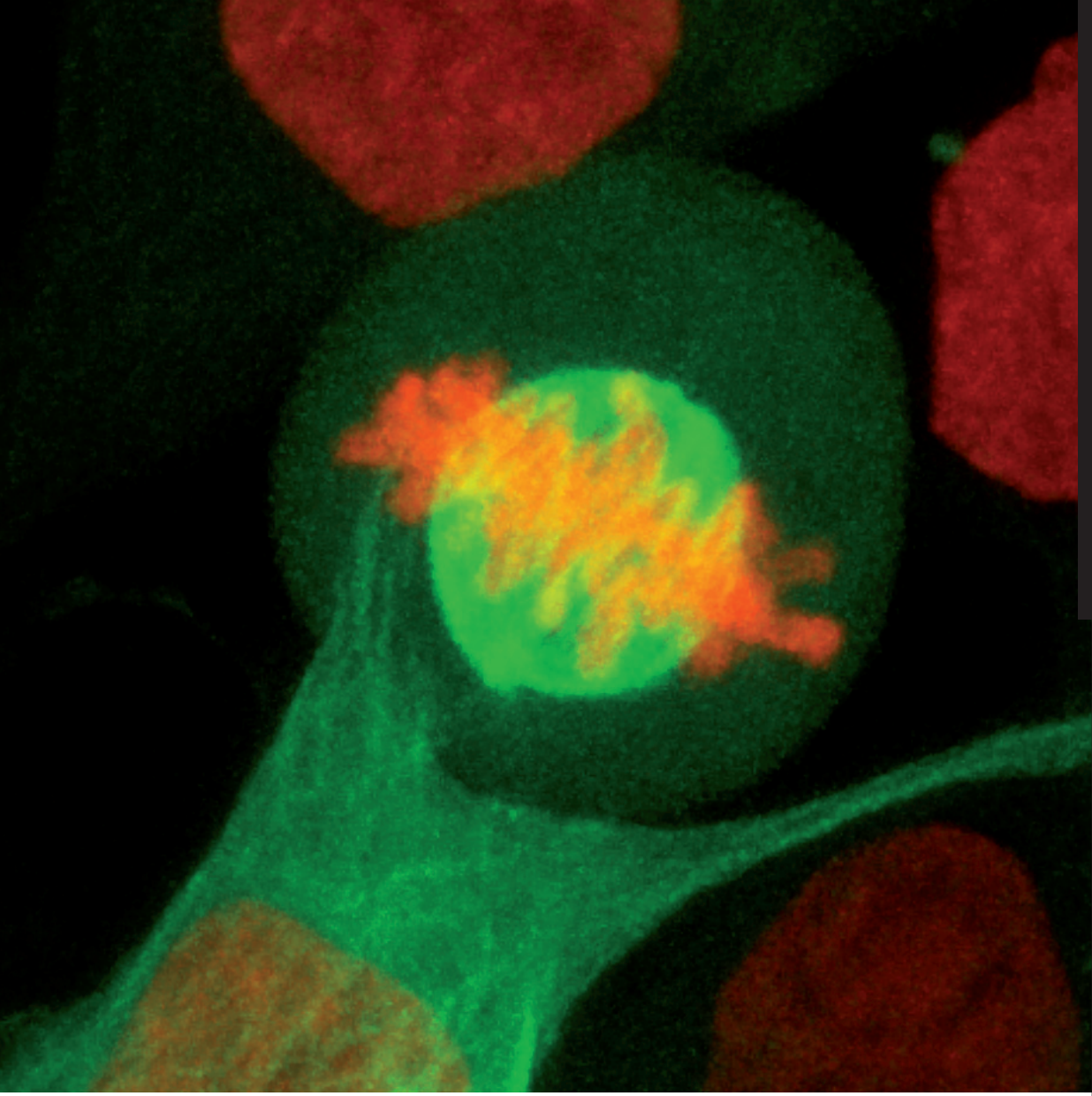
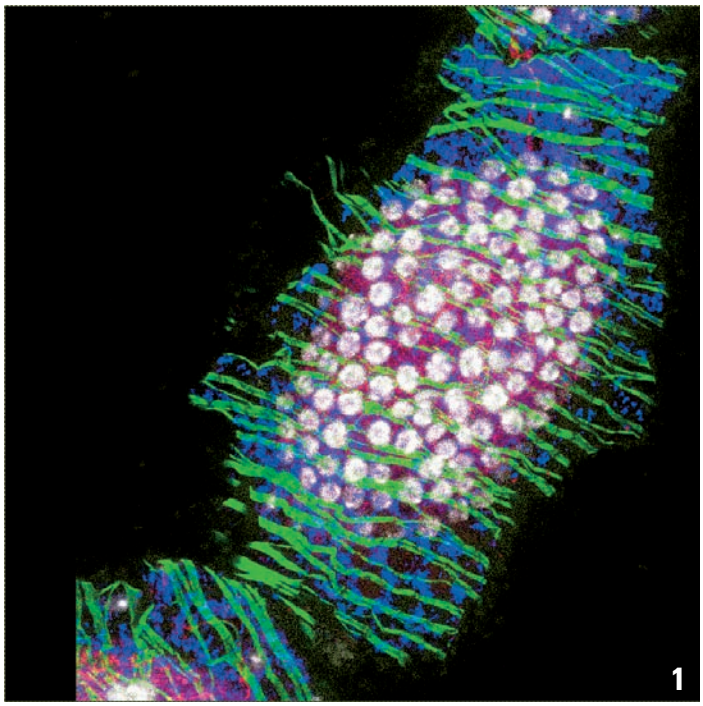


Leica HCS A

Amplify the Power of Imaging
High Content Screening Automation

Living up to Life

Leica
MICROSYSTEMS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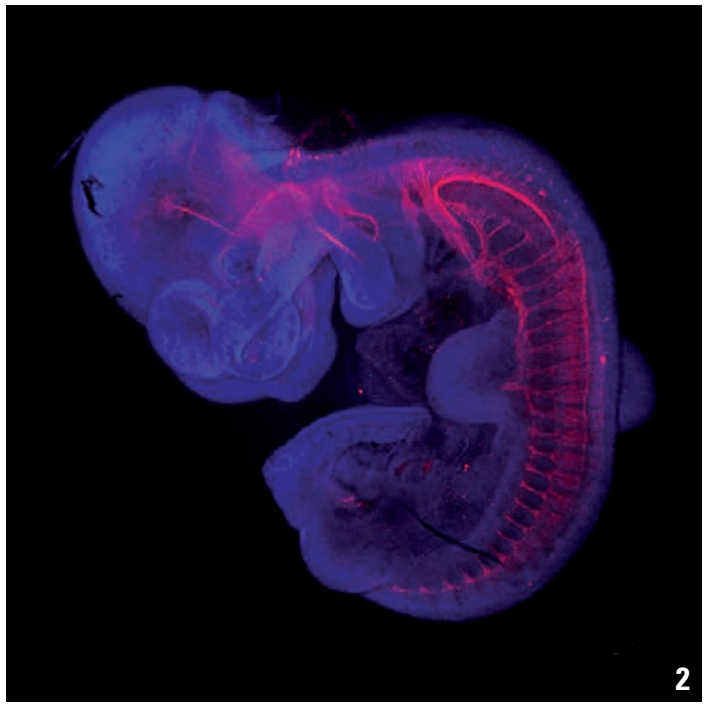
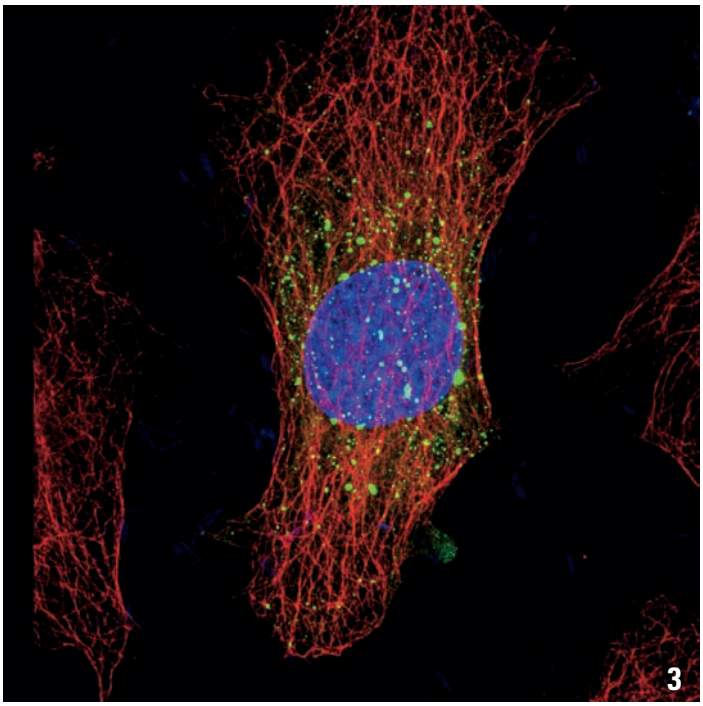

2

- **Amplify the power of confocal imaging with Leica HCS A**
- **Maximum flexibility for universal applications**
- **Easy-to-use automation provides efficient high content screening**
- **Powerful hardware for high resolution imaging and maximum content**

Leica Design by Christophe Apothéloz


3

High Content Screening (HCS) allows researchers to quickly change from descriptive to quantitative fluorescence imaging during an experiment.

