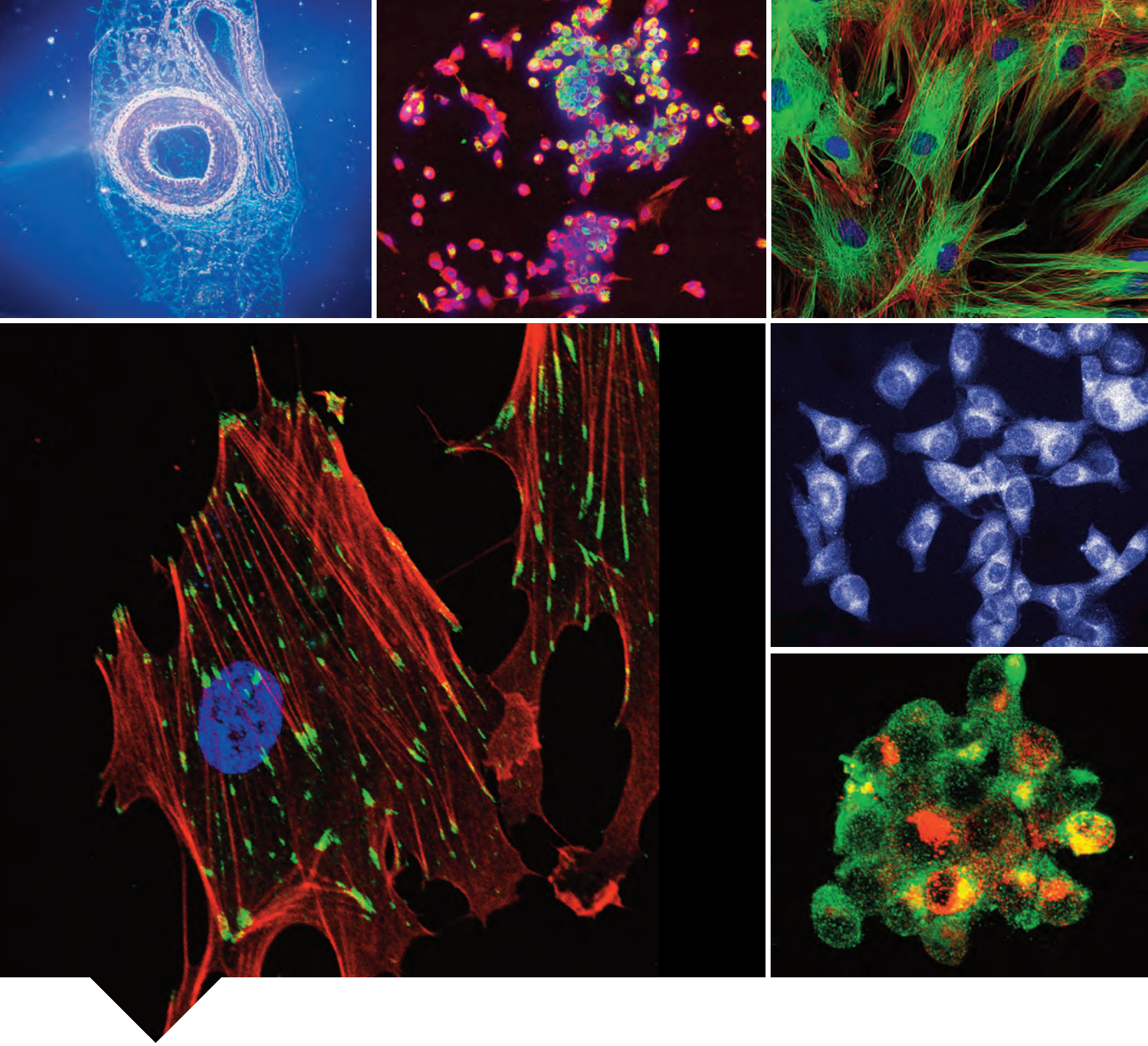

HIGH CONTENT SCREENING

UCD DUBLIN

High content screening:

A technology impacting both fundamental and translational science in the twenty-first century

