APPLICATION NOTE

High-throughput imaging assays using zebrafish, a model organism for human disease

Why zebrafish screening?

Recently, zebrafish-based screening has gained favor as an alternative to mammalian screening due to cost, throughput and reduced ethical concerns. Zebrafish are a useful model for drug development because of their high biological similarity to humans. Studies in ontogeny and organogenesis have shown that their primary organ systems are very similar to humans, and the synteny between zebrafish and *Homo sapiens* is as high as 70-80%.

Zebrafish are beneficial for screening assays because of their high fecundity, transparency of embryos for viewing organ structures, and ease of gene manipulation. Their small size allows embryos to be placed in microtiter plates and treated with compounds. Phenotypes can then be measured on a high content screening system.

The ImageXpress® High-Content Screening System provides optimal flexibility for acquiring high quality images with a large field of view. MetaXpress® High Content Image Acquisition and Analysis Software enables image analysis for a wide range of applications with simple workflows, and in combination with AcuityXpress High Content Informatics Software for data mining, this end-to-end solution dramatically improves throughput for zebrafish-based *in vivo* screening.

Zebrafish-based *in vivo* high content screening applications

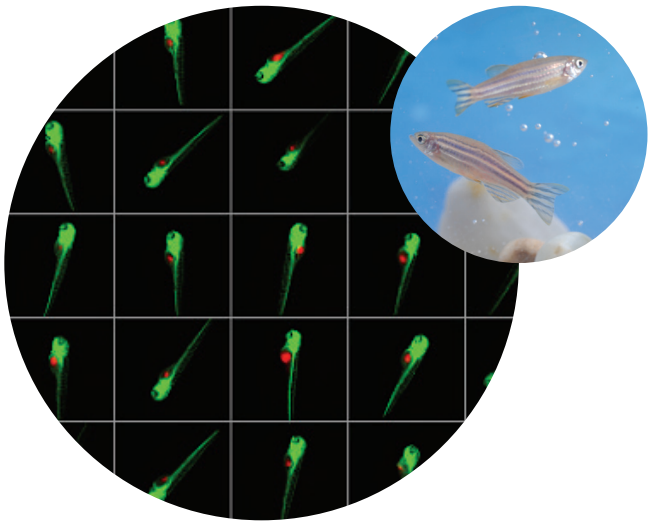
There has been a multitude of human disease models developed in zebrafish such as:

- **Metabolic syndrome:** obesity (visceral fat), dyslipidemia, fatty liver, glucose tolerance disorder
- **Xenotransplantation of human cancer cells:** tumor angiogenesis, distant metastasis
- **Circulatory disease:** cardiac failure, drug-induced arrhythmia
- **Central nervous system disorder:** deafness, visual disturbance, smell disorder, epilepsy, developmental disorder, sleep arousal disorder, myodystrophy (ALS)

Several examples of how high-content imaging assays can be used for investigating these disorders are shown here.

Benefits

- **Evaluate thousands of compounds in a few days in the complex 3-dimensional context of a whole organism**
- **Screen a wide range of disease and toxicity models**
- **Visualize and measure whole-body phenotypes in a single image**
- **Keep images in focus from head to tail with automated high-speed Z stacking**



The data presented in this application note was provided by Prof. Toshio Tanaka, Department of Pharmacogenomics, Mie University Graduate School of Medicine.

Modeling tumor metastasis after xenotransplantation of human cancer cells[1]

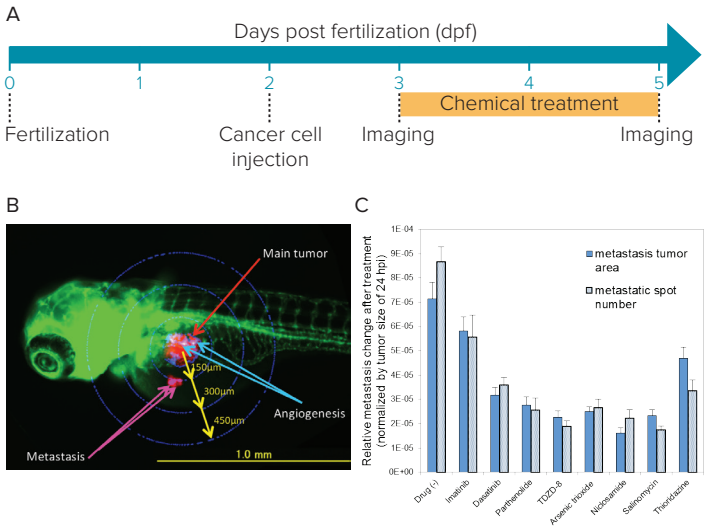


Figure 1. (A) Workflow for implanting human cancer cells in zebrafish. **(B)** Image of human leukemia stem cells labeled with Kusabira-orange (red) transplanted into Zebrafish embryo (green). **(C)** Measurement of tumor size and metastasis is graphed showing various levels of inhibition with different compounds.