High resolution imaging automation therefore answers complex questions in shorter time. It simplifies research work and efficiently reveals relationships within and between cells and organisms. Leica Microsystems offers a set of innovative tools to convert your high resolution confocal microscope into a high content imaging device.

Leica HCS A

High Content Screening Automation

Leica Microsystems provides a wide range of true spectral confocal imaging systems, known for brilliant image quality and maximum resolution. Combining high content with intelligent microscope automation greatly amplifies the power of an imaging system.

The value of an imaging system becomes more than the sum of its parts when the software platform LAS AF (Leica Application Suite Advanced Fluorescence) MATRIX M3 integrates them. A far higher number of experiments can be performed by automated sample screening. Quantification easily provides statistically relevant results. Unbiased automation concomitantly offers objective selection amongst those. In addition, standardized experiment results become reliable and provide comparable data.

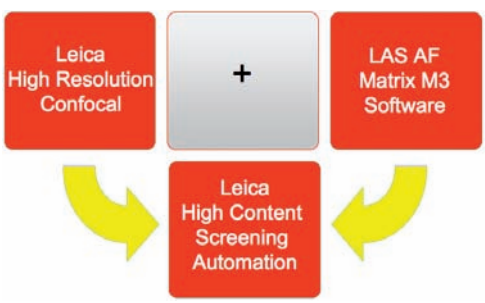
Leica Microsystems' automated high content screening speeds up experiment throughput and enhances laboratory capacity. Automation reduces routine microscopy and improves workflow. From automated routine image acquisition through to complex HCS experiments with on-the-fly image analysis, Leica HCS A is the right solution.

Leica HCS A fully integrates with your laboratory environment by open data interfaces matching established image analysis solutions. The Computer Aided Microscopy (CAM) tool empowers the team to freely program the imaging system and create specific protocols and workflows.

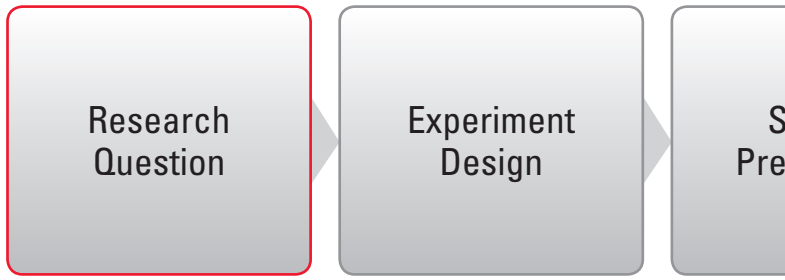
Customize the Leica microscope according to your actual needs and discover the unrivaled application flexibility for system biology, cancer research or environmental screening.

Features

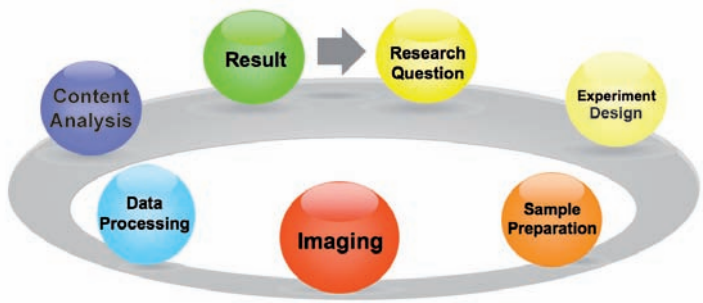
- High resolution imaging
- Time saving automation
- Open architecture
- Platform independent results
- OME data formats
- Perfect integration



Modern research is a continuous cycle of experiment design, set-up imaging, data handling, and analysis to discover live's processes and answer questions.



Amplify the Power of Imaging

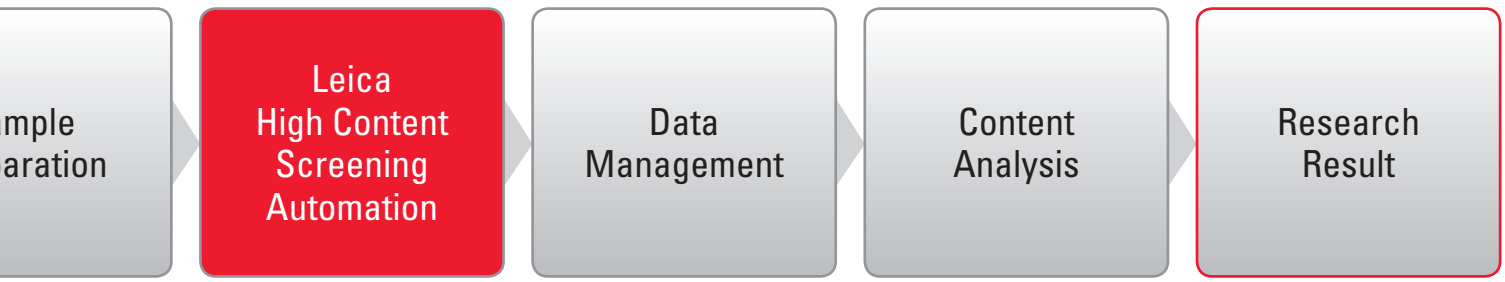


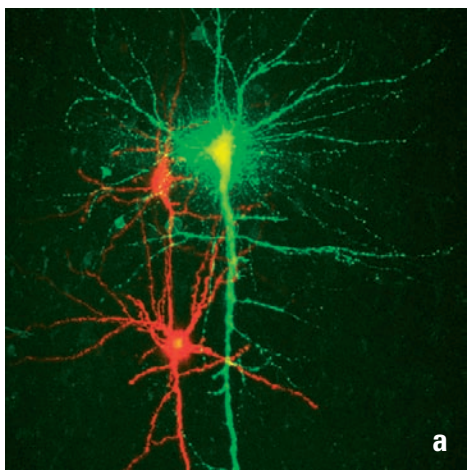
Automated Leica High Content Screening
High resolution imaging techniques answer many questions in modern life science. For high content screening, automation is essential to efficiently achieve results.

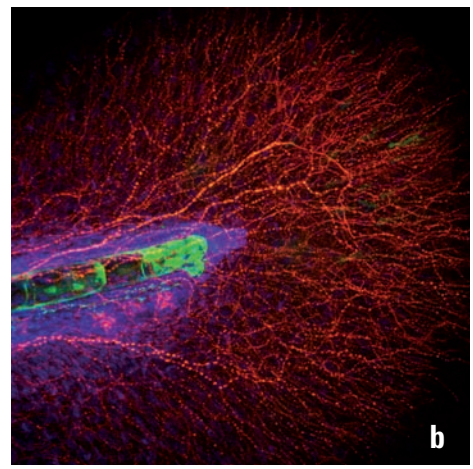
Intelligent Automation
Leica HCS A adds extensive automation capability to confocal microscopes and converts stand alone systems into fully-featured high content screening devices.

MultiPosition-MultiParameter experiment designs with autofocus and drift compensation provide maximum imaging flexibility and sharp imaging results.

By the power of open interfaces, the system becomes fully facility integrated. Leica generates Open Microscopy Environment (OME) and standard TIFF data for platform independent analysis. Existing algorithms and available image analysis programs can be used efficiently, saving costs and time.







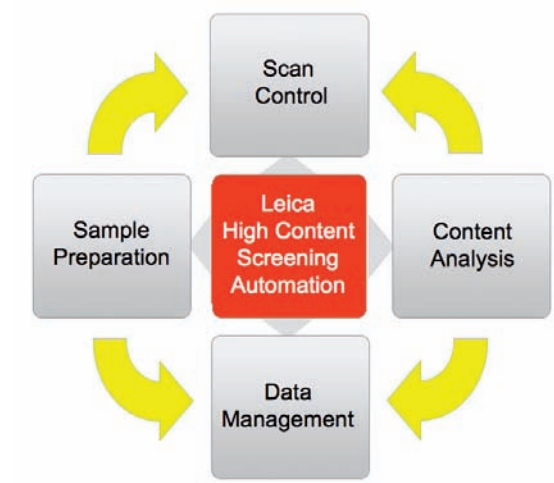
a Rat brain slice, small neuron network layer 5. Interneurons (Alexa 594, red) and Pyramidal Cell Oregon (Bapta 1, calcium sensitive, green). Courtesy of Dr. Thomas Nevian, Institute of Physiology, University of Bern, Switzerland.

b *Danio rerio* – Zebrafish – Nuclear and Acetylated α-Tubulin staining of 6 days flh:eGFP Zebrafish larvae Nuclei (Hoechst, blue), acetylated tubulin (red) and neurons (GFP, green). Courtesy of ICI Imaging Centre IGBMC, Illkirch, France.

Choose the adequate automation level: apply template automation with a mouse click or create your own sophisticated protocol. External programming languages from all operating systems can address the confocal microscope. Customize the imaging process according to your experiment design.

The open architecture of Leica HCS A enables image analysis to be directly connected with the ongoing imaging process. Switch imaging parameters instantly depending on analysis results and detect rare events when they happen.

Fast acquisition, on-the-fly analysis, and feedback system control add new dimensions of imaging automation to life science research. Four times higher acquisition speed, intelligent automation, plus seamless laboratory integration maximizes the power of Leica Microsystems' imaging systems and of your research.



Many companies offer dedicated imaging routines for dedicated assays only. Leica Microsystems provides standard solutions for routine experiments plus maximum flexibility to freely adapt imaging.