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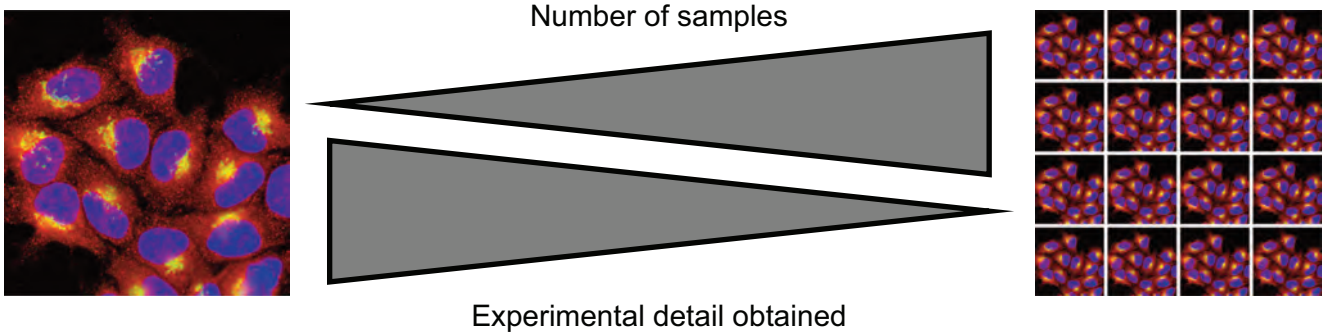
High content screening (HCS) is a remarkable technology that allows fully automated cellular imaging on a truly high-throughput scale. In the life and biomedical sciences it is now widely employed to solve questions in fundamental science, providing the research community with novel views on how cells function. In parallel, HCS is also playing an increasing role in the design and development of new therapeutics, providing key information to inform drug design and delivery. This article provides an introduction to this technology, and perspective on its future direction.

The technology of high content screening

The basic unit of all organisms is the cell. Ultimately it is cells that are responsible for all of our daily activities – walking, talking, visualising the world around us, intelligent thought. Therefore, gaining a greater understanding the cell and its regulation, and how each cell interacts with its neighbours in tissues and organs remains a fundamental goal for life scientists in the wider context of health and disease. While physiologists tend to consider this at the organism or tissue level, biochemists and cell biologists generally prefer to work with individual cells, on the premise that if they can understand the molecular complexity of a single cell growing in culture in the laboratory, then the information obtained can potentially be extrapolated to different cell types and perhaps the entire organism.

A landmark event for life scientists at the turn of the century was the sequencing of the human genome. Having access to the detail of the code of life provides us with the 'raw information' to understand how cells function. The 22,000 human genes encode proteins, each of which has a defined cellular function and a discrete network in which it operates. The challenge of course is how to decipher this network, and assign each protein to particular functions in the cell. Furthermore, there is also the need to be able to identify those proteins that make us susceptible to infection, or that act as drivers for specific diseases. For more than 350 years, microscopy has arguably been the primary tool for biologists to study cells and attempt to reveal how they function, although its application to study individual molecules was limited. In the latter half of the twentieth century a number of key limitations were overcome, principally through technologies allowing the introduction of fluorescent probes into cells, in turn providing a renaissance to microscopy. For the first time it became possible to rapidly and specifically highlight particular proteins, and visualise dynamic processes in live cells. Effectively, fluorescence

