

Assay Performance with IN Cell Analyzer 6000

Robert Graves*, Erwen Mei, Pavel Fomitchov, Dan Manning, Suzanne Hancock¹, John Picot, Jun Li, and Mark Campion

GE Healthcare, 800 Centennial Ave, Piscataway, NJ 08855; GE Healthcare, ¹Amersham Place, Little Chalfont, Buckinghamshire, England, UK, *e-mail: Robert.Graves@ge.com

Introduction

As High Content Analysis (HCA) gains prevalence in the research and drug discovery environment, researchers continue to ask more challenging questions from their assays. Imaging instrumentation and analysis software must keep pace with the evolving assay demands. The IN Cell Analyzer 6000 is the latest product in a family of HCA instruments designed to meet these challenges. It is a laser-based line scanning confocal imager that uses a novel sCMOS camera detection technology¹. Unlike conventional point scanning confocal microscopy, the IN Cell Analyzer 6000 images the sample line-by-line, and instead of a physical barrier to block out-of-focus light, it uses the electronic rolling shutter (RS) of an sCMOS camera as a virtual confocal slit². Since the RS width can be adjusted on-the-fly the user can control the degree of confocality, and is even able to simultaneously capture images from the same wavelength with different confocality settings.

Benefits of line confocal microscopy include removal of unwanted fluorescent background without significant loss of sample signal, and efficient Z-sectioning capability. This has been examined by imaging fluorescently labeled cells growing both as monolayer and 3D cultures. Throughout this study, results are compared to images of the same sample plates acquired using conventional lamp-based widefield microscopy (Nikon TE2000) and laser point scanning, using a Nipkow spinning disk installed on a modified Nikon TE2000.



Figure 1: IN Cell Analyzer 6000. Instrument is a bench-top design incorporating a similar footprint to IN Cell Analyzer 2000.

Imaging of Cells Grown as 3D Cultures

Imaging of cells growing in 3 dimensions provides special challenges for microscopy. To test this, CHO cells were cultured on plastic Cytodex beads of approximately 150micron diameter, stained with a nuclear dye and imaged by 3D sectioning.

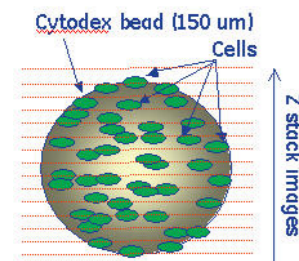


Figure 2: Schematic showing CHO cells growing on Cytodex beads. For 3D imaging, Z sections thru sample beads with 10micron steps were acquired. IN Cell Analyzer 6000 confocal and widefield imaging modes were used in the same run protocol.

The 3D projections of the IN Cell Investigator analysis results show reduced oversampling of nuclei and less distortion of bead morphology comparing analysis results from the confocal image stack (Fig.3G) versus the widefield image stack (Fig.3H).

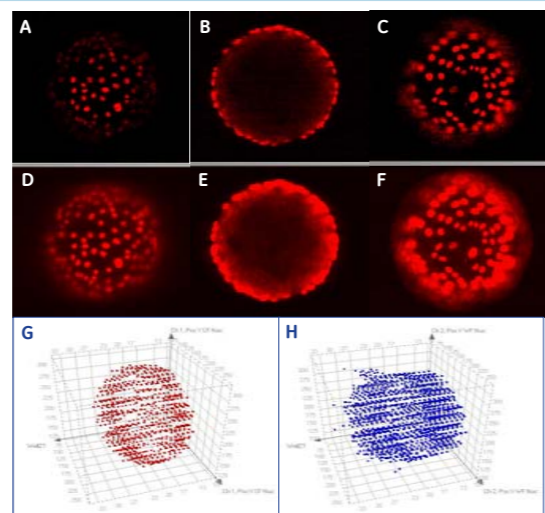


Figure 3: DAPI nuclear images of CHO cells on Cytodex beads. Sample 40x 0.6NA objective images of bottom (A), middle (B) and top (C) sections from 39 focal planes acquired with 10micron Z steps using IN Cell Analyzer 6000 in confocal mode. Images D-F show the same focal planes acquired in widefield mode in the same protocol. The images were analyzed for nuclear position and area using IN Cell Investigator for the confocal stack (G) and the widefield stack (H) and results are shown as 3D Spotfire plots of nuclear X, Y and Z position.

