Karlsruhe Institute of Technology
Institute of Functional Interfaces

X-Ray fluorescence microprobe analysis of marine macrofoulers

UNIVERSITÄT HEIDELBERG

T. Senkbeil[1,2*], T. Mohamed[1,2], A. Di Fino[3], R. Toscano[3], A. S. Clare[3], A. Rosenhahn[1,2]

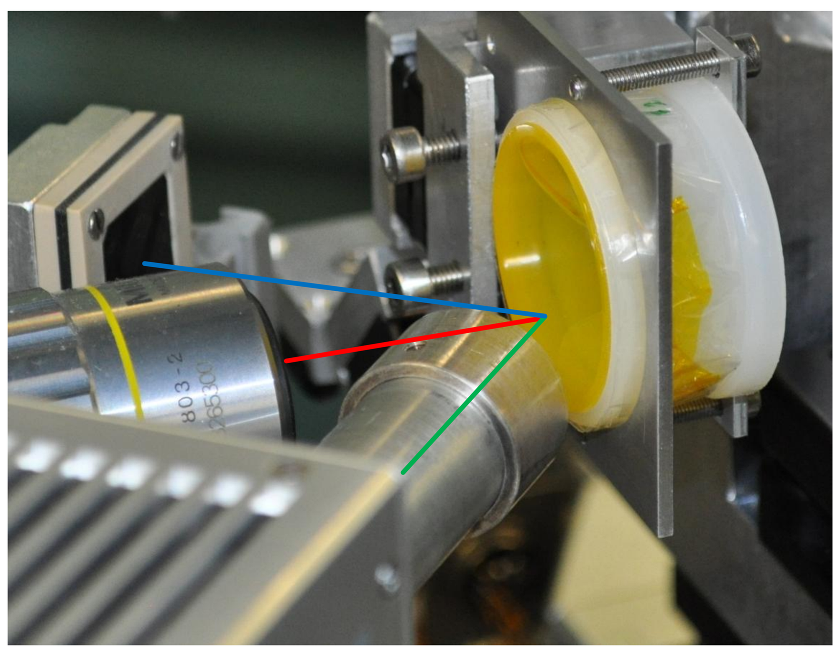
[1] Applied Physical Chemistry, Ruprecht-Karls-University Heidelberg, Im Neuenheimer Feld 253, 69120 Heidelberg, Germany

[2] Institute of Functional Interfaces, IFG, Karlsruhe Institute of Technology, PO Box 3640, 76021 Karlsruhe, Germany

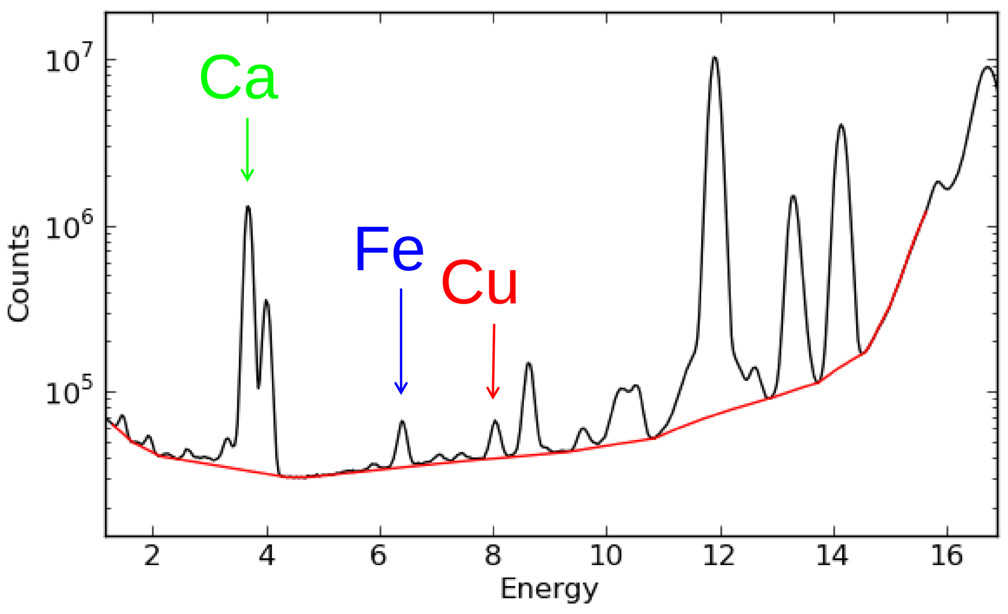
[3] School of Marine Science and Technology, Armstrong Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