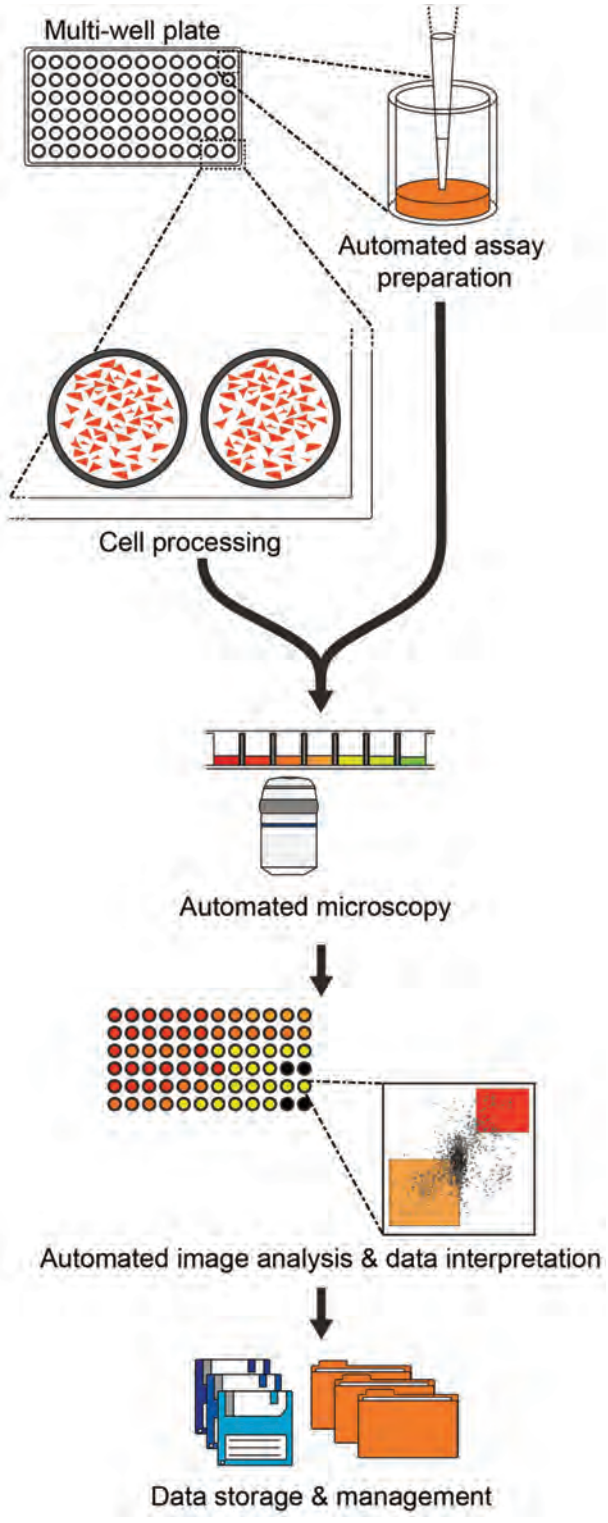


High content screening microscopy provides the capacity to image a large number of samples, and preserve the high level of detail within each

High content screening bridges the gap between image quality and quantity

microscopy became more sophisticated, and in parallel increasingly quantitative. Fluorescence imaging of cells has revealed the intricate detail of their architecture and the spatial and temporal arrangements of proteins encoded by the genome. However, microscopy was then faced with a new challenge, namely how to image on a truly high-throughput scale, yet still preserving image quality and the depth of information contained within. The automated microscopy that evolved from this conundrum is termed high content screening microscopy and analysis – HCS/HCA. This is a fusion technology, combining laboratory automation with sophisticated software routines capable of analysing images of millions of individual cells and extracting used-defined quantitative information – 'content' – for each cell. The real power of this approach is that it is now no longer essential to manually examine and curate each microscopy image, which in turn has the beneficial consequence of removing human bias.

HCS can in principle be applied to study any cell-based biological question. At its heart is typically an assay in multi-well plate format, designed with one or several fluorescent reporters that may change their intensity or distribution in the cell during the course of the experiment. Ultimately any changes must be detectable and be able to be quantified in a reliable manner. Another key facet of the technology is that as much of the experimental pipeline as possible should be automated. Laboratory automation has evolved massively in the last ten years, with small-scale and large-scale solutions available to suit both individual research laboratory groups and multi-national pharmaceutical companies.

