

Voucher Imaging and Analysis

Presented by
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Mark, Tag and Age Laboratory
Alaska Department of Fish and Game

Vouchers & Mark Assessment

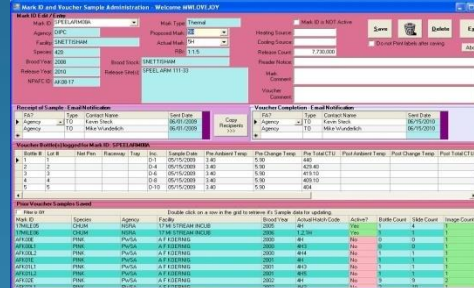
- Hatcheries submit vouchers from each mark group.
- Bottles are logged in by unique Thermal Mark Id (TMID).
- Vouchers are dissected, mounted, examined and imaged.
- Images are chosen that represent the variability of each mark group.
- Variability is quantified in terms of appearance, ring counts and distances.
- Images are measured, annotated and graded then submitted for approval before uploading to website.

Voucher Submission and Imaging Overview

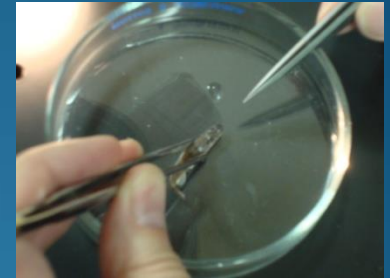
RECEIVE
VOUCHERS



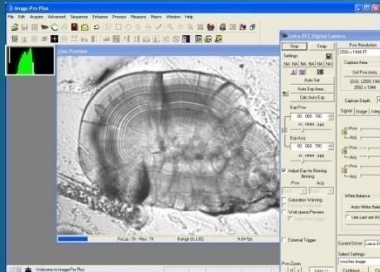
LOG IN
VOUCHERS



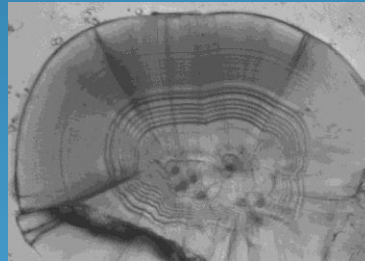
DISSECT AND
MOUNT VOUCHERS



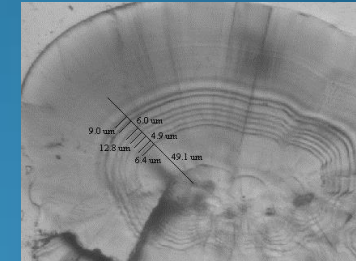
VOUCHER IMAGES
TAKEN AND EXAMINED



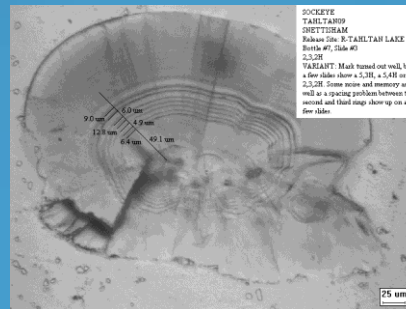
REPRESENTATIVE
IMAGES CHOSEN



IMAGES ARE LABELED,
ANNOTATED AND GRADED



FINAL IMAGES
ARE APPROVED
AND ENTERED INTO
ONLINE DATABASE



Receipt & Log In of Vouchers

- The Thermal Mark Lab uses a custom application to log samples into an ORACLE database by their unique TMID (thermal mark identification name).

Mark ID and Voucher Sample Administration - Welcome MWLOVEJOY

Mark ID Edit / Entry

Mark ID: SPEELARM08A Mark Type: Thermal Mark ID is NOT Active

Agency: DIPC Proposed Mark: 5H Heating Source: _____

Facility: SNETTISHAM Actual Mark: 5H Cooling Source: _____

Species: 420 RBr: 1:1.5 Release Count: 7,730,000

Brood Year: 2008 Brood Stock: SNETTISHAM Reader Notice: _____

Release Year: 2010 Release Site(s): SPEEL ARM 111-33 Mark Comment: _____

NPAFC ID: AK08-17 Voucher Comment: _____

Receipt of Sample - Email Notification

FA?	Type	Contact Name	Sent Date
Agency	TO	Kevin Steck	06/01/2009
Agency	TO	Mike Wunderlich	06/01/2009

Voucher Completion - Email Notification

FA?	Type	Contact Name	Sent Date
Agency	TO	Kevin Steck	06/15/2010
Agency	TO	Mike Wunderlich	06/15/2010

Voucher Bottle(s) logged for Mark ID: SPEELARM08A

Bottle #	Lot #	Net Pen	Raceway	Tray	Inc.	Sample Date	Pre Ambient Temp	Pre Change Temp	Pre Total CTU	Post Ambient Temp	Post Change Temp	Post Total CTU
1	1				D-1	05/15/2009	3.40	5.90	440			
2	2				D-4	05/15/2009	3.40	5.90	429.40			
3	3				D-6	05/15/2009	3.40	5.90	419.10			
4	4				D-8	05/15/2009	3.40	5.90	409.10			
5	5				D-10	05/15/2009	3.40	5.90	404			

Prior Voucher Samples Saved

Filter is Off Double click on a row in the grid to retrieve it's Sample data for updating.

