

**King's College London : Wohl Cellular Imaging Centre**

# **Batch Deconvolution using NIS-Elements**

How to create a macro to execute deconvolution in a folder and automatically save all the files

## Step 1) Make a Macro that has the deconvolution parameters you need for your files

### Requirements:

All files must have the same number and order of channels

All files must have the same z-step size

You will be running the same command across a set of nearly identical files.

### What can change?

Frame size (xy)

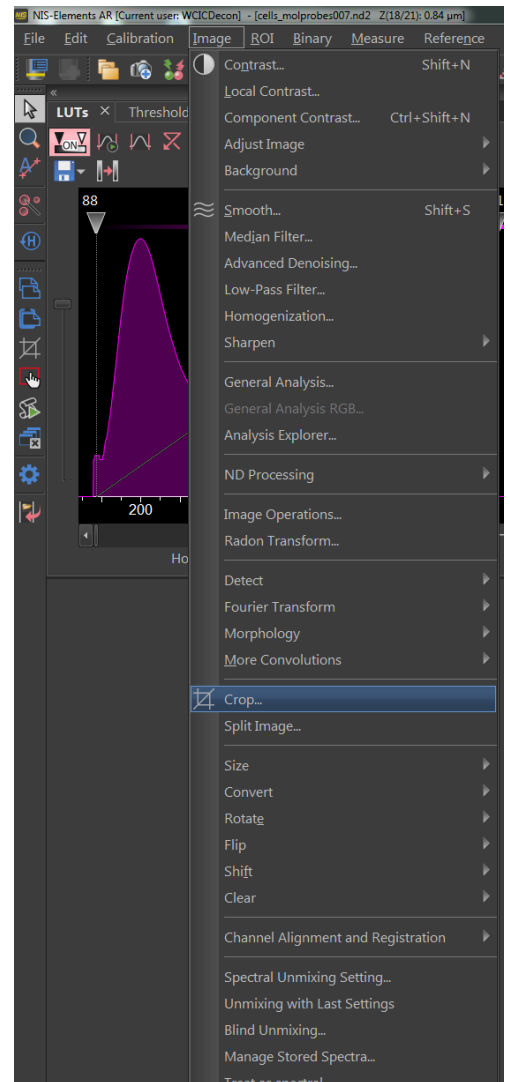
Number of timepoints (t)

Number of z slices (overall stack height)

### 1a)

Open one of your files to be deconvolved as part of the batch

Crop a sub section (this isn't strictly necessary but can save time)



