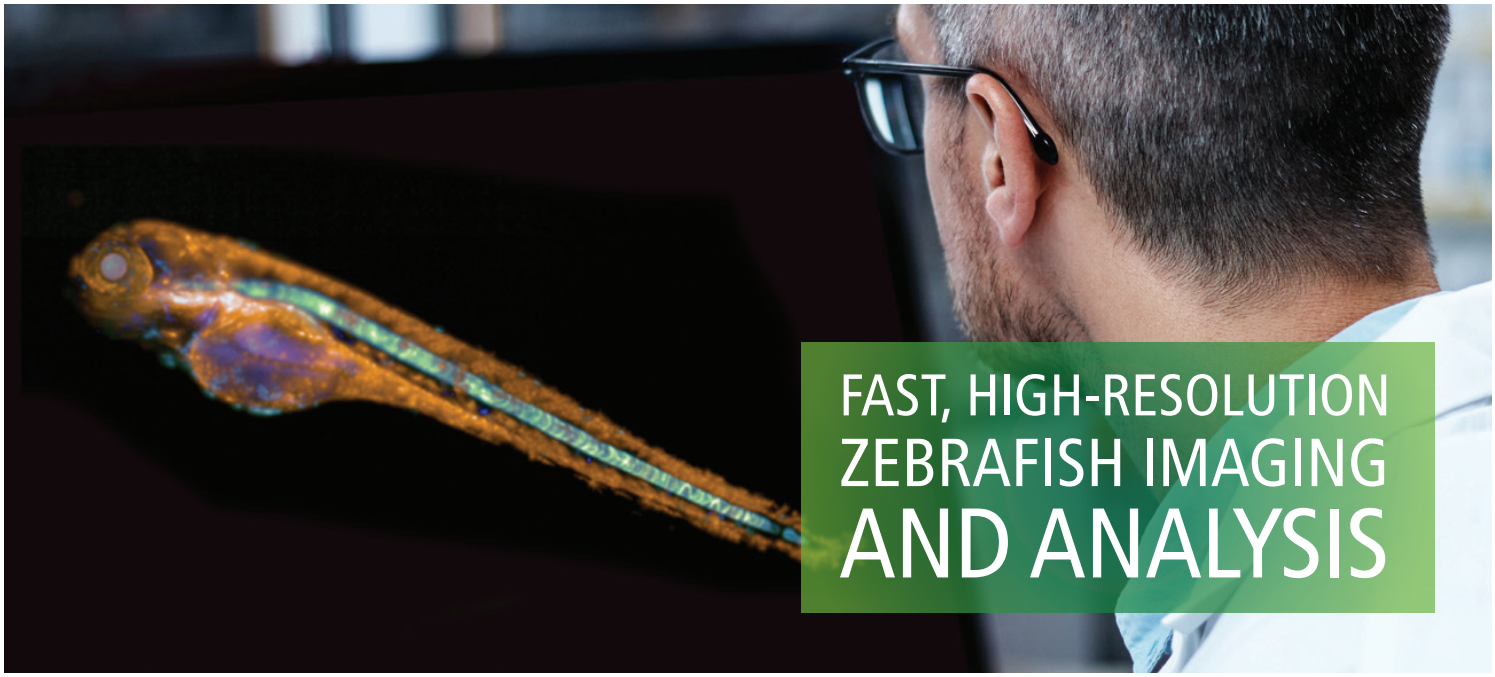

FAST, HIGH-RESOLUTION ZEBRAFISH IMAGING AND ANALYSIS

The zebrafish is an attractive model system for a variety of applications in basic research and drug discovery because of its similarities with human disease phenotypes. Many imaging based assays have been developed to measure phenotypic changes in zebrafish, but image acquisition and analysis are tedious and time consuming when performed manually. Automation of these processes increases throughput, whilst also enhancing data quality. Here, we show how the Operetta CLS™ and Opera Phenix™ high-content analysis and screening platforms streamline imaging and phenotypic analysis of zebrafish enabling you to focus on data evaluation rather than data generation.

