Inaugural winner of the GE Healthcare High-Content Analysis (HCA) Award

The annual GE Healthcare HCA Award, supported by BioTechniques, recognizes the outstanding contribution of HCA to scientific understanding and celebrates its positive impact on data quality and quantity, and in transforming the efficiency of research and discovery.

The winner of the first HCA Award is Dr. Michael Freeley from the Institute of Molecular Medicine, Trinity College, Dublin. Dr. Freeley was selected for his work with the IN Cell Analyzer 1000 in measuring a wide range of parameters related to cell shape and identifying which ones provide the most valuable information when T lymphocytes are exposed to a stimulus that triggers them to migrate. His work also demonstrated that using multiple parameters increases the sensitivity of hit selection, which helps in reducing the number of false positives. Dr. Freeley and his co-researchers are now using this approach to investigate the signaling pathways and proteins that regulate T lymphocyte migration. A better understanding of this movement could ultimately lead to treatments that block uncontrolled migration of T lymphocytes in autoimmune diseases such as multiple sclerosis, Crohn’s disease, and rheumatoid arthritis.

“We are delighted to receive the first GE Healthcare HCA Award”, said Dr. Freeley. “Quite simply, the scale of work that we performed could not have been done by manual means, and we estimate that the HCA approach has increased our throughput at least 20-fold. Capturing the same number of fields manually would have taken in the region of 400 h, with the IN Cell Analyzer image analysis software saving us a comparable amount of time”.

“The excellent quality of the entries certainly made judging difficult”, said Dr. Joe Trask, Head of Cellular Imaging Core at the Hamner Institutes for Health Services, and a member of the judging panel. “The winning entry showcased the immensely positive impact of HCA on research today and demonstrated how the IN Cell Analyzer’s technology saved invaluable time to produce high quality scientific data that, crucially, was also publishable”. Dr. Nick Thomas, Principal Scientist, Cell Technologies, GE Healthcare added: “This multiparameter approach has great potential to increase insights in a wide range of chemical and RNA inhibition screens”.

Entries were welcomed from scientists using IN Cell Analyzer instruments and reviewed by an expert scientific panel comprising Dr. Joe Trask, Head of Cellular Imaging Core at the Hamner Institutes for Health Services, Dr. Nick Thomas, Principal Scientist, Cell Technologies, GE Healthcare, and Dr. Patrick Lo, Associate Editor, BioTechniques. The HCA Award will be presented to Dr. Michael Freeley and his co-author Dr. Dara Dunican during an expenses-paid trip to the 50th American Society of Cell Biology Annual Meeting in Philadelphia.

An article based on Dr. Freeley’s winning entry is on pages 4–5 while a full report on his group’s research has been published in *J. Biomol. Screening*, 15(5), 541–555 (2010), which can be downloaded at http://jbx.sagepub.com/content/15/5/541.full.
High-content analysis using IN Cell Analyzer permits dissection of diverse signaling pathways for T lymphocyte polarization

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IN Cell Analyzer was used in an image based high-content analysis (HCA) approach to elucidate the signaling pathways that regulate integrin and adhesion receptor-mediated changes in T lymphocyte morphology. Multiparametric analysis of lymphocyte morphology that was based on intracellular staining of both the F-actin and α-tubulin cytoskeleton resulted in improved discrimination of morphological behavior compared to F-actin staining alone. Morphological and fluorescence intensity/distribution profiling of pharmacologically treated lymphocytes stimulated with integrin (LFA-1) and adhesion receptors (CD44) also revealed notable differences in their sensitivity to inhibitors. The procedure described here may be used in HCA strategies such as RNAi screening assays to elucidate the signaling pathways and molecules that regulate integrin/adhesion receptor-mediated T lymphocyte polarization.

Introduction
RNA interfering (RNAi) screening assays offer the potential to elucidate the signaling pathways that regulate integrin and adhesion receptor-mediated changes in T lymphocyte polarization. Of crucial importance, however, is the definition of key sets of parameters that will provide accurate, quantitative, and nonredundant information to flag relevant hits in such assays. In this study, we used the IN Cell Analyzer 1000 and associated software in combination with a panel of 24 pharmacological inhibitors (with known or suspected effects on the cytoskeleton), to define key sets of parameters that identify and quantitate the morphological changes that occur when T lymphocytes are stimulated through the integrin LFA-1.

