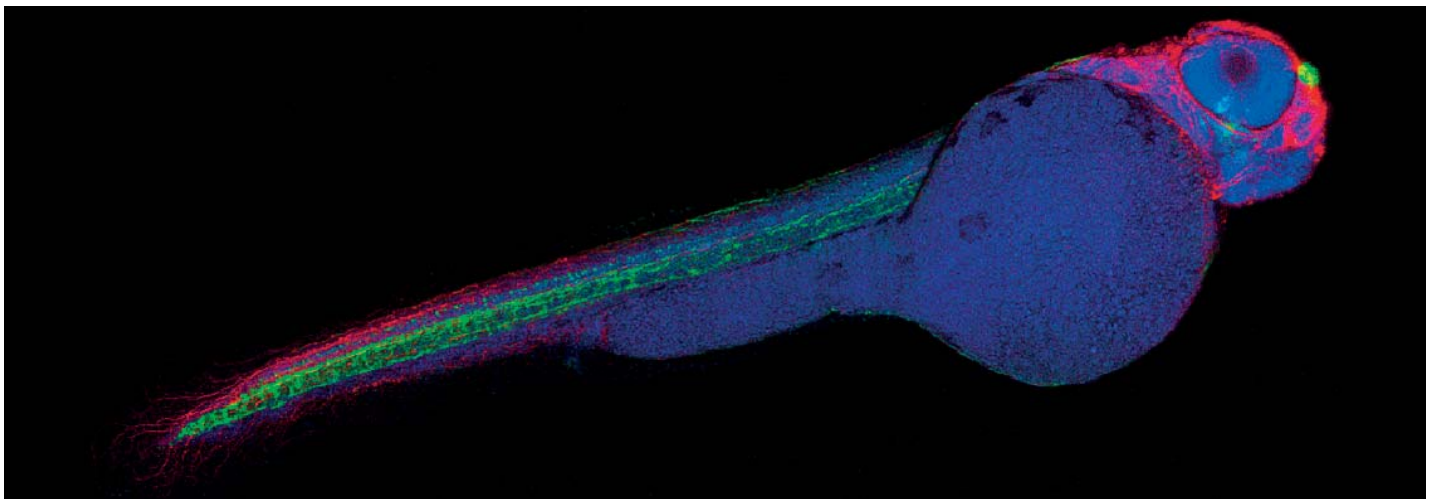
Leica HCS LSI

Explore the New Dimensions of Imaging

Zebrafish High Content Screening Automation

Living up to Life

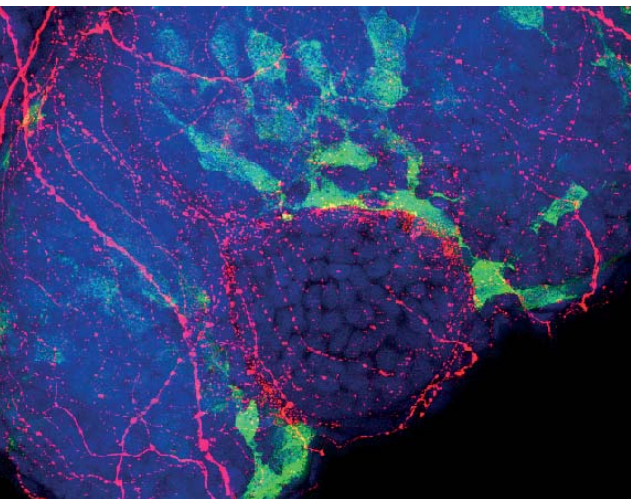
Leica
MICROSYSTEMS



Zebrafish *(Danio rerio)*

- High content screening in 4D by true confocal imaging
- From micro to macro with adaptive zoom optics
- Maximum flexibility for assay development
- Easy operation for fast results

Leica Design by Christophe Apothéloz



Zoom in on details: the Zebrafish eye

Zebrafish (*Danio rerio*) is a versatile model organism for a variety of assays: Embryos are transparent, organs develop rapidly, and embryogenesis is complete after 72 h. After a short generation cycle hundreds of eggs are present. Its high genetic homology to human makes *Danio rerio* a model for developmental studies, pharmacokinetics, and toxicological tests in fundamental research, biotechnology, and pharmacology.

Leica Microsystems introduces an innovative application solution to discover complex pathways from egg to whole organism *in vivo* at the highest resolution. Leica HCS LSI overcomes time-consuming microscopy work by intelligent automation and provides high content information from individual experiments to *in vivo* multi-well analysis in four dimensions.

Leica HCS LSI

Explore the new dimensions of imaging

With Leica HCS LSI, researchers can discover evolution at the highest resolution using true spectral confocal technology. Brilliant images reveal novel context details as the innovative optical zoom freely adapts to your field of interest.

Dynamic imaging meets intelligent high content screening automation: Explore new dimensions in life science and autonomously run multi-experiment analysis of large specimens in 3D and 4D.

Easily apply basic 3D scans and build digital models or explore spatio-temporal dynamics of organs during embryogenesis in extended 4D experiments. Time-saving routines generate unbiased data of statistical relevance that are transferred to automated image analysis.

Overcome any limitation by designing your individual screening automation protocol and perform image analysis on-the-fly. Apply remote control and link the image analysis to the CAM (Computer Aided Microscopy) programming interface: Optimize the screening process when it runs.

Leica HCS LSI offers the ultimate flexibility in acquisition, automation, and analysis. This application solution facilitates both the individual experiment and the upscaling to multi-dimensional models during assay development.

The fully automated Leica HCS LSI is a versatile, easy-to-use application solution from micro to macro scale that adapts to the high content screening automation needs of today and tomorrow.

Explore the New Dimensio

Leica HCS LSI – the application solution

Research Question → Experiment Design → Specimen Preparation → Le High C Scre Autor

Leica Microsystems combines maximum imaging flexibility and high content screening automation for multi-scale application assays.

A perfect start – Excellent images for optimal results
Leica HCS LSI true spectral confocal imaging answers questions in modern biology with comprehensive high resolution results: crystal clear images that yield maximum information.

Across the limits – Maximum flexibility for in vivo assays
Seamlessly adapt the optics and perfectly match every area of interest. Adjust the field of view up to 16 mm on demand – without objective change. Leica Microsystems' high quality macro objectives offer optimal conditions for *in vivo* analysis of large specimen. Precise, extended z-stacking capability makes perfect 3D experiments easy.

Choose the best – Confocal and widefield in one instrument
Freely choose the screening mode that best fits your experiment. Benefit from high resolution single point scanning confocal, or tune up to fast single color camera scans – multi z-acquisition is supported by both applications.

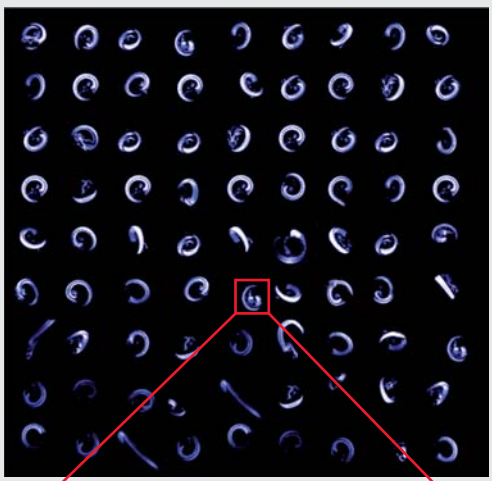
Discover the ease of use – Facilitate operation and save time
Eliminate extensive specimen preparation and insert the specimen in seconds – the system easily adapts to the imaging needs. Large working distance objectives and the spacious work area simplify plate handling. Complete automation allows optimal tuning and facilitates operation for reproducible results.

High content screening automation – From micro to macro, in 4D
Intelligent automation converts Leica HCS LSI into a full-featured screening robot. Apply template automation via a mouse click or design individual setups with computer-controlled zoom adjustments. Create MultiPosition-MultiParameter experiments with auto-focus and drift compensation quickly and easily – there is no need to be a programmer.

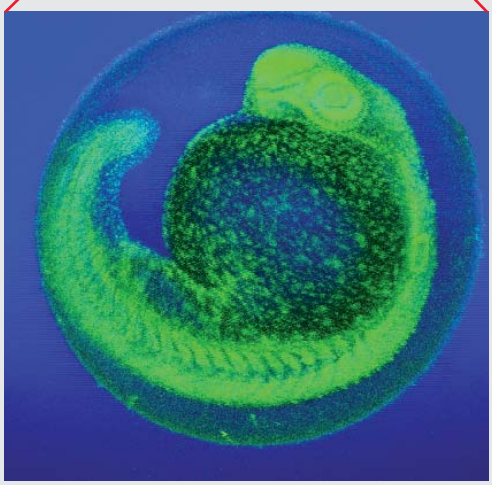
From egg to embryo

- High resolution confocal
- Micro and macro imaging
- Flexible system automation
- Open architecture
- Comprehensive image analysis

Multiwell plate screening



High content information



Zebrafish, Neurogenin – GFP. H2A (left). Courtesy of J. Legradi, U. Liebel, KIT Karlsruhe Institute of Technology, Germany.

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Data Management

Content Analysis

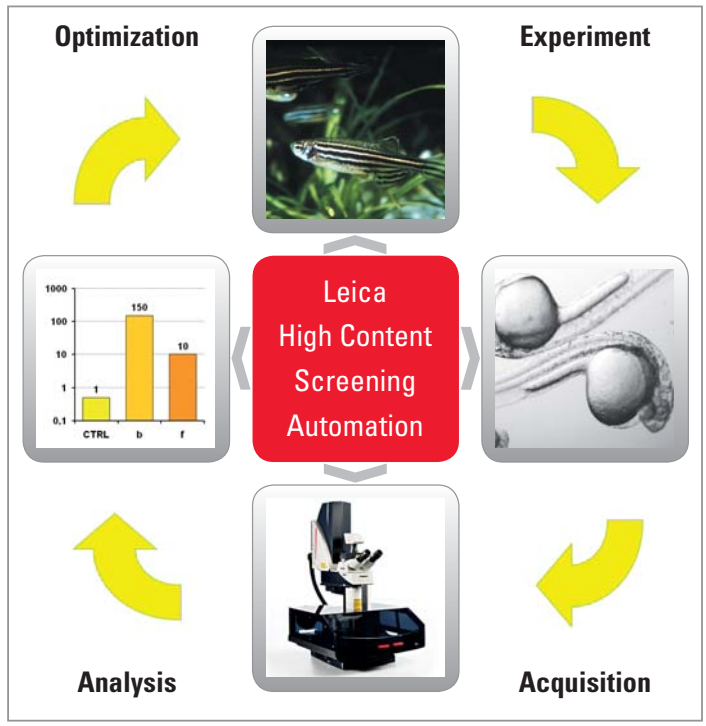
Research Result

Run unique experiments and easily apply new multi-scale assays. Leica HCS LSI is the first system that provides high content screening automation for large specimens in 3D and 4D.

Increase efficiency – Pre- and secondary scan in one system
Avoid empty data sets by automatically centering the objects of interest before the experiment runs. Scientists benefit from fast pre-screens to identify target objects on-the-fly. After this, high content data is automatically generated by zooming in, increasing the resolution, and multiplying the number of optical z-slices. The researcher's image analysis tools identify targets within the specimen, interact with the Leica HCS LSI via the CAM (Computer Aided Microscopy) programming interface, and automatically toggle between pre- and secondary scans.

