Metal concentrations in fish scales and otoliths

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THIRD PROJECT MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 19th May 2023

2.4. MEASUREMENT OF METAL CONCENTRATIONS IN FISH CALCIFIED STRUCTURES

Calcified structures will be cleaned by Milli-Q water and dried. Otoliths will be ground and polished. For each sample 4-6 scales will be prepared and mounted on small glass slides using adhesive tape and the scale with the most visible growth zones per sample will be analyzed. Measurement of metals will be conducted by connecting a laser ablation system (NWR193, Electro Scientific Industries) to an ICP-QMS (NexION 350D, PerkinElmer) and the laser lines will be taken through the middle of the hard tissues. Calcium, as a main element in the aragonite of otoliths and hydroxyapatite of scales, will be used as internal standard.

Gulnaz

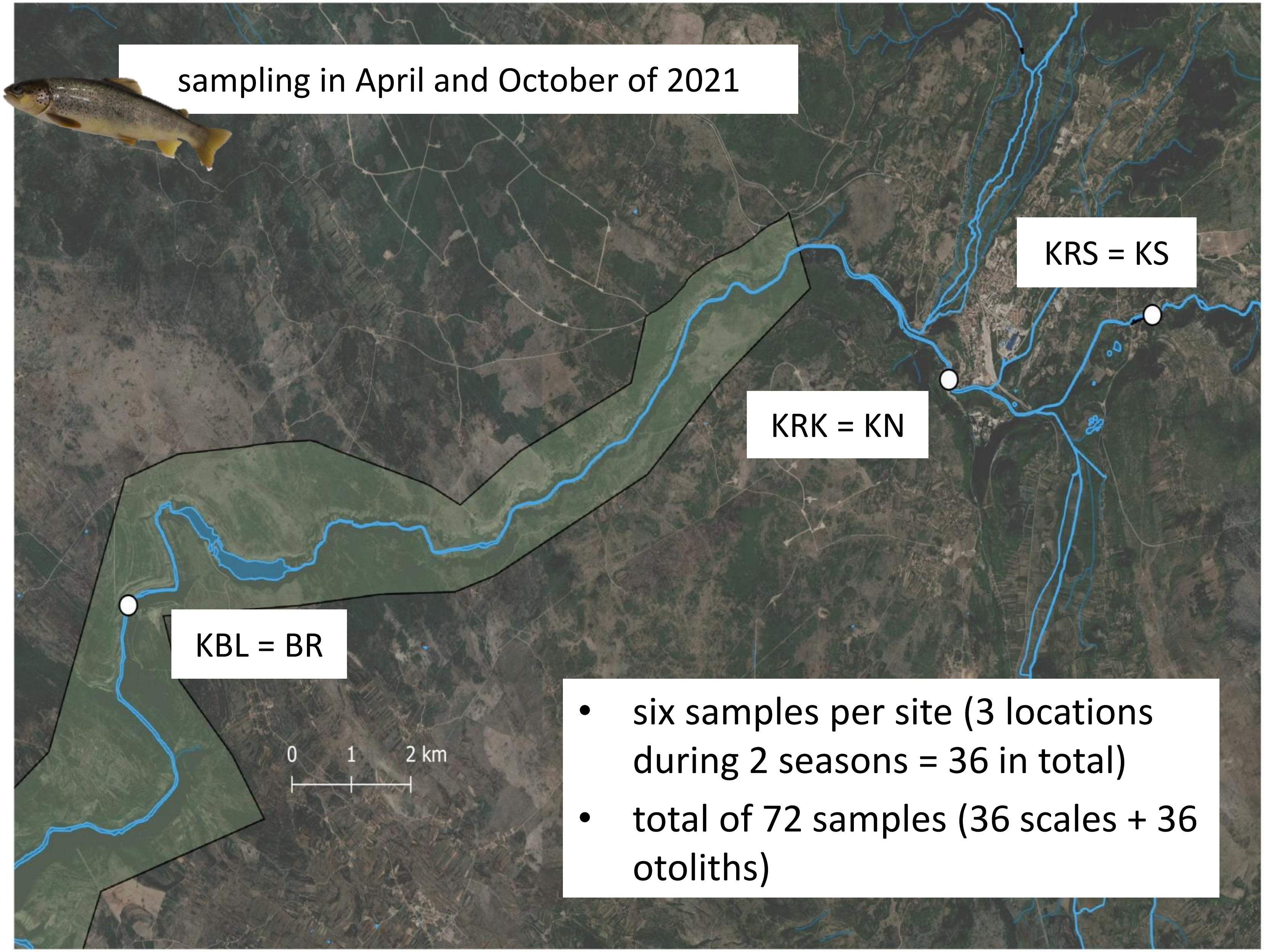
Stefan

Donata

Johanna

Thomas

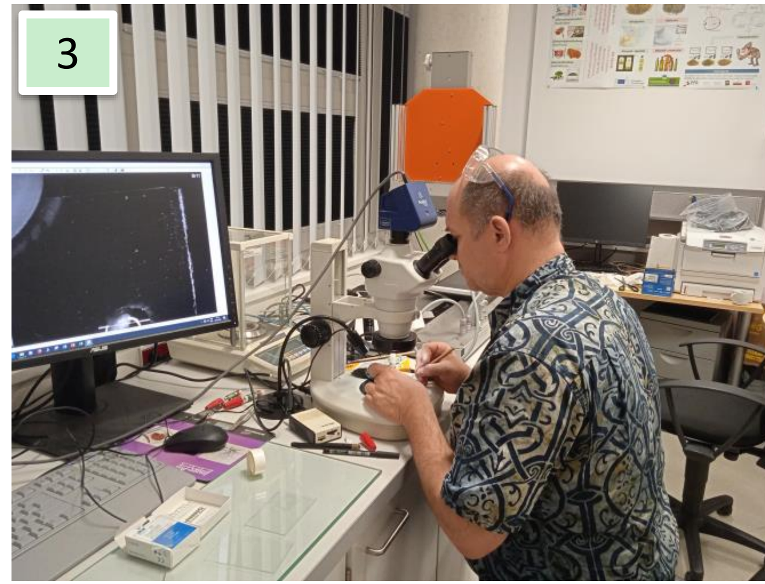
Andreas


sampling in April and October of 2021
KRS = KS
KRK = KN
KBL = BR
0 1 2 km
• six samples per site (3 locations during 2 seasons = 36 in total)
• total of 72 samples (36 scales + 36 otoliths)

