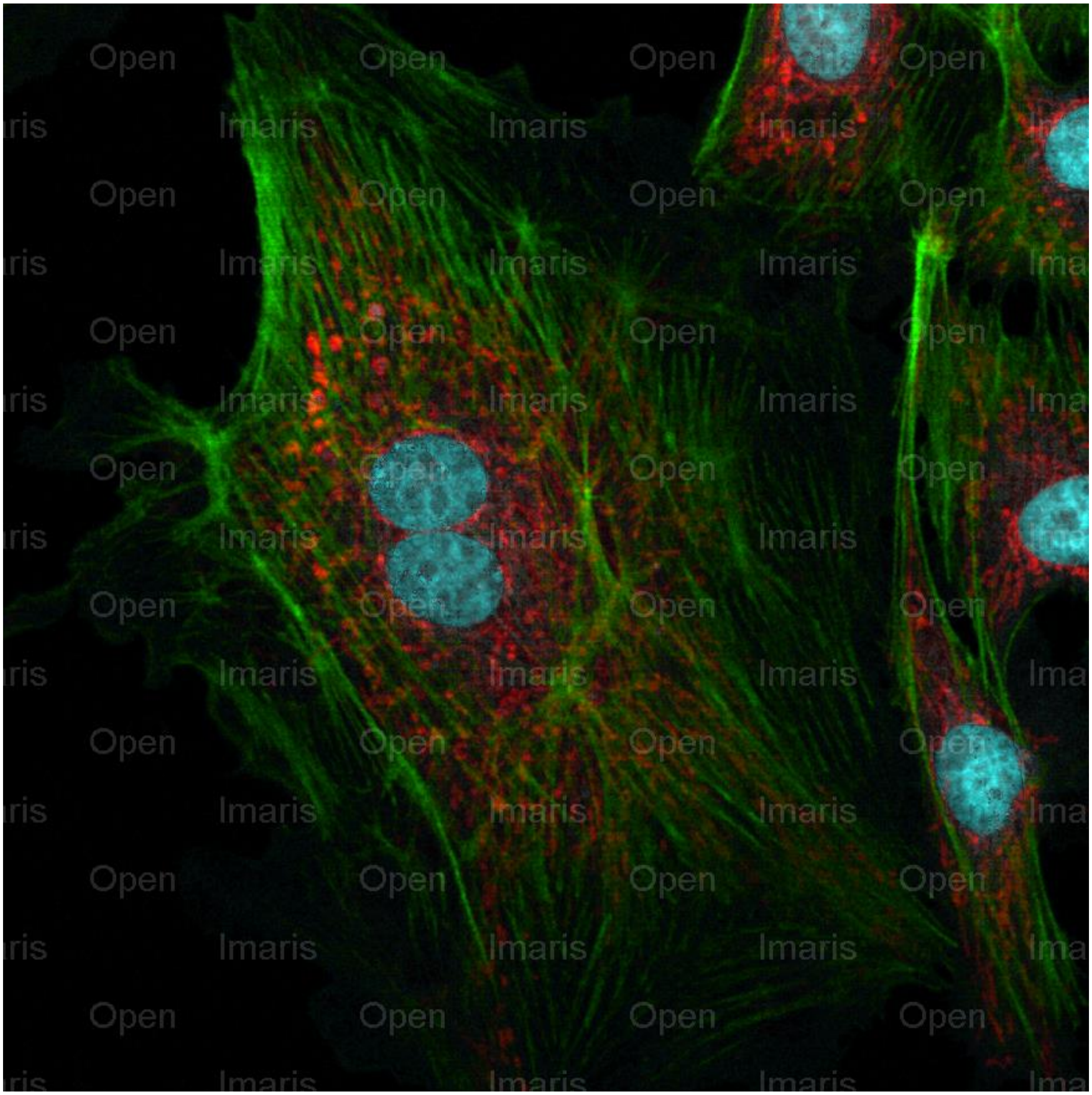
Infer channel names – Select if you want the output clusters to be named and colored according to the original names their channels had in Imaris. This will not necessarily work in cases where fluorophores overlap and double checking the names it gives channels is recommended. Default is true.

The progress of k-means will be displayed in a window which will pop up automatically after a minute or so when you start the XTension.