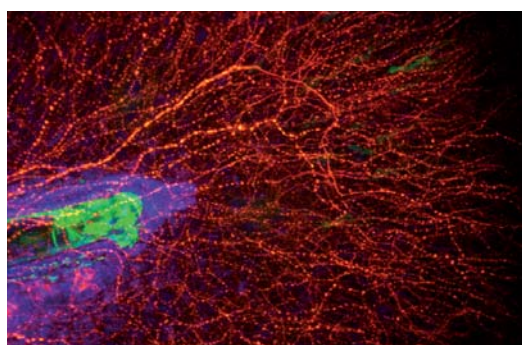
Quantify results – From image to data in multiple dimensions
Benefit from the power of open interfaces: As Leica HCS LSI creates Open Microcopy Environment OME.TIF data, existing image analysis algorithms can be used efficiently to save time and costs. Or, apply the novel approaches of Definiens' object recognition technology and analyze 3D volumes over time. The Leica HCS LSI is a single source application solution that combines high resolution with maximum flexibility and high content automation.

From embryo to results



The cycle of assay development: image acquisition, automation, and analysis create reproducible data.

High resolution for high content



Zebrafish, *Danio rerio* adult in native environment (left). Blood vessels in the fin at high resolution (right). Courtesy of D. Hentsch et al., IGBMC, Illkirch, France.

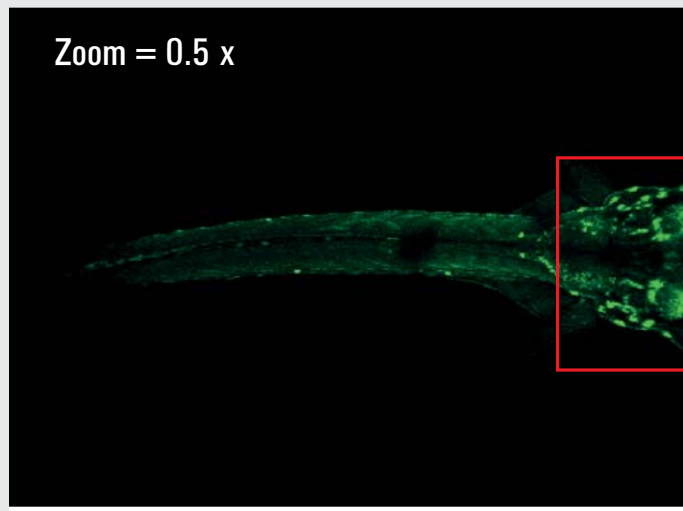
Explore the New Dimensions of Imaging

Leica HCS LSI – the system that adapts to the experiment

Zoom in: Automated 3D experiments

Zebrafish brain, *in vivo* imaging
Transgenic embryo, *Danio rerio*; GFP; Epithalamus; Optic Tectum. The images illustrate the zoom flexibility of Leica HCS LSI: The optical zoom is used first, than a combination of optical and confocal zoom is applied. The maximum projection of a 3D stack using a Leica Z6 APO A optical zoom and 5x objective is displayed.

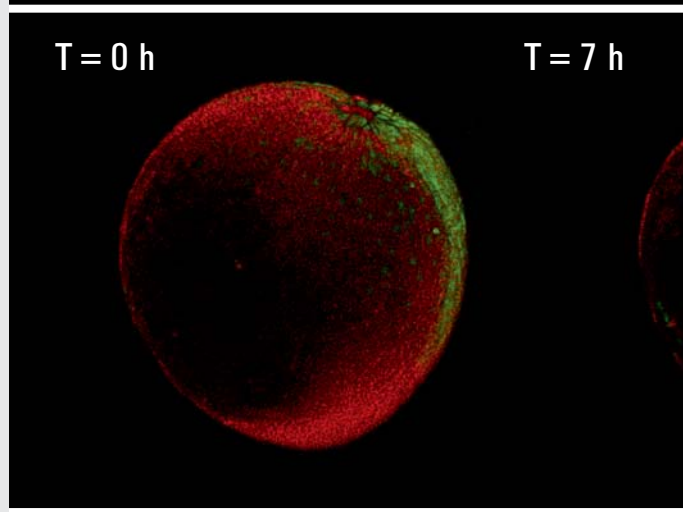
Courtesy of K. Palma, N. Guerrero, L. Armijo, ML. Concha, Laboratory of Experimental Ontogeny (LEO), S. Härtel, Laboratory of Scientific Image Analysis (SCIANLAB). Anatomy and Developmental Biology Program, ICBM, Faculty of Medicine, University of Chile, Santiago, Chile.



Advanced time lapse: 4D single well experiment

Zebrafish development
Tracking the development of life over time offers exciting insights for embryogenesis – all in the same specimen. From egg to embryo, obtain exciting views of organ development with Leica HCS LSI software and see the backbone formation during the growth of a zebrafish.

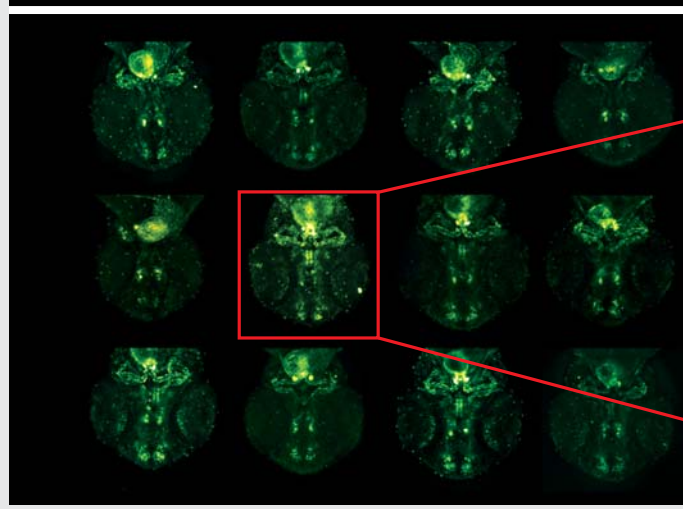
Zebrafish, novocord development, *Danio rerio*. Red: Rhodamine-dextran. Green: GFP, labeling of the notochord. Courtesy of Sophie Dal-Pra, Team B&C Thisse, Imaging Centre of IGBMC, IGBMC, Illkirch, France.

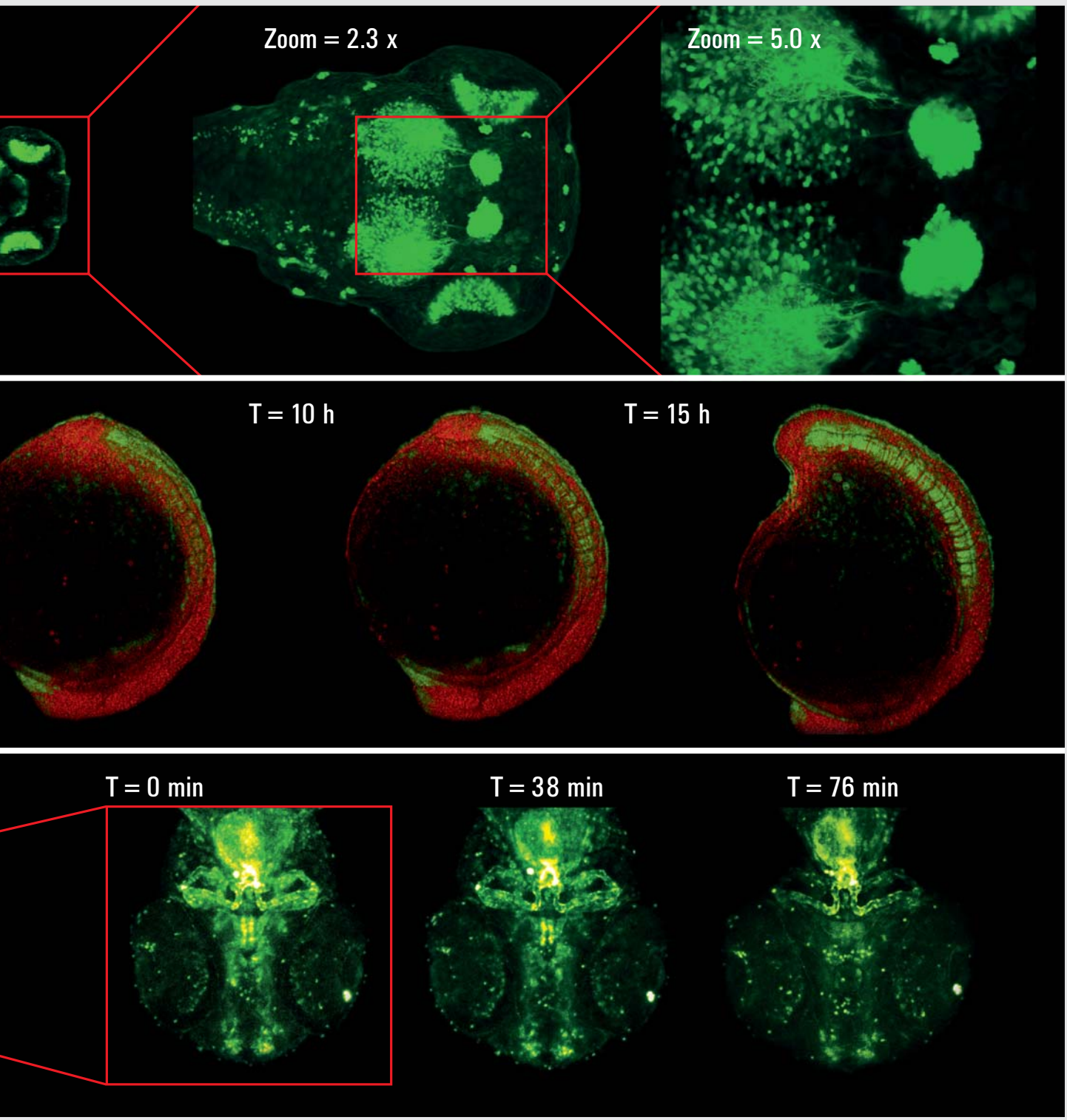


High content screening in 4D: The multiwell assay

Brain research
Automated 4D imaging discloses the spatio-temporal dynamics of fluorescent signals throughout development. 4D series of 2 day old zebrafish embryos (*Danio rerio*). Ventral views of embryonic brains of the ETvmat2:gfp stable transgenic line were automatically acquired every 38 min. GFP: Neurons in the brain and expression in heart and blood vessels. The embryos were arrayed in 96-well plates. The regions of interest (brains) were manually selected using the Leica Mark & Find mode and subsequently imaged at high resolution.

Courtesy of J. Gehrig, KIT – Karlsruhe Institute of Technology, Institute of Toxicology and Genetics (ITG), Karlsruhe, Germany.

