The three-dimensional elemental distribution profile within the freshwater crustacean *Ceriodaphnia dubia* was revealed at a spatial resolution down to 5 µm via a reconstruction approach employing state-of-the-art laser ablation-inductively coupled plasma-time-of-flight-mass spectrometry (LA-ICP-TOF-MS) and laboratory-based absorption micro-computed tomography (µ-CT).

**Introduction**

In serial 3D imaging mass spectrometry and LA-ICP-MS, 3D molecular ion or elemental distributions are reconstructed from serially imaged sections. In the reconstruction, the images of adjacent, independently recorded sections (Z-axis slices) are stacked and aligned to reflect the true 3D molecular or elemental profile in the sample. 3D LA-ICP-MS reconstruction approaches reported in literature are based on the sequential registration (automated alignment) of neighbouring slices (sequential slice registration – SSR). This approach operates under the assumption that:

- Some shared features are present in neighbouring slices.
- The relative positions of these features can be extrapolated along the Z-axis.

Hence, SSR is not applicable when the morphology of the sample changes too drastically from one slice to the next or when the sample’s central axis is not orthogonal to the cutting plane. SSR was compared to CSR (correlative slice registration): a new approach in which each Z-slice in the LA-ICP-MS data volume (a section) is registered relative to the corresponding slice in the µ-CT data volume, recorded a priori.

**Experimental part 1: Sample preparation (Figure 1A-E)**

- A juvenile *C*. *dubia* (300–3000000 µm in size) was exposed to elevated Cu (9.4 µg L⁻¹), Ni (3.9 µg L⁻¹) and Zn (25.2 µg L⁻¹) concentrations.
- B) Chemical fixation (para-formaldehyde), dehydration (EtOH), staining (Uranyl acetate) and embedding (Spurr’s resin).
- C) µ-CT analysis (1.5x1.5x1.5 µm³).
- D) Microtomy (5 µm thin sections).
- E) LA-ICP-TOF-MS imaging on every second section.

**Experimental part 2: Multidimensional registration (Figure 1F)**

Multidimensional image registration was performed to spatially align the 2D LA-ICP-TOF-MS images relative to the corresponding slices of the 3D µ-CT reconstruction. The µSSR obtained within the stain establishes a correlation between the 2 modalities.

- Detectable by both modalities (LA-ICP-MS and µ-CT) with good S/N ratio (Figure 3).
- The U stain fully penetrates the tissue.

**Experimental part 3: Segmentation (Figure 1I)**

The µSSR and µCP signal were used to assign each voxel to a volume-of-interest (VOI) representing a biological compartment or tissue. Cu, Zn, and Ni were quantified in each VOI (Figure 4).

**Conclusion**

- A series of 2D elemental images was acquired by LA-ICP-TOF-MS via serial sectioning in <24h using high-throughput low-dispersion LA-ICP-TOF-MS.
- Correlative µ-CT-guided slice registration (CSR) permits the accurate reconstruction of the 3D elemental distribution data when conventional methods such as SSR fail to reflect the tilted orientation relative to the cutting plane or the high level of depth heterogeneity (compare Figure 5A and Figure 5B with the µ-CT image in Figure 5D).

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