

**OLYMPUS**

## Cell-based genome-wide RNAi screening with the new scan<sup>^</sup>R Screening Station

In collaboration with the European Molecular Biology Laboratory (EMBL), Olympus has developed a fully automated and highly flexible microscope-based screening platform for life science applications.

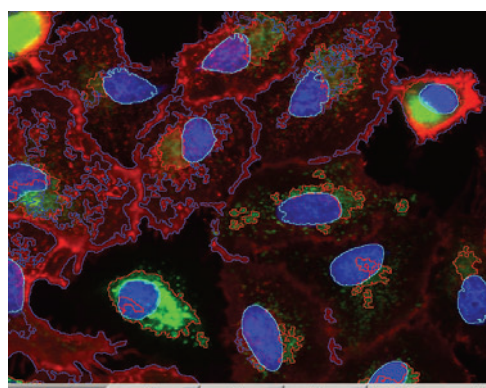
The need for scientific methods that allow large-scale functional studies of molecules in their natural environment is increasingly apparent. Automation in the handling and analysis of large sets of data must be considered. For this purpose Olympus has developed the scan<sup>^</sup>R Screening Station, on the basis of a custom-built screening setup that was the result of a close cooperation between Olympus and EMBL<sup>1</sup>. scan<sup>^</sup>R accommodates the demand for both throughput and reliability for functional cell-based assays and offers the flexibility needed for cutting-edge assay development.

scan<sup>^</sup>R is a modular microscope-based imaging platform for scientific screening and large-scale data analysis. Based on advanced microscope technology, scan<sup>^</sup>R can perform automated image acquisition and analysis with speed and precision. It is ideally suited for a wide range of applications and can be adapted and optimized for very different requirements. Moreover, it is the perfect system for the analysis of biological samples on a variety of formats such as multiwell plates, slides or any custom-built array. The scan<sup>^</sup>R analysis module provides complex image analysis and advanced data evaluation tools. Different particle and object detection functions can be selected and combined with segmentation for efficient and robust image analysis. From these detected objects, further in-depth cytometry-oriented analysis can be carried out. Complex multiparameter data analysis schemes can be set up easily by gating and classification.

Here we present two very different cell-based assays that show the broad application range and adaptability of the scan<sup>^</sup>R Screening Station.

### Intracellular analysis of protein transport in fixed cells

With the sequencing of the human genome and the annotation of 20,000–30,000 open reading frames, modern biomedical science now focuses on the identification, function, regulation and interaction of these potential genes. At the EMBL, the scan<sup>^</sup>R Screening Station was



**Figure 1** | Image screen shot following data acquisition using scan<sup>^</sup>R, showing the detection and separation of labels. The blue DAPI-stained nuclei are circled in cyan; the detected CFP-tagged VSV-G in the Golgi are circled in red, and the detected Cy5 VSV-G antibodies at the cell surface are circled in blue.

used to carry out a genome-wide RNA interference (RNAi) screen<sup>2</sup>, with the aim of identifying all human genes that are involved in or interact with the intracellular transport machinery.

The human small interfering RNA (siRNA) library was spotted onto glass slides, together with transfecting agents, in an array of 384 spots per slide<sup>3</sup>. HeLa cells were seeded onto the glass slides, where they were subsequently transfected. The spot diameter matched the field of view of a charge-coupled device (CCD) chip when used with a 10× objective. The advantage of this spot array is that 384 experiments, each corresponding to an siRNA spot, can be done on one 3 × 1 inch standard glass slide. Four of these slides are placed into a holder with the dimensions of a standard 96-well plate to allow for optional robotic loading.

The secretory marker protein ts045 vesicular stomatitis viral G protein (VSV-G), tagged with cyan fluorescent protein (CFP), was used as an intracellular transport indicator because it can be visualized as it is released from the endoplasmic reticulum and transported through the cell to the plasma membrane, where it was detected on fixed cells

**Konstantin Joanidopoulos**

Olympus BioSystems GmbH, Robert-Koch-Str. 9, D-82151 Planegg, Germany.  
Correspondence should be addressed to K.J. (konstantin.joanidopoulos@olympus-biosystems.com).