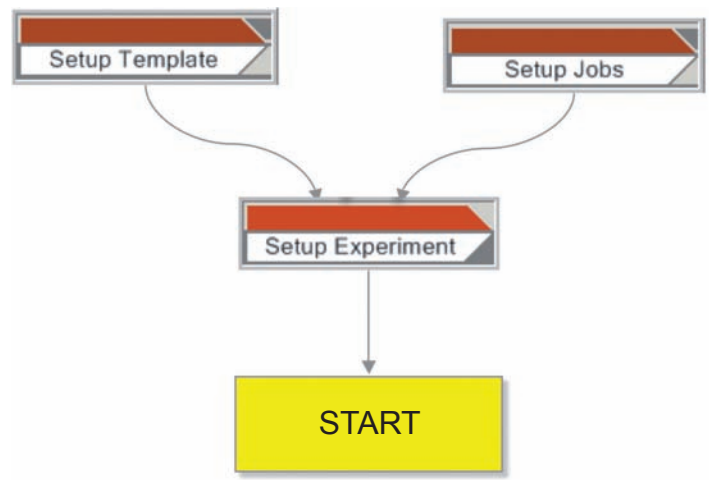
Easy-to-use Automation

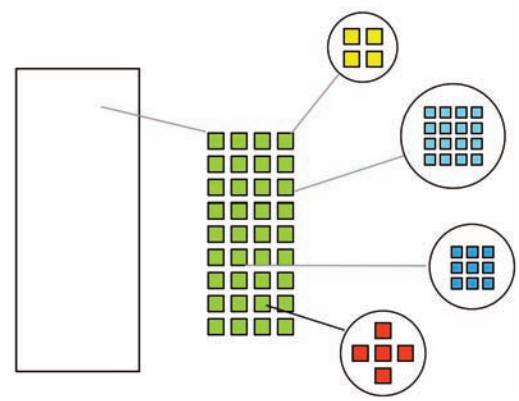
We Keep It Simple!

Features

- Smart user interfaces
- Workflow oriented wizards
- Predefined templates
- Easy adjustment
- Quick start

Wizards guide the user through an experiment in a streamlined way. Design follows function – benefit from clear user interfaces, ensuring fast training and the highest productivity.





Multifaceted specimen carriers are available for various applications; Leica Microsystems' scanning templates are easily customized.

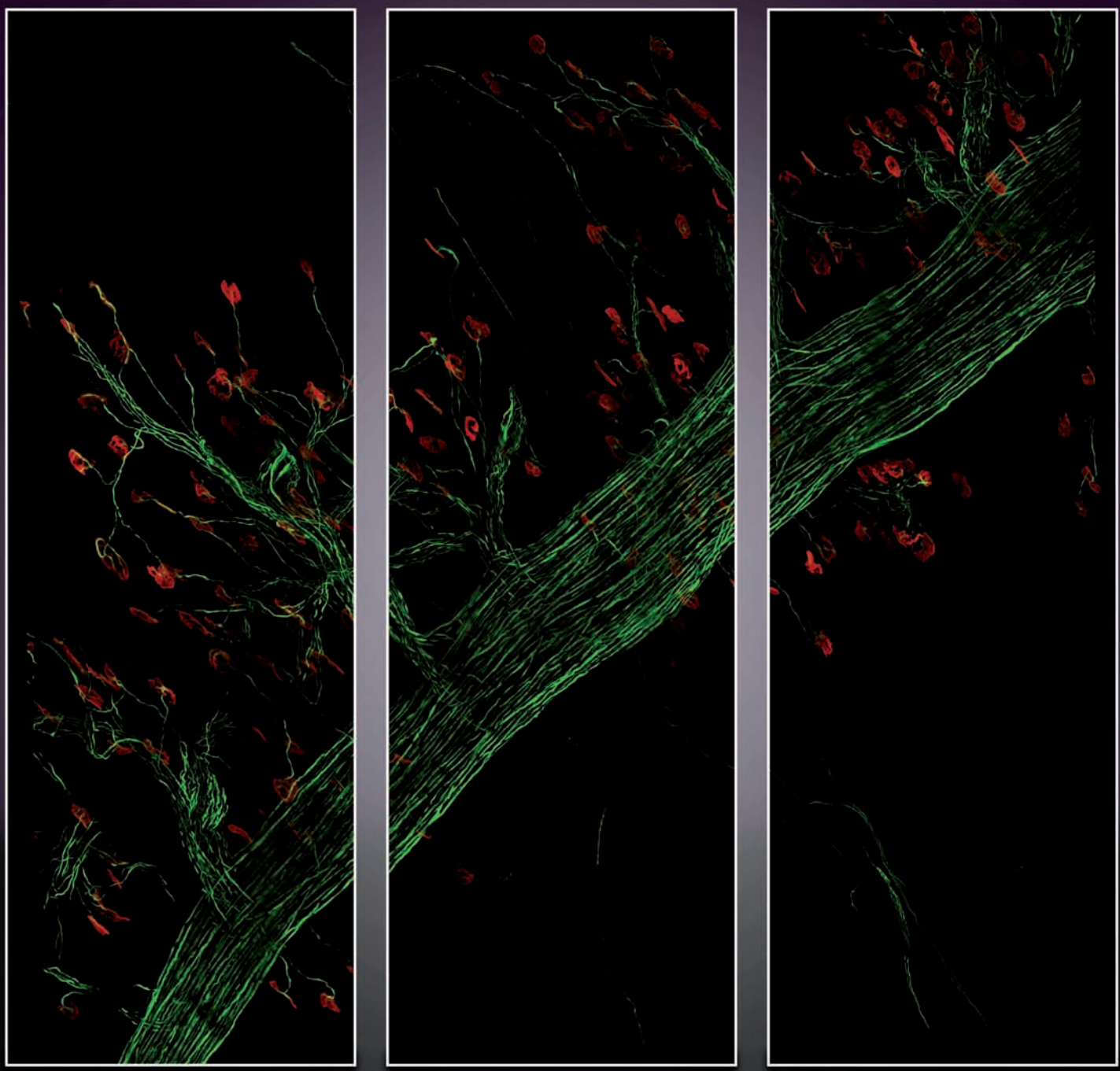
Predefined Scanning Templates

Place the specimen carrier on the microscope stage, enter the experiment ID, and move to the start point. Upload a pre-configured scanning template and fine-tune the scan job according to the experiment needs. With a click on the learn-button, all positions are automatically calculated and the experiment is ready to start.

The image data is continuously streamed on a local or network attached storage device, ready for immediate analysis.

Perfectly Timed Workflow

The operator stays in full control of the experiment and may stop or pause the screening at any time. The system provides continuous user feedback. By displaying all relevant process data on-screen, the laboratory workflow can be perfectly timed.



Mouse diaphragm muscle stained against neuro-filament 150. Mosaic: xyz: 5 x 5 x 101 images Green: Alexa Fluor 488-secondary antibody and acetylcholine receptors (Red: Alexa Fluor 647-alpha-bungarotoxin). Courtesy of Dr. R. Rudolf, Cellular Signaling in Skeletal Muscle, Karlsruhe Institute of Technology, Germany.

Automated High Content Screening Simplifies D

LAS AF software simplifies routines. Leica Microsystems' goal is to make daily work as easy as possible so researchers can concentrate on the results, not on the imaging process.

We Keep it Simple!

LAS AF MATRIX Mosaic

Fine details as well as an overview are important when evaluating experimental results. Today, simple routine tasks, such as stitching of individual images are challenging. Leica HCS A provides entirely new designed mosaic algorithms for excellent results at the push of a button.

Leica LAS AF MATRIX Mosaic generates large high content images fully automatically, providing both at the same time, overview and high resolution.

Mouse diaphragm muscle stained against neuro-filament 150. Mosaic: xyz: 5 x 5 x 101 images. (Green: secondary antibody coupled to Alexa Fluor 488) and acetylcholine receptors (Red: alpha-bungarotoxin coupled to AlexaFluor 647). Courtesy of Dr. Rüdiger Rudolf, Cellular Signaling in Skeletal Muscle, Karlsruhe Institute of Technology, Germany.

Get the Content!

LAS AF MATRIX Multiwell

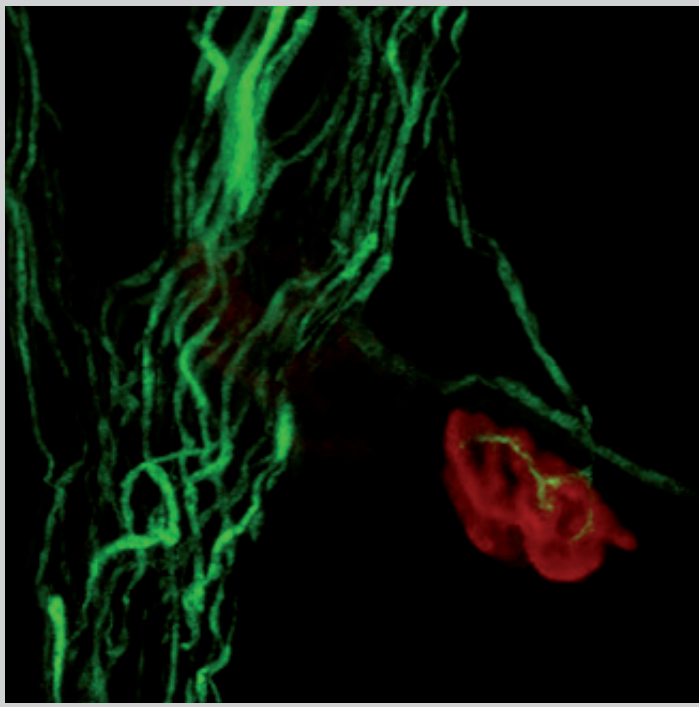
Answers to more complex questions require a larger number of sophisticated experiments. Leica HCS A supports frequently used multiwell plate formats to automatically study multi-dimensional experiments. Time resolved or concentration dependent tests unveil true biological context, more than a single snapshot.

