

# OLYMPUS

Your Vision, Our Future

Life science microscopes

scan<sup>IR</sup>

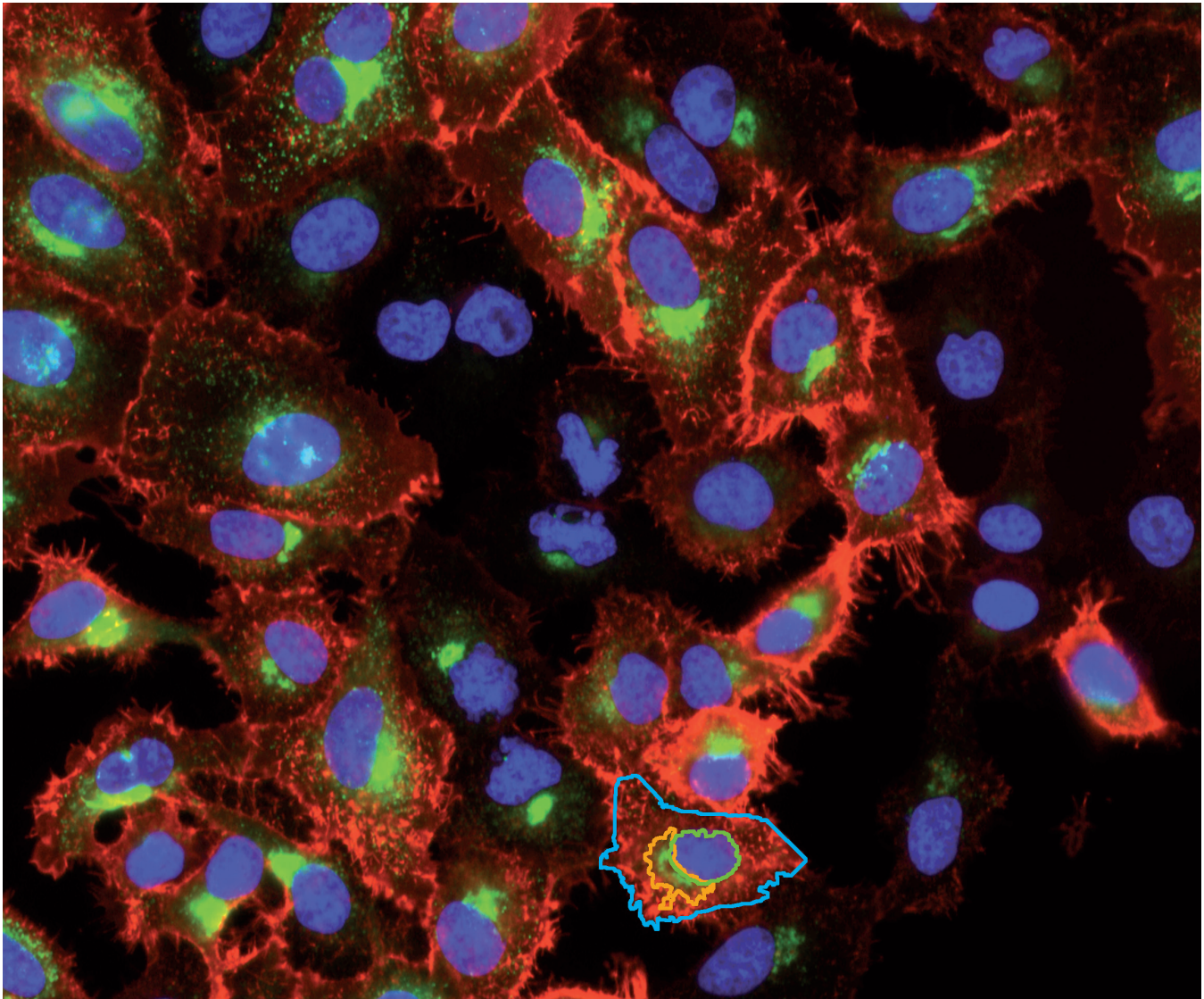
High-content screening

*High-content screening station for life sciences*



# HAVE A GOOD CHAT TO YOUR CELLS AND LISTEN TO ALL THEIR SECRETS

---



*Image courtesy of EMBL, Heidelberg, Germany.*

## High-content screening station for life science

For some challenges, hard work and motivation alone are not enough and the ideal equipment is essential. The scan<sup>^</sup>R screening station is the perfect solution to the challenge of microscope-based high-content screening. scan<sup>^</sup>R perfectly combines the modularity and flexibility of a microscope-based setup with the automation, speed, throughput, reliability and reproducibility demands of screening applications. The system is thus equally well suited to handling standard assays and assay development. Furthermore, the modular design allows the system to be adapted to the specific applications of an R&D lab as easily as to those of multi-user environments. scan<sup>^</sup>R was developed as a powerful screening station with an extremely broad application range on the basis of experimental screening systems designed by the European Molecular Biology Laboratory (EMBL).

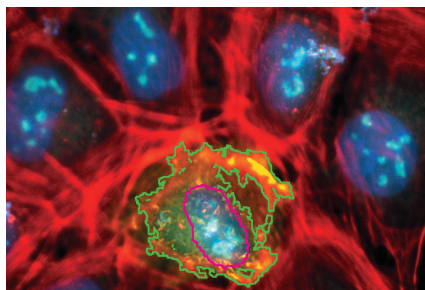