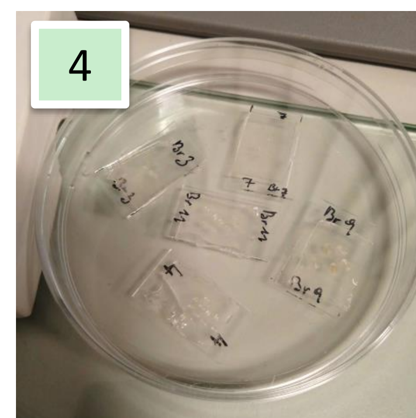
Sample preparation

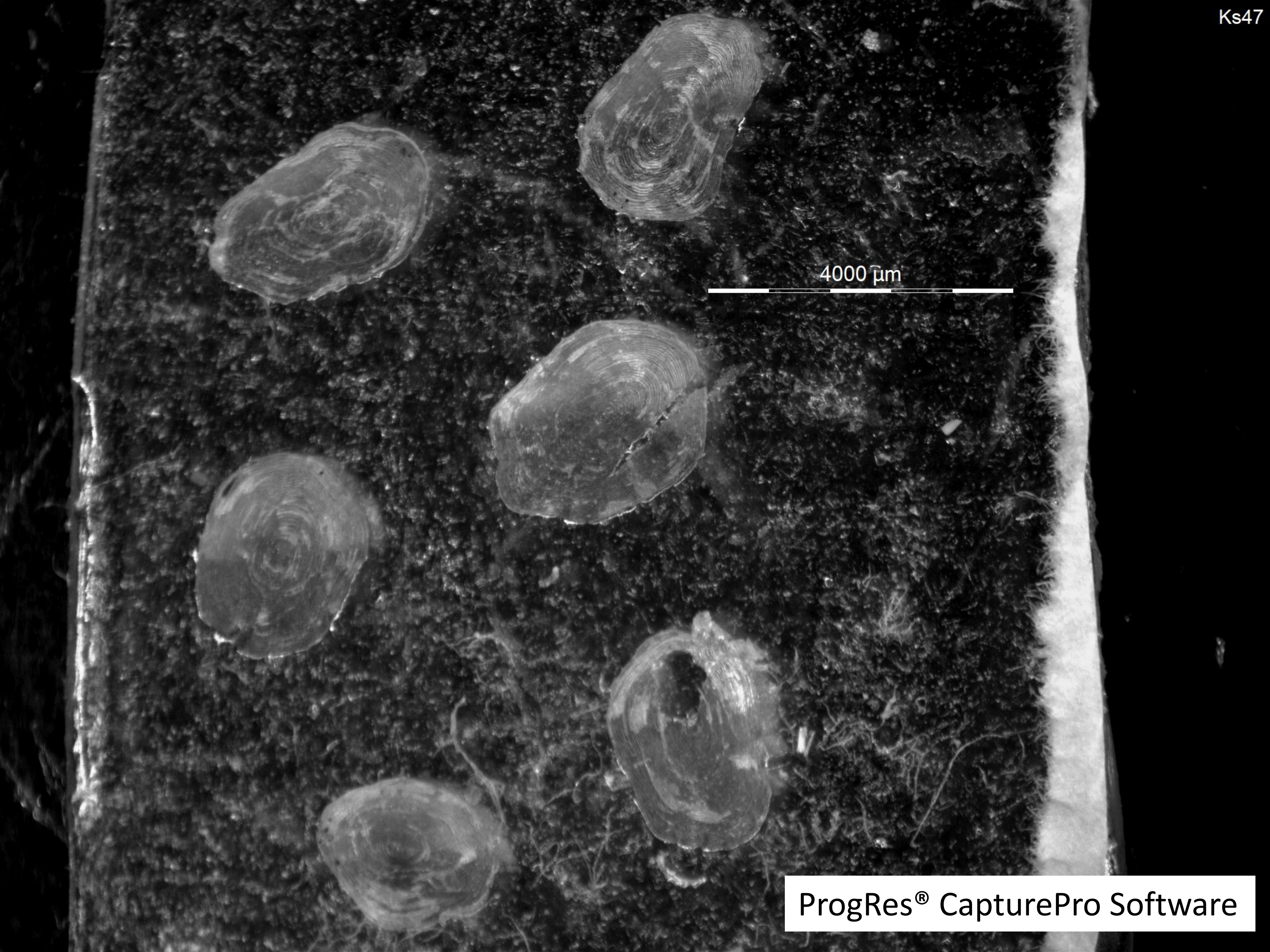
Preparation of scales

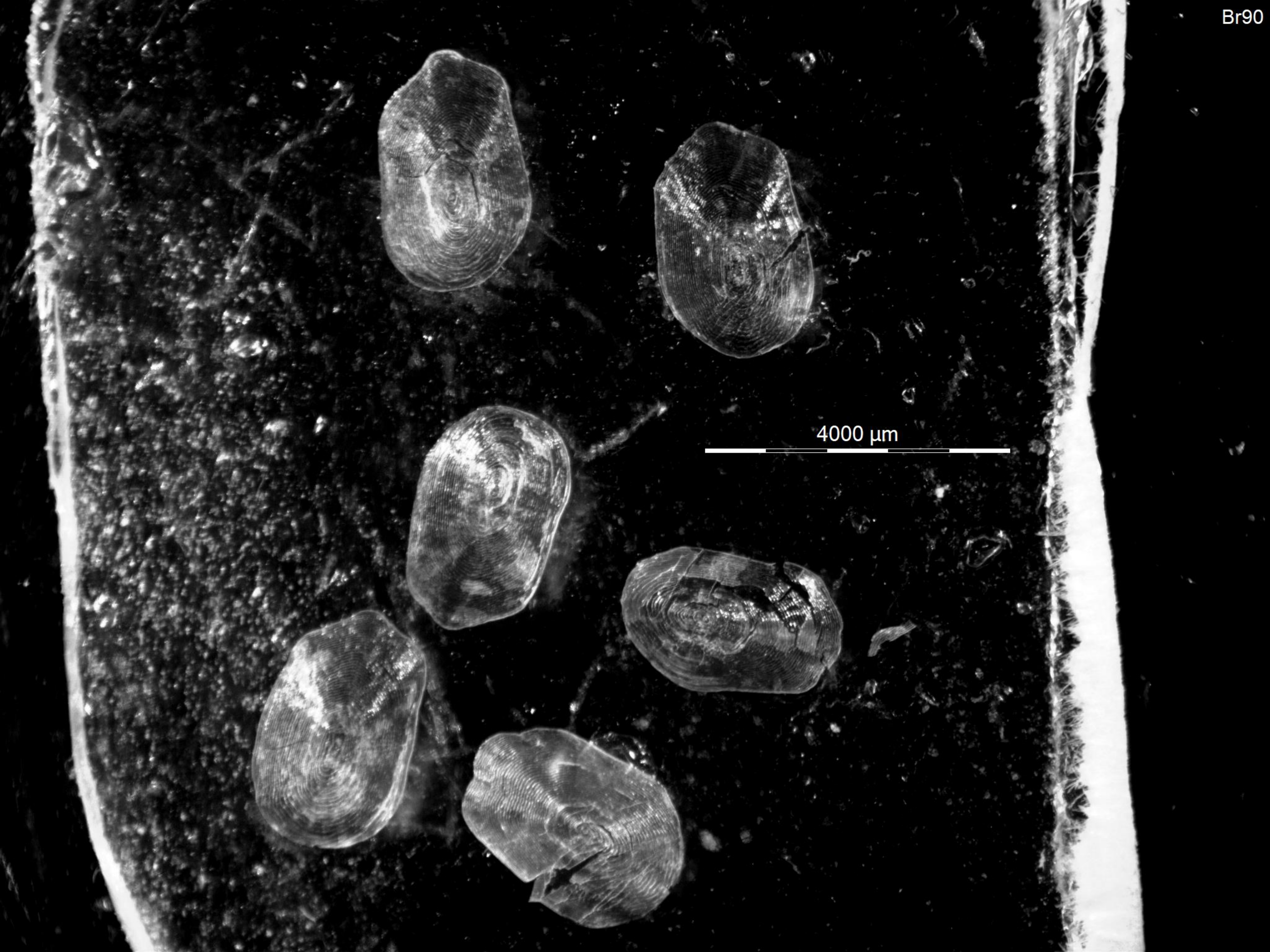
2

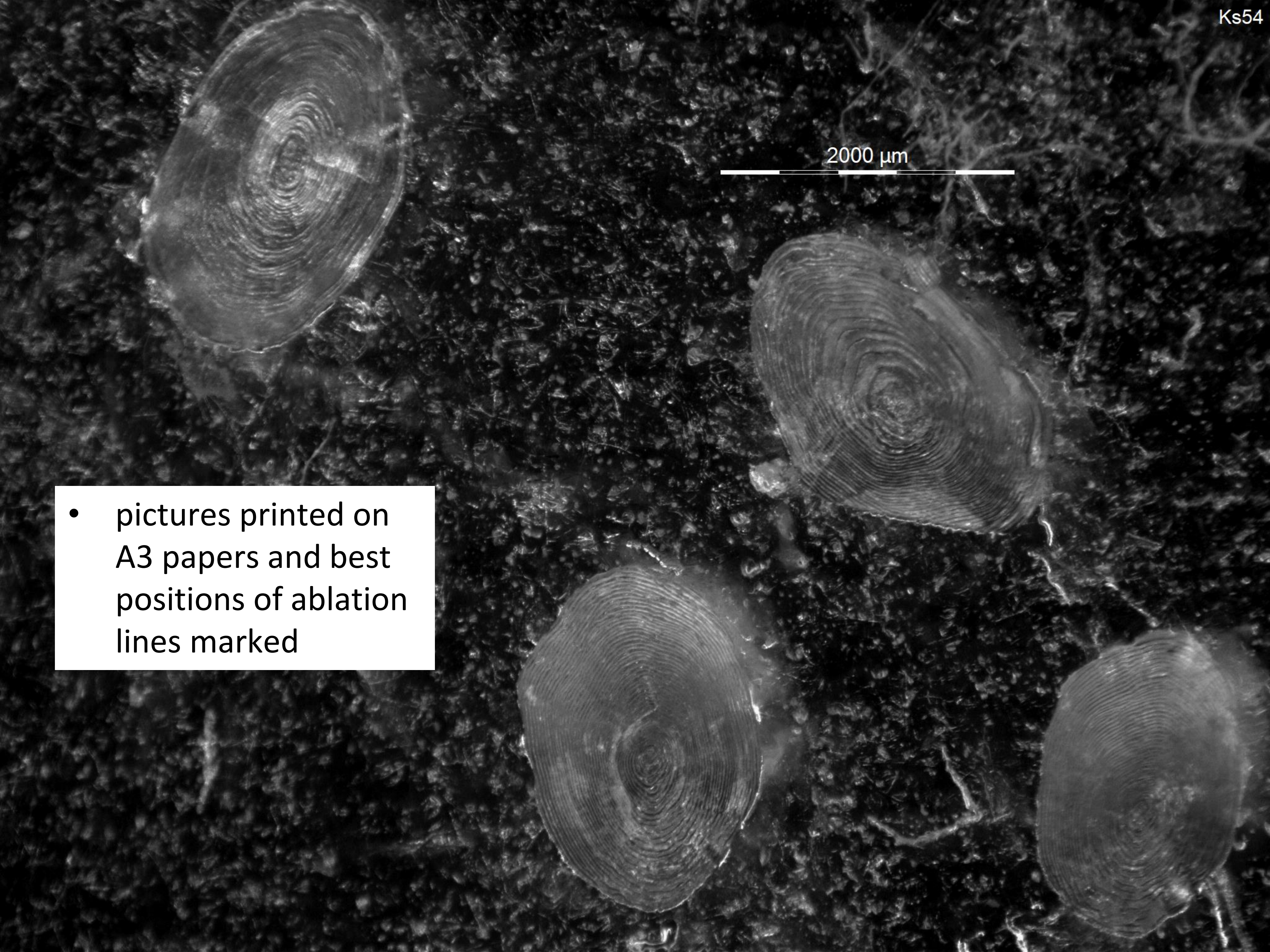


better to use paper envelopes/bags



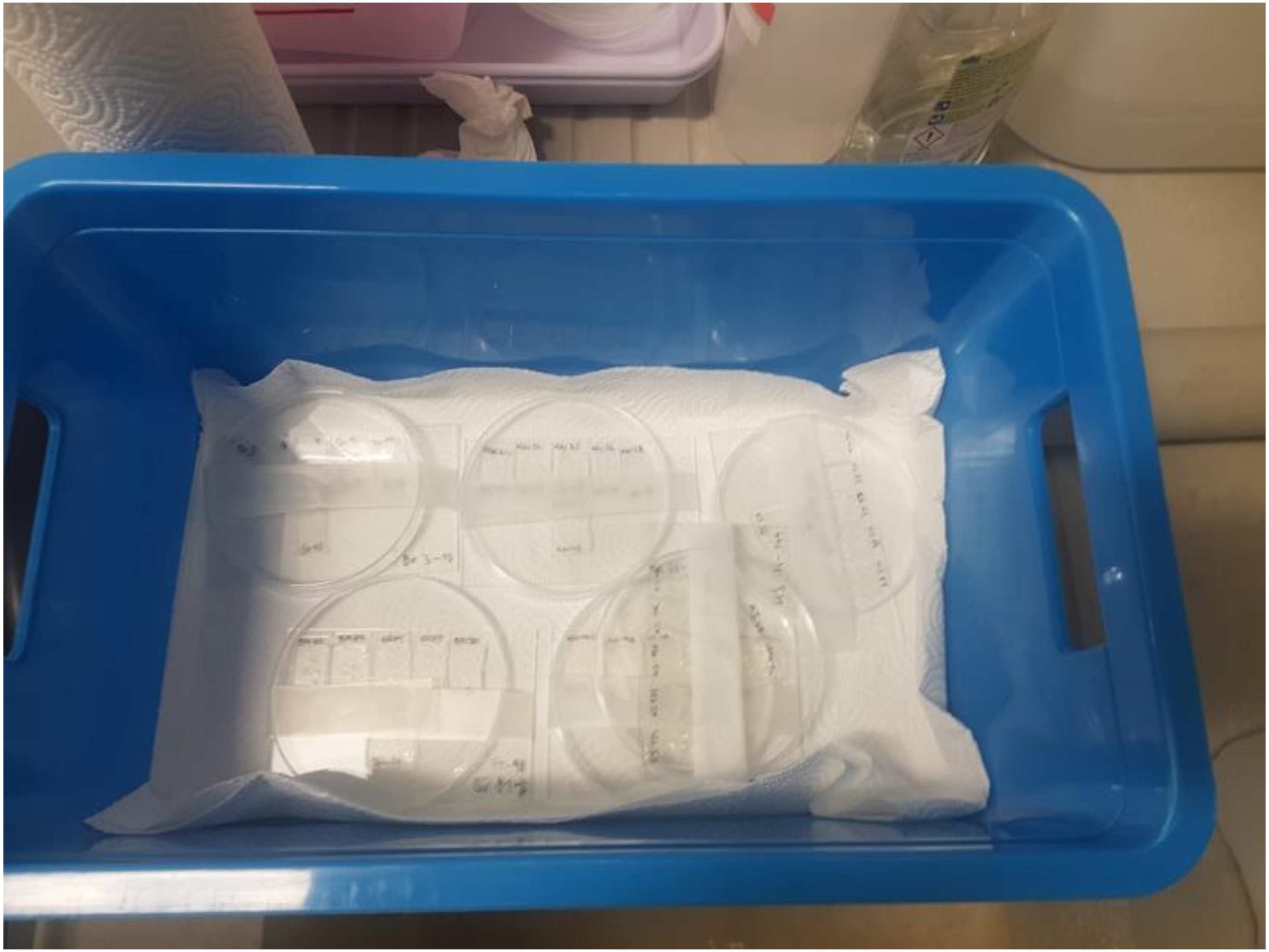

Ks47
4000 µm
ProgRes® CapturePro Software


Br90
4000 µm

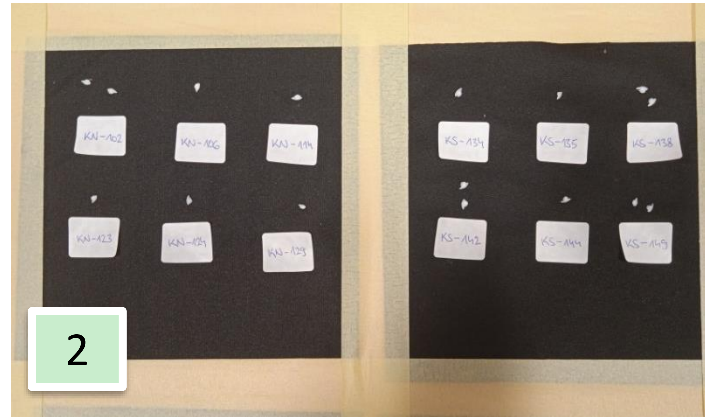


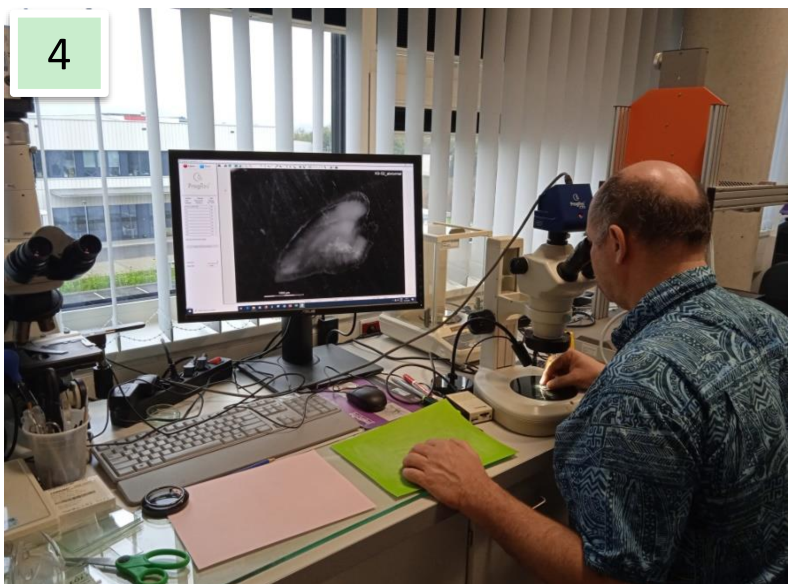
- pictures printed on A3 papers and best positions of ablation lines marked

