

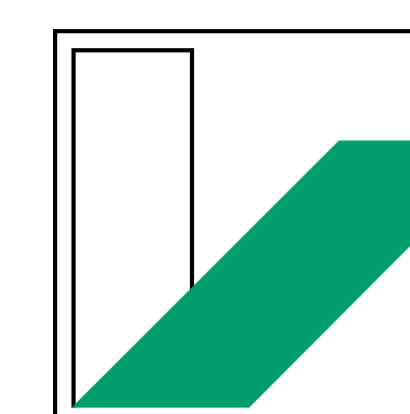
# MALDI mass spectrometry imaging workflows for the ecotoxicological model organisms *Daphnia magna*, *Danio rerio* and *Eisenia fetida*

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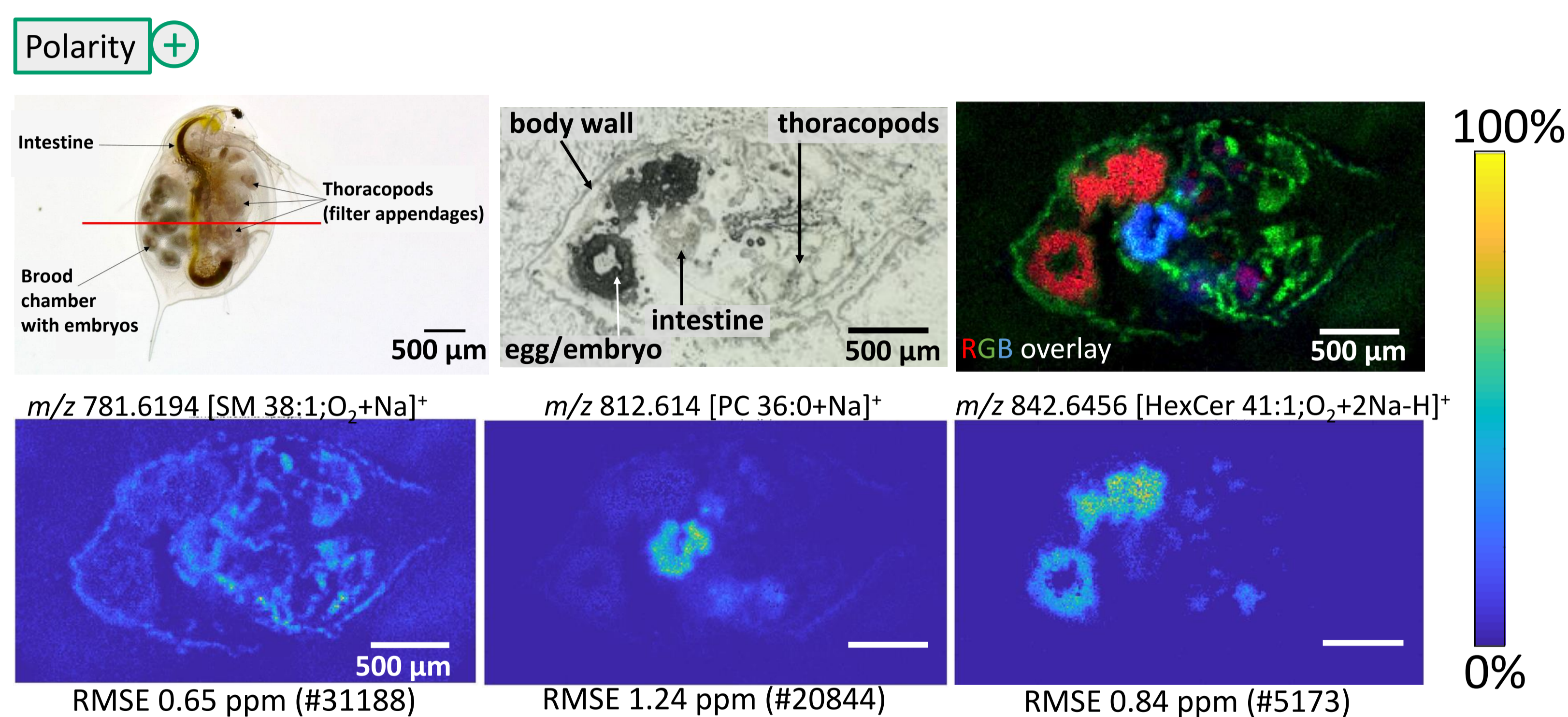


