Setting up Micro-Magellan for Device control

From the plug-in menu	, select Micro-Magellan	n and the unpopulated wind	ow opens
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🛃 Micro-Magella	n Beta	
Device status/cont	rol Setup multiple acquisitions Grids Surfaces	
Saving directory:	Browse	Open dataset
7 ataa ()	Explore sample	
Z-step (µm):	4 ← Channel Group:	
Explore!	titled Explore Acquisition	
Explore	Acquisition Settings	
	Space Channels Covaried Settings Drift Compensation	
Saving name: Untitled		
Cinducu		
L		
	Run acquisition	
Created by Henry I	Pinkard at the University of California San Francisco 2014-201 Configure device control Calibrate Help	

Select "Configure device control"

The window below will open, select devices to appear in the control window and the "nickname" that will appear

include	Property	Nickname	
	EOM1-TriggerInputLine		
1	EOM1-Volts	MaiTai Power	
	EOM2-Block voltage		
	EOM2-Description		
	EOM2-Name		
	EOM2-Sequenceable		=
	EOM2-SupportsTriggering		
	EOM2-TriggerInputLine		
1	EOM2-Volts	Chameleon Power	
	Violet-Block voltage		
	Violet-Description		
	Violet-Name		
	Violet-Sequenceable		
	Violet-SupportsTriggering		-
Entries wil	II appear in the Device status/control panel in the order th	at they are selected here	

Press "Ok" and the selected devices will appear in the control window

MaiTai Power 0		
Chameleon Power 0.102		1
Violet PMT 0.9		i ,
Blue PMT 0.9		i ,
Green PMT 0.9		
Yellow PMT 0.9		1
Red PMT 0.9		1
Far Red PMT 0.9		1
Core-Focus PIZStag	e	
PIZStage-Position 20		1

Setting up for Explore & Acquisition

In order for Micro-Magellan to acquire tiled images and assemble them, it must have an accurate pixel size calibration for the objective in use. If you have already calibrated your pixel size using the "Pixel size calibrator" plug-in from Micromanager, this should automatically propagate to Micro-Magellan. Before using Explore mode, verify that the calibration is correct by pressing "Calibrate".

🛓 Affine transfo	orm calibrator					x			
Pixel size Calibration: 25X Low-Res									
				e coordiantes to pix feature while fine tu					
Pixel size Rota	ation (degrees)	X Scale	Y Scale	Shear					
0.5518 um	92. 135 ≑	0.552 🌩	0.551 ≑	0.009 ≑	Apply				
			around the micro stimate these pa	scopes's field of vie rameters	w,				
		Sta	art	Capture					

If the fields are populated as above, Micro-Magellan has the necessary information. These parameters can be adjusted manually to fine tune stage movement for improved stitching.

If the fields are not populated (as below), you can run click Start and follow the wizard instructions to generate an affine transform.

🛓 Affine tra	ansfor	rm calibrator					- •	×
		Pixel	size Calibration	:				
			hear when translatir ons over a a contigu					
Pixel size 0.0 um	Rotat	tion (degrees) -1 🚔	Scale	Shear 0 🚔	Apply			
			tintive feature arou will attempt to estima		· ·	ew,		
			Start		Capture			

Using Explore Mode

Before exploring your sample you will need to enter your desired Z-step size and tile overlap. The channel group drop down is analogous to the channel group drop down within MicroManager's Multi-Dimensional Acquisition plug-in and will be pre-populated with the configurations groups you have created. Select the settings group that contains your channel configurations.

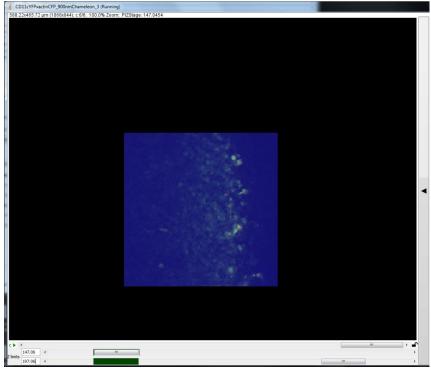
Saving directory:	Browse Open dataset
Explore sample	
Z-step (µm): 4 Channel Group: Tile overlap: 0 %	
Saving name: Untitled Explore Acquisition	
Explore!	

