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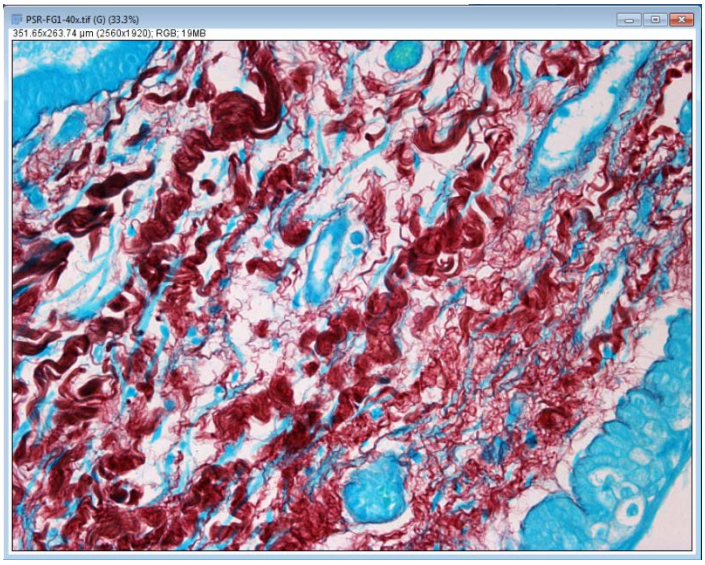
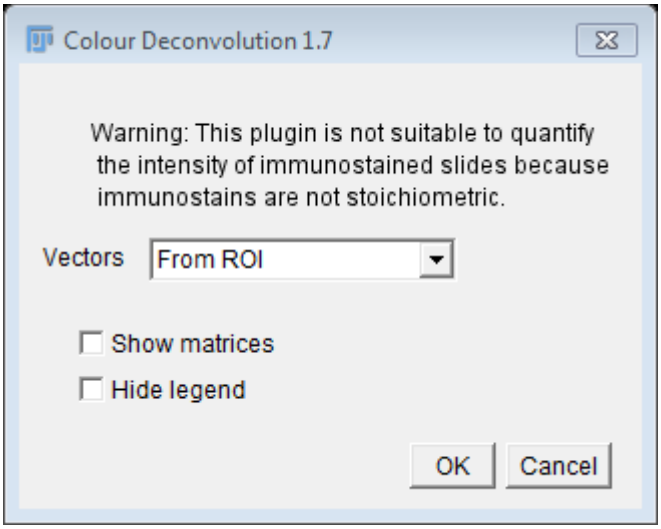
MICROSCOPY NEW ZEALAND INC. CONFERENCE WORKSHOP 2017

Creating a macro for colour deconvolution using ROIs. 31 January 2017

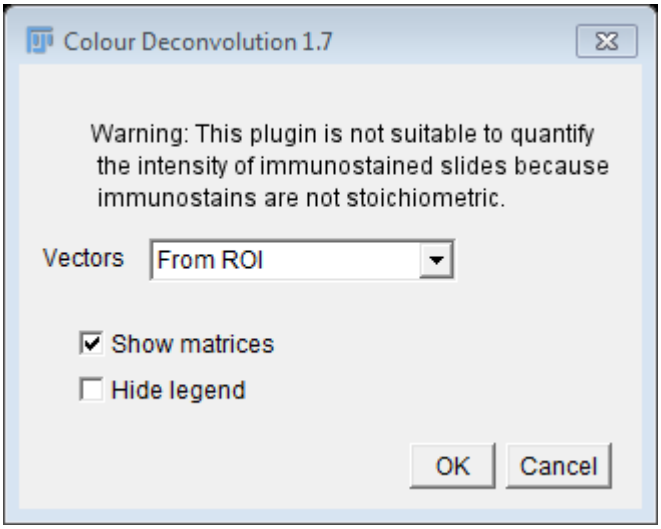
1. Open the image called **PSR-FG1-40.tif**;



2. Zoom up on the image using the **Magnifying tool** so that you have an area that has both of the stains clearly visible. You will need to sample from the image.
3. Make sure that you have the **Rectangle tool** or one of the other drawing tools selected on the tool bar.
4. Go to ***Image – Color – Colour Deconvolution***. The window below will appear;



