SR-µXRF of bone samples – a comparison of a confocal and a CXC setup

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1. Introduction

Using SR-µXRF to analyze trace elements in bone material has proven to be an effective method for determining trace element distributions in cartilage and bone. Nevertheless, for narrow bone structures (in the 2.5µm range thickness), such as tidemarks (the transition zones between non-calcified and calcified articular cartilage) and cement lines (the boundaries of osteons) a sub-micrometer resolution is desirable. Concentrations of the trace elements are very low and a compromise between viable count rate in an acceptable measurement time and resolutions has to be made.

2. Aim

To optimize the µXRF imaging of narrow bone structures, three human biopsy samples (seven areas of interest) were measured with two different setups: the confocal µXRF setup at the FLUO beamline at ANKA and the Color X-Ray Camera (CXC) setup at the BAMline at Bessy II.
These samples show higher Zn levels at the borders of osteons (the so called cement lines). Learing more about the structure of the cement line would allow more insight into the biology of bone metabolism. As the samples are thick cuts, the information depth needs to be limited.

3. Sample structure

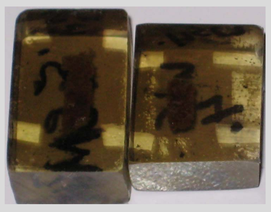

Figure 1: Two of the measured samples. Bone tissue embedded in PMMA

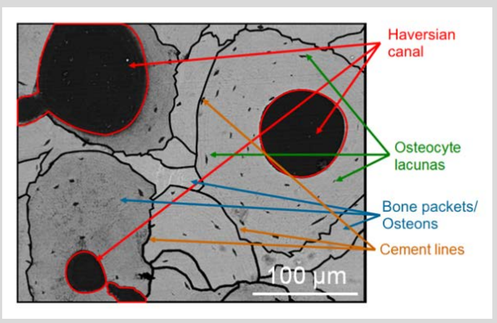

Figure 2: Cross section of cortical bone – qBEI close-up

4. Methods

Setup properties – ANKA

- Monochromator: W/Si Multilayer
- Excitation: 17keV
- Detector: 50mm² Vortex
- Resolution: 17µm x 12µm x 19µm

This setup works in scanning mode. The volume created by the overlap of the focal spots of the two polycapillaries (Fig. 4) is seen by the detector. The sample is moved through this volume.

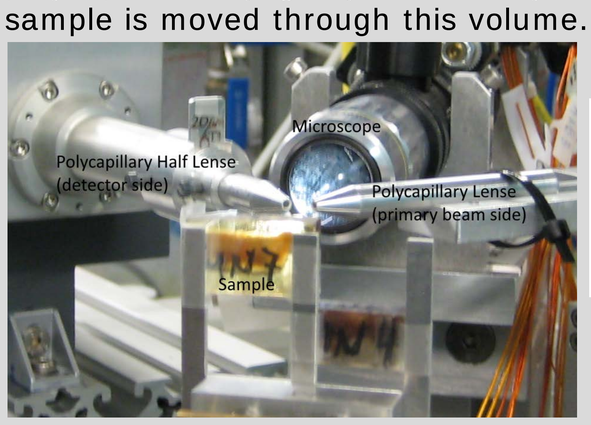

Figure 3: Confocal µXRF setup at the FLUO beamline at ANKA

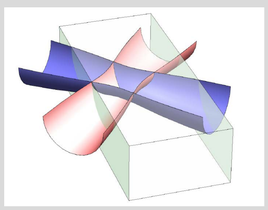

Figure 4: Overlap of the polycapillaries focal spots

Setup properties – BESSY II

- angle primary beam – sample: 2.5-5° (small angles are required to limit the information depth)
- Excitation: 12.6keV
- Detector: Color X-Ray Camera (CXC) [1]
- Resolution: 7µm x 7µm (1:8 polycapillary; depth resolution> 19µm)

The CXC is based on a spatial and spectral resolving pnCCD and a polycapillary. It can be used in full field mode. The resolution in the plane is defined by the used polycapillary. The depth resolution depends on the angle between primary beam and sample.

