Adipose Tissue Segmentation in Fiji



Joseph Roland, Ph.D.





BEFORE YOU BEGIN

Ensure that you have the most up-to-date versions of Fiji (<u>http://imagej.net/Fiji</u>) and the plug-in MorphoLibJ (<u>http://imagej.net/MorphoLibJ</u>)

Under "Help" in Fiji,		limage) Dipdatas	
choose "Updates"		Manage update sites	and the second sec
	Nan Name URL	Host	Directory on Host
Click the "Manage update sites" button on the bottom left of the ImageJ Updater window	Fiji-Legacy http://sites FiloQuant http://sites FracLac Suite http://sites FunImageJ http://sites Fuzzy logic and artificia http://sites GDSC http://sites GDSC-SMLM http://sites Hadim http://sites HDF5 http://sites	imagej,net/Fiji-Legacy/ imagej.net/FiloQuant/ imagej.net/FiloQuant/ imagej.net/FunImageJ/ imagej.net/Astartes91/ imagej.net/Rerger/ imagej.net/GDSC/ imagej.net/GDSC-SMLM/ imagej.net/Hadim/ imagej.net/Ronneber/	
	HistoJ Lite http://sites	.imagej.net/Pathomation/	
Check the IJPB plugins option	IBMP-CNRS http://sites IJ-OpenCV-plugins http://sites IJPB-plugins http://sites ilastik Import Export http://sites ImageJ_Latex http://sites ImageJ_ITK http://sites	. imagej.net/Mutterer/ . imagej.net/IJ-OpenCV/ . imagej.net/IJPB-plugins/ . imagej.net/Ilastik/ . imagej.net/Yul.liuyu/ . imagej.net/ImageJ-ITK/	
Click "Close"	ImageJ-MATLAB http://sites ImageScience http://sites ImagingBook http://sites ImagingBookEn1 http://sites	. imagej.net/MATLAB/ . imagej.net/ImageScience/ . imagej.net/ImagingBook/ . imagej.net/ImagingBookEn1/ . imagej.net/ImagingBookEn2/	
Allow Fiji to update	ImagingBookEn3 http://sites	.imagej.net/ImagingBookEn2/ .imagej.net/ImagingBookEn3/ .imagei.net/ImagingBookDe2/	
Restart Fiji	Add my site Manage update sites	Add update site Remove	Advanced mode Cancel

Step 1: Open FijiStep 2: Drag the file of interest into Fiji to open itStep 3: Convert the image to 8-bit grey if it is not already

💷 (Fiji Is Just	t) ImageJ	Same and		_ D X
File Edit	Image Process	Analyze Plugins	Window Help	
I.O.C	Туре	•	🛩 8-bit	>>>
Freehand sele	Adjust Show Info Properties Color Stacks Hyperstacks	Ctrl+I Ctrl+Shift+P	16-bit 32-bit 8-bit Color RGB Color RGB Stack HSB Stack	
	Crop	Ctrl+Shift+X	Lab Stack	



Step 4: Invert the image

Do this step if you are analyzing a bright field image. Skip this step if you are analyzing a fluorescent image.

📴 (Fiji	Is Just) ImageJ	-	
File	Edit Image	Process Anal	yze Plugins Window Help
	Undo	Ctrl+Z	2 877 Dev Stk LUT 8 8 3 >>
Angle t	Cut	Ctrl+X	
	Сору	Ctrl+C	
	Copy to Sys	stem	
	Paste	Ctrl+V	
	Paste Cont	rol	
	Clear		
	Clear Outsi	de	
	Fill	Ctrl+F	
	Draw	Ctrl+D	
	Invert	Ctrl+Shift+I	
	Selection		
	Options		



Step 5: Subtract background

🔟 (Fiji Is Just) ImageJ			
File Edit Image	Process Analyze Plug	jins Window Help	0
LO.CO/	Smooth	Ctrl+Shift+S	1 8 >>
Multi-point or point (rig	Sharpen		
	Find Edges		
	Find Maxima		
	Enhance Contrast		T
	Noise	•	
	Shadows		
	Binary		
	Math	•	
	FFT	•	
	Filters	•	-
	Batch	•	
	Image Calculator		
	Subtract Background.		
	Repeat Command	Ctrl+Shift+R	
	Calculator Plus		

Subtract Background Settings

Subtract Backgr	ound	×
Rolling ball radius:	20.0	pixels
Light backgrou	und	
Create backgr	ound (do	on't subtract)
Sliding parabo	biold	
Disable smoo	thing	
✓ Preview		
ОК	Cano	el Help



Step 6: Morphological Segmentation





Step 6: Morphological Segmentation - Settings



Step 6: Morphological Segmentation - Outcome

Joseph Roland, Ph.D.



Step 7: Display "Overlaid dams" option



Joseph Roland, Ph.D.

Step 8: Click "Create Image"



Step 9: Perform Gaussian Blur

📴 (Fiji Is Just) ImageJ	1			X
File Edit Image Angle tool	Process Analyze Plugins V Smooth Cl Sharpen Sharpen Find Edges Find Maxima Enhance Contrast Noise Shadows Binary Math FFT	Vindow Help trl+Shift+S	8 8	*
Convolve	Filters	× .		
Gaussian Blur Median Mean Minimum Maximum	Batch Image Calculator Subtract Background Repeat Command Ct	trl+Shift+R		

Gaussian Blur Settings





Step 10: Convert the image to 8-bit

匪 (Fiji Is Jus	t) ImageJ			_ D X
File Edit	Image Process	Analyze Plugins	Window Help	
0,0	Туре		🐓 8-bit	>
Freehand sele	Adjust Show Info Properties Color Stacks Hyperstacks	Ctrl+I Ctrl+Shift+P	16-bit 32-bit 8-bit Color RGB Color RGB Stack HSB Stack	
	Crop	Ctrl+Shift+X	Lab Stack	



Step 11: Threshold the image

	📴 (Fiji Is Just) ImageJ		
	File Edit	Image Process A Type	Analyze Plugins Window	v Help Lut Ø Ø Ø ≫
Brightness/Contrast	Ctrl+Shift+C	Adjust	×	
Window/Level		Show Info	Ctrl+I	
Color Balance		Properties	Ctrl+Shift+P	
Threshold	Ctrl+Shift+T	Color	•	
Color Threshold		Stacks	•	
Size		Hyperstacks	•	
Canvas Size		Crop	Ctrl+Shift+X	
Line Width		Duplicate	Ctd+Shift+D	
Coordinates		Rename	Gurshierd	

Threshold Settings

III Threshold	X
66.57 %	
	• 14
Mean 💽 B&W 💽	3
Dark background Stack histog	ram
Auto Apply Reset Set	



Step 12: Analyze Particles



Analyze Particles Settings

Size (pixel^2):	50-6000
Circularity:	0.00-1.00
Show:	Outlines 💌
Display resu	ults 🔽 Exclude on edges
Clear results	s 🔽 Include holes
☐ Summarize	F Record starts
Add to Mana	ager 🔽 In situ Show



Step 12: Analyze Particles - Output



Save this image, if you choose to.

Note that large open spaces, as well as cells which were joined together due to segmentation issues, are not counted.



Step 13: Export Results Data

Export your data in .CSV format

File	Edit	Font R	esult	s
	Save As	Ctrl+	s	Max
	Rename			14
	Duplicate			14
_	Duplicati	5		14
4	309	9.084	7	14
5	1005	8.576	7	14
6	239	9.820	7	14
7	504	8.740	7	14
8	1151	8 4 0 5	6	14