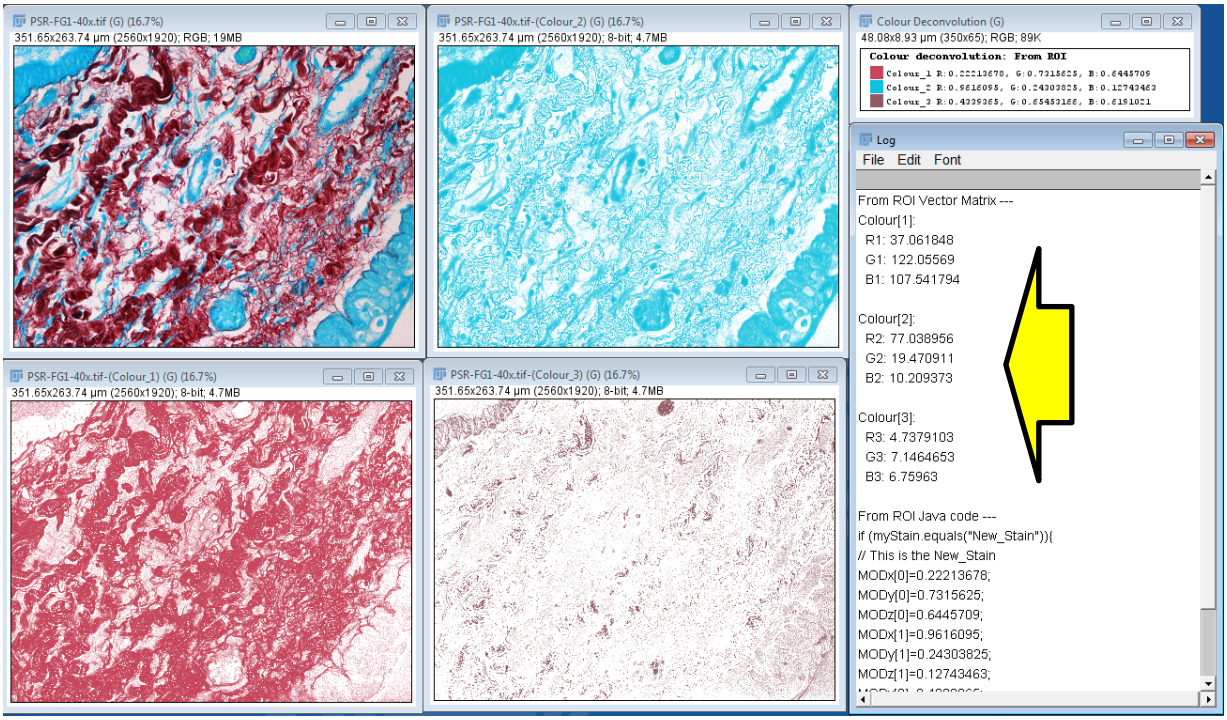
5. Select **Show matrices** as you will need the Vector information for the macro and click **OK**.



6. The message below will appear. Click **OK**, then draw a region of interest (ROI) over an area corresponding to the red (Picro-Sirius Red) staining. Right-hand mouse click when you have completed the ROI.
7. The next message will appear;

8. Click **OK** and draw an ROI for the second colour (green/blue). Right-hand mouse click when you have completed the ROI.

9. Click **OK** and draw an ROI for the third colour (usually in the white space). Right-hand mouse click when you have completed the ROI. Or just RHS mouse click without drawing an ROI.



10. Now that the colours have been separated, a macro can be written that uses the vectors detailed in the Log file. The values you need for the macro are the first set (**From Vector Matrix**). Save the **Log** file.

From ROI Vector Matrix ---

Colour[1]:

R1: 37.061848

G1: 122.05569

B1: 107.541794

Colour[2]:

R2: 77.038956

G2: 19.470911

B2: 10.209373

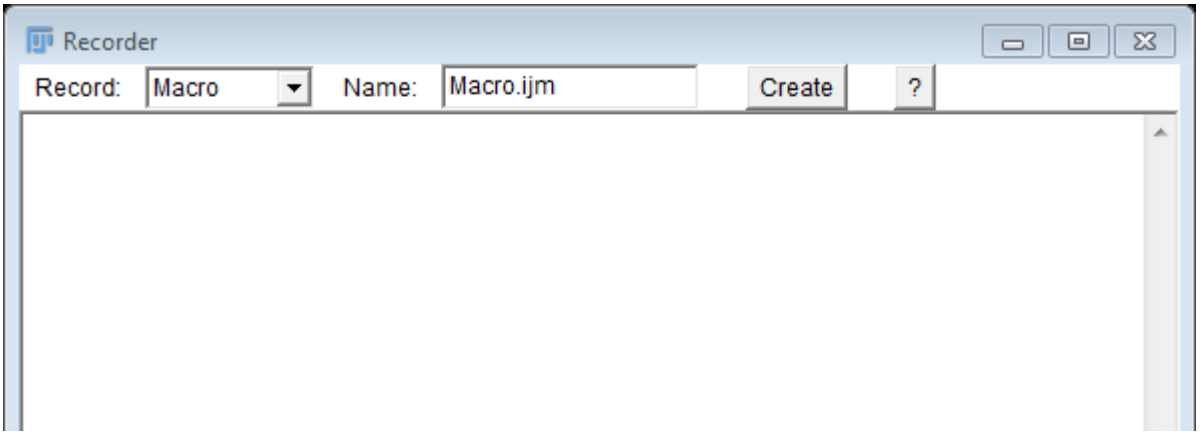
Colour[3]:

R3: 4.7379103

G3: 7.1464653

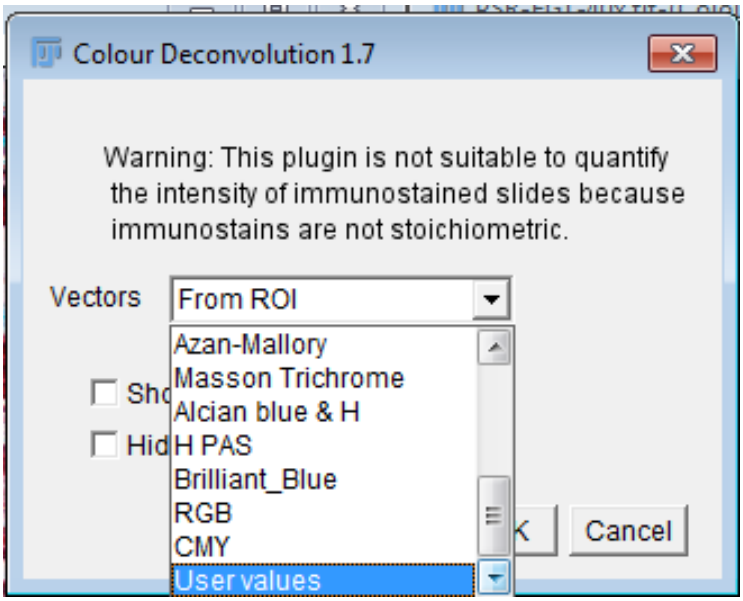
B3: 6.75963

11. Select the original image and go to ***Plugins – Macros – Record***. The **Macro Recorder** window will appear as below;

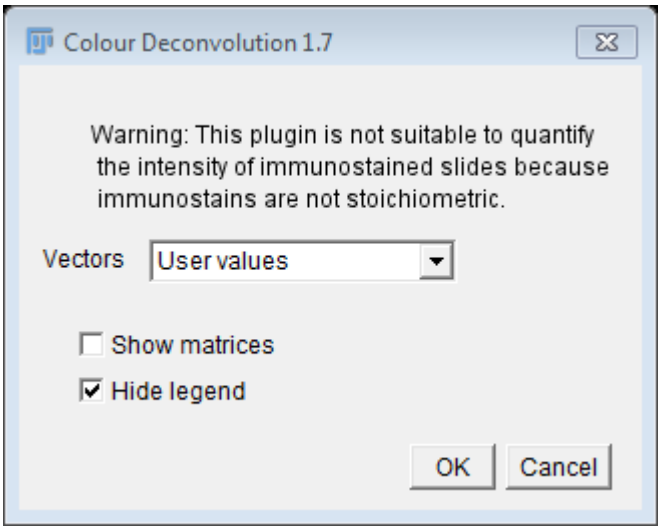


From now on, every action that you take with the mouse will be recorded and appear in the window.

