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## Developing a high-content imaging workflow to investigate the impact of environmental chemicals on intestinal macrophages in zebrafish larvae

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The gut is one of the primary sites for exposure with chemicals and hence a vulnerable target for toxic effects. We hypothesize that intestinal exposure to such chemicals can trigger gut inflammation. Macrophages, key innate immune cells, represent a promising readout for detecting immunomodulatory effects following chemical exposure. To investigate this hypothesis, we developed an open-source workflow for 3D high-content image processing and analysis of transgenic zebrafish larvae expressing mCherry under the macrophage-specific promoter *mpeg1*. We used a 96-well setup to acquire 3D confocal images of zebrafish larvae, which were processed through a semi-automatic workflow. The intestine is manually defined in transmitted light images using an ImageJ macro. This anatomical region was then mapped onto corresponding fluorescence channels for precise quantification in python. To further streamline the process, we are training a 3D nnU-Net for semantic segmentation of the larval body and gut based on partially annotated volumes. Preliminary results from the beta-version of our workflow demonstrate that we can detect changes in macrophage numbers following exposure to reference chemicals, such as dextran sodium sulfate and trinitrobenzene sulfonic acid. Our approach addresses natural biological variability by correcting for the inherent differences in macrophage distribution and fluorescence between individual larvae. Our workflow involves the detection of cells across both the whole body and the intestine of each larva, enabling the calculation of a ratio between intestinal and whole-body cell counts. This normalization strategy helps correct for individual differences in overall fluorescence and ensures that detected changes in gut macrophage numbers truly reflect inflammatory responses rather than natural variability. However, the development of this workflow is time-consuming and non-linear, so the involvement of various colleagues and experts is crucial for success. In the workshop, we would like to present the workflow in its semi-automated beta version and discuss possible streamlining measures to improve the workflow.

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**Session Classification:** Talks 1-5