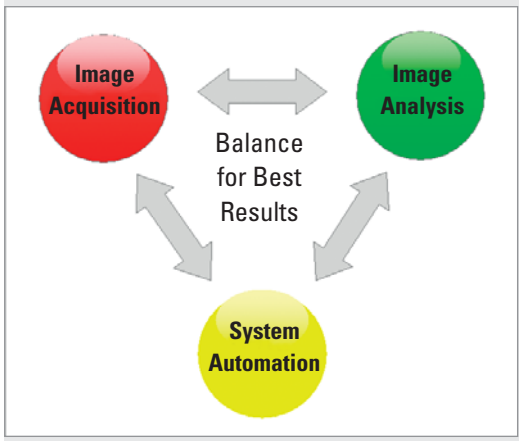


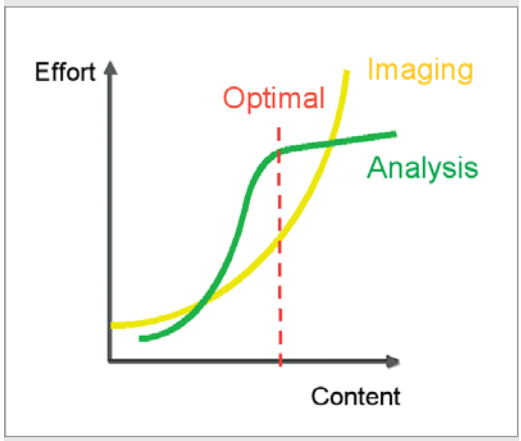

Zoom = 2.3 x
Zoom = 5.0 x
T = 10 h
T = 15 h
T = 0 min
T = 38 min
T = 76 min

Maximum Flexibility for Efficient Results

Balance for the best results

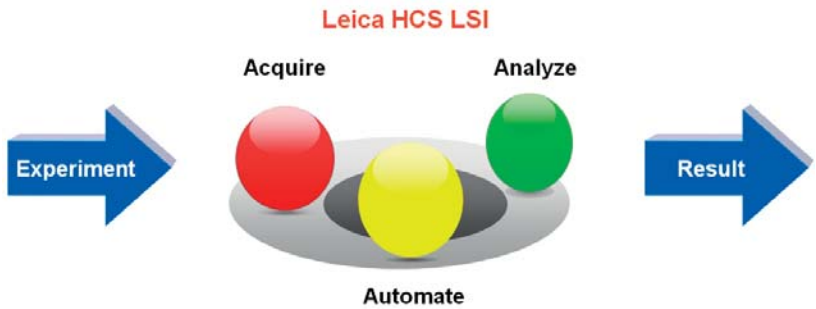
- Optimize image acquisition
- Choose the ideal automation level
- Automatically analyze details
- Choose the optimal parameter balance for efficient results





Optimize your screening results with Leica HCS LSI
High content screening is always a balance between experiment effort and desired results. It is not always about imaging speed but rather the smooth coordination of all elements, and finally the time to result. Good experiment quality is the first step to good results.

Basic research questions can be rapidly solved at lower resolutions, resulting in faster data analysis. For more complex experiments, the acquisition time, side effects on living samples, data storage capacities, and processing time need to be well balanced.



Leica HCS LSI offers the flexibility needed to ideally tune acquisition, analysis, and automation; and to efficiently gain optimal results.

Multi-scalar image acquisition
The hardware offers sensitive spectral detection that is freely tunable to optimize signal and minimize bleaching. The image size is optimally sized to the region of interest, avoiding excessive data storage without content. Three different z-modes add to the benefits of the system. For very fast experiments, the camera mode is always available.

Intelligent screening automation
The Leica Matrix M3 high content screening automation software fully automatically controls all components. Design individual programs for full control. Easily adapt the system to individual assays.

Adaptive image analysis
Image analysis software converts data into results. The existing solutions or the comprehensive Definiens Developer XD platform provide freedom and flexibility for excellent image quantification and statistically relevant results.

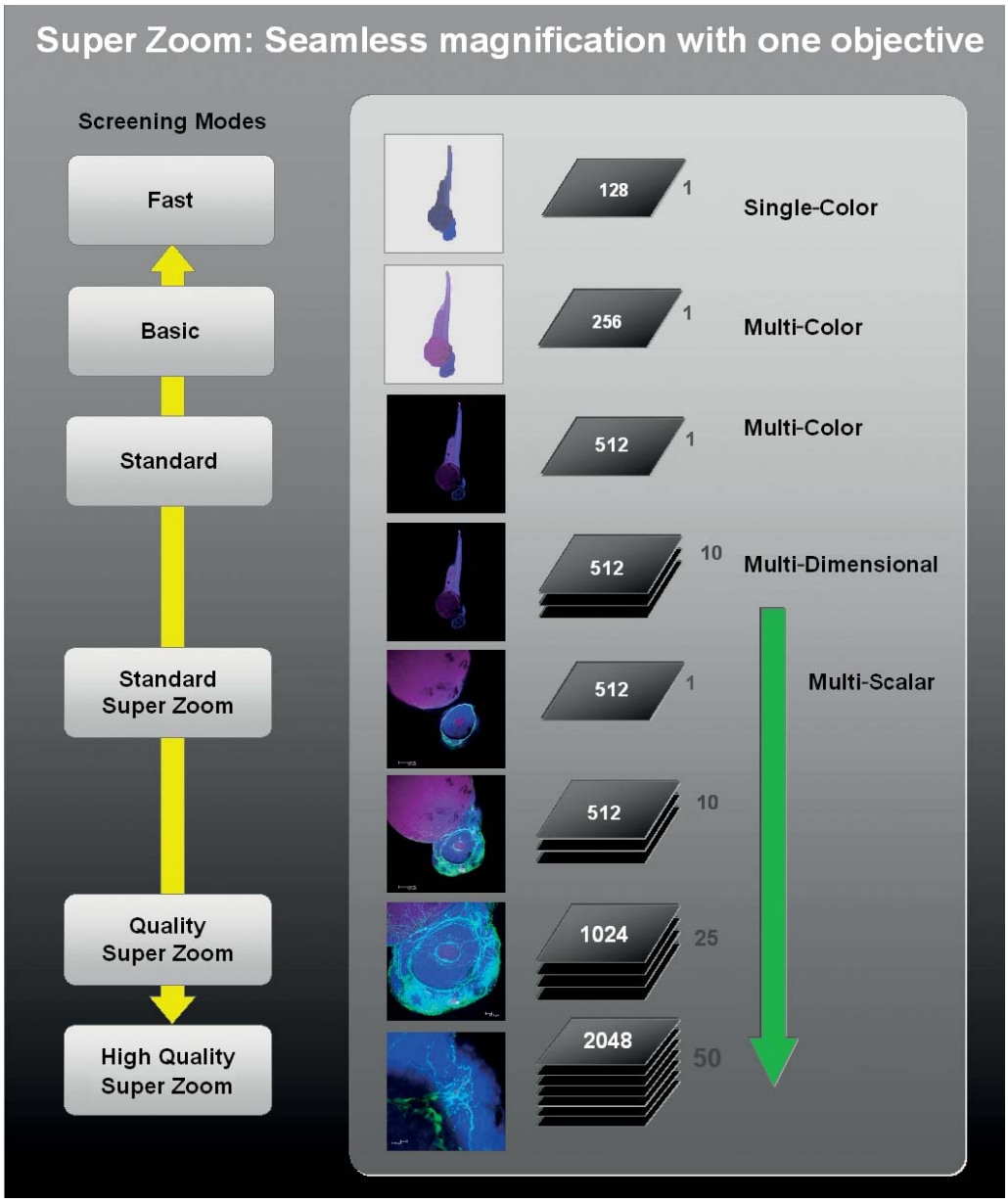
Easy Operation for Fast Success

A straightforward start with minimum training

The need to change objectives due to the lack of magnification, insufficient field of view with tedious readjustments, refocusing, and possibly still losing the area of interest, belongs to the past.

Leica HCS LSI is tuned in parallel and on-the-fly: Find the object, zoom in, and move to the position of interest. Adjust the fine focus and further zoom in – continuously!

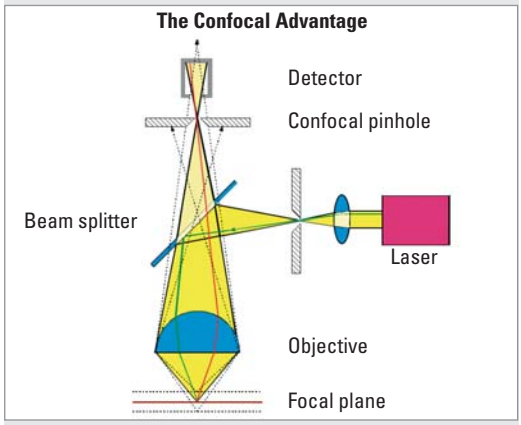
Enjoy handling efficiency: Operation is easy; results are quickly generated as all parameters can be intuitively and automatically adjusted. Illuminated knob controls allow the researcher to control the system in even dimly lit rooms.



The Leica HCS LSI – High

Highest sensitivity and precise hardware for rep

Excellent hardware



Crystal clear images by single point scanning confocal
Brilliant images at the highest resolution are achieved by scanning the specimen in thin optical layers, point by point. Leica HCS LSI detects fluorescence signals without stray light from adjacent objects.

Intelligent software reconstructs excellent 3D images, resolving the smallest details and providing maximum information. With minimum phototoxicity and low bleaching, Leica HCS LSI provides the best conditions for long term observations.

Easy sample insertion

Large working distance

Precise positioning

Spectral
Free band select
high signal efficien

True Confocal Point Scanner
High resolution imaging

Optical Zoom
Variable magnification

Macro Objective
Large field of view and working distance

Micro Objective
Use system as classic upright confocal

Transmitted Light Detector
Identify fluorescence in the contour

Precise Stages
Reproducible positioning and mosaicing

Large W
Easy operation a

Content Imaging Confocal

roducible results

Ultimate hardware flexibility for new experiments
Objects are easily inserted into the open workspace. Well plate handling is comfortable and safer. Freely tunable magnification, a variable field of view, and a large working distance provide maximum flexibility for *in vivo* observation from micro to macro.

Independent from fixed filters, the spectral confocal detector is freely tunable to provide highest signal efficiency. Leica TCS LSI offers reproducible and accurate results in confocal as well as in fast camera mode.