Mark ID	Species	Agency	Facility	Brood Year	Actual Hatch Code	Active?	Bottle Count	Slide Count	Image Count
17MILE05	CHUM	NSRA	17 MI STREAM INCUB	2005	4H	Yes	1	4	1
17MILE06	CHUM	NSRA	17 MI STREAM INCUB	2006	1,2,1H	Yes	1	1	1
AFK00E	PINK	PWSA	A F KOERNIG	2000	4H	No	0	0	1
AFK00L1	PINK			2000	4H3	No	0	0	1
AFK00L2				2000	4H4	No	1	1	1
		PWSA	A F KOERNIG	2001	4H	No	1	1	1
		PWSA	A F KOERNIG	2001	4H3	No	1	1	1
				2001	4H5	No	1	1	1
				2002	4H	No	0	9	0

Receipt & Log In of Vouchers, cont'd.

- Each bottle is labeled with a number starting with 1.
- This bottle number is entered with the hatchery's lot number and other information such as:
 - incubator,
 - sample date,
 - CTU's.

Mark ID and Voucher Sample Administration - Welcome MWLOVEJOY

Mark ID Edit / Entry

Mark ID: SPEELARM08A Mark Type: Thermal
Agency: DIPC Proposed Mark: 5H
Facility: SNETTISHAM Actual Mark: 5H
Species: 420 RBr: 1:1.5
Brood Year: 2008 Brood Stock: SNETTISHAM
Release Year: 2010 Release Site(s): SPEEL ARM 111-33
NPAFC ID: AK08-17

Receipt of Sample - Email Notification

FA?	Type	Contact Name	Sent Date
▶ Agency	TO	Kevin Steck	06/01/2009
Agency	TO	Mike Wunderlich	06/01/2009

*

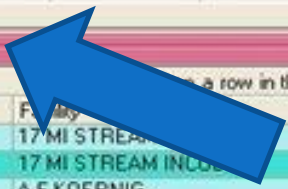
Voucher Bottle(s) logged for Mark ID: SPEELARM08A

Bottle #	Lot #	Net Pen	Raceway	Tray	Inc.	Sample Date	Pre Ambient Temp
▶ 1	1				D-1	05/15/2009	3.40
2	2				D-4	05/15/2009	3.40
3	3				D-6	05/15/2009	3.40
4	4				D-8	05/15/2009	3.40
5	5				D-10	05/15/2009	3.40

Prior Voucher Samples Saved

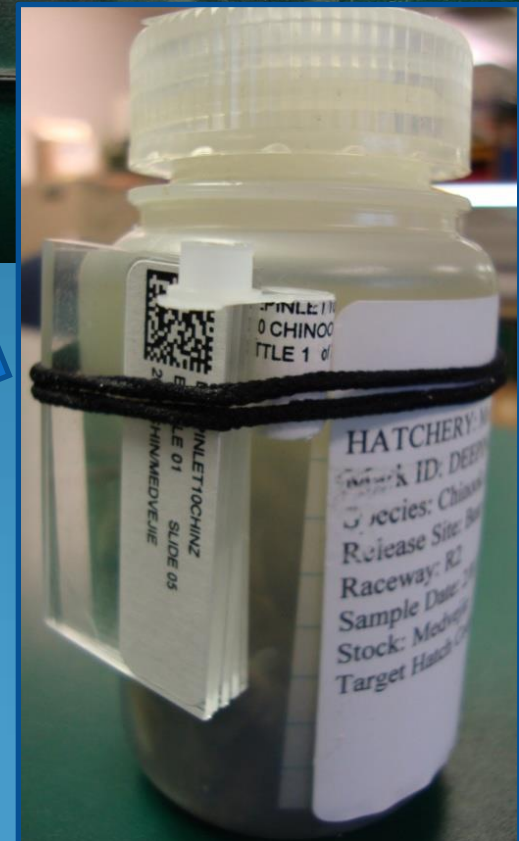
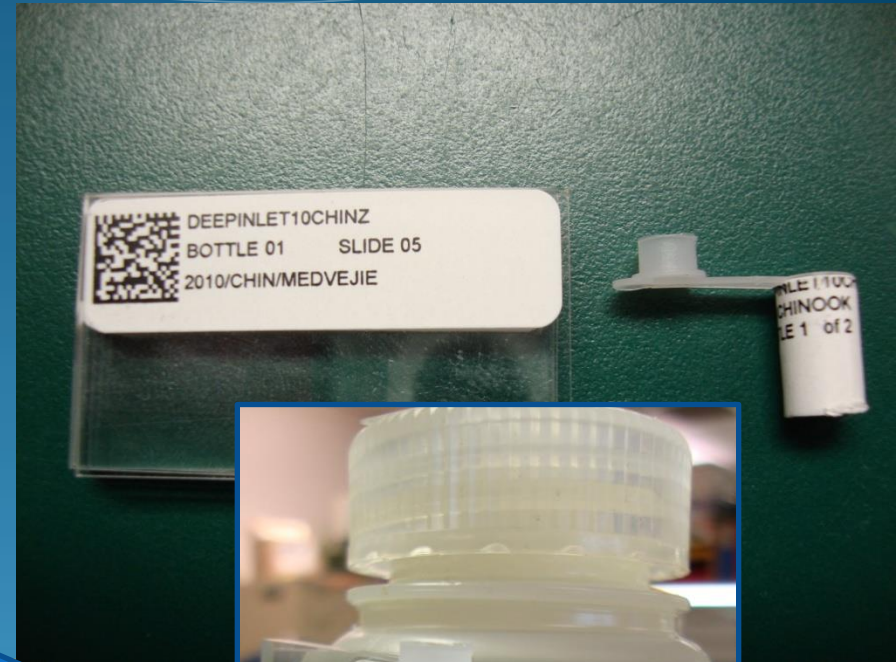
Filter is Off