## See your assay in action

scan<sup>^</sup>R incorporates advanced modular software and hardware, controlled through an easy-to-use interface which provides access to all image acquisition and image analysis parameters. The system is designed to produce multidimensional (X, Y, Z, t,  $\lambda$ ) images with great ease. Once acquired, images can be analysed automatically by the powerful scan<sup>^</sup>R image and data analysis software, which is based on a cytometry-oriented approach to handling and analysing huge numbers of multidimensional datasets. The scan<sup>^</sup>R analysis software permits full image processing (e.g. background correction), object and sub-object detection and parameter calculation, as well as sophisticated gating and classification schemes.

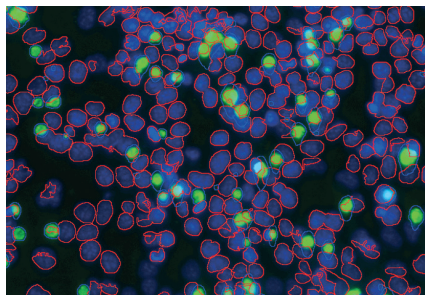
---



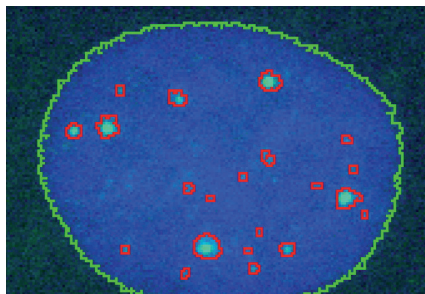
# EXPAND YOUR SCANNING HORIZONS



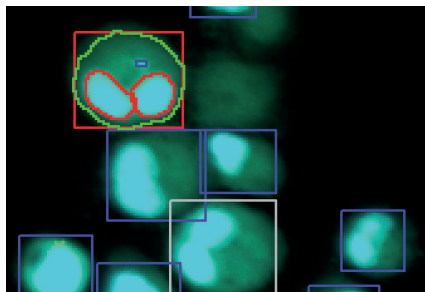
Genome-wide screen of cell arrays to identify novel genes involved in the intracellular transport mechanism. Image courtesy of Dr. R. Pepperkok, EMBL, Heidelberg, Germany.



Chlamydia trachomatis infection assay. Image courtesy of Dr. S. Hess, Max Planck Institute (MPI) for Infection Biology, Berlin, Germany.



Immunofluorescence staining of promyelocytic leukemia (PML) nuclear bodies in osteosarcoma cells.



