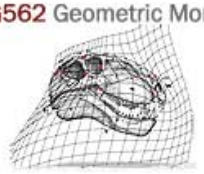

Collecting Landmarks

Important Requirements for Landmarks

Remember the following requirements for landmarks:

a. Each image must have the same number of landmarks;
b. The landmarks on each image must be in the same order;
c. Landmarks are ordinarily placed on homologous points, points that can be replicated from object to object based on common morphology, common function, or common geometry;
d. You may have to flip some images so that are not reversed left to right (e.g., if most of your images show the right side, flip left side images so that they mimic right side)

Collecting Landmark Coordinates with ImageJ

ImageJ – open-source software for image processing
http://rsbweb.nih.gov/ij/download.html

Point Picker plugin for ImageJ
http://bigwww.epfl.ch/thevenaz/pointpicker/

To install Point Picker follow these steps:

Mac OS X
1. Download the install image
2. Open the PointPicker.dmg
3. Copy the folder PointPicker to Applications -> ImageJ -> Plugins

Windows
1. Download the installation package
2. Unzip it
3. Copy the folder PointPicker to C:/Program Files/ImageJ/Plugins

To collect landmarks using *ImageJ*

1. Place all your images in a single folder by themselves
2. Start ImageJ
3. open the first image

Repeat the following steps for each image

4. Start the PointPicker plugin: Plugins -> PointPicker -> PointPicker

5. Choose the pen tool (the one with a +) to add points
6. Carefully place each of your points on the image, always in the same order. If you need to adjust the position, choose the move tool
7. When all points are placed, click the output button (the one with a piece of paper as the icon). Use the Show option.
8. Highlight all the data, copy it, paste it into Excel with one blank line above, and one blank line below
9. The x and y coordinates of your points are in the 2nd and 3rd columns.
10. Above the x-coordinates, enter the text "LM=" followed by the number of points (e.g. LM=5)
11. Below the x-coordinates, enter the text "ID=" followed by the taxon name (e.g. ID=Archaeopteryx)
12. Clear the data window (by closing or clicking the red circle (MAC))
13. Click the Return to ImageJ button (microscope icon)
14. Open next image
15. Repeat from Step 4 until all images are finished

	A	B	C	D	E	F	G	H
1								
2		LM=13						
3	0	260	6	1	0	1		
4	1	258	141	1	1	2		
5	2	259	167	1	2	3		
6	3	260	305	1	3	4		
7	4	259	388	1	4	5		
8	5	445	395	1	5	6		
9	6	403	263	1	6	7		
10	7	308	45	1	7	8		
11	8	297	168	1	8	9		
12	9	222	168	1	9	10		
13	10	217	51	1	10	11		
14	11	118	264	1	11	12		
15	12	76	394	1	12	13		
16		ID=Ateleaspis_tesselata						
17		LM=13						
18	0	192	6	1	0	14		
19	1	188	88	1	1	15		
20	2	188	111	1	2	16		
21	3	191	164	1	3	17		
22	4	192	206	1	4	18		
23	5	370	158	1	5	19		

Figure 1: screen shot from Excel showing what data look like at step 11 above.

Save the landmarks in TPS format

1. In Excel, highlight columns 2 & 3 from the first "LM=13" to the last "ID=Ateleaspis_tesselatai" and copy. For this lab that should be 165 rows with two columns.
2. In Word, use Paste Special to paste these rows as **plain text**. If you don't paste as plain text then you will have trouble importing the data into Mathematica.
3. Save the Word document **as plain text** somewhere convenient.
4. Import TPS file into Mathematica using the *tpsImport[]* function in the Polly Morphometrics add-in.

```
data = tpsImport["/Users/pdavidpolly/Documents/Data/Mandibles.TPS"];
```

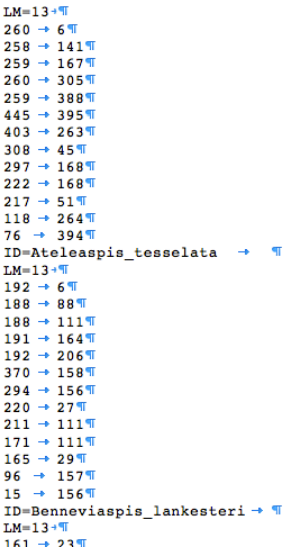

Figure 2: screen shot from Word showing what data look like in "TPS" format at step 2 above.