Automatic detection for high-resolution images

Easily find zebrafish in your wells

Locating zebrafish in your wells in order to image them using a microscope is time consuming. Harmony® high-content analysis software incorporates PreciScan™, an intelligent image acquisition tool that enables a fully automated, integrated workflow of low magnification pre-scan, image analysis and higher magnification re-scan to reduce acquisition times and data volume and to significantly speed up analysis. Zebrafish detection, on brightfield images, is made easy by PhenoLOGIC™, a tool for image segmentation using supervised machine-learning methods (Figures 1 and 2).

- Automatically detect zebrafish in microplate wells via supervised machine learning
- Increase throughputs through Intelligent image acquisition
- Obtain high-resolution confocal images with automated water immersion lenses
- Streamline and simplify image analysis with Harmony® imaging and analysis software

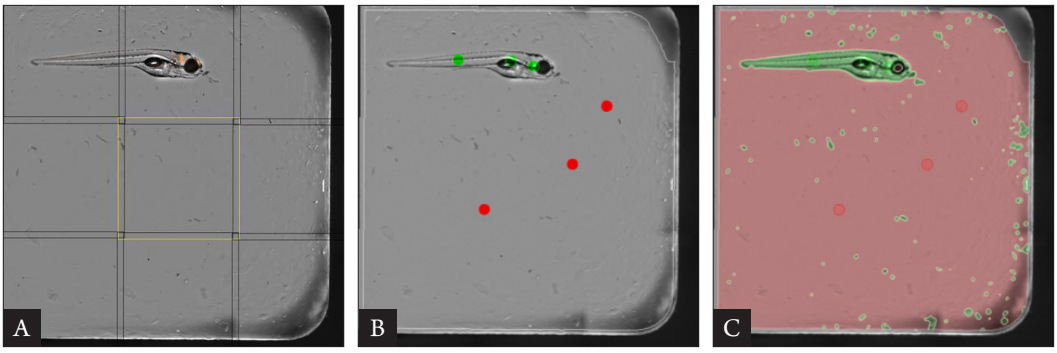

Figure 1. Automatic location of zebrafish using the PhenoLOGIC tools in Harmony. A - Brightfield pre-scan at 5x magnification. B - In this step, PhenoLOGIC is trained to distinguish between fish *(green spots)* and background *(red spots)*, based on texture. C - PhenoLOGIC based fish detection, which can easily be further optimized using shape based filtering to remove smaller mis-identified regions.

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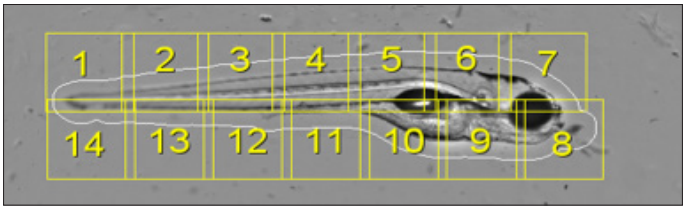
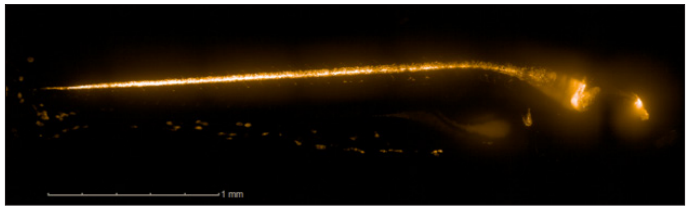


Figure 2. PreciScan positions the layout for the high-resolution re-scan on the fish detected in the pre-scan.



Re-scan at 20x (1.0 NA water immersion lens). Maximum projection of the confocal stack of 50 planes.

Acquisition time for the 700 images used to generate this image was <2 mins. Automated detection and acquisition means you can walk away whilst fish are detected in the remaining wells.