## Introduction

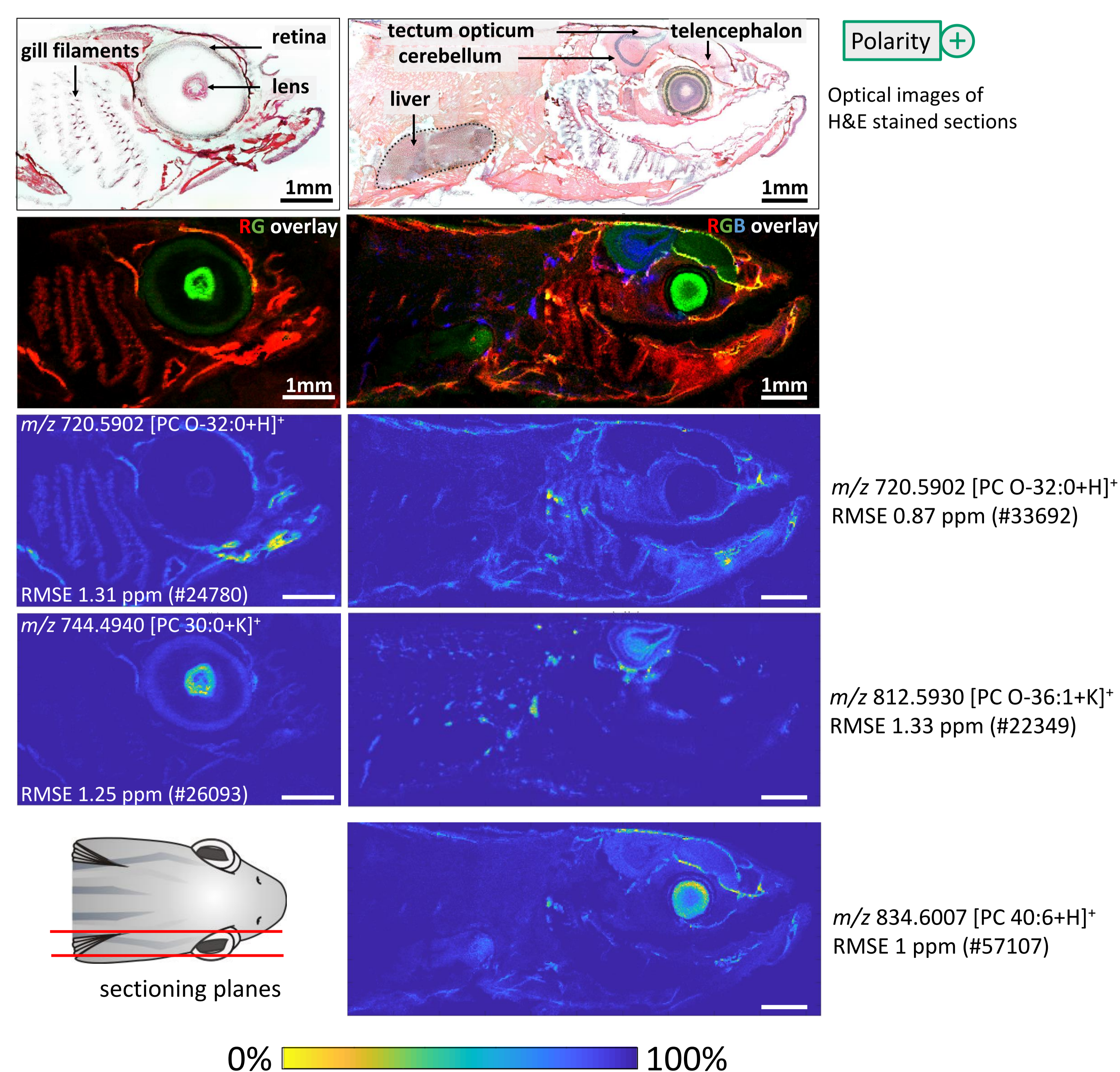
Environmental toxicology aims to understand the sources, fate and effects of chemicals released into the environment. A classical approach to understand toxic mechanisms involves the use of scientifically well-characterized model organisms<sup>[1]</sup>. In ecotoxicological studies targeting aquatic ecosystems, *Daphnia magna* (waterflea) and *Danio rerio* (zebrafish) are established model organisms<sup>[2]</sup> while the earthworm *Eisenia fetida* has proven to be a suitable candidate for the investigation of terrestrial ecosystems<sup>[3]</sup>. MALDI-MS imaging is a powerful technique for the visualization of molecules within tissue sections. However, for *E. fetida* and *D. rerio* only few and for *D. magna* no suitable MALDI-MS imaging workflow at high resolution in mass and space have been established. Major challenges pose the generation of sections with preserved tissue integrity as well as the choice of a suitable matrix application method to maximize signal intensity while minimizing analyte delocalization. We developed three MALDI-MS imaging workflows for the analysis of *D. magna*, *D. rerio* and *E. fetida* tissue sections with high-spatial resolution (5 to 25  $\mu\text{m}$ )<sup>[4,5]</sup>. This work presents ion images of structures & organs of the model organisms that can be utilized for future ecotoxicological studies.

