Enhancing Microplastic Detection: Non-Destructive 3D Visualization of Microplastics in Zebrafish via X-Ray Micro-Computed tomography

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Introduction

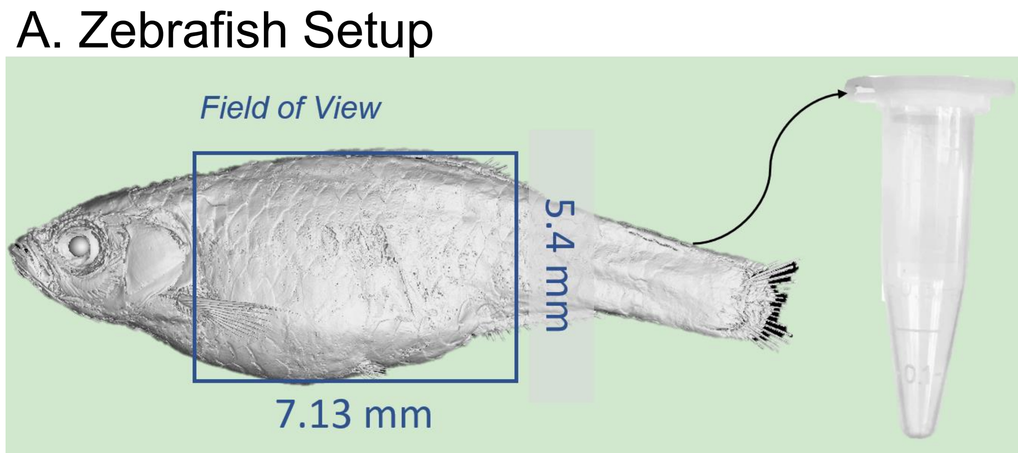
Plastic pollution is a pressing global concern, with microplastics (MPs) raising particular alarm due to their potential risks to ecosystems and human health. Once ingested or inhaled, MPs can accumulate in various tissues, making their detection critical for assessing biological impact. However, current analytical techniques, such as spectroscopic and pyrolytic methods, primarily focus on chemical composition and often require extensive sample processing, which can compromise spatial distribution data and introduce contamination risks. This study investigates X-ray micro-computed tomography (microCT) as a non-destructive approach for identifying MPs in biological tissues. Using zebrafish as a model, we demonstrate that microCT enables high-resolution, three-dimensional visualization of MP localization while preserving tissue structure. We successfully detected polyethylene MPs within the fish intestines by refining scanning parameters and employing contrast-enhancement strategies. Our results underscore microCT's potential as a powerful tool for studying MP accumulation in biological specimens, complementing conventional methods. The ability of microCT to maintain spatial distribution data opens new possibilities for exploring MP interactions in biological systems. With further refinement, this technique could advance environmental monitoring and biomedical research by offering more profound insights into MP exposure risks. Future efforts will enhance detection sensitivity for MPs of varying sizes, densities, and morphologies, broadening microCT's role in microplastic research.

