Electronic Supplementary Material (ESI) for Metallomics.

Supporting Information for

MicroXRF tomographic visualization of zinc and iron in the zebrafish embryo at the onset of the hatching period

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Sample Preparation Protocol

Zebrafish embryos were anaesthetized at 48 hours-post-fertilization in 0.02% Tricaine (Sigma, E10521), manually dechorionated, and fixed for 2 hours at room temperature with a 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PBS). After removing the fixative, the specimens were washed with PBS and transferred to disposable aluminum foil dishes. Embedding in Lowicryl K4M resin was performed according to the progressive lowering of temperature (PLT) method developed by Carlemalm *et al.* in 1982.[1,2] This technique involves dehydration with increasing concentrations of ethanol while progressively lowering the temperature from 0 °C to –35 °C. Specifically, the following steps were employed:

Ethanol (Vol/%)	**Temperature (°C)**	**Time (min)**
30	0	30
50	–20	60
70	–35	60
95	–35	60
100	–35	60
100	–35	60

Subsequent infiltration with Lowicryl K4M resin and UV-mediated polymerization (360 nm) was performed according to the following protocol:

K4M : Ethanol (Vol : Vol)	**Temperature (°C)**	**Time (min)**
1:1	–35	60
2:1	–35	60
pure K4M	–35	60
pure K4M	–35	overnight
UV polymerization	–35	24 hrs
UV polymerization	0	12 hrs

Attenuation Correction Based on Linear Scaling Factors

The Lowicryl resin used for embedding the specimen results in attenuation of both the excitation and fluorescence emission intensities in an energy-dependent manner. To compensate for the loss of photons due to matrix absorption, element specific linear scaling factors were derived and applied as follows:
According to the Beer-Lambert law (S1), the intensity I of the X-ray beam is attenuated as a function of the path length l in an exponential fashion with

$$I = I_0 \cdot e^{-l\mu} \tag{S1}$$

where I_0 corresponds to the incident beam intensity, and μ is the energy-dependent linear attenuation coefficient of the material. As the angular position of the specimen relative to the incident beam and emission detector is different for each tomographic projection, attenuation of the excitation and emission intensities for a given volume element will vary accordingly. For a cylindrically shaped matrix with diameter l, the attenuation of photons emitted at the center of rotation remains constant throughout all angular positions, and the intensity I of the emitted photons can be expressed as the geometric mean according to equation (S2)

$$I = I_0 \cdot e^{-\frac{l}{2}(\mu_{ex}+\mu_{em})} \tag{S2}$$

where μ_{ex} and μ_{em} are the linear attenuation coefficients for the material at the respective excitation and emission energies. To compensate for the attenuation of photons emitted at the axis of rotation, it would be thus sufficient to apply a linear scaling factor f according to equation (S3)

$$f = e^{\frac{l}{2}(\mu_{ex}+\mu_{em})} \tag{S3}$$

As with increasing distance from the rotation axis, the difference between actual and approximated elemental concentrations would scale exponentially, a simple linear attenuation correction offers only a useful approximation if the matrix exhibits a rather uniform shape and is characterized by short path lengths and small attenuation coefficients at the emission energies of the elements of interest. Concluding from previous attenuation simulations, which assumed a phantom embedded within a polymethyl methacrylate (PMMA) matrix with square-shaped cross section, the attenuation-corrected concentrations of Zn, Cu, and Fe for this size of specimens should be accurate within an error margin of 20% or better.[3]

Concentration Profiles of Zn and Fe Across the Yolk Region

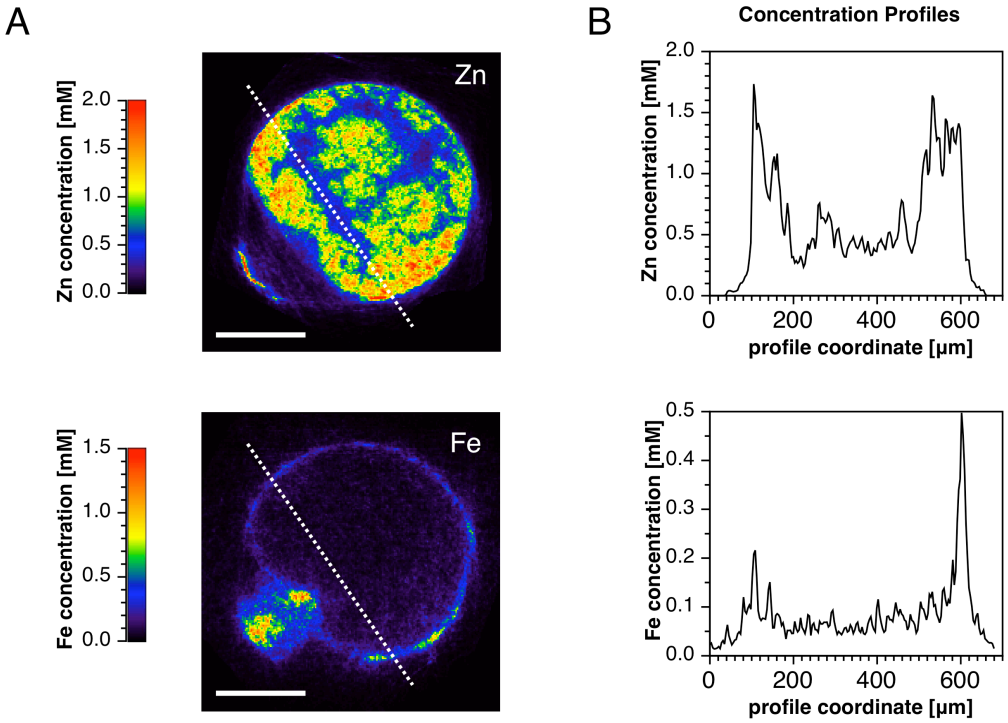

Figure S1: Estimated Zn and Fe concentrations in the yolk regions according to the reconstructed elemental densities using the iterative maximum likelihood expectation maximization (MLEM) algorithm. A. False-color concentration map. B. Concentration profile along the image traces indicated with dashed lines in panel A. Scale bar: 200 µm.

References:

(1) Armbruster, B. L.; Carlemalm, E.; Chiovetti, R.; Garavito, R. M.; Hobot, J. A.; Kellenberger, E.; Villiger, W., "Specimen preparation for electron microscopy using low temperature embedding resins", *J. Microsc.* **1982**, *126*, 77-85.

(2) Carlemalm, E.; Garavito, R. M.; Villiger, W., "Resin development for electron microscopy and an analysis of embedding at low temperature*", *J. Microsc.* **1982**, *126*, 123-143.

(3) Bourassa, D.; Gleber, S.-C.; Vogt, S.; Yi, H.; Will, F.; Richter, H.; Shin, C. H.; Fahrni, C. J., "3D imaging of transition metals in the zebrafish embryo by X-ray fluorescence microtomography", *Metallomics* **2014**, *6*, 1648-1655.