Leica DM6000 CFS

Confocal Fixed Stage System

The solution for applications
in live cell research,
from physiology to electrophysiology –
from single cells to whole organisms

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Leica TCS SP5 with integrated fixed stage microscope Leica DM6000 CFS

Features and Benefits

- Maximum workspace for manipulators and attached pipettes
- High collection efficiency due to short collection path before detectors
- Integrated change of magnification (0.35x, 1x, 4x) of camera port
- Remote control of all microscope functions via touch panel
- Special booster optic to fill the entrance pupil of the 20x1.0 objective
- "Dip-in" feature for immersing the objective
- Patented condenser drainage system
- High microscope and sample stability

Excellent Results Under All Conditions

Leica Microsystems sets a new standard with the integration of the Leica DM6000 CFS fixed stage microscope into the Leica TCS SP5 confocal platform.

Neurobiological research has a long history not only in measurements of single cells, but also of thick brain slices. Here more realistic results can be achieved due to the intact network. Ultimately, accessing the brain directly in a whole animal provides the most intact environment for conducting measurements on individual cells. So for optimal results, space for whole animal samples is an absolute must.

Electrophysiological research employs micropipette systems for recording electrical signals (patch clamp, whole cell recordings), electrical stimulation (intracellular stimulation, synaptic stimulation, soma-stimulation), dye injection and intracellular perfusion. To make such measurements effective, the microscope needs to have as much space as possible for micropipettes and other manipulators.

Searching for the perfect spot in the sample requires a large field of view, whilst the precise positioning of the micropipettes requires high magnification. Furthermore, high quality confocal image scanning requires a large numerical aperture for best performance. To fulfill these experimental prerequisites, a camera with an adjustable magnification and specially adapted objectives are needed. Any direct manipulation of the system potentially disturbs the delicate positioning of the micropipettes within the sample. This emphasizes the importance of a remote control for the imaging setup, providing convenient access to all relevant functions.

Finally, recording electrophysiological and imaging data with perfect synchrony is paramount for correct interpretation. Triggering image recording by external events and synchronizing the application of stimuli with image scanning down to the single line level helps realize sophisticated experimental setups. Online analysis of the resulting data allows on-the-fly adjustment of external parameters, helping the researcher to get everything just right.

The Leica TCS SP5 with the integrated fixed stage microscope Leica DM6000 CFS gives excellent experimental results under all conditions.

Variable Field at High Resolution

With the highest aperture objective 20x 1.0 NA, with the innovative, ergonomic and stable microscope stand Leica DM6000 CFS, with short coupled non-descanned detectors and with the fastest, most sensitive scanning system SP5, we enter a new dimension in imaging, from single cells to whole organisms.

The objective

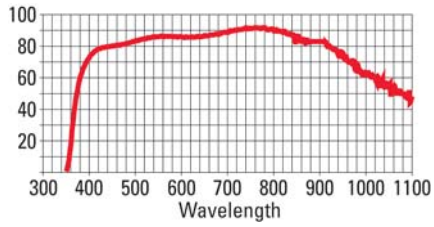
The new Leica HCX APO L 20x 1.0 water immersion objective offers both a large field and a high resolution with one single objective lens, making it unnecessary to change objectives between overview imaging for sample preparation and detail imaging for data recording.

In addition to optimizing the optical parameters, a number of physical, chemical and mechanical improvements have been integrated into the new HCX APO L 20x1.0 application objective: It is corrosion-free, chemically neutral and avoids diffusion of metal ions. Wettability is outstanding, thermal conductivity is minimal and magnetic fields at the front of the objective have been eliminated.

These new objective characteristics are possible by employing a special, extremely hard ceramic material for the whole front lens area. This material is resistant to mechanical damage and much better suited to the requirements of electrophysiology than typical objective metal sleeves.

A further advantage is the access angle of the objective, which is a measure of how easily manipulators can be fitted. This has been widened to 39 degrees, which is almost the limit of what is theoretically possible. In combination with a free working distance of 2 mm, the 20x objective is ideal for many applications.

Leica objective HCX APO L 20x1.0



Transmission HCX Apo L 20x/1.00 W

Features and Benefits

- 20x water immersion objective
- High numerical aperture (NA) of 1.0
- Free working distance (FWD) of 2 mm
- Optimized access angle of 39°
- Large objective focusing range of 13 mm
- Insulated ceramic tip
- Minimum conductivity
- High transmission in VIS and IR (see graph)
- Best DIC and Dodt contrast

Leica DFC350 FX Camera with parfocal magnification changer

Features and Benefits of the Leica STP6000

- Intuitive user interface
- Control of all microscope functions
- Z-focusing wheel for coarse and fine z-movements
- Freely programmable function keys
- Helps to avoid vibrations caused by manual operation

The microscope stand

But an objective alone doesn't make an electrophysiology setup. The Leica DM6000 CFS combines the specific needs of electrophysiological experiments with the optimized imaging performance of the DM series. With its focusing objective or nosepiece, it offers a highly stable and convenient optical platform for all fixed stage applications.

To cover the demand for large scale sample screening and fine needle approaches for electrophysiology, the DM6000 CFS has a special observation tube with 3 magnification positions (0.35x; 1x; 4x). This parfocal magnification changer allows a total change in magnification up to a factor of 12 without changing the objective.