Materials and Methods

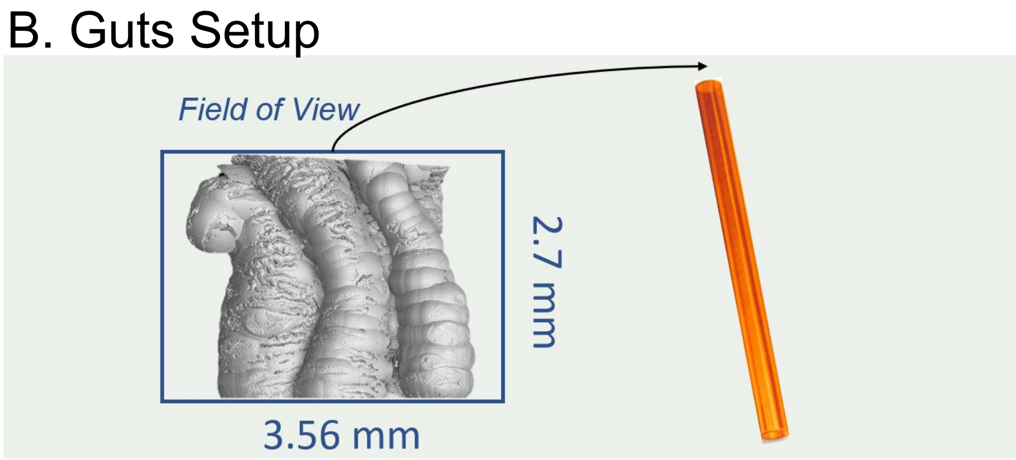
Spherical polyethylene (PE) MPs with a size range of **30–110 µm** (Cospheric LLC, USA) were used in this study. Adult **zebrafish** (Danio rerio) were selected as model organisms to evaluate the applicability of microCT for MP detection in biological samples. MPs were administered via intragastric gavage, where 2 mg of MPs in 100 µL ethanol was delivered directly into the gastrointestinal tract using an ultrafine pipette. After euthanasia, guts were dissected from half of the samples. **Whole zebrafish** and **dissected guts** were included as two optimization setups. Before microCT scanning, samples were **stained** with 1% iodine in 90% methanol for 12 hours at 4°C, followed by an ethanol dehydration series (30–90%) to minimize tissue shrinkage. Whole zebrafish were placed in 1.5 mL Eppendorf tubes, while dissected guts were positioned in 3 mm Kapton tubes embedded in 1% agarose gel for stabilization during scanning. MicroCT imaging was performed using the Nano3DX (Rigaku, Japan) with two optimized setups: one for whole zebrafish (voxel size: **4.2 µm**) and another for extracted guts (voxel size: **2.1 µm**).

Obtained datasets were processed in VG Studio Max 2023.4 (Volume Graphics GmbH, Germany) using a neural network-based segmentation workflow. MPs were identified using the Paint and Segment module, where manually labeled training data enabled automatic segmentation refinement. The segmented MPs were further analyzed for size and **spatial distribution** within the zebrafish **gastrointestinal tract**.