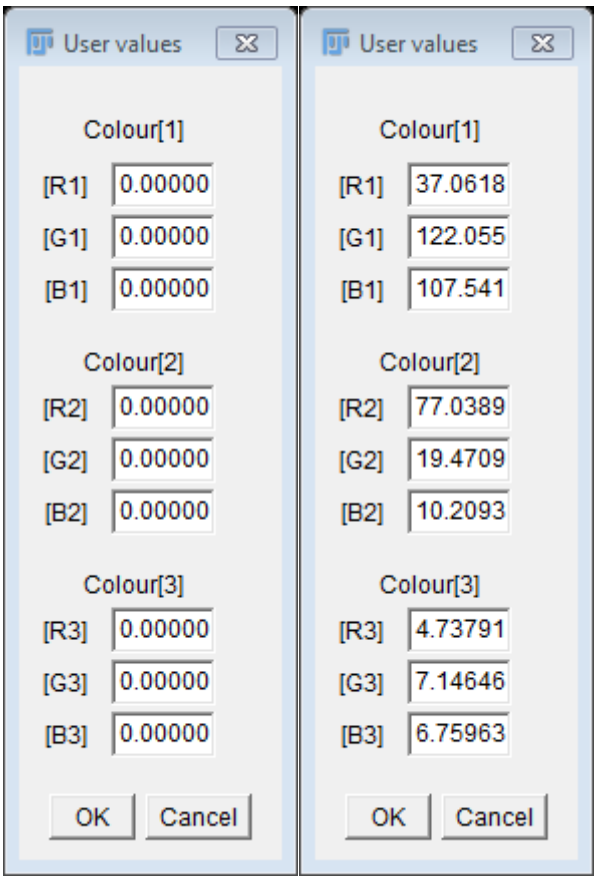
12. Go to ***Color – Colour Deconvolution*** and select ***User values*** from the drop-down box;



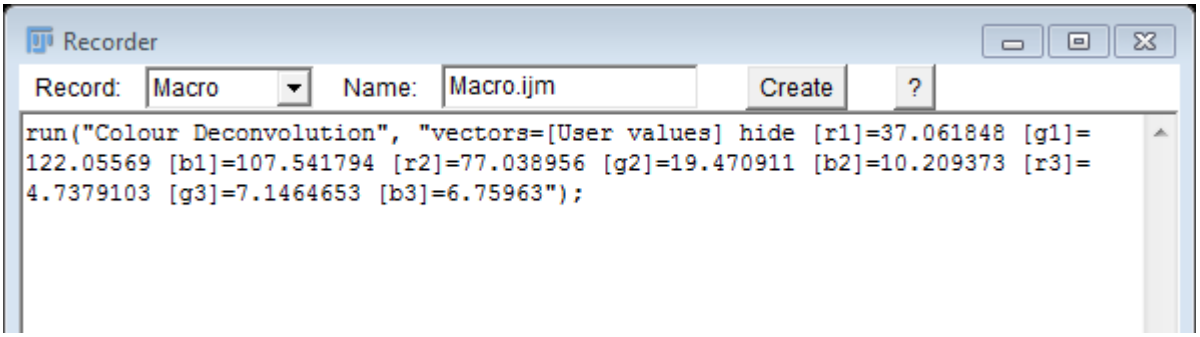
13. Select **Hide legend** but not **Show matrices** and click **OK**.



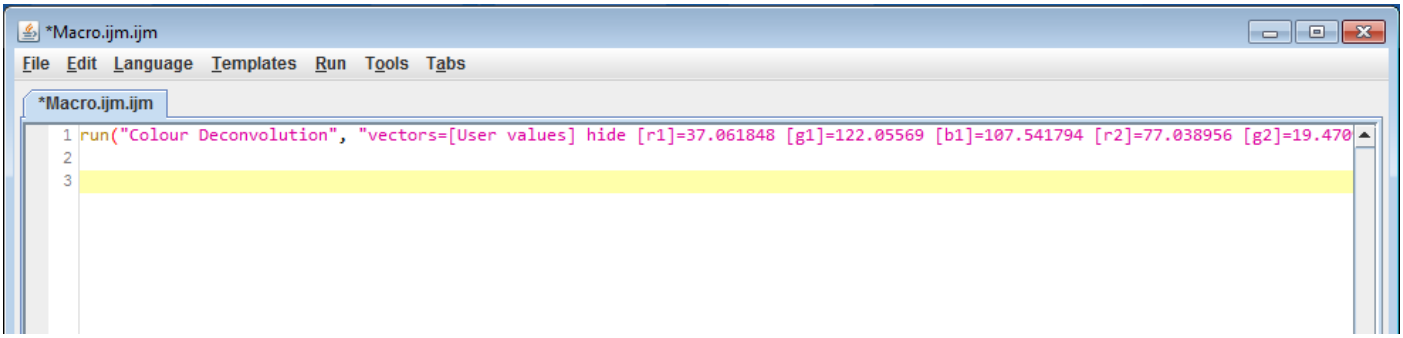
14. The window below will appear. Enter the values from the **Log** file that you saved, then click **OK**.



