

AUTOMATED IMAGING OF ZEBRAFISH EMBRYOS

OVERVIEW

The zebrafish core facility provides all users with a platform for automated imaging of zebrafish embryos. This platform, the ImageXpress Nano, is a plate-based imaging system in which zebrafish embryos (live/fixed) between 1 day and ca 10 days can be imaged in brightfield and/or fluorescence. Both single images and image stacks can be taken as well as time-laps and real-time movies recorded.

The zebrafish core facility can help you with mounting zebrafish, image acquisition and image analysis.

Quick facts:

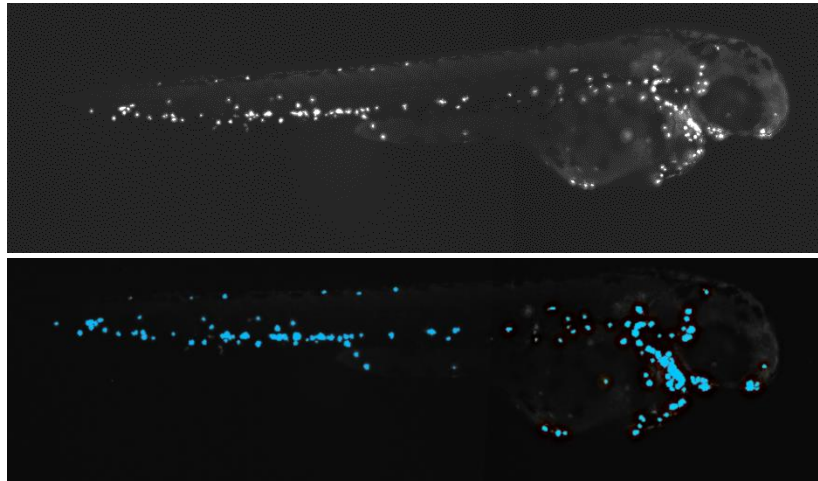
- Plate based system for automated imaging
- Imaging of stacks ("semi-confocal")
- 4x, 10x and 20x objective
- Fluorescence imaging (DAPI, GFP, texasRed, Cy3)
- Brightfield imaging
- Time-laps imaging
- Real-time imaging with up to 30 fps
- Analysis software for automatic analysis and quantification

POTENTIAL APPLICATIONS

In the following, potential applications are presented. Please contact the zebrafish core facility for more information.

IMAGING OF FLUORECENT CELLS

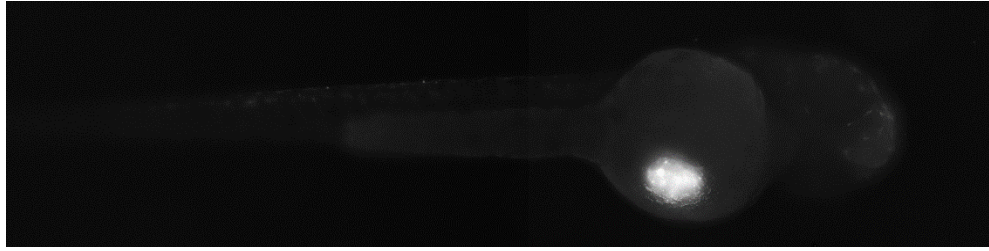
The imaging system can detect fluorescent cells in zebrafish embryos. Potential applications include monitoring cell migration, evaluating the effect of gene-modification on a specific cell population or studying the interaction of different cell types in a living animal. All images can automatically be analyzed and parameters such as the number of cells per single embryo can be quantified by a specific analysis software. In the examples to the right, transgenic fish expressing GFP in neutrophils have been imaged and a script in MetaXpress automatically identifies all fluorescent cells.



Living 2-day old Tg(mpx:GFP) embryo imaged with a 4x objective. The analysis software can automatically identify individual cells (compare the blue spots in the lower image to the position of the cells in the image upper). Parameters such as number of cells per embryo are automatically recorded and delivered in excel or similar.