Evaluation of Instrument Sensitivity

HeLa cells grown in 96-well plates were labeled with a dilution series of CellTracker™ Green and 2µM Hoechst 33258 (both from Invitrogen Molecular Probes), formalin-fixed and imaged with a 10x 0.45NA objective as indicated.

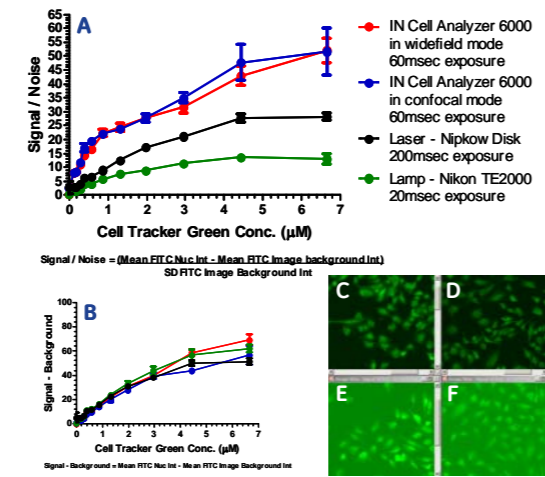


Figure 4: Sensitivity evaluation with IN Cell Analyzer 6000. IN Cell Investigator analysis of Signal / Noise (S/N) for images of cells labeled with Cell Tracker™ Green, and taken with various instruments (A). The S/N data was calculated from image stacks acquired giving equivalent cell signals by 12bit image analysis (B). Example images from the highest concentration of the label shown for IN Cell Analyzer 6000 in widefield mode (C), the same in confocal mode (D), laser Nipkow disk (E) and Nikon TE2000 lamp (F). All data +/-SEM (n=8).

The sensitivity study shows favourable Signal / Noise for the IN Cell Analyzer 6000 versus other instruments when imaging conditions giving comparable cell signals are used.

Background Removal (FYVE Assay)

The FYVE-finger protein domain binds to the inositol head group of phosphatidylinositol 3-phosphate (PI(3)P), a mediator of vesicular transport. We have used U2-OS cells expressing an eGFP-FYVE domains from human hepatocyte growth factor regulated tyrosine kinase substrate³. This construct localizes to PI(3)P on early endosome membranes in a PI3-kinase-dependent fashion and inhibitors of PI3-kinase such as Wortmanin induce a decrease in PI(3)P levels observed in endosomal membranes. The assay is analyzed for eGFP-FYVE domain vesicle (granule) content per cell using IN Cell Investigator analysis software.

