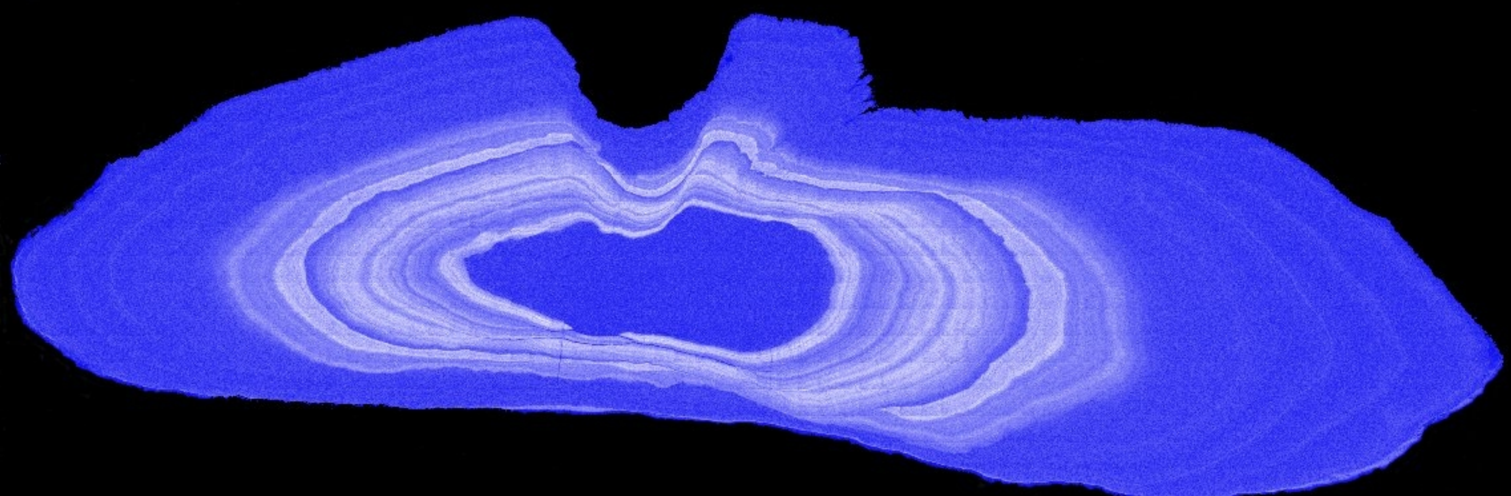
Strontium mark detection and other methods of otolith analysis available at the Advanced Instrumentation Laboratory, University of Alaska Fairbanks.

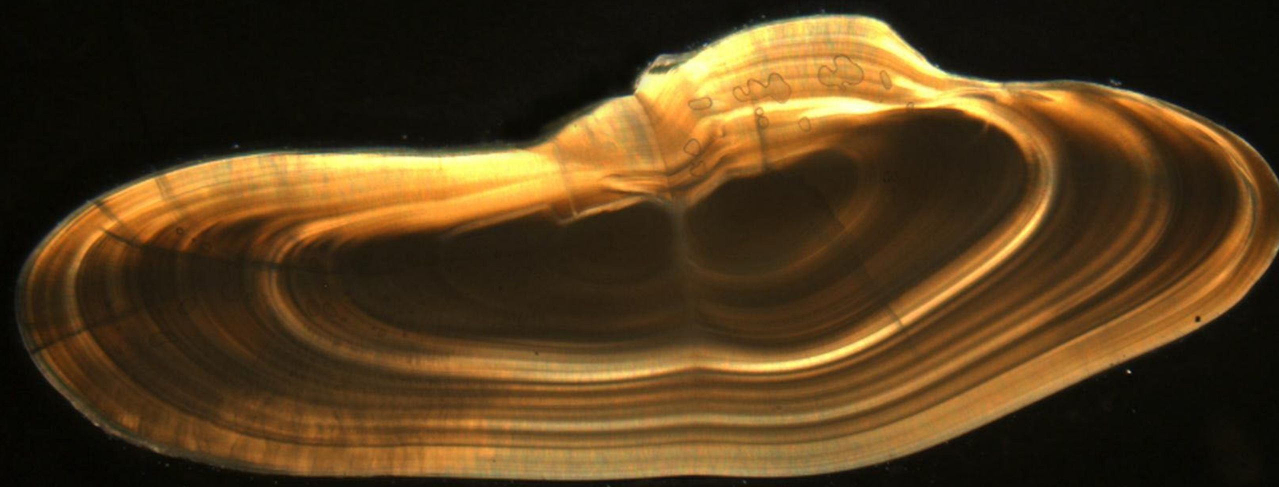
Ken Severin
Advanced Instrumentation Laboratory
University of Alaska Fairbanks

Advanced Instrumentation Laboratory
www.uaf.edu/ail



Many thanks to Randy Brown (USFW),
Anastasia Ilgen (UAF Chem and BioChem),
Dayna Norris (ex-ADFG),Moira Speer, and
Karen Spaleta (AIL)