Monitoring inhibition of angiogenesis

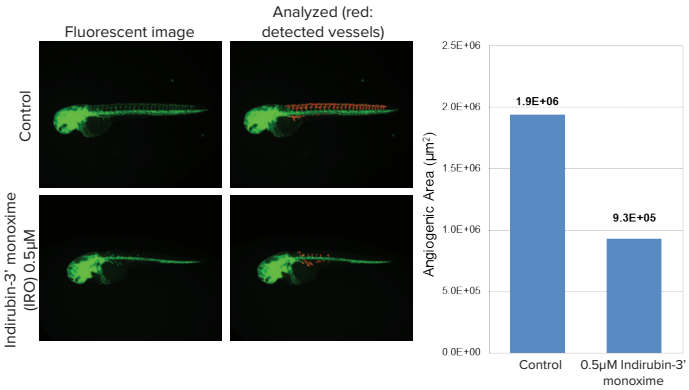


Figure 2. Zebrafish expressing GFP in vascular endothelial cells were exposed to compounds for 12-48 hours. The intersegmental vessels, thin dorsal and ventral vessels emerging from the main trunk, were measured using the Angiogenesis Tube Formation application module of MetaXpress Software. Inhibition of angiogenic vessel growth is evident upon compound treatment.

Cardiac function analysis

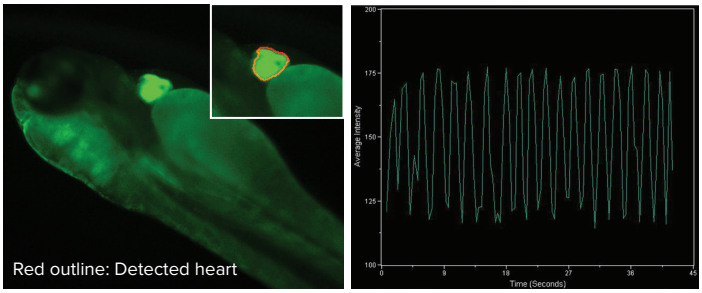


Figure 3. Cardiac function in transgenic zebrafish with cells expressing a GFP-tagged protein in the heart is measured by outlining the heart as a region-of-interest and using time-lapse imaging to measure the change in area or intensity over time as the heart beats. MetaXpress displays the intensity fluctuations over time.

Gene knockdown quantitation[2]

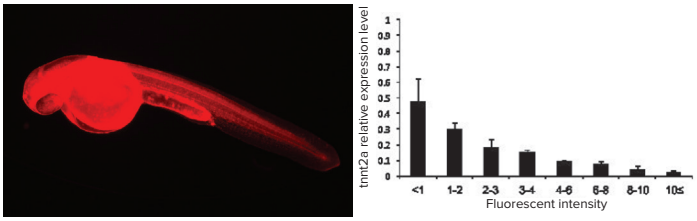


Figure 4. Lissamine fluorescence was used to correlate the amount of injected morpholino antisense oligonucleotide (MO) with the level of gene knockdown.

Measuring ototoxicity

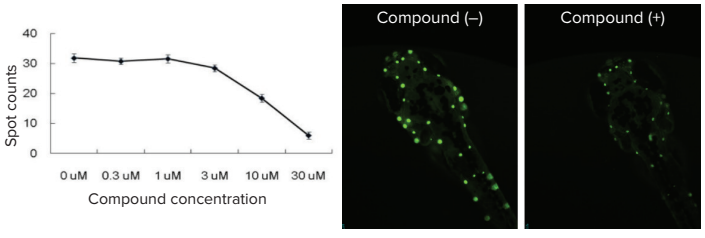


Figure 5. The destruction of zebrafish hair cells has been used an indicator of ototoxicity. After treating zebrafish with toxic compounds known to cause ototoxicity, fluorescently-labeled hair cells were measured using a spot-counting algorithm.