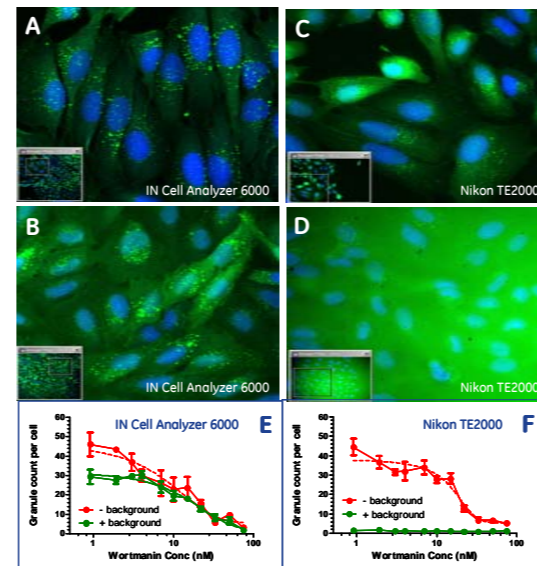


Figure 5: eGFP-FYVE domain assay imaged with introduced background. 20x 0.45NA objective images from IN Cell Analyzer 6000 in confocal mode for wells containing PBS (A) or PBS with 0.5µM fluorescein (B). The lamp-based Nikon TE2000 was used to image equivalent wells containing PBS (C) or PBS with 0.5µM fluorescein (D). The plots show a titration of the PI3-kinase inhibitor Wortmanin in a 96-well plate with wells imaged in PBS (red) or PBS with 0.5µM fluorescein (green) on IN Cell Analyzer 6000 in confocal mode (E) or on Nikon TE2000 (F). Results of average eGFP-FYVE granule count per cell are shown as means of 4 wells +/-SEM.

The effective removal of background fluorescence using the IN Cell Analyzer 6000 in confocal mode resulted in maintenance of the granule count per cell from the image analysis (Fig.5F). This was further examined using the same assay in a mock chemical screen. A 96-well plate was set up in which wells were spiked with a chemical inhibitor (Wortmanin) predicted to result in reduced granule count per cell, or wells spiked with fluorescein which could potentially show as false positive "hits" thru interference with the image analysis of granule counts in the cells.

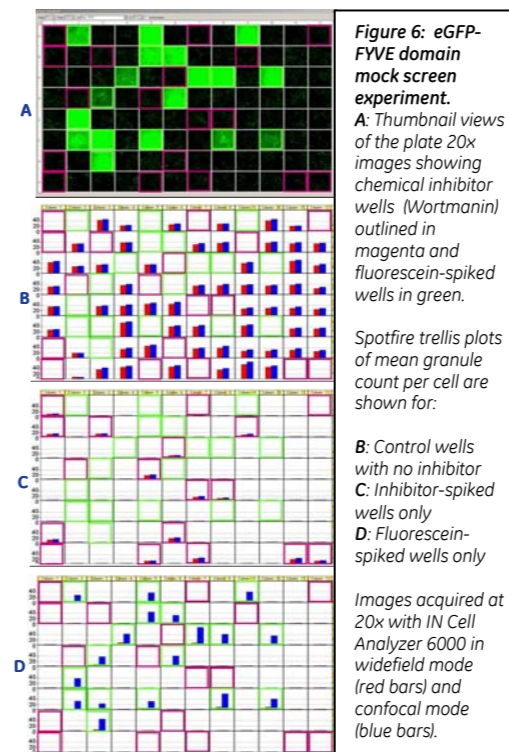


Figure 6: eGFP-FYVE domain mock screen experiment.

A: Thumbnail views of the plate 20x images showing chemical inhibitor wells (Wortmanin) outlined in magenta and fluorescein-spiked wells in green.

Spotfire trellis plots of mean granule count per cell are shown for:

B: Control wells with no inhibitor
C: Inhibitor-spiked wells only
D: Fluorescein-spiked wells only

Images acquired at 20x with IN Cell Analyzer 6000 in widefield mode (red bars) and confocal mode (blue bars).

The results in Fig.6 show the use of the IN Cell Analyzer 6000 in confocal mode "rescues" false positive hits from the effect of introduced background fluorescence.

Whole-well Imaging Applications

A feature of the IN Cell Analyzer 6000 is the large field-of-view (2.32mm²) afforded by the use of the sCMOS camera allowing whole-well imaging with 2x and 4x objectives.