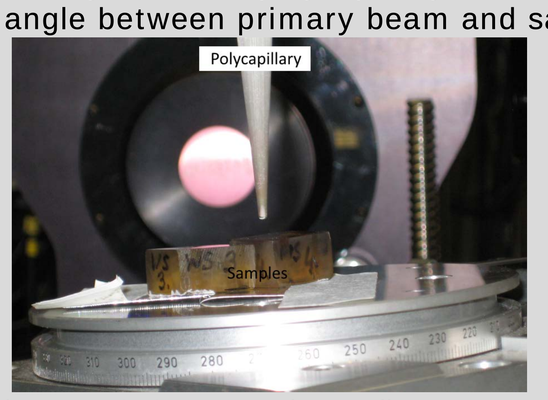

Figure 5: CXC setup at the BAMline at Bessy II

5. Results

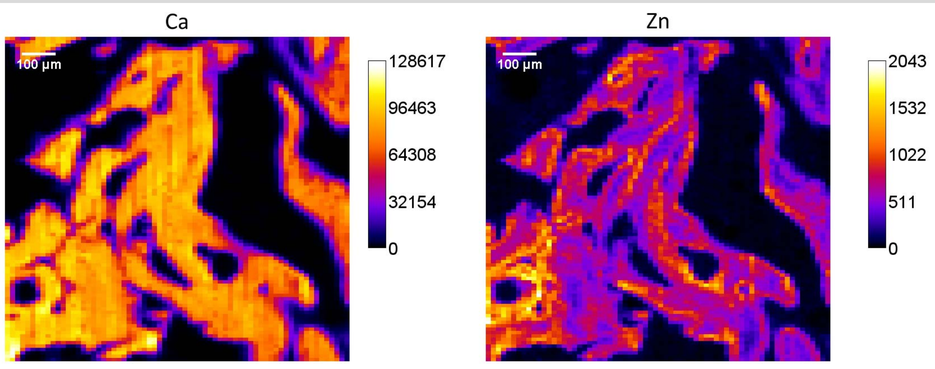

Figure 6: Ca and Zn map of sample WSX2 Area A measured at ANKA. Measurement time: 2s/pixel (3.6h for the complete map)

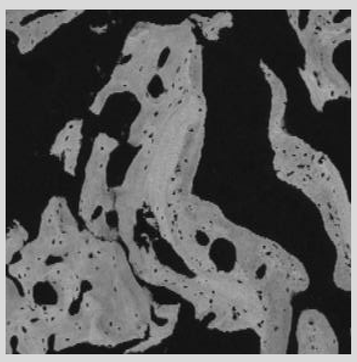

Figure 7: qBEI of WSX2 Area A

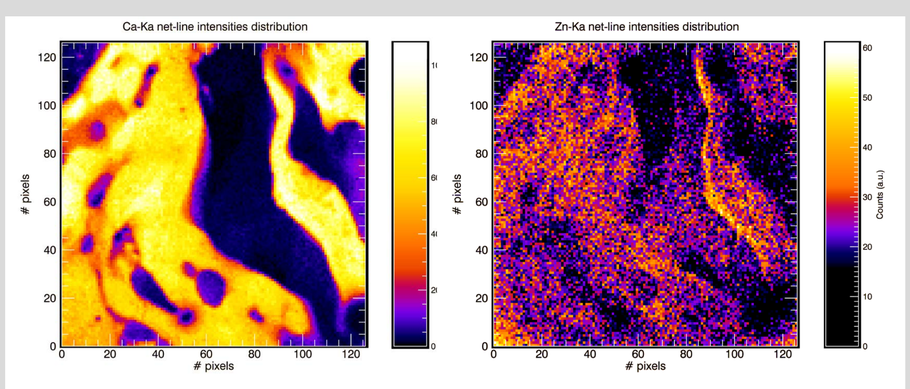

Figure 8: Ca and Zn map of WSX2 Area A measured at Bessy II Measurement time: 5.5h; Angle between primary beam and sample: 2.5°

Although the maps measured at Bessy II (Fig. 8 and 11) have the higher resolution in the plane the Zn is seen much clearer in the maps measured at ANKA (Fig. 6 and 9). For the Bessy maps measured with an angle of about 2.5° between sample and primary beam (Fig. 8) the count rate is too low to recognize any structure in the Zn distribution. For the maps measured under 5° (Fig. 11) the count rate is higher, but we get the signal with a lower depth resolution which makes it harder to correlate the maps with the bone structure seen in the qBEI images.