Pressing "Explore!" will open a new window:

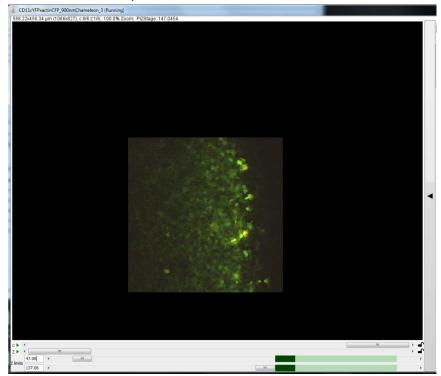
CD11cYFPxactinCFP_900nmChameleon_3 (Running)				and the second second		
588.22x475.10 µm (1066x861); c:1/6; 100.0% Zoom; PIZStage: 147.0454				and the second second		
			C 🕄 🙂	Animation FPS: 7 🔄 📝 Move scrollbars or	new image	
			In the Inc. Inc.			
			Status Ord Barface Exp	ore		
			Use	Configuration	Exposure	
				Input-0-NewPreset	1.0	1
				Input-0-NewPreset	1.0	
				Input-2-NewPreset	1.0	1
				Input-3-NewPreset	1.0	3
				Input-4-NewPreset	1.0	
					1.00	
			Contrast			
			Contrast	Metadata		
			Scale Bar Top-Left	+ White + Sync channels 🕑 Slow his		
			Display mode: Compos	site 🔹 🔄 Autostretch 🗌 ignore %	0.4 🕀 🚺 Log hist	
			Local Contraction			
			Input-0-NewPreset	1		^
			Evilia Autoria			
			Hist, range:			
			the second secon			
			Min: Max			
			Mills: Max			
			Input-1-NewPreset			258
			M Input-Invenireset			
			Ful Auto			
			Hist, range:			H
			Camera Depth 👻			
			Min: Max			
				1		256
			Input-2-NewPreset			200
			Hist. range:			
			Camera Depth 👻			
			Min: Max			
						256
			Input-3-NewPreset			
			Full Auto			
			Hist. range:			
			Camera Depth +			
			Min: Max			
Z imits 47.06 4	H.	,				-
147.06 +	87	,				144

On the left side is your image area; a field of view will highlight blue as you hover over it. On the right, are look-up tables (LUTs) for each channel and several other acquisition set-up parameters. To acquire an image in that area click to select (area will highlight green during active selection), once your selection is highlighted magenta, click again to confirm. You can click and drag to select multiple fields of view to be imaged.

The "Z limits" scroll bars below the image window control what Z-locations will be imaged, and will adjust automatically to accommodate the selected Z-step size.



Once you have begun to image, the Z-plane currently being displayed will appear dark green in the Zlimits scroll bars. Other acquired Z-planes will appear light green. In the example below, we have already collected a Z-stack (light green) and are extending our acquisition by acquiring additional Z-planes above what we have already.



You can review acquired images by scrolling with the "Z" bar, and right-clicking and dragging to navigate in the XY plane. You can also zoom in and out by using the mouse scroll wheel with the image window selected.

Once you have explored your sample and would like to select areas to image over time, with more optimized parameters, better Z-resolution, etc. you can use either the "Grids" or "Surfaces" tab above the LUTs to do so.

Grids

In the Grids tab, you can define an N x N grid, which will appear over your explored image. With the Grids tab active, you can drag this grid over the area you would like to image. You may generate multiple grids of different sizes by pressing "New Grid", and then move them to different areas.

	ODDICK & A & OF	
	ation FPS: V Move scrollbars on new image	
Status Grid Surface Explore		
Current grid:	New Region 1	
New Grid Rows:	5 Columns: 5	
CD11cYFPxactinCFP_900nmChameleon_3 (Running)		
2352.88x1825.35 µm (1066x827); c:6/6 z:8/16; 25.0% Z	oom, M25tage:117.0464	
		•
€ € 47.06 € Ш		: 5
imits 137.06 <		,

All of the grids generated in explore mode will populate the "grids" tab at the top of the Micro-Magellan main window, next to the Device status/control tab. If you are creating multiple grids, it may be helpful to toggle back to the Micro-Magellan main window, and in the select and rename the grids with their identifying features (ie: airway, duct, tumor).

