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High Content Screening in zebrafish embryos using automated image and video analysis –advancements and challenges

Zebrafish embryos are frequently used as an experimental model in the assessment of toxic effects. They are considered as alternatives to animal testing, since early life stages are not considered as "protected"stages by recent European regulations on animal welfare. An important read-out in effect assessment of chemicals in the zebrafish embryo is the assessment of morphological alterations. Typically, this has been conducted by microscopical observation and user-based scoring. This resulted in a bias in the effect assessment and difficulties to derive effect concentrations for chemical effect in a non-cumulative way for individual phenotype. To derive an unbiased quantitative assessment of morphological phenotypes we developed the software FishInspector (freely available via https://codebase.helmholtz.cloud/ufz/tb3-cite/biotox/FishInspector/-/tree/v1.87). By initial user annotation of the coordinates of structural features such as contour, eye, yolk, otoliths, mouth tip, notochord, pericard, fins and swimbladder in lateral and dorsoventral position, appropriate models were trained for an automated detection. The coordinates of the detected features were then used to derive quantitative metrics for concentrations response analysis, such as eye size, body lengths or otolith-eye distance (and many more). The first versions of FishInspector used models trained in MATLAB, based on a SegNet architecture with a pretrained VGG-19 encoder from the Deep Learning Toolbox. However, these models required downsizing of images and the derived annotations required relative intense user editing. Therefore, we revised the FishInspector software to incorporate PyTorch-based models trained in Python. The High-Resolution Network (HRNet), a state-of-the-art segmentation model, was integrated to address the multilabel segmentation task. While FishInspector is still coded in Matlab it is now interacting with a Python environment to annotate the structural features. An accessory software (MultiLabelFishTrainer) has been developed for a user-friendly training of Python models. Furthermore, we established a software (FishCardiologist) to analyse short 15-second videos from lateral position in order to extract the heart rate and to obtain an image of the cardiovascular system based on video frame subtractions. By combining these image analysis tools with the assessment of embryonic movement patterns, over 200 chemicals have been analysed so far and examples of a comparative analysis will be demonstrated. In the zebrafish workshop we would like to discuss major challenges in image analysis, such as assessment of quantitative analysis of complex structures (e.g. cardiovascular system) and the training of additional models.

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