X-RAY FLUORESCENCE MICROPROBE ANALYSIS

X-Ray fluorescence is a spectroscopy technique routinely used to study the elemental composition of samples. An X-ray source is used to illuminate the sample and the emitted X-Ray fluorescence (XRF) spectrum is detected. Fitting this spectrum with the known positions of the XRF peaks of the elements in the sample yields the concentrations of these elements.
Combining a small X-ray focus size with a scanning sample stage turns this spectroscopy technique into a valuable imaging tool which provides elemental distribution maps of the sample.

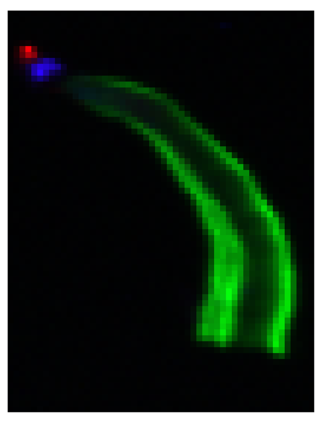


Filled liquid cup mounted in a sample holder. The monochromatic X-ray beam (blue line) is focused on the organism settled on the Kapton window film. The emitted X-ray fluorescence signal is detected at a right angle (green line) with an silicon drift detector. An optical microscope aligned to the X-ray micro-focus at an 45° angle (red line) is used to align the sample for the scans.



Integrated spectrum for a tubeworm scan showing the XRF peaks of Ca, Fe and Cu quantified to obtain the elemental mapping of these elements. The spectrum was evaluated using the XRF analysis software package PyMCA [1], developed at the ESRF (European Synchrotron Radiation Facility, Grenoble, France)

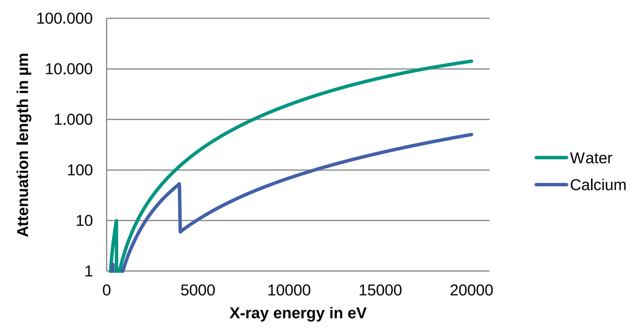
Optical micrograph of tubeworm species *Ficopomatus enigmaticus*