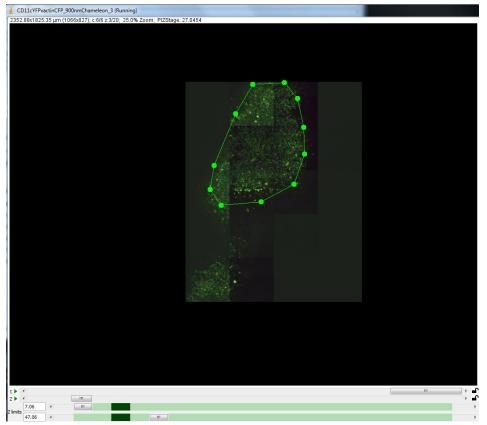
Surfaces

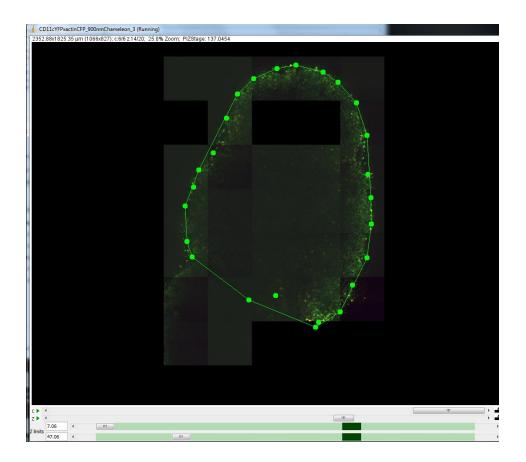
The surfaces tab allows you to define the morphology of the sample by defining the edges at multiple Zplanes; Micro-Magellan then interpolates between the outlines to generate a 3D representation of the sample which can then be used to adjust which fields are acquired to avoid collecting empty space, or vary hardware settings in a sample dependent manner.

To begin drawing your surface, first select (surface above/below)

Status Grid Surface Explore
Current Surface: New Surface 1
New Surface V Footprint V Interpolation V Tiles Above surface V
Above surface Below surface

Here we have selected Surface Below, so we begin at the top of the sample and add points around the perimeter, then move the Z-slider down and adjust the outline.





Once you are satisfied with grids or surfaces generated, you can return to the main Micro-Magellan window to set up acquisition.

All of the grids and surfaces generated in explore mode will populate the "grids" and "surfaces" tabs at the top of the Micro-Magellan window, next to the Device status/control tab

Device status/control Setu	p multiple acquisitions Grids	Surfaces				
Name	XY Device	# Rows		# Cols	Width (µm)	Height (µm)
New Region 1	XYStage	2		2	1029.0699	1029.0699
New Region 2	XYStage	3		3	1530.7286	1530.7286
New Region 3	XYStage				1530.7286	1029.0699
Device status/control Set	in multiple acquisitions Grids	Surfaces	-	Delete all		
Name	XY Device	J ļ	Z Device		XY padding (µm)	# Positions
New Surface 1	XYStage		Focus		0.0	12
- Delete all Save Load						

Acquiring Images

There are a series of tabs in the lower half of the Micro-Magellan window to help manage acquisition set up.

The "Save" tab allows you to define the name of the acquisition, and uses the same directory as the explore mode. To set up a simple, single area acquisition, enter a name for the file.

In the "Time" tab, you can enter time interval between image acquisition and the total number of time points to acquire.

Saving	Time	Space	Channels	Covaried	Settings	Drift Compensation
🔽 Tim	e points					
Numbe	r	1				
Interva	al	0 🌲	ms	•		

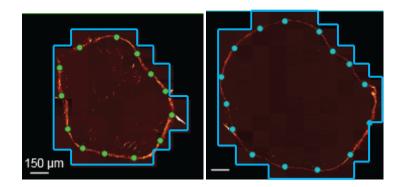
In the "Space" tab there are several options for defining the volume you want to image. In a simple Zstack you enter start and end Z positions, and either the grid or surface to define XY location and positions.

Saving Time Space Channels Covaried Settings Drift Compensation							
☑ Z-step (µm): 1 ☆ Tile overlap: 5 ☆ %							
Simple Z stack	O Volume between two surfaces	Within distance from surface					
Z-start (µm) 0 🖨	Z-start 0 → µm above v Z-end 0 → µm below v	Z-start 0 x µm above Z-end 0 x µm below					
Surface/Grid XY footprint:	XY positions from: Top surface	Surface:					
	Surface/Grid footprint:						

If you have created surfaces, you can also elect to image the area between two defined surfaces with a buffer on either side.