Identifying neurotoxicity

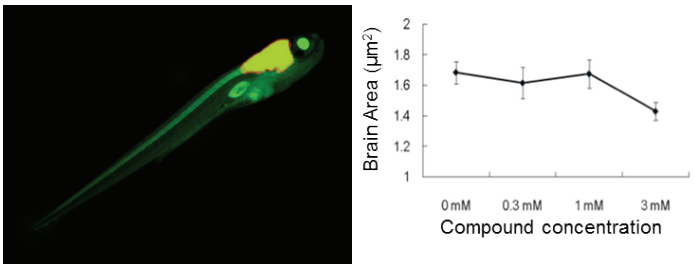
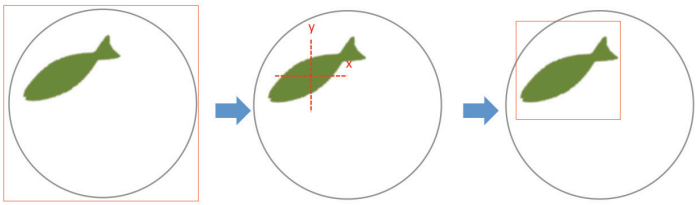


Figure 6. Zebrafish with fluorescently-labeled neurons (yellow in image) were treated with neurotoxic compounds and the brain area affected by the exposure was measured as an indicator of neurotoxicity. At a high dose of compound the measured fluorescent brain area was significantly smaller than in the untreated control embryos.

Targeted image acquisition of a specific area of the well or zebrafish

For objects that may not be in the same area of every well, targeted imaging can be used to pinpoint the object of interest using low magnification and then return to those coordinates to acquire a higher magnification image.

A



1. Image whole well with one snapshot using a 2x objective.
2. Detect the position of the sample (fish).
3. Move the stage so that the centroid X, Y is in the center of the sample.
4. Change the objective to a higher magnification and acquire one site with Z stacking and combine into a single best focus image.

B C

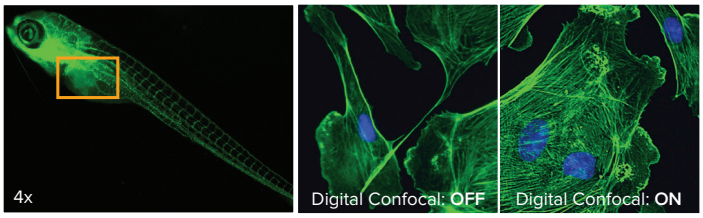


Figure 7. Entire fish imaged in one field-of-view. **(A)** To achieve a completely in-focus image, multiple z-planes were acquired and combined using a best-focus algorithm. **(B)** The area-of-interest (orange outline) was then re-acquired at high magnification. **(C)** The Digital Confocal feature is an on-the-fly deconvolution method that can be used to improve resolution and facilitate more accurate segmentation of subcellular features.

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Summary

Zebrafish embryos are a valuable vertebrate model for translational research. Using the ImageXpress High-Content Screening Systems to acquire images of zebrafish in focus from head to tail with a large field of view and high-speed z-stacking provides the ability to measure phenotypes characteristic of disease or toxicity. High-throughput *in vivo* imaging of zebrafish in combination with MetaXpress High-Content Analysis software empowers laboratories to increase their throughput dramatically and screen thousands of compounds in just a few days. Set up and run automated imaging screens to monitor inhibition of angiogenesis, quantitate gene knockdown, and measure ototoxicity and neurotoxicity with this end-to-end solution.

References

1. Zhang, B., et al., Quantitative phenotyping-based in vivo chemical screening in a zebrafish model of leukemia stem cell xenotransplantation, *PLoS One,* 2014 Jan 15; 9(1).
2. Umemoto, N., et al., Fluorescent-based methods for gene knockdown and functional cardiac imaging in zebrafish, *Mol Biotechnol,* 2013 Oct; 55(2): 131-42.
3. Kanungo, J., et al., In vivo imaging and quantitative analysis of changes in axon length using transgenic zebrafish embryos. *Neurotoxicol Teratol,* 2011 Nov-Dec; 33(6): 618-23.
4. Diekmann, H., et al., Characterization of optic nerve regeneration using transgenic zebrafish. *Front Cell Neurosci,* 2015 April 9; 9:118.
5. Huan, H., et al., High-throughput screening for bioactive molecules using primary cell culture of transgenic zebrafish embryos. *Cell Rep,* 2012 Sep 27; 2(3):695-704.