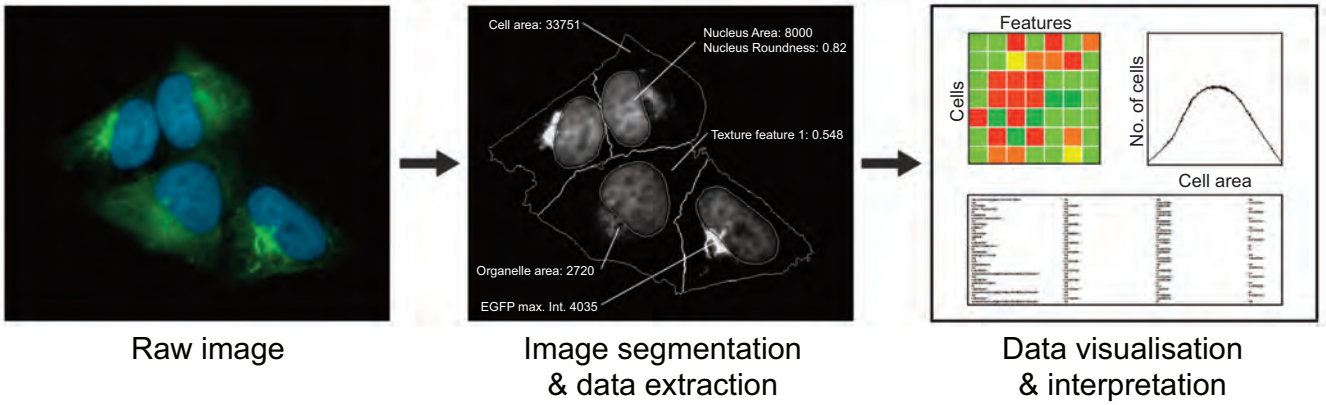


The high content screening pipeline

Automation can be at the level of liquid handling, cell preparation, reagent dispensing, and of course the automated imaging and analysis; but in each case strict quality control is required to ensure that each sample of series assays is processed in a highly parallel manner. HCS assays deliver image data on a massive scale, with individual screening campaigns producing data that run into the tens of terabytes. At the core of the technology is the analysis component – not only does every image need to be analysed, but indeed every cell in every image needs individual quantification, thereby preserving the 'content'. Early HCS experiments tended to use low power microscope objectives, as typically the readouts from the assays were relatively simple, for example total fluorescence intensity of a marker in question, and as such the spatial detail was less important. However more recently HCS experiments have become increasingly sophisticated, with advanced software routines measuring intricate detail of fluorescence distribution in cells and indeed in individual cellular structures. Analysis routines can be combined with machine learning to automatically classify cell phenotypes into defined populations. Furthermore, when the analysis software is running in real-time alongside the image acquisition it is also possible for the automated microscope to return to a particular well position to acquire further high-resolution images of particular phenotypes identified in the cells. HCS technology and the image analysis possibilities continue to evolve at a rapid rate, and in a way are only limited by the creativity of assay design.

Applying HCS in fundamental scientific research

It is clear that HCS can be applied to address a huge range of fundamental questions about cell



High content screening facilitates automated image analysis and easy data visualisation

behaviour and function. As mentioned above, mammalian cells display a complex internal architecture, with one fundamental premise being that all the cells' components operate in distinct locations with specific distributions. Certain material also periodically needs to move from one location to another (including between the inside and outside of the cell), and any defects in this can have serious consequences for the cell. These observations all provide opportunities for assays to be designed to monitor change. Similarly most cells at steady state have a defined size and shape, features that also lend themselves well to automated analysis and quantification. Using fluorescent reporters, typical cellular parameters that HCS assays measure can broadly be divided into three categories:

- morphological (area, width, length, circularity)
- intensity-based (number, average, distribution, variation, co-localisation)
- texture (granularity, topography).

Many successful HCS campaigns have therefore utilised basic cell biology knowledge, combined with these analysis possibilities to design assays to identify proteins and networks regulating particular cell functions. Examples include the protein networks required to control cell division, migration, signalling, the internalisation or secretion of molecules, and cell death, to name but a few. Other exciting microscopy screens have examined the mechanisms and pathways used by bacteria and viruses to enter target cells and initiate infection. All of these examples can be classified as either basic cell functions, or challenges that cells are likely to encounter, and so HCS presents itself as a marvellous technological tool to systematically probe how such events are regulated. Although this represents fundamental scientific research, it is inevitable that the findings from these screens will ultimately have consequences with respect to our wider understanding of cell health. As more proteins (and their dysfunction) become linked to disease, it is also clear that these data will inform possible interventions.

