Otolith Aging and Analysis

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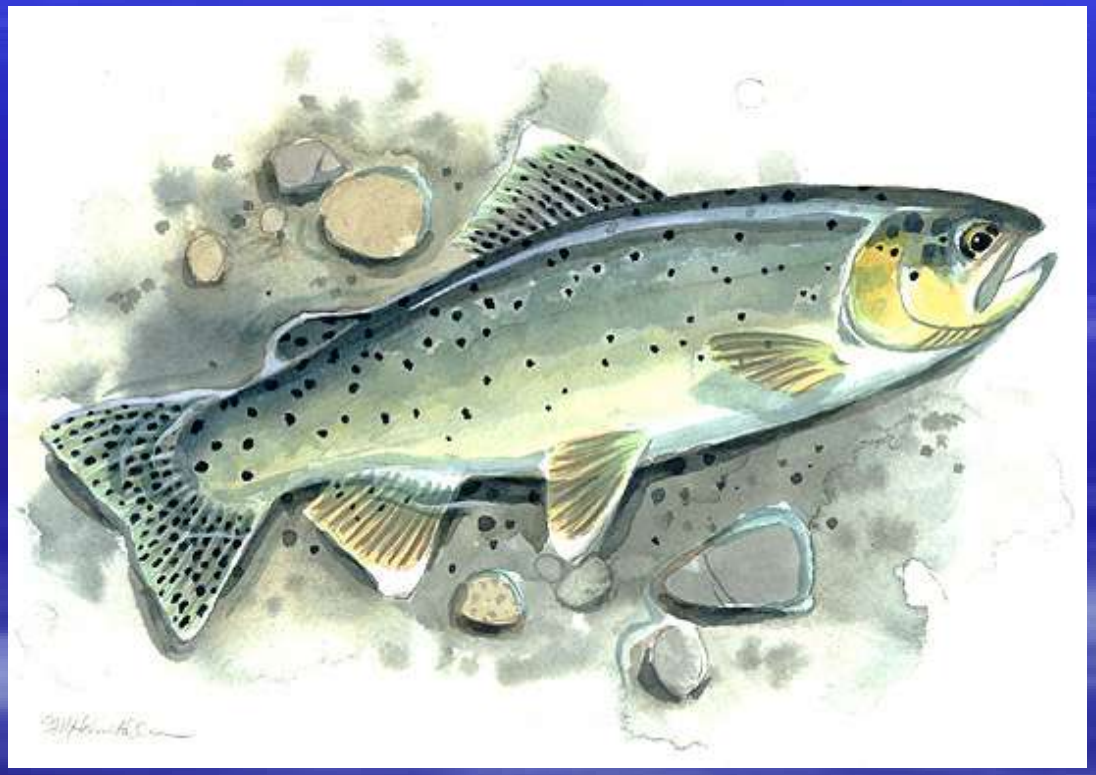
Project Goals

- Create a program that calculates a fish's age by anaylzing digital images of it's otoliths.

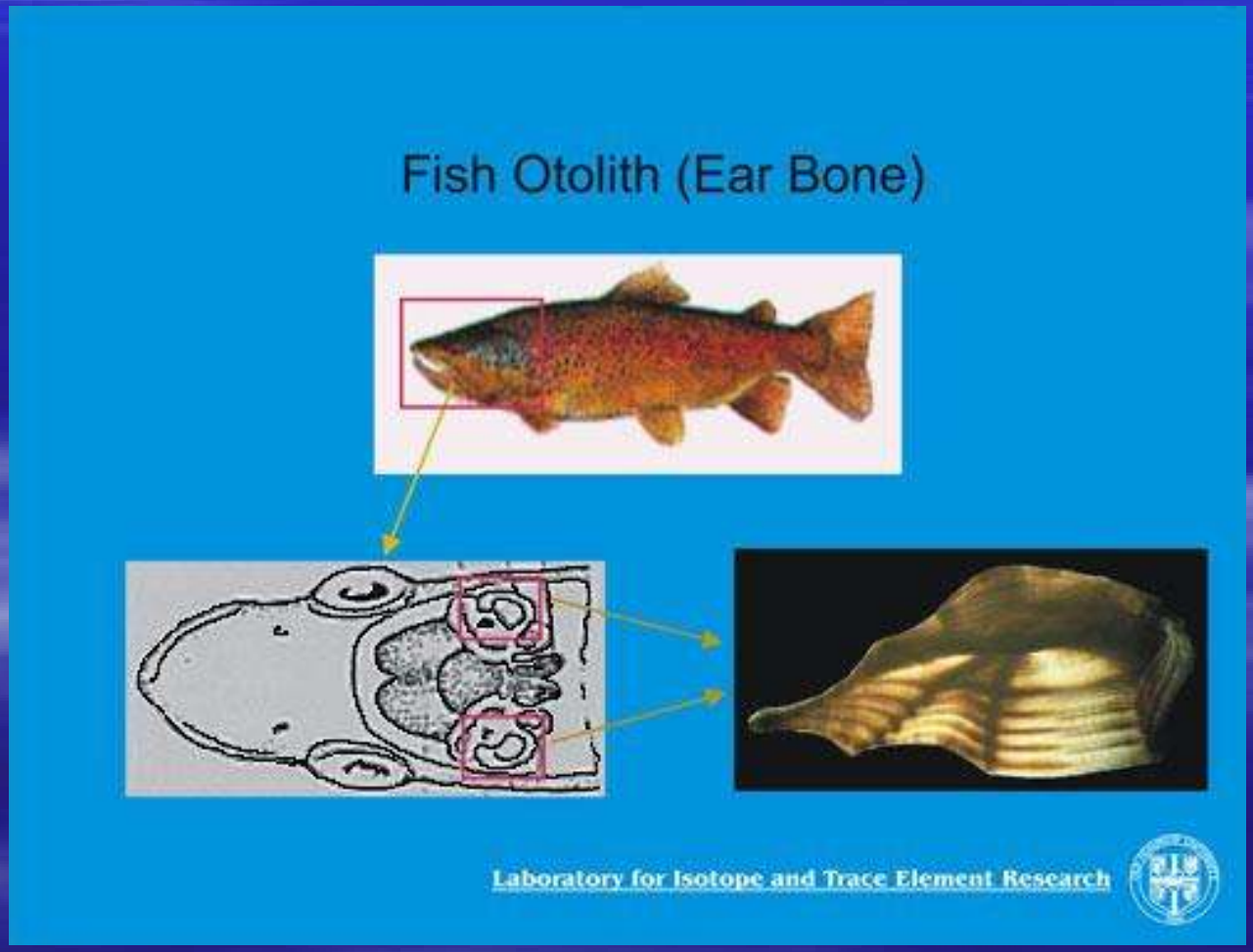
Structures used to age fish

- Scales
- Bones
- Fin Rays
- **Otoliths**



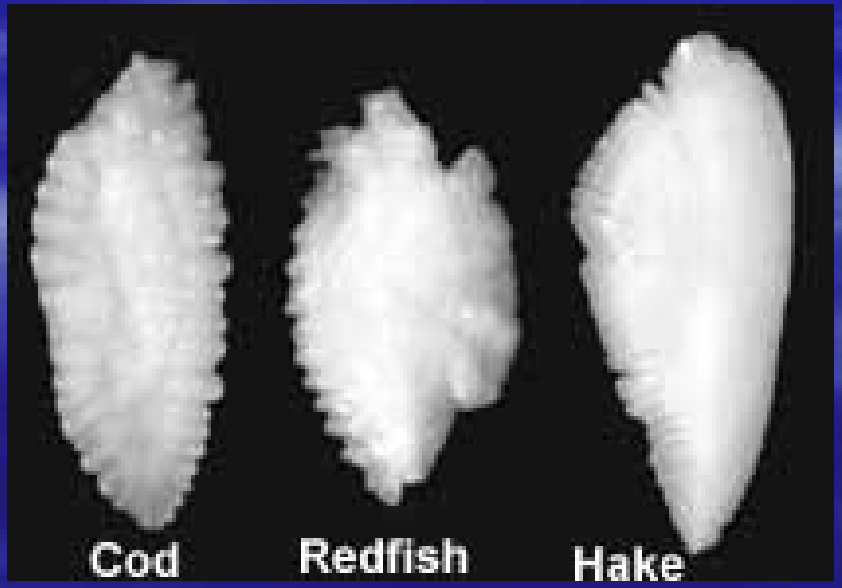
Otoliths

Otoliths are small calcified structures used for balance and hearing in fish.