for photographs and help

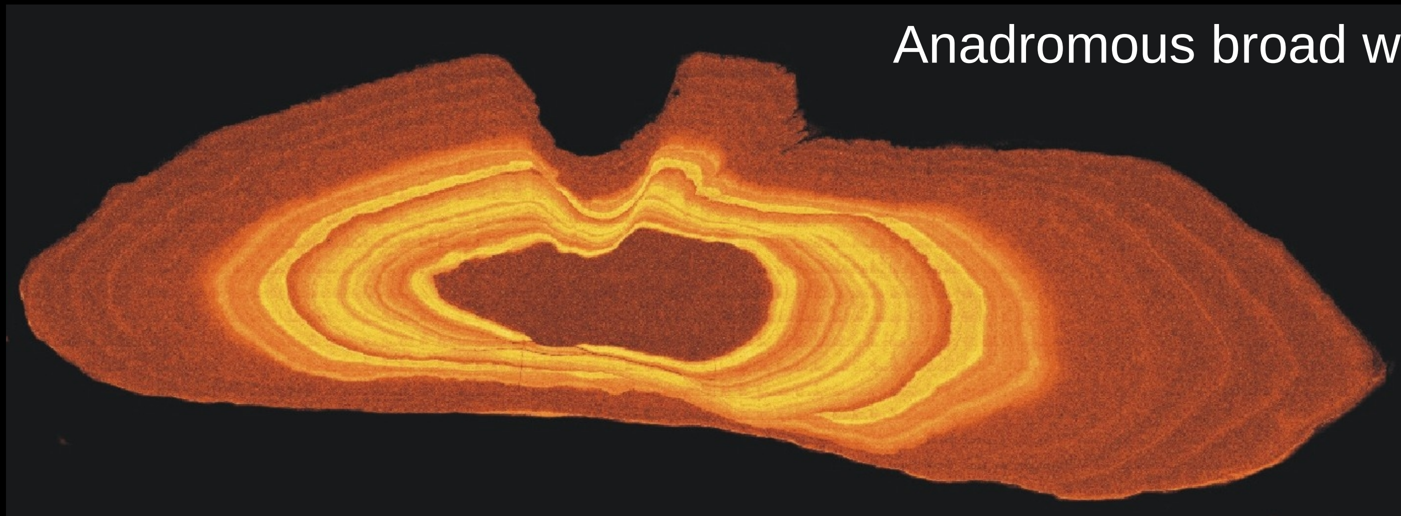


Dorsal

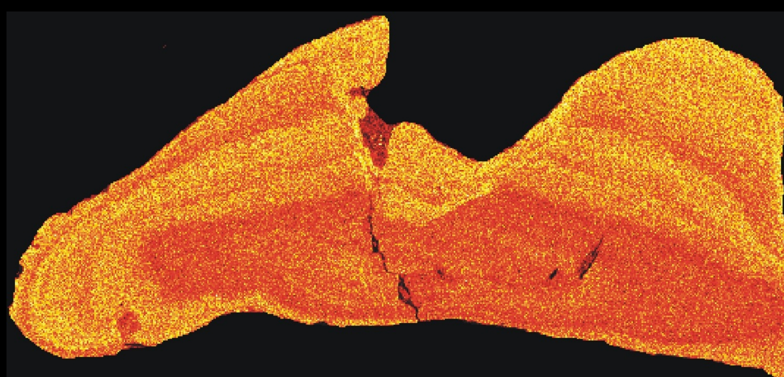
Posterior

Anterior

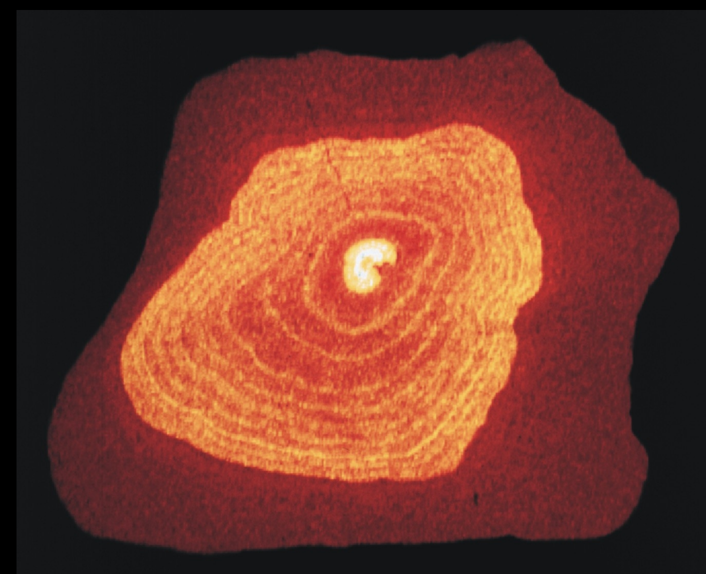
Ventral



Anadromous broad whitefish



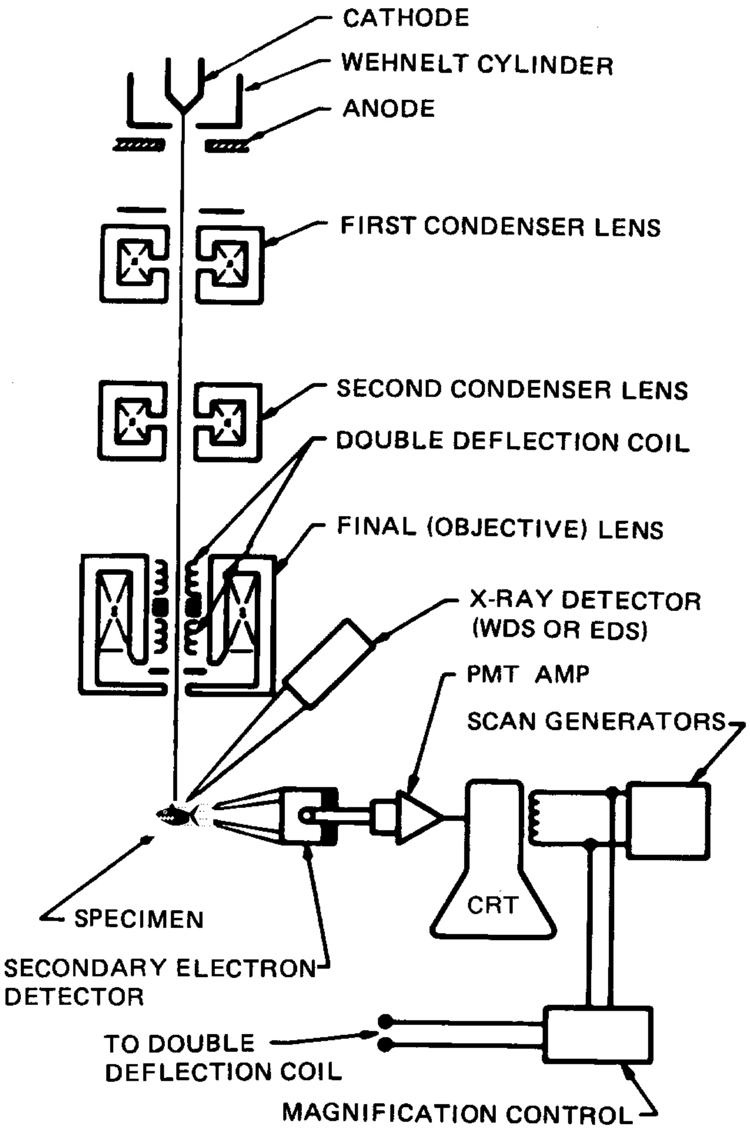
Catadromous Atlantic tarpon

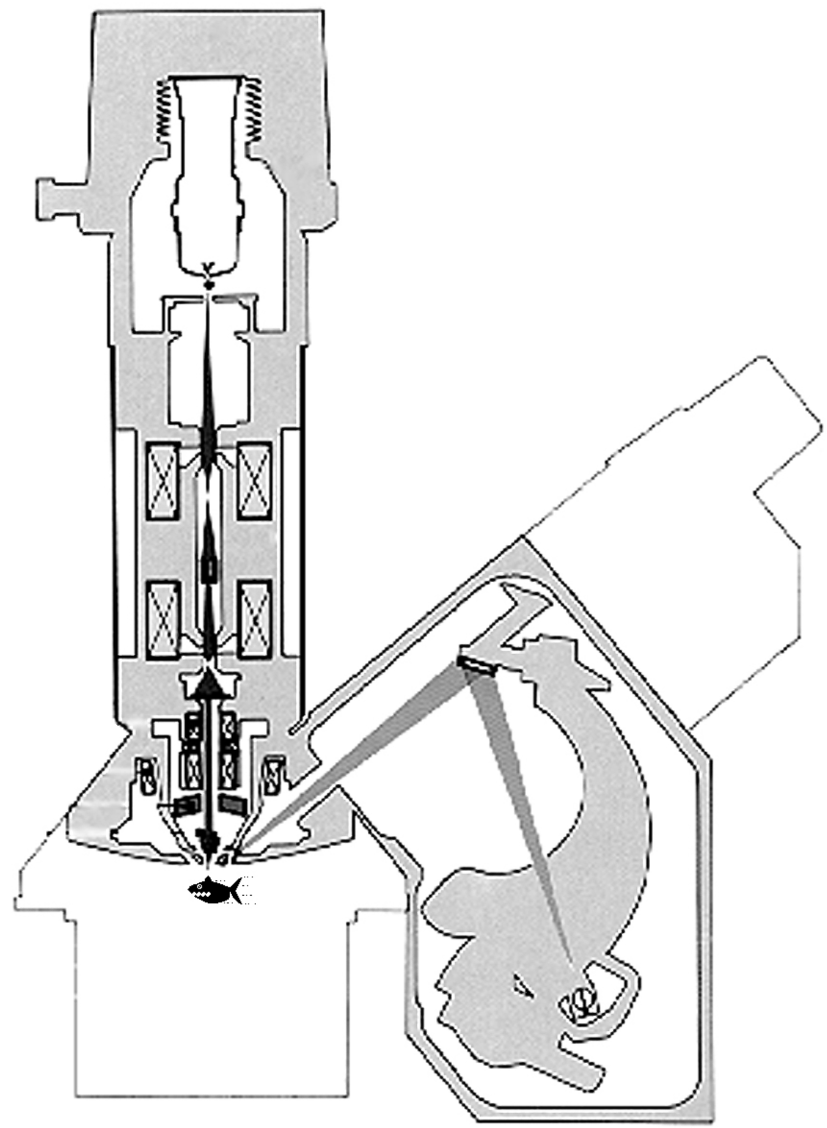


Catadromous European eel

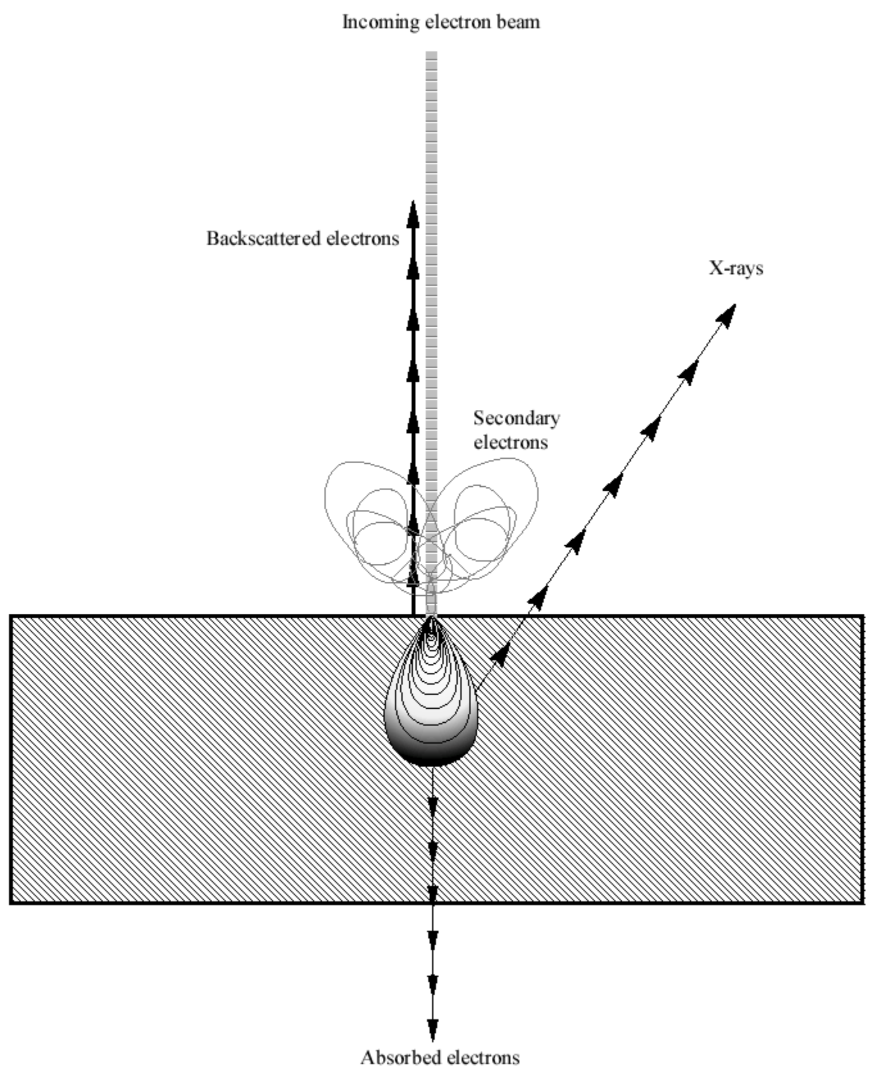
The technology:

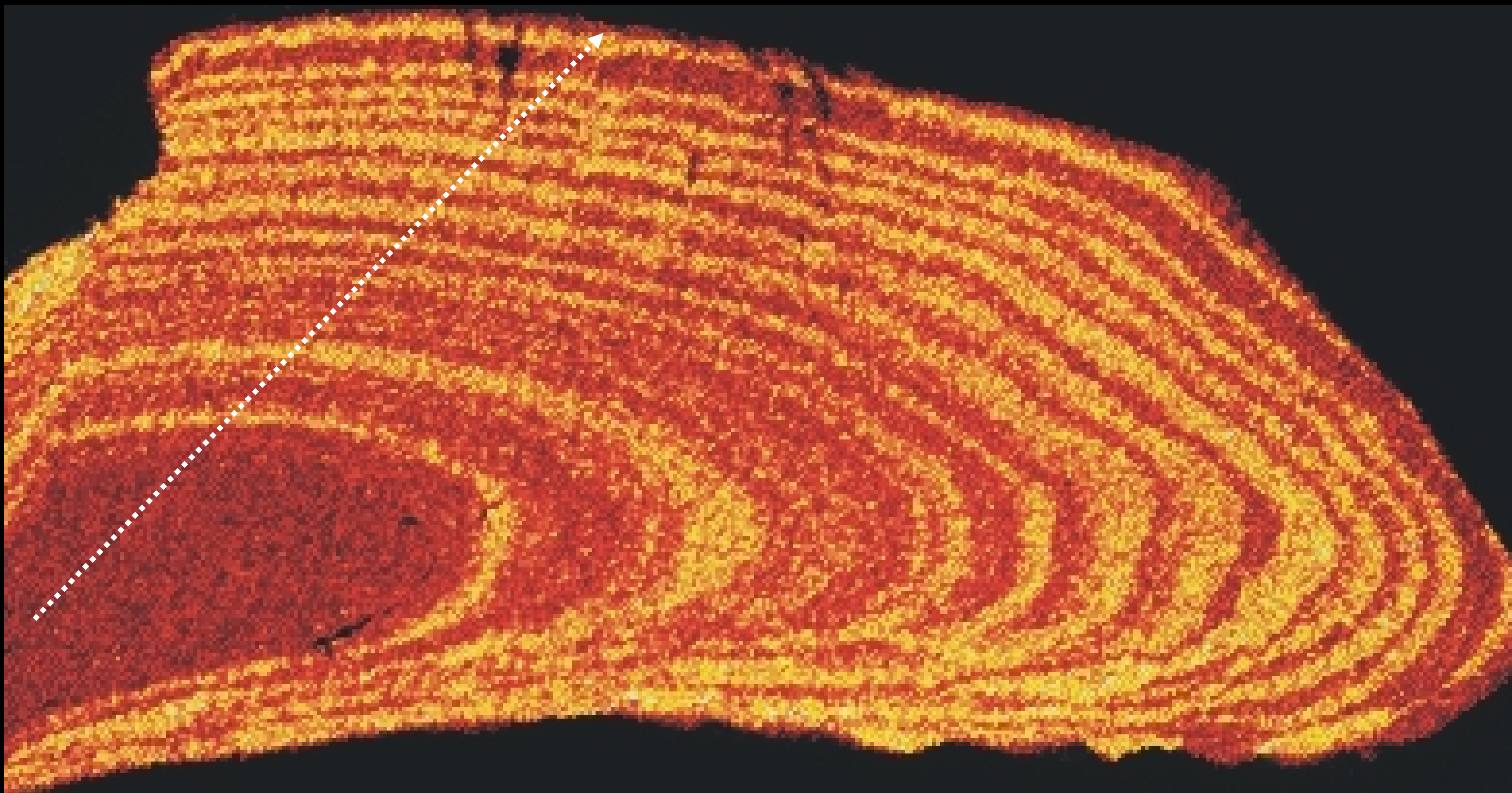
EPMA (Electron Probe Micro Analysis)
or EMPA (Electron Micro Probe Analysis)

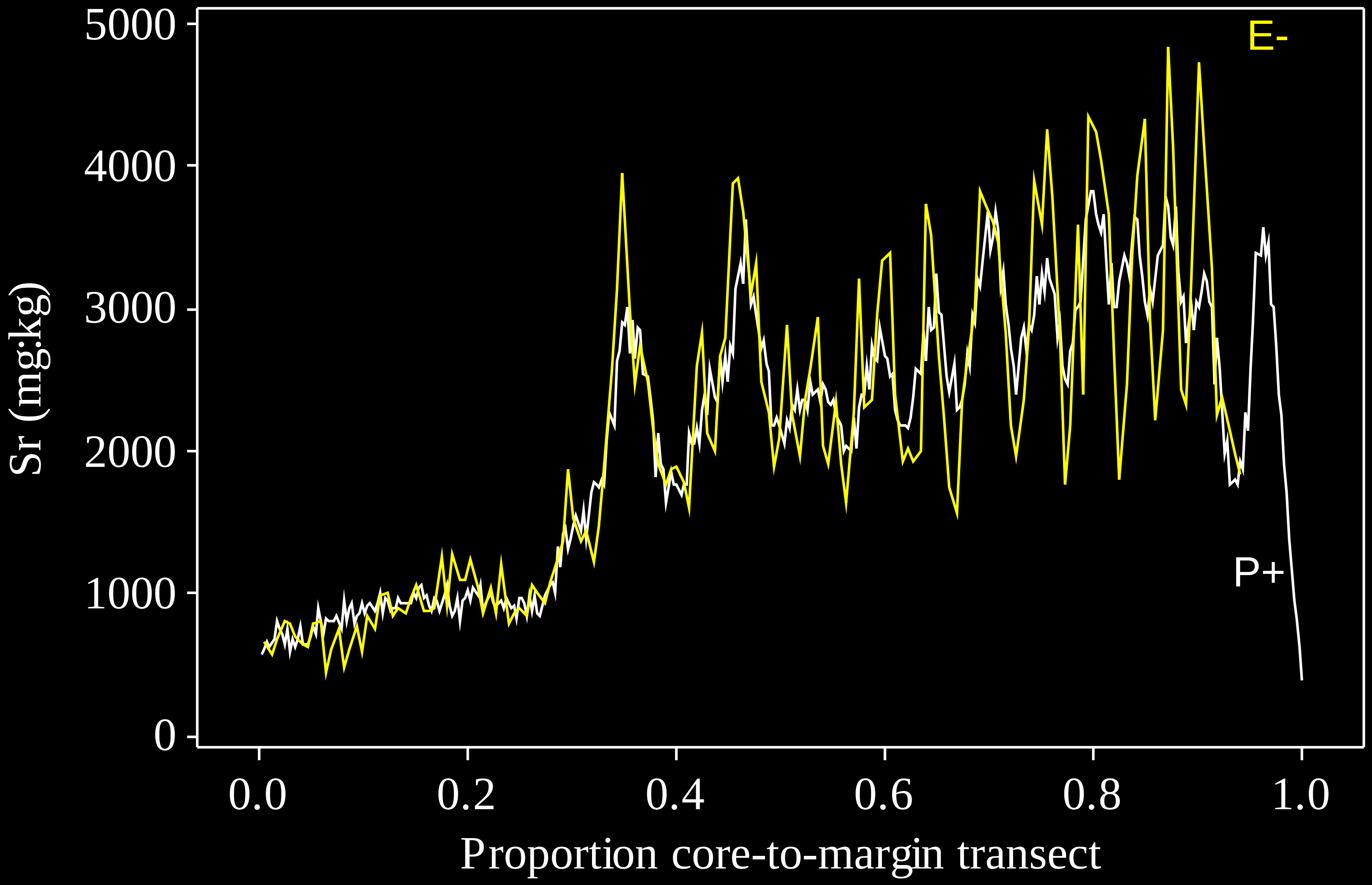




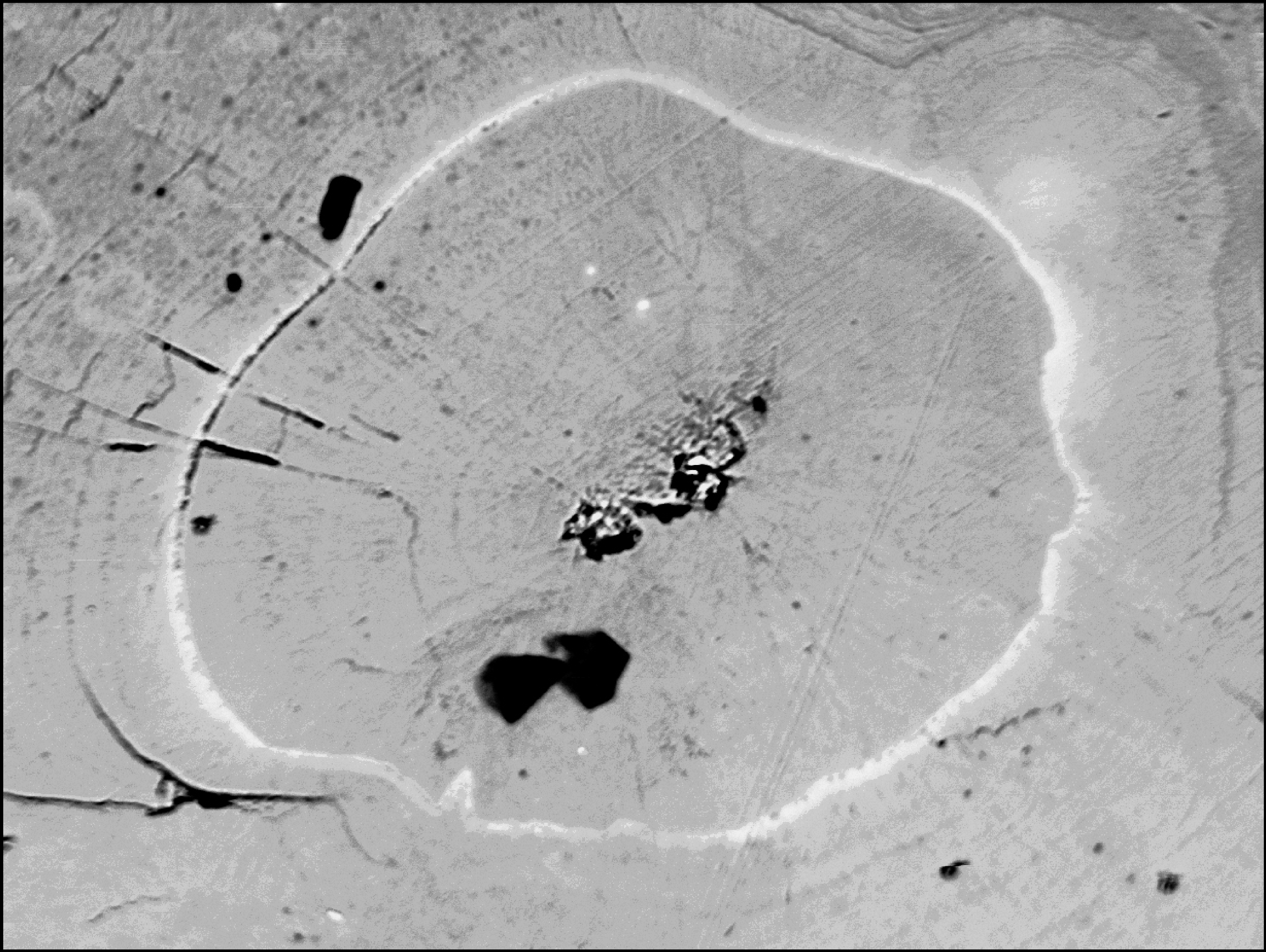

OF THE ELEMENTS
SARGENT-WELCH
EDAX
PHOENIX


Incoming electron beam
Backscattered electrons
X-rays
Secondary
electrons
Absorbed electrons





E-
P+
5000
4000
3000
2000
1000
0
0.0
0.2
0.4
0.6
0.8
1.0
Sr (mg:kg)
Proportion core-to-margin transect



