A plate of live fish!

The inaugural Tecan Detection Award, designed to celebrate our customers' innovation and ingenuity, received many strong entries. A very close contest eventually saw Dr Jeff Mumm from Georgia Health Sciences University (GHSU), US, awarded second place for his work on reporter-based drug screening using Tecan's Infinite® M1000 and live zebrafish!

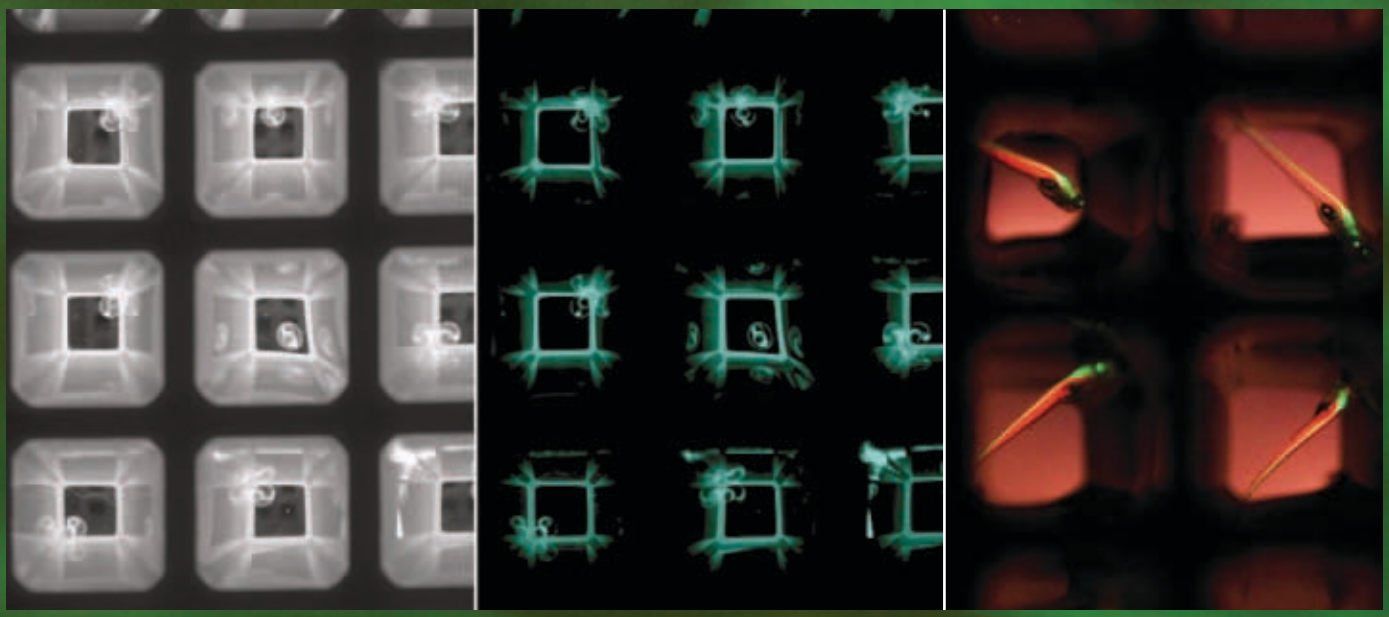
Performing *in vivo* HTS assays using the Infinite M1000: Dr Jeff Mumm (background), Steven Walker (at microscope) and Junko Ariga

Scientists in the Department of Cellular Biology and Anatomy at GHSU have developed a high throughput system for reporter-based drug screening assays in living zebrafish disease models. Dr Jeff Mumm, Assistant Professor at GHSU, explained: "Quantitative microplate reader systems have revolutionized the pace of drug discovery, enabling the development of reporter-based *in vitro* and *in silico* assays that allow high throughput screening (HTS). This strategy has resulted in dramatic increases in compound hit rates in recent years. However, biological validation – confirmation of the benefit of drug candidates in living disease models – has become a bottleneck in the drug discovery process due to a lack of HTS-compatible *in vivo* assay platforms. The zebrafish is a vertebrate model system that is amenable to both disease modeling and HTS methodologies, offering unique advantages to the drug screening process. Quantifying reporter levels in living zebrafish provides a versatile means of alleviating the biological validation bottleneck. Accordingly, we have developed a simple, rapid and cost-effective method for detecting fluorescent reporter changes in individual zebrafish disease models over time."

"My research focuses on regeneration, and our laboratory develops models for degenerative disease states. We are predominantly studying retinal neuron regeneration, investigating how various sub types of retinal neurons can be regenerated, and trying to identify the factors that allow regeneration to occur. However, the techniques used for our research are also being applied to other high profile diseases, such as diabetes and Parkinson's disease, looking at how to regenerate insulin-producing beta cells in the pancreas and dopaminergic neurons respectively. By building a system that enables us to study localized loss of specific cell types, we can develop more targeted therapeutic approaches that allow us to intervene in a more specific manner than globally-induced proliferation of stem cells."

"Using a nitroreductase-based system of targeted cellular ablation – a methodology borrowed from cancer therapeutics – an enzymatic activity of a type not normally present is introduced into the cell types that we want to kill. Initially the enzyme is completely innocuous, but, once a prodrug is introduced, the enzyme converts this into a toxin, enabling us to very specifically kill certain cells without affecting the surrounding environment. We have adapted this method to the zebrafish, with the intention of extending regenerative studies from the tissue level, where we're regenerating nerve, bone and muscle, to the level of individual cell types within tissues."

Dr Mumm continued: "To study regeneration you need a time-resolved assay that allows you to trace when the cells are present, when they have disappeared and when they have returned. We needed a microplate reader that was capable of accurately detecting fluorescent signals in zebrafish and chose Tecan's Infinite M1000. The Infinite M1000 provides us with z-focusing capabilities and a superb signal-to-noise ratio (>500:1), increasing our assay sensitivity by nearly an order of magnitude and allowing us to look at signals that have a much lower intensity. This is very important with, for example, beta cell regeneration, where cell numbers are quite low in larval stage fish. Although we could study older fish, the number of adult fish that can be screened is significantly lower than the number of juvenile or larval stage fish; we use 96-well plates for larval stage fish but would need to use six-well plates for adults. We have been able to increase the assay robustness by performing ratiometric



Individually arrayed, 1 day old transgenic fish embryos (left & center), and 5 day old transgenic fish larvae (right)

measurements of two different fluorophores, one linked to the targeted cell type and another to a neighboring control cell population – we're getting very, very good z-factor scores – and the Quad4 Monochromators™ technology is key to this."

"Using the Infinite M1000, we have been able to quantify the loss and regeneration of targeted cells in zebrafish disease models, as well as small molecule-induced changes in disease-linked molecular signaling pathways. Importantly, high signal-to-noise ratios allow us to monitor changes in individual fish, which accounts for wide reporter level variance across populations by normalizing signals to each individual's ground state, and enabling us to detect long-term changes over several days. We have optimized the system by characterizing autofluorescence issues and identifying the reporter variants that provide optimal signal-to-noise ratios, and are developing customized multi-well formats suited to HTS applications." Dr Mumm concluded: "The Infinite M1000 has allowed us to develop a simple, cost-effective, automated quantitative method which will benefit a wide variety of high throughput chemical and/or genetic screens, using fluorescent and/or luminescent reporter detection in live zebrafish."

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To find out more on Tecan's Infinite M1000, visit **www.tecan.com/InfiniteM1000**

To find out more about the Department of Cellular Biology, Georgia Health Sciences University, visit **www.georgiahealth.edu/som/cba**