

A Compact, Modular System for Miniaturized FLIPR[®] Assays

Calcium mobilization assays are a vital part of many laboratories' screening campaigns, from those underway in the largest pharmaceutical companies to those carried out in smaller biotech companies and academic institutes. The Molecular Devices FLIPR^{TETRA}[®] (Fluorometric Imaging Plate Reader) system provides a reliable and flexible screening solution for identifying compounds with activity against drug discovery targets, such as GPCRs and ion channels.

In recent years, such screening laboratories have come under increasing pressure to screen higher numbers of compounds more rapidly and at less cost. Labs are meeting these challenges in two ways: 1) by using automation technology to increase productivity and provide hours of unattended operation; and 2) by reducing assay volumes, and running assays in higher-density well plates to achieve significant cost savings.

This application note describes how the technology of **HighRes Biosolutions** and **Molecular Devices** (now part of MDS Analytical Technologies) can be combined to meet these challenges, delivering a flexible and robust solution for automated, miniaturised FLIPR screening.



HIGHRES BIOSOLUTIONS NANOCELL KEY FEATURES:

- HighRes MicroDocks and turntables provide flexibility and modularity over a very small footprint
- Industrial-grade robot allows for reliable picking and placing of labware
- Cellario scheduling software with full error recovery and over 80 device drivers
- Can be easily expanded at any time by linking a second NanoCell to an existing system
- Operates on a standard system setup for extremely fast project turnaround
- Features high quality construction with Trespa surfaces
- Can be fitted with a range of enclosures and safety options



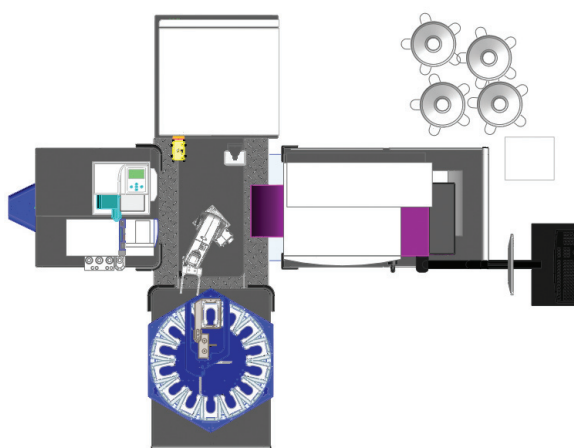
MOLECULAR DEVICES FLIPR^{TETRA} SYSTEM KEY FEATURES:

- Provides reproducible and robust data for Calcium screens as well as other assays
- New aequorin camera option allows for both fluorescent and luminescent detection
- Easy to use software supports a wide variety of applications and skill sets
- Configuration flexibility facilitates system expansion
 - *User exchangeable fluidics and optics*
 - *Field upgradable camera, automation, and cell suspension option*
- Smooth and seamless integration with robotic systems

An Example NanoCell for FLIPR^{TETRA} Screening

FLIPR^{TETRA} NanoCell Inventory List:

- 1 x Denso Robot
- 1 x Random Access Incubator
- 1 x HighRes MicroServe
- 1 x Molecular Devices FLIPR^{TETRA}
- 1 x Molecular Devices Aquamax DW4
- 1 x Single-Reagent Non-Contact dispenser
- 1 x Automated Plate Centrifuge
- 1 x Lid Hotel
- 1 x Barcode Reader



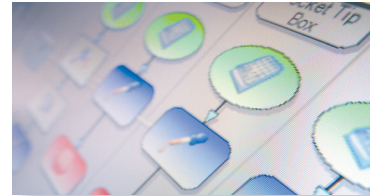
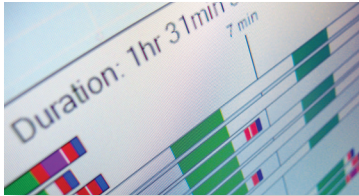
Example Workflow for FLIPR Agonist Assay:

- **Molecular Devices Aquamax DW4** removes growth medium from adherent cells in assay plate, and then dispenses FLIPR dye to all wells
- **Automated plate centrifuge** spins assay plates to remove air bubbles and ensure efficient mixing
- **Environmentally controlled incubator** stores assay plate at 37°C, 5% CO₂ for one hour
- **HighRes MicroServe™** feeds intermediate compound plates and tip boxes in and out of the system
- **FLIPR^{TETRA}** transfers compounds to assay plate with simultaneous real-time kinetic imaging. The effect of the potential agonist compounds on the cells is measured and data transmitted to a predefined destination.



System Options:

- This exact system configuration can also be used to prepare adherent cells in assay plates for the next batch of FLIPR screening. The non-contact reagent dispenser can add cells in growth medium to assay plates that can then be stored overnight at 37°C, 5% CO₂ in the incubator. The cell plates would be ready for FLIPR processing the next morning.
- This system is compatible with labs that are supplied with "assay-ready" acoustic dispensed plates. The plates (containing only nanoliters of compound) can either be manually loaded into the MicroServe – or for labs with other HighRes systems, the empty MicroServe can be undocked and wheeled to a separate compound delivery system, filled with assay-ready plates, and then wheeled back to the NanoCell for FLIPR screening.