15. The **User values** will be used for the **Colour Deconvolution** and will appear in the **Macro Recorder** window.

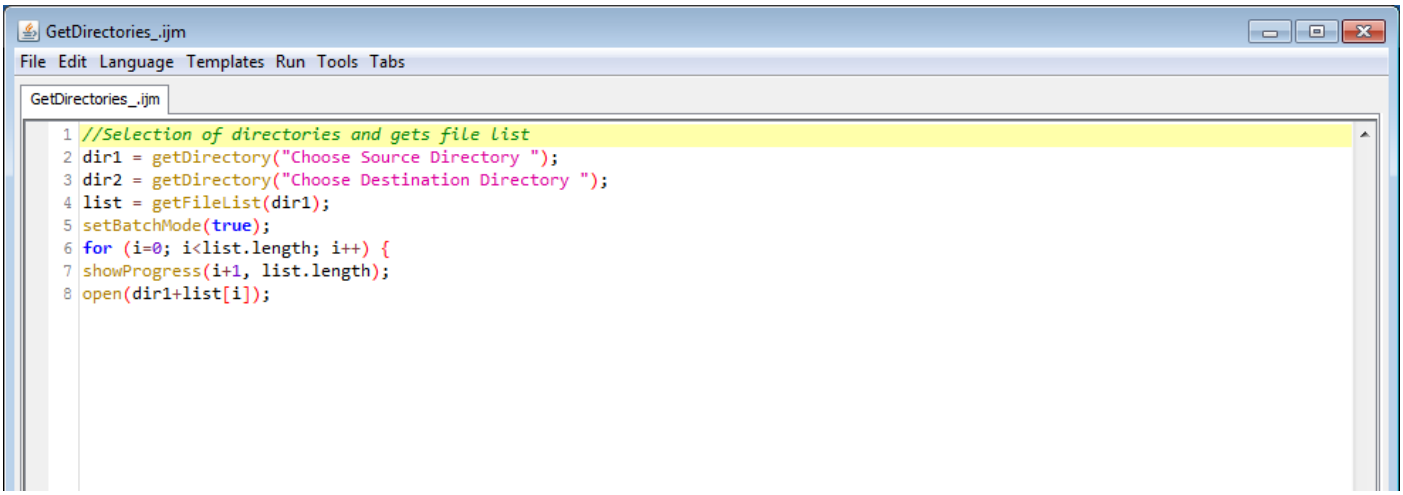


16. Click **Create** to create the Macro.

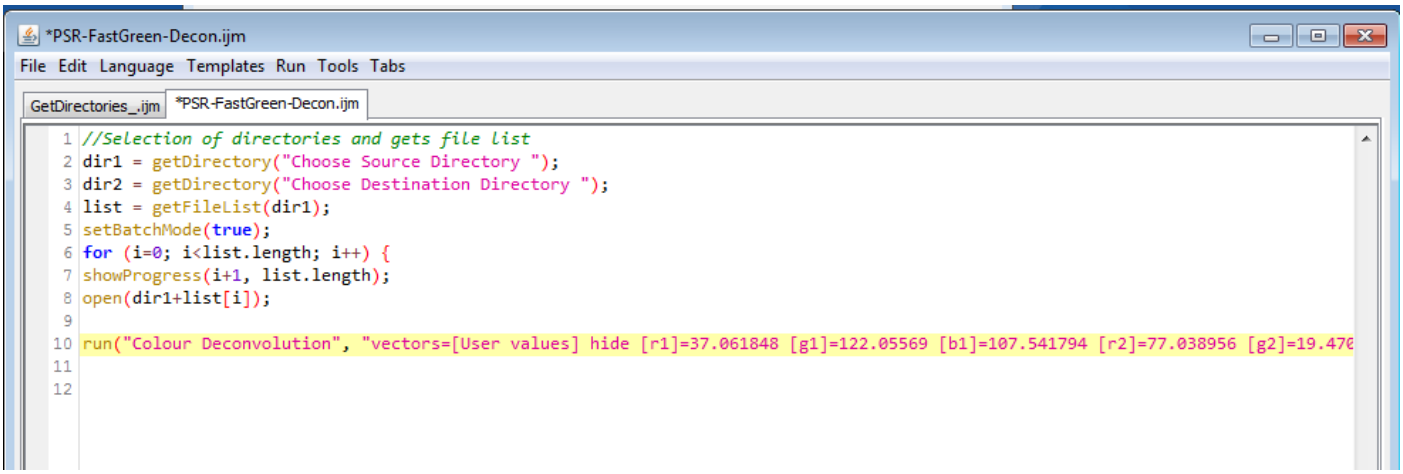


17. Save the macro with extension **.ijm**.

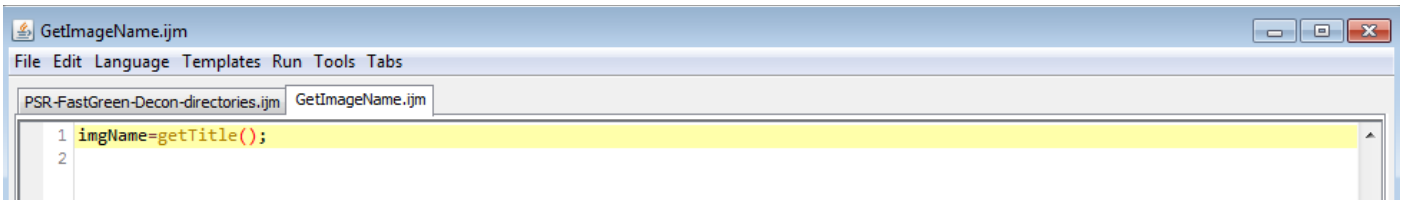
18. To make the macro suitable for batch processing, we need to add some additional code to specify the Input (source) and Output (destination) directories.
19. Open the file called **GetDirectories_.ijm**;



20. Select all of the text (***Ctrl-A***) and copy it.
21. Click on the tab for the macro you just saved. Add some blank lines by placing your cursor at the start of ***Line 1*** and pressing **Enter** a few times;
22. Then click at the start of Line 1 and go to ***Edit – Paste*** (***Ctrl-V***);



23. Leave one blank line before the **run("Colour Deconvolution"…)** command but remove any additional blank lines by backspacing or using the delete key.
24. Change the **Comment** in Line 1 (green text) to reflect the changes to the macro and save the macro with a new name.
25. Now you need to add in some code to save the output files. Open the text file called **GetImageName.ijm;**



26. Select the text and copy it. Paste it into the blank line. This code will get the original image name which you need to make the macro generic/flexible (i.e. not reliant on specific file names);