Elemental mapping of Ca, Fe and Cu obtained from 68x52 spectra

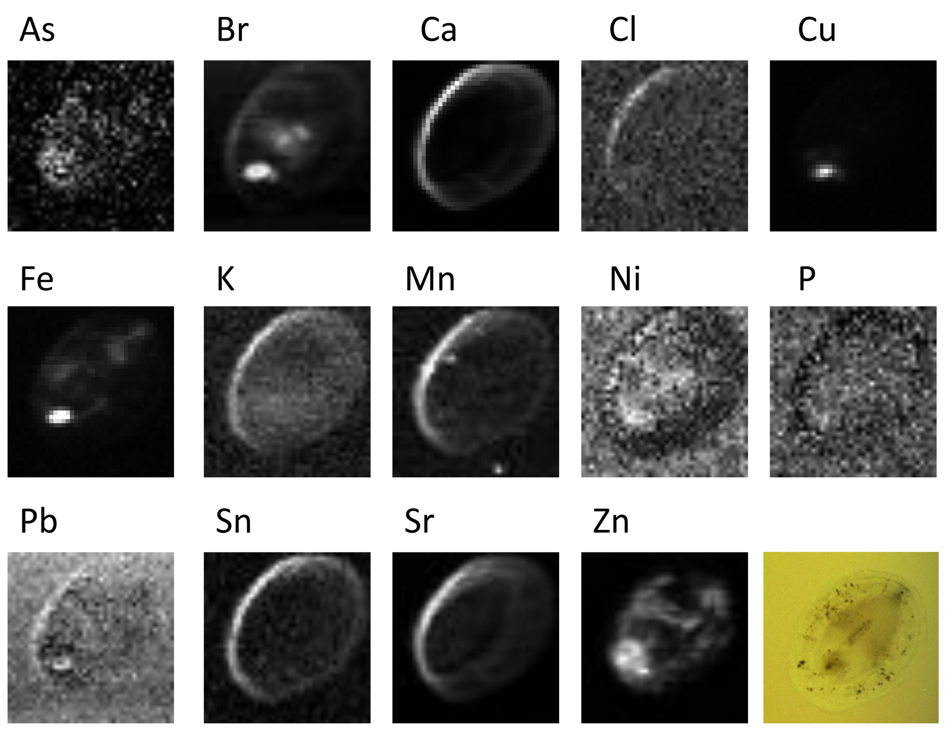
The measurements were performed at the XRF microprobe beamline FLUO at ANKA [2], KIT, Germany using the following parameters:

- Bending magnet beamline providing a monochromatic 17 keV X-ray beam
- A scanning step size of 15 µm was chosen to match the focus size of the capillary focussing the beam on the sample.
- Unfocused detection of the XRF signal yields an projection-type map of elemental distributions. The depth visibilty of different elements depends on the penetration depth of the X-ray energy corresponding to the XRF line.
- Confocal scans using another capillary in front of the detector allow for a depth-resolution similar to the lateral resolution. Confocal measurements are severely limited by the signal intensity which is orders of magnitude lower compared to an unfocused detection.

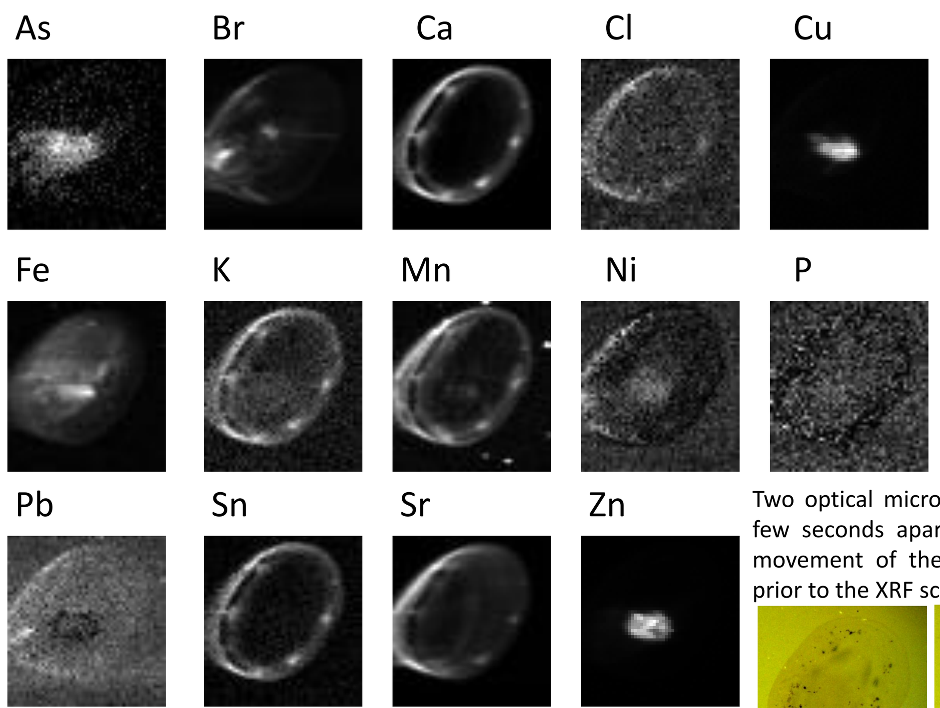


Attenuation lengths of water and calcium. The XRF signal from lighter elements is attenuated stronger with increasing depth. This directly effects the depth visibility and has to be taken into account when comparing distribution maps of different elements. Based on data from [3]