To draw the correct conclusion, meta data is very important. LAS AF MATRIX Multiwell links meta data with the image to provide comprehensive information and enables researchers to go back to the single imaging results of each well at any time. The Leica HCS A data model secures the proper results generated from acquisition to future image analysis.

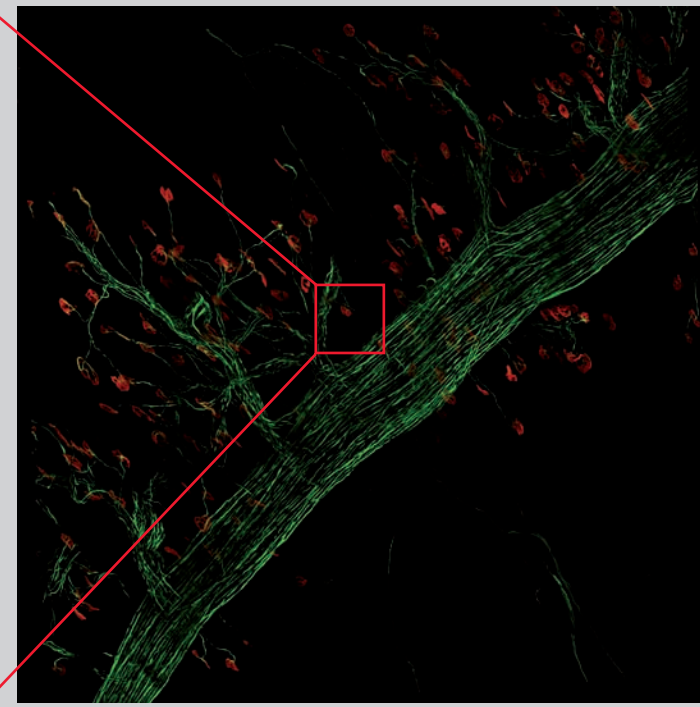
Zebrafish, *Danio rerio*, Neurogenin - GFP. H2A
Courtesy of J. Legradi, Dr. U. Liebel, KIT Karlsruhe Institute of Technology, Germany.

aily Routines

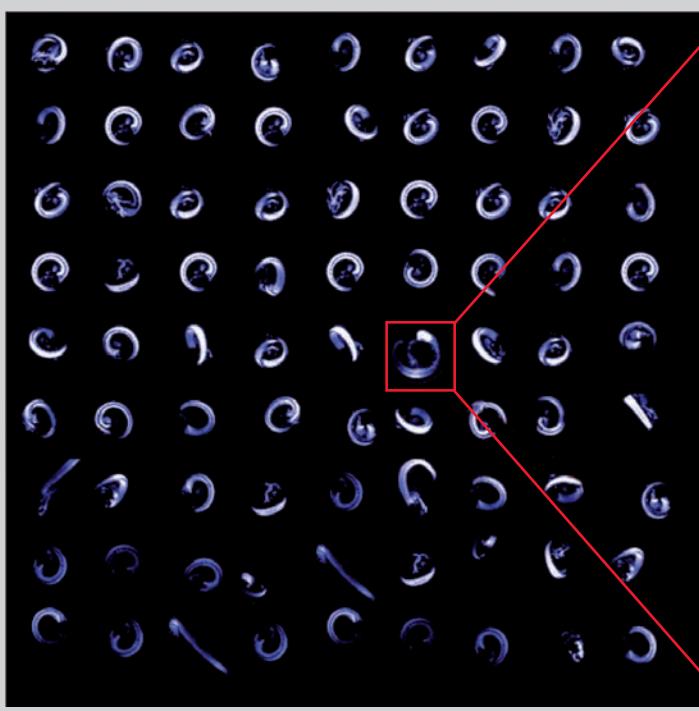
High Resolution Single Image



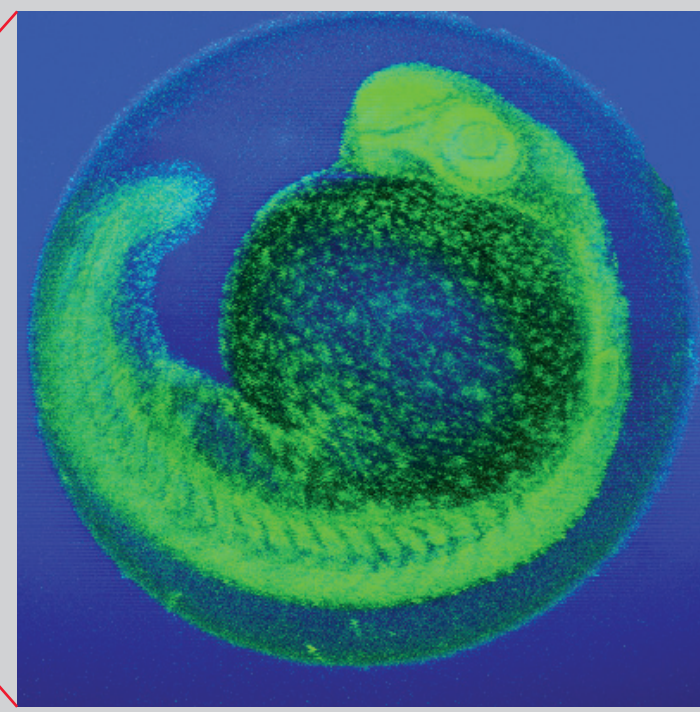
High Content Mosaic



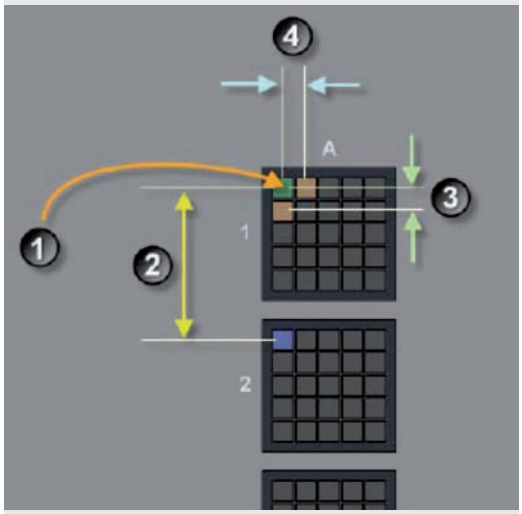
Fast Multiwell Plate Screening



High Content Information



Gain Flexibility



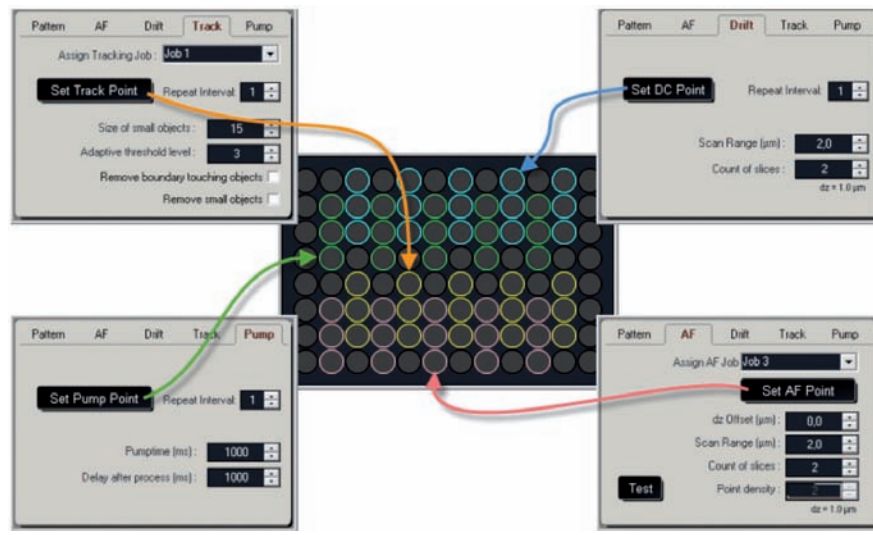
Make it fit: adjustment flexibility for scanning templates.

Flexible scanning conditions, even on the smallest scan field level, match a full variety of experiments.

Adjustable Scanning Templates
No need to be a programmer – new scanning templates can be designed at micron scale to easily fit chamber slides, multiwell plates or simple spotter arrays. Once defined, the templates are ready to use for all applications and can even be conveniently shared between laboratories or communities.

Imaging without Limits – MultiJob and MultiPositioning
Feel free to combine a variety of individual scan jobs for any area of interest of the specimen.

The MultiJob – MultiPosition function provides maximum flexibility for your experiments. Several jobs, such as low resolution prescans or multicolor 3D acquisition, can be freely combined. Zoom in and out automatically. Individual settings can be adjusted for each position. From basic routine up to the most complex experiments, Leica HCS A greatly extends the spectra of applications.



Automated color coding assigned to the wells above provides easy control of multiple scan jobs.

Trigger In – Automated Control
This function allows scan jobs immediately to start on external trigger signals. *Trigger In* provides new opportunities for external events to control internal process steps.

Water Immersion Objective Control – Never Run Dry
A sufficient supply of immersion fluid is highly important for long-term observations. Water dispense volume, timing, and position can be controlled to maintain excellent optical conditions based on environmental conditions.