Mark ID	Species	Agency	Facility
17MILE05	CHUM	NSRA	17 MI STREAM
17MILE06	CHUM	NSRA	17 MI STREAM INCO
AFK00E	PINK	PWSA	A F KOERNIG
AFK00L1	PINK	PWSA	A F KOERNIG
AFK00L2	PINK	PWSA	A F KOERNIG
AFK01E	PINK	PWSA	A F KOERNIG
AFK01L1	PINK	PWSA	A F KOERNIG
AFK01L2	PINK	PWSA	A F KOERNIG
AFK02E	PINK	PWSA	A F KOERNIG
AFK03E	PINK	PWSA	A F KOERNIG



Receipt & Log In of Vouchers, cont'd.

- Slide, vial, tray and slide box labels are printed out.
- Slide and vial labels are affixed and attached to their corresponding voucher bottle.



Voucher Preparation for Imaging

Other Necessary Supplies:



• Dissecting Microscope

• Thermoplastic Cement

• Cooling Tray

• Hot plate

• Vial Holder

• Water

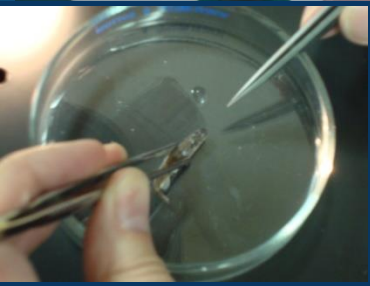
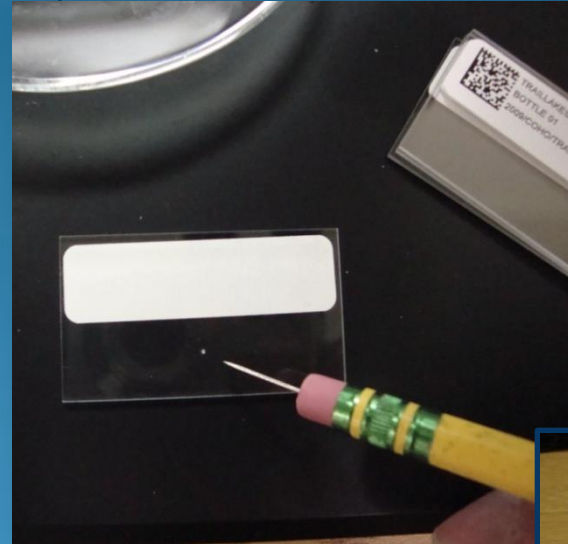
• Ethanol

• Forceps

• Probe

Voucher Preparation for Imaging, cont'd...

5 otoliths, one per slide are mounted onto the slide using thermoplastic cement.



Approximately 25 left otoliths are dissected for each voucher bottle.

20 otoliths are placed in storage vials with a 10% Ethanol solution



Voucher Preparation for Imaging, cont'd...

Once dissected, otoliths are ground by hand to expose the thermal mark using:

- Lapping film with varying grit: 0.3 um to 9 um.
- Water
- Compound Microscope



Grinding

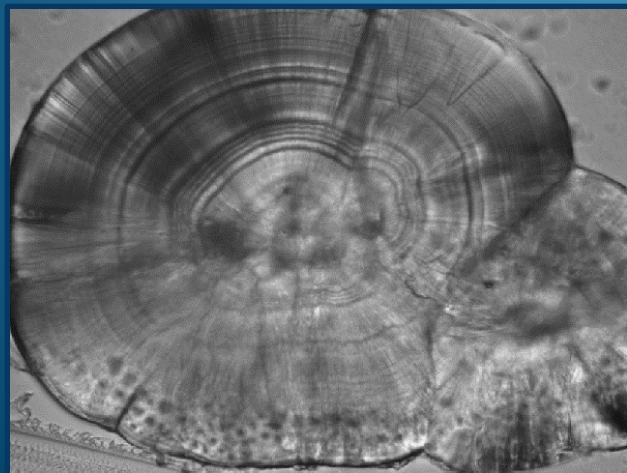
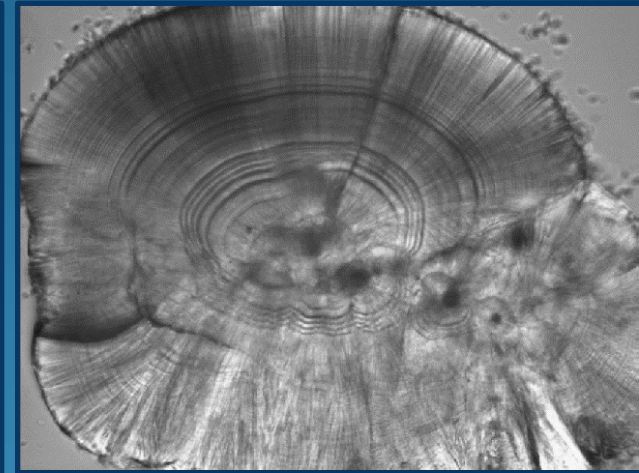
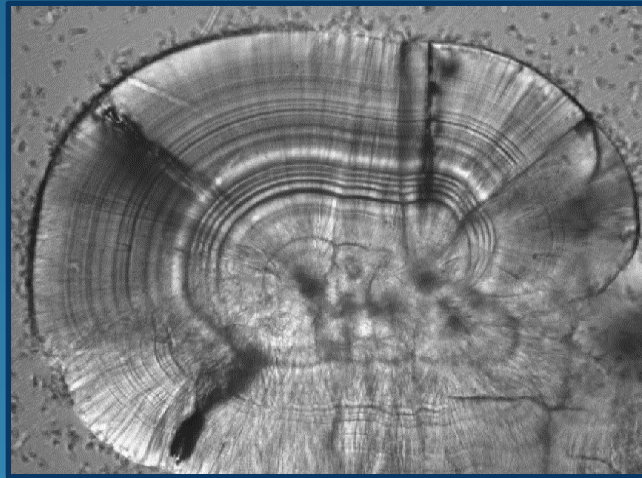
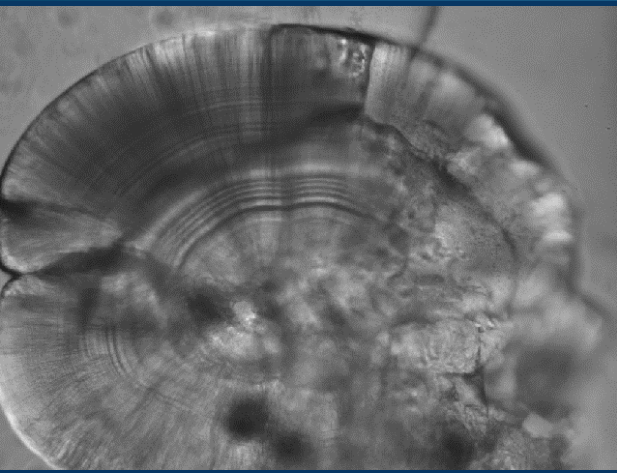


Polishing

Voucher Imaging

Multiple Images are taken for each TMID

- 5 slides from each bottle are reviewed and imaged:



Voucher Measurements

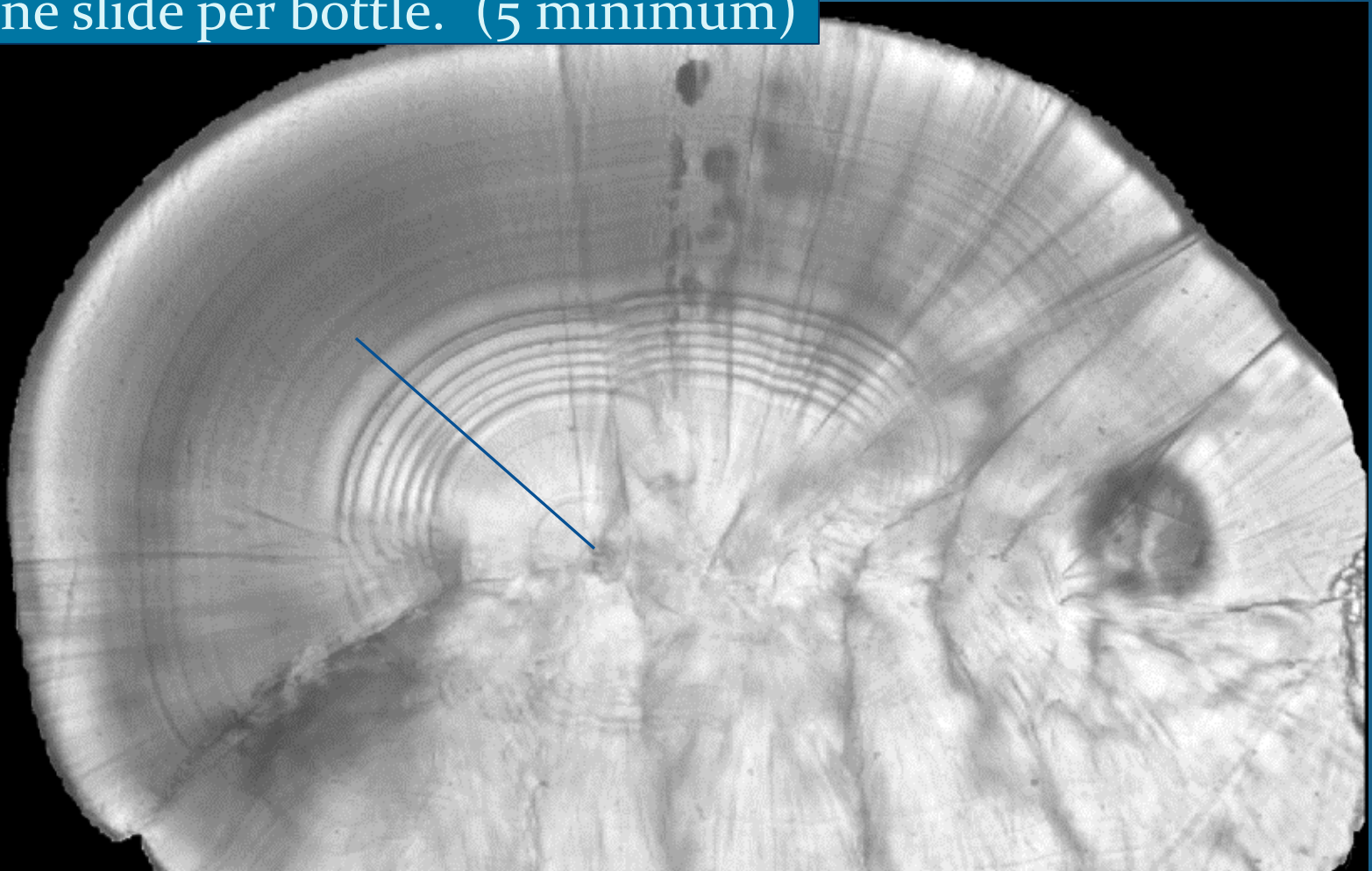
The screenshot displays the Image-Pro Plus software interface. The main window shows a grayscale image of a circular specimen with concentric rings. Two measurements are indicated with blue lines and text: **D3: 18.53232 um** and **D2: 51.81650 um**. The software's menu bar includes File, Edit, Acquire, Advanced, Sequence, Enhance, Process, Measure, Macro, Window, and Help. The toolbar contains various icons for image manipulation. Floating windows include 'Annotation' and 'Measure distance'. The 'Measure distance' window shows a scale bar. The 'Annotation' window has a 'Show' checkbox and 'Start with default Text' option. The 'Measure distance' window shows a scale bar. The 'Annotation' window has a 'Show' checkbox and 'Start with default Text' option. The 'Measure distance' window shows a scale bar. The 'Annotation' window has a 'Show' checkbox and 'Start with default Text' option.

Measurements are taken using Image-Pro Plus.

Gray Scale 9(503,676 bytes), Zoom:100% 221, 14 0 W.H: 823,612 Image: 100Micrometer25x (um) System: 100M

Voucher Measurements

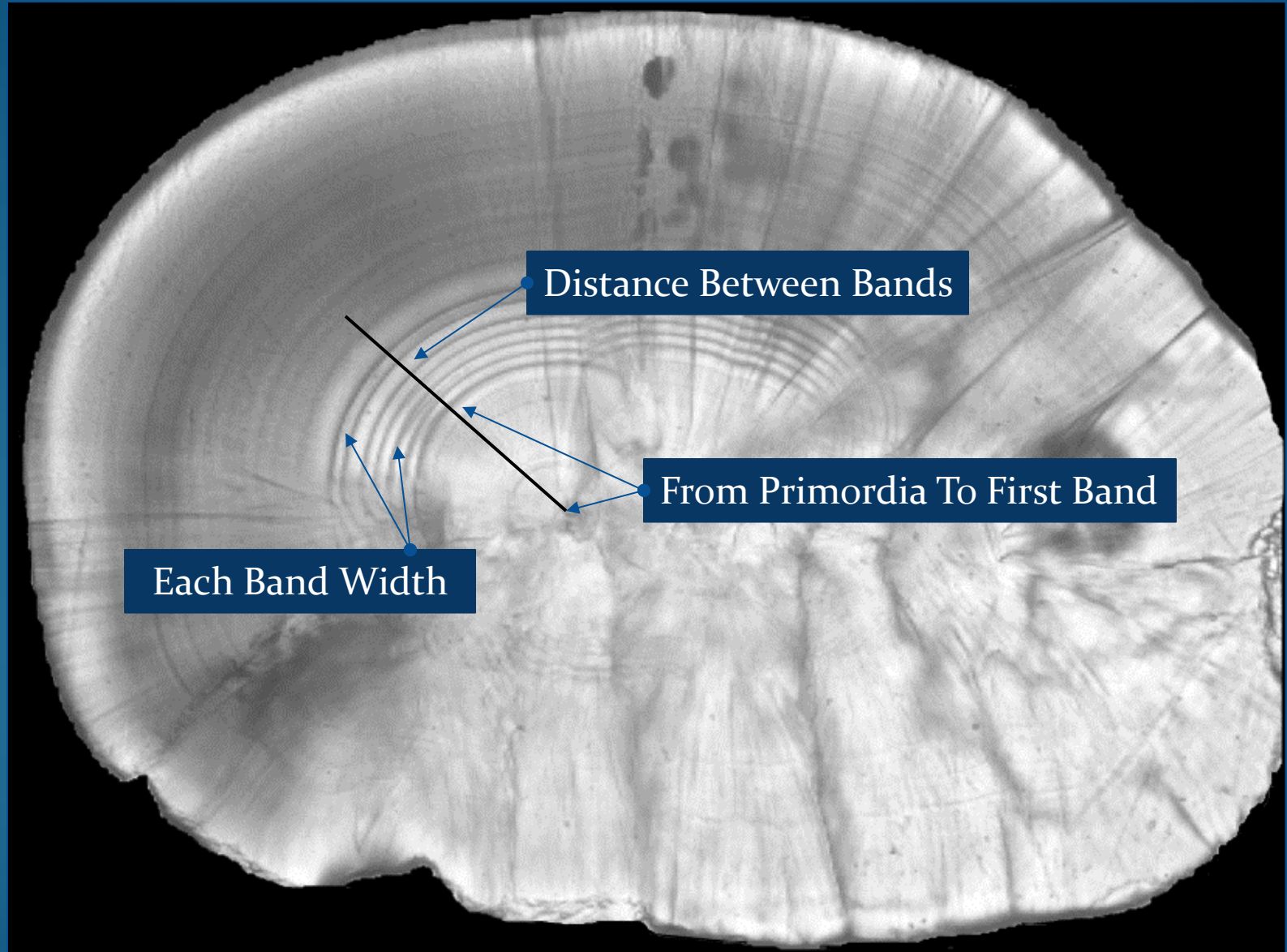
- Distance measurements are taken for *at least* one slide per bottle. (5 minimum)