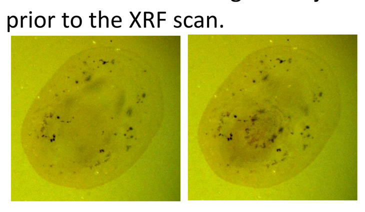
BARNACLE MEASUREMENTS



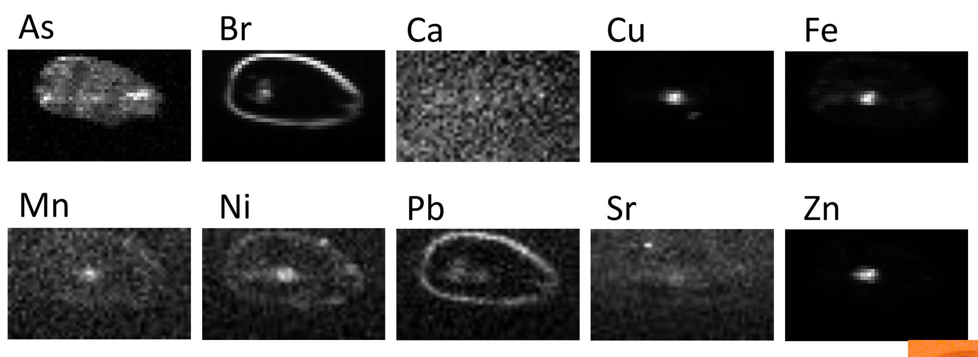
Elemental distribution maps and optical micrograph of a barnacle (*Elminus modestus). The* scan area measures 585 µm x 600µm. The barnacle cyprid had been settled on Kapton 6 days prior to the measurement. The outer shell contains Ca, K, Mn, Sn and Sr. In the antennula region As, Br, Cu, Fe and Zn can be found. Ni and Pb are there present as well, albeit in minute concentrations.



Two optical micrographs taken a few seconds apart illustrate the movement of the organism just prior to the XRF scan.

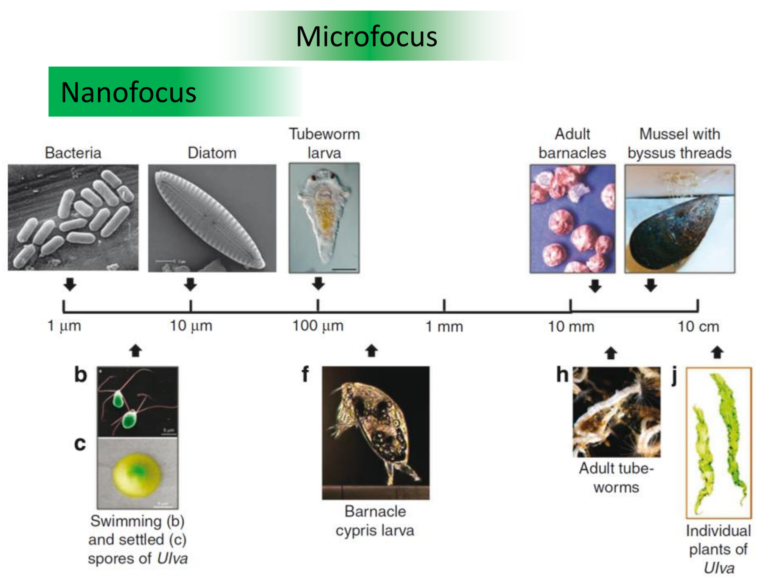


Elemental distribution maps of a barnacle (*Balanus Improvisus).* The barnacle cyprid had been settled on Kapton 8 days prior to the measurement. The size of the scan area is 750 µm x 810 µm. Ca, K, Mn, Sn and Sr can be found in the outer shell. The distribution of these elements correlates quite strong. Cu, Fe and Zn are located in the antennula region, as well as As and Ni in lower concentrations.