Automated water immersion objective

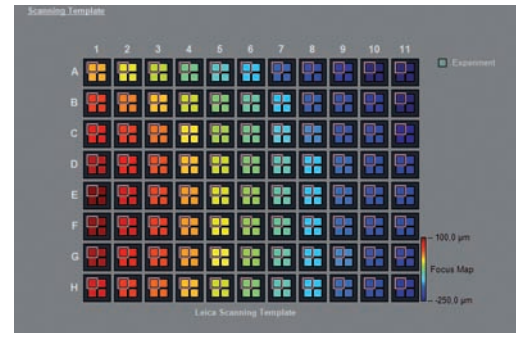
Autofocus Routines
Five autofocus algorithms are available, optimized for different setups. The suitable routine is selected from a pull-down menu. After the initial scan, the software automatically creates a focus map with true sample topology. This map is used for fast, accurate z-positioning during the scan. According to the size and planarity of the samples, the optimal number and positions of the autofocus points can be freely defined.

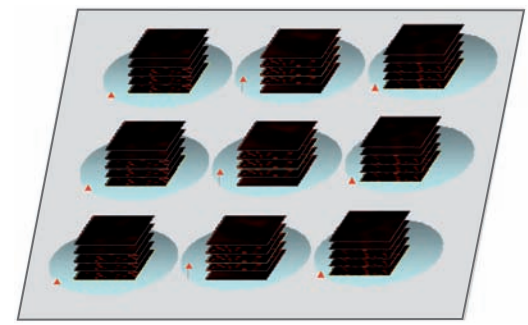
Z-Drift Compensation
Live specimens can grow in long-time measurements, changing the z-position of interest. Microscope conditions can change due to temperature shifts. The algorithm adjusts the focus independently over time and provides sharp images throughout the experiment.

Tracking Algorithm
As live organisms may change their xy-position, the center of intensity is calculated at each scan. If the single target is moving, the software automatically repositions the object of interest to the center of the objective, providing the best imaging conditions.

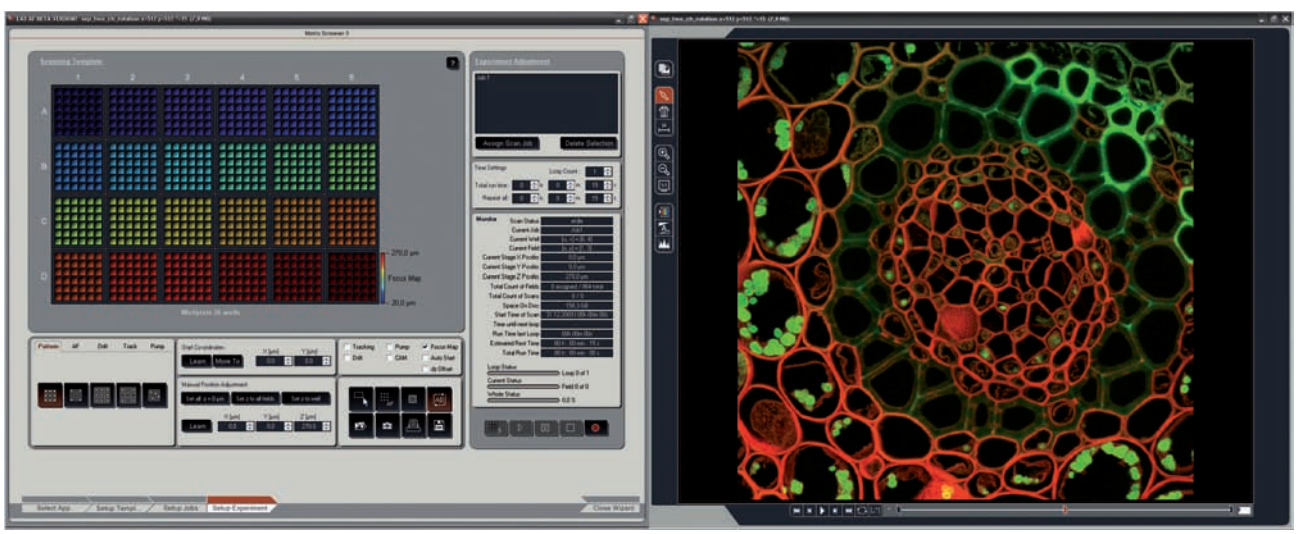
Review On-the-Fly
Data is stored at predefined locations on a local hard disk or network storage device (NAS) via a TCP/IP protocol. Experiment data flows into a ring buffer to ensure an unlimited stream of images enters the specified target folder.

The advantage: data analysis or review of image data is performed immediately. Image analysis starts when the experiment starts and provides fast results. Fast feedback loops automatically communicate with the microscope as it scans to automatically modify the scanning parameters. This enables researchers to detect rare events at the moment they happen.





Autofocus Procedure
Z-stack images are acquired at freely selectable positions. The focus positions are determined and stored in a color coded focus map.



Automated High Content Screening for Quan

Detect Rare Events On-the-Fly!

LAS AF MATRIX Developer Suite
Highly complex assay designs are now possible with LAS AF MATRIX Developer Suite. The Mitocheck project (1), conducted at the EMBL in Heidelberg, is an excellent example of comprehensive and flexible automation: The self-acting process automates mitosis identification.

Pre-Scan – Object Identification
At first, a fast, low resolution pre-scan of sample plates is routinely performed to identify the events of interest (1). After each scan, OME-image data are streamed on-the-fly to a buffer to be distributed online. The image information is stored on a network attached storage device (NAS) or server to be processed with the researcher's image analysis (2).

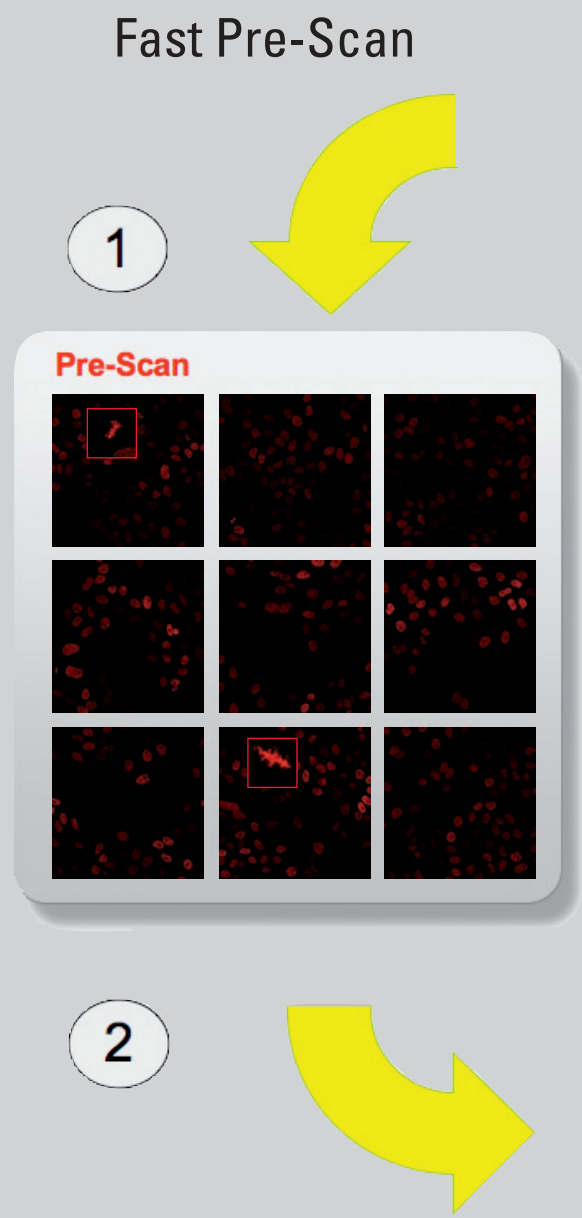
Fully automated, the pre-scan data is segmented, object features are extracted, and the target cells are classified. The object is selected and can clearly be identified by the meta data of the confocal microscope (3). The position of interest is reported via a network protocol to the Computer Aided Microscopy (CAM) interface, which now automatically starts additional imaging protocols (4).

High Content – Object Scan
In the second phase, high resolution scans start to acquire time resolved behavior of the target cells only at the reported positions. The imaging mode automatically switches from pre-scan to high resolution acquisition. Now, multiple z-positions and additional channels are applied. Only the positions marked during the pre-scan are scanned. After each cycle, a pre-scan is repeated to find new cells switching into mitotic phases, and the process starts again.

The method is highly efficient: all mitosis events can be identified and scanning of non-target cells is avoided. Toxic bleaching of cells that are not in the mitosis phases is minimized. Thus, scanning speed is maximized at reduced laser exposure.