IMAGING OF XENOTRANSPLANTS

Zebrafish embryos transplanted with fluorescent tumor cells can easily be imaged using our robotic imaging system. Potential applications can be recording of

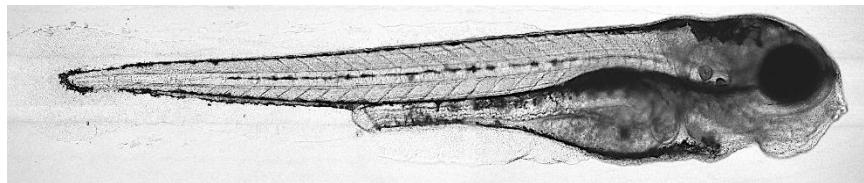


Living 48 hpf embryo transplanted with fluorescent cancer cells into the perivitelline space. Image taken with the 4 x objective. The analysis software can be programmed to assess tumor size, nu

tumor size over time or imaging the interaction of tumor cells with fluorescent host cells. Furthermore, tumor cell migration can be followed over time. The analysis software can automatically quantify the tumor size, hence an increase or decrease of tumor burden can automatically be documented.

CHEMICAL EXPOSURE AND DRUG DISCOVERY

Zebrafish embryos are mounted in specific 96 well plates in which they can be exposed to chemical libraries. The imaging plates are of similar size as usual 96 well plates and can therefore be

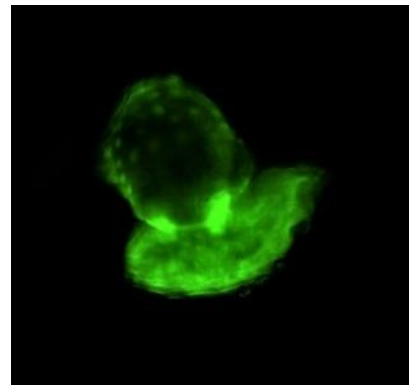


Living 96 hpf embryo imaged with the 4x objective

used in any liquid handling system. The zebrafish embryos remain in the 96 well plates before, during and after imaging. Therefore, the exposure to chemicals is, unlike other zebrafish imaging solutions on the market, not disrupted during the imaging process. Potential applications could be studying the effect of small molecules on embryonic morphology or physiology like heart-beat frequency (see below).

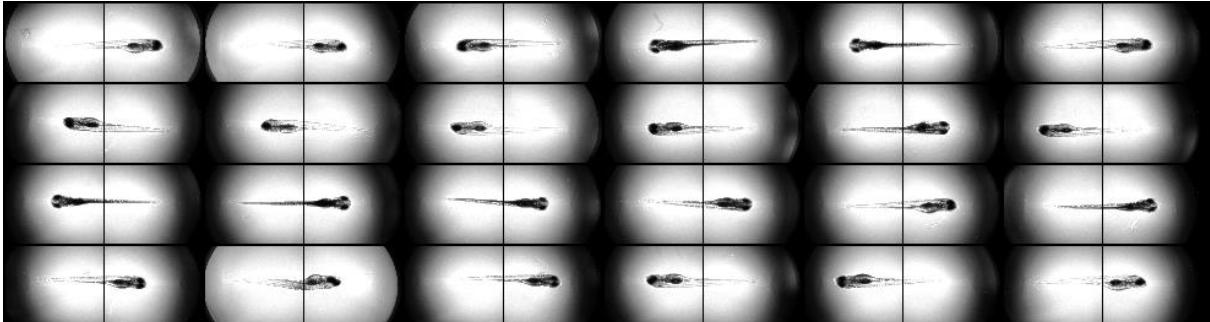
QUANTIFICATION OF HEART-BEAT

The automated imaging system allows to image and quantify physiological parameters like heart-beat frequency. Potential applications could be analysis of small molecules on heart-beat frequency or blood flow. To the right a picture of a 3 day old zebrafish embryo expressing GFP in the heart. The atrium, valve and ventricle are clearly visible. The heartbeat can be filmed with > 30 fps.



MOUNTING OF EMBRYOS

The zebrafish core facility provides 96-well plates specifically designed for zebrafish imaging. Those plates are coming with an agarose mold which keeps the embryos fixed in position for either dorsal or ventral imaging. Zebrafish embryos between 1 and ~10 days can be quickly mounted and the addition of tricaine or similar prevents any movements.



3-day old zebrafish embryos mounted into 96 well imaging plate. An agarose slit secures a ventral or dorsal position of the sedated embryos.

SERVICE FEE FOR AUTOMATED IMAGING

- **Imaging fee including set-up of imaging, imaging and export of images:**
 - o First plate per screen: 300 kr/plate
 - o For every additional plate: 100 kr/plate
- **Analysis fee including programming of the analysis module, analysis and data export:**
 - o First plate per screen: 300 kr/plate
 - o For every additional plate: 100 kr/plate
- **96 well imaging plate (Falcon imaging plate with agarose slits for either dorsal or ventral mounting of zebrafish \geq 48 hpf); plate can be reused: 200 kr/plate**