Otolith Structure

- Each fish has three pairs
  - Sagittae
  - Lapilli
  - Asteriscii
- Different shapes and sizes



Formation of Annuli

- Otoliths have continuous growth. So as new material is added to the outside surface the older material is preserved providing a record of the fish's life.
- Otoliths form daily rings which during periods of slow growth pile up and form annular rings.

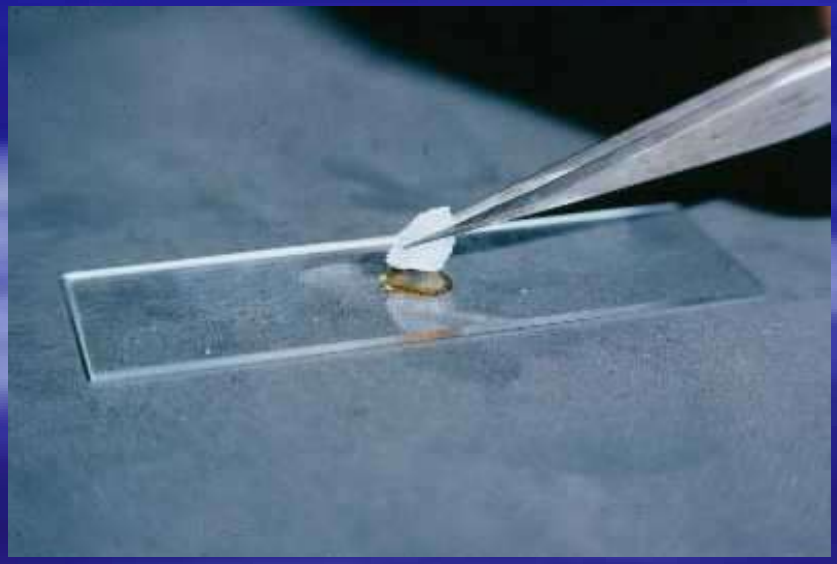
How Biologists Use Otoliths

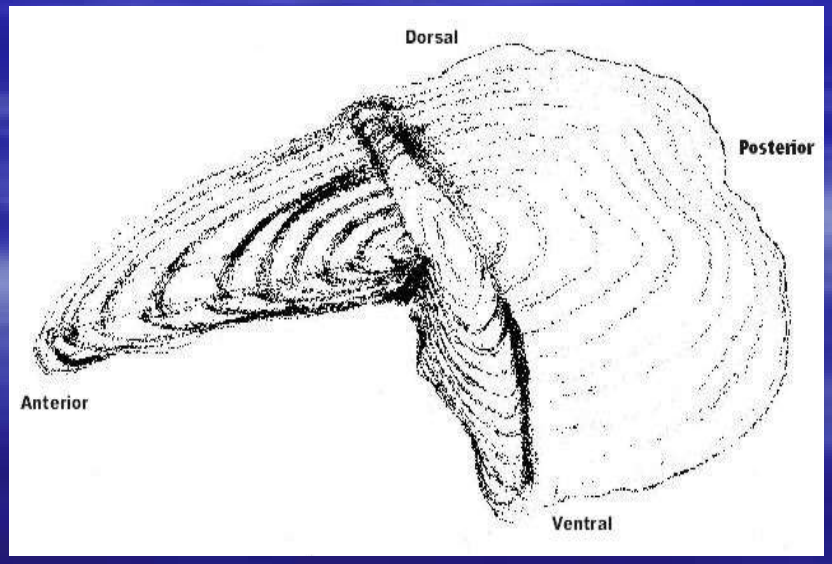
- Temperature history (Patterson et al. 1993)
- Anadromy (Secor 1992)
- Migration Pathway (Thresher et al. 1994)
- Stock Identification (Edmonds et al. 1989)
- Used as a natural tag (Campana et al. 1995)
- Age Validation (many publications)

Otolith Preparation

(Very time consuming process.)

- Step one: Mounting on slide
- Step two: Grinding until translucent





Examples of images

Matlab

Three Parts

- Image enhancement
- Methods for counting and measuring distance between annuli
- Backcalculations

Image Enhancement

- Rgb2gray intensity image

Image Enhancement

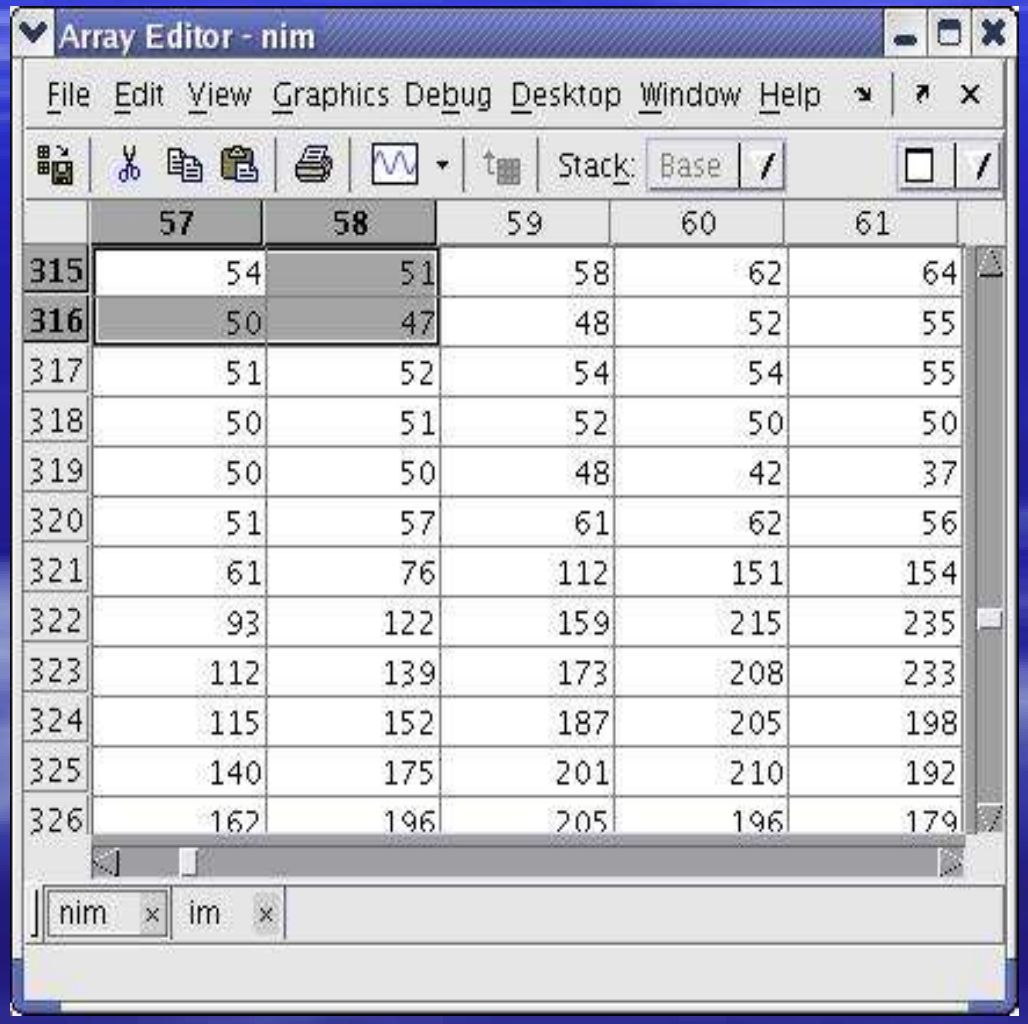
- Adapthisteq
- Transforms pixal values using contrast-limited adaptive histogram equalization (CLAHE)