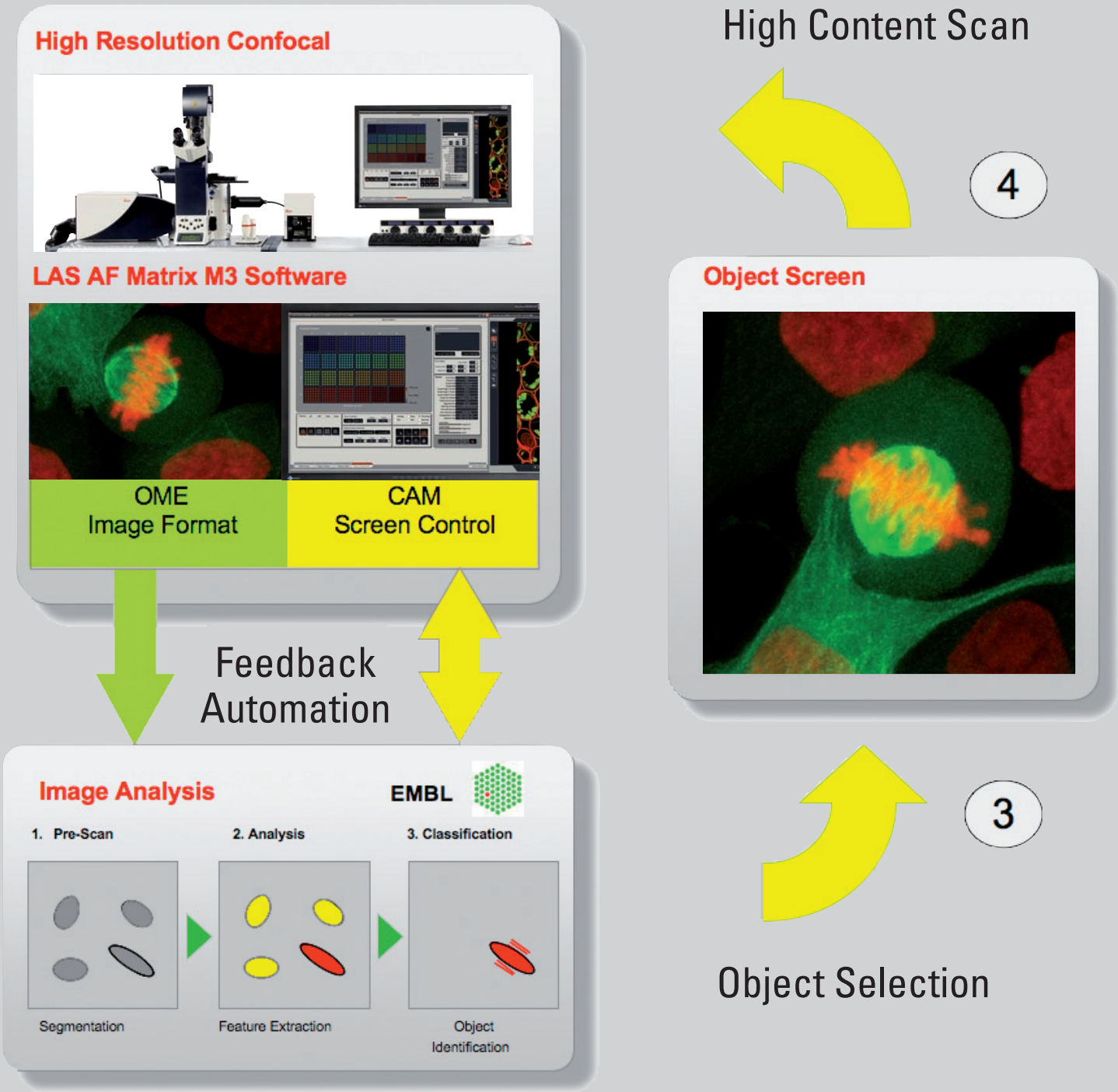
Microtubule secondary screen: scrambled sRNA Tubulin (green), H2B (red). Leica TCS SP5. Objective: 63x oil (pre-scan); zoomed, maximum projection: 30 x 0.4 µm slices, 2 channels (high resolution). Courtesy of Christian Conrad, EMBL, Heidelberg, Germany.
(1) Mitocheck Project: www.mitocheck.org.

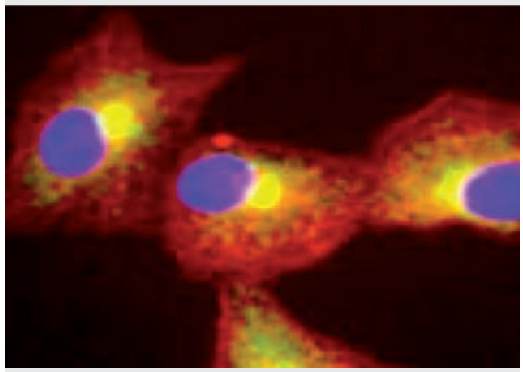
High Content Screening wi

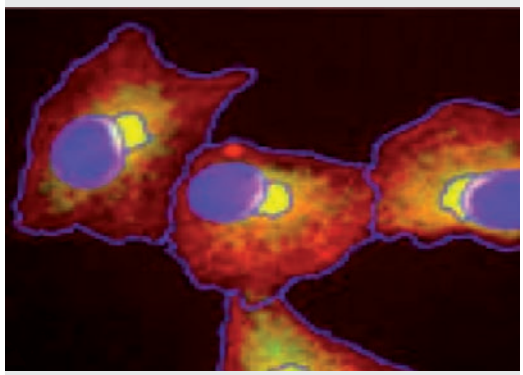


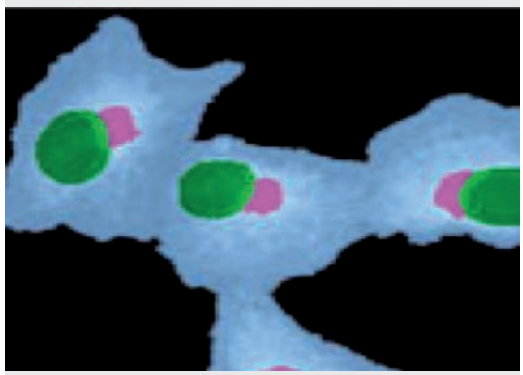
titative Assays

th Interactive System Control: Fully Automated Mitosis Acquisition









Automated image analysis of multiple assay by DEFINIENS Cellenger®, Courtesy of Dr. R. Pepperkok, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

The Perfect Match

Data Interfaces

Open architecture for truly platform independent information exchange in an interactive environment – this is the goal attained by the new Leica HCS A data model.

Experiment Meta Data Administration
Experiment IDs, description, and meta data can be entered manually or by barcode. Additional experiment information can be added to the existing XML meta data file by external programming to provide comprehensive result data sets.

Platform Independent Results Distribution
Leica HCS A imaging formats can be used platform independent on Apple MAC™ OS, Microsoft Windows® or LINUX[1] platforms. The new Data Exporter provides OME-TIFF image files automatically, containing binary image data plus XML meta data structure.

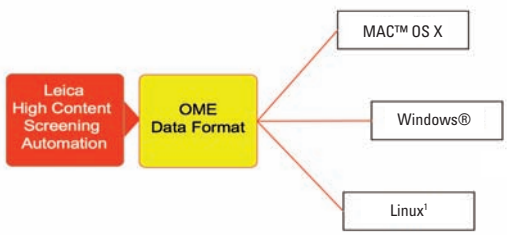
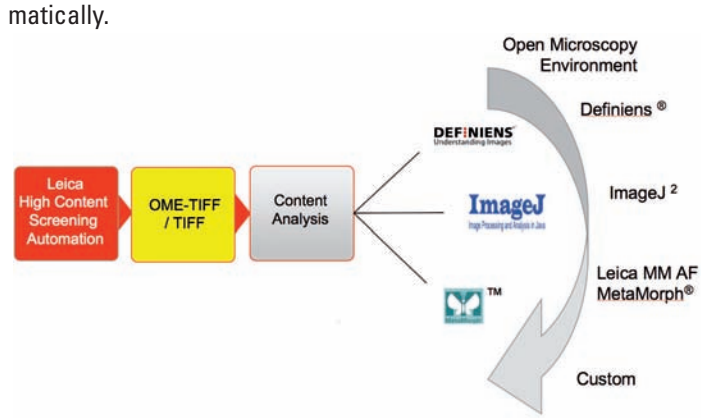


Image Analysis

Leica HCS A export formats are easily imported into all modern image analysis solutions such as e.g. DEFINIENS Cellenger®, ImageJ[2], or MetaMorph®. Ensuring full compatibility to modern analysis platforms provides new options for target recognition, analysis and decision-making. Researchers benefit from applying existing algorithms or may create new ones to analyze data automatically.

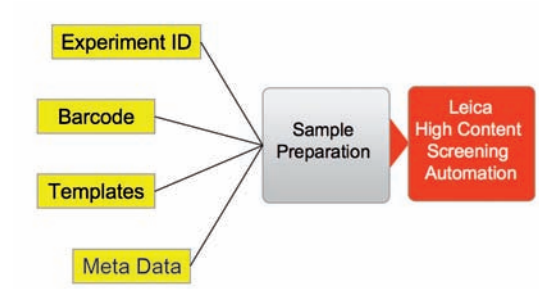


Modern analysis tools offer solutions to identify, count, and measure target cells to obtain statistically relevant results. In addition, DEFINIENS Cellenger® quantifies relationships between target objects even in 2D or 3D. End users are free to choose among local, server-based, or clustered node analysis methods to maximize throughput and efficiently achieve high content screening results.