Periodic Table of the Elements

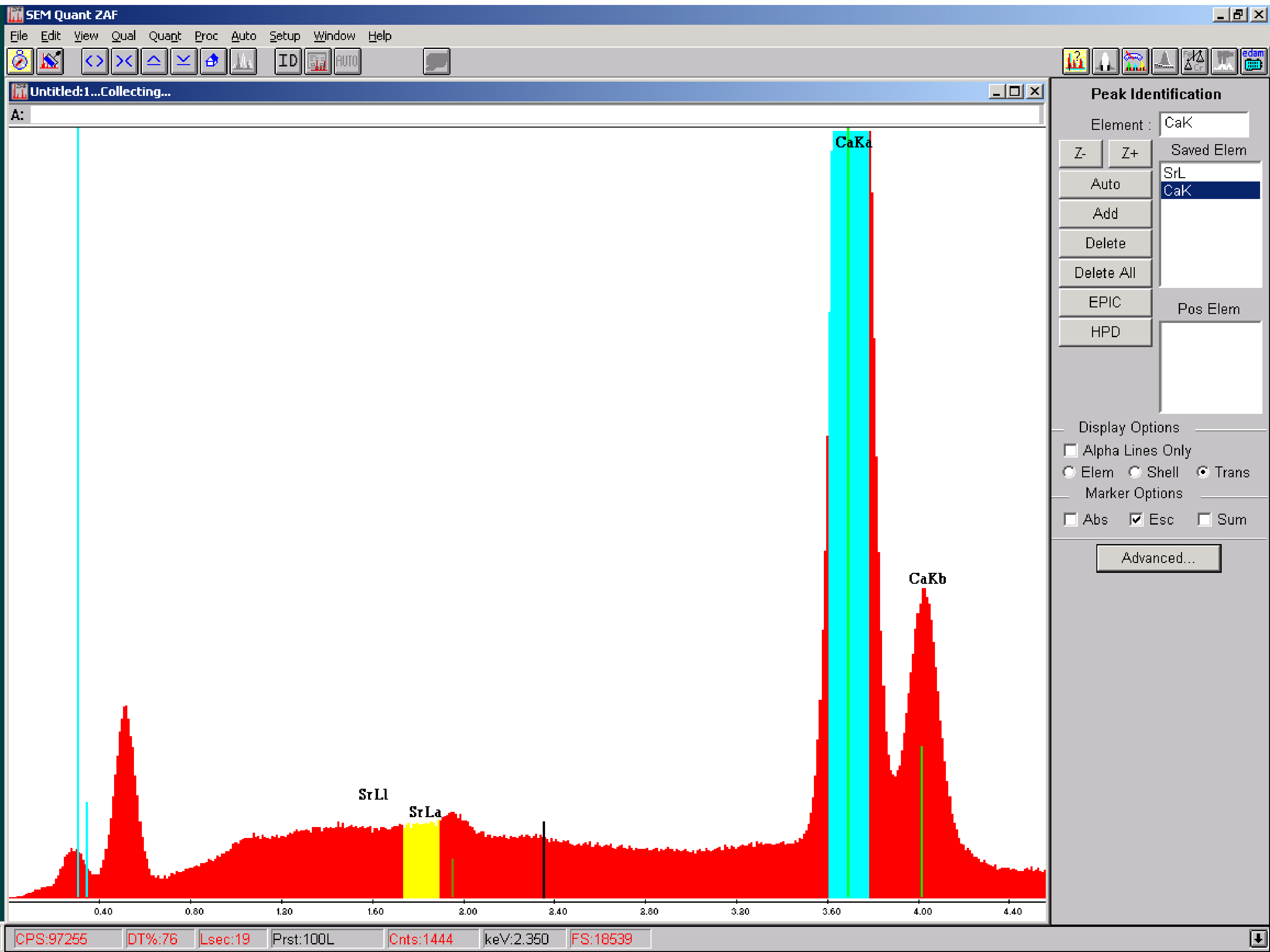
- hydrogen
- alkali metals
- alkali earth metals
- transition metals
- poor metals
- nonmetals
- noble gases
- rare earth metals

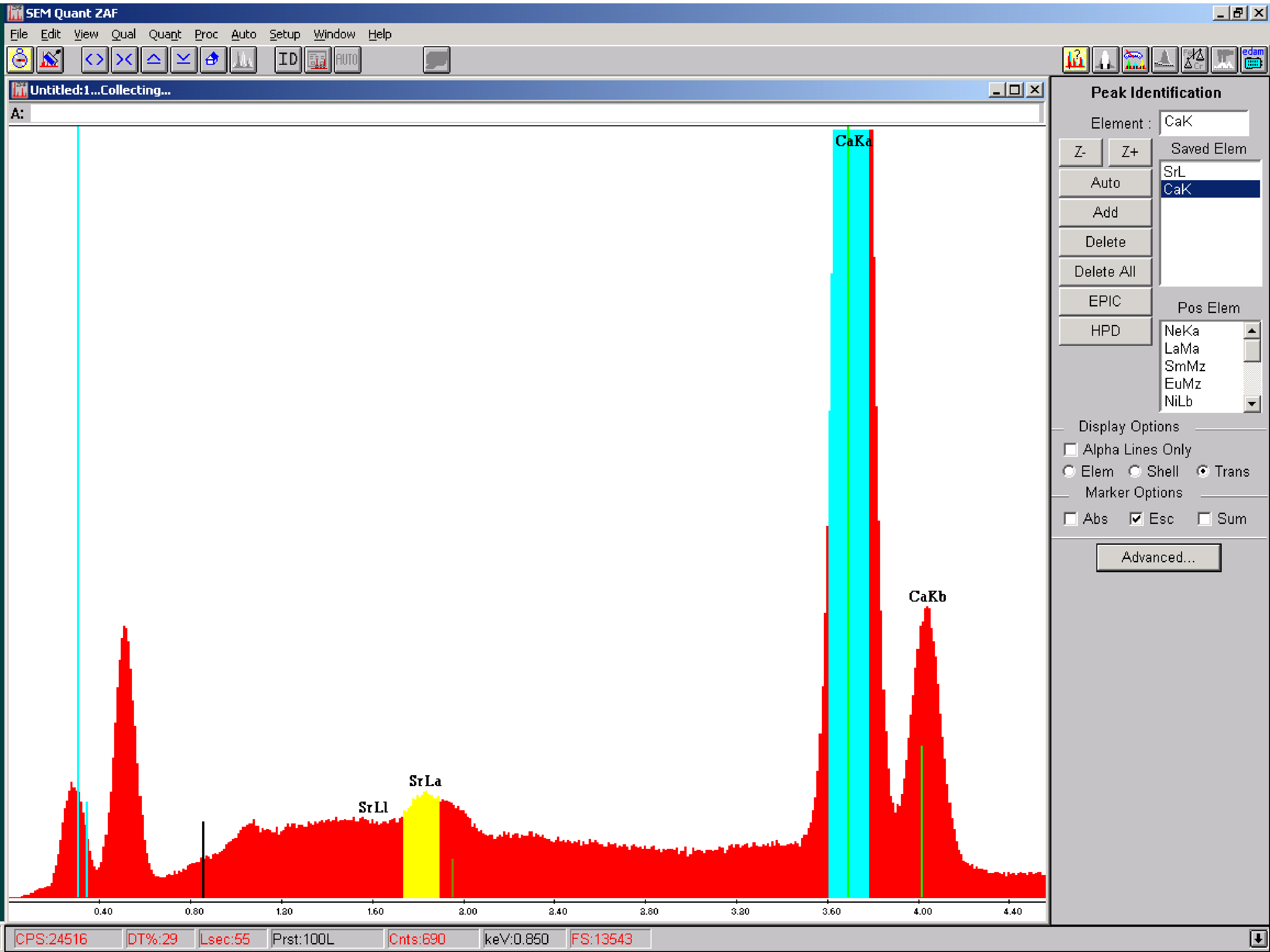
1 H																	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	57 La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	89 Ac	104 Unq	105 Unp	106 Unh	107 Uns	108 Uno	109 Une	110 Unn								

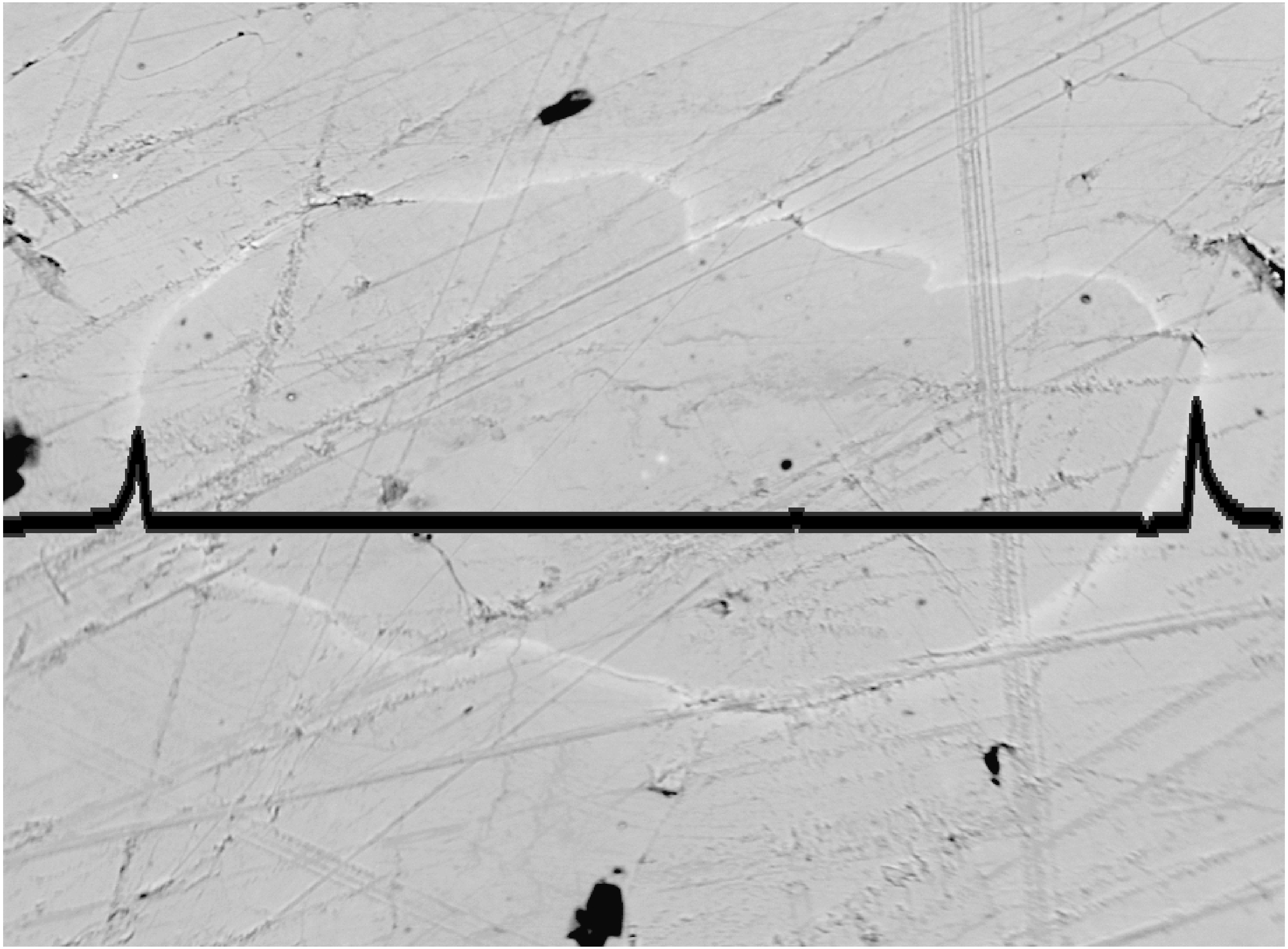
58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

13-APR-2008 Hend Socke No 0
r200.0µ BSE
15.0kV
150


13-APR-2008 Hend Socke No 0
r100.0µ BSE 15.0kV 400


SEM Quant ZAF
File Edit View Qual Quant Proc Auto Setup Window Help
ID
AUTO
Untitled:1...Collecting...
A:
CaKa
CaKb
SrLl
SrLa
0.40
0.80
1.20
1.60
2.00
2.40
2.80
3.20
3.60
4.00
4.40
Peak Identification
Element : CaK
Z- Z+
Saved Elem
SrL
CaK
Auto
Add
Delete
Delete All
EPIC
HPD
Pos Elem
Display Options
Alpha Lines Only
Elem Shell Trans
Marker Options
Abs Esc Sum
Advanced...
CPS:97255
DT%:76
Lsec:19
Prst:100L
Cnts:1444
keV:2.350
FS:18539


SEM Quant ZAF
File Edit View Qual Quant Proc Auto Setup Window Help
Untitled:1...Collecting...
A:
CaKa
CaKb
SrLa
SrLl
0.40 0.80 1.20 1.60 2.00 2.40 2.80 3.20 3.60 4.00 4.40
Peak Identification
Element : CaK
Z- Z+
Saved Elem
SrL
CaK
Auto
Add
Delete
Delete All
EPIC
HPD
Pos Elem
NeKa
LaMa
SmMz
EuMz
NiLb
Display Options
Alpha Lines Only
Elem Shell Trans
Marker Options
Abs Esc Sum
Advanced...
CPS:24516 DT%:29 Lsec:55 Prst:100L Cnts:690 keV:0.850 FS:13543



File Edit View Insert Format Tools Data Window Help

B10 | Yes

Copper River Commercial Common Property Sr SEM Results from sockeye salmon otoliths.

Period:	**15**	
Date Read:	**7/15-16/2009**	(mm/dd/yyyy)

Otolith#	Marked	Comments from Dayna Norris (dayna.norris@alaska.gov) *[visual inspection /* **microprobe**
1	Yes	
2	Yes	
3	Yes	
4	Yes	
5	No	
6	Yes	
7	No	
8	No	
9	No	
10	Yes	
11	No	
12	Yes	
13	Yes	
14	Yes	
15	Yes	
16	Yes	
17	Yes	
18	Yes	
19	Yes	
20	Yes	
21	Yes	

Page 1

Sheet1 Sheet2 Sheet3

Sheet 1 / 3 PageStyle_Sheet1 STD Sum=0 Average= 125%

To get to the final product takes:

- Mark Application
- Sample Collection
- Sample Preparation
- Sample Analysis
- Report Preparation
- Report Submission

But I'm not sure that's all

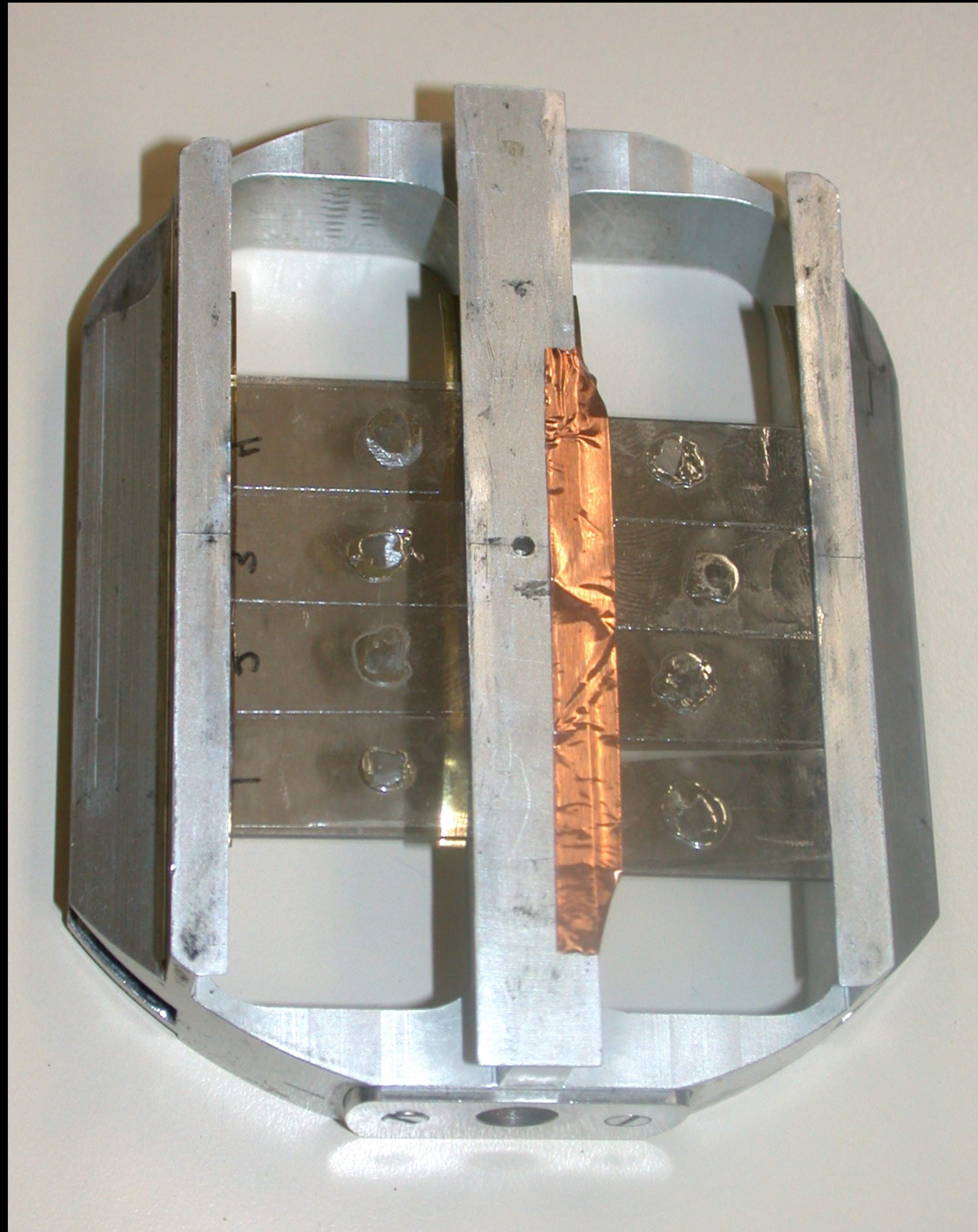
To get to ~~the final~~ a product takes:

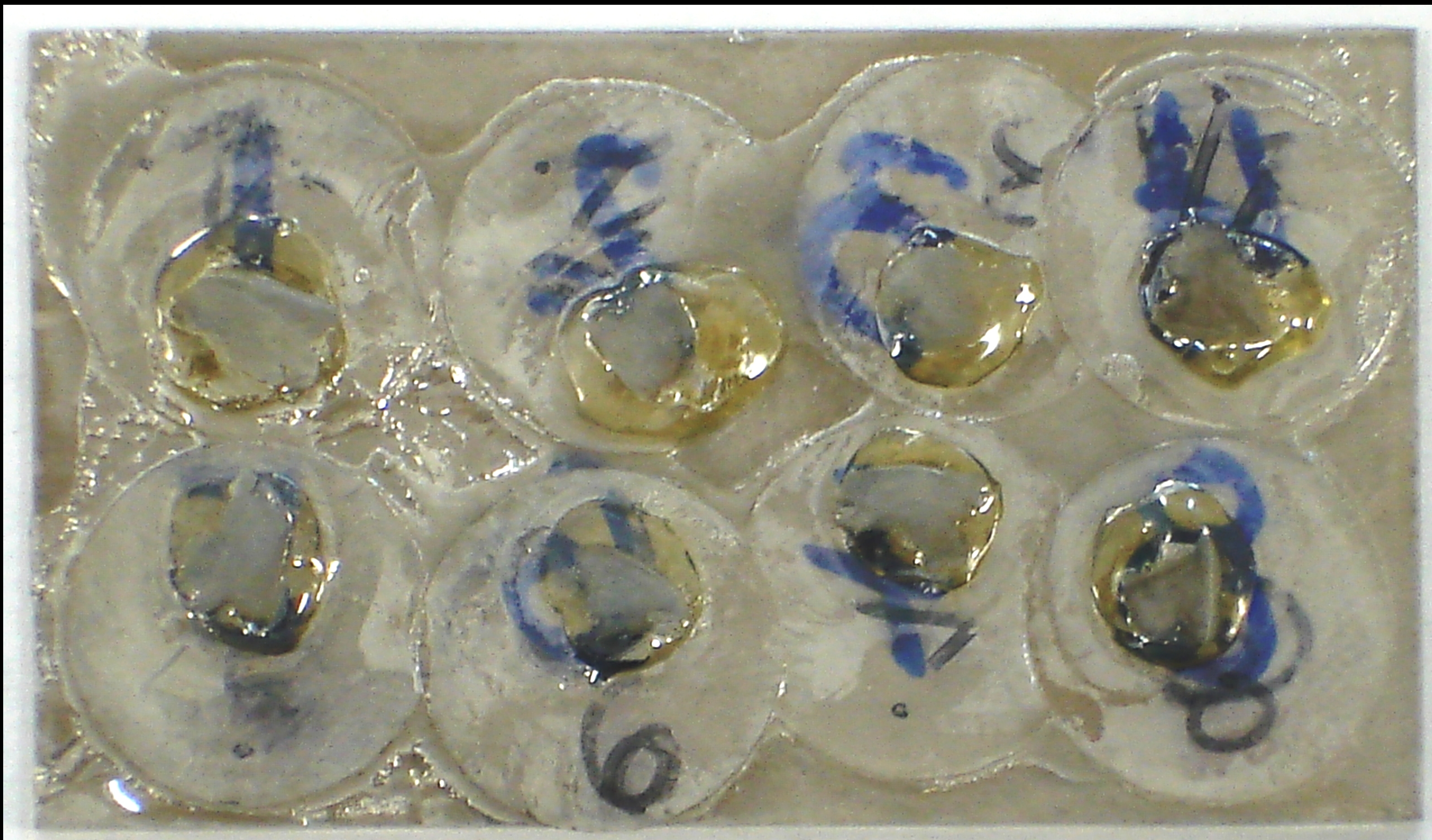
- Mark Application
- Sample Collection
- Sample Preparation
- Sample Analysis
- Report Preparation
- Report Submission

But I'm sure that's not all

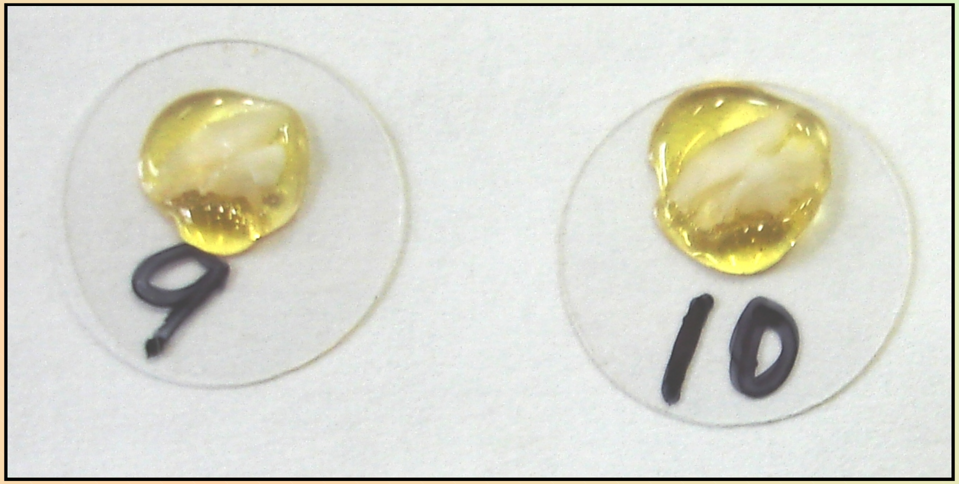
For EM Work the sample should:

- Have the mark exposed
- Be fairly flat and smooth
- (Be electrically conductive)
- Be mounted systematically





Mount otoliths onto cover slips



- Glue should be a clear pool when ready
- Mount sulcus down
- ~~Do not leave on hot plate too long to avoid bubbling~~

Mount cover slips onto slides

Cover slips are mounted on the slide with wax

Wax is used because:

- it has a lower melting point than the glue
- it holds the cover slip in place while grinding
- comes off easily after cover slip is removed

Grind and polish otoliths

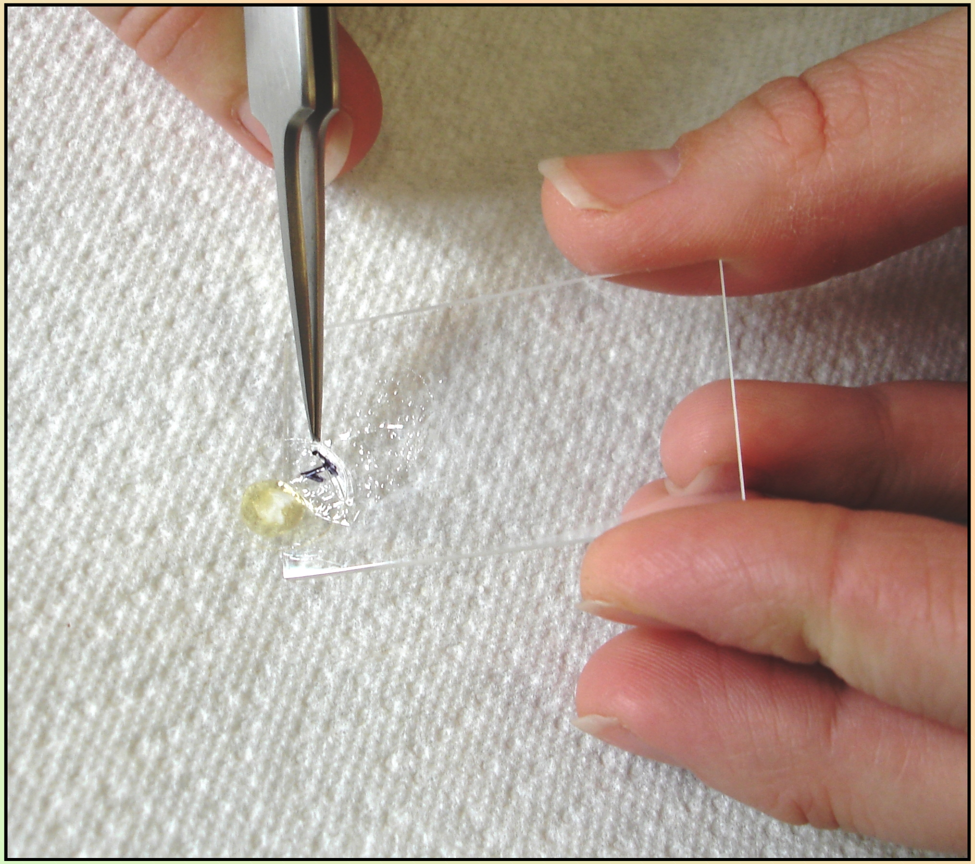
Grinding

- 500-grit SiC paper
- 250 rpm wet grinder

Polishing

- 4000-grit SiC paper
- 250 rpm wet grinder.