## IMAGING AND FLUORESCENCE FLUORESCENCE MICROSCOPY

by a cyanin 5 (Cy5)-conjugated antibody<sup>3</sup>. Cell nuclei were stained with DAPI. The scan<sup>^</sup>R screening platform was thus used to observe deviations in distribution and localization of intracellular and membrane-bound VSV-G. High-resolution three-color image acquisition was done with the motorized Olympus IX81 inverted microscope. The powerful, object-oriented software permits rapid auto-focusing to maximize the number of cells in focus using a CCD camera. The system also incorporates the advanced MT20 illumination system with parallel operation capabilities, an 8-position filter wheel to switch between chosen filters, a 14-position attenuator shutter for illumination intensity control and a very fast shutter. The MT20 offers stabilized intensity for quantitative measurements and fiber-coupled optimized illumination. A novel real-time controller allows precise synchronization and reduces photobleaching to a minimum.

The Olympus scan<sup>^</sup>R software consists of two modules—one for acquisition and hardware control and one for image and data analysis. The modules are completely independent and can run on the same computer or on separate computers. In both cases image and data analysis can be performed 'online' in parallel to image acquisition.

The scan<sup>^</sup>R analysis module excels in data analysis and evaluation with a multistep procedure that can extract multiparameter image contents. For the automated analysis of the transport assay just described, DAPI-stained nuclei were identified via an object-oriented detection algorithm. Starting from the nucleus, two additional particle detection algorithms were applied—one for the detection of the CFP-tagged intracellular VSV-G protein and the other for the detection of Cy5-conjugated antibody specific to the extracellular domain of VSV-G (Fig. 1). From the detected objects, parameters such as intensity and

geometric information were extracted for analysis. Calculations can be performed on these values, and 'derived parameters' were created. Subsequently these parameters can be plotted on one-dimensional histograms or two-dimensional scattergrams, and data populations can be selected and gated. This powerful approach is commonly used in flow cytometry for the analysis of large data sets and was adapted to the analysis of image data.

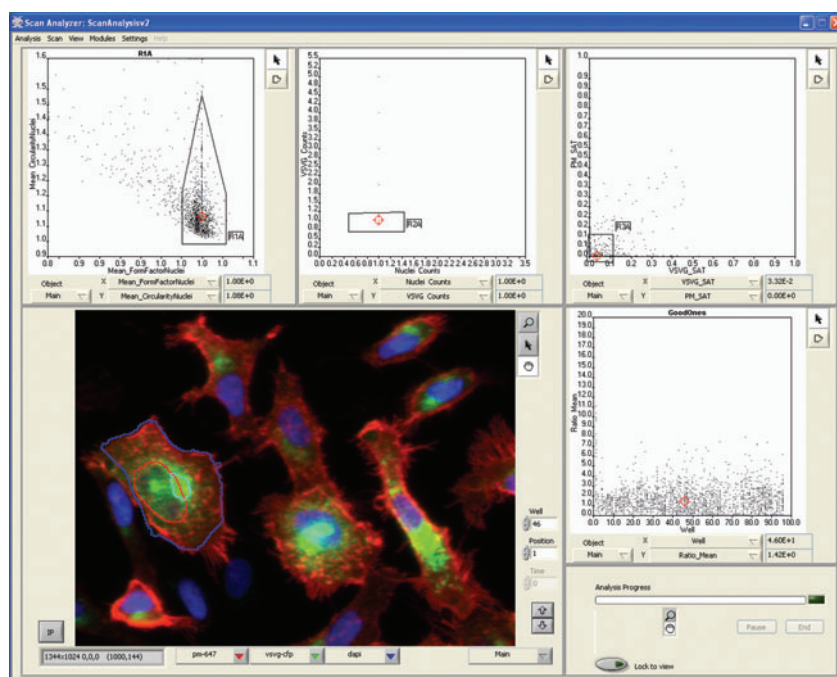
By gating, only cells that match specific criteria were selected for further analysis. For the analysis of the VSV-G assay, elliptical to round nuclei were selected for further analysis to exclude overlapping nuclei and irregularly shaped staining artifacts. The second gate dictated that only cells with a single area of VSV-G labeling are considered, thus preventing quantification from neighboring cells. The final gate removed cells with pixel saturation >10%, to prevent biased results. Finally, the ratio of the intensity of the CFP-tagged VSV-G intracellular signal and the membrane-associated signal from the Cy5-conjugated antibody specific to the extracellular domain of VSV-G was calculated and plotted for every cell on every spot. The quantification summary for the entire plate or array was finally displayed, and spots with altered VSV-G distribution could be detected immediately. Each data point is linked to the respective image, allowing immediate 'quality control' of the applied image and data analysis procedures (Fig. 2).