Segmentation of binucleated cells and micronuclei counting. Image courtesy of Department for In-Vitro Toxicology, Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany

Designed for fully automated image acquisition and data analysis of biological samples, scan^R can handle many different formats, e.g. multi-well plates, slides or custom-built arrays. Its unmatched flexibility and open design make it equally adept at routine and advanced applications. With its powerful analysis module for biological functional assays, it is the perfect tool for assay development and high-content screening. scan^R provides complex image analysis and advanced data evaluation, enabling it to complete a whole range of standard and bespoke assays.

## Research fields

The system handles fixed and live cells with equal ease and is therefore the perfect screening platform for a wide cross-section of research. scan^R specifically targets the requirement for quantitative imaging and image analysis in modern cell biology, systems biology and medical research. For example, cancer research involves investigations on many levels, such as DNA damage, cell proliferation and cell cycle analysis. Novel genome-wide screens using RNA interference are a further key application to which scan^R is specifically adapted (developed in close cooperation with the EMBL).

For drug screening, a great many protocols have been developed to show the biochemical effects of compounds at a cellular level. For example, drug-induced changes in levels of gene expression can be accurately assessed using a range of standardised assays. scan^R also excels at routine screens measuring apoptosis, micronuclei or DNA fragmentation (comet assays). Additionally, scan^R covers a huge range of screening applications (selected examples are listed below) used in many research areas.

## Examples of cellular screening assays:

- Cell counting
- Gene expression
- Intracellular transport
- Translocation
- Cell proliferation
- Promyelocytic leukemia (PML) body assay
- Bacterial infection assay
- Cell cycle analysis
- Protein localization and co-localization
- Cell array screens
- Multicolour assays
- Rare event analysis
- Automated FISH analysis
- Fluorescence analysis in tissue sections
- Live cell assays including kinetic analysis and gating on resulting · response curves
- Micronuclei and comet assays

# ADVANCED ACQUISITION



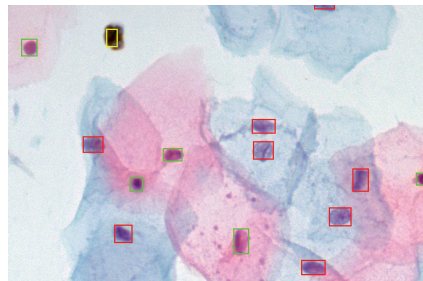
Automated, high throughput. Cellular assays in microplates, slides and self-defined cell arrays.



MT20 illumination. Highly stabilized system for reproducible and fast fluorescence excitation.



U-RTC. The hyper-precise real-time controller ensures that all scan^R devices and components are perfectly synchronized and parallelized to guarantee the necessary precision for high-throughput image analysis.



Fluorescence or transmitted light images can be used for automated segmentation.

The Olympus scan^R software features an intuitive graphical user interface based on a strictly workflow-oriented approach. This ensures simple handling in daily operation, reliable and stable image acquisition and straightforward system configuration. The scan^R system design focuses specifically on quantitative measurements and on easy, reliable repeatability and flexibility, addressing the needs of scientific screening and assay development.

## Expandability

**A** scan^R is based on the latest Olympus high-end IX83 inverted microscope, providing the utmost flexibility, robustness and stability for a research microscope setup, and can be fitted with a large set of optional devices. It handles all standard assay formats, such as microplates and slides, with ease and can also be configured to accept any custom designs such as spotted arrays and biochips. With these capabilities, scan^R can be precisely adapted for use in a vast range of applications, and is highly suitable for multi-user environments.

## Highly stabilized illumination

**B** The MT20 illumination system developed by Olympus has, at its core, a highly stabilized xenon or mercury/xenon burner, a fast filter wheel, an achromatic and absolutely reproducible attenuator, as well as a fast shutter with an exceptional on/off time of less than 1 ms. The MT20 is coupled to the microscope by a flexible quartz light guide, eliminating the effects of heat and vibration, and has optimized optics for homogeneous illumination. Based on the proven power of the MT20 illumination system and the real-time controller described below, highly reproducible, consistent image data are generated and photobleaching is reduced to a minimum.