Saving Time Space Channels	Covaried Settings Drift Compensation	
☑ 3D Z-step (µm):	1 Tile overlap: 5 %	
© Simple Z stack Z-start (µm) 0 →	Volume between two surfaces Z-start	© Within distance from surface Z-start 0 → µm above
Z-end (µm) 0	Z-end 0 v µm below New Surface 2 v	Z-end 0 × µm below
Surface/Grid XY footprint:	XY positions from: Bottom surface Top surface	Surface:
	Description of the second seco	

Imaging within a distance from a defined surface allows you to collect non-cuboidal volumes by using the edges of your surface to choose the XY positions that are collected at each Z-plane (example of XY footprints below). By selecting a Z-distance below a surface to image, you can also avoid collecting "blank" data in deep areas of the sample where there is no information due to scattering.



The "Channels" tab allows you select which of the available channels to collect, and the exposure time for each.

Saving Time Space Channels Covaried Settings Drift Compensation 2Photon settings					
Channel group:					
Use	Configuration	Exposure	Color		
-	Input-0	1.0			
1	Input-1	1.0			
1	Input-2	1.0			
1	Input-3	1.0			
	Input-4	1.0			
1	Input-5	1.0			

The "Covaried Settings" tab allows you to vary one hardware setting depending on the state/value of another hardware device/setting.

Saving	Time Space Channels Covaried Settings	Drift Compensation	
Cova	riant pairings	Interpolation points	
Active	Pairing (Independent Variable : Dependent Variab	le)	٦
	+ - Save	Load +	-

The "+" button on the right brings up the window below, allowing you to select one hardware setting as the independent variable, and a second as the dependent variable.

Select one from each list to create pairing			x
Independent variable		Dependent variable	
FarRed-Name		EOM1-TriggerInputLine	
FarRed-Sequenceable	1	EOM1-Volts	
FarRed-SupportsTriggering	1	EOM2-Block voltage	1
FarRed-TriggerInputLine	1	EOM2-Sequenceable	
FarRed-Volts	1	EOM2-TriggerInputLine	1
PIZStage-Description	1	EOM2-Volts	
PIZStage-Name	1	Violet-Block voltage	1
PIZStage-Position		Violet-Sequenceable	
PIZStage-StepSizeUm	1	Violet-TriggerInputLine	
XYStage-Acceleration	1	Violet-Volts	
XYStage-Description	1	Blue-Block voltage	=
XYStage-MaxSpeed	1	Blue-Sequenceable	-
XYStage-SCurve		Blue-TriggerInputLine	
XYStage-StepSizeX_um	1	Blue-Volts	
XYStage-StepSizeY_um	1	Green-Block voltage	
XYStage-TransposeMirrorX	1	Green-Sequenceable	1
XYStage-TransposeMirrorY		Green-TriggerInputLine	1
ResTrigger-ClosedPosition		Green-Volts	1
ResTrigger-Description		Yellow-Block voltage	1
ResTrigger-Name	1	Yellow-Sequenceable	1
ResTrigger-OutputChannel		Yellow-TriggerInputLine	1
ResTrigger-Sequenceable	1	Yellow-Volts	1
ResTrigger-State	1	Red-Block voltage	1
ResTrigger-SupportsTriggering	1	Red-Sequenceable	1
ResTrigger-TriggerInputLine	-	Red-TriggerInputLine	-
ОК		Cancel	

In this example, we have selected the Z stage position as the independent variable, and our EOM (laser power) will vary depending on its position allowing us it increase laser power as we get deeper into our sample.

Saving) Time	Space	Channels	Covaried Settings	Drift Compensation	
Cova	riant pairir	ngs				Interpolation points
Active	Pairing (I	independ	ent Variable	: Dependent Variab	le)	
	+	-		Save	Load	[+]

Once you have selected the two variables, clicking the "+" button on the right under Interpolation points allows you to enter settings for them. Micro-Magellan interpolates and applies values between the points entered.

If you have defined surfaces, you can also choose to vary hardware settings based on surface data.