Detector
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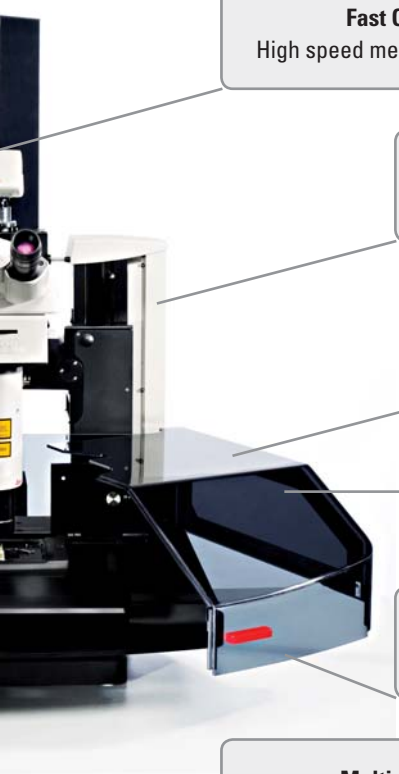
cy at low bleaching



Fast Camera Imaging
High speed measurements or pre-scans

Perfect Z-focusing
Precise and large Z-range: 10 nm to 150 mm

Environmental Control
Best condition for long term observations

Colored Climate Chamber
Light protection and stable climate

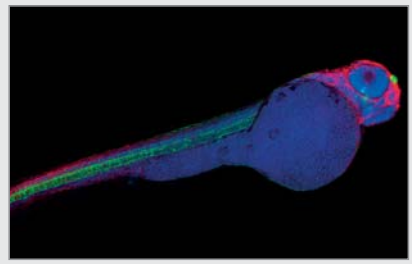
Wing Doors
Easy sample handling

Multiple Stage Inserts
Commonly used specimen carriers fit easily

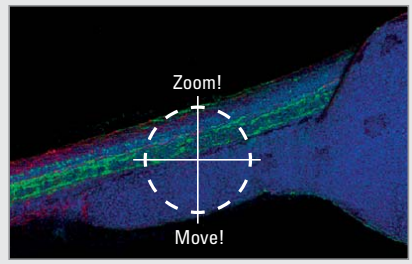
rkspace
d sample access

Efficient Operation

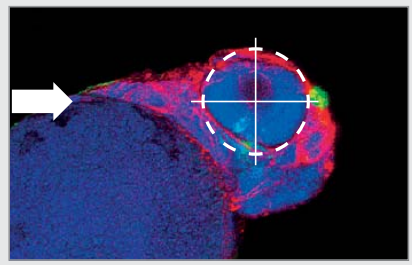
1. View all: overview



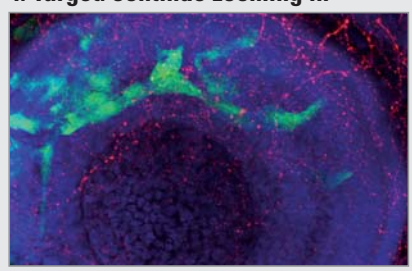
2. Adjust: zoom continuously



3. Find: move to target



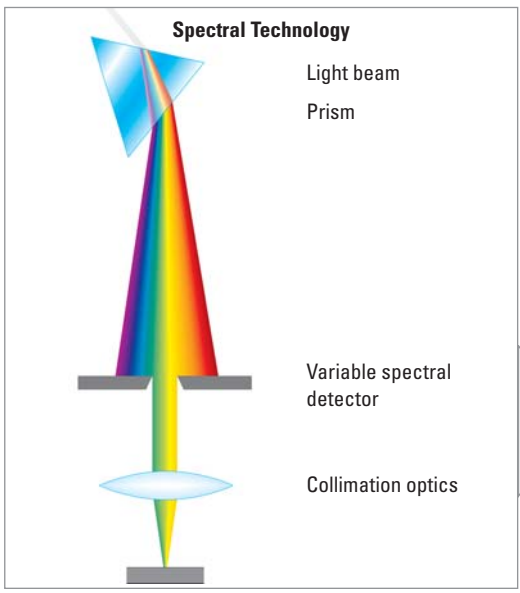
4. Target: continue zooming in

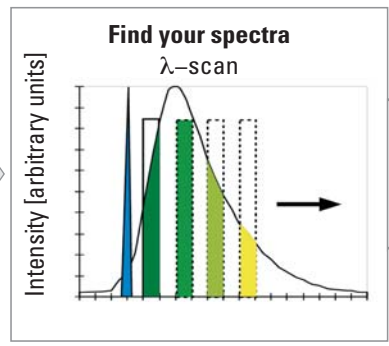


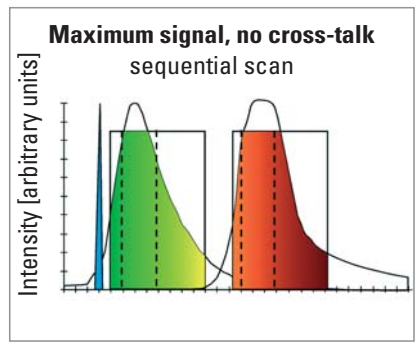
Innovative Optics for Best Results

Legendary expertise in optical solutions

Leica TCS LSI spectral detection







Spectral detection – highest signal efficiency

Ultra high sensitivity is achieved with Leica Microsystems' spectral detection technology. The seamless tunable detection range, independent of the limits of fixed filter barriers, provides ultimate detection flexibility. Benefit from the freedom to use many different dyes without filter changes.

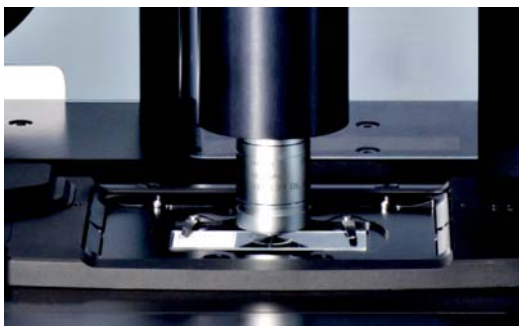
Capture more light: Open the detection window and reduce laser power by AOTF (Acousto Optical Tunable Filter) – both are infinitely variable – and maximize the signal with minimum bleaching. The combination of a high performance glass prism with a selected high dynamic photomultiplier offers maximum signal efficiency to precisely detect even the weakest signals. Channel multiplexing prevents any crosstalk of dyes and results in excellent dye separation. Higher signal, less averaging, and less phototoxic impact on the specimen are clear advantages of the efficient, directly connected spectral detector.

Tunable optical zoom from 0.57x to 9.2x with macro objective

Optical zoom – perfect adaptation

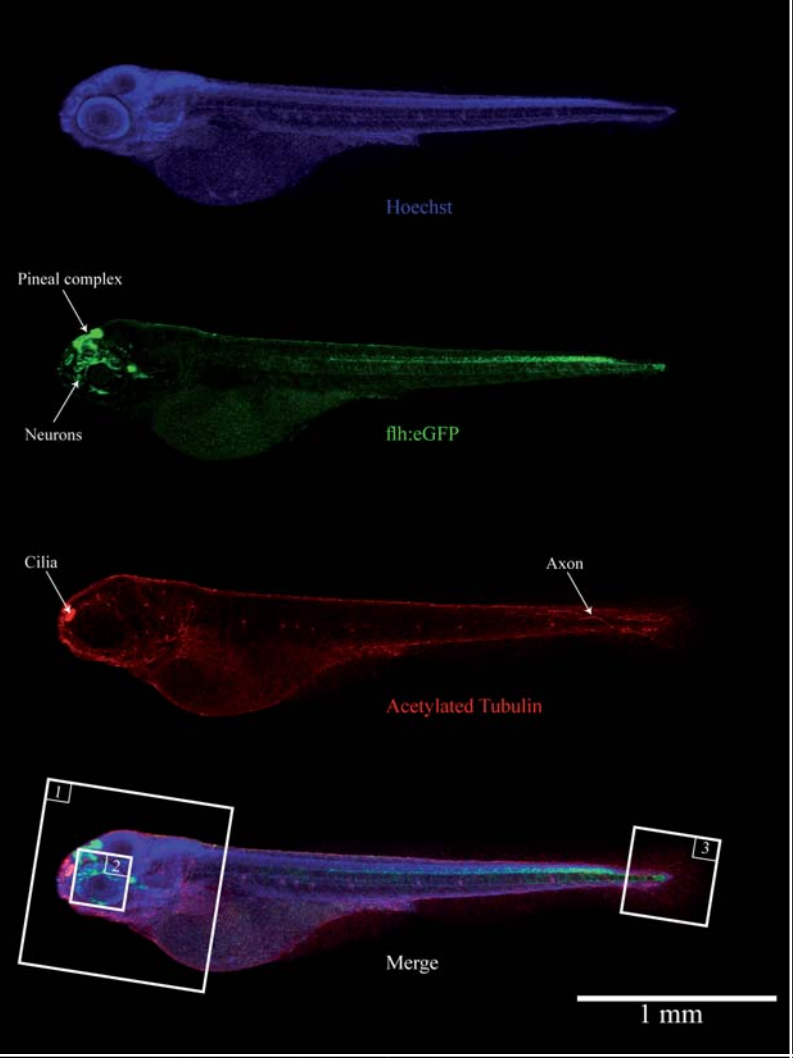
Overcome the limitations of fixed objectives by freely adjustable the optical zoom. Perfectly adapt the field of view to the specimen – with a button click. The fully motorized optical zoom enables seamless magnification control without objective change. Achieve a total zoom range of more than 240x by combining optical and confocal zoom.