Open the 3D Deconvolution GUI

Select the parameters you want

Ideal method – Blind or Richardsonlucy

Iterations – no more than 20 usually needed

### **Output – choose keep current document**

Normally this isn't recommended – for batch deconvolution it is necessary

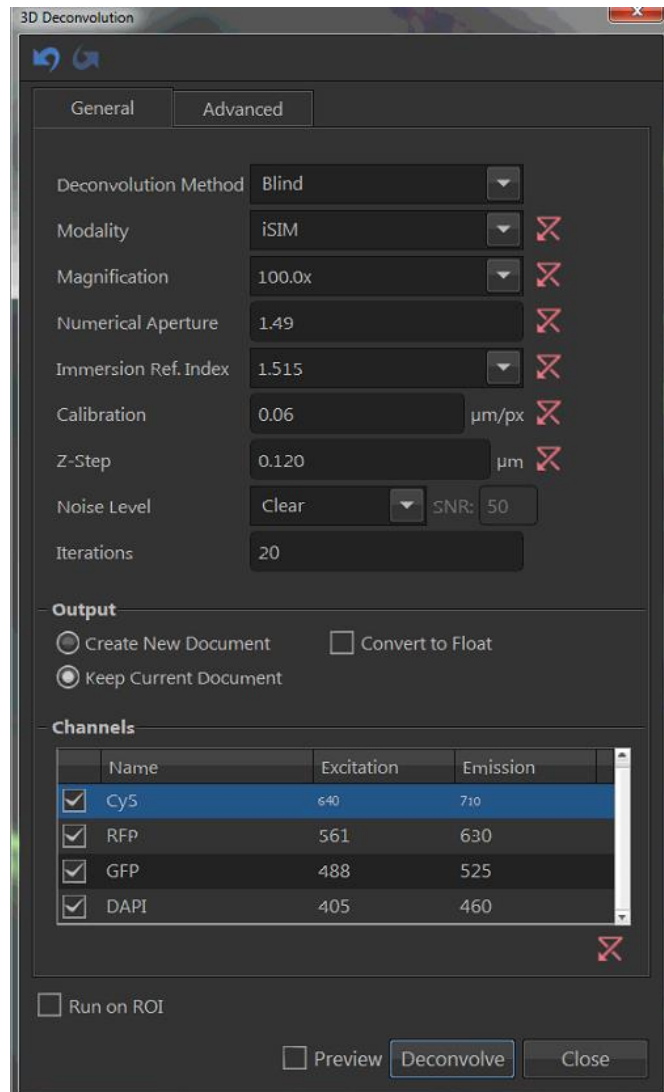
You will probably need to input the values  
For the excitation and emission wavelengths  
If you are deconvolving iSIM data