## Results

### MALDI-MS imaging of a whole *D. magna* section at 10 $\mu\text{m}$ pixel size<sup>[4]</sup>



### MALDI-MS imaging of *D. rerio* sagittal sections at 25 $\mu\text{m}$ pixel size<sup>[4]</sup>



## Materials and Methods

Cryosectioning of samples was conducted on a CM3050 S from Leica (Nussloch, Germany).

- Cryosectioning**
- D. magna***
    - cultivated in an artificial M4-medium and daily fed with green algae
    - to prevent the carapace from fracturing during cryosectioning, daphnids were allowed to swim in a vial filled with 8% gelatin solution before embedding.
    - whole-body sections (thickness: 18  $\mu\text{m}$ ) of embedded daphnids were sectioned at  $-27^\circ\text{C}$ .
  - D. rerio***
    - Wildtype *D. rerio* were embedded in 3% CMC and sectioned with 20  $\mu\text{m}$  thickness at  $-15^\circ\text{C}$ .
  - E. fetida***
    - Earthworms were anesthetized using 7% magnesium chloride to enhance muscle relaxation and subsequently embedded in 3% CMC.
    - Sectioning was performed at  $-20^\circ\text{C}$  with a thickness of 20  $\mu\text{m}$ .

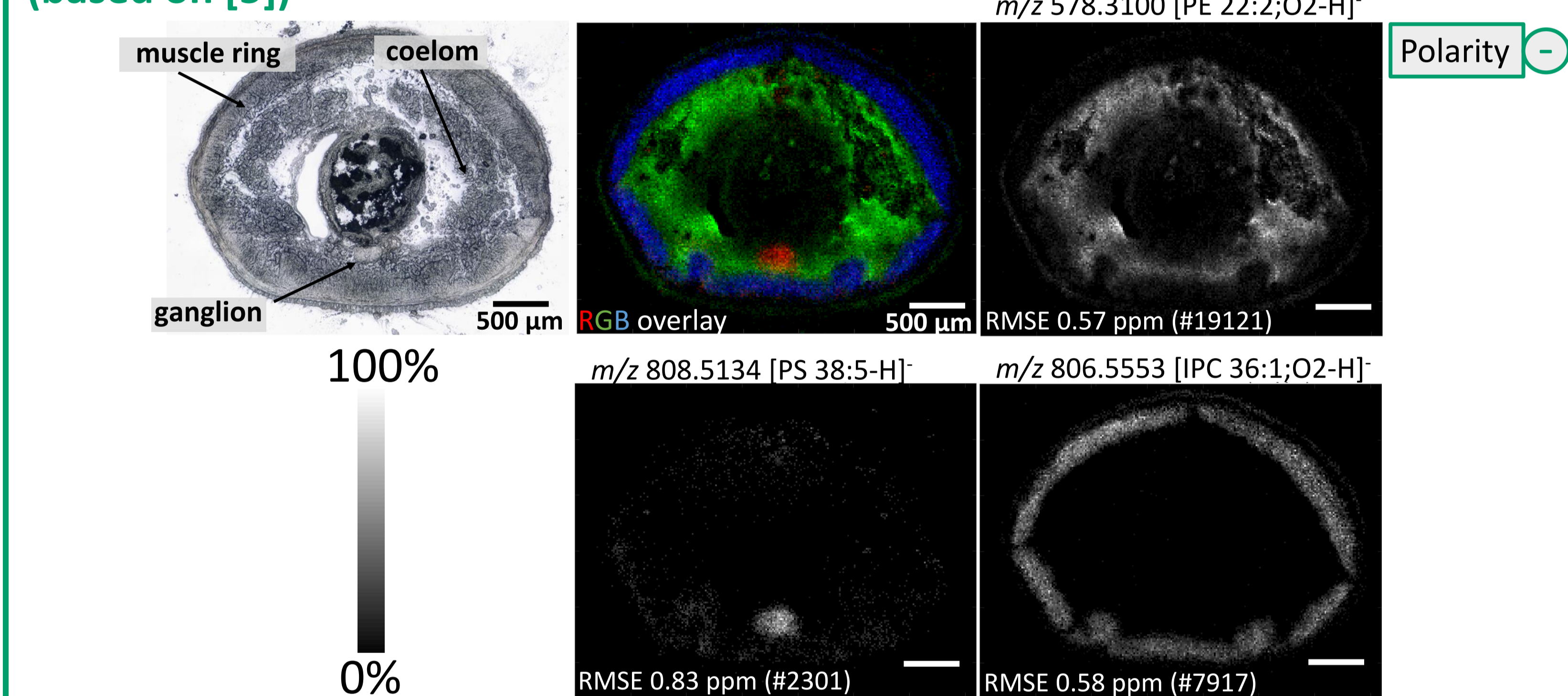
**Matrix application**

Sections were spray-coated with p-nitroaniline (pNA) using a home-built semi-automatic sprayer. For *D. rerio* & *D. magna* sections, pNA solutions (5 mg/mL) were prepared with acetone/water (3:1, v/v). *E. fetida* samples were sprayed with 10 mg/mL pNA (acetone/water (1:1, v/v)).