Image Enhancement

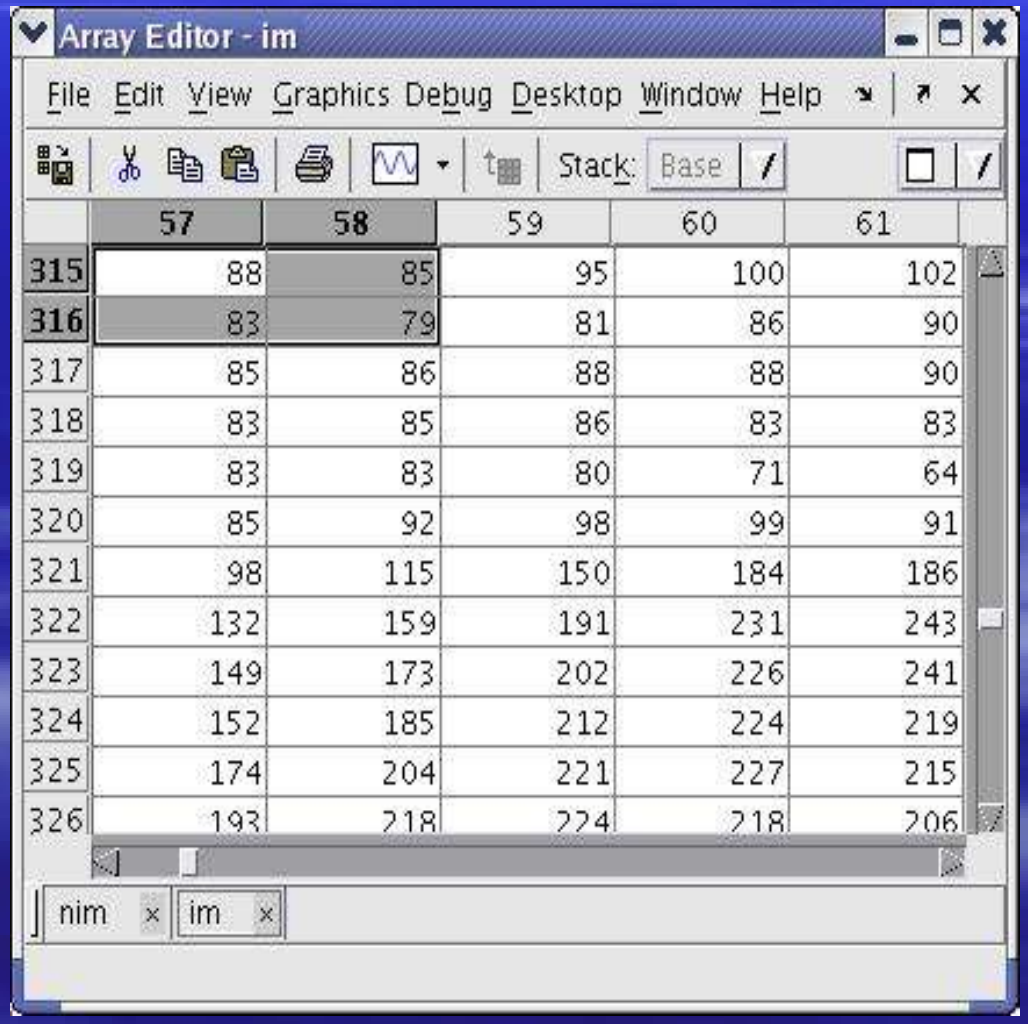
- Subdivides the image into n×m blocks, calculating the histogram of each such block.
- For each block, a histogram equalization is formed, which transforms the intensity values so that they are apporixmately similar.
- Adapthisteq parameter “Numtiles” allows user to select n×m block.

Image Enhancment

Example 'Numtiles', [2 2]



	57	58	59	60	61
315	54	51	58	62	64
316	50	47	48	52	55
317	51	52	54	54	55
318	50	51	52	50	50
319	50	50	48	42	37
320	51	57	61	62	56
321	61	76	112	151	154
322	93	122	159	215	235
323	112	139	173	208	233
324	115	152	187	205	198
325	140	175	201	210	192
326	162	196	205	196	179



	57	58	59	60	61
315	88	85	95	100	102
316	83	79	81	86	90
317	85	86	88	88	90
318	83	85	86	83	83
319	83	83	80	71	64
320	85	92	98	99	91
321	98	115	150	184	186
322	132	159	191	231	243
323	149	173	202	226	241
324	152	185	212	224	219
325	174	204	221	227	215
326	193	218	224	218	206