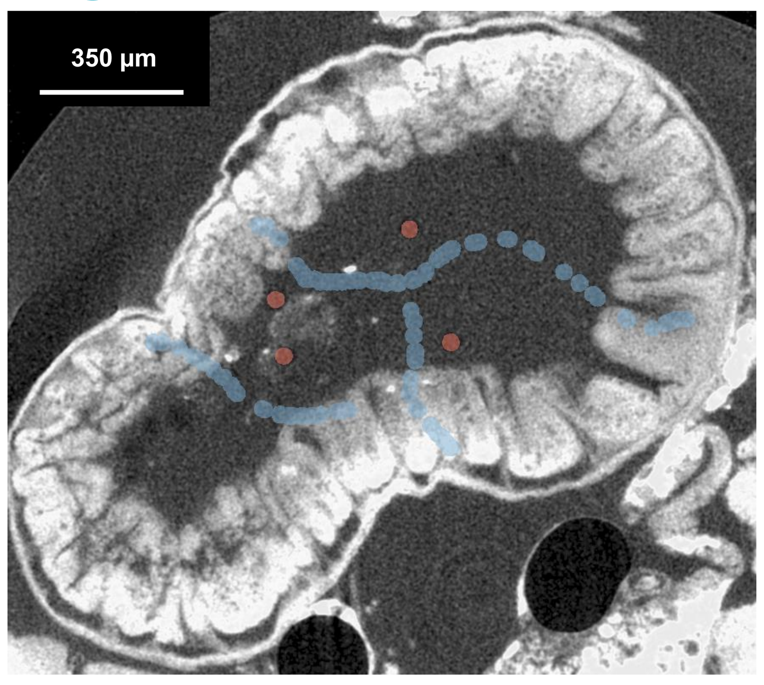
A. Zebrafish Setup



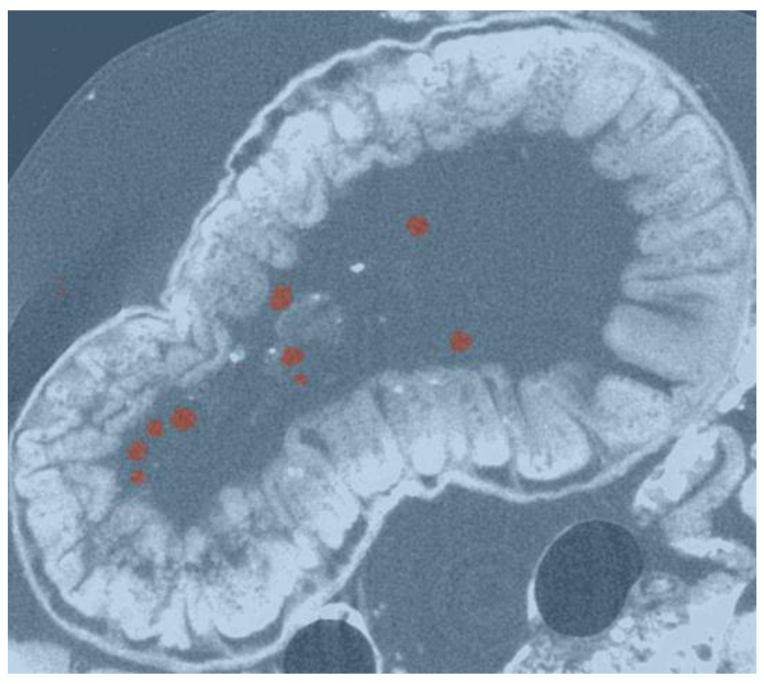
B. Guts Setup



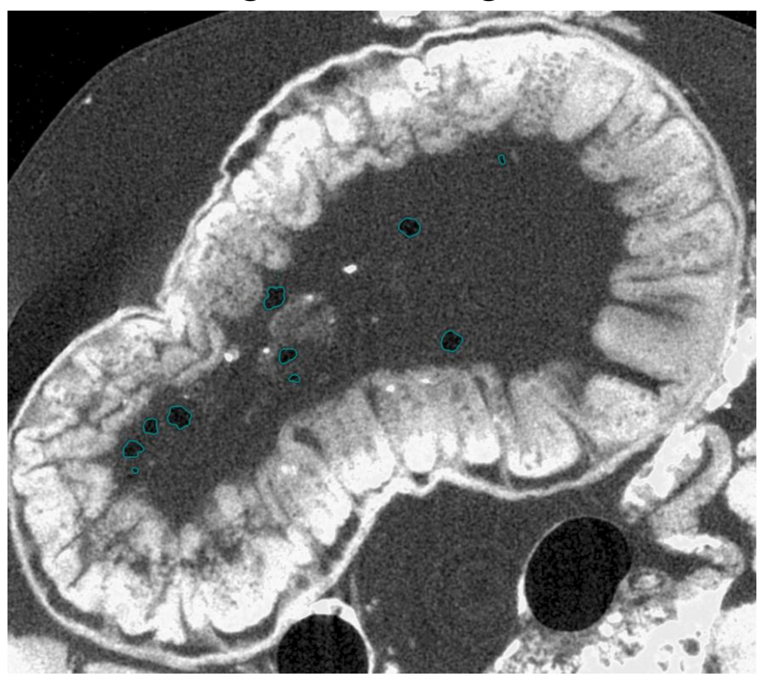
Segmentation



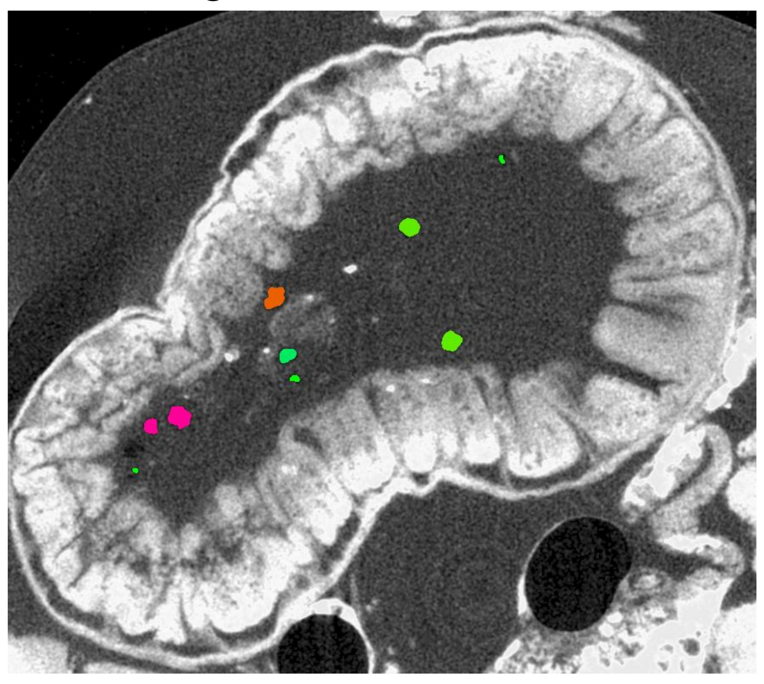
2. Labeling for AI Segmentation



3. AI Segmentation



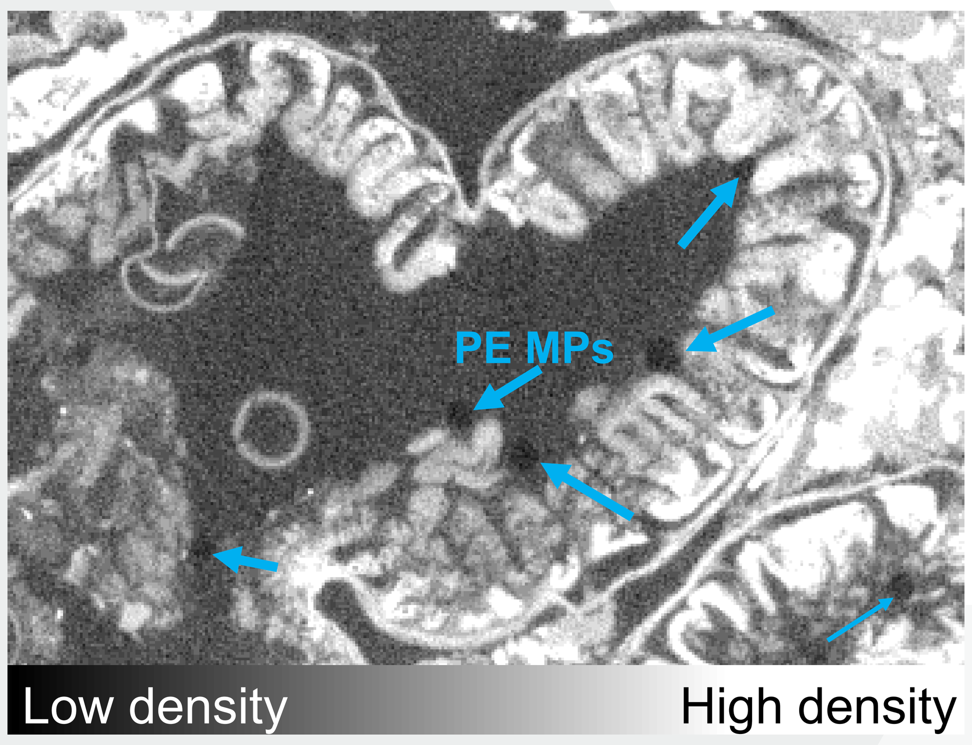
4. Final Segmentation



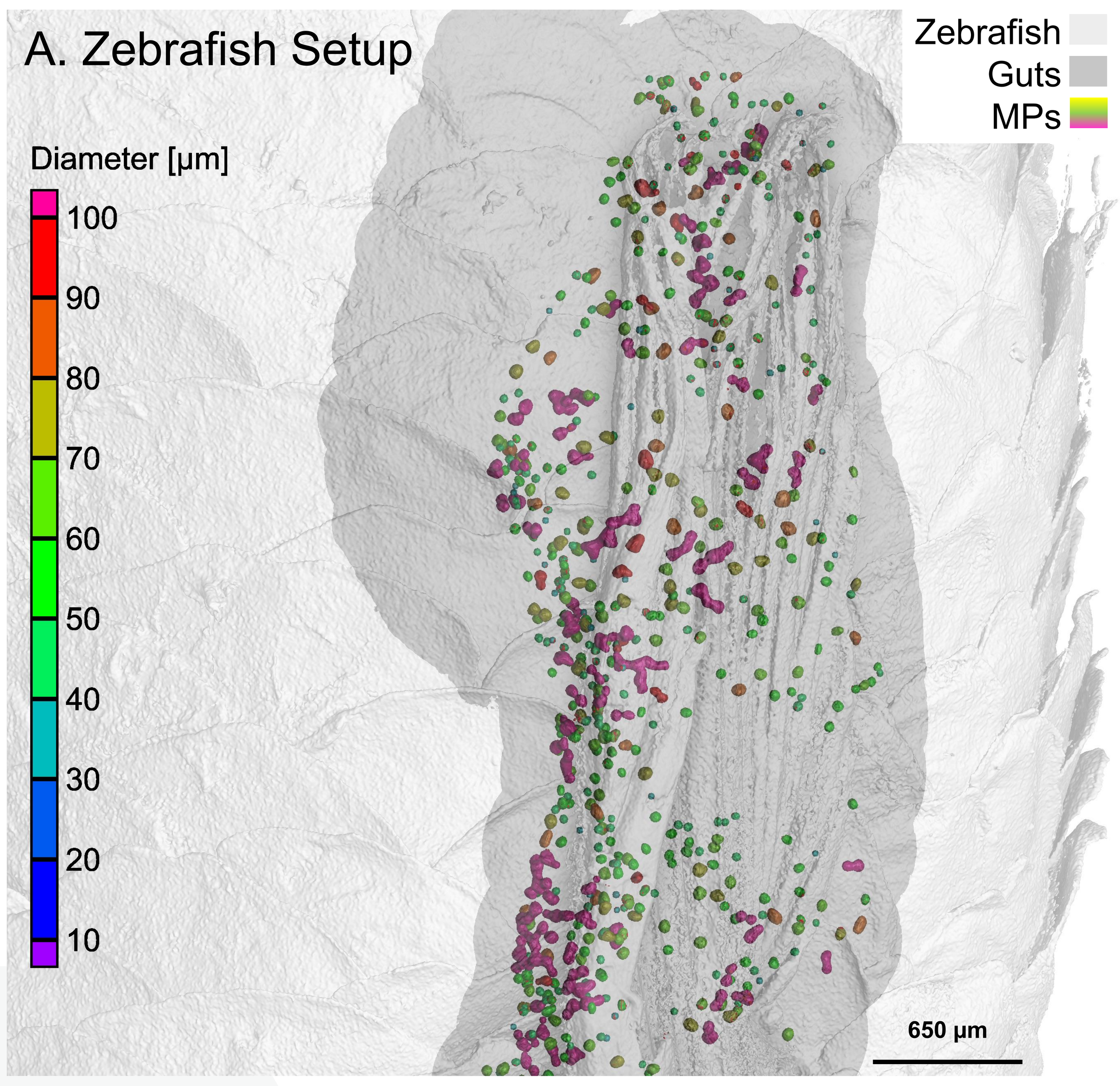
5. Quantification

Results

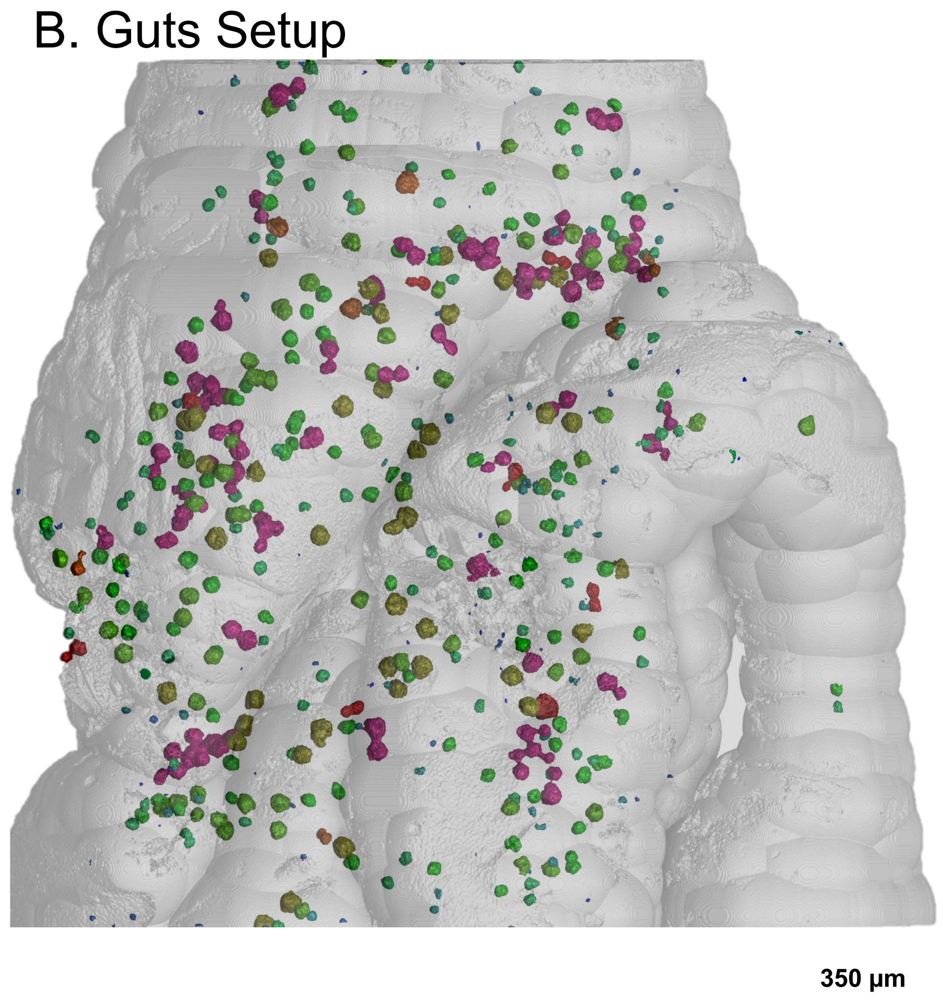
Visualization of MPs in obtained data Since microCT visualizes materials based on their **densities**, the PE MPs appeared as **darker** voxels