- A transect line is placed at ~"10:00" starting at the left-most primordia and extending past the last thermal mark.

Voucher Measurements

Distance Measurements (μm) are taken for:



Voucher Measurements

Mark ID, Bottle Number and Slide Number are selected using the *Read Specimen Detail* application.

Corresponding measurements are entered into the application.

Manually Select Specimen to Read

Mark ID: TAHLTAN07 Bottle Number: 1 Slide Number: 2

Mark ID Details

Brood Year: 2007 Agency: DIPC Facility: SNETTISHAM Species: SOCKEYE

Specimen Characteristics

Band #	to this Band	Band Width
1	54.80	1.50
2	3.80	4.20
3	5	9.10

Temp Shift Direction: Proposed Mark: 1,2n,3H

Thermal Mark Schedule: Observed Mark: 1,2n,3H

Mark Grade: A Actual Mark: 1,2n,3H

Make Observed Mark the Actual Mark

Mark Image: C:\Documents and Settings\mwlovejoy\Local Browse Clear Image Variant: 0

Profile Image: Browse Clear Clear Form on Save

Read Comment: Photo

AutoMagically Retrieve Specimen to Read by double clicking on a row in the grid Filter is Off

Double click Slide# to view details. To delete a slide, highlight a row then press 'Del'. Slide deletions will be processed immediately.

Mark ID	Bl#	Sl#	Mark Grade	Obs. Mark	Var#	Profile	Date Read	Read Comment
TAHLTAN07	1	2	A	1,2n,3H	0	N	1/5/2009 9:	Photo
TAHLTAN07	2	1	A	1,2n,3H		N	1/12/2009	
TAHLTAN07	3	4	A	1,2n,3H		N	1/13/2009	
TAHLTAN07	4	1	A	1,2n,3H		N	1/13/2009	
TAHLTAN07	5	4	A	1,2n,3H		N	1/13/2009	
TAHLTAN07	6	3	A	1,2n,3H		N	1/13/2009	

Voucher Annotation

Using Image-Pro, the image is annotated with the measurements.

SOCKEYE
SWEETHEART08
SNETTISHAM
Release Site: SWEETHEART LK
111-35
Bottle #1, Slide #4
5,2H
Nice Mark all slides look similar,
with no variants.

A label is generated with the Read Specimen Detail application. This label displays all pertinent information about that sample.