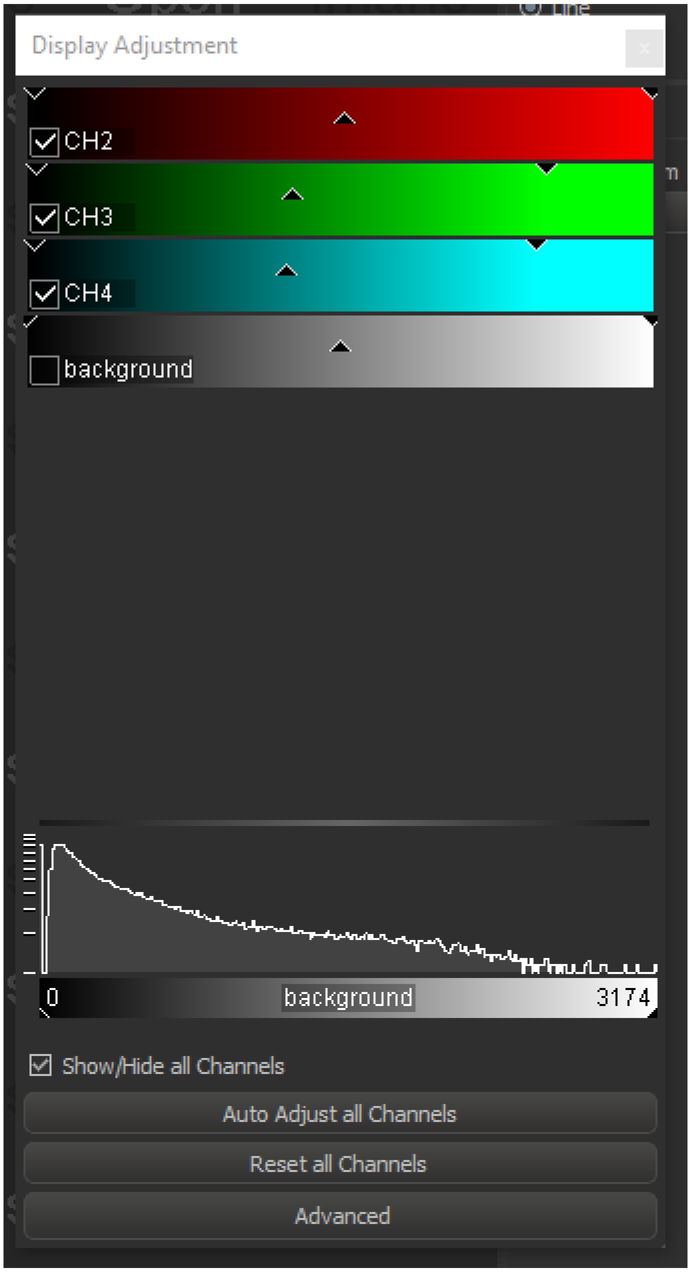


Spectral Unmixing:Running K-Means...

When finished, the XTension will write a new image to your Imaris workstation with the separated channels. (Note how the cell nuclei no longer show up in the green channel in this example.)



You can manually change the appearance of the channels with Imaris's Display Adjustment tool which can be brought up with ctrl+d on Windows or with *Edit -> Show Display Adjustment*. Here you can adjust channel intensities and turn off the background channel by unchecking it's box. It won't change the data, just its display in Imaris.



☑ CH2

☑ CH3

☑ CH4

☐ background

☑ Show/Hide all Channels

3.4 Background Removal Only

The *Background Removal* option uses *k*-means to label each pixel as being background or not. It then removes the background pixels and leaves the rest of the pixels untouched. It does not require any selection of options.

3.5 Co-localization

This spectral unmixing method can also be used for detecting co-localization of fluorophores in an image. If you wish to use the spectral unmixing algorithm to create a separate channel for pixels where co-localization is occurring, add one “fluorophore” for each co-localization combination when asked how many fluorophores you want to unmix. For example, if you have two channels, - CH1 and CH2 - and wanted to separate pixels that are purely CH1, purely CH2 and a mix of CH1 and CH2, you would tell the algorithm to unmix three fluorophores. The co-localization of CH1 and CH2 will have its own spectral signature and be treated as a unique fluorophore. This will only work if there are instances in your image where CH1 and CH2 both exist apart from one another.

4 Other useful XTensions

4.1 An online community where user-created XTensions are created and discussed can be found here: http://open.bitplane.com/ If you are looking for functionality that Imaris doesn’t already have, this is a good place to check to see if there is a solution.

4.2 You can also integrate XTensions from Python or Fiji / ImageJ into Imaris. They are generally less popular and worse documented than MATLAB XTensions but they are a possibility.