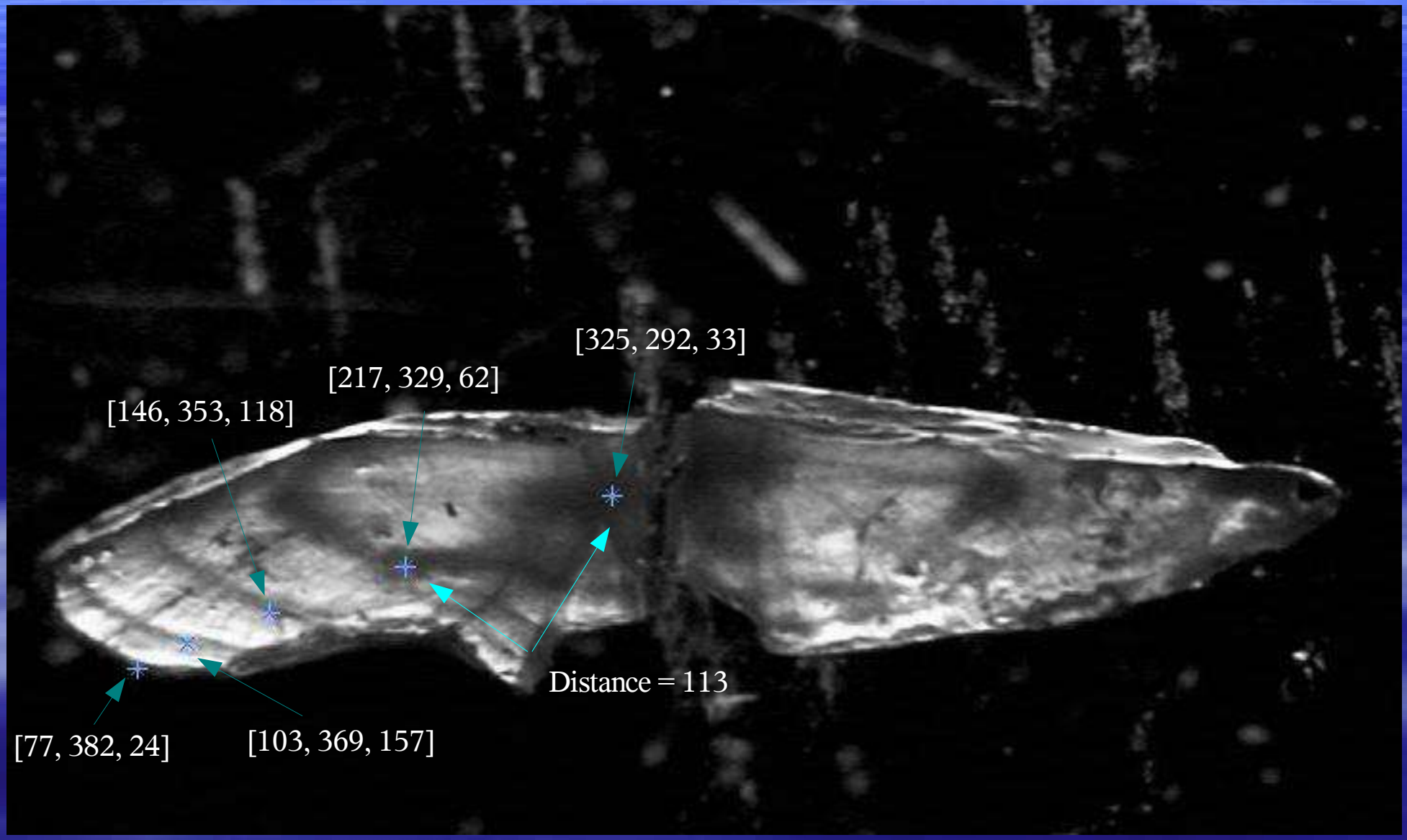
Measuring Annuli

- Manually
  - Allows user to click on each annuli.
  - Keeps count.
  - Measures annuli distance.
- Semi-automatic
  - User selects area of otolith to count.
  - Keeps count.
  - Measures annuli distance.

Measuring Manually

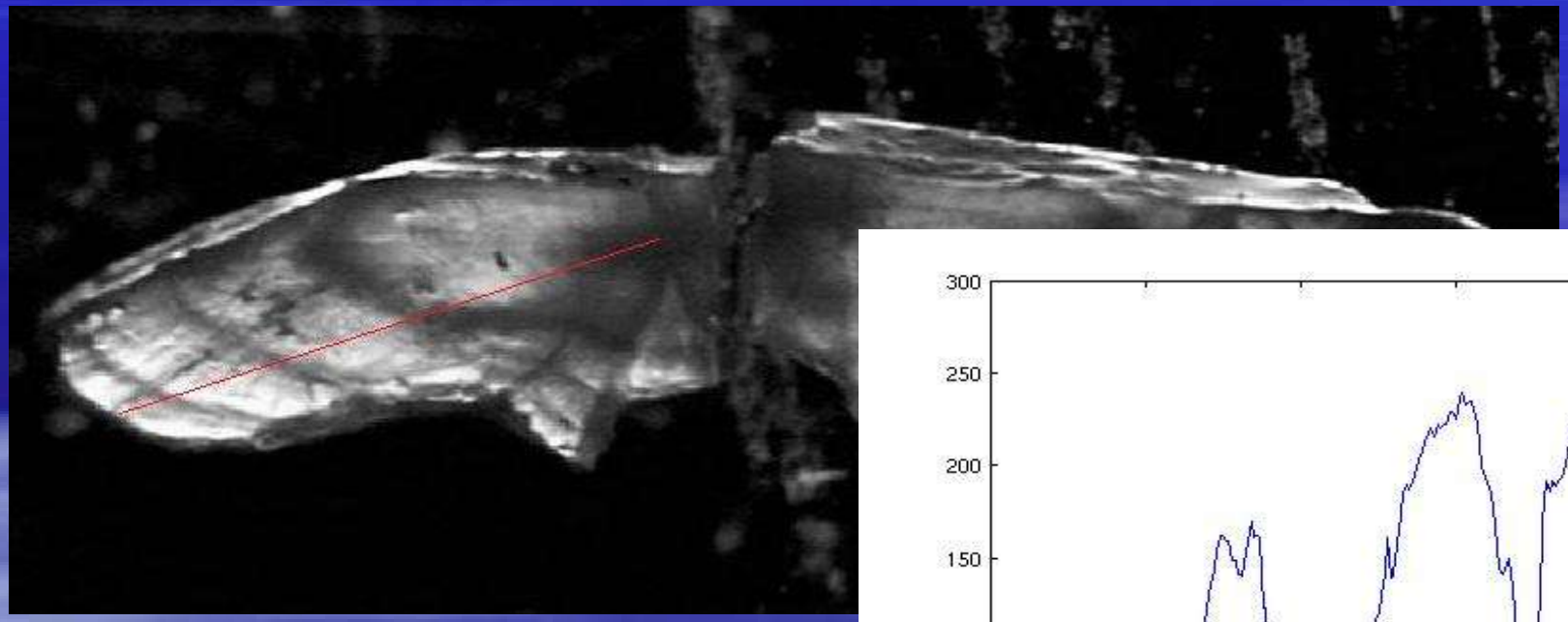
- Impixel lets user select any point on the image by clicking mouse.
- Outputs [x, y, intensity].
- Count the number of annuli and measure distance from each point to focus of otolith.