Cellario for FLIPR:

Cellario™ is HighRes' control software package for NanoCell. It uses a combination of resource availability and events to dynamically schedule automated runs. The four main areas of Cellario allow the operator to design protocols, create labware orders against those protocols, perform detailed pre-run simulations and then run their chosen protocol live on the robot system.

SOFTWARE FEATURES FOR FLIPR:

Simple Protocol Design

- Benefit from a simple drag and drop approach to protocol design
- Build flow diagrams, including all of the required process steps and device operations
- Use multiple threads (e.g. cell plate and compound plate) to achieve efficient use of the NanoCell

On- and off-line use of FLIPR

- Schedule fully automated runs, including FLIPR liquid transfer and read operations
- Use HighRes' custom device driver to control FLIPR in off-line mode also. This can be very useful for instrument maintenance and assay development.

Thread Ratios

- Assign ratios to different labware threads to account for varying usage rates (e.g. one FLIPR tip box might get used for every five cell plates)
- Receive on-screen prompts while the order is being created

System Advantages:

Compatible - Since NanoCells are compatible with larger HighRes systems, simply use HighRes' modular docking technology to transfer materials and equipment between a NanoCell and a separate HighRes system – smoothly, quickly, and intact.

Flexible - Use the docking stations to reconfigure the system with other types of devices best suited to the experiment being conducted (e.g. Molecular Devices' IsoCyte™ high content imager).

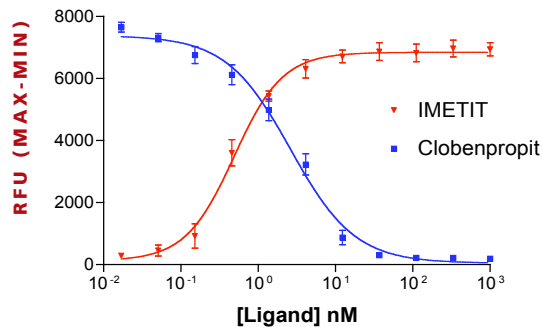
Reliable – and yet Compact – Rely on industry-grade lab automation, over a very small footprint.

Efficient – To get the most out of the system, use Cellario to schedule additional protocols (e.g. cell plating) that can run during overnight periods.

Expandable – As your screening requirements evolve, link one NanoCell to a second one at a later date to expand your system. Relying on Cellario, used in conjunction with plate exchange stations, pass plates from one NanoCell to the other as part of one integrated process.

FLIPR^{TETRA} System Histamine H3 Receptor Agonist and Antagonist Assays

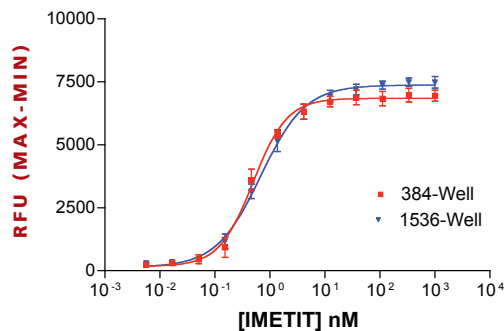
Figure 1. Fluorescent calcium flux assay demonstrating Histamine H3 receptor agonism by IMETIT in CHO cells transfected with Histamine H3 receptor. Clobenpropit antagonism of IMETIT EC80 response is also shown.



Agonist	EC ₅₀	Z at EC ₈₀
IMETIT	0.48 nM	0.89
Antagonist	IC ₅₀	Z at IC ₅₀
Clobenpropit	2.6 nM	0.71

FLIPR^{TETRA} System 384-well to 1536-well agonist assay comparison

Figure 2. Agonism response of Histamine on CHO H3 cells using FLIPR Calcium 4 Kit compared on FLIPR^{TETRA} System in 384-well format and 1536-well format using the 384 pipettor head and 1536 pipettor head respectively.



	EC ₅₀ (nM)	Z at EC ₈₀	S:B
384-Well	0.51	0.8	3.8:1
1536-Well	0.68	0.72	3.9:1

TETRAcyler exchanges microplates between the FLIPR's internal plate stage and the HighRes robot



Miniaturizing a 384-well assay to 1536-well format on the FLIPR^{TETRA} System

The FLIPR^{TETRA} offers unmatched flexibility to easily accommodate changing laboratory environments. The system features:

- User-configurable excitation and emission optics to provide the flexibility to handle a wide range of assays
- User-exchangeable pipette heads and pin tools to accommodate different throughput requirements
- Field upgradable fluorescence/luminescence camera for increased assay flexibility
- Field upgradable cell suspension option for assay format versatility
- Easy integration with external automation to significantly improve research productivity and effectiveness

Over the years, FLIPR systems have become the industry standard for calcium mobilization assays (Figure 1). By programming the FLIPR 384 pipettor head to pipette into quadrants of a 1536-well plate or directly using the 1536 pipettor head with proprietary elastomeric technology, you can increase your screening throughput while benefiting from assay miniaturization. Figure 2 demonstrates that equivalent performance can be achieved when converting a 384-well assay to 1536-well format.

Whether performing assay optimization off-line or high-throughput screening on line, the FLIPR^{TETRA} instrument integrated on the NanoCell system provides a reliable, flexible combination designed to meet your most critical laboratory requirements.

The Building Blocks for Discovery.

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