*PSR-FastGreen-Decon-directories.ijm

File Edit Language Templates Run Tools Tabs

*PSR-FastGreen-Decon-directories.ijm | GetImageName.ijm

```
//Runs colour deconvolution for all images in a folder stained with Picro-Sirius Red/Fast Green
dir1 = getDirectory("Choose Source Directory ");
dir2 = getDirectory("Choose Destination Directory ");
list = getFileList(dir1);
setBatchMode(true);
for (i=0; i<list.length; i++) {
showProgress(i+1, list.length);
open(dir1+list[i]);

run("Colour Deconvolution", "vectors=[User values] hide [r1]=37.061848 [g1]=122.05569 [b1]=107.541794 [r2]=77.038956 [g2]=19.476
```

27. Now you need to add in some code for closing the images we don't need and saving the ones we want to analyse later. Open the file called **CloseImagesSaveSelected.ijm**

CloseImagesSaveSelected.ijm

File Edit Language Templates Run Tools Tabs

*PSR-FastGreen-Decon-directories.ijm | GetImageName.ijm | PicroSiriusRed-FastGreen-Deconv.ijm | CloseImagesSaveSelected.ijm

```
//Closes images you don't need, saves the images you want
selectWindow(imgName + "-(Colour_3)");
close();
selectWindow(imgName +"-(Colour_1)");
title = getTitle();
saveAs("TIFF", dir2 + title);
close();
selectWindow(imgName + "-(Colour_2)");
title = getTitle();
saveAs("TIFF", dir2 + title);
close();
}
```