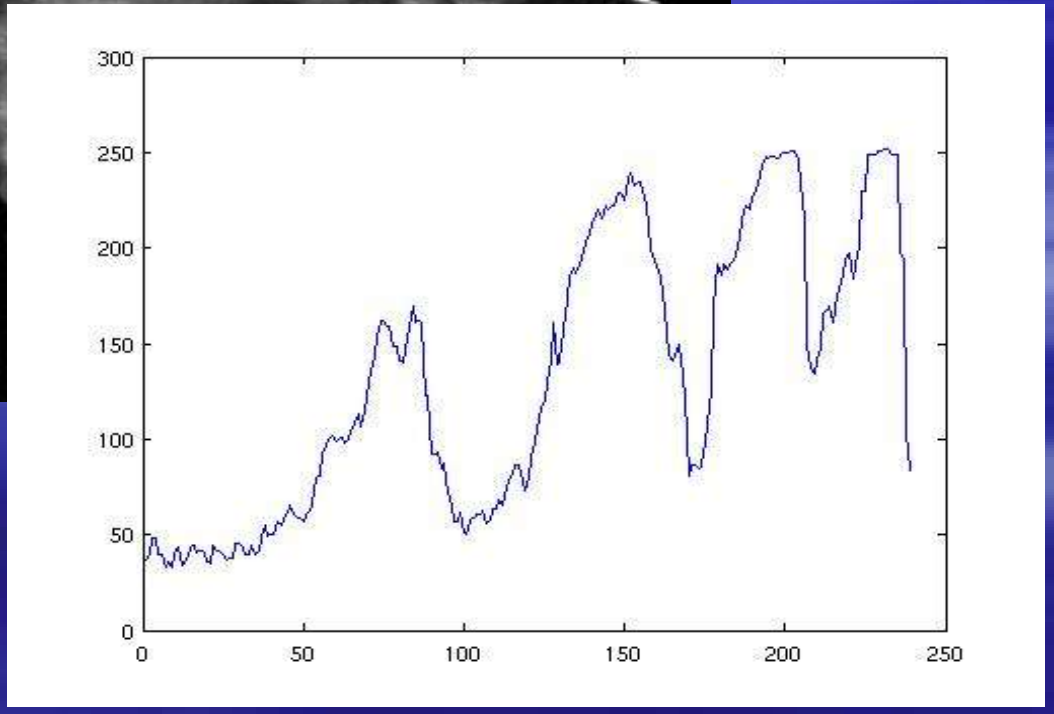
Measuring Manually



Semi-automatic Measuring

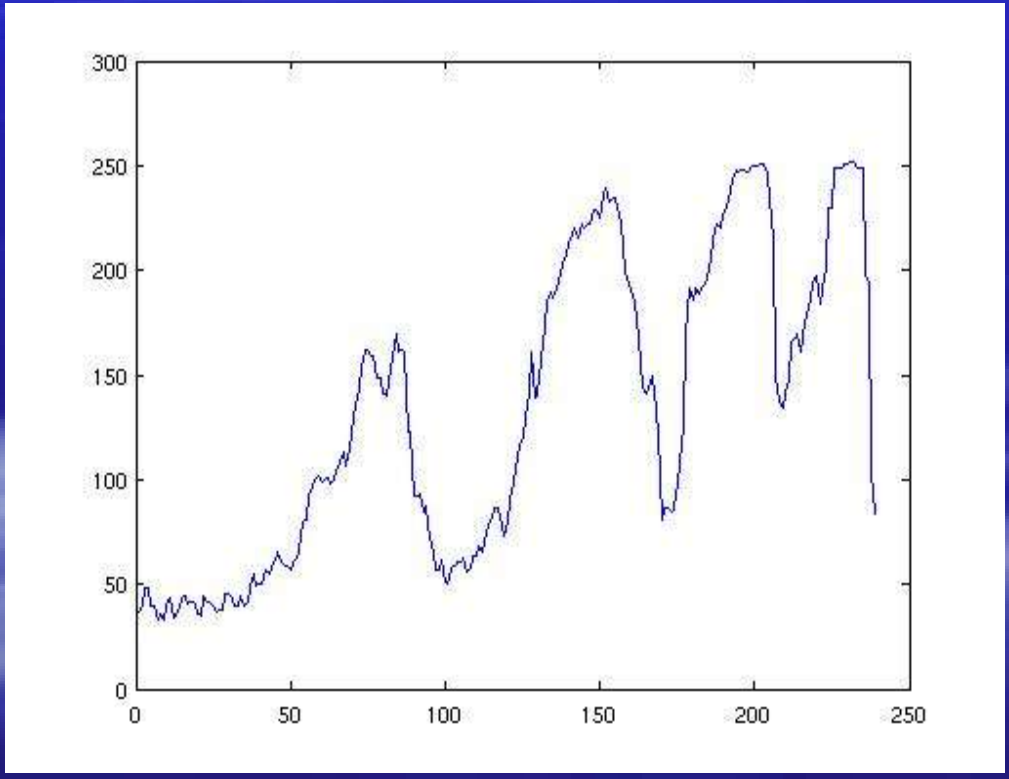
- Improfile
  - Computes the intensity values along a line or multiline path in an image.



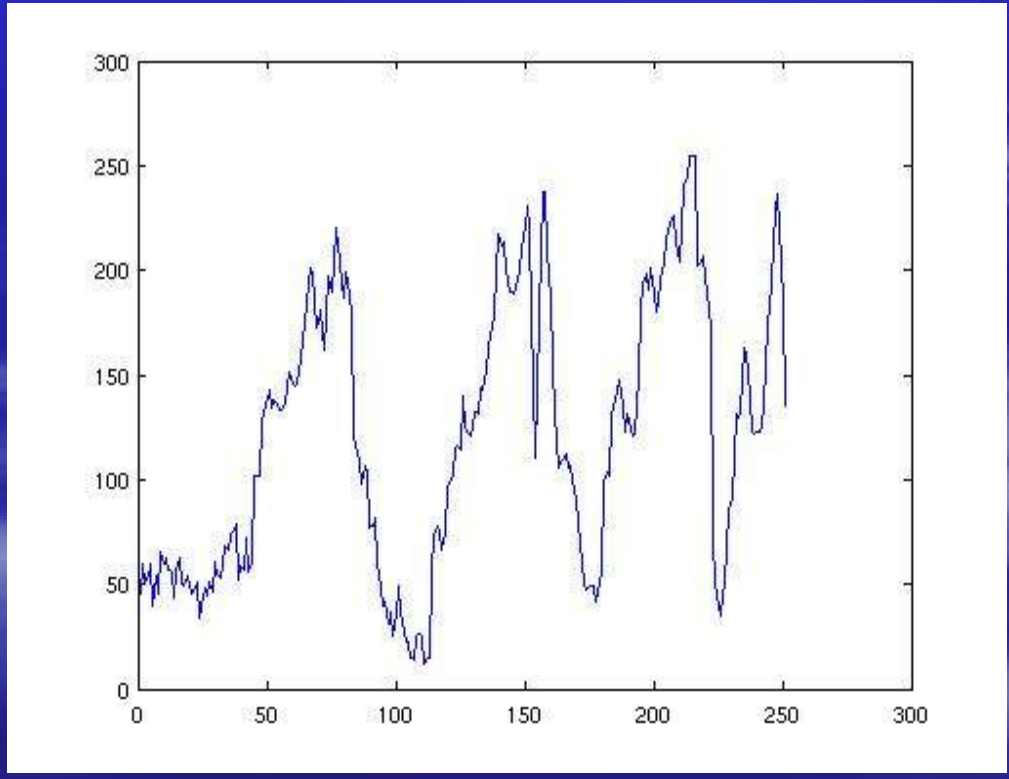


Measuring Annuli

- Profiles of images before and after enhancement.



Large Mouth Bass Age 3
“ No adjustment ”



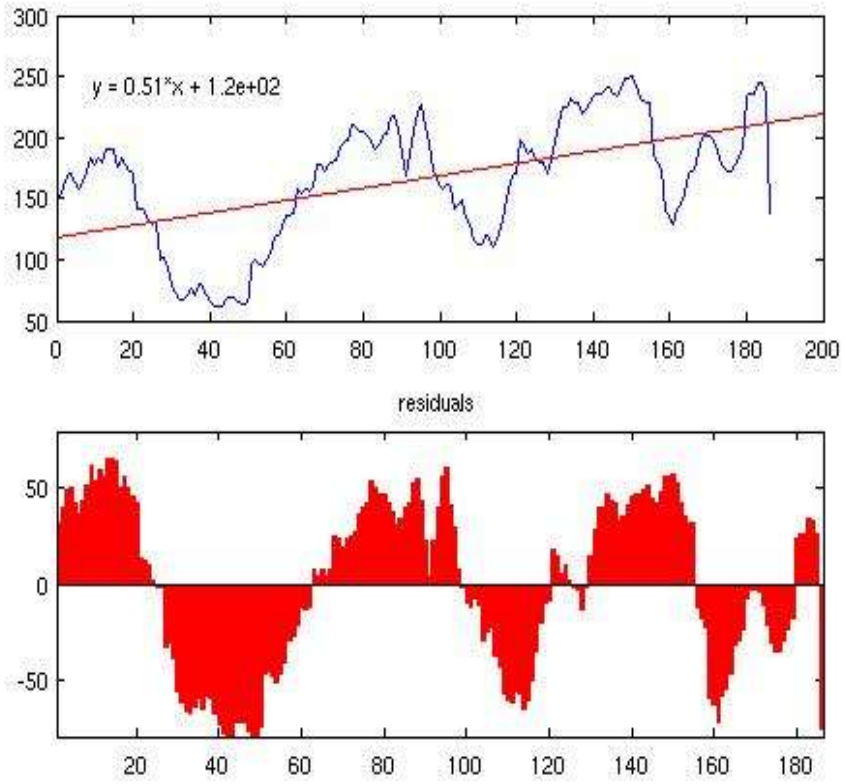
Large Mouth Bass Age 3
“ 'Numtiles', [20 20] ”