Collecting landmarks in Mathematica

If you are dexterous you can collect your landmark data directly in *Mathematica* using the Get Coordinates tool (see *howto/GetCoordinatesForPointsInAPlot* in the *Mathematica* Documentation Center).

2. Place all of your images in the same folder by themselves
3. Use the SetDirectory[] function to select the folder. You can use Insert -> File Path to make this easier, but you have to (a) select an image in the folder and (b) remove the name of that image from the path as follows:

```
SetDirectory["/Users/pdavidpolly/Documents/Mandibles/image1.jpg"]
SetDirectory["/Users/pdavidpolly/Documents/Mandibles/"]
```

4. Save the image file names in a variable using the FileNames[] function. Selection of files can be controlled by giving an argument that specifies which file names to load. * means any character, so "*.JPG" would load all files ending in capital JPG, "DSCN*" would load all files starting with DSCN.

```
filenames = FileNames["*.JPG"]
```

5. Import the images into a list.

```
imgs = Table[Import[filenames[[x]]], {x, Length[filenames]}];
```

6. Display all the images, or selected ones.

```
imgs
```

```
imgs[[1]]
```

```
imgs[[1;;5]]
```

7. Activate the "Get Coordinates" tool by right clicking on one of the images, or by starting Drawing Tools under the *Window* menu and choosing the cross-hair tool.

8. Select landmarks by clicking on the image with the tool. You may need to enlarge the image by resizing it or by choosing a magnification option from the right click menu. Click Ctrl-C (Cmd-C on Mac) or Copy from the Edit menu to copy the coordinates.

   Note: remember to copy the coordinates before choosing another tool or entering text in the Notebook otherwise the points will disappear.

9. Paste the landmarks into the Notebook, and repeat for each image. You will need to format them by placing a set of curly braces "{" around the entire collection of landmarks and separating the landmarks from each image with a comma.

```
lands = {
    [Paste landmarks here] ,
    }
```

   This will result in the following:

```
lands = {
    {{1221.5`, 653.5`}, {1167.5`, 539.5`}, {1238.5`, 438.5`}, {1049.5`, 469.5`}},
    {{806.5`, 633.5`}, {850.5`, 633.5`}, {1083.5`, 830.5`}, {1156.5`, 631.5`}}
    }
```

Collect landmarks with tpsDig (PC only)

1. Install *tpsUtil* and *tpsDig* programs from Rohlf's morphometrics site (http://life.bio.sunysb.edu/morph/)

2. Place all images in a single folder by themselves.

3. Use tpsUtil to create a new data file from images.
   a. Select operation.
   b. Select folder by clicking on one of the images.
   c. Select output file by choosing folder and giving the file a new name.
   d. Confirm which images you want under Actions.
   e. Click "Ok" to create empty file.

4. Open empty data file in tpsDig. Your images should appear in the program.
   a. Use cross-hair tool to add landmarks;
   b. Right click to delete landmarks;

c. Use arrow tool to move landmarks;
d. Use arrow buttons to move to next image;
e. Use + and – buttons to enlarge or reduce image on screen;
f. Use “Save data” to save coordinates into TPS file (overwrite existing data).

5. Import TPS file into Mathematica using the *tpsImport[]* function in the Polly Morphometrics add-in.

```
data = tpsImport["/Users/pdavidpolly/Documents/Data/Mandibles.TPS"];
```