## Hyper-precise control

**C** The extreme demands for robustness, reliability and throughput are met by the perfect device synchronization and parallelization of the unique real-time controller (U-RTC) from Olympus.

## More dimensions

The advanced features of scan^R allow truly multi-dimensional screening. Time-lapse Z-stack images can be recorded at any number of locations on multi-well plates, slides or custom formats, using all available observation methods, such as fluorescence or brightfield, as well as contrast-enhancing methods, such as differential interference contrast (DIC) or phase contrast.

## Software autofocus

Fast and accurate autofocus is crucial for successful automated image acquisition. scan^R provides different autofocus algorithms to meet the demands of extremely variable biological samples. The scan^R 'object-based' autofocus uses specific features to distinguish relevant cellular objects from cell debris, dirt or dust. 'Image-based' autofocus is available for highly variable samples; this focuses on the basis of information from the entire field of view. Image acquisition can be performed with Z-offset with respect to the autofocus plane, so that structures located in different focal planes can be imaged in focus.

# ANALYSIS AND VISUALISATION

The huge amount of data you can now collect from your assays creates a need for coherent and careful automated quantitative analysis. The powerful scan<sup>^</sup>R analysis software package is completely independent of the scan<sup>^</sup>R acquisition software. This enables both modules to be installed on either the same or different workstations, connected via a local network. In both cases, image and data analysis can be performed “online” at the same time as acquisition, or “offline” on previously-acquired datasets.

By collecting a relevant amount of data, you are also increasing the number of analytical techniques which can be performed on your samples. These can be as simple as counting labeled cells on display or as complex as ratiometric feature-based analysis of multi-labeled objects and sub-objects in different cell types or cell compartments. Image analysis is carried out as a logical multi-step procedure consisting of image processing, object detection, feature extraction and data analysis by means of gating and classification. A workflow-oriented user interface guides users through each step.

## Image processing

Before nuclei, cytoplasm and other sub-cellular objects are contoured, the raw images are pre-processed if necessary. For example, Adaptive Size Background Correction is used to remove heterogeneous background and shading automatically and rigorously whilst retaining the relevant intensity information.

## Object detection and analysis

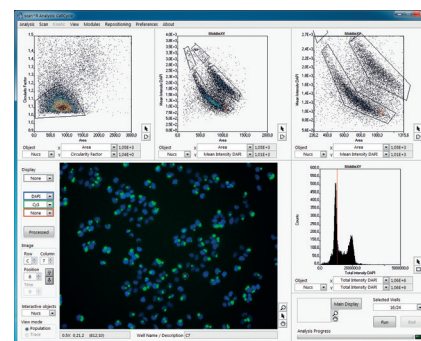
Powerful object detection modules are optimized to segment nuclei, cells or other structures. Several detection algorithms can be selected and adapted to the objects of interest. Based on this, features relevant to segmentation can be selected from a list of over 100 object parameters. The selected parameters are extracted for evaluation and classification. Additional mathematical operations can be performed on the parameters. This highly flexible data output enables the scan<sup>^</sup>R system to be used for almost any current and future cell-based assay.

## Gating and classification

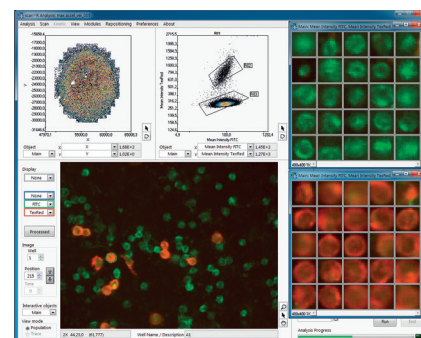
scan<sup>^</sup>R specifically excels in subsequent data analysis and evaluation. For this purpose, the powerful data analysis concepts which are successfully applied in cytometry are adapted to suit the specific demands pertaining to the analysis of large image datasets. The multi-dimensional image data generated are displayed in two-dimensional scatter plots or one-dimensional histograms, from which clustered data populations of interest can be selected using graphical tools. Selected datasets can be repeatedly gated for further investigation. Gates from different plots can be combined with Boolean operators to create complex classification schemes. Since scan<sup>^</sup>R is compatible with the Olympus xcellence rt live cell imaging system, gated objects can be imported into xcellence rt - to perform an automated rare event analysis, for example.