Counting Annuli

- Polyfit p = polyfit(x,y,n)
  - Finds the coeffecients of a polynomial p(x) of degree n that fits the data.
  - Linear p = polyfit(x,y,1)
  - Quadractic p = polyfit(x,y,2)

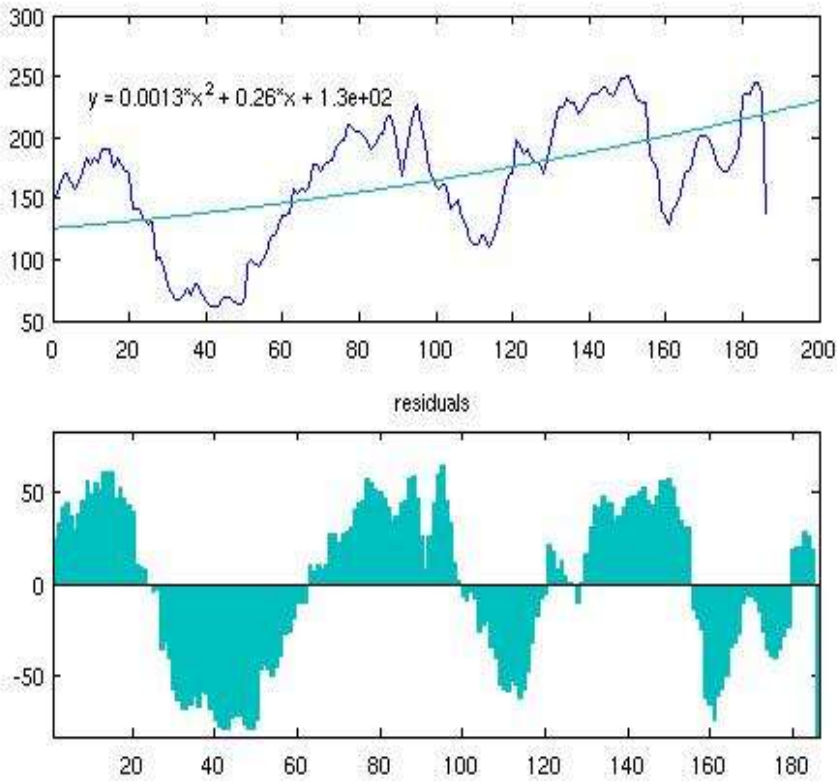
Examples

- Linear



Large Mouth Bass Age 3

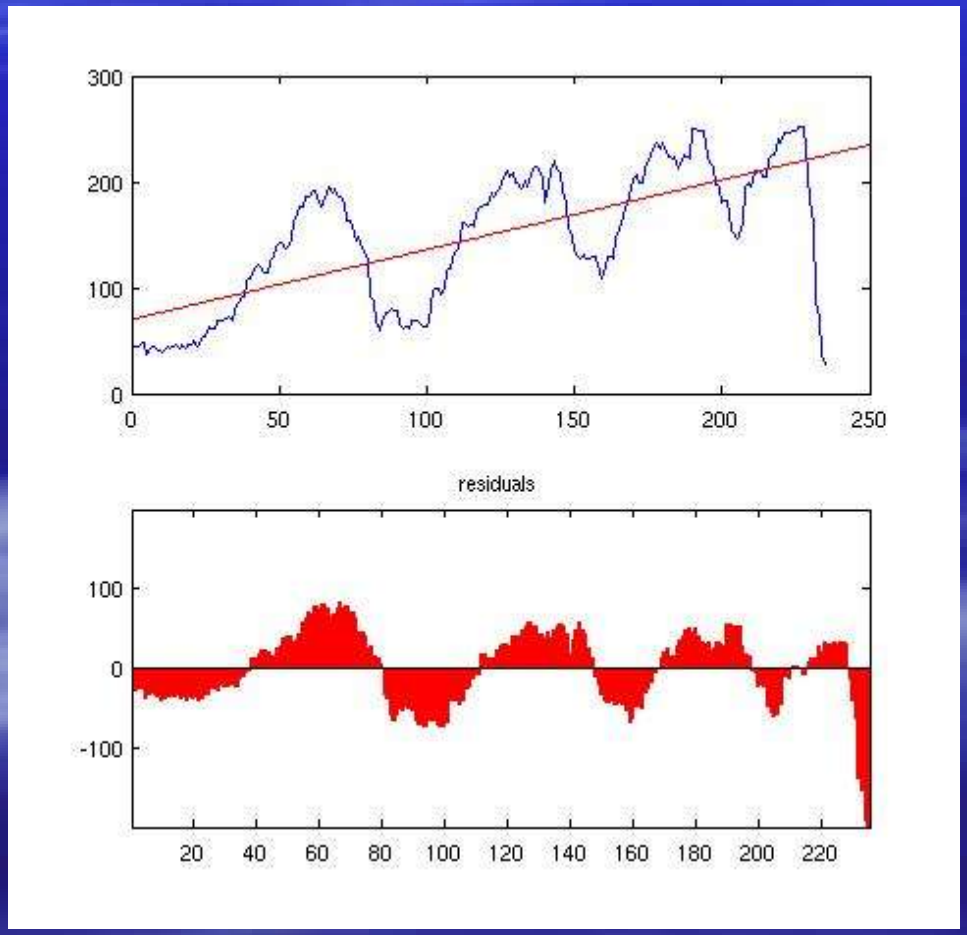
- Quadratic

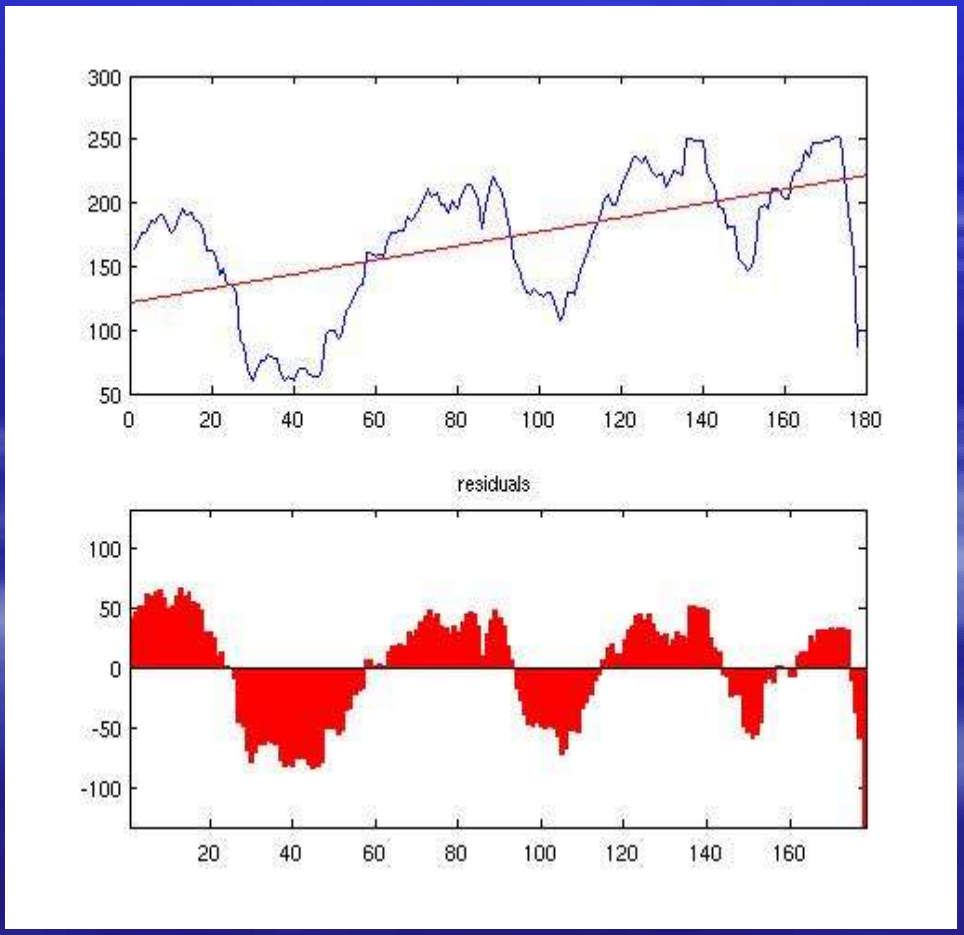