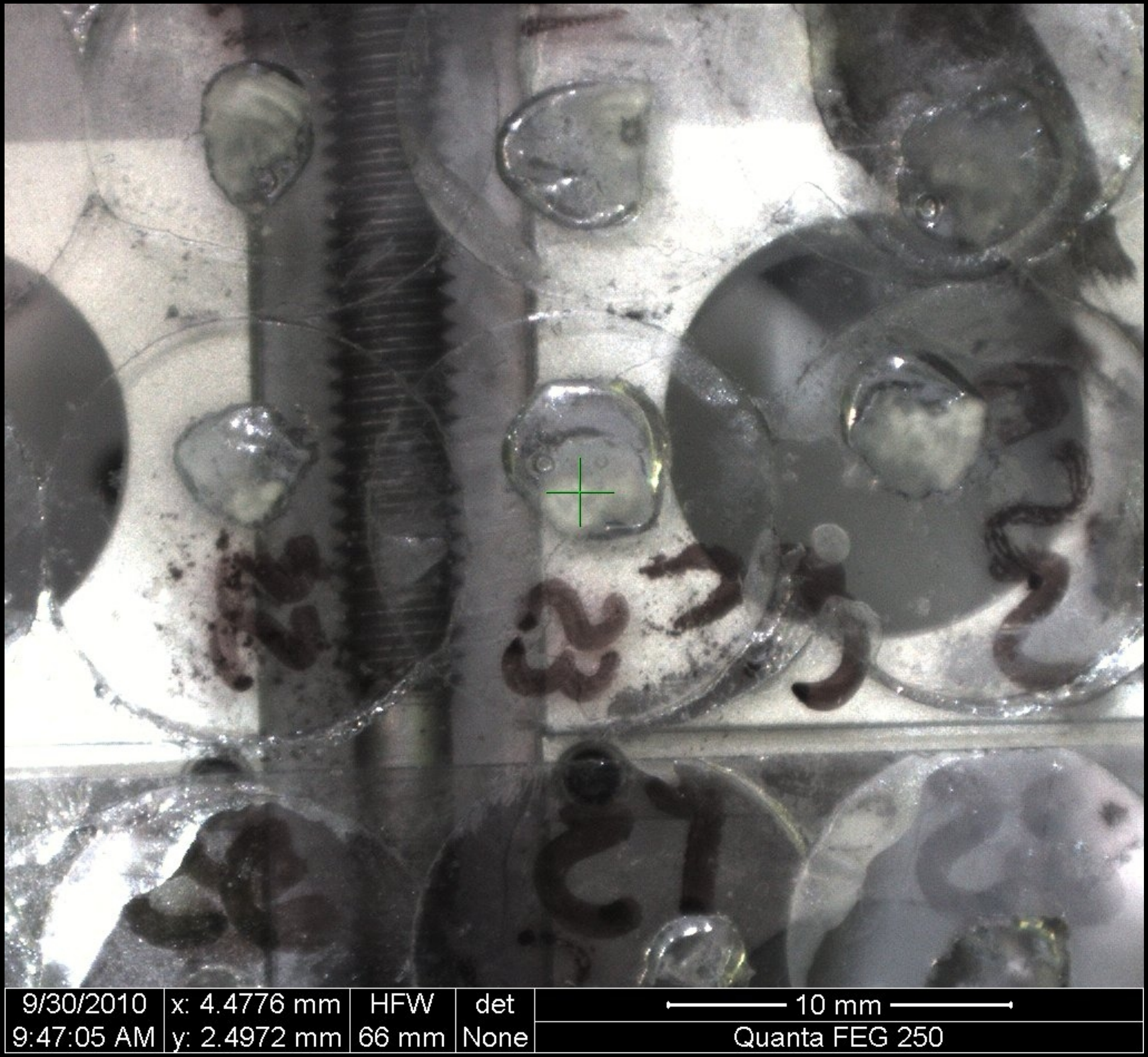
Remove cover slips from slides

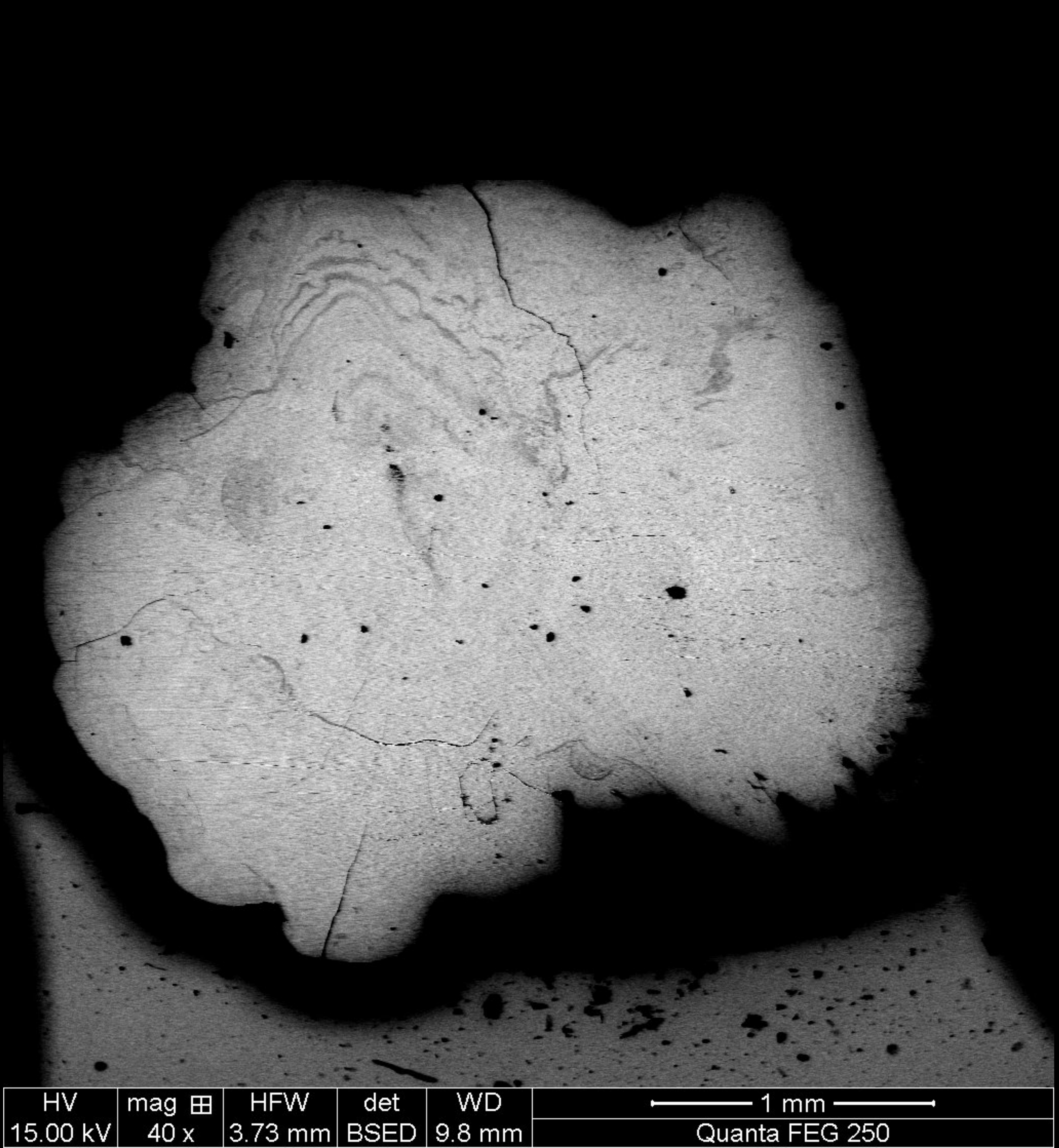


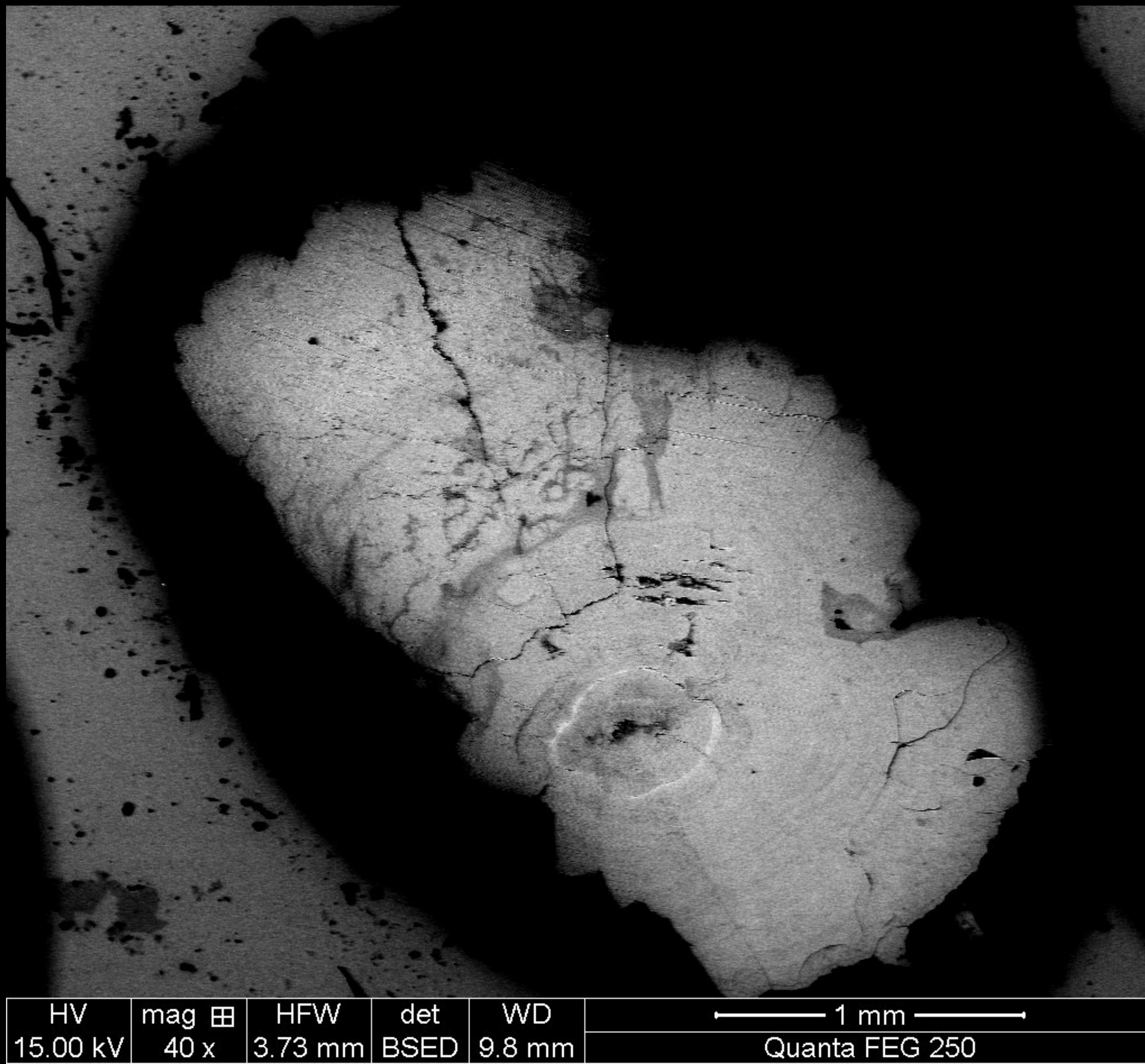
- Melt the wax but not the glue
- Gently push across a paper towel to remove excess wax
- Allow to dry
- DO NOT touch the surface of the otolith

Mount cover slips onto final slide

- DO NOT touch surface
- Blob super glue on slide
- Place cover slip in glue
- Make sure otolith is close to center of slide
- Allow to completely dry-approx. 2 hours


9/30/2010
9:47:05 AM
x: 4.4776 mm
y: 2.4972 mm
HFW
66 mm
det
None
10 mm
Quanta FEG 250


HV
15.00 kV
mag ⊞
40 x
HFW
3.73 mm
det
BSED
WD
9.8 mm
1 mm
Quanta FEG 250


HV
15.00 kV
mag ⊞
40 x
HFW
3.73 mm
det
BSED
WD
9.8 mm
1 mm
Quanta FEG 250

ICP-MS Capabilities and Hyphenated Techniques

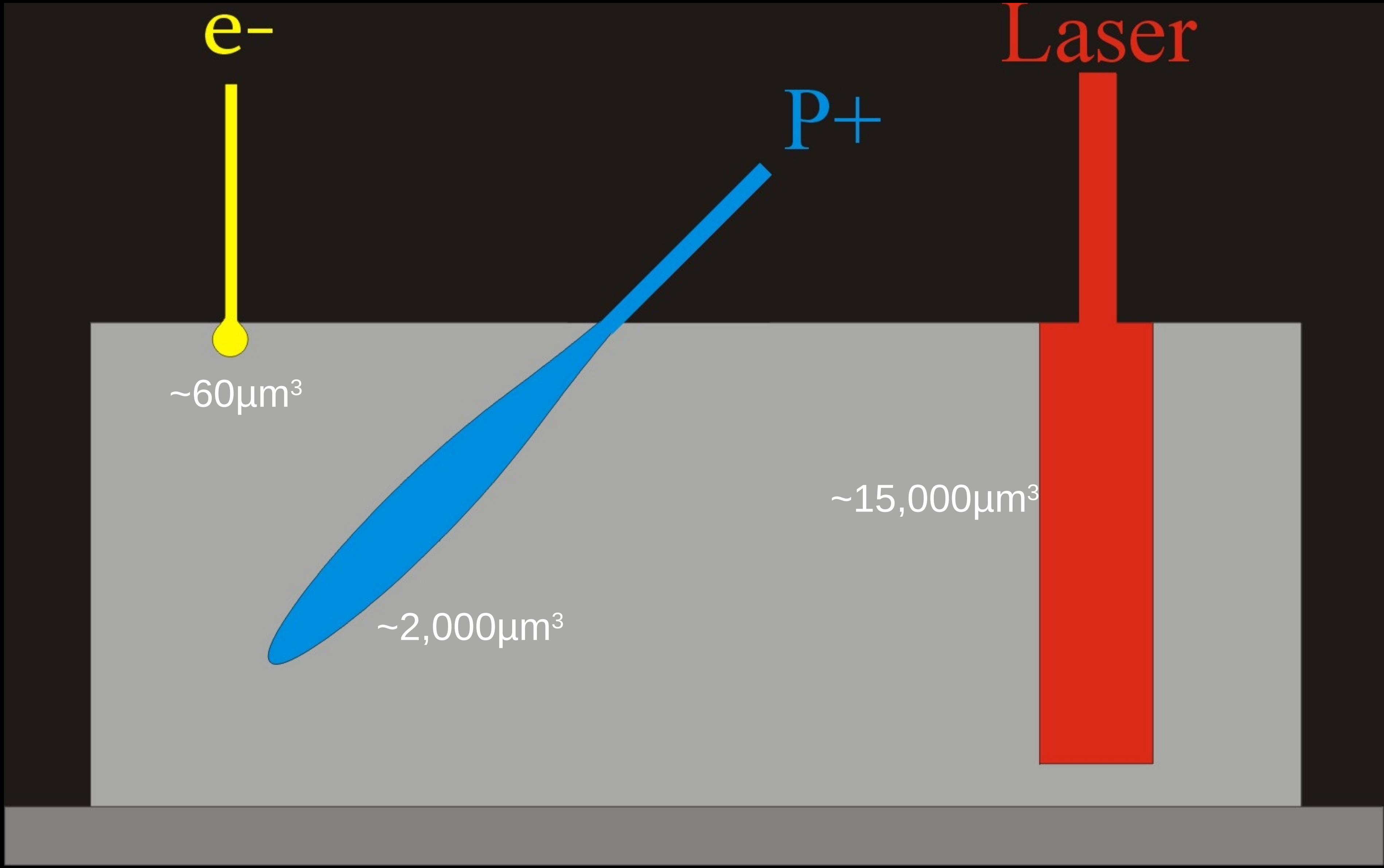
Capabilities
- Multi-element detection (metals and non-metals)
- DL – down to ppt
- Fast analysis times (full elemental suite in ~4 min)
- Isotopic information

Hyphenated ICP-MS
- LC (liquid chromatography)
- LA (laser ablation)