Cy5 – exc 640 – emm 710

RFP – exc 561 – emm 630

GFP – exc 488 – emm 525

DAPI -exc 405 – emm 460



**DON'T FORGET YOUR ADVANCED PARAMETERS LIKE WHICH REFRACTIVE INDEX TO USE FOR SPHERICAL ABBERATION CORRECTION!!!!**

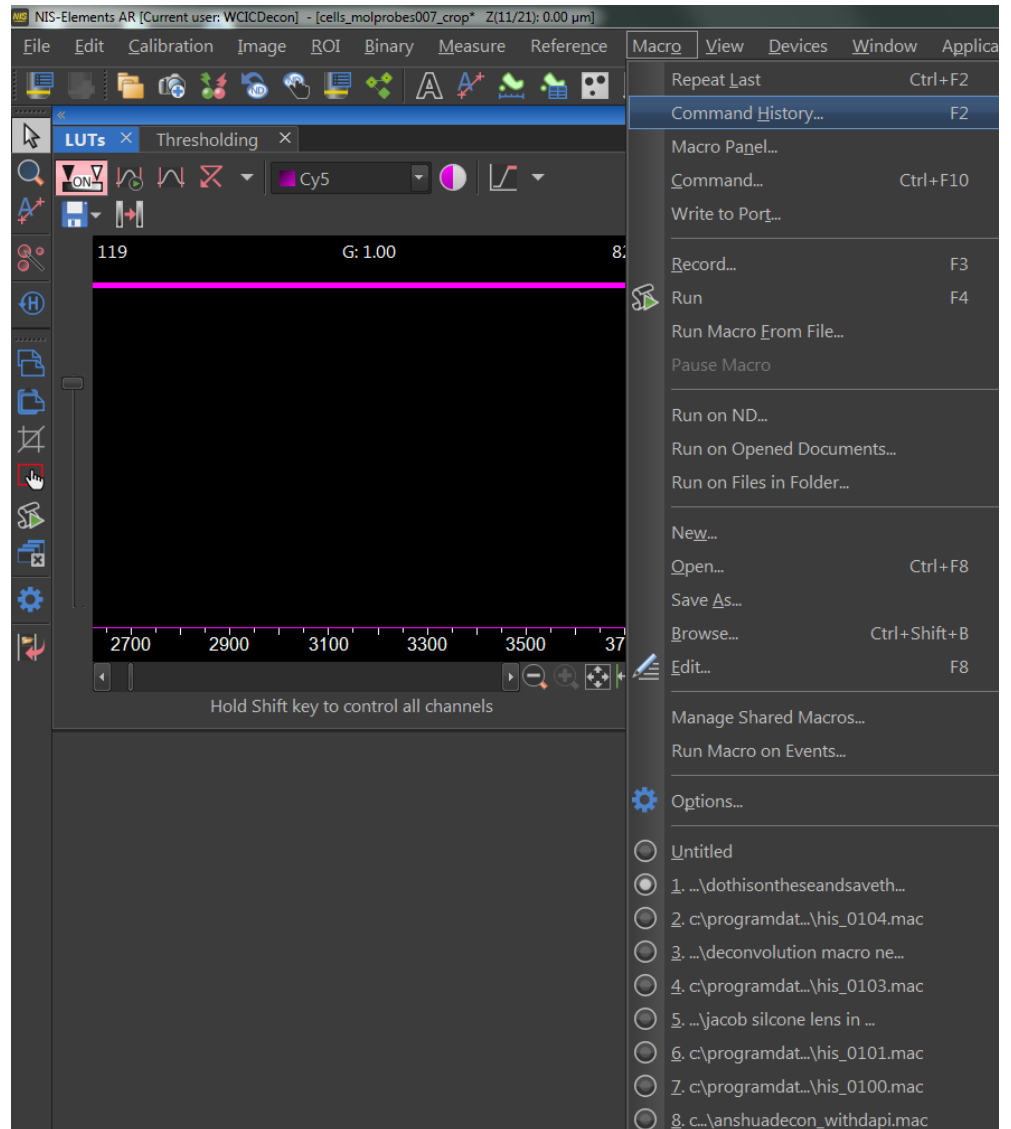
Run the deconvolution

## 1b) – Make a macro to run on all your files

Do not save the file that was just created – you are about to run this all again

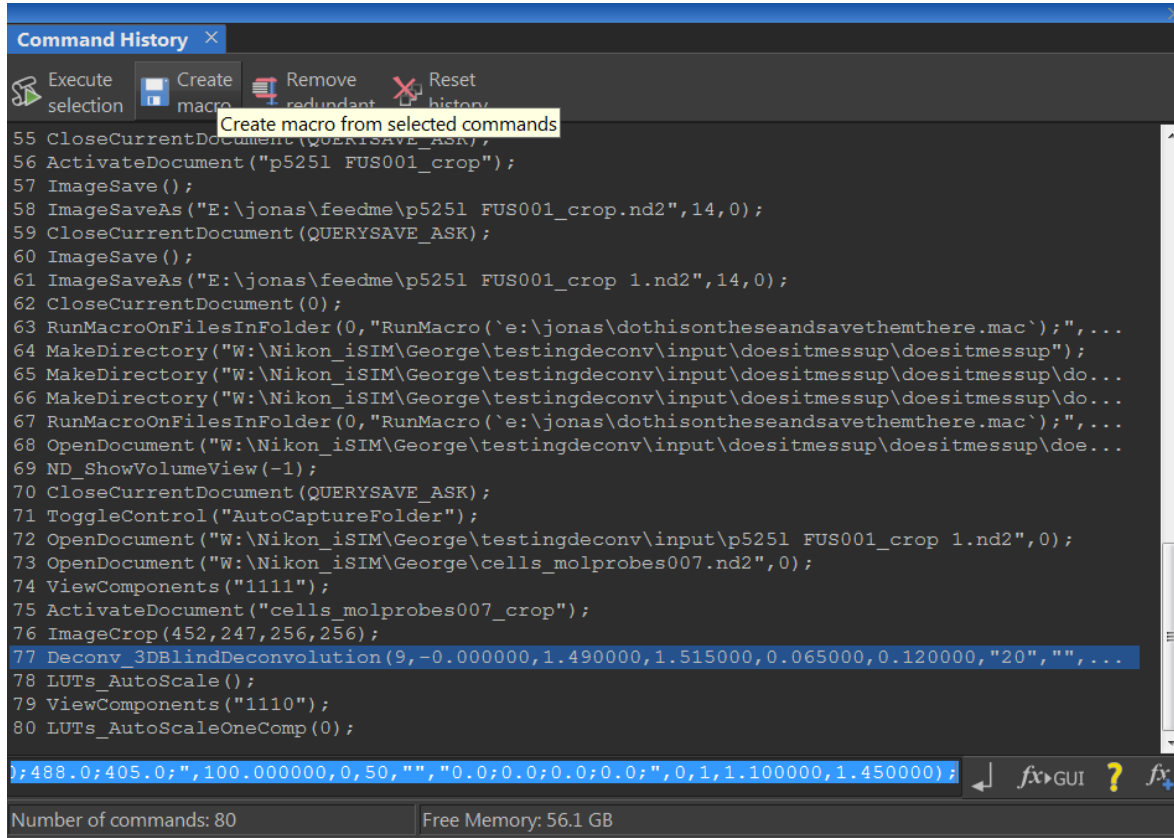