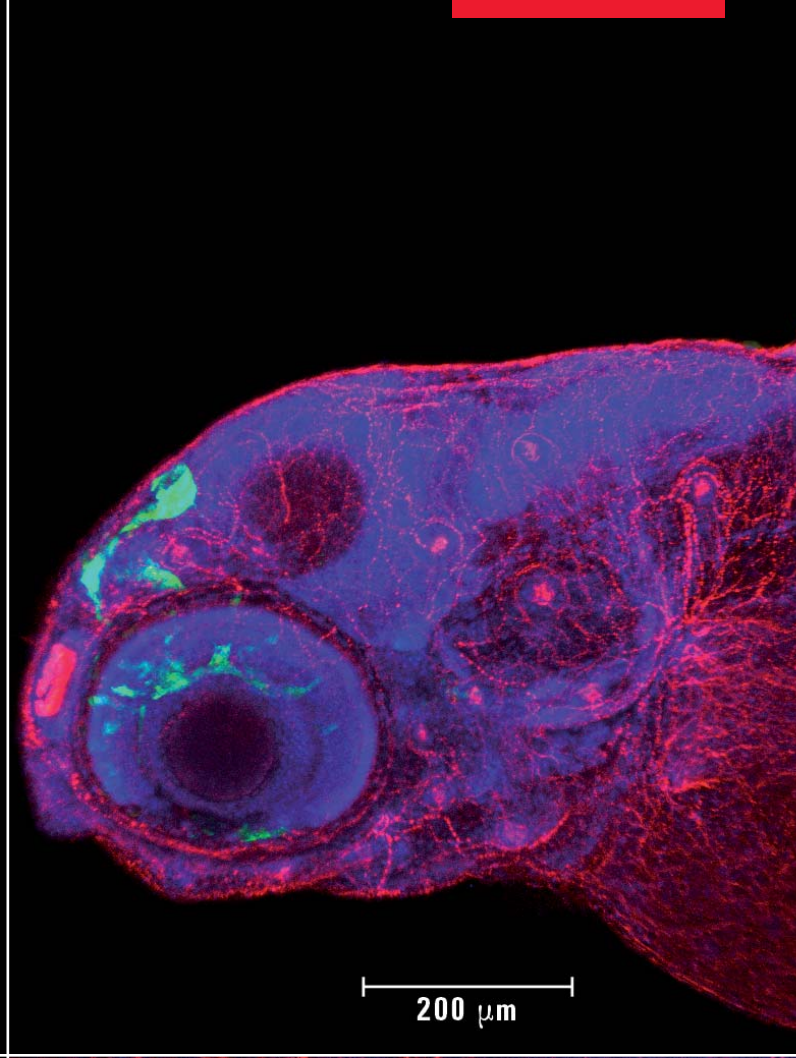
Adapter with classical micro objective

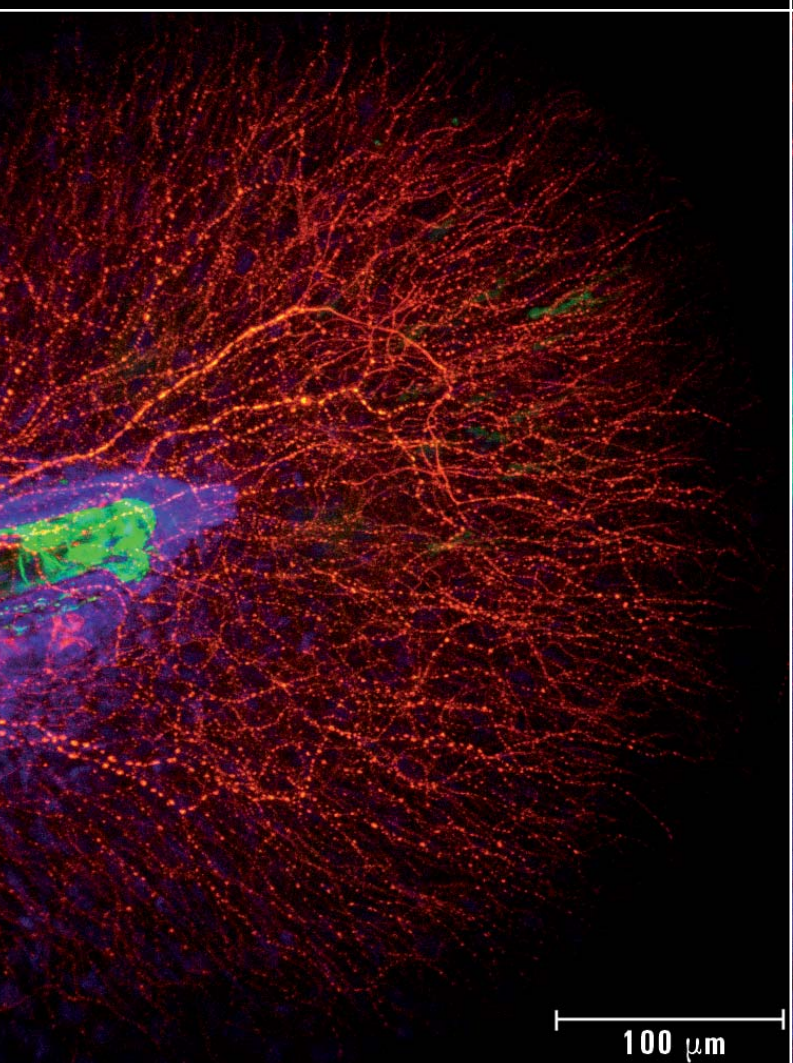


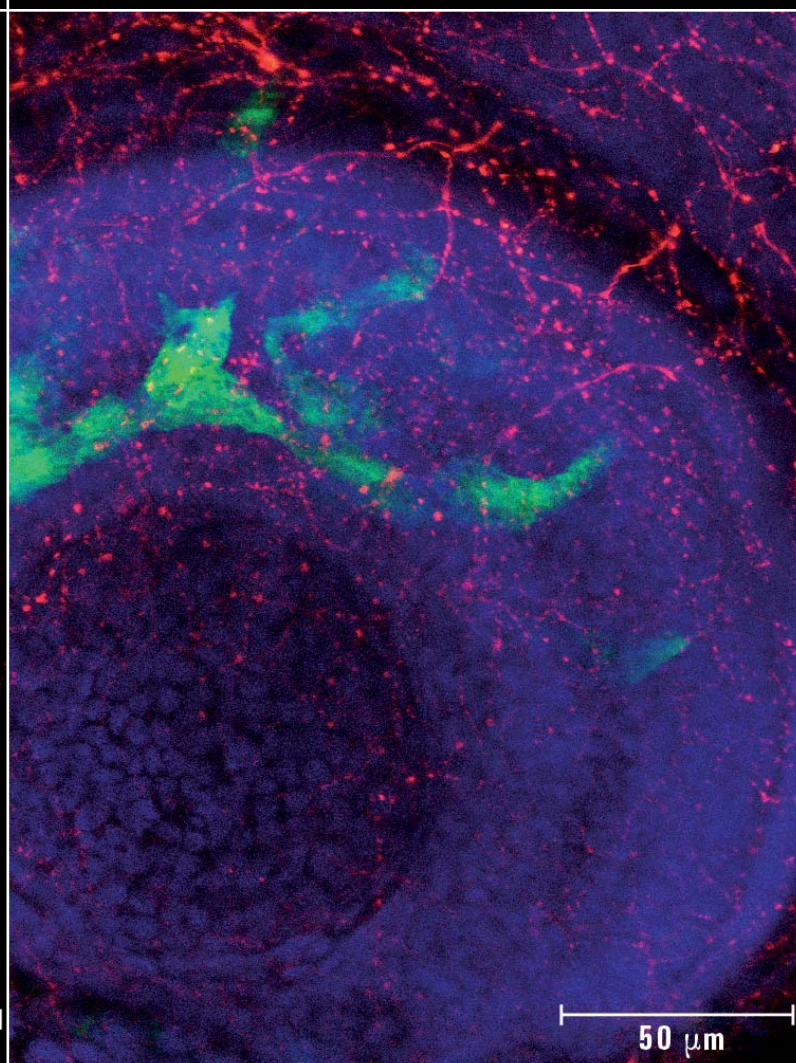
Micro and macro objectives – always the ideal resolution

The crucial advantages of macro objectives are the large field of view plus an enormous working distance for imaging large specimens at high resolution. Benefit from the two-in-one solution and convert Leica HCS LSI into a standard upright confocal by using the high numerical aperture micro objectives to clearly detect even sub-cellular structures.


Hoechst
Pineal complex
Neurons
flh:eGFP
Cilia
Axon
Acetylated Tubulin
1
2
3
Merge
1 mm


200 µm

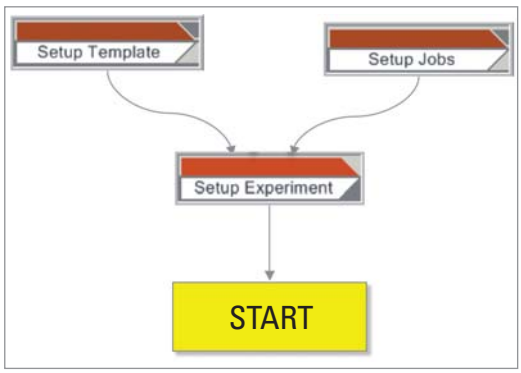

100 µm


50 µm

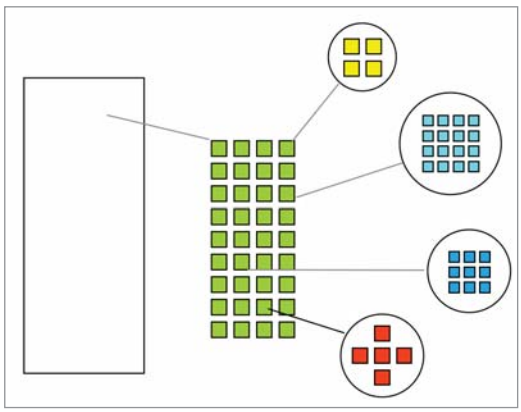
High Content Screening Automation

Optimize screening with easy and intelligent automation

Three steps to start



Free adjustable scanning template



Make it fit: flexible adjustment of scanning templates.

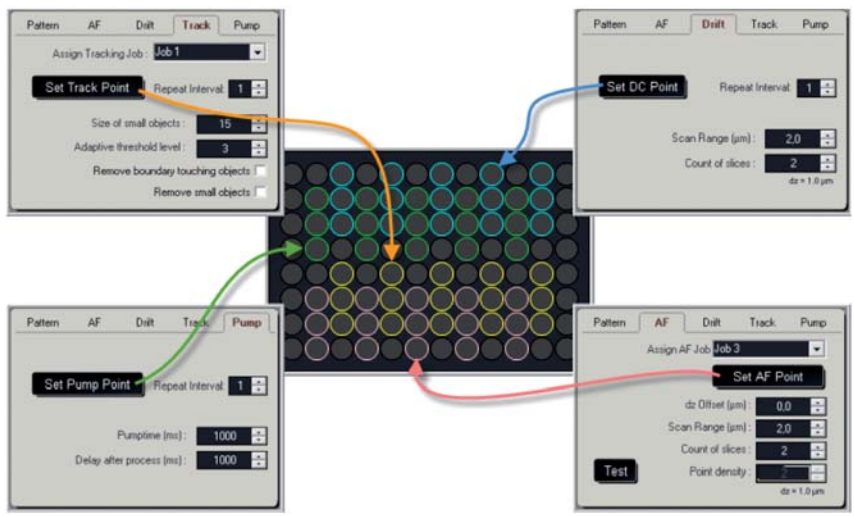
Many companies offer dedicated imaging routines for dedicated assays only. Leica Microsystems provides standard solutions for routine experiments plus maximum automation flexibility for elaborate experiments.

Easy automation – we keep it simple
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.

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. No need to be a programmer – new scanning templates for various chamber slides or multi-well plates are easily created. Once defined, the templates are ready to use for all applications and can even be shared between laboratories.

PLACE → LOAD → TEACH → START

Imaging without limits – MultiJob and MultiPositioning
Feel free to combine a variety of individual scan jobs for any area of interest within the specimen. The MultiJob-MultiPosition function provides maximum flexibility.



Several jobs, such as low-resolution pre-scans or multi-color 3D acquisition, can be freely combined. The zoom in and out function is software-controlled and individual settings can be adjusted for each position. From basic routines up to the most complex experiments, Leica HCS A greatly extends the spectra of applications.

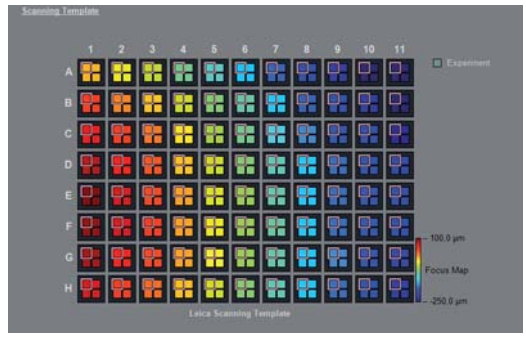
Autofocus routines
Five autofocus algorithms are available, optimized for different setups. The suitable routine is selected from a pull-down menu. After the initial scan, the software automatically creates a focus map with true specimen topology. This map is used for fast, accurate z-positioning during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

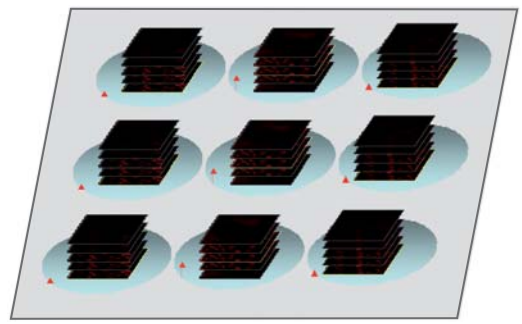
Z-drift compensation
Live specimens can grow in long-time measurements, changing the z-position of interest. Microscope conditions can change due to temperature shifts. The algorithm adjusts the focus independently over time and provides sharp images throughout the experiment.