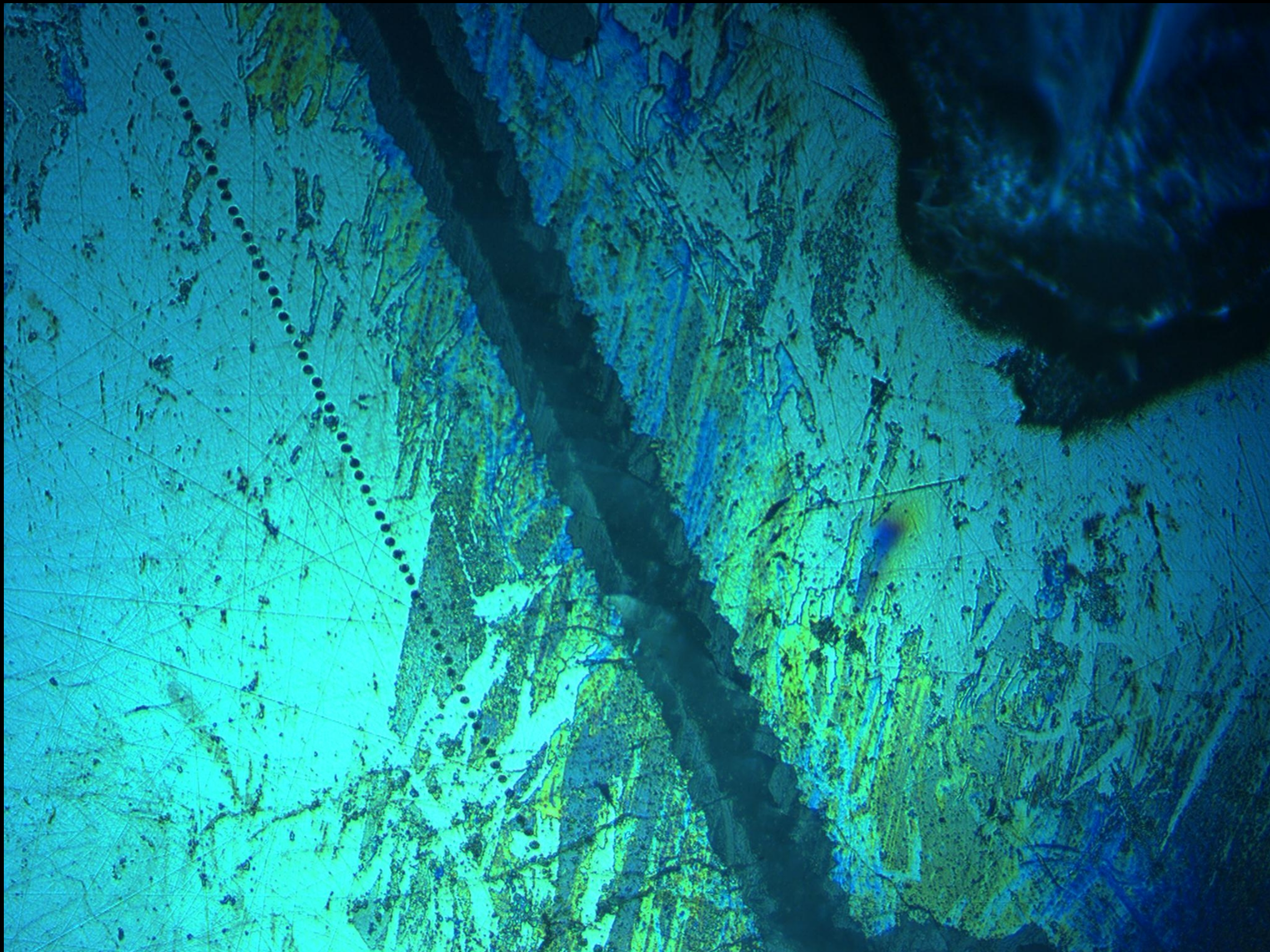
Agilent Technologies

photo by Karen Spaleta

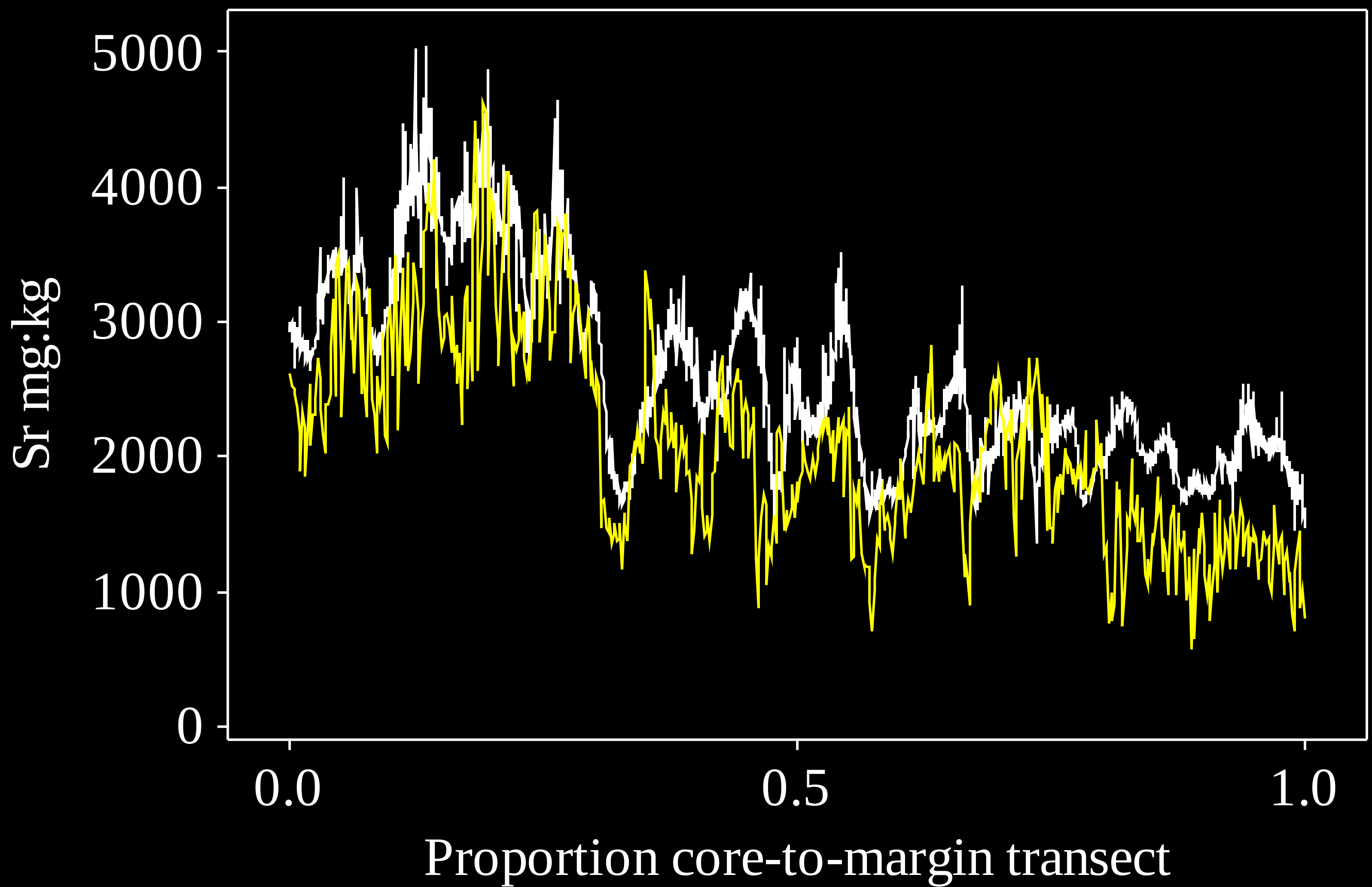
Probe qualities	Electron	Proton	Laser
Mechanism	ionization	ionization	ablation
Linear resolution	~5 μm	~5 μm	variable
Data type	X-ray counts	X-ray counts	Isotope counts
Count time	25 s/point	3-4 s/point	<1 s/point
Penetration depth	3 μm	35-50 μm	50-100 μm
Spatial resolution	high	medium	low
Surface sensitivity	high	low	low
Detection level	>100 mg/kg	~1 mg/kg	<1 mg/kg

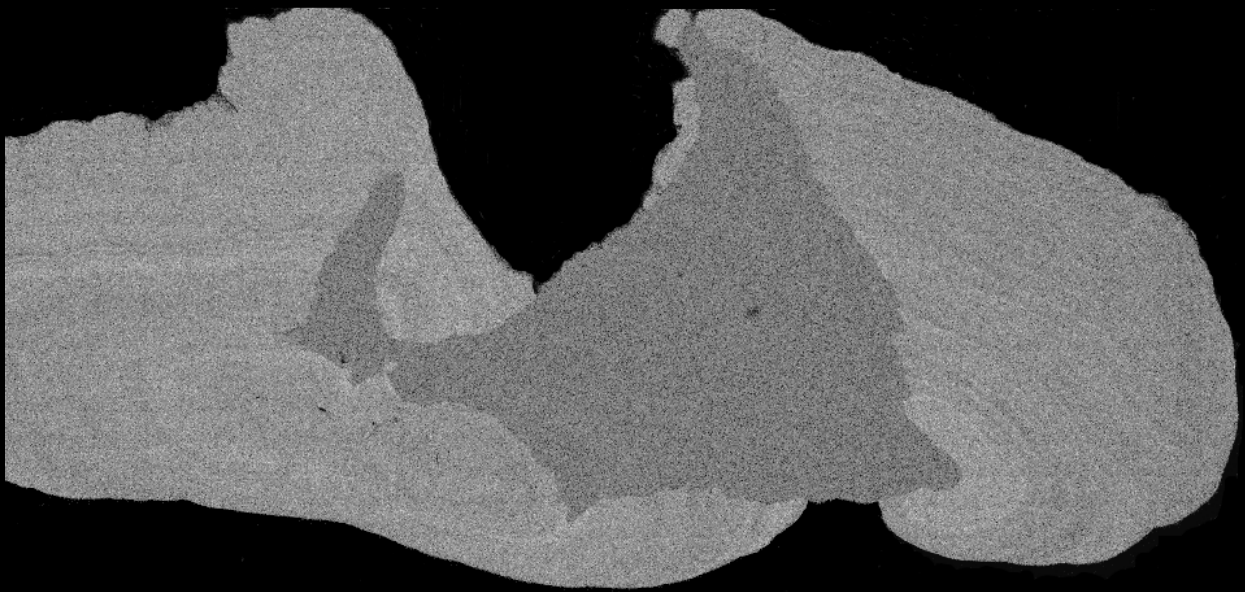


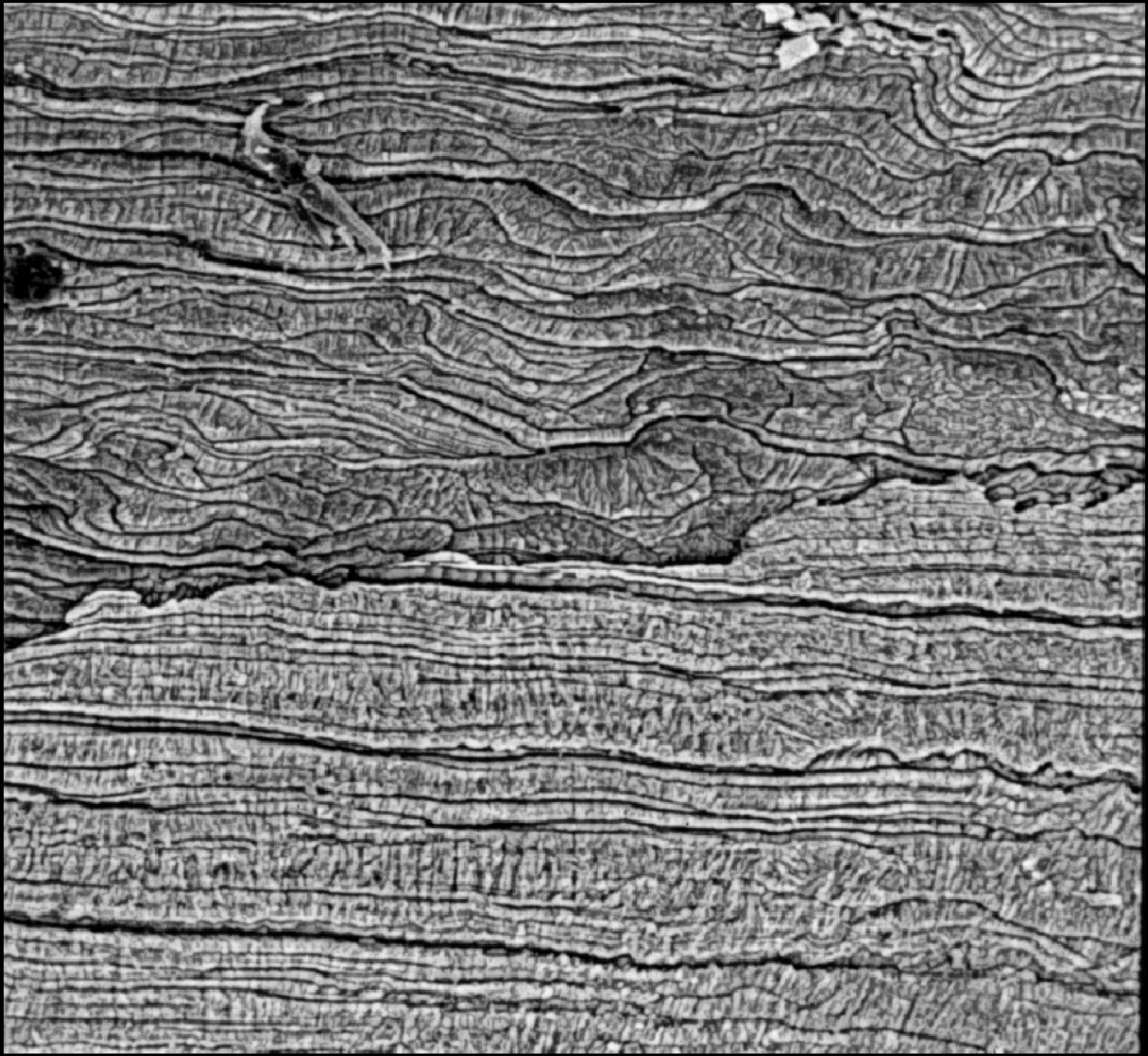
Approximate volumes of material examined per sample point



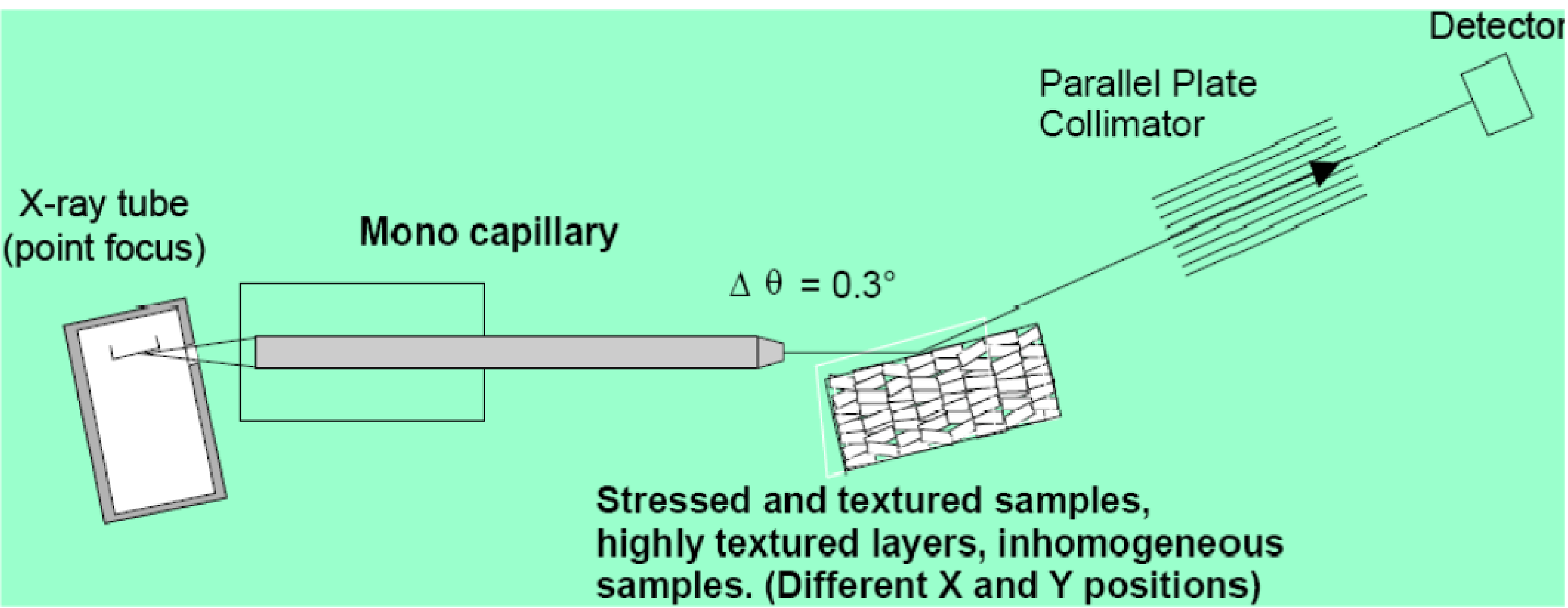
Laser (white) and electron (yellow) Sr distribution data for Barramundi