Potential impacts of HCS in translational science

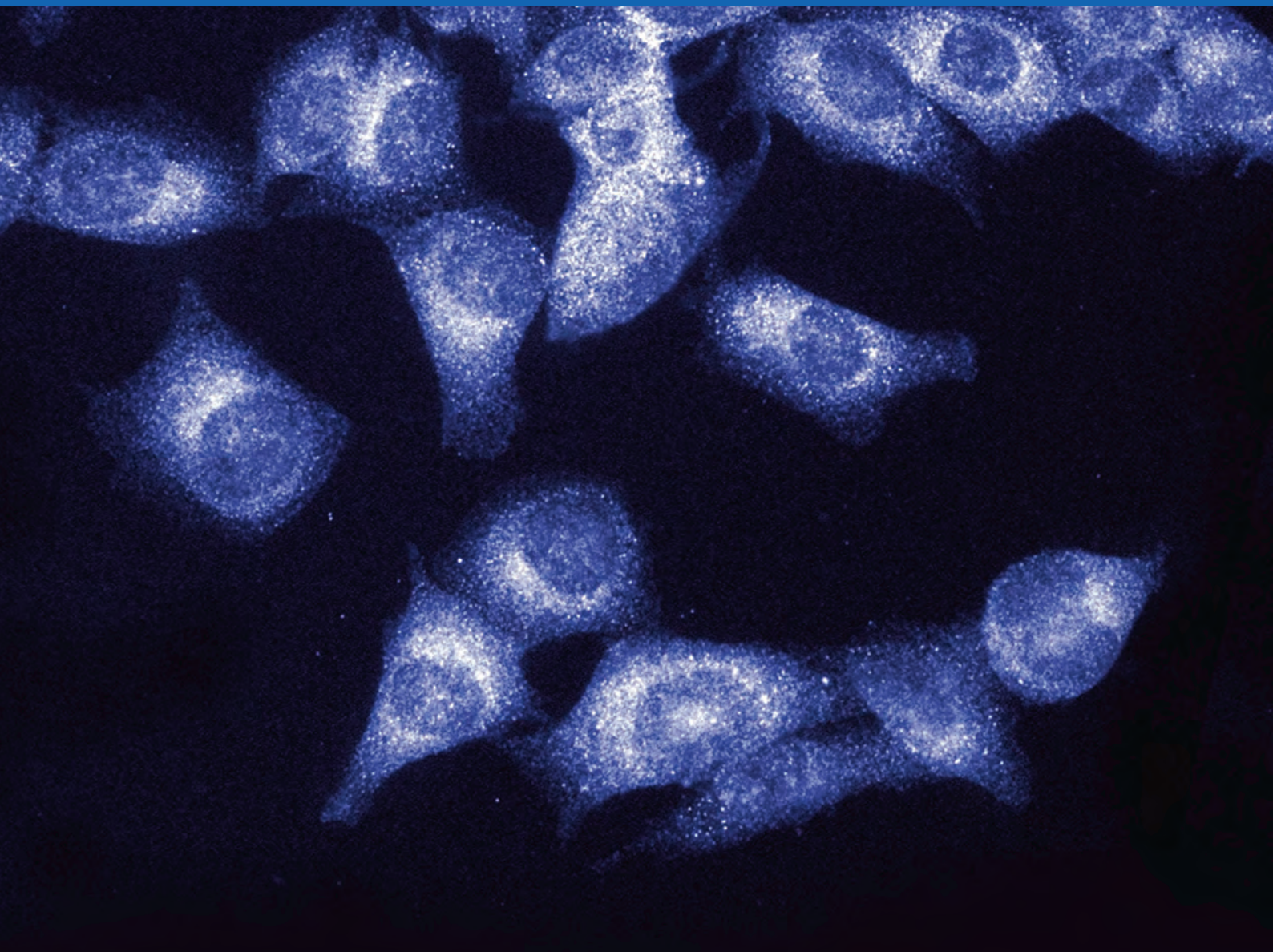
Interestingly HCS has its roots in the pharmaceutical, rather than the academic sector. For many years the identification of novel drugs and their targets was hindered by the lack of information coming from traditional high-throughput screening of compound libraries, largely because they employed simple colorimetric assays which were unable to reveal sub-populations of cells within a single well of a multi-well plate producing differing responses or phenotypes. Microscopy-based imaging of each well was the obvious solution to this problem, and hence automated imaging and analysis of cells was developed and became established technology. In the pharmaceutical sector it is now clear that even subtle differences between how cells respond to compounds is critical information that can be used to inform design and dose of the potential therapeutic.

HCS has also found a role for itself in pre-clinical toxicity studies. This is an important development because cell-based assays can reveal a number of toxicity-related phenotypes in cells – for example changes in the shape of the nucleus, alterations in mitochondrial potential, permeability of the cell membrane – all in parallel in a single experiment (so-called 'multiplexing'). The application of HCS to toxicity studies not only enhances the information that we have about a potential therapeutic agent, but its use also significantly reduces the amount of animal testing required. Another emerging translational application of HCS is in the area of drug delivery. It is becoming clear that even after the development of a new drug, its ability to be targeted correctly is not guaranteed. HCS is playing a role in screening for drug carriers and formulations that provide optimal targeting and delivery to particular cell types, again information that is incredibly valuable for the smarter design of therapeutics.