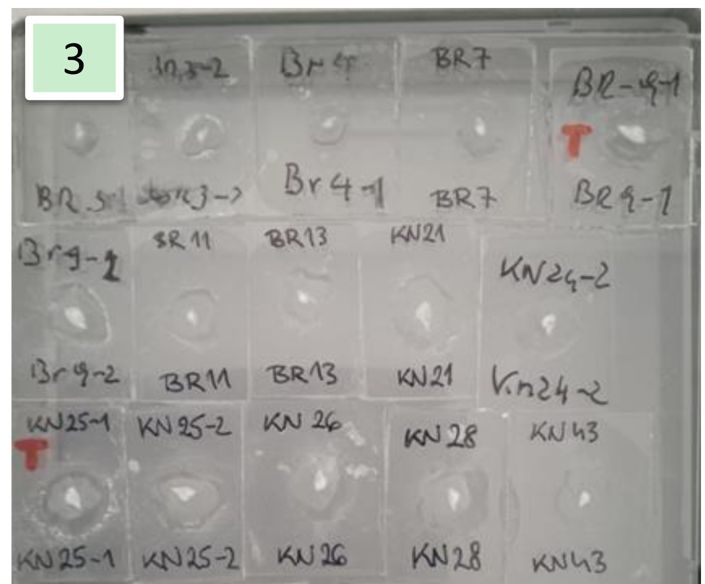

Br98
2000 µm



Preparation of otolithes

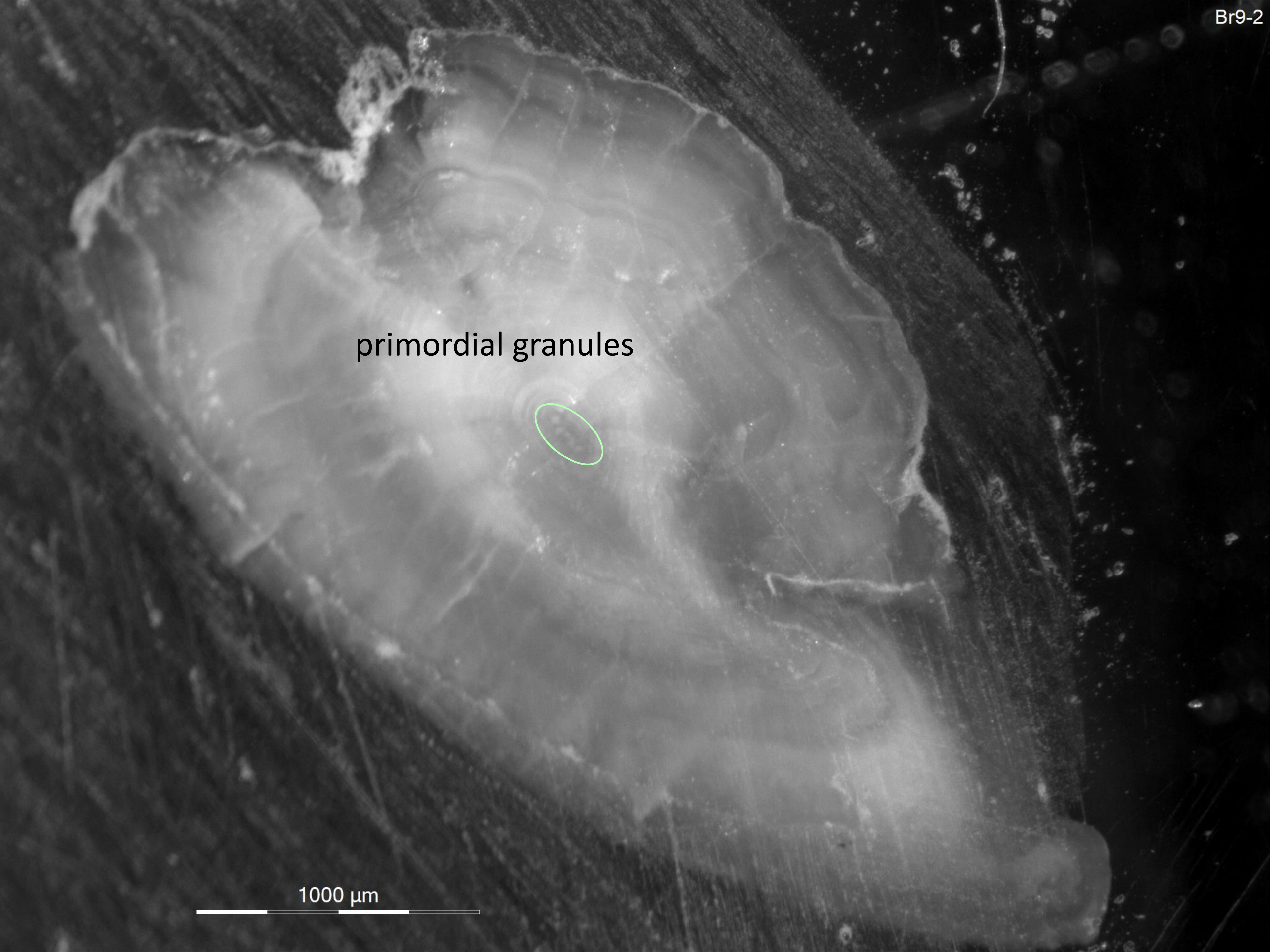


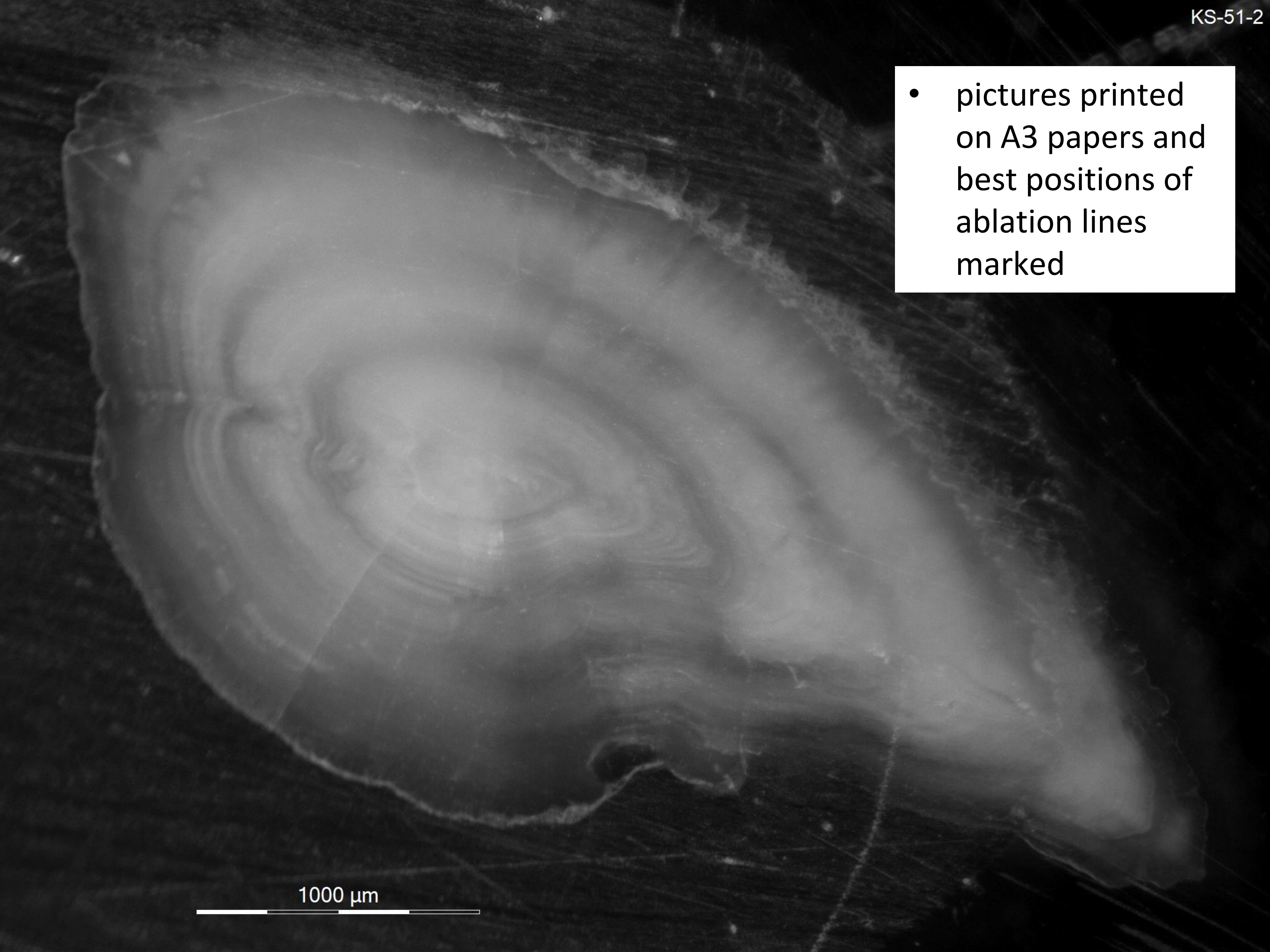




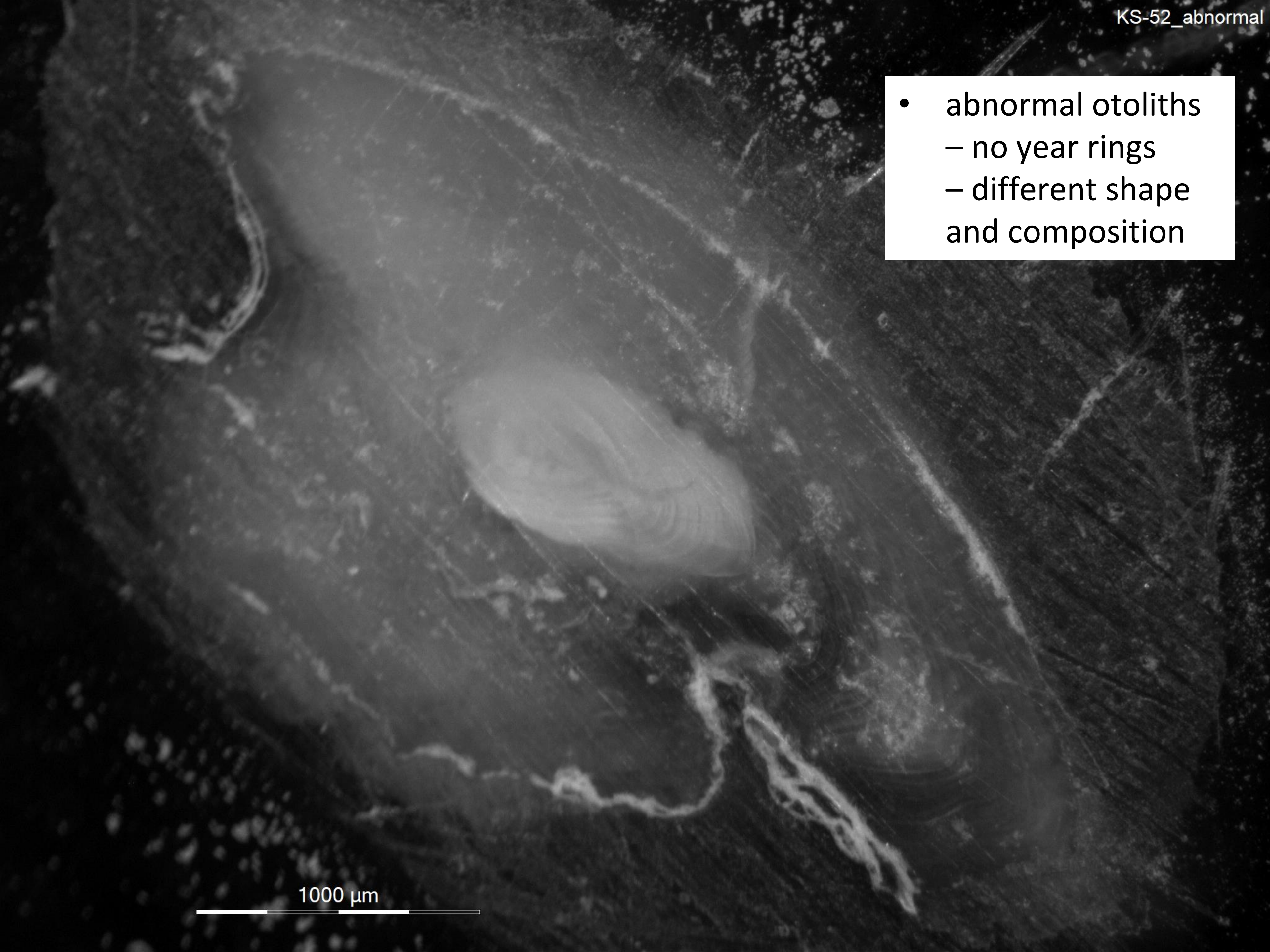
Krazy glue

lapping films of 30 µm and 3 µm for grinding and polishing

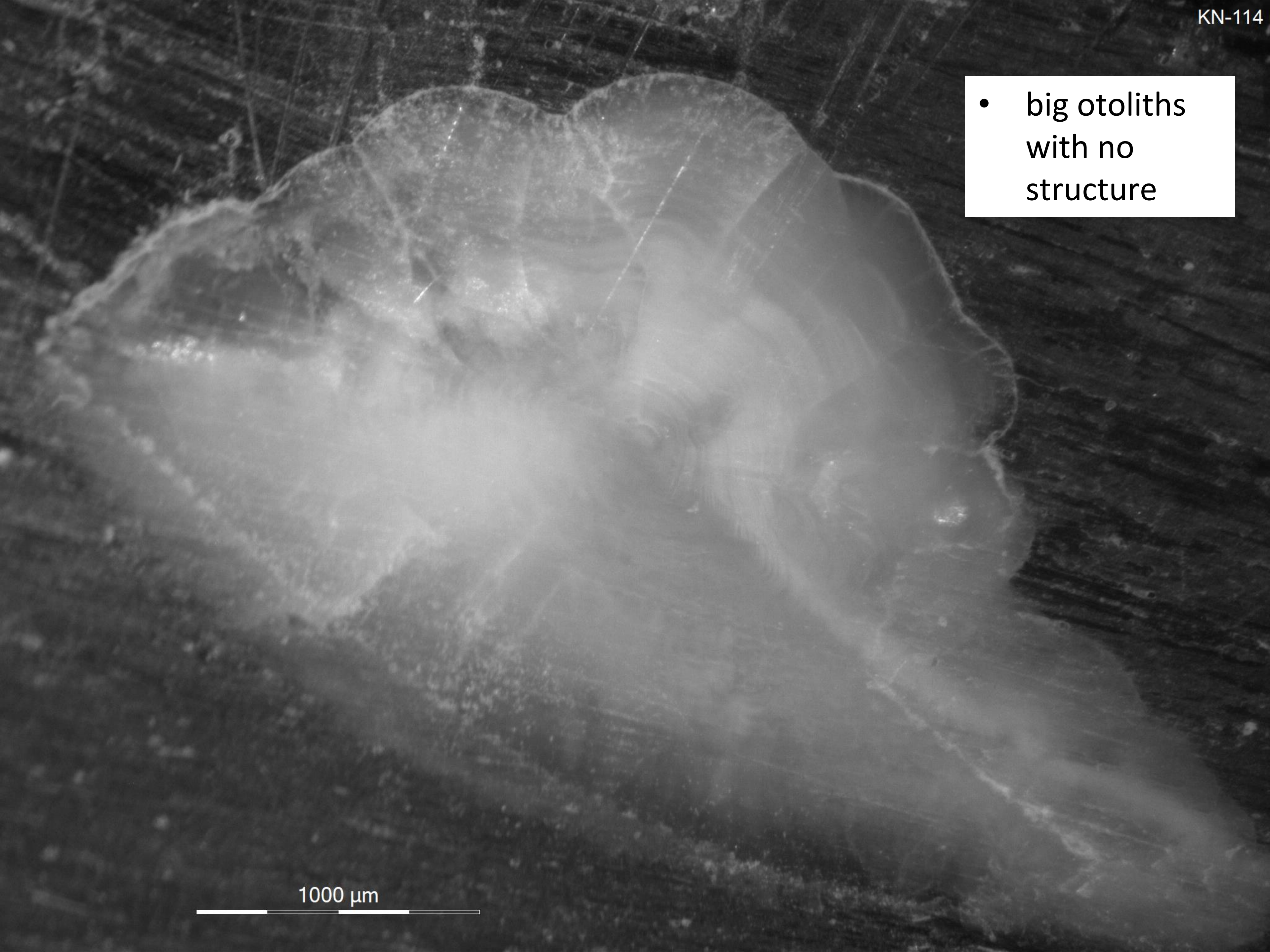

Br9-2
primordial granules
1000 µm



- pictures printed on A3 papers and best positions of ablation lines marked



- abnormal otoliths
  – no year rings
  – different shape and composition

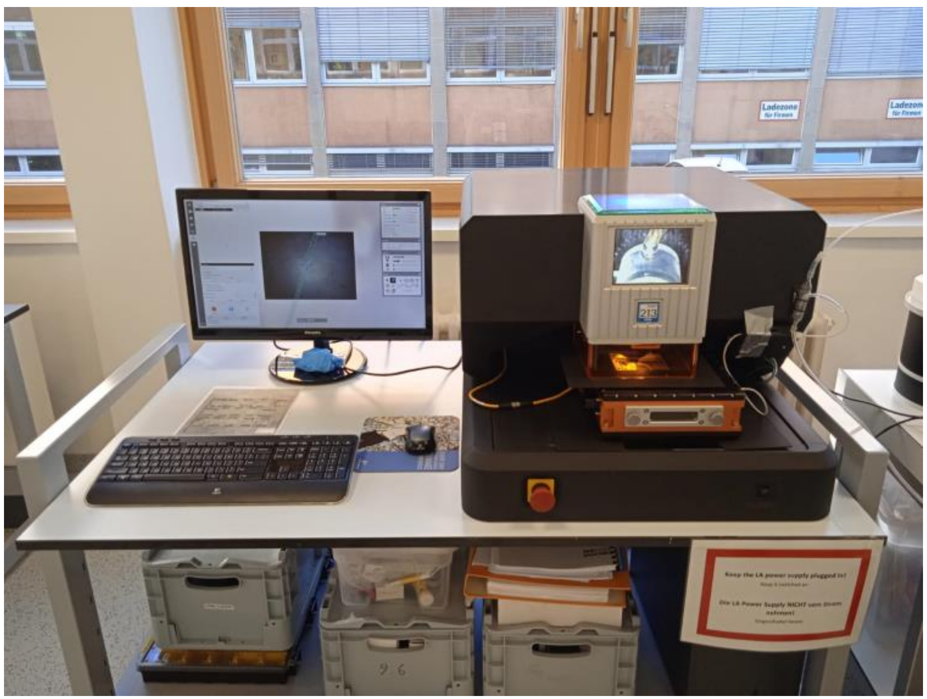


- big otoliths with no structure



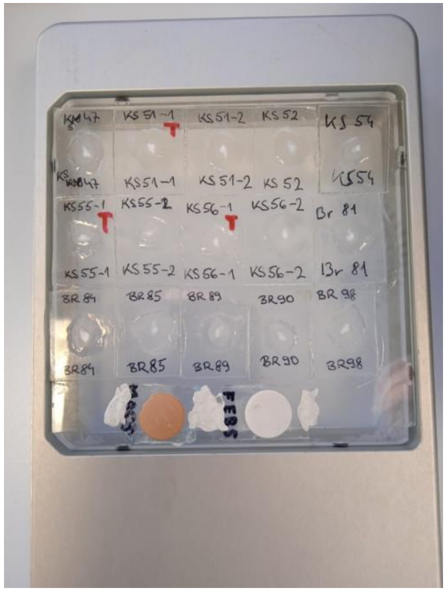


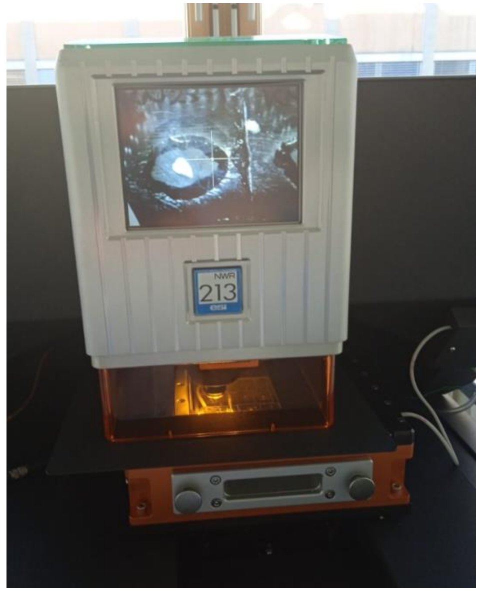
Measurements on LA-ICP-MS

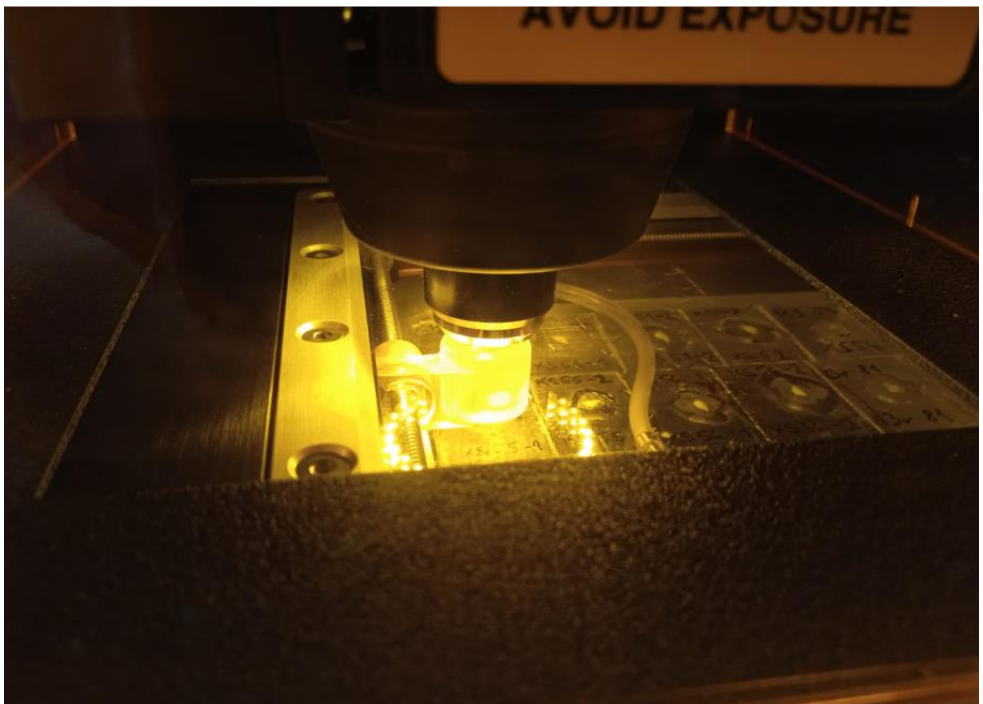


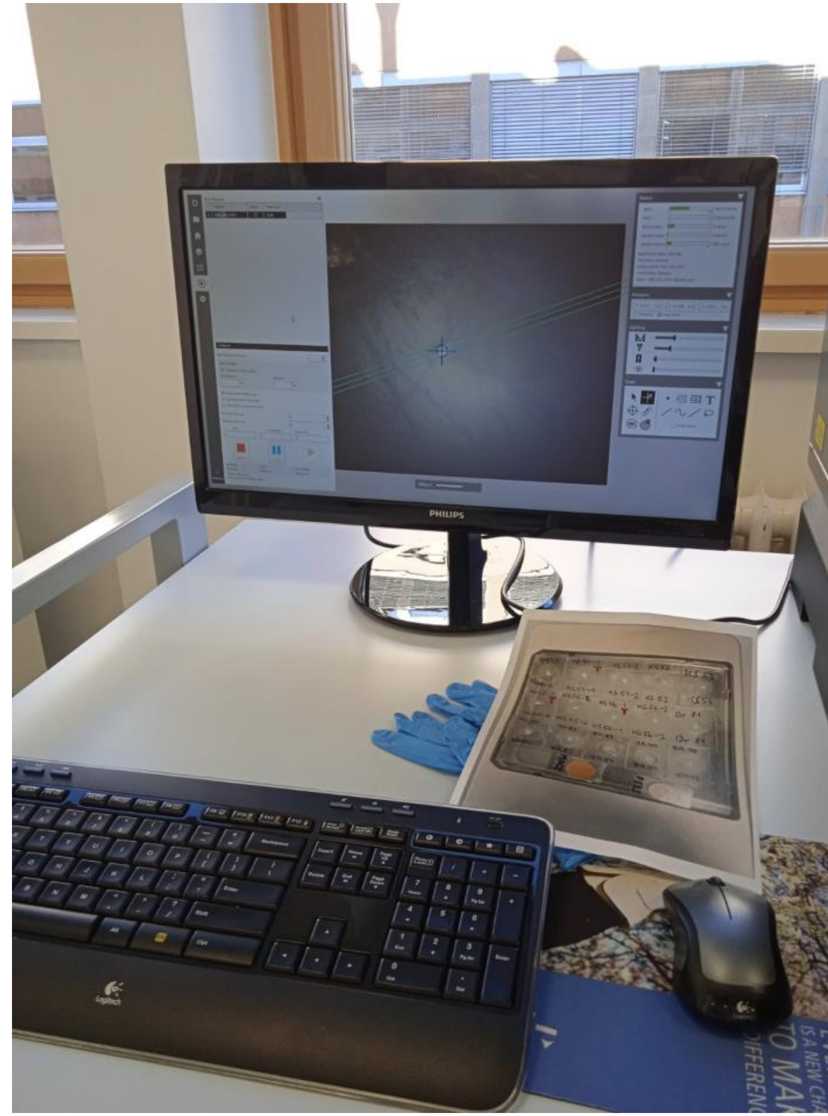
ESI NWR 213 Laser-Ablation System

Agilent 8800 ICP-MS-CRC-MS


KS 51-1
KS 51-2
KS 52
KS 54
KS55-1
KS55-2
KS56-1
KS56-2
Br 81
BR 84
BR 85
BR 89
BR 90
BR 98


NWR
213


AVOID EXPOSURE


PHILIPS

Scales

NIST SRM 1400 (Bone ash, National Institute of Standards and Technology, Gaithersburg, MD, USA) and **NIST SRM 1486** (Bone meal, National Institute of Standards and Technology, Gaithersburg, MD, USA)

Laser (NWR213, ESI)		Unit
spot size	50	µm
speed	5	µm/s
rep rate	10	Hz
energy output	15	%
energy		mJ
line scan distance (x-direction)	600	µm
distance between lines (core)		µm
warm-up (delay)/gas blank	30	s
wash-out (delay)	30	s
carrier gas	He	
carrier gas flow rate	0,900	L/min
fluence		J cm2
line time	120,0	s
transfer time		s
shutter (IVA / XYR)	IVA	

(20 s for samples)

ICP-QQQ-MS (8800, Agilent)	Value	Unit
RF power	1550	W
nebulizer gas flow		L/min
auxillary gas flow		L/min
plasma gas flow		L/min
total acquisition time/integration time		s
wash out/buffer time	30,00	s
sum time (whole sample)	180	s
total acquisitions/line	#DIV/0!	

