

## Optimas 6.5

### General Methods

First open up Notepad and save a blank spreadsheet – with a meaningful title e.g. otolith increment recruits Jan 09. Then close this file.

Next start up Optimas

Go to **File**

**Open Image file** – find the photograph you want to work on

Note: every image needs to have a scale bar in the photograph (if the photographs are taken in the histology lab a scale bar will be on the photograph, if you have taken the photographs in McCormick's lab you will have done this manually, the scale must be visible in the photograph you want to analyze)

Then go to **Data**

**Calibrate Spatial**

Then go to the **Add/Edit** tab

You can **change the name** to something more meaning full e.g. scale bar

Also you need to **change the units** depending on the resolution and scale bar in the photograph e.g. change from centimeters to mm, or micrometers, nano etc

Select **calibrate single distance**

Note:

You will calibrate on the image a known distance from the scale bar that was initially associated with the photograph, the program then uses this to make measures based on number of pixels.

**Click Calibrate**

With the line mouse **click release** at the beginning of the scale bar and **click again** at the end to trace the scale bar. Then **right click**, a window will pop up.

**Enter the line length** (known scale bar length) in mm, or mico, nano etc

**Press ok**

Double **click** on **Scale bar**

Close tab

Go to **Data**

**Point Morphometry**

**Select the show reference box** – the reference point is the point you want to make the measurements from – it may be a certain check mark in the otolith that you want to measure growth from i.e. recruitment mark, or sex change mark etc.

Click **Move Reference** – this allows you to place the reference point in the correct position.

Click **Mark Points** – this allows you to mark the points you want to measure the distances between i.e. the increments between the rings on the otolith.

Work from the **reference point out**

Once you finish marking all the points, **Right click**

Go to **File**

**Open data file** – find the data file you saved earlier in Notepad e.g. otolith increment recruits Jan 09

A **Warning** window will pop up saying that the data file already exists, press on **Append** (if you press **Replace** you will lose all data previously recorded on the file).

On the **Header information** window, write down the **Title** of the otolith measurements. Again give **meaningful names** when saving the data. **Press ok**

Press on the **Save Data** tab – if you have already saved data in this file from other otolith measurements simply push amend when prompted.

Follow the same procedure if you wish to do Line Morphometry or Area Morphometry.

Line Morphometry

**Data**

**Line Morphometry**

**Draw Line** – to keep the line straight click once release and click again at the end of the measurement, **right click** to take you to the data tab.

You can either **manually write down** the measurement **or save to the data file** using the procedure mentioned above

Remember to **always check** the **measurements** are in the **correct units**

And **remember** you will need to **calibrate each image**

**Area Morphometry**

Press **Draw or trace an area** (third icon on the Menu). This allows you to trace an area, i.e. if you wanted to look at the **shape** of the **otoliths; symmetry/asymmetry**. Before doing this, the otolith has to be in contrast with the background (i.e. white otolith on black background).

**Click** on a starting point on the edge of the otolith. Then press **Ctrl + click** next to the starting point (but still on the edge of the otolith). The program will start tracing the area of the otolith.

Press **Ctrl** for the area to be traced fast.

Go to **Data, Area Morphometry**.

Same as above.

**Make measurements of the**

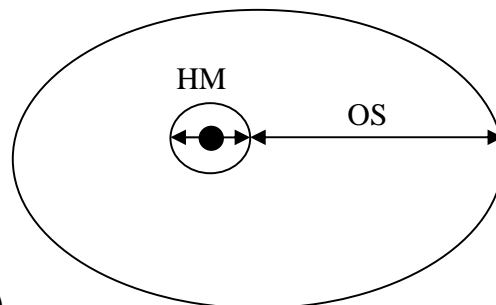
**Hatch Mark (HM)** – the nucleus and the area to the first ring.

This 'hatch mark' is usually circular in shape, and after which  
The increments start to become elliptical.

(Using line Morphometry)

**Otolith size (OS)** – the nucleus ring to the outer edge (longest axis)

(Using line morphometry)



**Increment widths – distance between the rings. We try and use the longest axis here because it will minimize the measurement error. It doesn't really matter which axis of the otolith cross section you choose, you just have to be consistent within and among otoliths you expect to compare.  
(using point morphometry)**