Independent variable		Dependent variable	
Surface data: New Surface 1Vertical distance below at XY position center		ora forreamerazzi manaposezi	
Surface data: New Surface 1Minimum vertical distance below at X1 position	-6	EOMShutter-DAC1	
Surface data: New Surface 1-Maximum vertical distance below at XY position	-111	EOMShutter-DAC2	
Surface data: New Surface 1-Relative power for curved surface	Ξ	EOMShutter-State	
Surface data: New Surface 2Vertical distance below at XY position center	-	EOM1-Block voltage	
Surface data: New Surface 2Vertical distance below at X1 position center Surface data: New Surface 2Minimum vertical distance below at X2 position		EOM1-Sequenceable	
Surface data: New Surface 2Maximum vertical distance below at XY position	-	EOM1-TriggerInputLine	
Surface data: New Surface 2Relative power for curved surface	-	EOM1-Volts	
Surface data: New Surface 2Relative power for curved surface Surface data: New Surface 3Vertical distance below at XY position center	-	EOM2-Block voltage	
	-	EOM2-Sequenceable	
Surface data: New Surface 3Minimum vertical distance below at XY position	-	EOM2-TriggerInputLine	=
Surface data: New Surface 3Maximum vertical distance below at XY position	-	EOM2-Volts	
Surface data: New Surface 3Relative power for curved surface	-	Violet-Block voltage	-
Surface data: New Surface 4Vertical distance below at XY position center	-	Violet-Sequenceable	
Surface data: New Surface 4Minimum vertical distance below at XY position	-	Violet-TriggerInputLine	
Surface data: New Surface 4Maximum vertical distance below at XY position	_	Violet-Volts	
		Blue-Block voltage	
Surface data: New Surface 5Vertical distance below at XY position center		Blue-Sequenceable	
Surface data: New Surface 5Minimum vertical distance below at XY position		Blue-TriggerInputLine	
Surface data: New Surface 5Maximum vertical distance below at XY position		Blue-Volts	
Surface data: New Surface 5Relative power for curved surface		Green-Block voltage	
Group: Acquisition		Green-Sequenceable	
Group: Active EOMs		Green-TriggerInputLine	-
Group: Enable Channels		Green-Volts	-
Group: Resolution		Yellow-Block voltage	-
Group: System	Ŧ	Vellow-Sequenceshle	
ОК		Cancel	

For a given tissue type and morphology, these parameters may be optimized and saved for use across samples. To load a saved set of interpolation points and settings, click "load" and select the saved parameter file. You will be prompted to select which surface you would like to assign the settings to, and which surface parameter you would like to use.

<u>با</u>		x
Associate	with surfaces	
New Surf	ace 1	
New Surf	ace 2	
	e lika i li avaz ne a	-1
	tical distance below at XY position center	-
	tical distance below at XY position center imum vertical distance below at XY position	
	ximum vertical distance below at XY position	
	ative power for curved surface	

Drift Compensation

Drift compensation allows you to use a single channel that represents the static features of your sample, and use it to correct for Z drift.

Saving	Time	Space	Channels	Covaried Settings	Drift Compensation
📝 Acti	vate cro	ss-correla	ation based d	rift compensation	
Use ch	nannel:	Input-0		•	
Maxmimum displacement (um):					
Drift c	ompensa	ation Z de	vice: PIZSt	age 🔻]
E Se	et initial (position			

Setting up multiple acquisitions

To acquire more than a single grid or surface at a time, select the "Setup multiple acquisitions" tab. Click "+", this will add a line with the same name as is in the "Saving" tab below. Enter the parameters for this acquisition in the other acquisition settings tabs.

🔏 Micro-Magellan Beta	X
Device status/control Setup multiple acquisitions Grids Surfaces	
Order Name Status	
1 Demo_surface	
+ - Movet Movet Interleave Deinterleave Run all	
Saving directory: D: Waitin Browse Open dataset	
Explore sample	
Z-step (µm): 4 📩 Channel Group: Channel 🗸 Tile overlap: 0 📩 %	
Saving name: Untitled Explore Acquisition	
Explore!	
Acquisition Settings	
Saving Time Space Channels Covaried Settings Drift Compensation	
Saving name:	
Demo_surface	
Run acquisition	
Created by Henry Pinkard at the University of California San Francisco 2014-2015 Configure device control Calibrate Help	

Clicking "+" again will add another line, it will appear with the same name. Highlight the second (new) acquisition and change its name in the "Saving" tab below, and change necessary parameters in the other acquisition settings tabs.