Isotopes	Abundance	Integration time (s)
13C		0,2
23Na		0,1
24Mg		0,1
27Al		0,1
43Ca		0,05
44Ca		0,05
55Mn		0,1
56Fe		0,1
57Fe		0,1
59Co	100%	0,1
63Cu	69,15%	0,1
66Zn	27,73%	0,1
75As	100%	0,1
88Sr	82,60%	0,1
111Cd		0,1
114Cd		0,1
133Cs		0,1
138Ba		0,1
202Hg		0,1
205Tl	70,40%	0,1
208Pb	52%	0,1
238U		0,1

Otoliths

FEBS-1 (Otolith Certified Reference Material for Trace Metals, National Research Council Canada) and **MACS-3** (Calcium carbonate standard, United States Geological Survey, 189 USA)

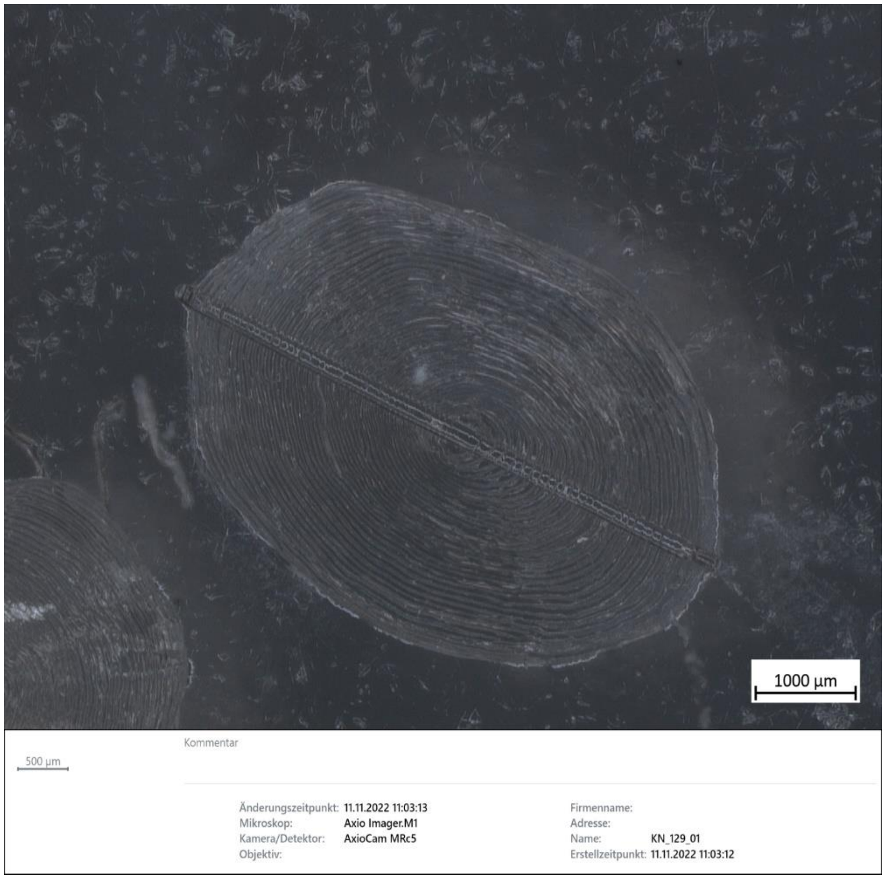
Laser (NWR213, ESI)		Unit
spot size	40	µm
speed	2	µm/s
rep rate	20	Hz
energy output	45	%
energy		mJ
line scan distance (x-direction)	300	µm
distance between lines (core)		µm
warm-up (delay)/gas blank	30	s
wash-out (delay)	30	s
carrier gas	He	
carrier gas flow rate	0,900	L/min
fluence		J cm2
line time	150,0	s
transfer time		s
shutter (IVA / XYR)	IVA	

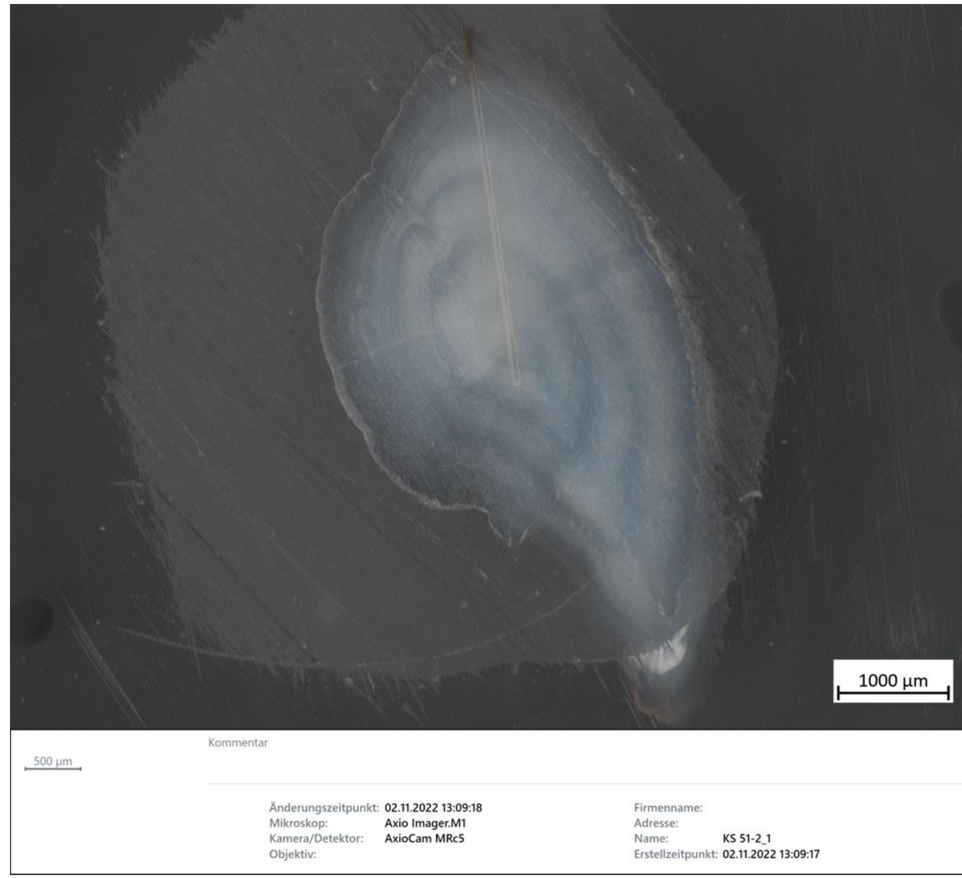
ICP-QQQ-MS (8800, Agilent)	Value	Unit
RF power	1550	W
nebulizer gas flow		L/min
auxillary gas flow		L/min
plasma gas flow		L/min
total acquisition time/integration time		s
wash out/buffer time	30,00	s
sum time (whole sample)	210	s
total acquisitions/line	#DIV/0!	

Isotopes	Abundance	Integration time (s)
23Na		0,1
24Mg		0,1
27Al		0,1
43Ca		0,05
44Ca		0,05
55Mn		0,1
56Fe		0,1
57Fe		0,1
59Co	100%	0,1
63Cu	69,15%	0,1
66Zn	27,73%	0,1
75As	100%	0,1
88Sr	82,60%	0,1
111Cd		0,1
114Cd		0,1
133Cs		0,1
138Ba		0,1
202Hg		0,1
205Tl	70,40%	0,1
208Pb	52%	0,1
238U		0,1

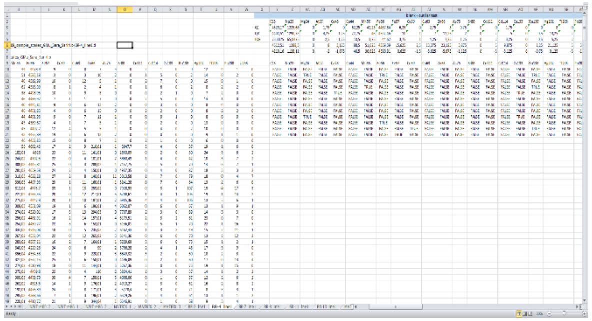
Data processing

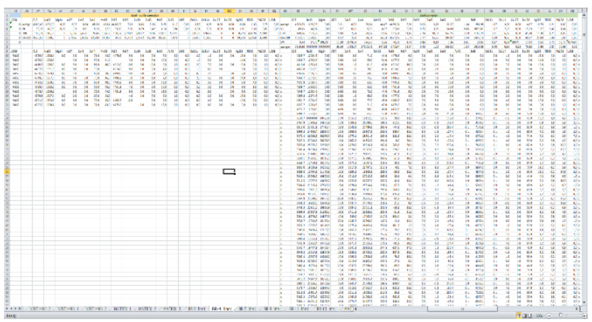
- pictures taken under stereomicroscope after ablation of samples

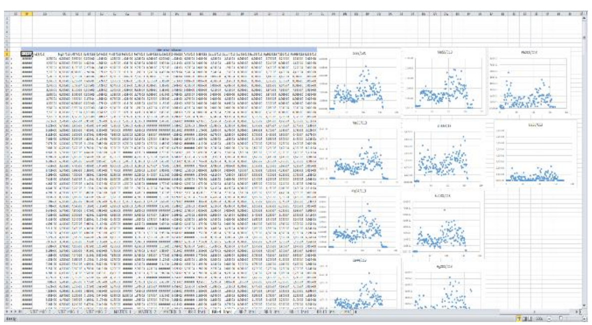




Scales

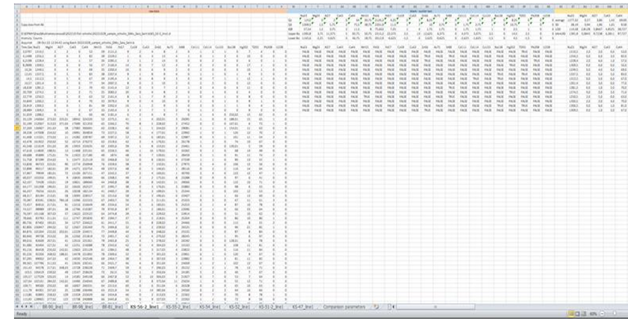


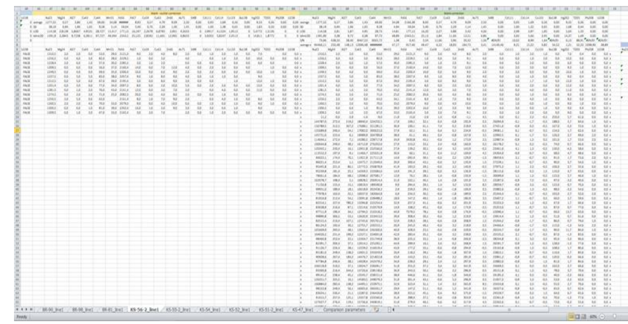


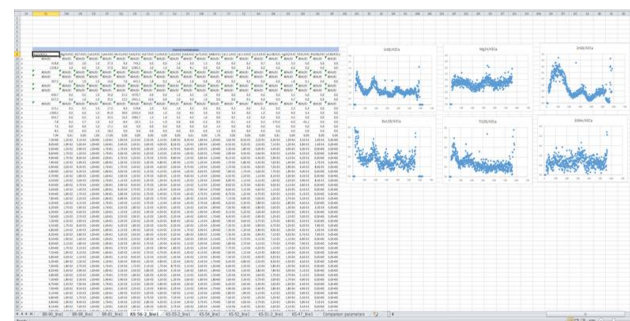


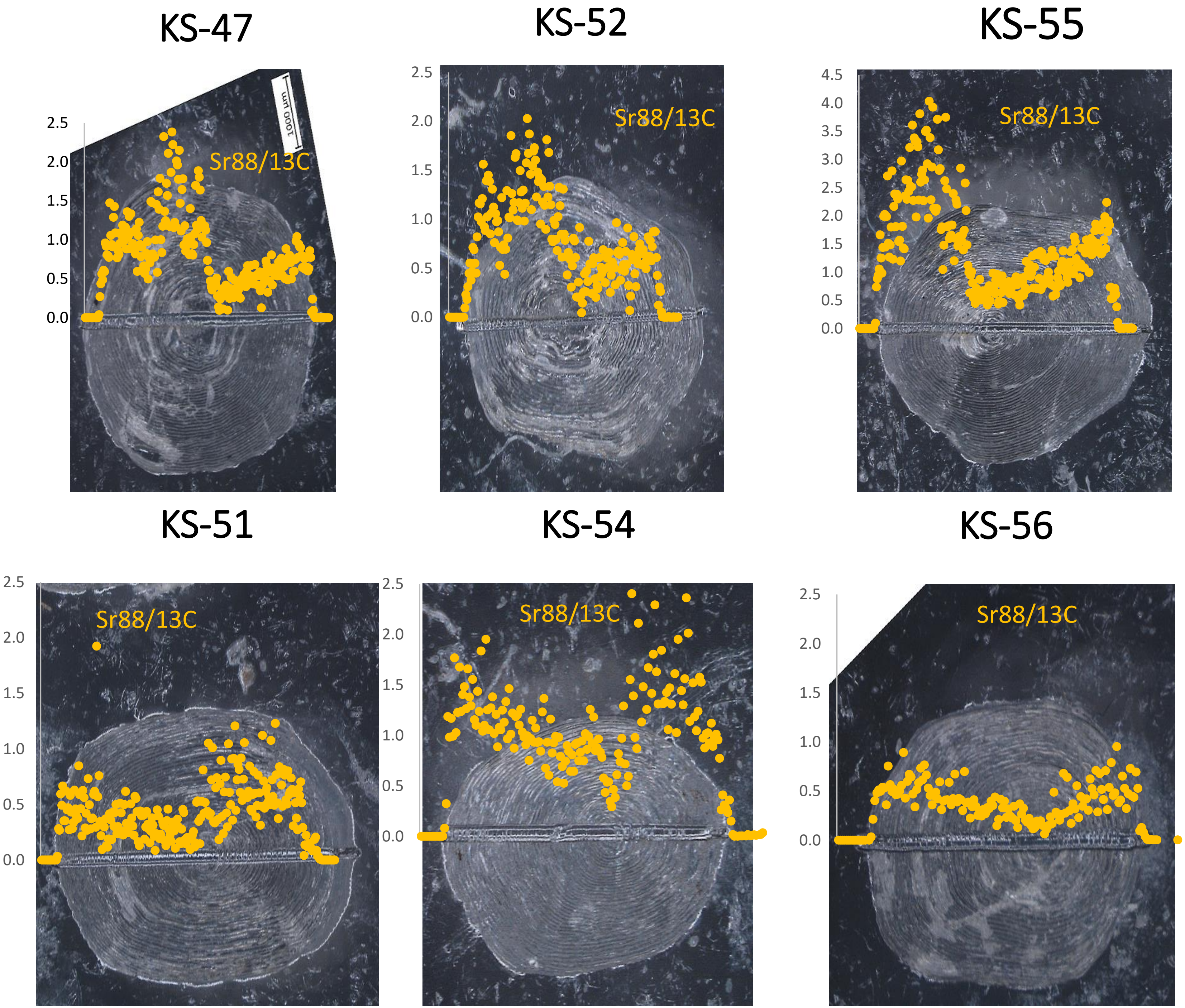
- blank – outliers replaced with average
- average blank value for each element subtracted from sample values
- **scales** - internal normalization to **^{13}C**
- **otoliths** - internal normalization to **^{43}Ca**
- intensities plotted for each element to find trends along ablation lines

