### **IASS Guide to ImageJ**

ImageJ is a freely available piece of software for generating numerical data from images. Though simple to use, it has been cited 1000's of times in many areas of scientific research in peer reviewed papers.

Please follow the link on the IASS webpage to download the correct version for your own PC:

http://imageanalysis.weebly.com/resources.html

The application was developed for medical applications, to allow various sorts of cells within tissues to be labelled. In recent years the applications have been greatly varied. In the context of ROBEX and the Remote Exploration of Extreme Environments, its main applications are for:

- 1) Determining **linear distances** in images. (Lengths of ice cracks on Europa, lengths of animal tracks, distances between animals)
- 2) Determining **areas** within images (Areas of a seafloor type, areas made up of lunar highlands or lowlands)
- 3) Quantifying **points** in an image or image area. (e.g. Craters on the moon, starfish on a seabed, burrows in mud)

These three simple tasks are demonstrated here.

#### **Opening the ImageJ interface:**

As soon as you start up ImageJ, you get this menu bar appear:

👱 ImageJ			23
File Edit Image Process Analyze Plugins Window Help	)		
	\$	*	*
Magnifying glass (or use "+" and "-" keys)			

ImageJ window GUI.

#### Opening an image:

Go to 'File', select 'Open', navigate to the image you want. If the image is big, it may well be viewed at less than 100% size. Change this in 'Image'>'Zoom'

#### 1) Determining pixel distances:

The image above shows the 5<sup>th</sup> tool highlighted. This is the line tool. With it selected, press and hold the left mouse button to draw a line onto the image, releasing the button where you

want the line to end, as shown:



After releasing the button, go to 'Analyze', 'Measure'... this give the following table popup:

₫ R	d Results								
File	e Edit	Font							
	Area	Mean	Min	Мах	Angle	Length	<u> </u>		
1	202	128.461	34.781	208.570	-47.017	200.943	E		
							+		
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The length of the line, in pixels is given.

If further lengths are required, repeat the measurement procedure. The initial line will vanish from the image, but the new length will appear in the results window below the last measurement:

⊈ Re File	esults Edit	Font					
	Area	Mean	Min	Max	Angle	Length	
1	202	128.461	34.781	208.570	-47.017	200.943	
2	197	127.441	34.269	205.217	-56.634	196.367	
							E
•						ш	

# 2) Determining pixel areas:

To determine the area of a feature, in pixels, the 3<sup>rd</sup> or 4<sup>th</sup> tools can be used, the 'polygon selector' and the 'freehand selector'. Here we use the 'polygon selector' to draw the outline of a crater by left clicking a chain of lines around the crater rim. When the last line returns to the initial box (the start of the first line) the polygon is completed, as here:



To determine the area, go to 'Analyze', 'Measure'...

	🛓 Results									
	File Edit Font									
	Length	Angle	Мах	Min	Mean	Area				
	200.943	-47.017	208.570	34.781	128.461	202	1			
	196.367	-56.634	205.217	34.269	127.441	197	2			
E	0	0	229	7	117.247	6281	3			
	III						•			

The latest measurement is placed in the Results window below the others.

## 3 Quantifying points within an image

The procedure to count points within an image is a little different. Here in this example we will quantify the numbers of shrimp on various types of seafloor:



The shrimp are the bright dots. The red material is 'Bubblegum coral' and the grey background is 'Seabed' – for our purposes.

To open the image in ImageJ, go to 'File','Open' and navigate to your image.

Next, go to 'Plugins', 'Analyze', 'Cell counter'... the following window should pop up:

🛓 Cell Cour	nter					
Counters		Actions				
O Type 1	0	Keep Original				
O Type 2	0	Initialize				
O Type 3	0	Add				
O Type 4	0	Remove				
O Type 5	0	Delete				
O Type 6	0	Delete Mode				
O Type 7	0					
O Type 8	0	Results				
		Reset				
		Show Numbers				
		Show All				
		Save Markers				
		Load Markers				
		Export Image				
		Measure				

The left hand column, 'Counters' shows that 8 sorts of point can be counted. For this example, Type 1 will be shrimp on 'Bubblegum coral', Type 2 will be shrimp on 'Seabed'.

The first thing to do is press 'Initialize' in the window. This may cause the image to resize, so you may have to go to 'Image','Zoom' on the main ImageJ interface to correct this.

Next, select the 'Type 1' radio button and mark all the shrimp on 'Bubblegum coral'. Next, select the 'Type 2' radio button and mark all the shrimp on 'Seabed'.

After doing this, you should see an output like this:



Pressing the 'Results' button will export the counts to the 'Results' window, thus:

🛓 Resu	ults								
File	Edit For	nt							
Slice	Type 1	Type 2	Туре З	Type 4	Type 5	Type 6	Type 7	Туре 8	
Total	30	12	0	0	0	D	0	0	E
									*
×						111			1

 $\ldots \mbox{And}$  that is it for the basic operations of ImageJ.