## Immediate quality control

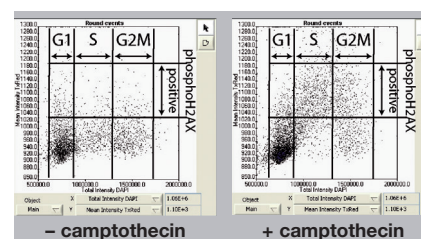
Images and objects are reciprocally linked to any data point related to them. Clicking on a data point loads the relevant image into the display window and highlights the object in question. Clicking on an object in the image display window highlights the related data points in the scatter plots and histograms. A gallery view of all images of a selected or gated data population can also be created to allow a direct and visual comparison of larger image sets with relevant information.



Cell cycle distribution of chlamydia trachomatis-infected HeLa cells.\*



Diagnostic screening of tumour markers in cytospin preparations\*\*



Correlation between H2AX phosphorylation and the cell cycle phase in a DNA repair assay.\*\*\*

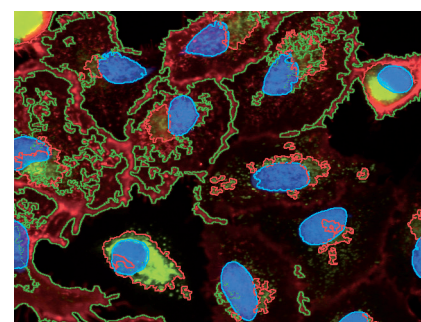


Image screenshot detail following data acquisition by scan<sup>^</sup>R demonstrating the detection and separation of labels.\*\*\*\*

\* Image courtesy of Dr S. Hess, Max Planck Institute (MPI) for Infection Biology, Berlin, Germany.

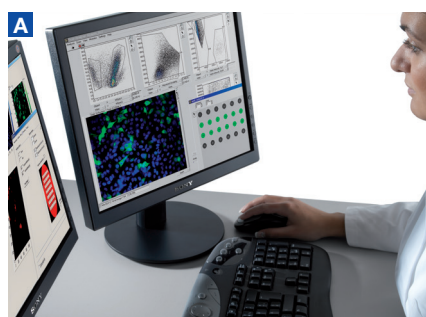
\*\* Courtesy of Dr M. Jäger, Clinical Research, TRION Research GmbH, Martinsried, Germany.

\*\*\* Courtesy of Dr F. Buchholz, MPI for Molecular Cell Biology and Genetics, Dresden, Germany.

\*\*\*\* Courtesy of Dr. R. Pepperkok, EMBL Heidelberg, Germany.



# FLEXIBLE MODULE OPTIONS

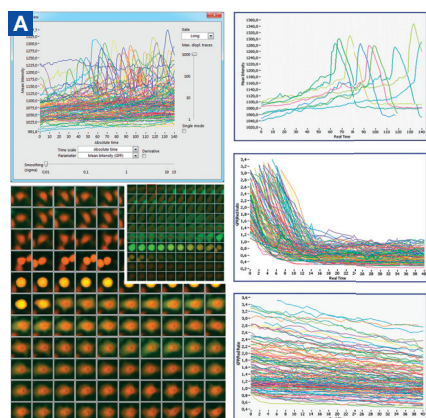


Automated image analysis runs at the same time as acquisition, or uncoupled on a separate analysis system.

scan^R was designed not only to satisfy the specific speed, endurance and reliability requirements of a fully automated high-content screening system, but also to provide unmatched flexibility and adaptability coupled with extensive expansion capabilities. This enables scan^R to match perfectly the specifications of any application and budget.