28. Select all (Ctr-A) and copy. Paste the text at the end of your macro.

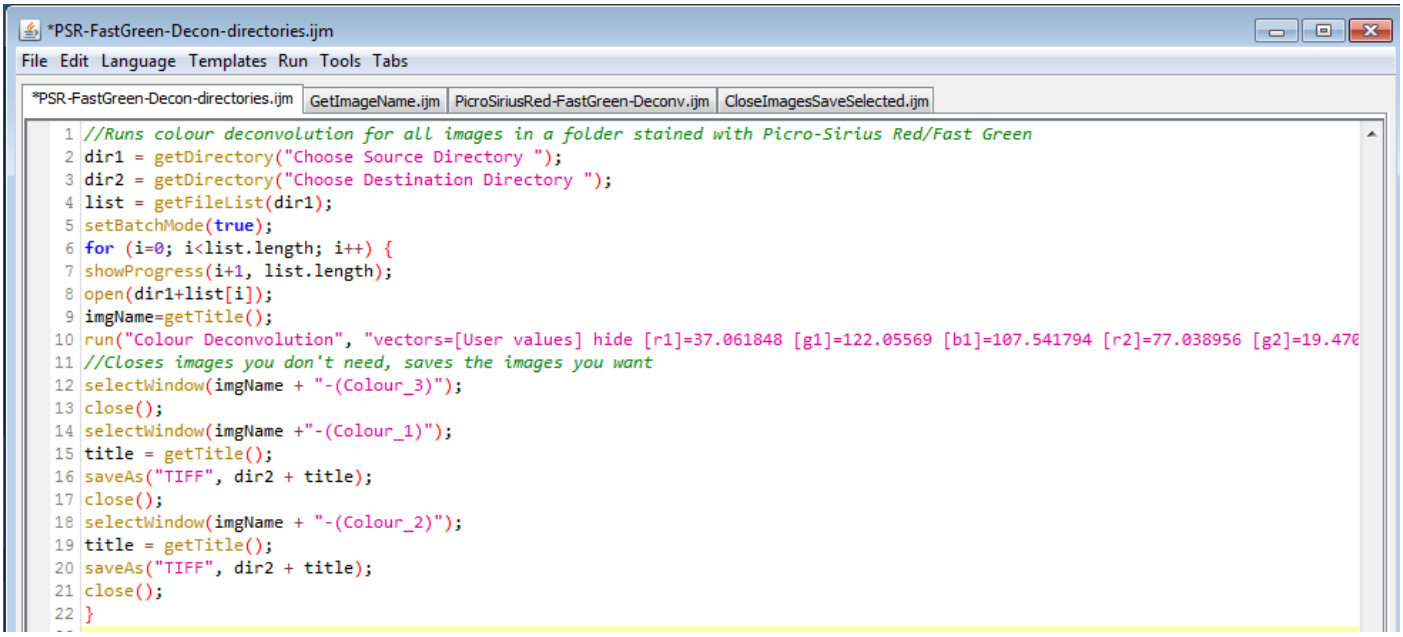
*PSR-FastGreen-Decon-directories.ijm

File Edit Language Templates Run Tools Tabs

*PSR-FastGreen-Decon-directories.ijm | GetImageName.ijm | PicroSiriusRed-FastGreen-Deconv.ijm | CloseImagesSaveSelected.ijm

```
//Runs colour deconvolution for all images in a folder stained with Picro-Sirius Red/Fast Green
dir1 = getDirectory("Choose Source Directory ");
dir2 = getDirectory("Choose Destination Directory ");
list = getFileList(dir1);
setBatchMode(true);
for (i=0; i<list.length; i++) {
showProgress(i+1, list.length);
open(dir1+list[i]);
imgName=getTitle();
run("Colour Deconvolution", "vectors=[User values] hide [r1]=37.061848 [g1]=122.05569 [b1]=107.541794 [r2]=77.038956 [g2]=19.476
//Closes images you don't need, saves the images you want
selectWindow(imgName + "-(Colour_3)");
close();
selectWindow(imgName +"-(Colour_1)");
title = getTitle();
saveAs("TIFF", dir2 + title);
close();
selectWindow(imgName + "-(Colour_2)");
title = getTitle();
saveAs("TIFF", dir2 + title);
close();
}
```

29. Save the macro with a new name. This is the final step.



30. Now click the **Run** button at the bottom of the **Script** window to test out the macro.