Single object 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.

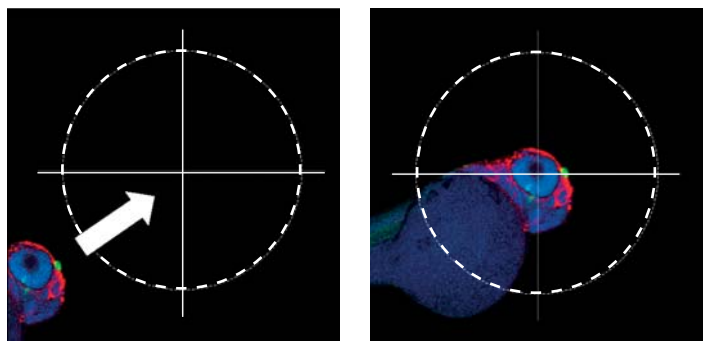
Review on-the-fly
Data is stored at predefined locations on a local hard disk or network storage device (NAS) via TCP/IP protocol. Experiment data flows into a ring buffer to ensure that an unlimited stream of images enters the specified target folder. The advantage: data analysis or review of image data is performed immediately. Image analysis starts as soon as the experiment starts generating fast results.

Fast feedback loops between the system and the analysis during the scanning process ensure the quick change of scanning parameters according to the target of interest.





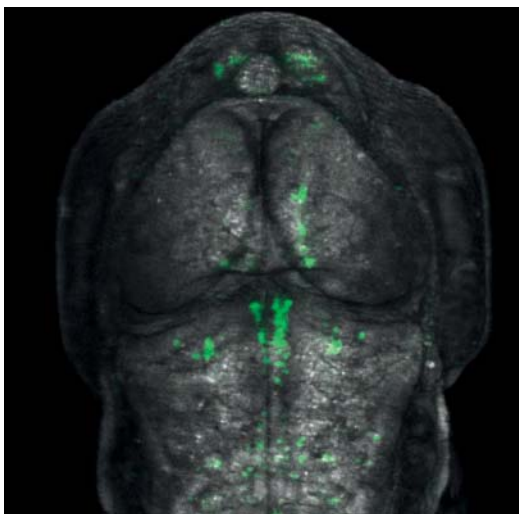
Autofocus procedure
Z-stack images are acquired at freely selectable positions. The focus positions are determined and stored in a color-coded focus map.



Tracking algorithm application of Leica HCS LSI to center objects

Automation Control

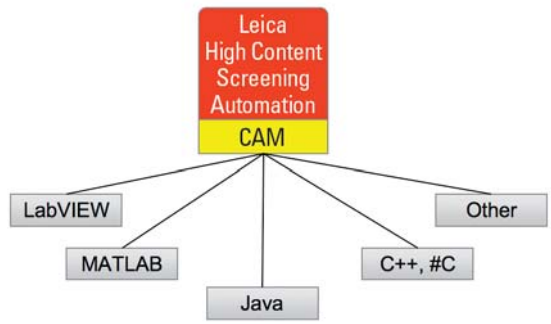
Computer aided microscopy – customize your imaging system



Dorsal view of a brain of a 2-day-old transgenic zebrafish embryo injected with rhodamine dextrane to visualize tissue structures. Green: GFP expression, monoaminergic neurons. Grey: rhodamine-dextrane.
Courtesy of J. Gehrig, KIT Karlsruhe Institute of Technology, Institute of Toxicology and Genetics (ITG), Karlsruhe, Germany.

Active interaction based on clear decisions is the key for success in science. Computer Aided Microscopy (CAM) is the tool for automated and immediate control of your confocal.

Get the power
The new CAM programming interface of Leica LAS AF MATRIX M3 software offers remote control of Leica HCS LSI by LabVIEW™, MATLAB™ or script-based programming languages. Individual imaging jobs are started rapidly and interactively, based on the decisions of image analysis, external trigger events or time loops.



Immediately after image acquisition, the data streams to a storage device to be retrieved by the analysis tools for processing. A moment later, target cells are clearly classified and marked by 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.

Data Interfaces

The perfect match to your laboratory

An open architecture for truly platform-independent exchange of information in an interactive environment – this is the goal of 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 data sets.

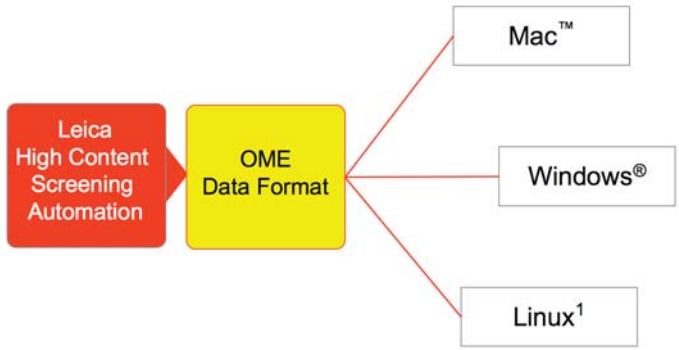
Platform-independent results

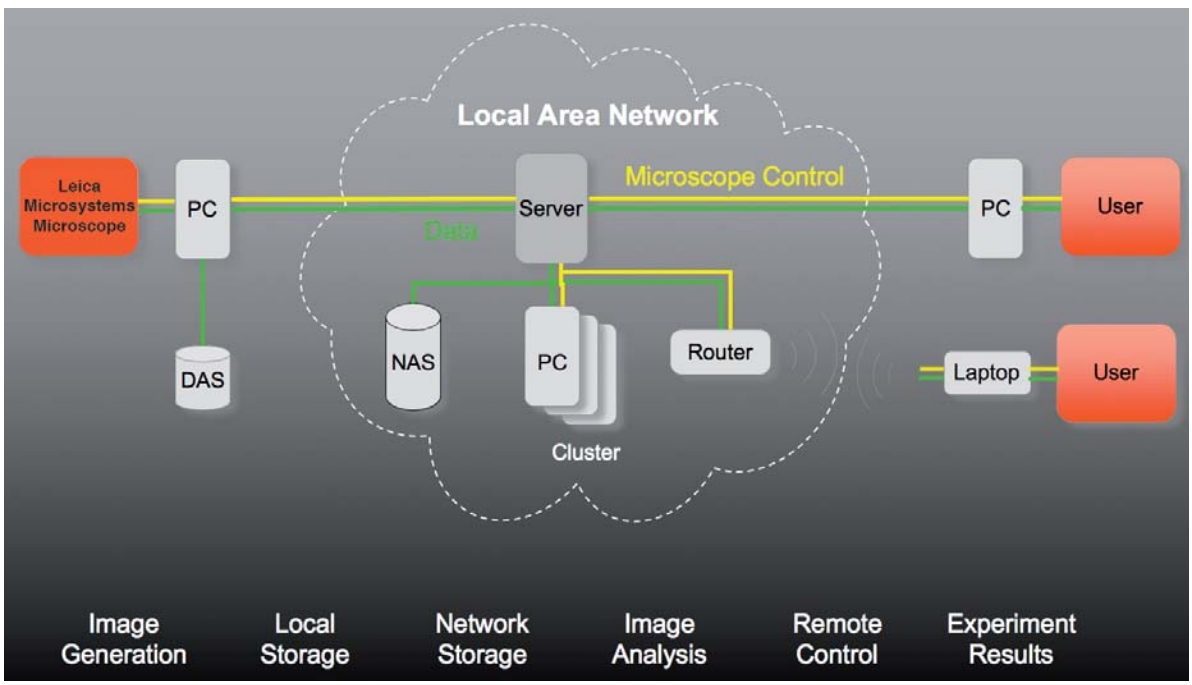
Leica HCS A imaging formats can be used platform independently on Apple MAC™ OS, Microsoft Windows® or LINUX[1]. The new Data Exporter automatically provides OME .TIF image files, which contain binary image data plus XML meta data structure.

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 now 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, meta data can be combined with the results of Leica HCS A using external programs.

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





Automated Image Analysis

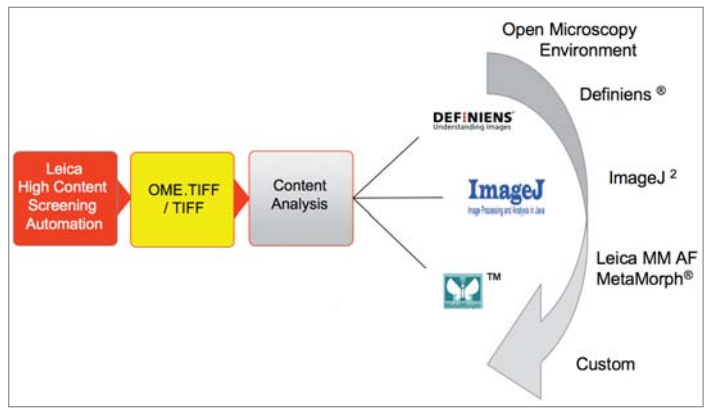
Turning images into data

Flexible data generation

- Freely adjustable image acquisition
- Individual automation
- Unrestricted analysis

Automated Image Analysis
Images stored in the Leica HCS A export formats can easily be imported into Definiens®, ImageJ[2], or MetaMorph®, etc. to ensure full compatibility to modern image analysis software platforms. Researchers can use existing algorithms and new routines to analyze the data in an automated way.

Freely choose among local, server-based and even clustered image analysis tools to achieve result efficiently.



Modern image analysis software using Cognition Network Technology analyses image data fully automatically in 2D or 3D context based. Target components are identified, measured, and counted to provide statistically relevant information as the result of High Content Screening Automation.

Definiens Developer XD available as Leica Edition
The Leica Edition is a portal within the Definiens Developer XD that offers all the advantages of analyzing multi-dimensional image data of zebrafish from egg to embryo. The image analysis software provides a comprehensive toolbox for the entire process from rapid prototyping to the deployment of fully automated image analysis routines.

To quantify objects in 2D, 3D or 4D time lapse data sets, Definiens established a new dimension of image analysis: The Cognition Network Technology® is a context-sensitive network of structures of interest that enables automated tracking of cellular movements, volume measurements, and the quantification of developmental processes in whole organisms with high reproducibility.

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-like 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.

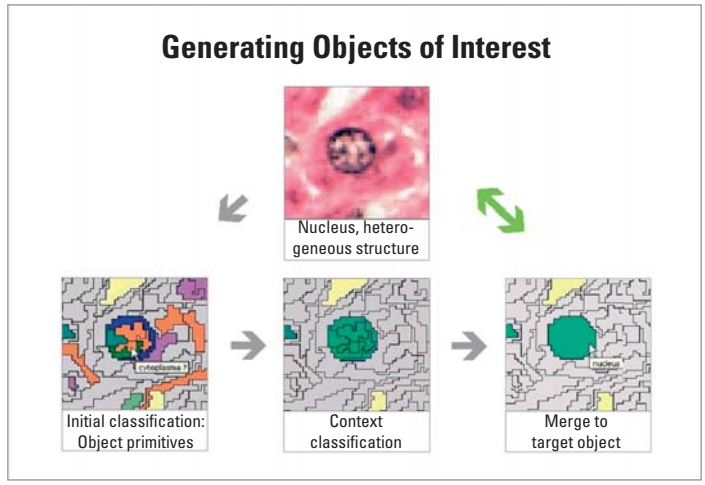
LabVIEW™ is a registered trademark of NI National Instruments Inc. MATLAB™ is a registered trade-mark of The MathWorks™, Inc.Java™ is a registered trademarks of Sun Microsystems, Inc. C++ is a programming language standardized by ISO. C# is a programming language developed by Microsoft, Inc.