in the dataset due to their lower density compared to the agarose gel. This contrast enabled the detection and segmentation of MPs in obtained data. Segmentation was **successful** using both setups.



A. Zebrafish Setup



B. Guts Setup



Limitations and Conclusions

MicroCT enables **non-destructive**, **high-resolution** MP visualization in biological samples, providing **spatial insights** unavailable with conventional methods. This study serves as a **proof of concept**, demonstrating that microCT can **track targeted MPs** of known type (spherical PE) within zebrafish tissues. MPs as small as **30 µm** were successfully detected, allowing for the assessment of their distribution in the gastrointestinal tract. However, MPs at the nanoscale can cross cell barriers, posing significant biological risks. At this stage, microCT does not yet offer the resolution needed to detect such nanoparticles, but future advancements in imaging technology may bridge this gap. While microCT **does not provide chemical identification**, it can complement spectroscopic methods to enhance MP characterization. Further research is needed to adapt this methodology for more environmentally relevant MPs, including different shapes, compositions, and smaller particle sizes. This methodology is best suited for **tracking MPs in biological systems**, offering valuable insights into their **accumulation** and potential health impacts.

Scan for more information.

REFERENCES

[1] Parobková, V., et *al*. (2025). Advancing microplastic detection in zebrafish with micro computed tomography: A novel approach to revealing microplastic distribution in organisms. Journal of Hazardous Materials, 488, 10.1016/j.jhazmat.2025.137442.

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