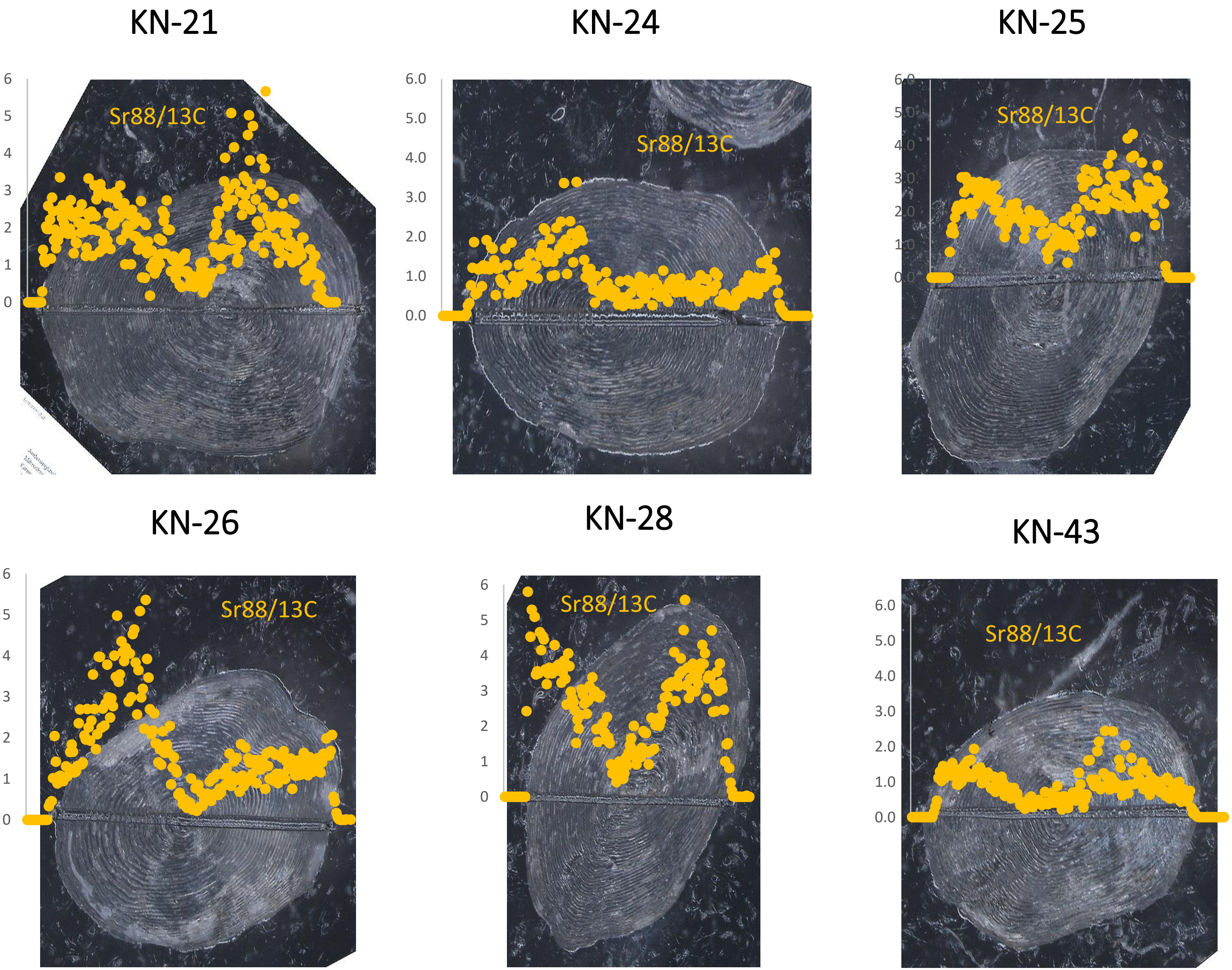
Otoliths





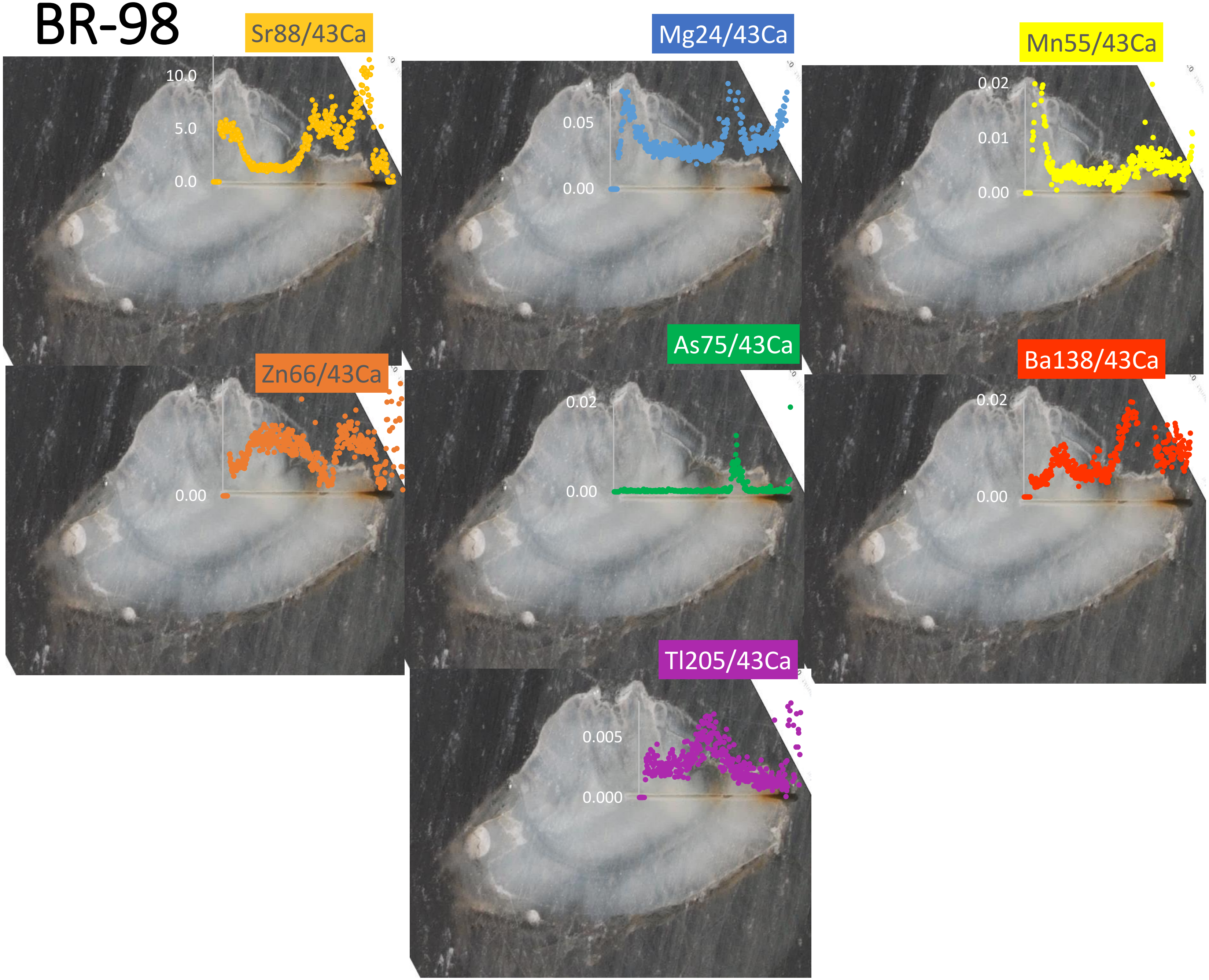



KS-47
1000 µm
Sr88/13C
2.5
2.0
1.5
1.0
0.5
0.0
KS-52
Sr88/13C
2.5
2.0
1.5
1.0
0.5
0.0
KS-55
Sr88/13C
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
KS-51
Sr88/13C
2.5
2.0
1.5
1.0
0.5
0.0
KS-54
Sr88/13C
2.5
2.0
1.5
1.0
0.5
0.0
KS-56
Sr88/13C
2.5
2.0
1.5
1.0
0.5
0.0

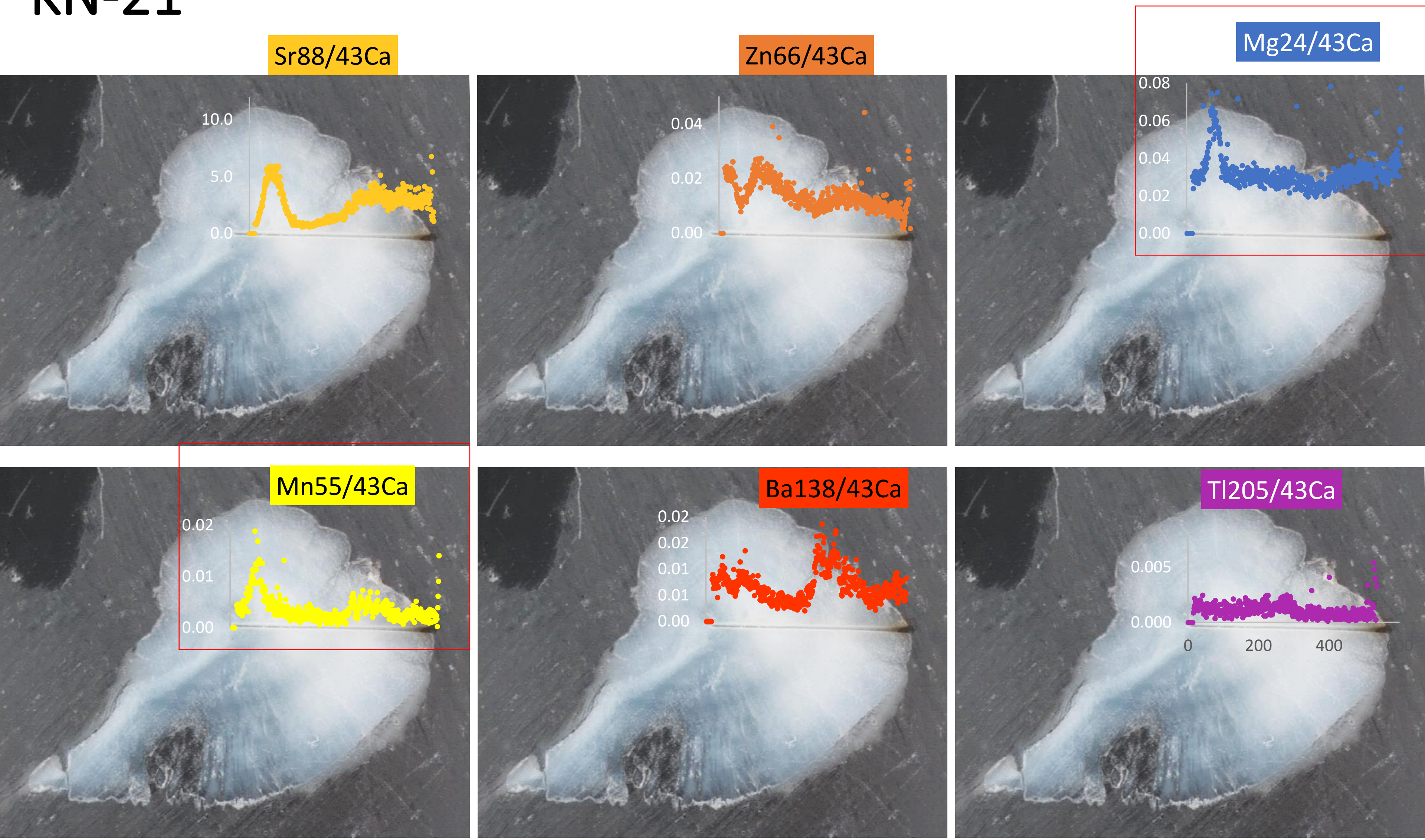


KN-21
Sr88/13C
KN-24
Sr88/13C
KN-25
Sr88/13C
KN-26
Sr88/13C
KN-28
Sr88/13C
KN-43
Sr88/13C