2.8 um
3.2 um
9.5 um
51.2 um

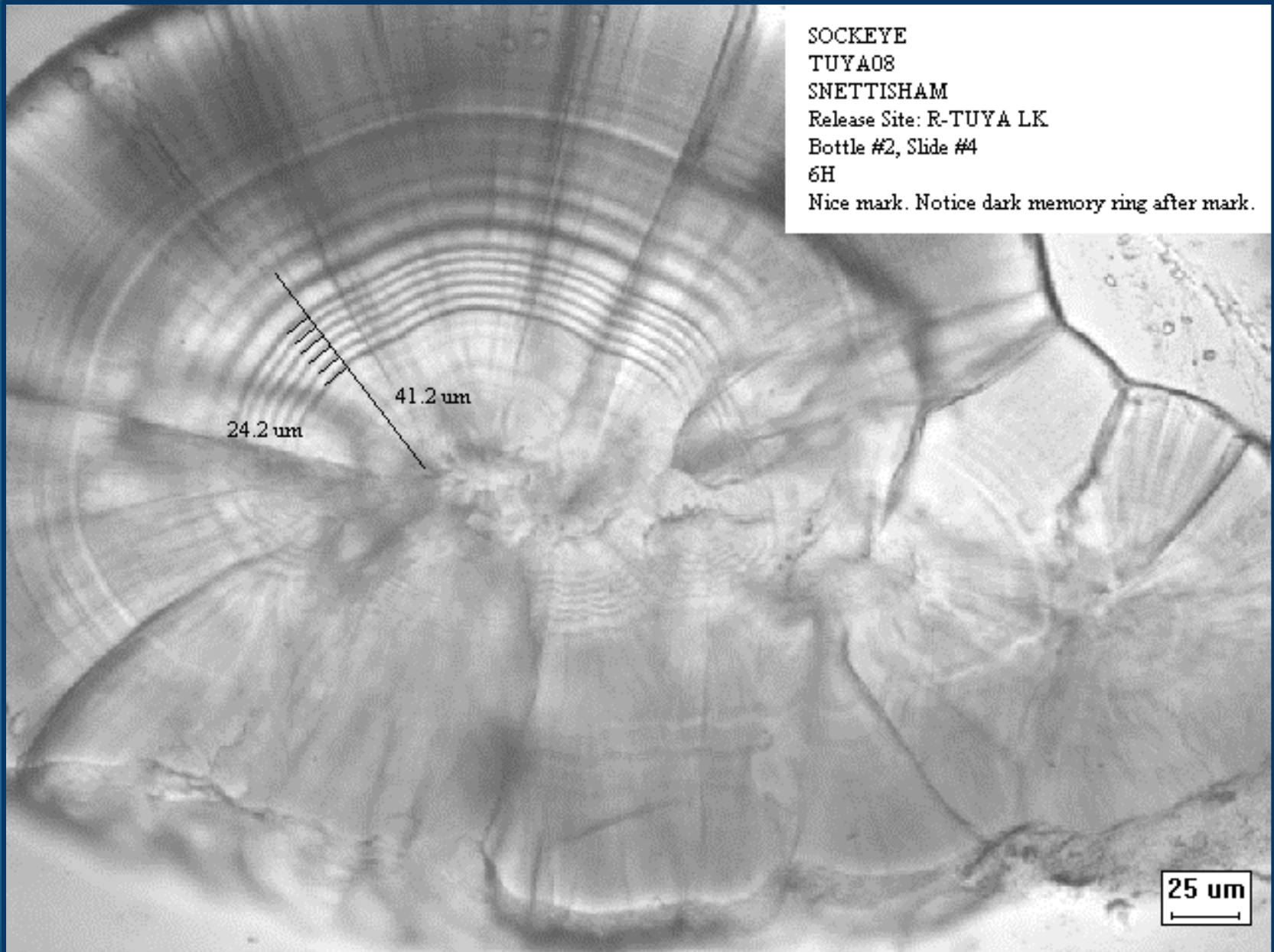
A calibration marker is placed.