Large Mouth Bass Age 3

Counting

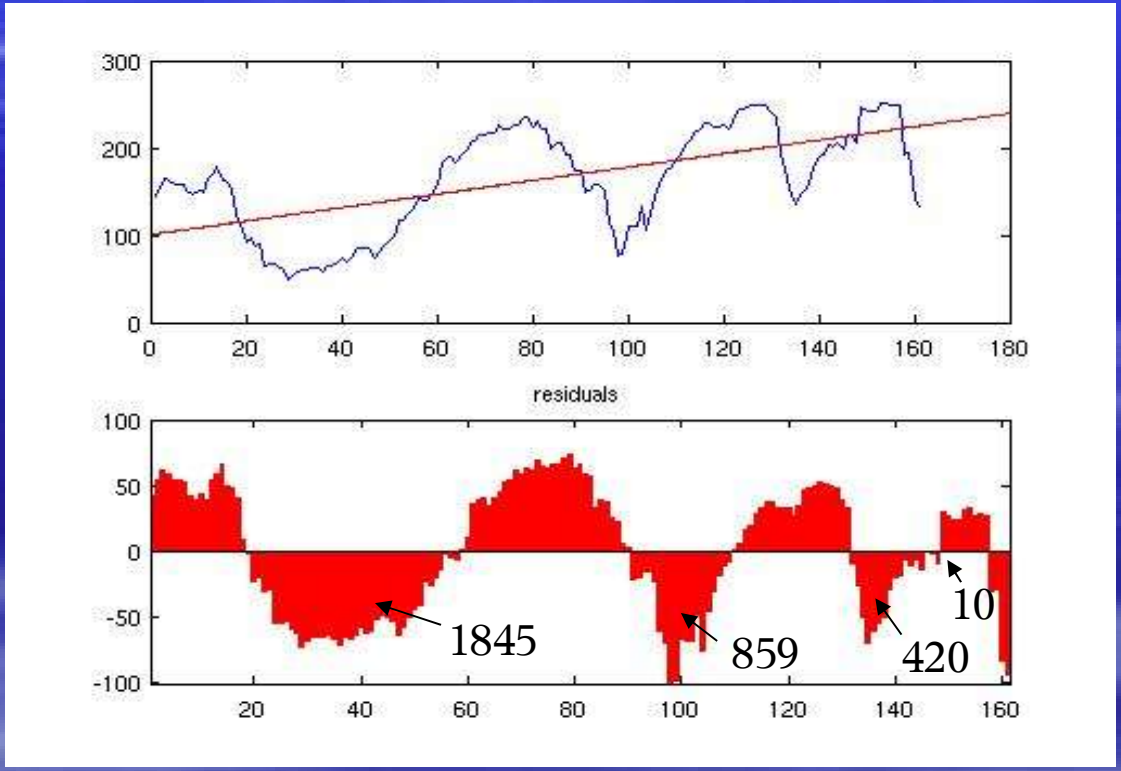
- Calculate area under polyfit line
- For better polyfit disregard first area plotted by focus





Counting

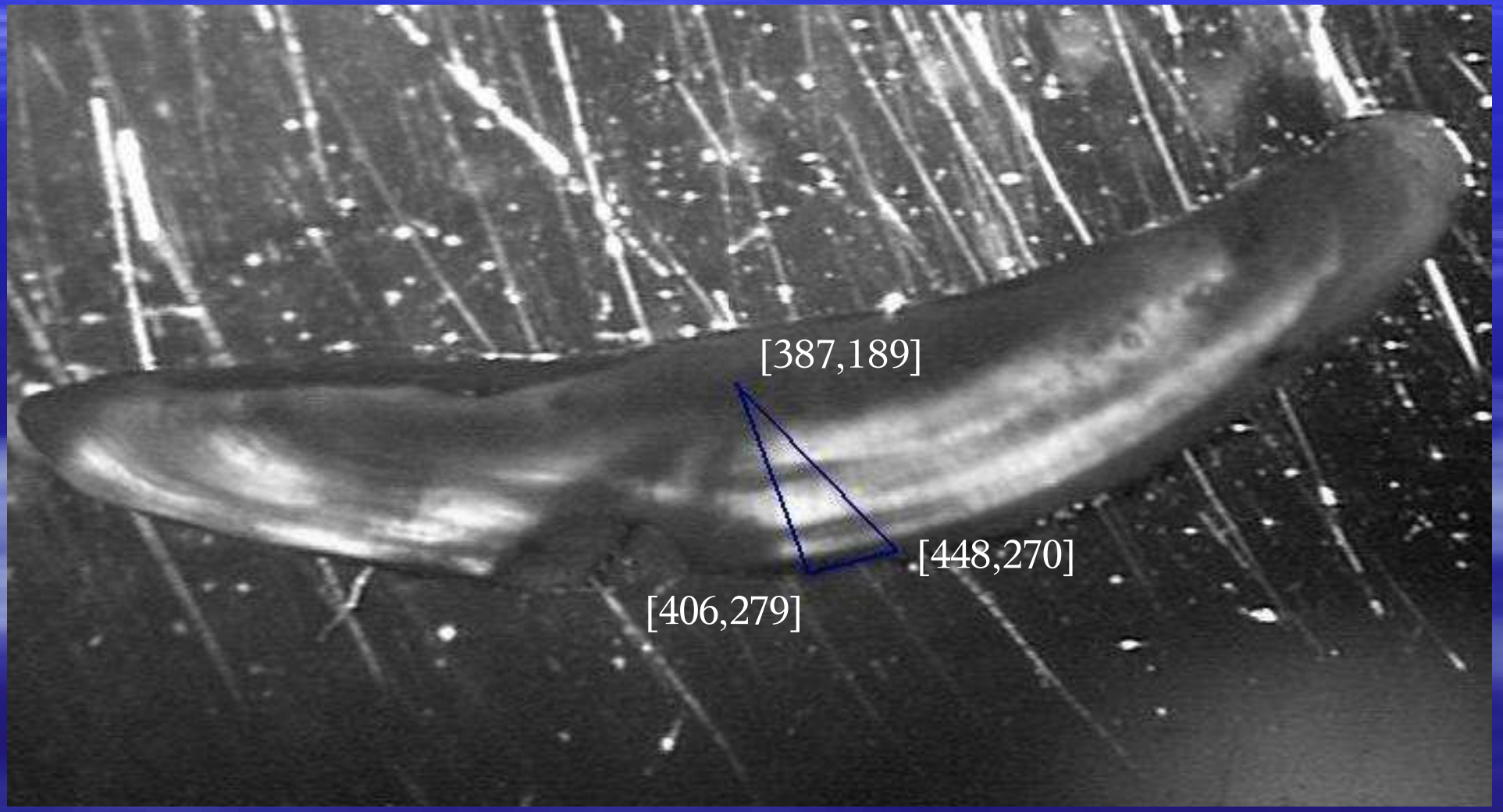
- Measure residuals below the polyfit.
- Take average residual and multiply it by a stringency factor between 0.1 and 0.5.