Automatic quantification of phenotypes

Analyze gene expression patterns

In this experiment, zebrafish are transfected with four fluorescent proteins. The four markers are multiplexed on the Operetta CLS high-content analysis system and the images (Figure 3) can be analyzed.

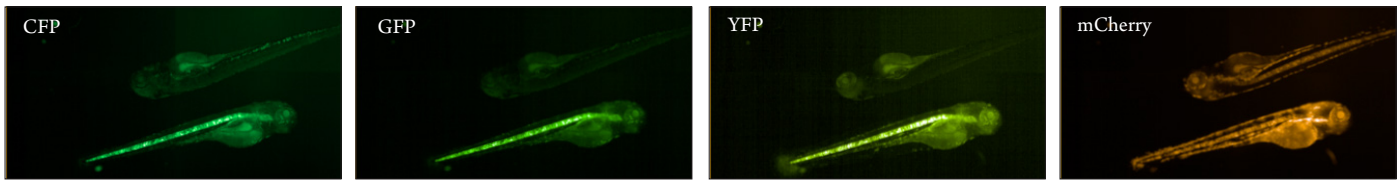


Figure 3. Zebrafish expressing CFP, GFP, YFP and mCherry-tagged proteins. Samples supplied by CCRI, Vienna.

Automatically and accurately determine morphological features

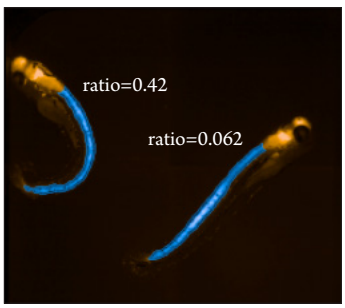


Figure 4: Zebrafish stained with a single fluorescent dye enabling determination of head:tail ratio.

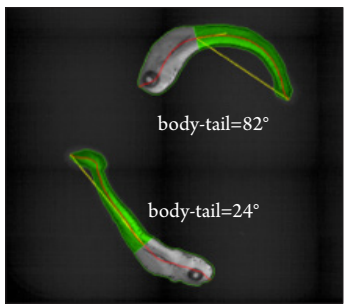


Figure 5: Automatic detection of tail axis.

Changes in intensity, morphology and texture can be measured easily using the building block approach of Harmony imaging and analysis software. The example (Figure 4) shows that a single fluorescent dye can be used to identify different body segments. The head is identified based on width, enabling the backbone to be detected. An equivalent ellipse is used to describe the shape of the fish backbone and estimate its curvature.

In a similar manner, the tail axis (Figure 5) is automatically detected and provides an unbiased method for the detailed assessment of tail curvature.

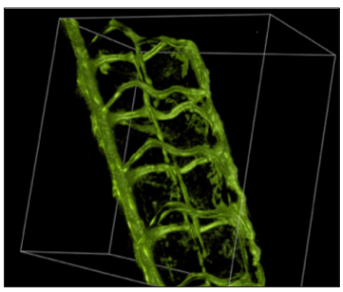


Figure 6: This image shows a 3D rendering of blood vessels in a zebrafish tail recorded with the 20x water immersion lens.

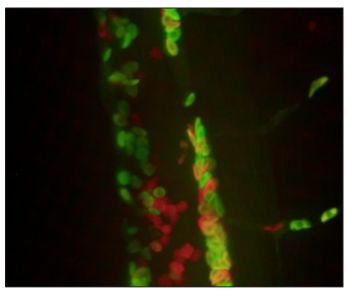


Figure 7: Individual red blood cells expressing GFP and RFP are visible. 63x magnification, 1.15 water immersion lens.

See fine detail, even in 3D

Harmony software provides tools for visualization of your samples in 3D. The Opera Phenix and Operetta CLS high-content analysis systems provide a range of magnification options from 1.25x to 63x and are equipped with automated water immersion lenses to reduce distortion as you image deeper into the sample, as well as to increase sensitivity.

Acknowledgements: Figures 1-6: Samples supplied by CCRI, Vienna, Figure 7: Sample supplied by Kamil Jastrzebski, IIMCB, Warsaw

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