## Time-Lapse Cytometry – measure kinetic parameters

**A** The scan^R kinetic module allows objects to be classified by their kinetic properties. Tracking curves are evaluated on the basis of values (mean static parameters such as intensity, area, ratio, shape factor, etc.) measured over time. The curves are condensed into single characteristic values. Finally, kinetic parameters can be plotted in 1D or 2D histograms and populations can be gated based on their specific kinetic properties.



Time Lapse Cytometry. Impression of kinetic analysis.

## A tale of two systems

scan^R is compatible with the Olympus xcellence rt live cell imaging system, since both systems are based on the same hardware components. The xcellence rt software can run on the scan^R workstation. The same setup can thus be used both as the dedicated scan^R screening system and also as the xcellence rt live cell imaging system.

## Plate-loading robot and integration interface

**B** The scan^R platform supports a plate-loading robot. The robot facilitates fully automated screening of multi-well plate stacks and extends screening to 24/7 operation. scan^R offers continued support for integration into automated sample preparation lines, e.g. scriptable interfaces for liquid handling.

## IR laser autofocus – IX83 ZDC

The latest Olympus infrared laser-based autofocus system, which interferes with neither fluorescence imaging nor cell viability, is available for scan^R to maximise autofocus accuracy, reliability and speed. The laser perfectly complements the distinct autofocus options of the software to ensure the best focus accuracy and reliability.

## Interfaces and System Integration

scan^R provides interfaces for integrating the acquisition system into automated preparation lines and liquid handling. Moreover, the analysis software package provides interfaces which allow the addition of new custom-built image processing and object detection modules for programming by the user in National Instruments LabVIEW® and Vision graphical development environments.

## Customisation

The scan^R team of application specialists will be happy to customise your system to suit your needs and applications precisely.