Go to Macro Menu

Open command history

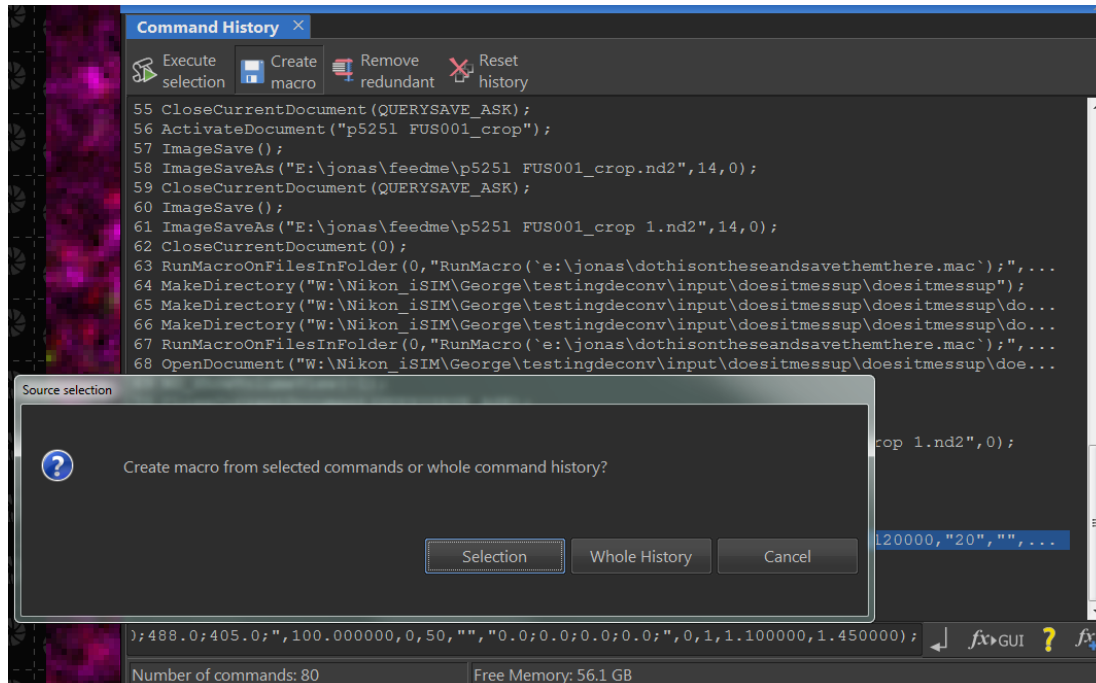


Find the deconvolution command near the bottom of the list & select it

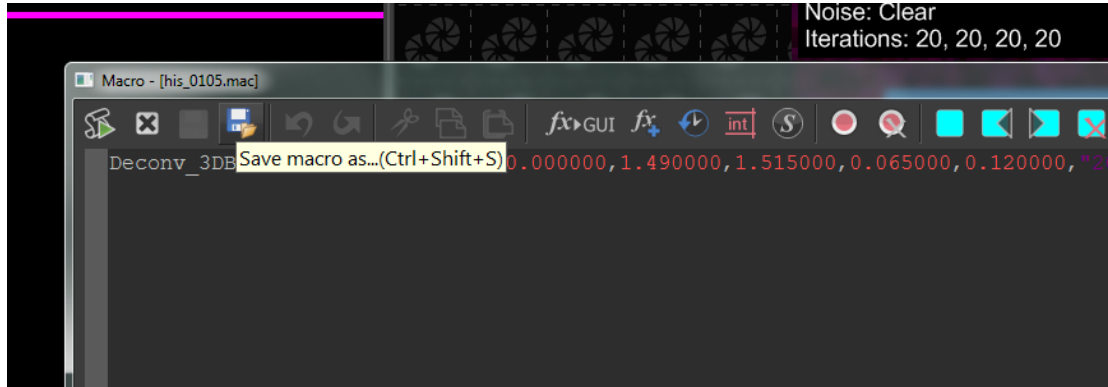
Then click on create macro (as shown below)



Choose selection



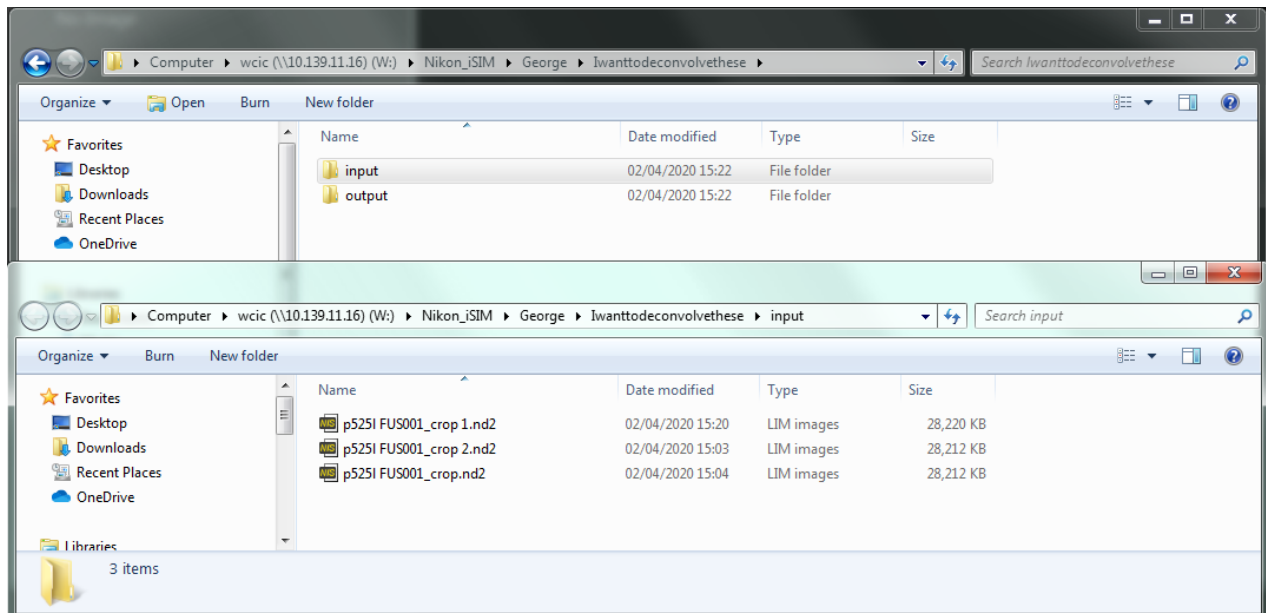
A new window should appear and you can now save the macro (somewhere you can find again)



You should now have a .mac file that can be ran on multiple files in a folder

## Step 2 – create an output folder outside the folder of data you are going process

We are going to direct the software to take everything from one folder, run the macro, and save it to another folder as it goes. If you put the output folder inside the input folder it will infinitely loop redoing the macro on the same file over and over if you don't remember to untick the 'include subfolders' option later.