Elemental distribution maps and an optical micrograph of a recently settled barnacle (*Balanus Improvisus)* in the metamorphosis stage.
The scan area measures 750 µm x 450 µm. This scan has been performed using a confocal setup and hence shows only the elements present directly at the surface. Br and Pb are the first elements present in the initial phase of the outer rim formation, when Ca has not yet been incorporated. Br, Cu, Fe, Mn, Ni and Zn can be found in the antennula region, while As is distributed all over the contact area.

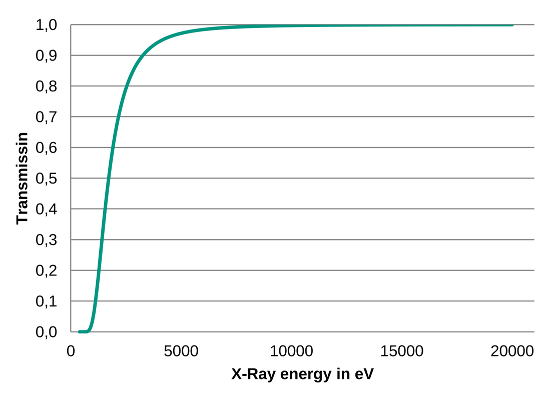
LENGTH SCALES



Length scales of marine biofouling organisms [4]. While a microfocus beamline (like the FLUO beamline at ANKA) is sufficient for macrofoulers, the analysis of microfoulers requires the reduced focus size of a nanofocus beamline.

IN SITU SAMPLE ENVIRONMENT

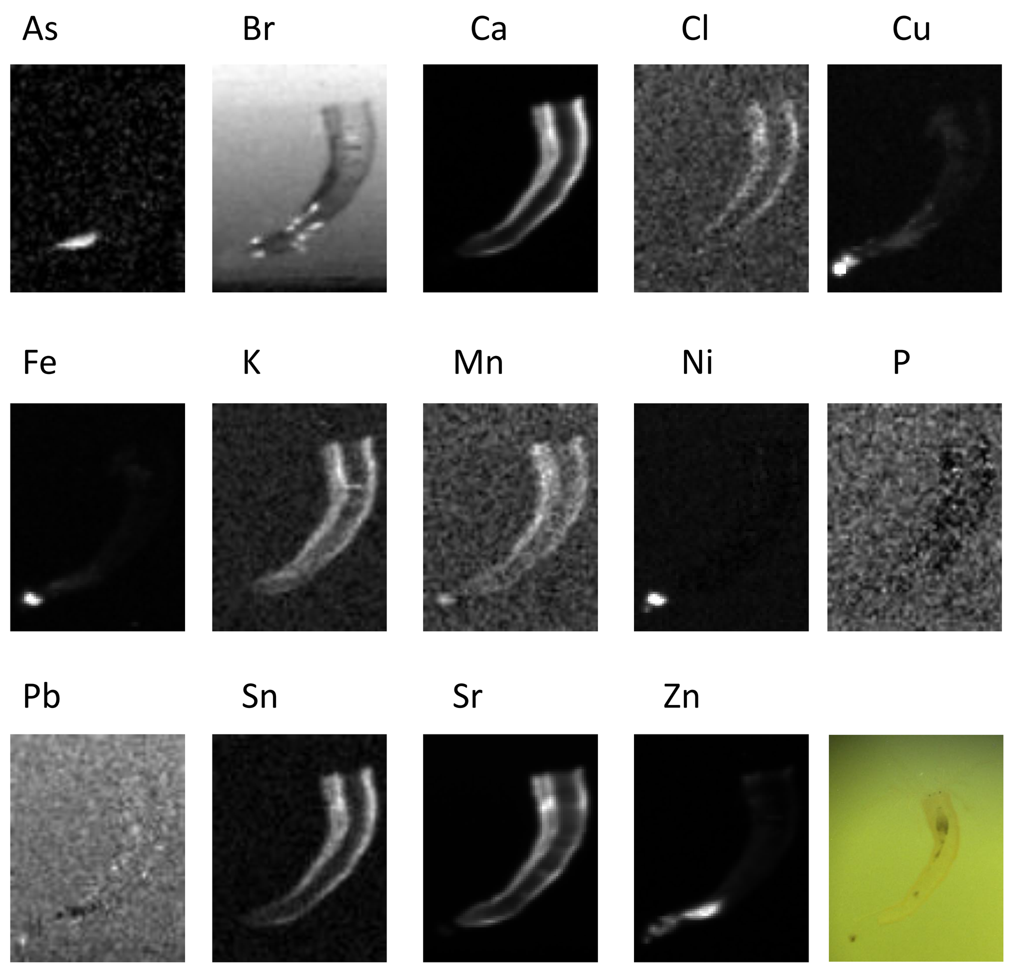
We use 40 mm Disposable Closed X-Ray Cells from SPEX SamplePrep. The cells consist of a polyethylene snap-ring and cup with snap-post vent and reservoir. After settling the biofouling organism on a 8 µm Kapton film, the reservoirs are filled with the medium or artificial sea water and the Kapton film is placed on top of the cell. Afterwards the cells are sealed with the snap-ring. Now the water-tight sample can be placed in a sample holder and the XRF microprobe scans can be performed.