Method: mono-capillary microdiffraction phase analysis

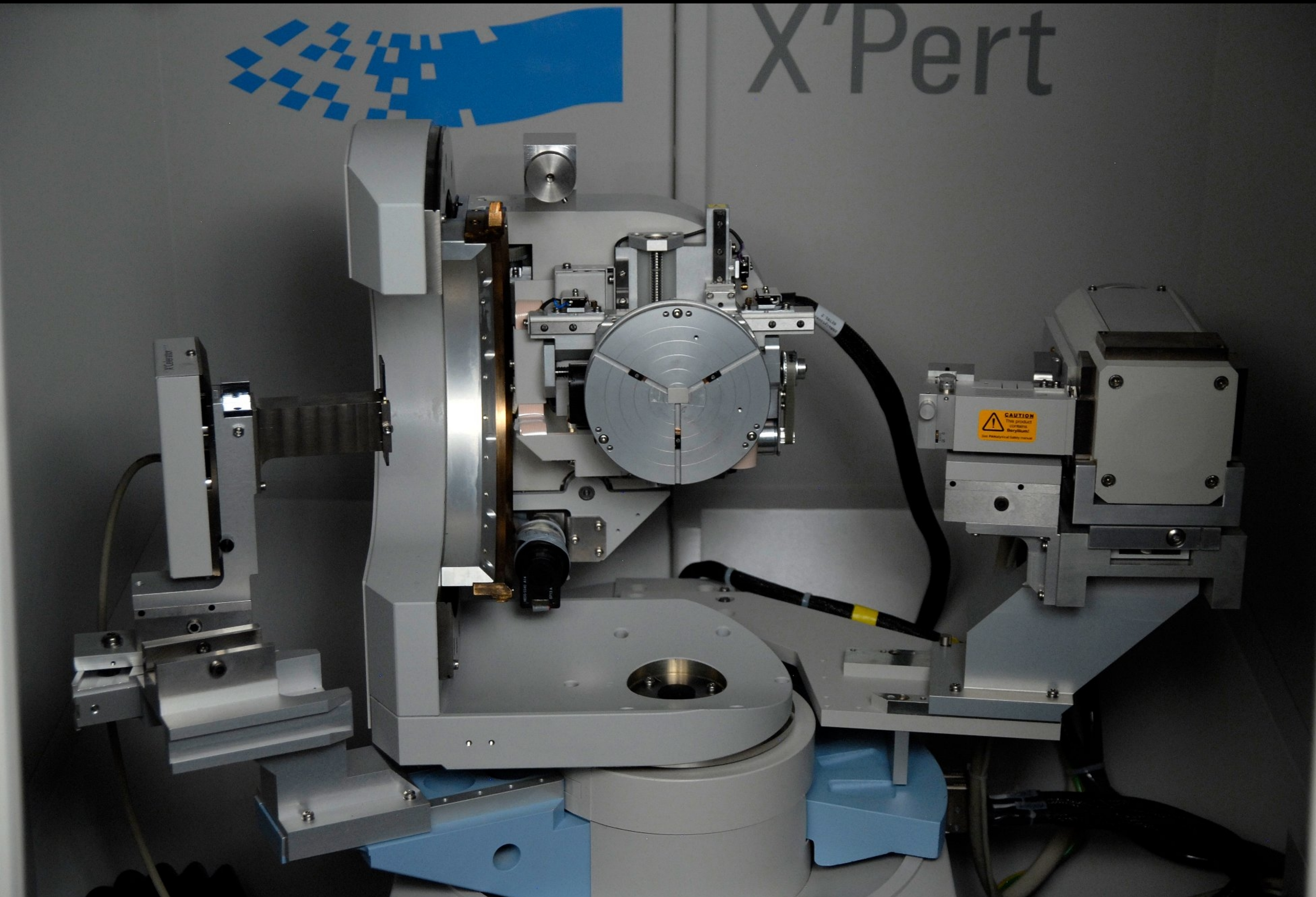


From CHEM 693 Fall 2009 lecture #7 notes

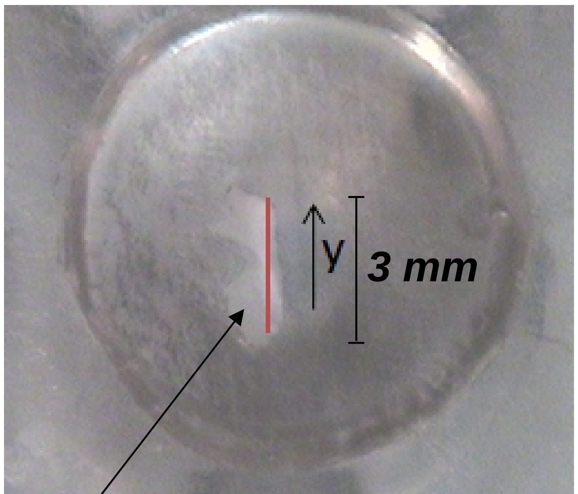
Resolution ~150 μm

Challenges:

- ***Alignment of X-ray beam and camera***
- ***Finding Z-coordinate so that X-ray beam penetrates sample***


X'Pert
CAUTION
This product
contains
Beryllium!

Results: mixed sample

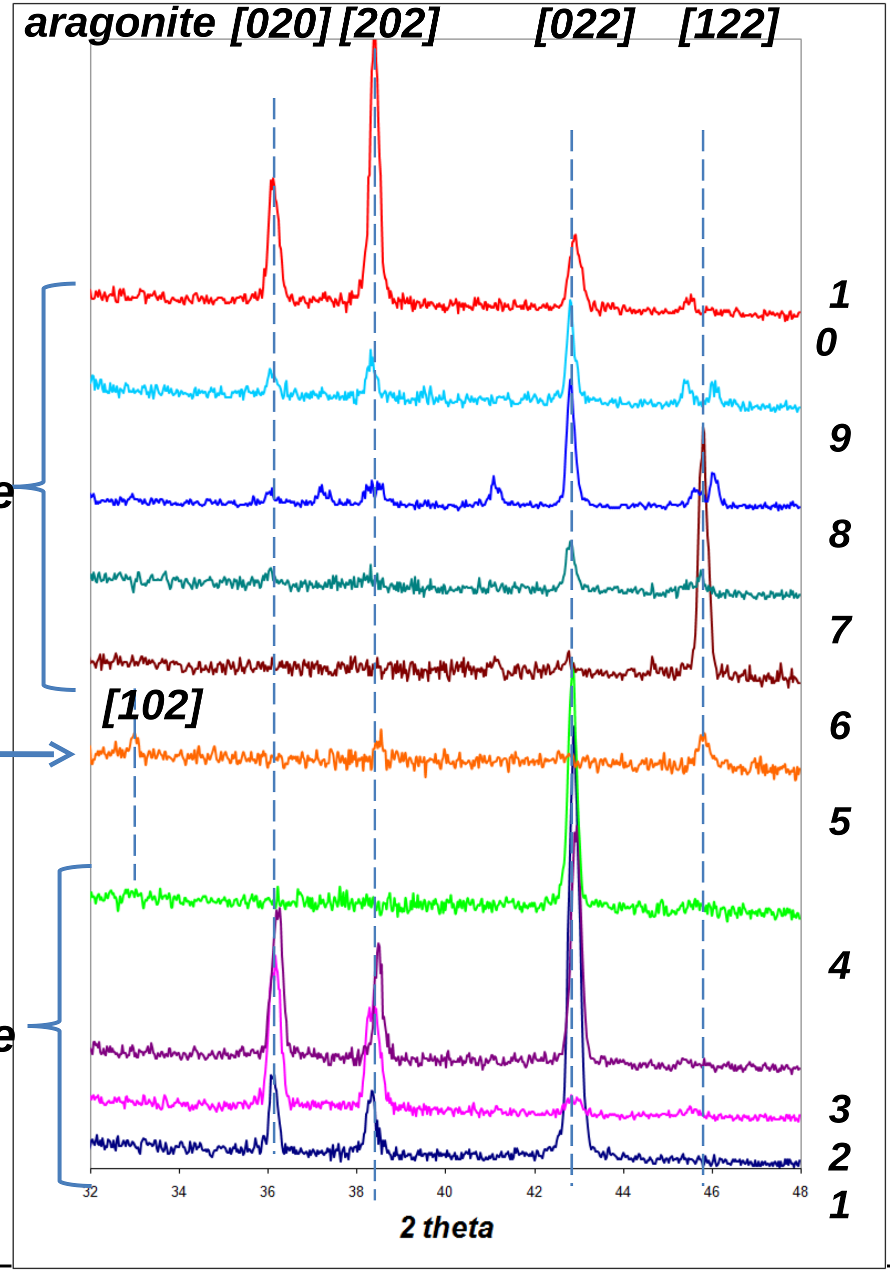


Sheefish otolith

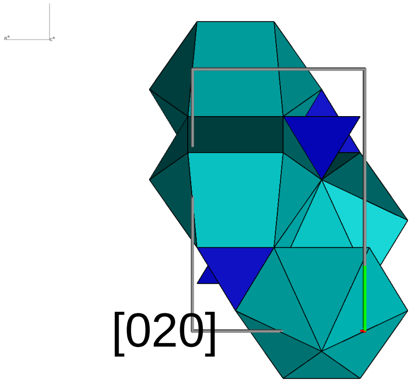
aragonite

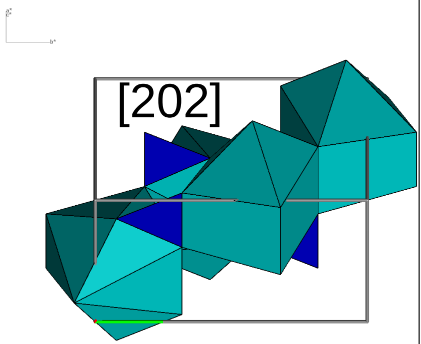
vaterite

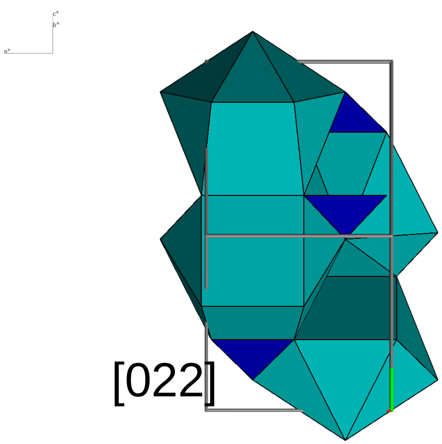
aragonite

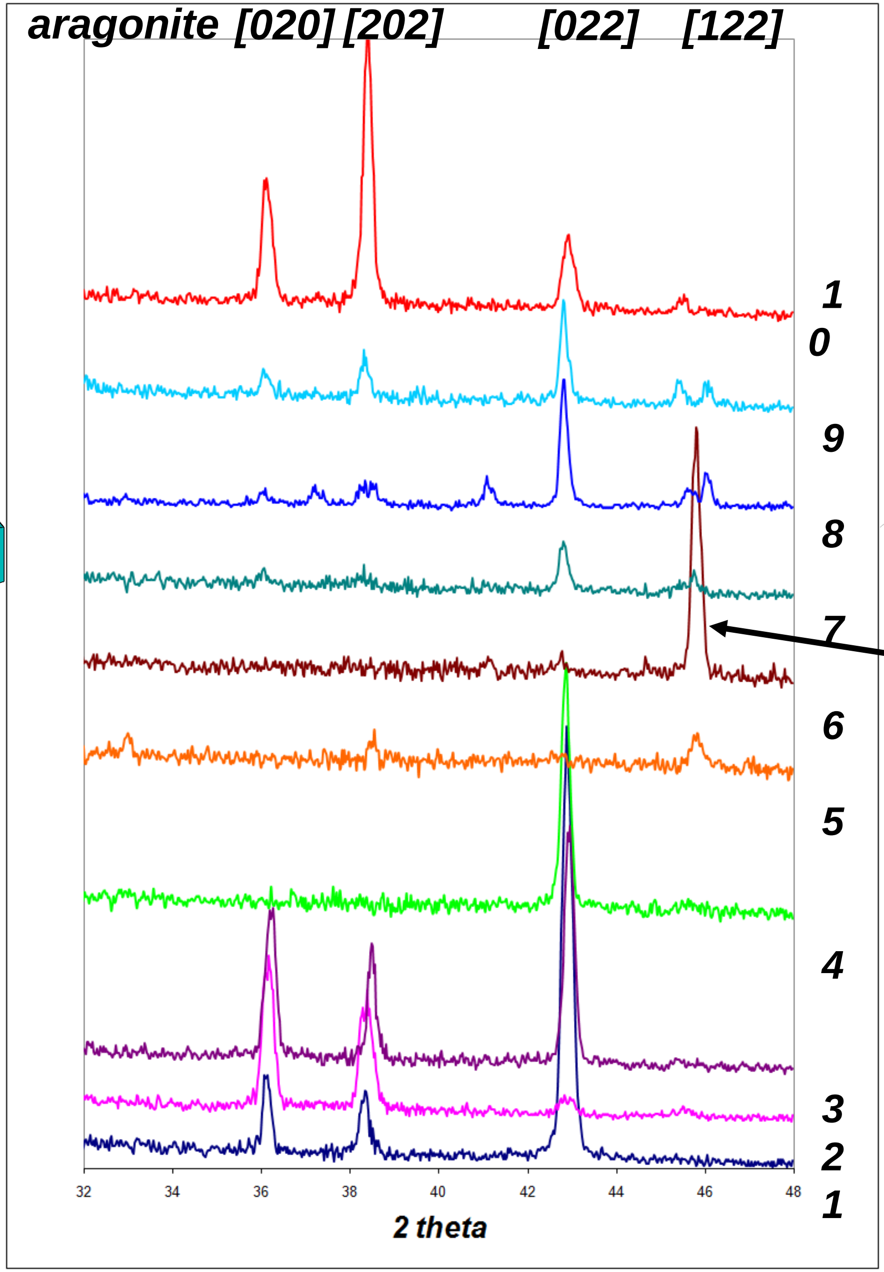


Results: mixed sample

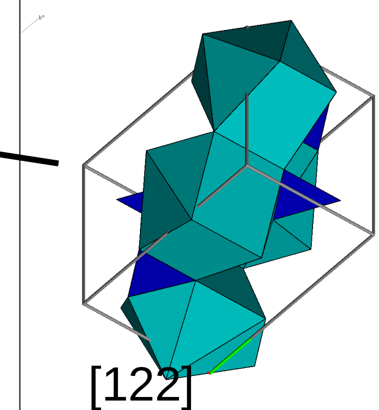








The orientation of aragonite crystals is different along the scan line



X-rays are perpendicular to the page

Some thoughts I've had after the talks on Tuesday

- Assumptions that marked fish act the same as wild fish
- “Compatible with hatchery procedures”
- “Save money in mark assessment”
- Human Error

and probably most important

- What is the question that the interpretation of the mark is supposed to answer, and how good does the answer have to be?

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Advanced Instrumentation Laboratory

The Advanced Instrumentation Laboratory at the University of Alaska Fairbanks is a multi-instrument resource for the state of Alaska. It specializes in surface and elemental analysis as well as electron microscopy. In addition to the instrumentation it also houses support sample preparation facilities. AIL is located on the UAF campus in the Reichardt Building.

Please forgive us as this page is under construction. You will find several blank pages, and, given our skill with web editing, some things that don't appear quite as nice as we would like.

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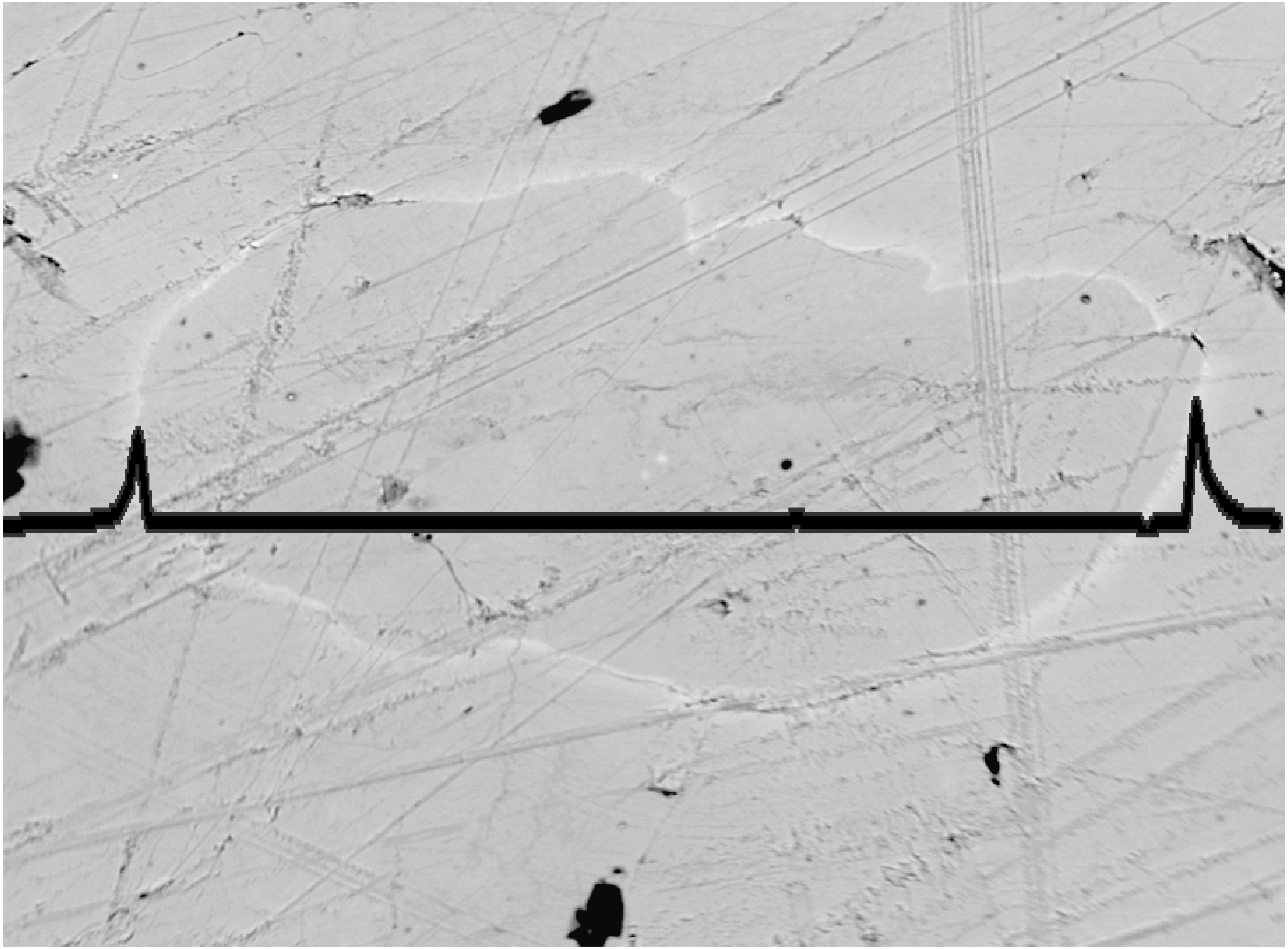


Artifacts: Si-escape peak; Si internal fluorescence peak

There are 2 exceptions to the previous neat explanation of how the Si(Li) detector works. Si-escape peaks are artifacts that occur in a small % of cases, where the Si ka X-ray generated in the capture of the original X-ray escapes **out of the detector** (red in figure). Since this X-ray removes 1.74 keV of energy, the signal generated (electron-hole pairs) by the incident X-ray will be 1.74 keV LOW. This will produce a small peak on the EDS spectrum 1.74 keV **below** the characteristic X-ray peak. Another artifact is the Si internal fluorescence peak, which occurs if an incident X-ray is absorbed in the Si "dead" layer (green region). This region is "dead" to production of electron-hole pairs, but Si ka X-rays can be produced here which then end up in the "live" part of the detector, and result in a small Si ka EDS peak.