Transparency of High Content Data

Data Model

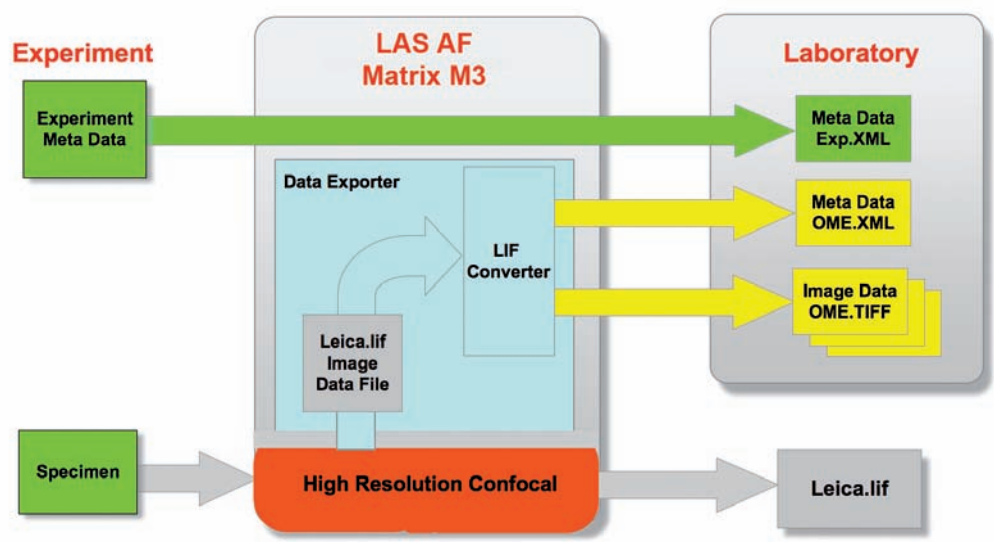
In the past, the original imaging and meta data had to migrate through a myriad of different conversion formats before ending up in a condensed Excel or Word document. Loss of data due to conversion is a problem of the past. The Leica export format follows the conventions of well-defined and well-formed structures, and can be read by all modern software platforms. Data conversion is no longer necessary. Transformation problems, data mix-up or transcription errors are avoided and processing time is saved. Additionally, even meta data can be combined with the results of Leica HCS A using external programs.

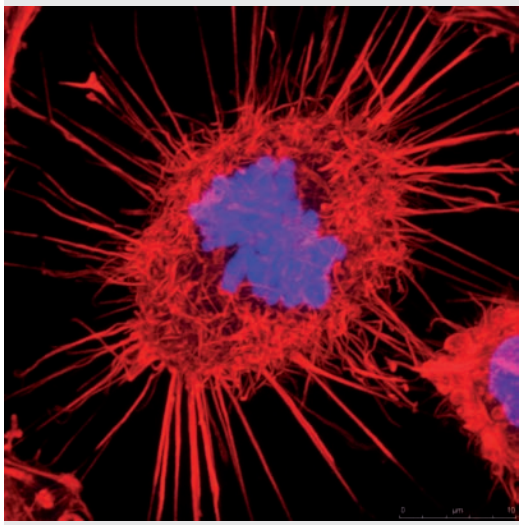


With Leica LAS AF MATRIX M3, researchers now respond to questions quicker as a clear picture of the experiment and result data is always provided. For the entire research chain from sample preparation to confocal parameters to future image analysis data, never lose any information within this scalable data model.

Easy Collaboration - The Power of Networking
Collaborative sharing of data is the key to understanding the complete content. Leica HCS A evolves its full power in a network environment. Information is exchanged in a LAN via basic TCP/IP protocols from the imaging system to network attached storage and analysis tools. Collaborative sharing of scientific data between laboratories in your facility provides an extensible, time saving way to achieve fast results.

Data Model:





Fibroblasts Nuclei (DAPI, blue) and Actin (Phalloidin-TRITC red). Courtesy of Dr. G. Giese, MPI for Medical Research, Heidelberg, Germany.

Annotations:
MAC™ OS X is a registered trademark of Apple® Inc. Windows® is a registered trademark of the Microsoft® Corporation. (1) Linux is a free Unix-type operating system originally created by L. Torvalds with the assistance of developers around the world.

Definiens® is a Registered Trademark of Definiens AG. (2) ImageJ is a public domain Java image processing program inspired by National Institutes of Health, NIH Image for Windows®, Mac™ OS, Mac™ OS X and Linux. MetaMorph® is a Registered Trademark of MDS Analytical Technologies. Huygens Professional® is a Registered Trademark of SVI Scientific Volume Imaging.

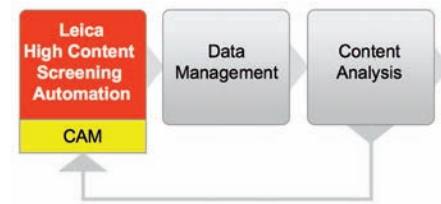
(3) Open Microscopy Environment (OME) is a multi-site collaborative effort among academic laboratories and a number of commercial entities that produces open tools to support data management for biological light microscopy. Designed to interact with existing commercial software, all OME formats and software are free, and all OME source code is available under GNU public copyleft licenses. OME is developed as a joint project between research-active laboratories at the Dundee, NIA Baltimore, and Harvard Medical School and LOCI.

LabVIEW™ is a registered trademark of NI National Instruments Inc. MATLAB™ is a registered trademark of The MathWorks™, Inc. Java™ is a registered trademarks of Sun Microsystems, Inc. C++ is a programming language standardized by ISO. C# is a programming language developed by Microsoft, Inc.

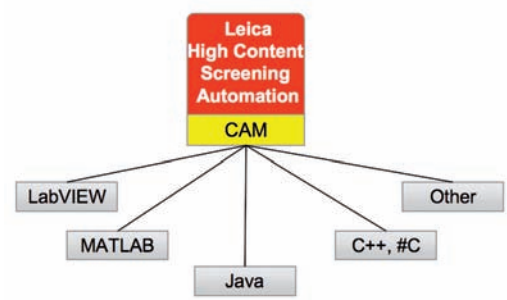
The Benefit of Integration – Excellent Results
Leica HCS A provides full connectivity within every laboratory environment by overcoming format barriers. Using existing software solutions saves time and money. Implementation is fast and ensures excellent research results for high content experiments.

Automation Control via LAN

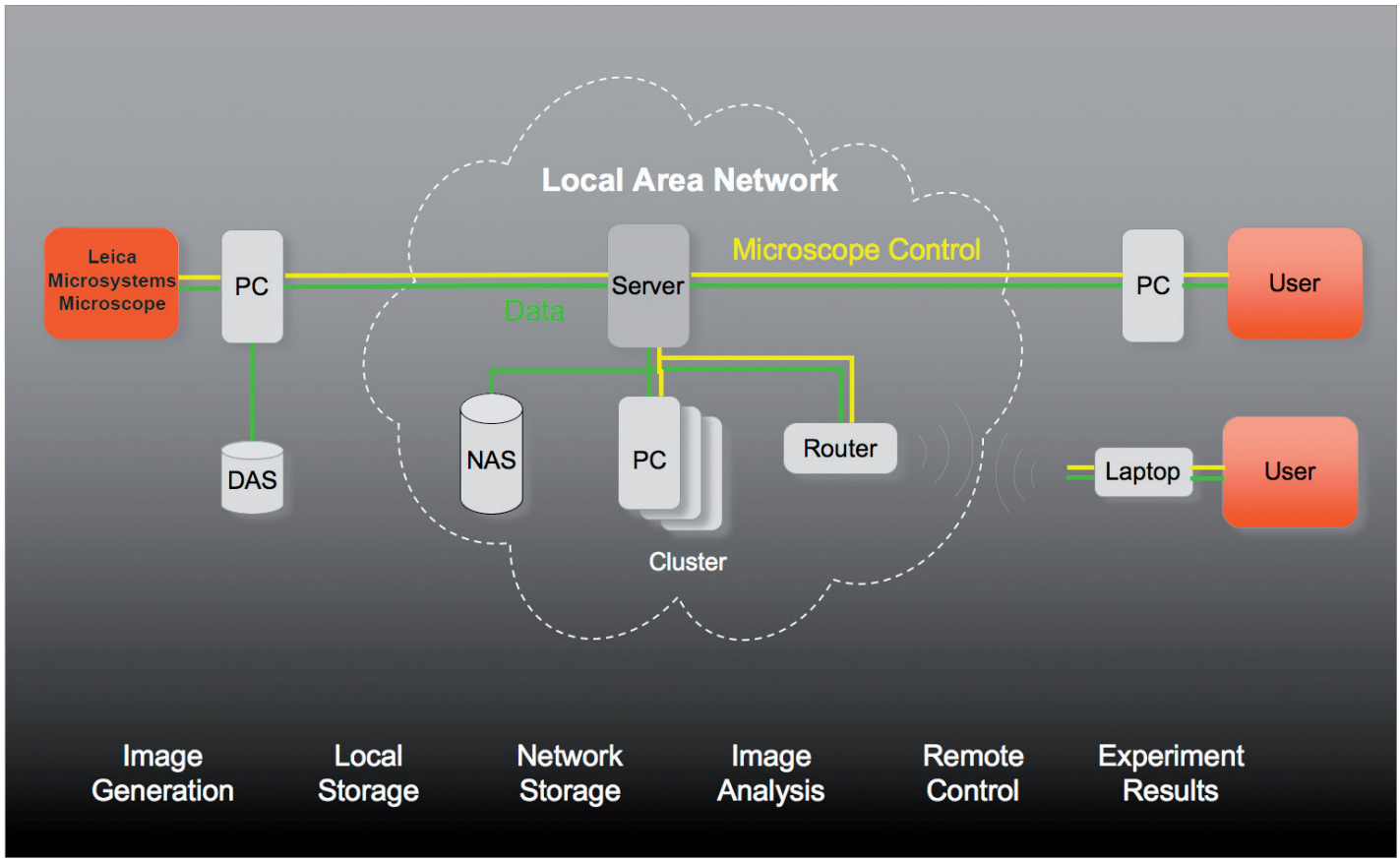
Computer Aided Microscopy – Customize Your Imaging System
Active interaction, based on clear decisions is the key for success in science. Computer Aided Microscopy (CAM) provides the tool to immediately control a confocal instrument by full automation. A few, easy-to-learn commands are sufficient to get you started.



