

## Analysis of Transiently Transfected Cell Populations on the EIDAQ™ 100 High Throughput Microscopy (HTM) System

A novel high throughput microscopy transient transfection assay (HTM-TTA) is described using the EIDAQ™ 100 HTM system from Q3DM. Dynamic data-driven mining creates defined cell-by-cell subpopulations without physical sorting to facilitate rapid design and testing of new subcellular assays. Here, the impact of dynamic data mining was established through development of a ligand-dependent androgen receptor (AR) trafficking assay. As an example, validated results of nuclear-cytoplasmic translocation of transiently transfected populations using the EIDAQ™ 100 & CytoShop™ Cell Analysis Software are presented with representative fluorescent images and dose response curves.

### Introduction

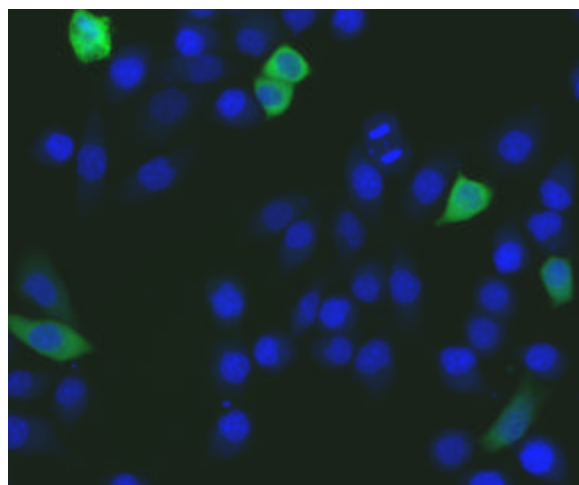
Dynamic data mining combined with HTM improves productivity of assay design and screening. Automated high throughput/high-resolution microscopy creates a potentially overwhelming number of cellular and subcellular measurements. Cellular heterogeneity adds complexity, can hinder screen significance and complicate assay design. A large suite of cell-by-cell subcellular parameters used for combinatorial gating enables rapid culling together of the best fluorescence, morphometry, translocation and pattern measurements for a new assay.

Here, transiently transfected GFP-AR plasmids were introduced into HeLa cells and the AR subcellular distribution was examined for nuclear response to the antagonist OH-Flutamide. The heterogeneous and limited transfection in the expression levels of the cell population hindered quantification of an antagonist response. While no significant responses could be determined in examination of the entire cell population, a well-defined subpopulation of low level expressing transiently transfected cells responded consistently to ligand concentrations.

Cell cycle phase and metabolic state dependent expression are additional examples where subpopulation assays may enable more productive screening. Thus, while image-based subcellular imaging can at first appear to increase assay complexity, its combination with powerful dynamic data mining tools enables rapid development of new subcellular assays.

### Experimental Methods

Experiments were performed using HeLa cells transiently transfected with GFP-AR expression plasmids. Cells were then exposed for two hours to an 11-point range in ligand (OH-Flutamide) concentrations followed by addition of Hoechst solution.



**Figure 1:** Population of HeLa cells transiently transfected with AR-GFP plasmid. Transfection manifests itself in a wide range of AR expression. *Biology courtesy of Dr. Michael A. Mancini and Baylor College of Medicine.*