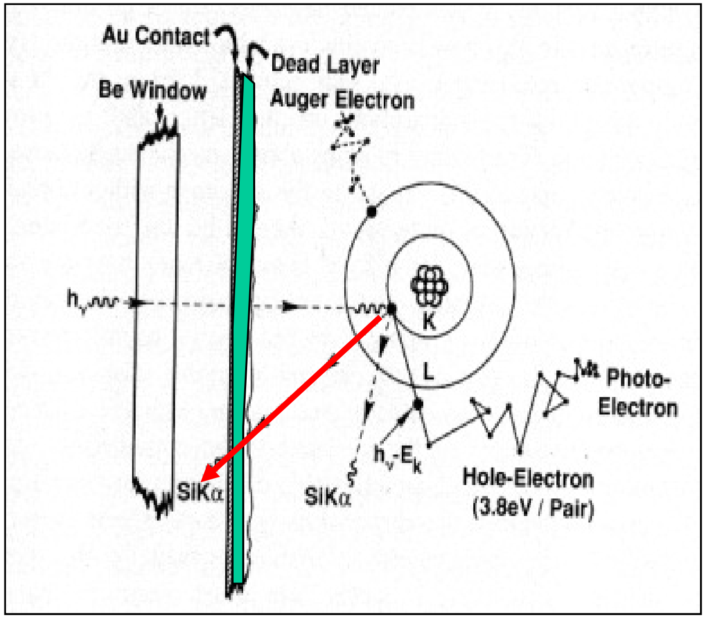
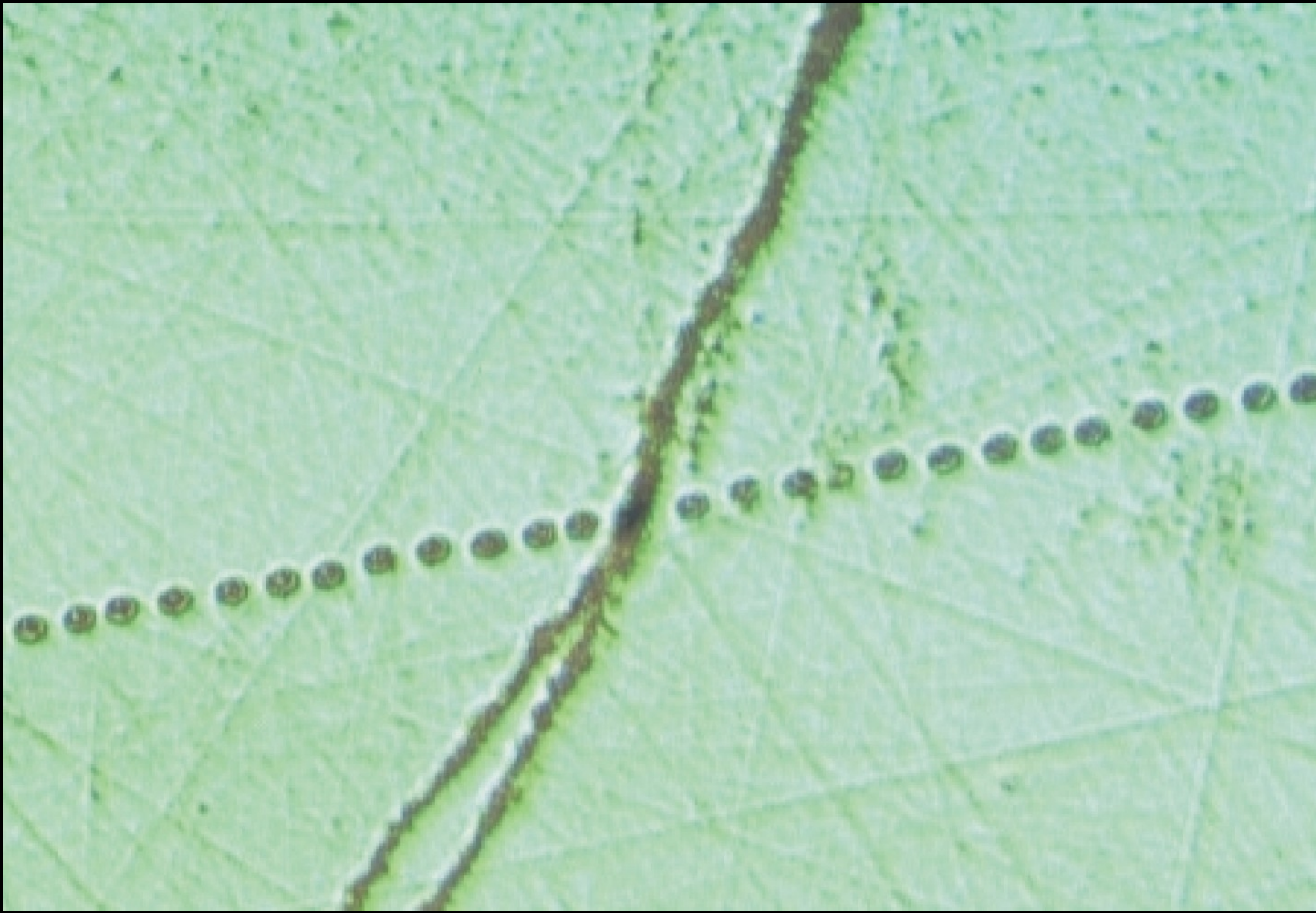
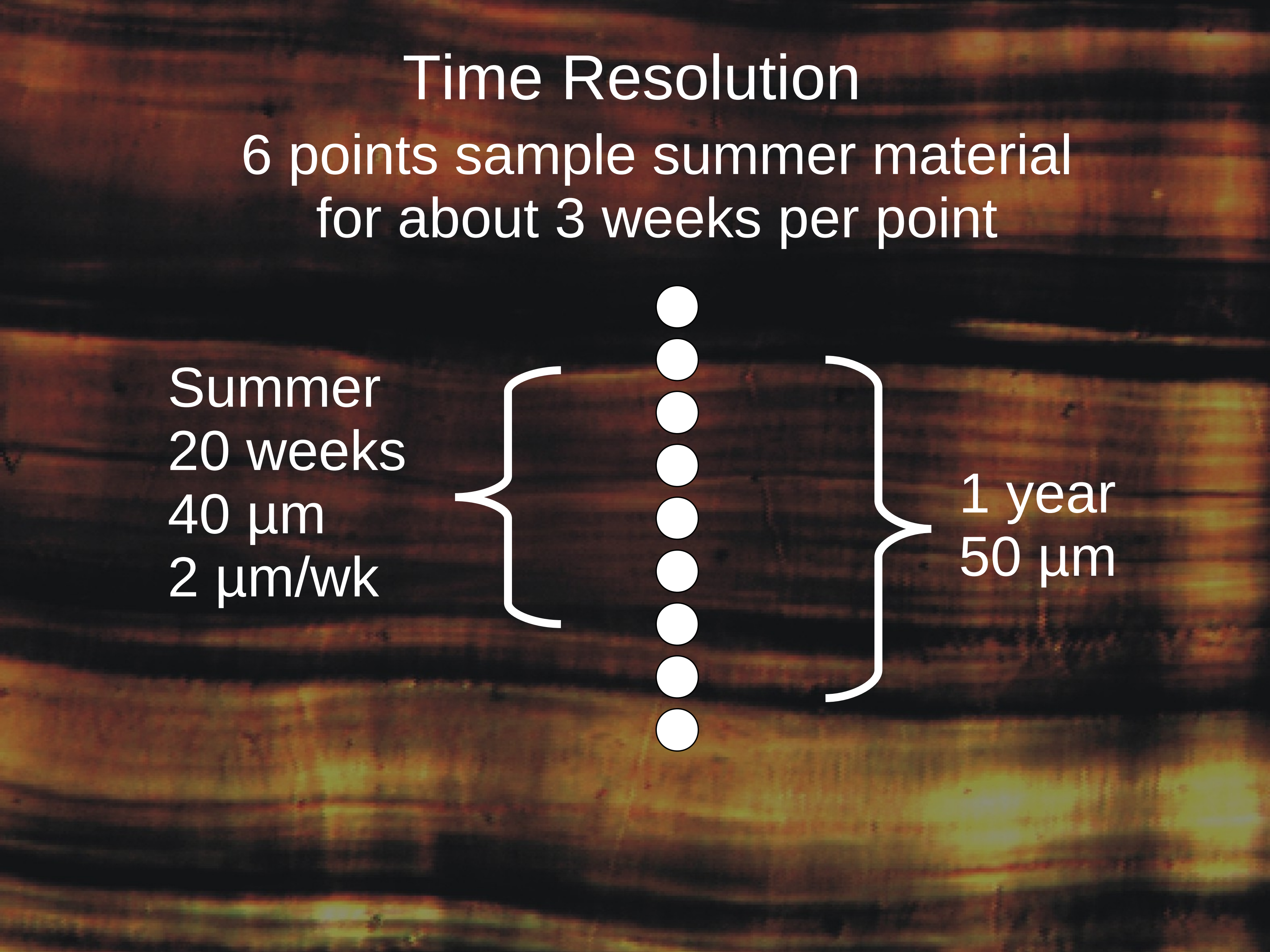
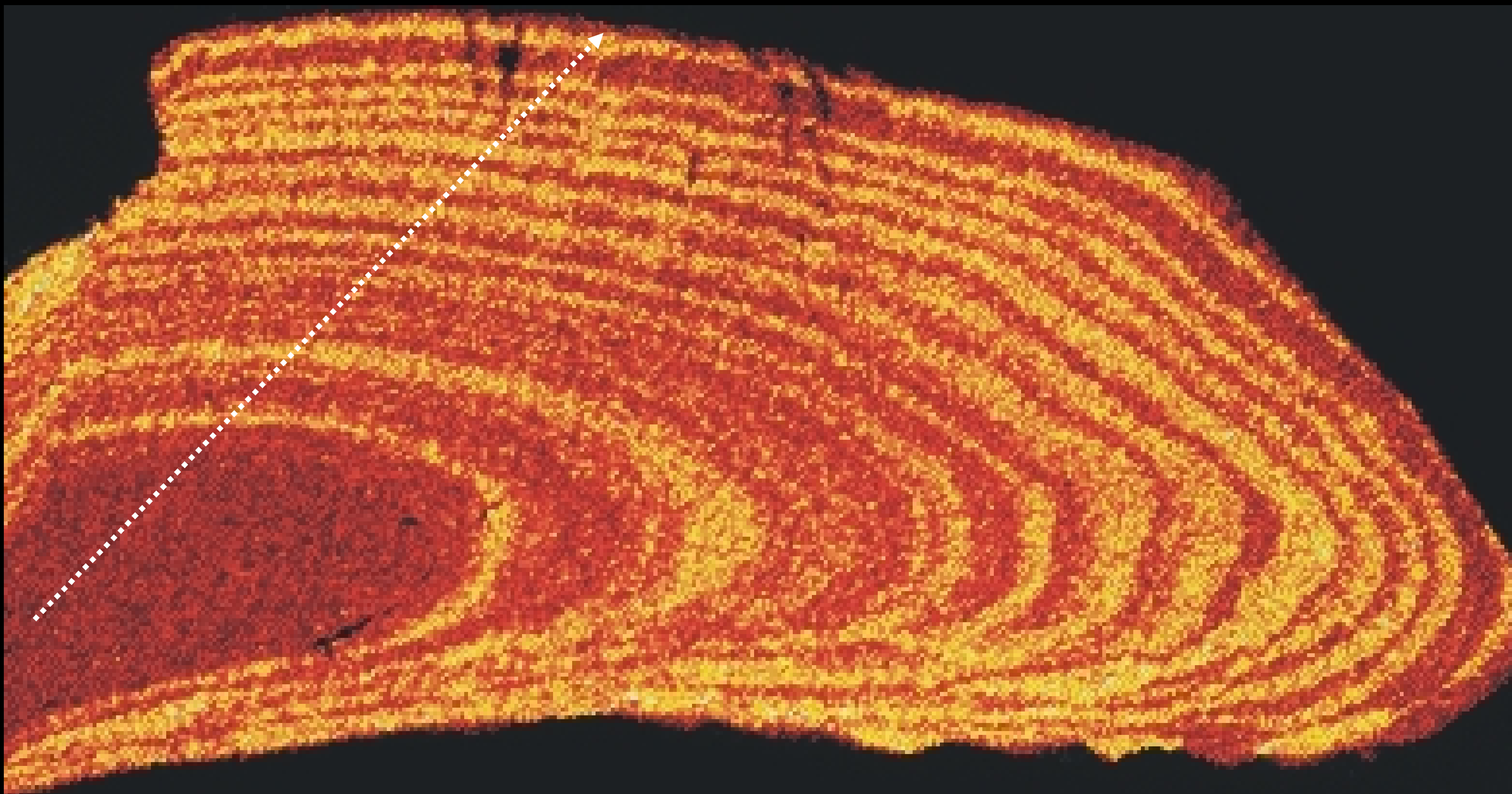


Fig 5.22 Goldstein et al




Time Resolution
6 points sample summer material
for about 3 weeks per point
Summer
20 weeks
40 µm
2 µm/wk
1 year
50 µm



A Comment:

- Until we understand how and why trace elements are incorporated in otoliths, we will be working with correlations rather than causality. This may not be a problem for marked otoliths where we know what we are putting into otoliths, but will slow the interpretation of natural elemental variability.

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