Get the Power
The new CAM-interface offers remote control of confocal systems, such as the Leica TCS SP5, TCS SPE or TCS LSI by LabVIEW, MATLAB or script based programming languages, for example. Individual imaging jobs are started interactively and fast, based on the decisions of image analysis, external trigger events or time loops.



Immediately after image acquisition, the data streams into the analysis tools for processing. A moment later, target cells are clearly classified and marked by the spatial position. Following the program, the instrument may now start a zoom-in or high resolution scan for more detailed observation. Due to the high speed of the process, even rare events are no longer lost.

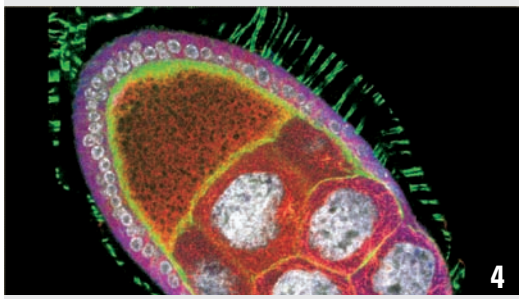


Overcome the limits of static experiments and immediatelly start individual imaging processes based on the results of image analysis. Unbiased algorithms perform objective target selections and automatically perform statistically relevant screens. Leica HCS A provides all the tools needed for excellent high content screening results.

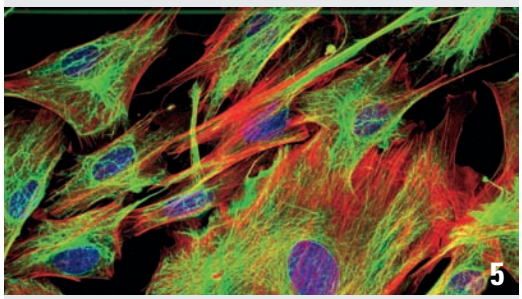
System Administration

Leica HCS A evolves to its full power in a network environment. The systems follows standard rules of LAN administration to best fit into the facility's IT structure. The local administrator is always in full control of the system as LAS AF MATRIX M3 uses assigned permissions.

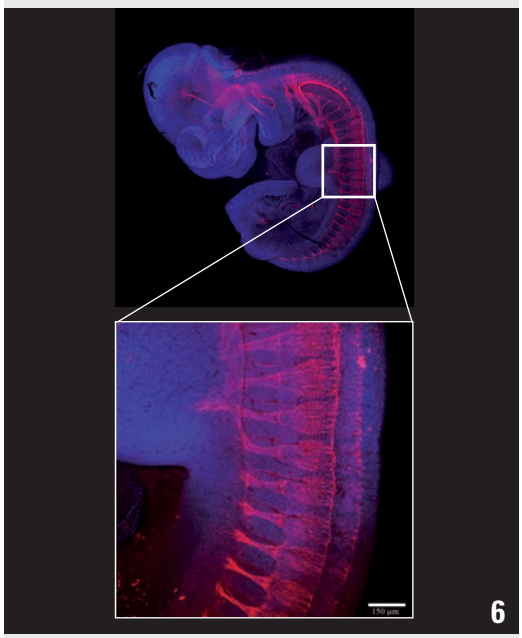
Standard remote system tests can be started by any user to ensure uptime and reproducible results. Additionally, Leica Microsystem offers technical service support by RemoteCare© instrument diagnosis via a secure internet protocol to minimize downtime.



Drosophila, Leica TCS SP5



Fibroblasts, Leica TCS SPE



Mouse embryo, Leica TCS LSI

Powerful Platforms

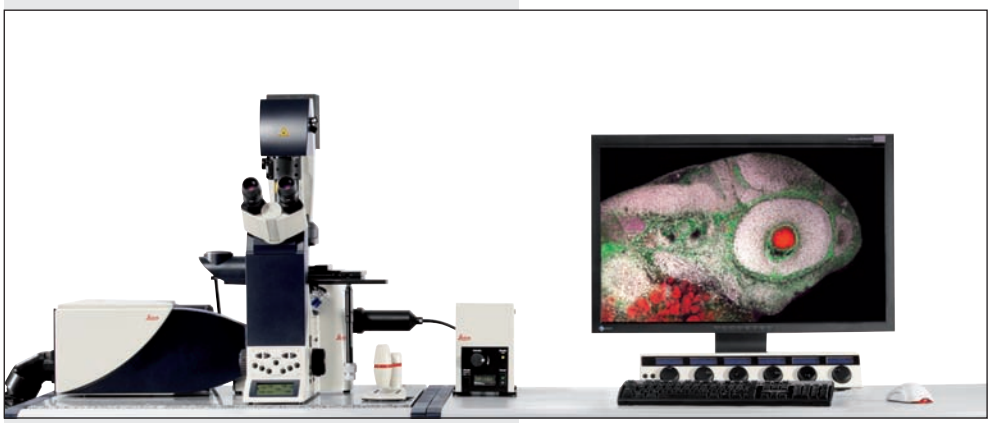
Hardware for High Resolution Imaging

Leica Microsystems, known for excellent high resolution image quality, offers three instrument platforms for automated high content screening. Choose the best tool for your applications.

Leica TCS SP5
The only broadband confocal is a universal high-speed platform for parallel multi-channel micro imaging. Leica Microsystems' AOBS (Acousto-Optical Beam Splitter) technology provides full spectral detection and highest transmission.

Leica TCS SPE
The compact, robust confocal system is cost-effective and extremely easy to use. The system performs sequential multi-channel micro image acquisition analysis up to eight colors. A special glass prism and precise spectral selectors provide maximum spectral efficiency.

Leica TCS LSI
The platform provides high resolution imaging from micro to macro. The combination of confocal and optical zoom offers maximum flexibility for specimens up to 16 mm. Sequential multi-channel micro plus macro image acquisition analysis up to eight colors. A special glass prism provides the transmission for spectral analysis.



Leica TCS SP5

Leica TCS SPE

Gain Scientific Advantage

Intelligent Automation

Leica HSC-A Platforms	TCS SP5	TCS SPE	TCS LSI
Content Resolution	●●	●●	●●
Experiment Flexibility	●●	●	●●
Imaging Speed	●●	●	●
Ease of Use	●	●●	●
Micro Imaging	●●	●●	●●
Macro Imaging			●●

The Leica HCS A technology provides an excellent platform for fast, efficient high content screening results.

Leica combines highest resolution for all specimen sizes with LAS AF MATRIX M3 automation software. Maximum application flexibility for all automation levels creates unrivaled freedom for experiment designs of today and tomorrow.

Standardized interfaces integrate Leica HCS A platforms with every laboratory environment in an optimal way.

Open, well-defined architecture and Open Microscopy Environment (OME) formats are compatible with many image analysis technologies. The perfect match between scalable export formats and platform independent device control via Computer Aided Microscopy (CAM) interfaces creates far more value than the sum of each component's benefits. Obtain more results in shorter time – efficiently and statistically verified.

Gain scientific advantage from Leica HCS A for high content screening and amplify the power of imaging.

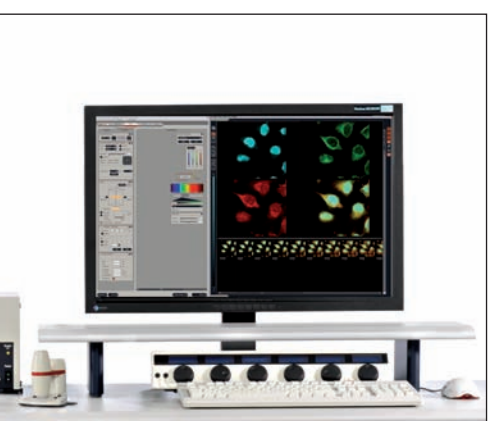
Acknowledgements
We gratefully acknowledge the scientists cited in the brochure and below for providing images:

1, 4 *Drosophila melanogaster* (egg chamber)
Green: Actin, Alexa 488-Phalloidin; Red: Cortex, Egalitarian; Red Blue: hnRNP, Cy5; Grey: Nuclei, DAPI.
Courtesy of Sonja Lopez de Quinto, Florence Besse and Oliver Hachet, EMBL, Heidelberg, Germany.

2, 6 Mouse embryo
Nuclear and neurofilament staining of mouse embryo (10.5 days post coitum). Courtesy of ICI, Imaging Center of IGBMC, Strasbourg, France.

3 COS 7 cells
Green: uncharacterized protein, GFP; Red: α-Tubulin, Cy3; Blue: Nuclei, DAPI. Courtesy of Prof. Wei Bian, Cell Research Center, Institute of Biochemistry and Cell Biology, SIBS, CAS, Shanghai, China.

5 Mouse fibroblasts
Green: F-Actin, FITC; Red: Tubulin, Cy5; Blue: Nuclei, DAPI. Courtesy of Dr. Günter Giese, Max Planck Institute for Medical Research, Heidelberg, Germany.



Leica TCS LSI

“With the user, for the user”
Leica Microsystems

Leica Microsystems operates globally in four divisions, where we rank with the market leaders.

• Life Science Division

The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems’ customers at the leading edge of science.

• Industry Division

The Leica Microsystems Industry Division’s focus is to support customers’ pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

• Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

• Medical Division

The Leica Microsystems Medical Division’s focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life**.

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