BR-98

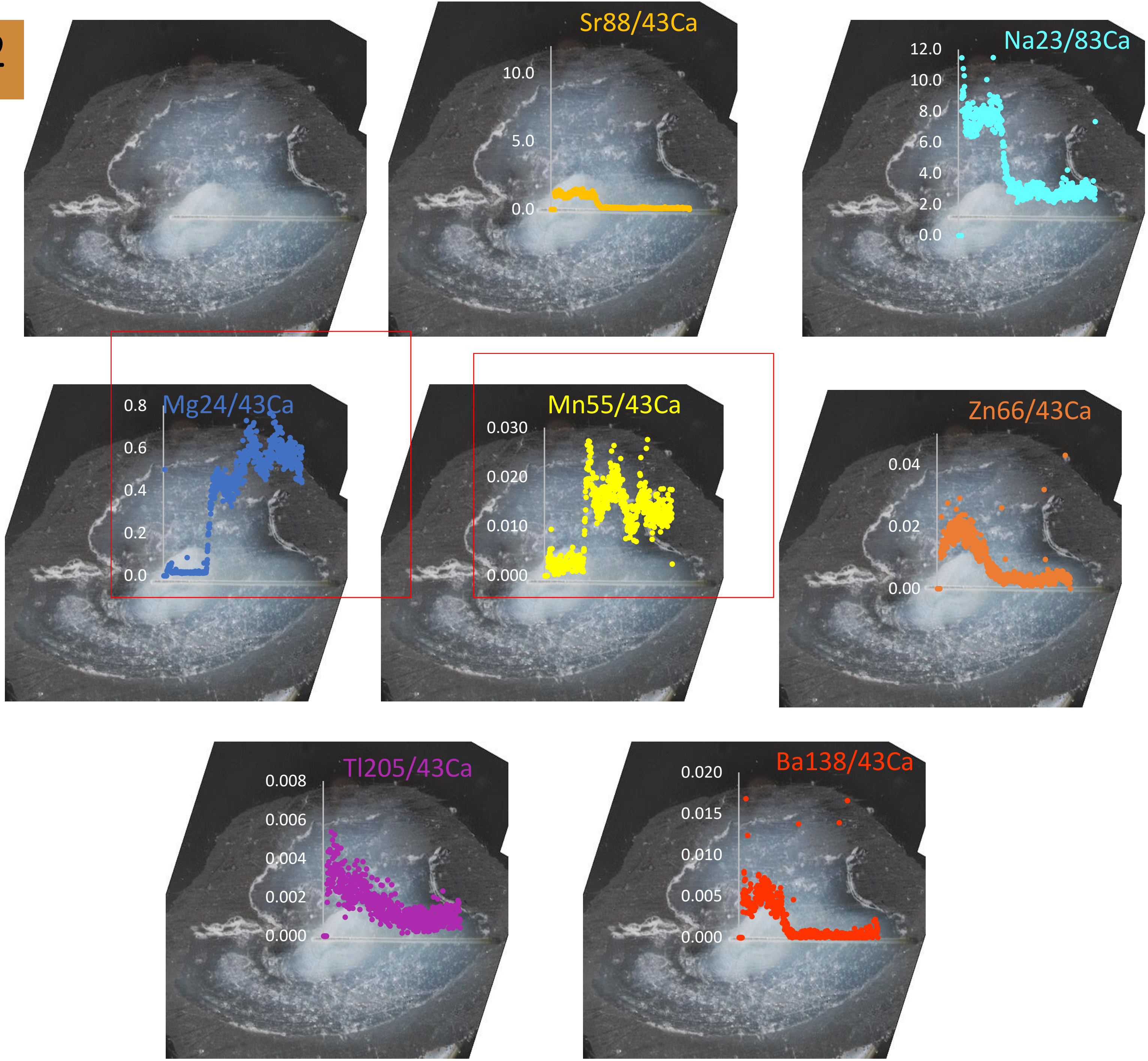

Sr88/43Ca
10.0
5.0
0.0
Mg24/43Ca
0.05
0.00
Mn55/43Ca
0.02
0.01
0.00
Zn66/43Ca
0.00
As75/43Ca
0.02
0.00
Ba138/43Ca
0.02
0.00
Tl205/43Ca
0.005
0.000

KN-21



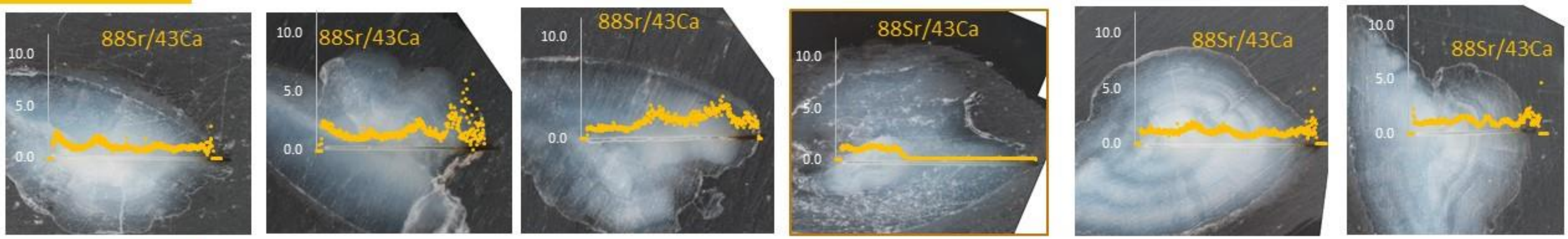
Sr88/43Ca
10.0
5.0
0.0
Zn66/43Ca
0.04
0.02
0.00
Mg24/43Ca
0.08
0.06
0.04
0.02
0.00
Mn55/43Ca
0.02
0.01
0.00
Ba138/43Ca
0.02
0.02
0.01
0.01
0.00
Tl205/43Ca
0.005
0.000
0
200
400

KS_52

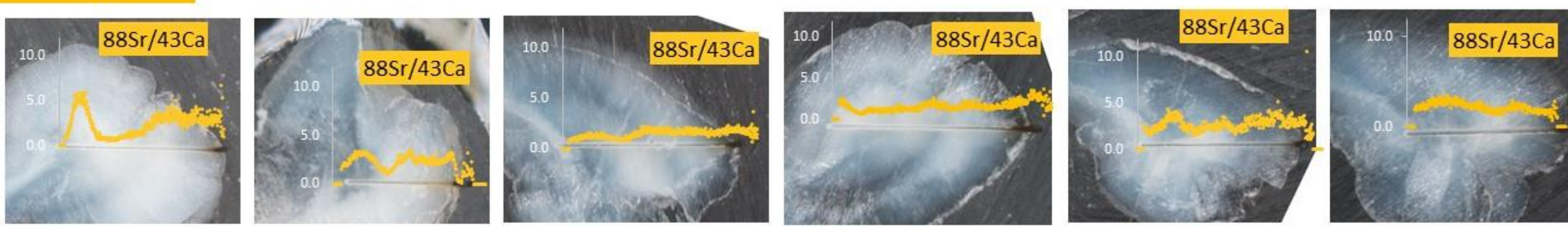

Sr88/43Ca
10.0
5.0
0.0
Na23/83Ca
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Mg24/43Ca
0.8
0.6
0.4
0.2
0.0
Mn55/43Ca
0.030
0.020
0.010
0.000
Zn66/43Ca
0.04
0.02
0.00
Tl205/43Ca
0.008
0.006
0.004
0.002
0.000
Ba138/43Ca
0.020
0.015
0.010
0.005
0.000