Greater productivity with multiparametric quantitative analysis
Prior to using the IN Cell Analyzer 1000, our image capture and analysis procedure was extremely slow and laborious, requiring a considerable amount of time to manually capture, process, and quantitate each set of images. Our study, using the IN Cell Analyzer 1000 in combination with a panel of pharmacological inhibitors, allowed us to (a) rapidly capture high-resolution quality images in an automated fashion in a cell population-based manner, and (b) perform a multiparameter-based analysis of the captured image data set to define key morphological and fluorescence intensity parameters that provide rapid, accurate, and quantitative data. This enabled the effects of these inhibitors on cytoskeletal-mediated lymphocyte polarity to be evaluated. A study of this size could not have been performed manually as it would have been unfeasible in terms of the amount of time (and therefore cost) required.

Fig 1. Multiparametric morphological analysis of LFA-1-mediated T lymphocyte polarization using IN Cell Analyzer 1000. White arrows indicate F-actin-rich lamellipodia.
**Deeper insights for more informed decisions**

We determined that multiparametric analysis of lymphocyte morphology based on intracellular staining of both the F-actin and α-tubulin cytoskeleton resulted in improved discrimination of morphological behavior compared to F-actin staining alone (Fig 1). Multiparametric analysis therefore increases the sensitivity and confidence in detecting effects of different treatments on lymphocyte morphology, which may have otherwise been missed using single parametric analysis (Fig 2).

![Fig 2. Using multiple parameters increases the sensitivity of hit identification and morphology discrimination.](image)

We also defined a key number of fluorescence intensity/distribution parameters that provided valuable information on the intracellular distribution of the F-actin and α-tubulin cytoskeleton in T lymphocytes. When combined with morphological parameters, these fluorescence intensity distribution parameters may permit dissection of cytoskeletal components that are perturbed as a consequence of inhibition of signaling pathways. We clustered the morphological and fluorescence intensity profiles based on similarity and identified five major morphological and fluorescence intensity subtypes for cells stimulated with anti-LFA-1. Such clustering allows one to infer relationships between different treatments on the T lymphocyte polarization process. Our clustering analysis highlighted similarities and yet notable differences between lymphocytes in their sensitivity to pharmacological inhibitors following stimulation with anti-LFA-1 and anti-CD44, which implies the signaling pathways that are activated downstream of these receptors are different.

**Conclusions**

This approach has great potential for drug or RNAi screens that involve complicated morphology readouts. To date, most large-scale screens tend to use single readouts because of the difficulty of combining them in an easily interpretable way. Our research demonstrates that the combination of a relatively small number of IN Cell Analyzer 1000 morphology and fluorescence intensity parameters and their visualization in heat maps (not shown) can overcome this problem. By using a parameter profile that corresponds to easily identifiable cell or cytoskeletal morphologies, the heat maps can be quickly scanned for interesting hits. The heat maps can be colored to filter out values that do not reach a cutoff threshold, thus speeding the hit identification process. The parameters we used allow a high level of discrimination between morphologies, and this permits hits to be grouped together on the basis of similar effects, giving valuable information on potentially common pathway targets. The data (not shown) demonstrate that using multiple parameters increases the sensitivity of hit selection and allows a statistically more stringent cutoff to be used, in turn potentially helping to filter out false positives. The identification of key parameters that we have characterized will have important use in wider based screening strategies such as larger pharmacological libraries or whole-genome RNAi screening studies and therefore may have significant implications for understanding the mechanisms underlying cell migration in inflammatory processes.

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A full report on this research has been published in *J. Biomol. Screening* 15(5), 541-555 (2010) and can be downloaded at [http://jbx.sagepub.com/content/15/5/541.full](http://jbx.sagepub.com/content/15/5/541.full)

More information on the IN Cell Analyzer platform, applications, and product support is found at [www.gelifesciences.com/incell](http://www.gelifesciences.com/incell)