Outlook

The ever increasing role that HCS technology plays in the life sciences is impressive, however it is a technology that must continue to evolve to changing needs and the increasing demands of researchers. One area that is seen as particularly challenging is in the use of more complex three-dimensional cell culture models. The power of HCS lies in its ability to extract detailed quantitative information from single cells, information that accurately portrays the state of that cell. One criticism however is that the majority of cell types used in HCS experiments are effectively two-dimensional, and do not significantly resemble cells found in the organism from which they were originally derived. It is now relatively straightforward to culture clusters of cells in three dimensions, for example as 'cysts' or 'spheroids', but they suffer from the problem that each cell in the cyst is small, and therefore the quality of spatial information that can be extracted is poor. Such cell models will undoubtedly add extra value, particularly in pharmaceutical research, but imaging and image analysis difficulties will need to be overcome.

Another area that HCS needs to address is its standardisation. A wide variety of hardware and software solutions are available, but to date there have been few attempts to standardise image types and image analysis algorithms and protocols across different platforms. This would be highly beneficial, as it would enable more efficient data mining from the large image sets publically available, and as a consequence the extraction of even more 'content' from the images. Nevertheless, HCS appears to be a technology that is now fundamental to so many strands of biological research, and for the foreseeable future it is here to stay as part of the search for answers to the big questions in the life sciences. ■



It's the 'content' of cells that matters in biomedical research

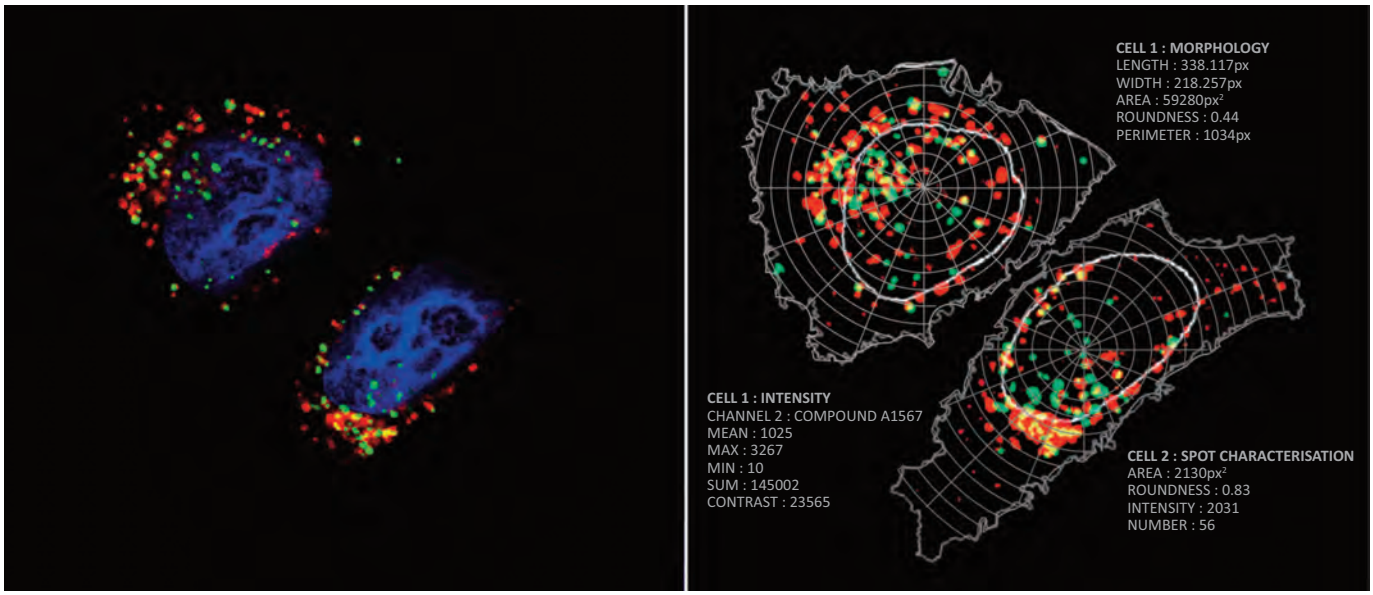
One of the most remarkable things about life on earth, in all its forms, is how cells often only tens of microns in diameter have evolved to carry out the variety of tasks that they do. In multicellular organisms the situation is even more complicated, as different cell types need to work together in an orchestrated manner in functional units such as tissues and organs to maintain the health of the organism. Ultimately therefore, it is the function of individual cells in our body that determines our health, and our susceptibility to disease and infection. The discipline of cell biology serves to understand how cells work, and importantly what goes wrong in cells to cause disease. It is a discipline, with associated technologies, positioned at the centre of all fundamental biomedical research.