25 um

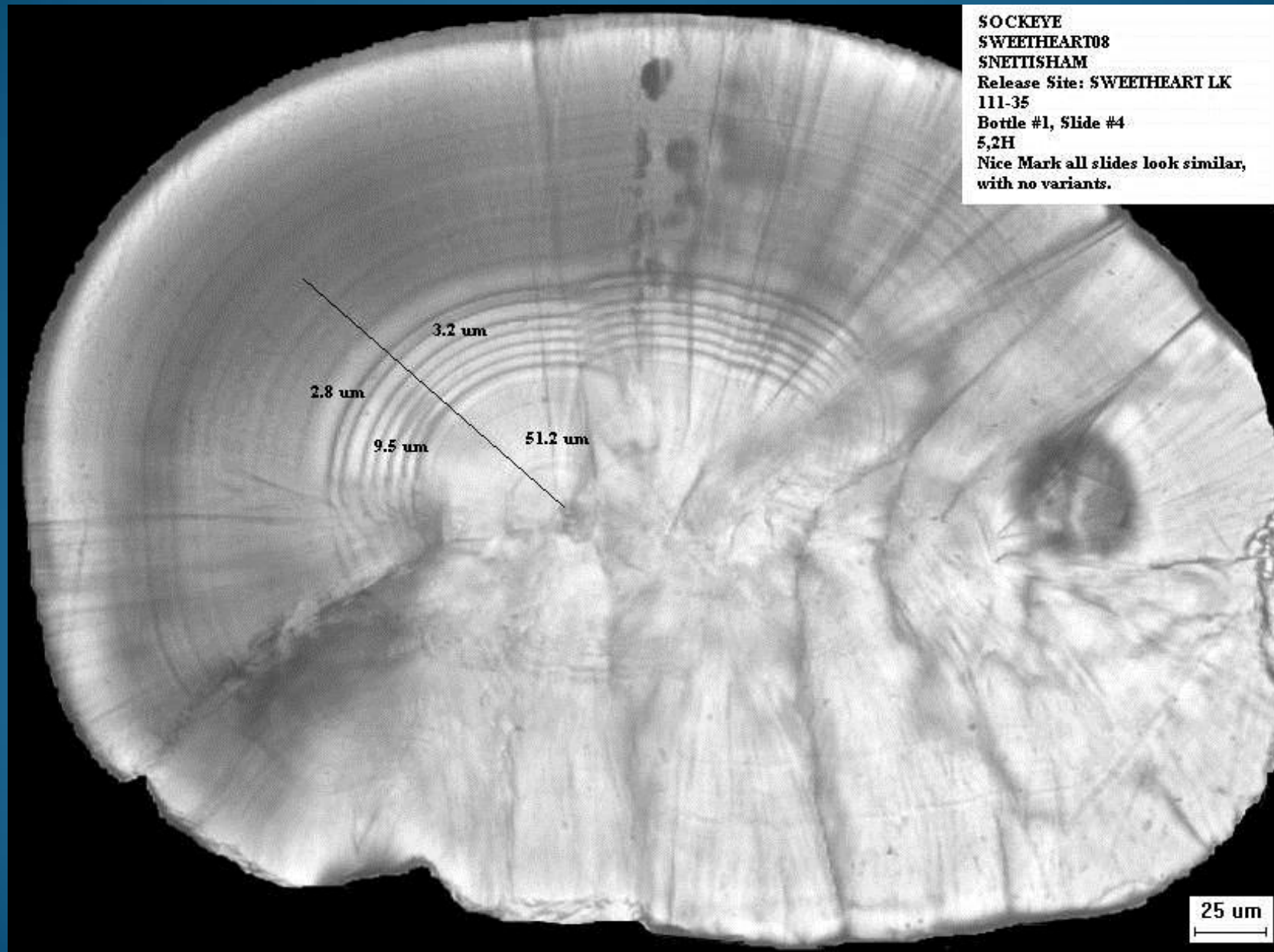


Final Voucher Image

SOCKEYE
TUYA08
SNETTISHAM
Release Site: R-TUYA LK
Bottle #2, Slide #4
6H
Nice mark. Notice dark memory ring after mark.



Final Voucher Image



SOCKEYE
SWEETHEART08
SNETTISHAM
Release Site: SWEETHEART LK
111-35
Bottle #1, Slide #4
5,2H
Nice Mark all slides look similar,
with no variants.

25 um

Voucher Quality

Read Specimen Detail - Welcome MWLOVEJOY

New Read Scan or Type a Mark ID:

Voucher Comment:

Manually Select Specimen to Read
Mark ID: TAHLTAN07 Bottle Number: 1 Slide Number: 2

Mark ID Detail
Brood Year: 2007 Agency: DIPC Facility: SNETTISHAM Species: S...EYE

Specimen Characteristics

Band #	to this Band	Band Width
1	54.80	1.50
2	3.80	4.20
3	5	9.10
*		

Temp Shift Direction: Proposed Mark: 1,2n,3H
Thermal Mark Schedule: Observed Mark: 1,2n,3H
Actual Mark: Mark Grade: A

Mark Image: C:\Documents and Settings\mwlovejoy\Local... Image Variant: 0
Profile Image: Clear on Save

Read Comment:

AutoMagically Retrieve Specimen to Read by double clicking on a row in the grid Filter is Off

Double click Slide# to view details. To delete a slide, highlight a row then press 'Del'. Slide deletions will be processed immediately.

Mark ID	Blt#	Slid#	Mark Grade	Obs. Mark	Var#	Profile	Date Read	Read Comment
TAHLTAN07	1	2	A	1,2n,3H	0	N	1/5/2009 9:	Photo
TAHLTAN07	2	1	A	1,2n,3H		N	1/12/2009	
TAHLTAN07	3	4	A	1,2n,3H		N	1/13/2009	
TAHLTAN07	4	1	A	1,2n,3H		N	1/13/2009	
TAHLTAN07	5	4	A	1,2n,3H		N	1/13/2009	
TAHLTAN07	6	3	A	1,2n,3H		N	1/13/2009	

The thermal mark observed in the majority of samples is entered.

The mark quality is then assessed and a "Mark Grade" is entered.



Voucher Quality

Mark Grade	Certainty it is marked	% Actual mark is identifiable	How Does it fit the proposed mark?	Consistency among bottles	Noise?
A	Excellent	95%	Perfect	Excellent	None
B	Very Good	75%	80% or better of lots fit proposed mark	Good, all similar	Little
C	Good	50%	60% or better of lots fit proposed mark	1 or 2 lots inconsistent from others	Some
D	Maybe	30%	30% or better of lots fit proposed mark	3 lots inconsistent from others	Noise, maybe 1 extra ring
E	None	<30%	No lots match proposed mark	Difficult to Identify	Lots of noise and extra rings

“Otie,” our lab mascot!