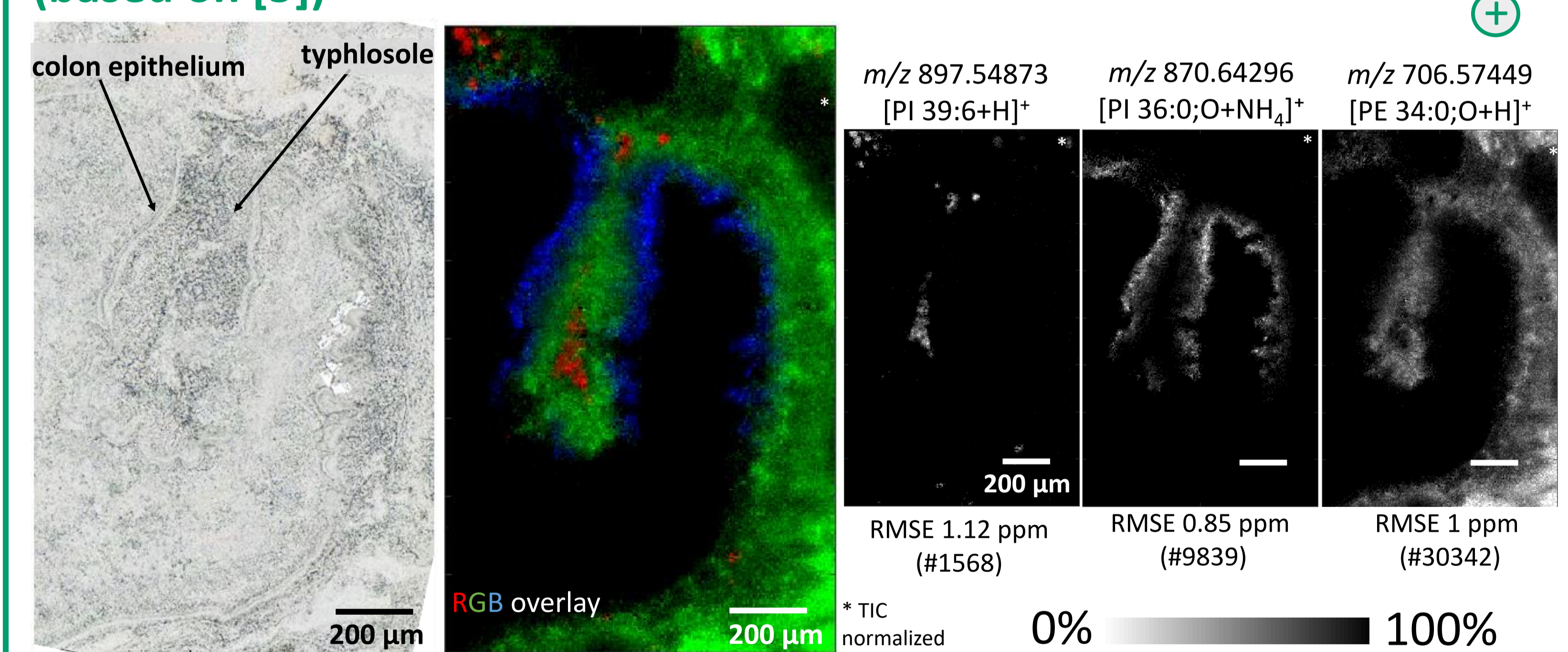
**MALDI-MSI**

Measurements were conducted using a Q Exactive HF (Thermo Scientific, Bremen, Germany) with a mass resolution of 200 000 at  $m/z$  200 (FWHM). The instrument was coupled to an atmospheric pressure-MALDI ion source (TransMIT, Gießen, Germany).

### MALDI-MS imaging of a whole *E. fetida* section at 15 $\mu\text{m}$ pixel size (based on [5])



### MALDI-MS imaging of the *E. fetida* colon region at 5 $\mu\text{m}$ pixel size (based on [5])



## Conclusion

- Development of high-resolution MALDI-MS imaging workflows for the ecotoxicologically relevant model organisms *D. rerio*, *D. magna* and *E. fetida*.
- The presented cryosectioning protocols preserved the tissue integrity of the samples.
- D. magna***
  - Analysis revealed distinct lipid distributions in embryos/eggs, the carapace lining and parts of the thoracic legs.
- D. rerio***
  - Measurements revealed distinct lipid-distributions showing anatomical structures of the eye, the brain, gill filaments and the liver.
- E. fetida***
  - Detection of prominent histological features such as the typhlosole, the ventral ganglion, the colon epithelium and the muscle ring.

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