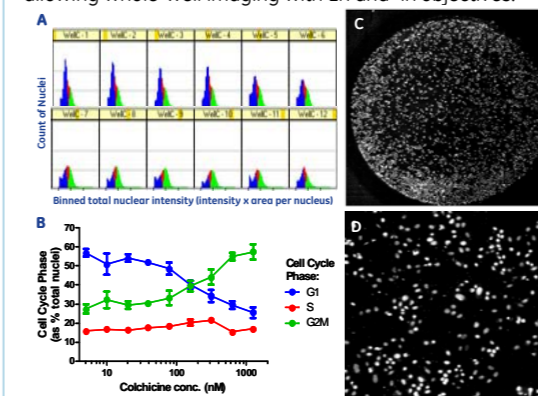


Figure 7: Cell cycle analysis of HeLa cells stained with Hoechst 33258. DNA content histograms (A) from IN Cell Investigator analysis were used to calculate cell cycle phases across a titration of the mitotic inhibitor Colchicine (B). All images acquired with a 2x 0.1NA objective allowing single-field whole-well capture in a 96-well plate (C) at single cell resolution (D). The image in (D) is a digital zoom of the 2x whole-well image. All results means of 8 wells +/-SEM.

With 384-well plates, single-field whole-well image capture is possible with the 4x 0.2NA objective. We have used this objective in a study of human umbilical vein endothelial cell (HUVEC) migration using Oris™ Pro 384-well Cell Migration Assay Plates (collagen I coated) from Platypus Technologies.

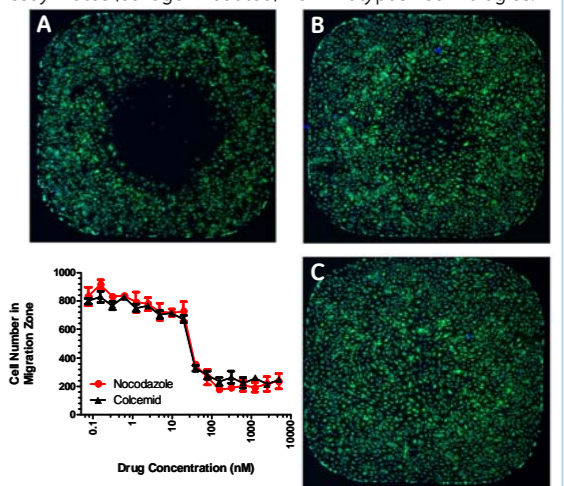


Figure 8: HUVEC migration in Oris™ Pro 384-well cell migration plates. Sample images acquired with a 4x 0.2NA objective are shown for wells treated for 24 hours with high (A) medium (B) and low (C) concentrations of the mitosis inhibitor Colcemid. Cells are stained with Hoechst 33258 and Cell Tracker™ Green. The plot shows mean cell count in the migration zone from 4 replicate wells per dose (+/-SEM).

Conclusions

- ❖ A unique laser line confocal imaging technology is demonstrated which provides user-adjustable confocality via protocol-driven software inputs.
- ❖ A variety of applications have been demonstrated using a wide range of objectives (2x, 4x, 10x, 20x and 40x) and imaging modes (confocal and widefield).
- ❖ The sCMOS camera large FOV enables single-field whole-well imaging with a 2x objective in 96-well plates and the same with a 4x objective in 384-well plates.
- ❖ Experimental results for rejection of fluorescent background demonstrate the utility of this technology for various High-Content Analysis applications including chemical screening studies.
- ❖ The IN Cell Analyzer 6000 sensitivity compares favourably with lamp-based widefield microscopy and laser-based spinning disk (NipKow) technology.
- ❖ 3D sectioning is demonstrated with a 40x objective for cells grown on 3D spheres (Cytodex), and confocal imaging provides for more accurate analysis of the 3D stacks compared to widefield imaging.

References

1. US patent 7335898 - P. Donders, C. Zarate, P.A. Fomitchov, "Method and apparatus for fluorescent confocal microscopy", General Electric, 02/26/2008
2. "Confocal Scanning Optical Microscopy and Related Systems" by Gordon S. Kino, Timothy R. Carle, Academic Press.
3. Quantitating translocation of an endosomal PI 3-kinase sensor using IN Cell Analyzer 2000, GE Healthcare Application Note 28-9538-33 AA