$^{88}Sr/^{43}Ca$ SPRING

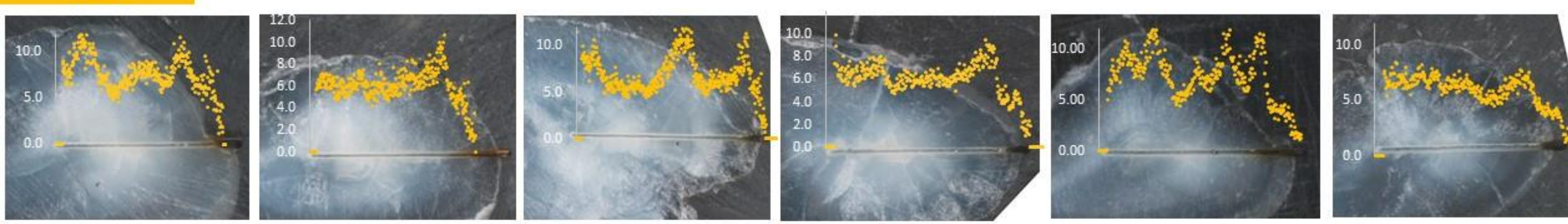
KRS



KRK

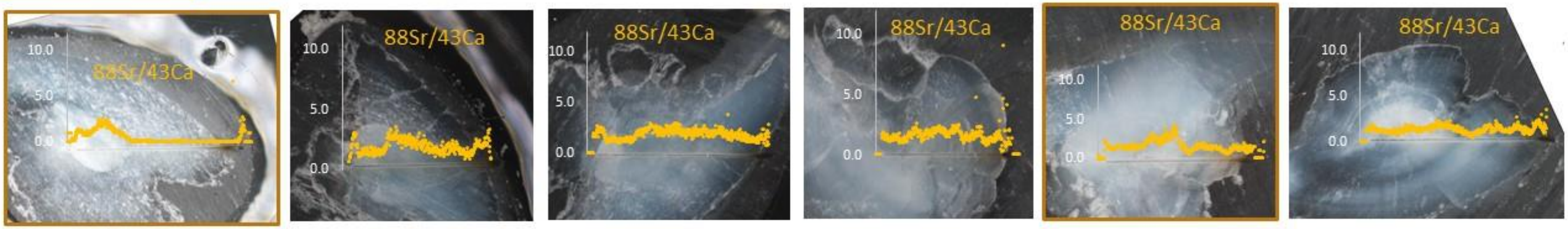


KBL

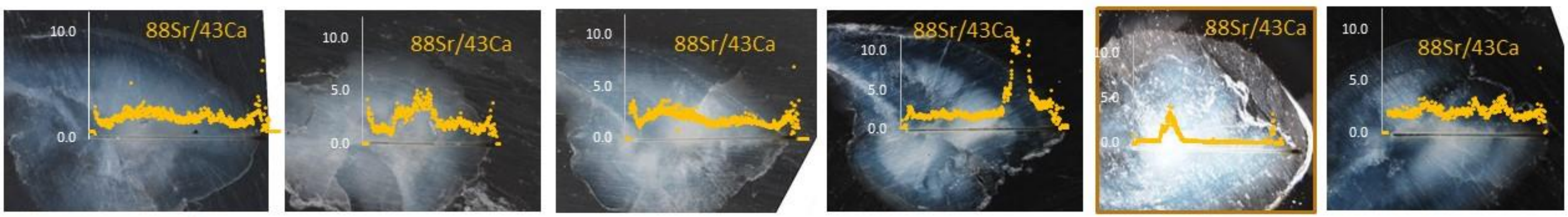


$^{88}Sr/^{43}Ca$ AUTUMN

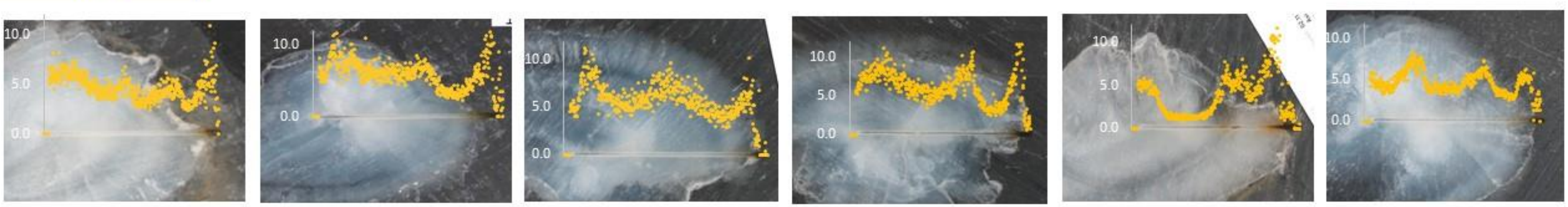
KRS



KRK



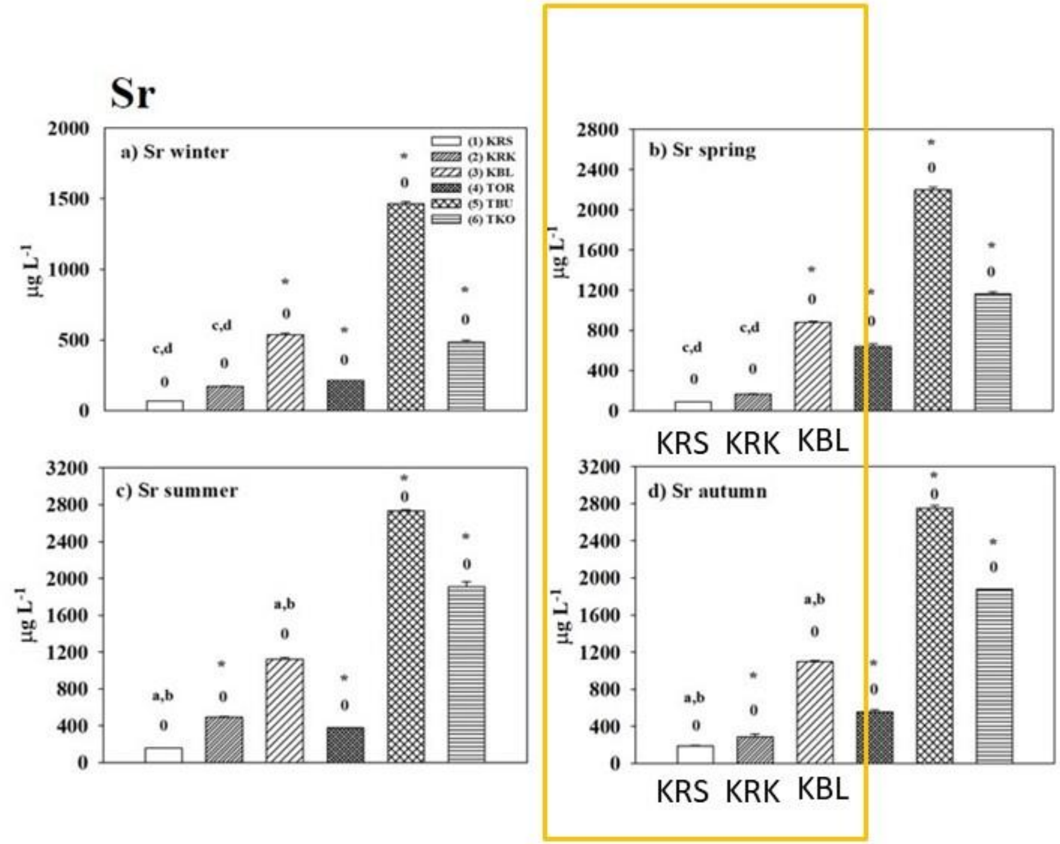
KBL



- KBL > KRK = KRS

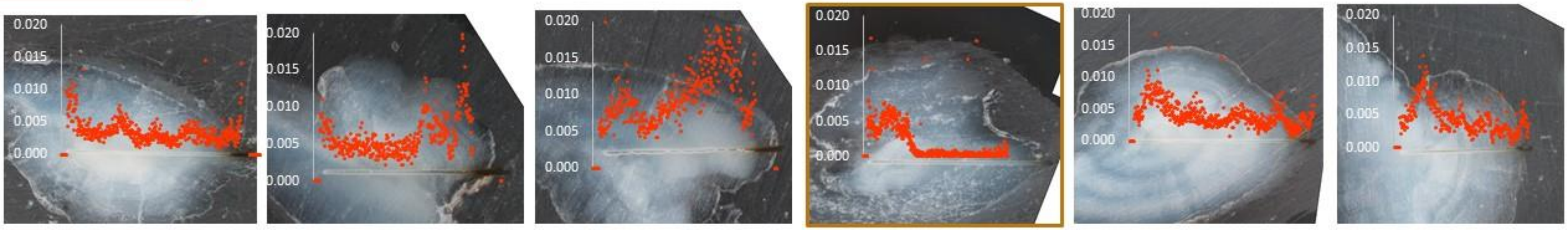
water

- the same trend in both seasons

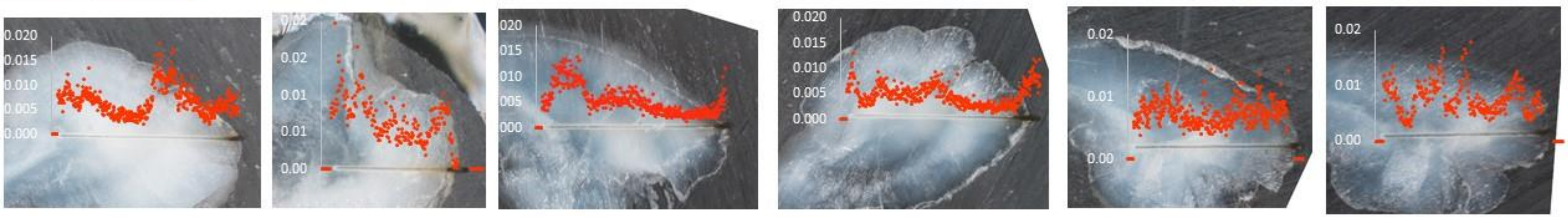


$^{138}Ba/^{43}Ca$ SPRING

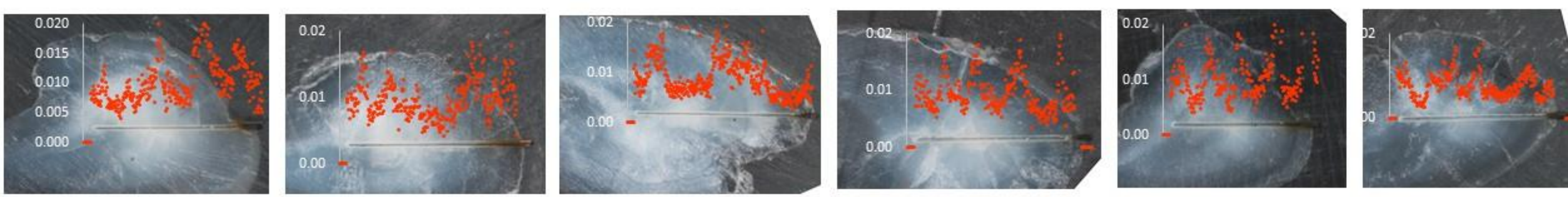
KRS



KRK

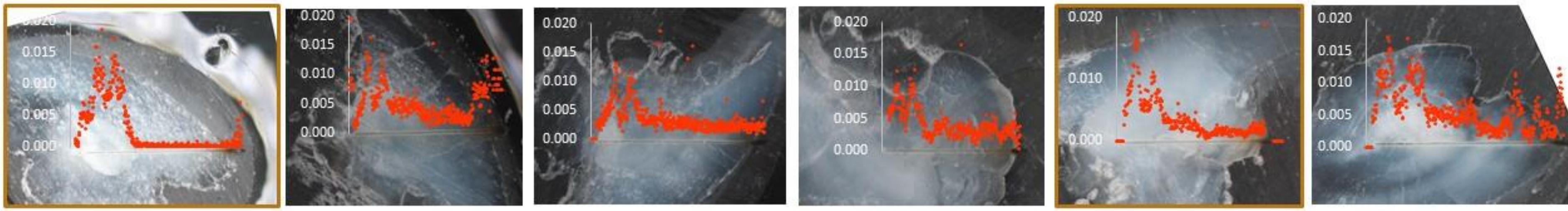


KBL

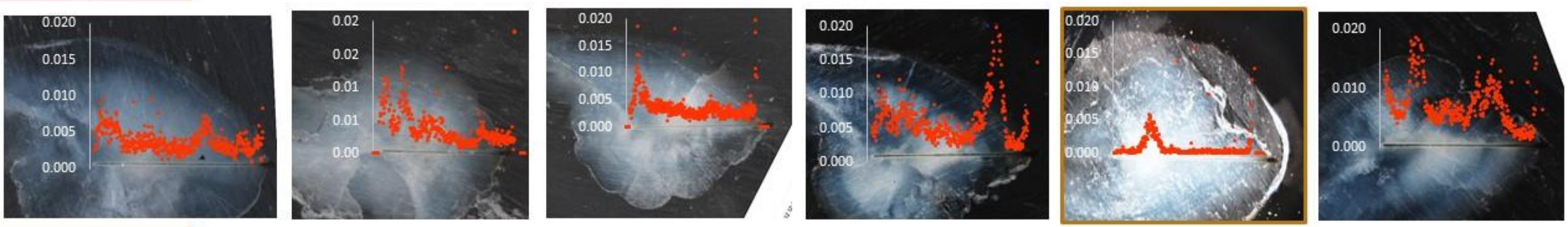


$^{138}Ba/^{43}Ca$ AUTUMN

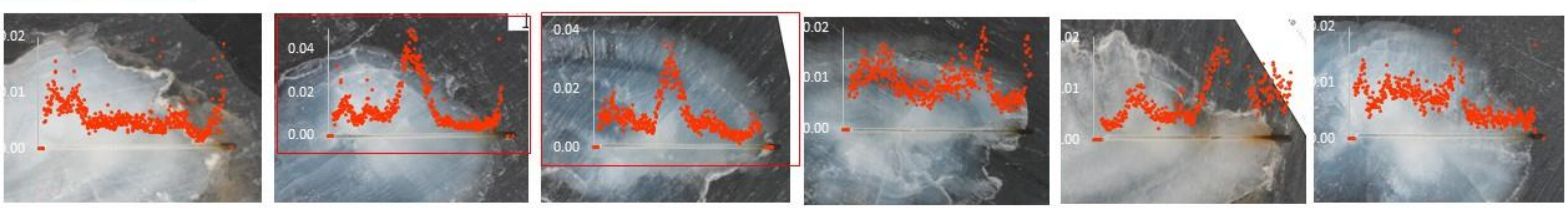
KRS



KRK



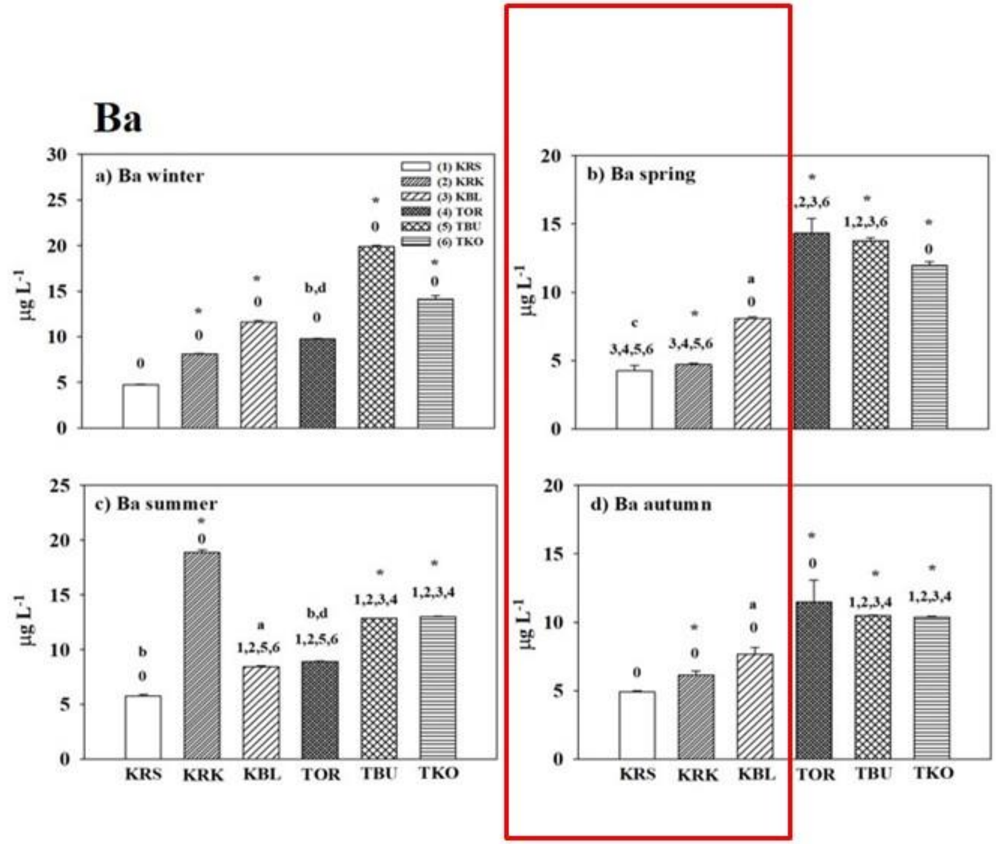
KBL



- KBL > KRK = KRS
- differences between locations smaller than for Sr

water

- the same trend



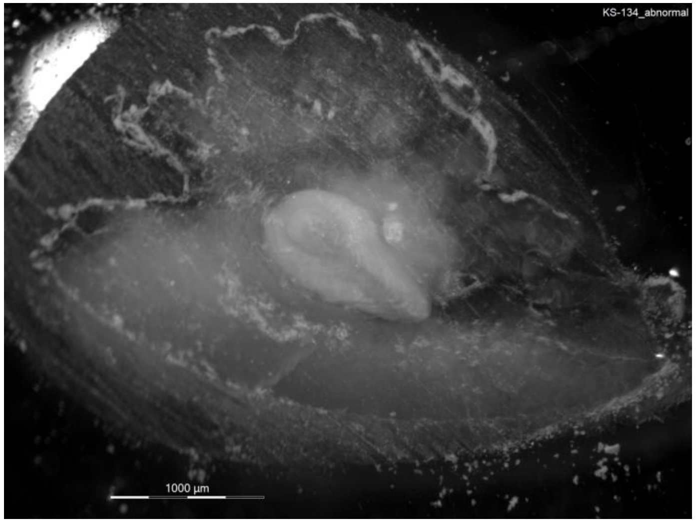
Next steps

- comparison between otoliths and scales
- digestion of remained scales and measurement of metal concentrations
- comparison of element ratios (Sr/Ba, Zn/Ca, …) in water and fish tissues
- separation of normal and abnormal otoliths
- finding seasonal pattern in normal otoliths with year rings
- quantification of metal concentrations

Stocked fish?

- **replacement scales** – injuries, <u>loss of scales</u> in a hatchery (Fiske, P., Lund, R. A., & Hansen, L. P. (2005). Identifying fish farm escapees. In *Stock identification methods* (pp. 659-680). Academic Press.)

- **abnormal otoliths** – different otolith chemistry and shape than in wild populations (no variations in temperature and food availability) – fast growth due to environmental control leads to <u>development of vaterite instead of aragonite</u> (Reimer, T., Dempster, T., Wargelius, A., Fjelldal, P. G., Hansen, T., Glover, K. A., ... & Swearer, S. E. (2017). Rapid growth causes abnormal vaterite formation in farmed fish otoliths. *Journal of Experimental Biology*, *220*(16), 2965-2969.)



Thank you for your attention!

THIRD PROJECT MEETING

Integrated evaluation of aquatic organism responses to metal exposure: gene expression, bioavailability, toxicity and biomarker responses (BIOTOXMET)

Zagreb, 19th May 2023

HRZZ Croatian Science Foundation · ANDRIJA STAMPAR TEACHING INSTITUTE OF PUBLIC HEALTH · MONTAN UNIVERSITÄT · FFoQSI · BIOTOXMET