### The challenges of live cell analysis

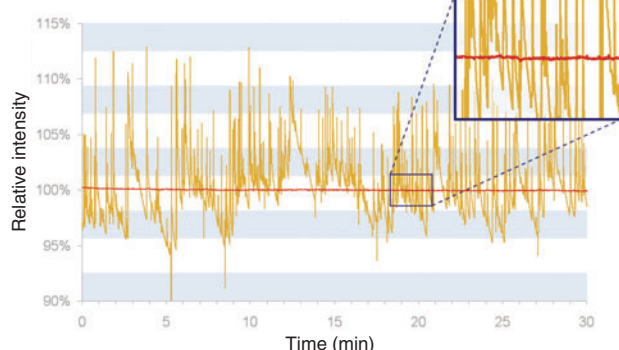
There has been a steady progression from the use of fixed cells for end point analysis to live cell research, where it is possible to study dynamic events and follow the fate of specific molecules and cell components *in vivo*.

Mitosis is one of the most dramatic events in the life cycle of a cell. To test all presently known human genes for their involvement in mitosis, the Mitocheck project group at EMBL has worked out—using scan<sup>^</sup>R—an image-based live cell screening protocol to analyze the frequency and dynamics of mitosis in siRNA-transfected cells. Cell division is observed in a HeLa (Kyoto) H2B-green fluorescent protein reporter cell line, which is growing on siRNA arrays.

Because cell division occurs only with a frequency of 24 h and lasts about 60 min, the cell cultures are observed for 48 h and images are captured from 1,536 spots every 30 min. This allows a quantitative observation of cell behavior. Initially a three-dimensional focus map of the slide arrays is taken and then repeatedly applied to the slides with maximum speed, without refocusing. Such accuracy is achieved through a specially modified and highly stabilized incubator, developed by EMBL, in addition to the high-precision hardware of the scan<sup>^</sup>R Screening Station.



**Figure 2** | Gating, classification and data evaluation by multiple selection of data subpopulations according to specific criteria.



**Figure 3 |** Fluorescence illumination stability. Comparison of the burner light output, normalized to the average intensity (100%). Red, MT20; yellow, standard illuminator with mercury burner.

High-frequency image acquisition is an important requirement for long-term, live cell experiments. The scan<sup>^</sup>R hardware is perfect for such applications because it incorporates the Olympus illumination system MT20. The MT20 features a highly stabilized xenon or xenon-mercury burner for constant illumination intensity, a precondition for quantitative imaging and automated image analysis (Fig. 3). Additionally, the 8-position filter wheel, the 14-position attenuator, and the fast shutter with millisecond closing time, are fully synchronized with the CCD camera. This ensures that exposure of the sample with light is reduced exactly to the exposure time of the CCD camera and bleaching and phototoxicity are reduced to the absolute minimum.

### Flexibility and performance

The scan<sup>^</sup>R Screening Station for Life Science is a highly integrated, comprehensive modular system that can incorporate additional hardware and software components for assay-specific tasks covering a multitude of disciplines. scan<sup>^</sup>R can be used to investigate a plethora of cellular functions via single- or multi-color assays, including gene expression, intracellular transport and location, bacterial infection,

cell proliferation and cell cycle analysis. It is thus also possible to carry out extensive drug screening in complex cell cultures.

In addition to the advanced imaging capabilities already discussed, scan<sup>^</sup>R boasts comprehensive software that allows detailed experimental design and analysis. Therefore, cell-based assays can be rapidly developed and tested using the associated software. The format manager contains prestored, editable plate formats, providing arbitrary screening patterns for use on plates, slides, and arrays. Plate-loading robots can also be incorporated to increase throughput in a fully automated environment, and long-term observation of living cells can be achieved through the addition of the Olympus cell<sup>^</sup>cubator, with sensitive temperature, humidity and CO<sub>2</sub> control as well as a high-efficiency particulate air sterile-filtered closed airflow.

### Conclusion

The scan<sup>^</sup>R system is successfully applied for the identification of relevant transport genes, using RNAi and the localization of encoded protein during intracellular transportation via multicolor labeling. The Olympus scan<sup>^</sup>R is a microscope-based screening platform for efficient acquisition of high-quality imagery and, importantly, the automated collection and analysis of bioinformatics data. The modular nature and open platform design of the scan<sup>^</sup>R allows the user to customize the apparatus for individual experimental design.

Additional information on the Olympus scan<sup>^</sup>R system and the screening assays described is available on our company website (<http://www.olympus-europa.com/microscopy>).

### ACKNOWLEDGMENTS

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