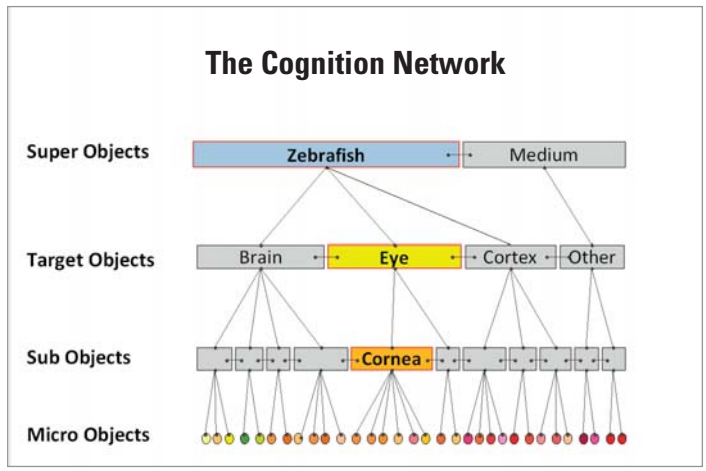
Optimal results by hierarchical object recognition
In addition to basic pixel based analysis, intermediate objects and their interrelationships are analyzed, providing a unique level of object recognition. By storing objects, sub-objects, and their relationships in a clear hierarchy, automated extraction of information comes close to the accuracy of the sophisticated human segmentation and recognition process.

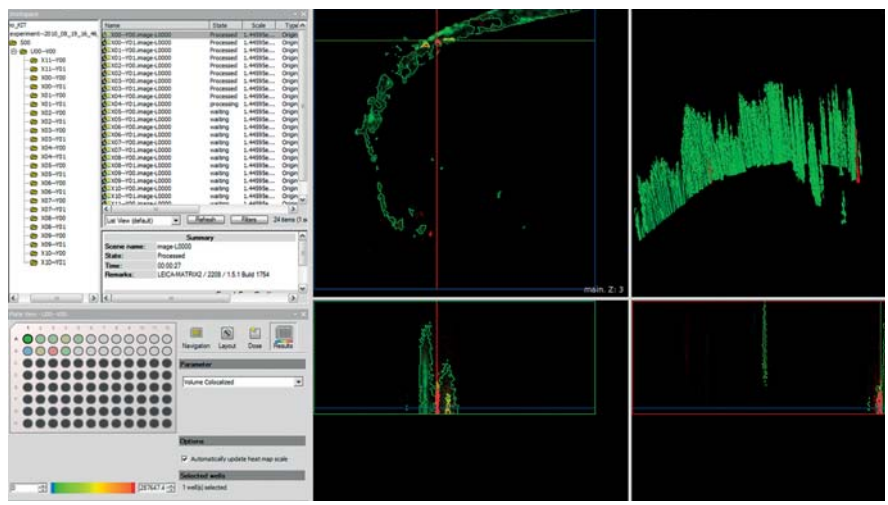
New experimental approaches become reality and exceed the information level of classic 2D segmentation. 3D object analysis or 4D object tracking in multiple volumes over time is feasible, opening up an entirely new area of assay development.

Turning multi-dimensional images into data
One button click imports all OME.TIF data generated by LAS AF Matrix M3 as well as the Leica lif-formats. Proper meta data handling ensures consistent spatial and volume information. Comprehensive sets of tools and algorithms are provided to generate and modify rulesets quickly according to upcoming demands. The final results are exported as basic data files or can be easily displayed in individual reports.

Plug-in technology
Open-source analysis plug-ins can be adapted and shared between the community to quickly and efficiently solve dedicated analysis. The Zebrafish colocalization module, developed at KIT Karlsruhe Institute of Technology, is a valid example of turning straightforward 4D image data into quantitative results.





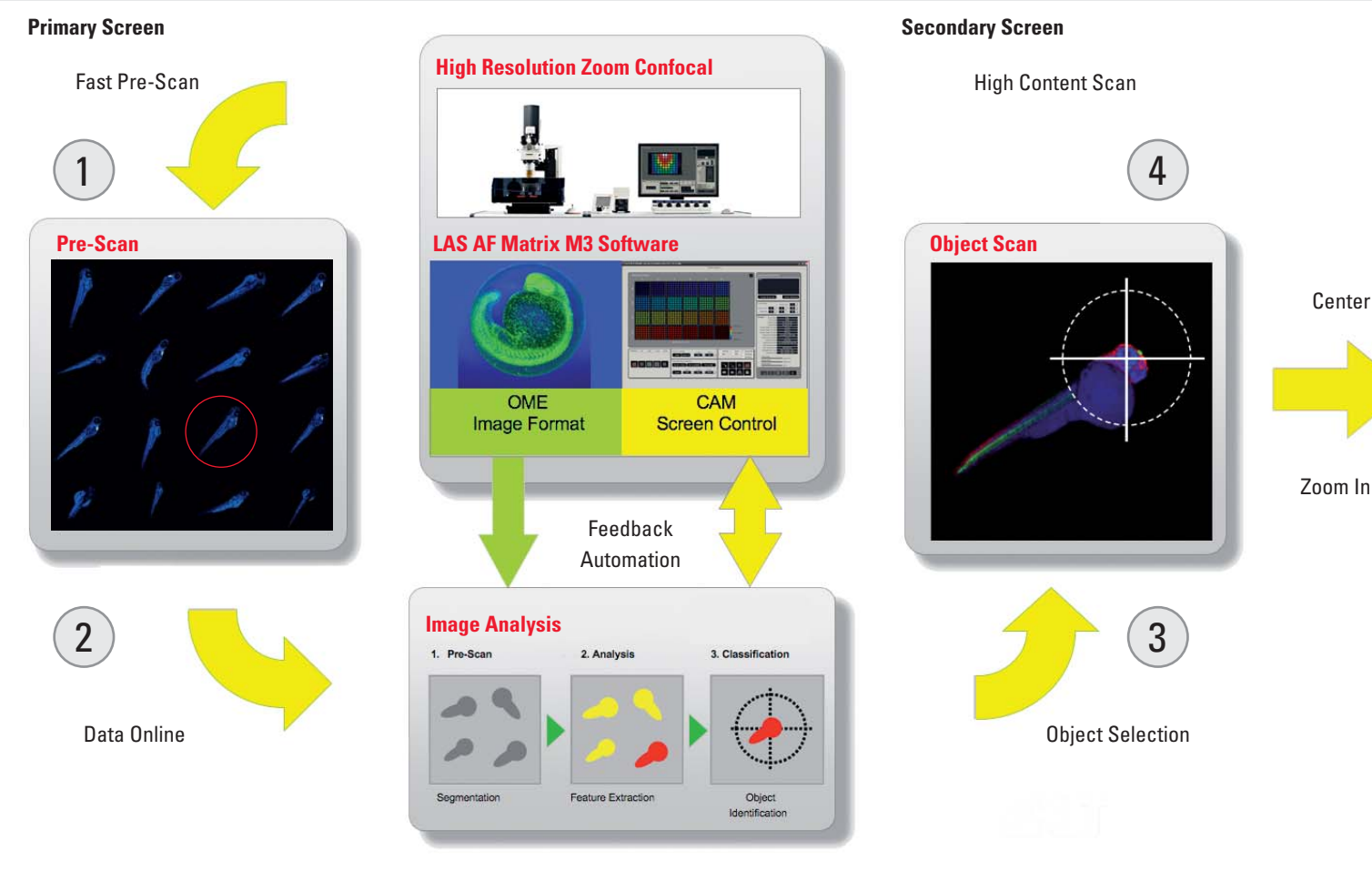


The Zebrafish colocalization module.
Import function and slider threshold.

Courtesy of M. Reischl, KIT Karlsruhe Institute of Technology, Germany and K. Hartmann, Definiens.

Leica HCS LSI High Content Screening Automati

The smart solution for complex experiments



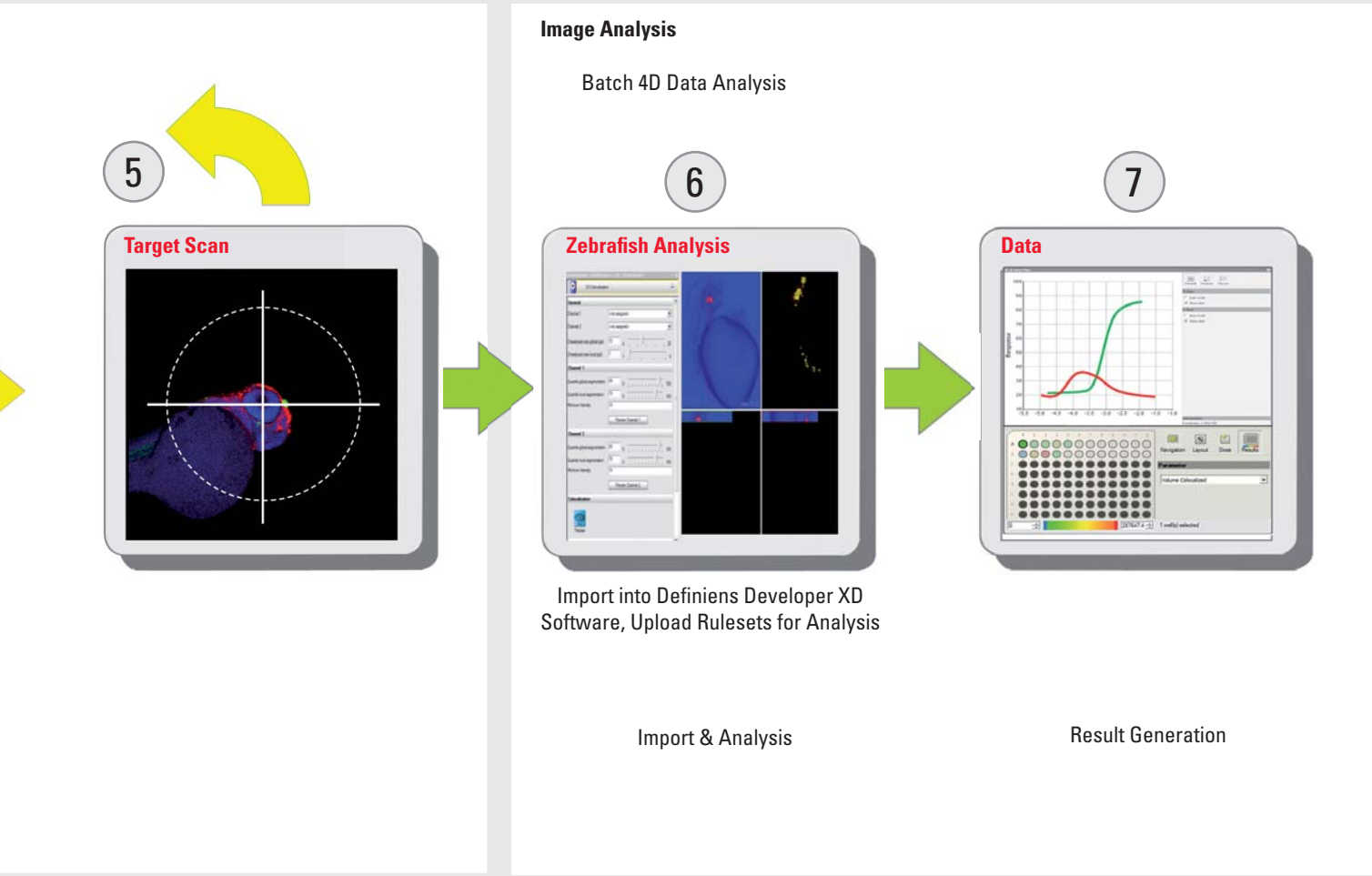
Intelligent screening of large objects
High Content Screening Automation of large objects at high resolution can be performed with the new Leica HCS LSI application solution. At Karlsruhe Institute of Technology (KIT), one of the large Zebrafish Centers in Europe, highly complex experiments are excuted with excellent results. The general setup illustrates the potential to screen transgenic embryos of *Danio rerio* automatically executed in multi-color mode.