Median = 640
Stringency = 0.3
Anything less that 192 will not be counted as an annlus.

Semi-automated Aging

- Select an area of interest.



Measuring Annuli

- Takes Width at edge of area selection to calculate number of rays.

Backcalculations

- Growth backcalculations are one of the most powerful applications of the otolith and are used to estimate fish length at a previous age.
- Backcalculations are a relationship between otoliths and fish length.
- Three Models
  - Frasier-Lee
  - Biological Intercept
  - Weisburg

Frasier-Lee (Regression Model)

$$Li = a + (Lc - a)(Si / Sc)$$

- *Li = BCL at annulus i*
- *Lc = length at caputre*
- *Si = otolith radius to annulus i*
- Sc = total otolith radius
- a = correction factor (used only when aging with scales otherwise = 0)

Biological Intercept Model

- Modified version of the Frasier-Lee model.
- Accounts for systematic variation in fish length. Otoliths of slow-growing fish tend to be larger and heavier than fast-growing fish of the same size.
- Biological Intercept can be determined by simple measurements of fish and otolith size in newly-hatched larvae in the laboratory

Biological Intercept Model

$$Li = Lc + (Lc - L0)(Si - Sc)/(Sc - S0)$$

- Li = BCL at annulus i
- Lc = lenth at capture
- Si = otolith radius to annulus i
- Sc = total otolith radius
- L0 = correction factor for body length
- S0 = correction factor for otolith length

Weisberg Model

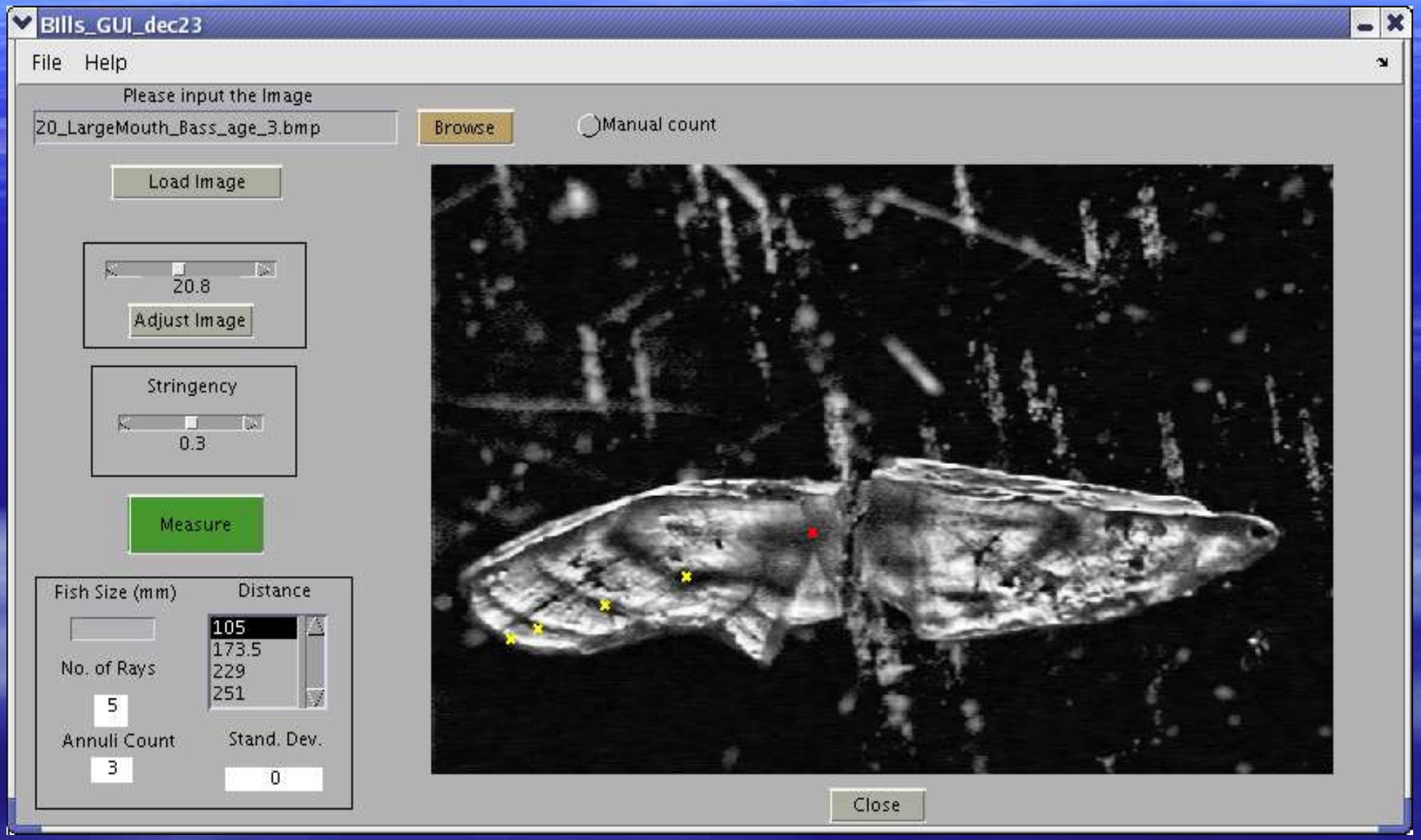
Expected annual growth increment = Age effect + Year effect x Year Interaction

- Uses a linear model to separate age- and year-specific effects on otolith growth.
- Similar to that of a two-way analysis of variance, where age of fish and growth year are factors assigned to the response variable of average increment width in a growth year for a given age of fish.
- The year interaction describes how environmental conditions affect fish of different ages.

Age1 + Year1993 = 1.7
Age2 + Year1994 = 1.4
Age3 + Year1995 = 0.9
Age1 + Year1994 = 1.9
Age2 + Year1995 = 1.2
Age3 + Year1996 = 0.7

Year	Age1	Age2	Age3
1993	1.7		
1994	1.9	1.4	
1995		1.2	0.9
1996			0.7

Graphical User Interface



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Mathworks website: http://www.mathworks.com

Otolith Research Laboratory

Bedford Institute of Oceanography

http://www.mar.dfo-mpo.gc.ca/science/mfd/otolith/english/home.htm

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