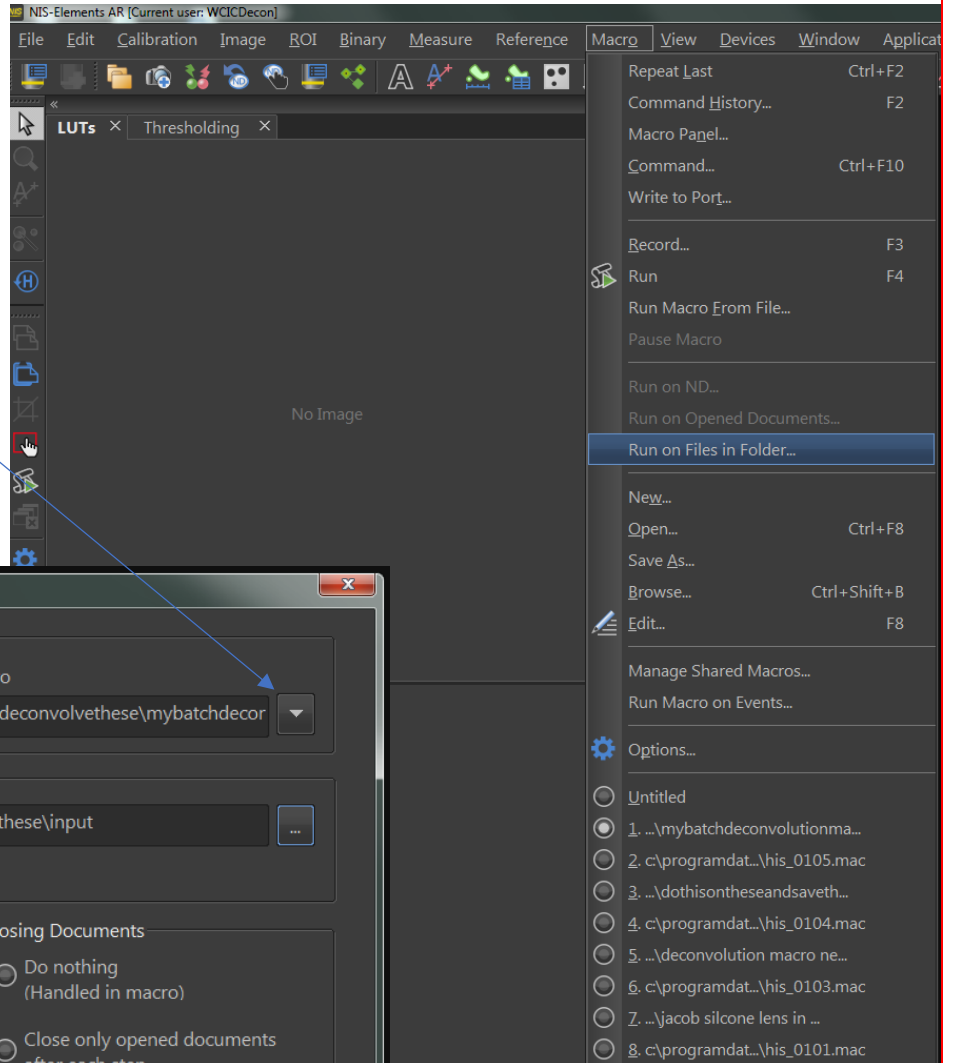
I would suggest that you keep things clear and have a folder with all the files to run one place and clearly name the output folder

## Step 3 – Run macro on all files in folder

Open the Macro Menu  
Select Run on Files in Folder  
(as shown right)

The GUI will open (as below)

Select the Command/Macro option  
Click the drop down arrow on  
Choose the 'RUN macro option'  
Find your macro



Set the input folder (probably sensible to untick 'Include Subfolders') – This go very wrong if not

Select the option 'Save in to different Folder' in the Saving Documents sub field

In the Closing Documents Sub field select close all documents after each step

Click Run and be more productive. Many files will take a while to complete.