Primary scan – identify target on-the-fly
In practice, centering the zebrafish areas of interest in well plates is challenging. A fast initial pre-scan is performed at low magnification to identify the position of the target objects. The fast confocal mode with transmitted light detection easily enables the user to identify fluorescent markers in the specimen outline.

The OME-imaging data is stored locally or streamed to a network attached storage device (NAS). To automate the workflow, the "Mark & Find" mode of LAS AF Matrix M3 is used to easily center the regions of interest (ROI). Intelligent automated workflows can be established via the CAM Computer Aided Microscopy Interface by external programing languages like e.g. MATLAB™ to perform pre-analytical steps. Target ROIs in the Zebrafish can be automatically detected by the external software, sending the stage coordinates to the Leica HCS LSI for ROI centering. These processes can be performed either semi- or fully automated.

Secondary scan – high content acquisition in 3D
After centering, the optical zoom is seamlessly tuned to perfectly match the field of view with the region of interest. The secondary scan, with a high number of optical slices at high resolution, is performed with more channels *in vivo* in 4D.

on for Quantitative Zebrafish Assays



Intelligent automation – increase experiment efficiency
Primary and secondary scans are automatically alternated in one instrument due to the highly flexible system optics. Phototoxic effects and bleaching are minimized as the pre-screen can be performed at lower laser power. Only the target areas are imaged during the high-resolution scan, which eliminates unnecessary light exposure to the entire larvae during time-lapse observations. This method is highly efficient, increases specimen lifetime, and enables long observation times.

The iterative approach ensures two advantages: Only true targets are selected for the secondary screen. The amount of imaging data is reduced to the relevant information. In the secondary screen, the advantage of the optical zoom over fixed objectives becomes apparent.

Maximum content is covered per image, gaping space is minimized and content information is maximized.

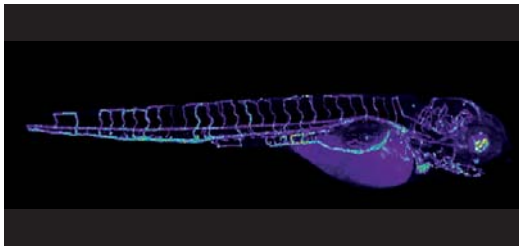
Image analysis – turning experiments into data
All data is uploaded into the Definiens Developer XD Leica Edition for batch processing. Image analysis is performed fully automatically. An open source plug-in developed at KIT and Definiens enables 4D colocalization analysis of *in vivo* zebrafish data. Experiment results are immediately visible on screen or can be exported for future publication.

Courtesy of U. Liebel, J. Gehrig, R. Peravali, M. Reischl, KIT Karlsruhe Institute of Technology, Institute of Toxicology and Genetics (ITG), Karlsruhe, and K. Hartmann, Definiens, Germany.

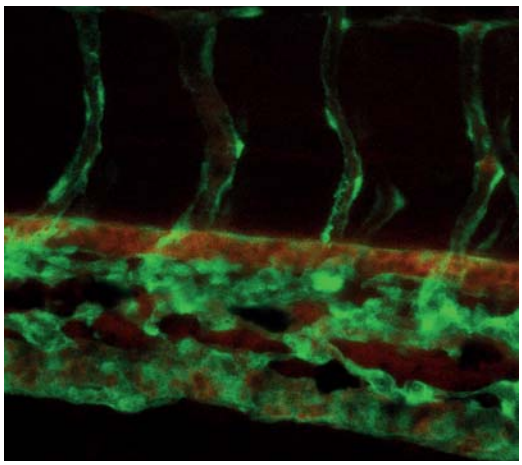
New Opportunities for Research

Ultimate flexibility from image acquisition to analysis

New applications



Blood flow visualization by fast camera imaging in zebrafish larvae. CD41-GFP expression in thrombocytes. Time-lapse image of thrombocyte circulation to illustrate the vascular system. Courtesy of P. Herbomel, Institut Pasteur, Paris, France.

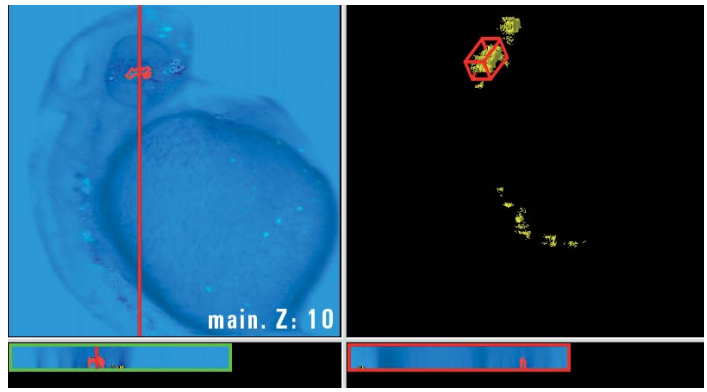


Zebrafish, *Danio rerio* adult in native environment.
Blood vessels in the fin at high resolution.
Courtesy of D. Hentsch et al., IGBMC, Illkirch, France.

The Leica HCS LSI platform offers a variety of methods to streamline experiment flow from high content imaging to quantitative results.

As an example, colocalization events can be analyzed in 4D data sets to uncover the signal development per volume over time. Even fast movement such as blood flow can be measured *in vivo* in zebrafish embryos.

The application range is not restricted to a specific model organism. High content screening experiments can as well be performed with other specimens, such as *C. elegans* or *Drosophila sp.*. Leica HCS LSI provides excellent conditions for individual analysis up to multi-scalar measurements in multi-wells for entirely new experiment designs.



4D analysis: colocalization in zebrafish.
Courtesy of M. Reischl, KIT Karlsruhe Institute of Technology and K. Hartmann, Definiens.

The Advantages at a Glance

Explore the new dimensions of imaging!

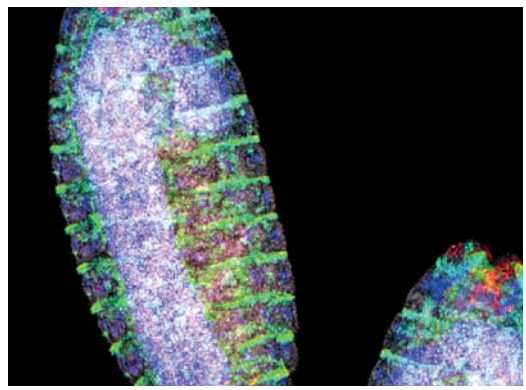
Leica HCS LSI – the innovative application solution
- Acquisition, automation and analysis on one platform: Well aligned for best results
- Maximum flexibility: Easily fit the system to your experiment, from micro to macro
- Scalable: Perfectly automate individual experiments. Assay development and high content screening
- Get unbiased results fast and efficiently: Gain scientific advantage and amplify the power of imaging

True spectral confocal technology – high content in 4D
- Spectral analysis from 430 to 750 nm optimizes quantum efficiency
- Robust solid state lasers 405-488-561-635 nm for reproducible results
- Environmental chamber with large workspace provides optimal specimen conditions
- Multidimensional time lapse acquisition: Scale Z from 10 nm to 150 mm

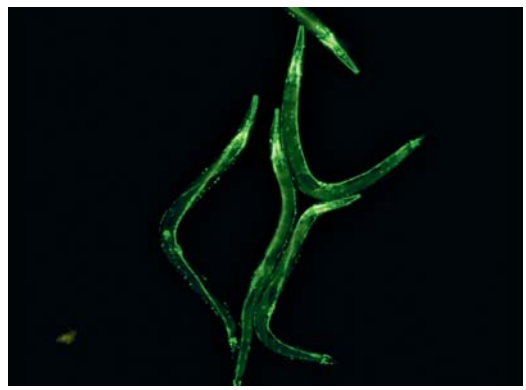
Adaptive optical zoom – discover the ease
- Infinitely adjustable magnification without objective change
- Scale the field of view perfectly to the specimen size
- Large working distance, maximum 16 mm field of view
- Seamless positioning in xyz

Intelligent automation – increase efficiency
- Pre-screen and secondary screen in one system saves operation time
- Well plate screening, auto focus, single object tracking optimize assay development
- Easily assign multiple jobs to multiple positions to accelerate the upscale process
- Results control the experiment: Trigger the system by image analysis and benefit from the external device control via Computer Aided Microscopy (CAM) interface

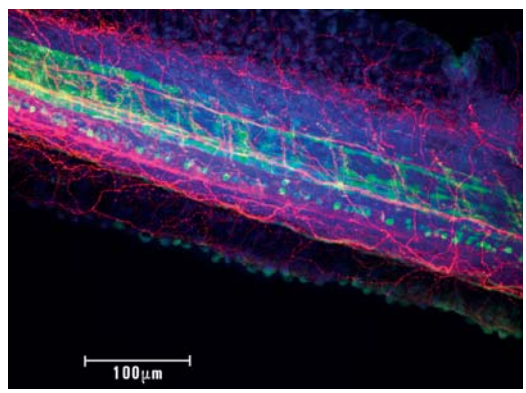
Optional image analysis – quantify your experiments
- Open and standardized interfaces for perfect laboratory integration: OME.TIF export optimally connects to existing algorithms
- Sophisticated – Leica Edition of Definiens Developer XD: meta data import, object recognition, and report out
- Multidimensional analysis and freely programmable



Neuron development in *Drosophila sp.* embryo., labeled with Dapi, GFP, Cy3, Cy5, maximum projection.
C. elegans nervous system, GFP expression.



C. elegans. Nervous system, GFP expression.



Danio rerio, tail. Fine structure of blood vessels in the tail at high resolution. Courtesy of D. Hentsch et al., IGBMC, Illkirch, France.

“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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