X-Ray transmission of an 8 µm Kapton film. The window film shows a high transparency for energies > 2 keV. Energies below this threshold are not relevant for the analysis as they are not accesible with the silicon drift detectors used in microprobe XRF analysis. Based on data from [3]

Kapton window films are used as an inert substrate. They feature a high transparency for X-rays in the relevant energy range. The films have been checked to be free of contaminations with heavier elements which might interfere with the XRF measurements. On the other hand, this substrate shows a high bio-compatibilty: Marine biofoulers readily settle on this substrate, which is a prerequisite for the experiments.

TUBEWORM MEASUREMENTS



Elemental maps and an optical micrograph of a tubeworm (*Ficopomatus enigmaticus*). Ca, K, Mn, Sn and Sr are present in the outer shell, while Cu, Fe, Mn and Ni are only present at the tip of the organism. As and Zn can be found inside the tube. Br has a rather high background as it is present in the medium. Additionally there are a few Br hotspots at the narrow end of the tube. Scan area: 765 µm x 1005 µm

DISCUSSION & OUTLOOK

General

- Scanning X-ray fluorescence microprobe analysis is a valuable tool to investigate the elemental composition of marine biofoulers. Especially the distribution of metals is easily accesible with this method and sheds light on their role in the process of initial attachment and curing of adhesives.
- The liquid cup sample environment is suited for in situ investigations of various settled biofouling organisms.
- First successful experiments using this in situ sample enviroment have been performed at ANKA, but these experiments were limited by the synchrotron/beamline performance.
- Different elements show various hot spots inside tubeworm and barnacle samples. These high elemental concentrations have to be correlated to the biological processes taking place at these sites.

Outlook

- Increased photon flux is desirable for confocal, interfacial sensitive measurements.
- A reduced focus size is needed for analysis of bacteria (e.g. *Cobetia marina*) and microfoulers (e.g. *Navicula perminuta*) and the initial attachment disks of macrofoulers like barnacle larvae.
- Higher photon energies would allow for access of heavier elements (e.g. iodine K-edge at 28.6 keV)
- Based on our first results obtained at ANKA we applied for beamtime at X-Ray fluorescence beamlines at other synchrotron sources providing a higher performance in terms of focus size and flux. A first proposal for experients at the P06 beamline at PETRA III (DESY, Germany) has been accepted.

LITERATURE

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COLLABORATIONS

Clare group, Newcastle, UK (Barnacle and tubeworm culture);
Callow group, Birmingham, UK (Navicula culture);
Lopez group, Duke, USA (Cobetia culture)

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Email: Senkbeil@uni-heidelberg.de