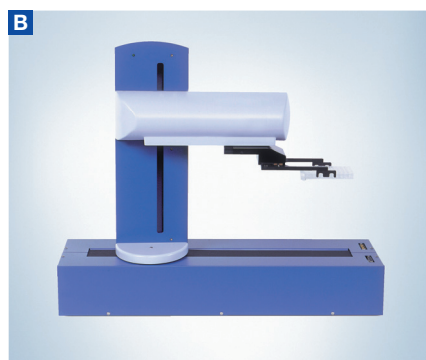


Plate loading robot systems. Supported by scan^R

## Specifications scan<sup>^</sup>R

Item	Specification
<b>scan<sup>^</sup>R screening system</b>	<p>Microscope-based screening system platform for life science applications</p> <p>Flexibility: system configuration can be adapted precisely to suit the application</p> <p>Performance and endurance: a highly integrated system concept and real-time synchronization unites the advantages of an open platform with the demands of screening applications for throughput and reliability</p>
<b>Microscope frame</b>	<p>Olympus inverted microscope IX83</p> <p>Motorized stage, Märzhäuser SCANplus IM 120x80 for IX83</p>
<b>MT20 fluorescence illumination system</b>	<p>Short arc burners, 150 W, xenon or mercury-xenon</p> <p>Highly stabilized light output, intensity fluctuations &lt; 0.1%, feedback loop stabilized burner current</p> <p>Fast filter wheel with 8 filter positions for standard 25 mm optical filters which are easy to exchange without tools</p> <p>Filter switch &lt; 58 ms (adjacent positions)</p> <p>Attenuation, 14 levels from 1 % to 100 %, achromatic, high level of repeatability</p> <p>Attenuation switch, min. 58 ms (adjacent positions)</p> <p>Shutter, on/off time &lt; 1 ms</p> <p>Operation: all modes in parallel</p>
<b>Transmitted light illumination options</b>	<p>Transmitted light illumination for visual inspection only (no transmitted light screening)</p> <p>Transmitted light illumination for screening and visual inspection including fast shutter (transmitted light screening supported)</p> <p>Optional DIC (differential interference contrast) or phase contrast</p>
<b>Hardware control and system synchronisation</b>	<p>Real-time controller with additional CPU, independent of the OS of the imaging PC</p> <p>Temporal resolution: 1 ms</p> <p>Timing precision: &lt; 0.01 ms</p> <p>Multi-task acquisition with hardware switch (Z-position, exciter filter, etc.)</p> <p>Precise camera control via external trigger</p>
<b>Camera options</b>	<p>Hamamatsu ORCA R2, high-sensitivity cooled CCD camera, recommended for long exposure times</p> <p>Hamamatsu ORCA-ER, high-sensitivity cooled CCD camera, recommended for long exposure times</p> <p>Hamamatsu ORCA-AG, high-sensitivity cooled CCD camera, recommended for long exposure times</p> <p>Hamamatsu C8484, high-sensitivity CCD camera</p> <p>Hamamatsu C9100-13 intensified and EMCCD camera</p>
<b>Objectives options</b>	<p>Objectives for "thin" (0.1 – 0.2 mm) substrates, cover slips and glass bottom plates (2x, 4x, 10x, 20x, 40x, 60x, 100x)</p> <p>Objectives for "thick" (~1 mm) substrates, plastic bottom plates and slides (2x, 4x, 10x, 20x, 40x, 60x)</p> <p>Phase contrast objectives for "thin" (0.1 – 0.2 mm) substrates, cover slips and glass bottom plates (10x, 20x, 40x)</p> <p>Phase contrast objectives for "thick" (~1 mm) substrates, cover slips and glass bottom plates (10x, 20x, 40x)</p>
<b>Filter sets</b>	<p>Single-band filter sets (specifications as requested)</p> <p>Multi-band filter sets (specifications as requested)</p>
<b>scan<sup>^</sup>R system software</b>	<p>2 independent software modules: scan<sup>^</sup>R acquisition software and scan<sup>^</sup>R analysis software</p> <p>The software modules can be installed on the same or on different workstations (Windows XP, Windows 7)</p>
<b>scan<sup>^</sup>R acquisition software</b>	<p>Automated image and data analysis for standard assays and assay development</p> <p>Workflow-oriented configuration and user interface</p> <p>Variable powerful software autofocus procedures which can be combined with an optional IR laser hardware autofocus, 2-step coarse and fine AF, object-based AF, image-based AF</p> <p>Plate manager with predefined formats (slides, multi-well plates) and editing interface to create and edit customized formats (spotted arrays)</p> <p>Online display</p> <p>Time-lapse screening, Z-stack screening, multi-colour screening (no limit on number of acquisition channels)</p> <p>User interaction: pause, resume, reconfigure software autofocus</p> <p>Support for integration into automated sample preparation lines, e.g. scriptable interfaces for liquid handling.</p>
<b>scan<sup>^</sup>R analysis software</b>	<p>Automated image and data analysis for standard assays and assay development</p> <p>Online and offline multi-core analysis with support</p> <p>Image processing, image analysis and particle detection, parameter extraction and calculation</p> <p>Cytometric data analysis, gating and classification</p> <p>Direct link between data points, objects and images</p> <p>Assays based workflow and advanced scientific assay development functionality</p>
<b>Computer</b>	Imaging computer (latest generation PC), Windows 7 Professional
<b>Additional options</b>	<p>Time-Lapse Kinetic Analysis module</p> <p>IR laser hardware autofocus based on the ZDC of the latest Olympus IX3 microscope series</p> <p>Plate-loading robot</p> <p>IX81S1F-3 frame, IX81S1F-ZDC2 frame, one-deck IX83P1ZF and two deck IX83P2ZF</p> <p>Two-deck IX83P2ZF with encoded magnification changer IX3-CAS</p> <p>Customization: hardware, software, assays</p> <p>Service contract</p> <p>Additional scan<sup>^</sup>R analysis workstation</p> <p>2<sup>nd</sup> license for scan<sup>^</sup>R analysis software</p>
<b>2 systems in one setup</b>	Can be combined with xculture rt live cell imaging system