"Run all" will sequentially acquire all acquisitions listed.

If you would like to collect time data in different areas concurrently (rather than completing a time lapse in one area, and then beginning the second time lapse in another area), select "interleave" and then "Run all"

🔔 N	licro-Magellan Beta	
Dev	ce status/control Setup multiple acquisitions Grids Surfaces	
Ord	rr Name	Status
1	Demo_surface	
1 2	Demo_grid1	
	+ - Movei Interleave Deinterleave	Run all

Line Scanning Systems

If you are using Micro-Magellan on a line scanning system, this plugin replaces the previous "100X TwoPhoton" plugin.

Because Micro-Magellan uses its own acquisition engine within MicroManager, when switching between using the Snap/Live buttons and Micro-Magellan, you must switch the BitFlowCamera-

IntegrationMethod device parameter. For Snap and Live modes, select "FrameAverage"; for Micro-Magellan select RawFramesToCircularBuffer.

🛓 Device Property Browser	
Show cameras Refresh! Show shutters Show stages Show stages Show discrete changers Show other devices Show other devices	properties
Property	Value
BitFlowCameraX2-Binning	1
BitFlowCameraX2-CenterOffset	.1 ()
BitFlowCameraX2-ChannelOffsets	113300
BitFlowCameraX2-Deinterlace	On +Unwarp
BitFlowCameraX2-Description	BitFlow camera adapter
BitFlowCameraX2-EnableChannels	111111
BitFlowCameraX2-FrameIntervalMs	0
BitFlowCameraX2-InputChannel	0
BitFlowCameraX2-IntegrationMethod	RawFramesToCircularButter
BitFlowCameraX2-Name	FrameAverage
BitFlowCameraX2-ProcessingTimeMs	RawFramesToCircularBuffer
BitFlowCameraX2-TransposeCorrection	0
BitFlowCameraX2-TransposeMirrorX	1
BitFlowCameraX2-TransposeMirrorY	0
BitFlowCameraX2-TransposeXY	1

🦌 Micro-Manager 1.4.23 20150817 - C:\MMConfigFiles\BIDC_MMconfig_Gen3_Magella 💶 💷 🗮 🏧									
File Tools Plugins Help									
Snap	Camera settings		Configuration sett	ings Save	•				
Live	Exposure [ms]	2	Group	Preset					
Album	Binning	1 👻	Acquisition	Magellan	•]				
	01-14-1		Active EOMs	Magellan					
Multi-D Acq.	Shutter EOM	IShutter 👻	Enable Channels	Snap and Live					
🥏 Refresh	Auto shutter 🔽 Open		Resolution	25x Hi-Res					
Please cite Micro-M	lanager so funding v	vill continue!	System						
ROI Zoo	om Profile /	Autofocus							

We simplify this for users by creating an "Acquisition" configuration with presets.

On startup, Micro-Magellan looks for the presence of a Bitflow board in the configuration file. If you it finds one (or multiple), it also loads a 2Photon Settings tab in the Micro-Magellan main window.

Saving Time Space Channels	Covaried Settings	Drift Compensation	2Photon settings	
Image construction method	Offsets			
Frame average	Ch0 -14			
🔘 Rank filter	Ch1 -13			
Rank: 0.9 荣	Ch2 -7			
Frame summation	Ch3 -10			
💿 Burst mode	Ch4 -10	_		
	Ch5 -8			

This tab gives you several options for image construction/processing. All options here pull the number of frames used from the "Exposure [ms]" field in the main MicroManager window.

The offsets fields allow you do adjust the interleave offset for each channel individually, although maximum difference between any two channels is 9 pixels.

Outputs

Micro-Magellan will create a directory with the name entered in the "saving" tab. Within this directory are subfolders with downsampled versions of the acquired images, these are used to create a "zoomable" image. Within each folder is a single TIF image for each XY position acquired, which stores all the channels, Z-planes, and time points for that XY location. These TIF files can be automatically stitched and saved as either an Imaris (.ims) file, or output to a FIJI window using the Imaricumpiler plugin for FIJI found here:

http://biomicroscopy.ucsf.edu/mediawiki/index.php?title=Analysis_Software_Repository