Since the mid-seventeenth century microscopy has been the primary tool for scientists to reveal the structure and organisation of cells, both in isolation and in their 'social' context. In the late

twentieth and early twenty-first centuries however, the widespread application and integration of fluorescence technologies with microscopy has provided new opportunities to reveal the innermost workings of cells. Fluorescence microscopy allows researchers to potentially view not only the cellular organelles, but also the billions of molecules – in particular proteins – that work together to provide the cell with its functionality. Therefore, in this post-genome sequencing age, how can we assign discrete function to each of the 22,000 human genes and the proteins that they encode? Furthermore, how can we identify those proteins that can cause particular disease, and those proteins that can have protective properties? Carrying out such experiments in intact and preferably living cells has obvious benefits, but clearly the scale of such experiments is challenging. Simply visualising each protein in turn (and molecular techniques in principle make this possible) requires 22,000 individual microscopy images, and so without considering any further complexity of the experiment or replicates we would need to image almost every well from 230 96-well plates in a consistent manner. Even if this is achievable, the next problem becomes one of how to interpret the images, and in such a way that we can objectively compare them. The issues are experimental scale and complexity of information.

In the last ten years this experimental approach has become a reality, and these barriers are being overcome, encompassed in a technology termed 'high content screening and analysis' – HCS / HCA. This is a fusion technology, combining lab automation, particularly in terms of the microscopy, with sophisticated software routines capable of analysing images of millions of individual cells ('high throughput') and extracting user-defined quantitative information ('content') for each cell. Since its development, labs around the world have embraced its power to address both fundamental cell biology questions and applications relevant to human health and disease. HCS can and has been used to rapidly screen massive libraries of chemical compounds to identify leads with desired cellular phenotypes, to identify host factors associated with virus infection, and reveal new triggers for cancer cell development. For cell biologists it has proved to be a particularly powerful technology when combined with RNA interference (RNAi), a molecular technique that allows researchers to inactivate genes and the proteins that they encode in a systematic manner. Carrying out RNAi experiments in an HCS format effectively allows us to dissect the function of each gene / protein in turn with respect to a particular biological question, with the output being images of cells revealing the phenotype, and also their quantitative analysis.

In the Cell Screening Lab at UCD (www.ucd.ie/hcs) we have been developing and applying HCS strategies for a number of years to address questions related to how cells transport material (cargo) between their various internal organelles. Understanding how these membrane transport processes work is of vital importance, as all cargo inside cells will only facilitate cell function if it is located in the correct place. For example, signalling receptors at the cell surface are actually synthesised and assembled in internal membranes of the endoplasmic reticulum, requiring transport through intermediate organelles for further processing prior to delivery to the cell surface. Many human diseases are associated with mistargeting of such receptors – cystic fibrosis is a well-known example. Our ongoing mission is to use RNAi at a whole genome scale to systematically dissect how such transport pathways are regulated, and ultimately to use this information to gain insight into how they can be manipulated. Improving drug



Left: image of two cultured human cells fluorescently labelled for various subcellular structures. Right: example of 'content' that can be extracted from these cells

delivery efficacy into cells is one good example of how this approach can be utilised. Ultimately therefore, we believe that our HCS approaches provide critical information about cell organisation that can be exploited by many branches of biomedical research.

There are of course both technological and political challenges to overcome if HCS is to continue providing valuable data to the scientific community. From a technological perspective there is a move towards use of more complex 3-dimensional multi-cell type models, which although may better represent the in vivo situation, they are more difficult to image and precisely quantify, requiring confocal HCS technology. Politically, HCS is a relatively expensive technique, both in terms of its hardware and the reagents needed to carry out large-scale screens. In the current challenging environment of research grant availability, funding bodies often prioritise more advanced or applied projects that might return gain in the short term. Ignoring HCS projects would be foolish, as they show real promise to deliver advanced cell biology knowledge that will inform and drive the direction of future biomedical research. ■

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