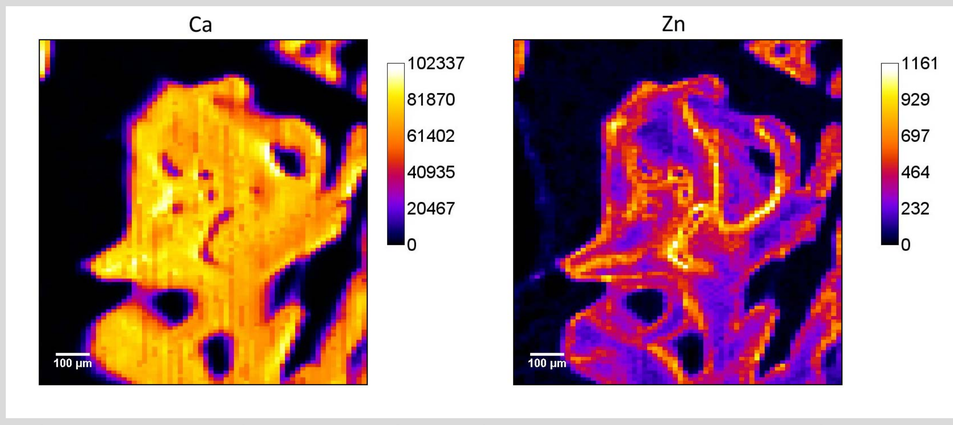

Figure 9: Ca and Zn map of sample WSX4 Area A measured at ANKA. Measurement time: 2s/pixel (3.6h for the complete map)

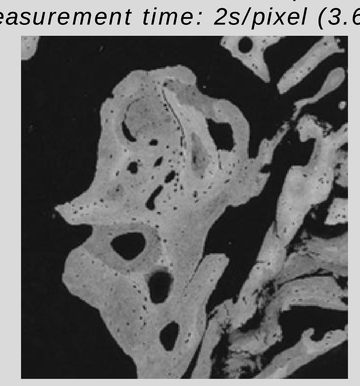

Figure 10: qBEI of WSX4 Area A

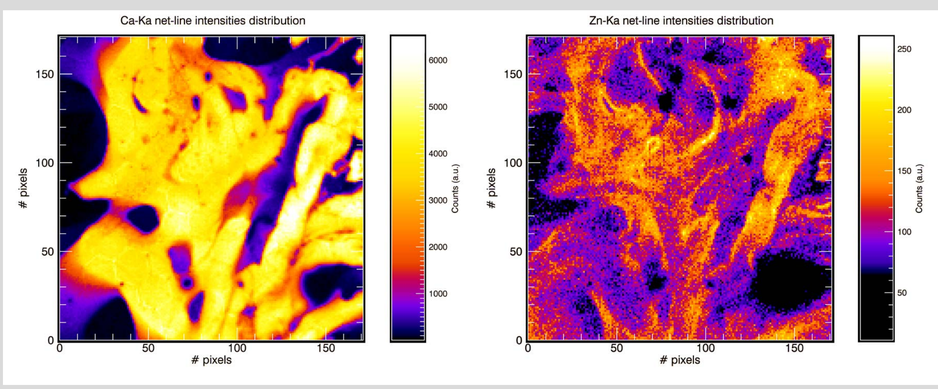

Figure 11: Ca and Zn map of WSX4 Area A measured at Bessy II. Measurement time: 18h; Angle primary beam – sample 5°

Conclusions and Outlook

1. Although the resolution of the CXC setup at the BAMline is higher than of the confocal µXRF setup at the FLUO beamline the count rates are too low to take advantage of that.
2. Enlarging the angle between primary beam and sample to obtain higher Zn count rates worsens the depth resolution excessively.
3. To investigate narrow bone structures further a combination of confocal µXRF and TXM (Transmission X-ray Microscopy) with thin samples could be fruitful.

References

1. O. Scharf et al., Analytical chemistry 83.7 (2011): 2532-2538.

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