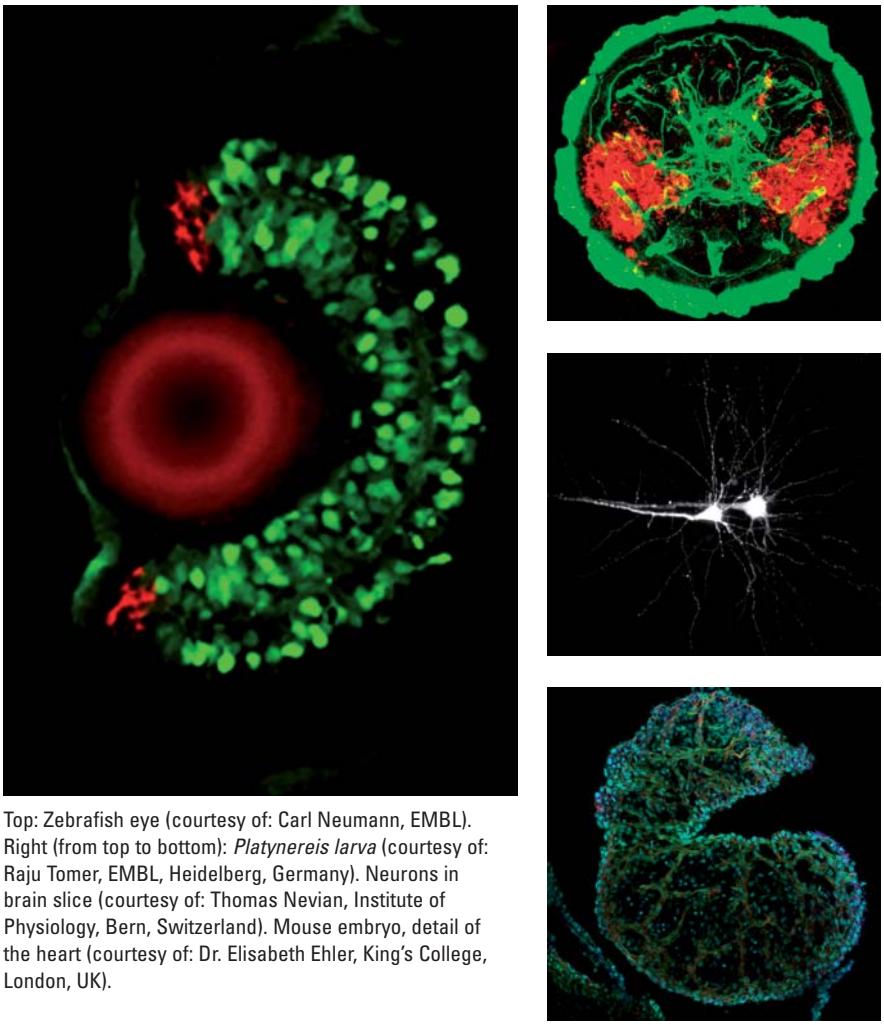
The specimen is completely decoupled from the microscope, both mechanically and electrically. The microscope provides a high degree of free space for application-specific sample holders, bath chambers and several manipulators at a time. In this way, the sample and the patch electrodes can be moved below the fixed optical axis of the microscope. This allows the researcher to scan the entire dendritic tree and follow the axons of fluorescently labeled cells. The DM6000 CFS is also designed for use with a separate third-party fixed platform for holding micromanipulators and other devices, as well as an x,y-translation stage.

To avoid the need for direct manipulation of the microscope stand which could cause accidental vibrations, the external control panel Leica STP6000 (Smart Touch Panel) controls all the microscope functions. A touch screen and freely programmable function keys allow quick and easy operation without adversely affecting the measurements.

Large range of applications

Even though the new 20x 1.0 objective makes electrophysiology with one single objective possible, sometimes this isn't enough. In developmental biology, there is a clear trend towards imaging even larger organs and organisms, calling for different special application objectives. The Leica DM6000 CFS offers maximum flexibility in a multi-user environment to cater to everyone's needs. A high precision adapter is available, allowing to replace the single objective with an electronic 6x objective nosepiece. The patented change of objectives works vibration-free, with automatic power switch-off to avoid disturbing measurements. For each objective, the focus position can be programmed. Thus, by simply pushing a button, a quick change between the magnifications can be achieved even in the near infrared without losing the area of interest, implementing automatic parfocality.

Exchangeable nosepiece



Top: Zebrafish eye (courtesy of: Carl Neumann, EMBL). Right (from top to bottom): *Platynereis larva* (courtesy of: Raju Tomer, EMBL, Heidelberg, Germany). Neurons in brain slice (courtesy of: Thomas Nevian, Institute of Physiology, Bern, Switzerland). Mouse embryo, detail of the heart (courtesy of: Dr. Elisabeth Ehler, King's College, London, UK).

Features and Benefits

- High precision adapter for switching between single objective and objective revolver
- 6 objective nosepiece positions
- Patented electronic nosepiece turret
- Automatic power switch-off after objective change
- Automatic electronic parfocality

Orientation and Contrast

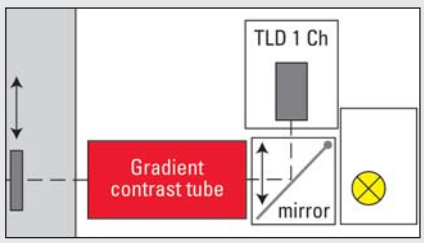
Features and Benefits of Gradient Contrast

- Usable both with camera (IR-video microscopy) and scanner (IR-scanning gradient contrast, IR-SGC)
- Optical elements are outside the fluorescent light path, allowing highest possible photon collection efficiency of two-photon microscopy
- Alignment-free overlay of IR-SGC and fluorescence images
- Scanner patching with IR-SGC – no need to patch in camera mode

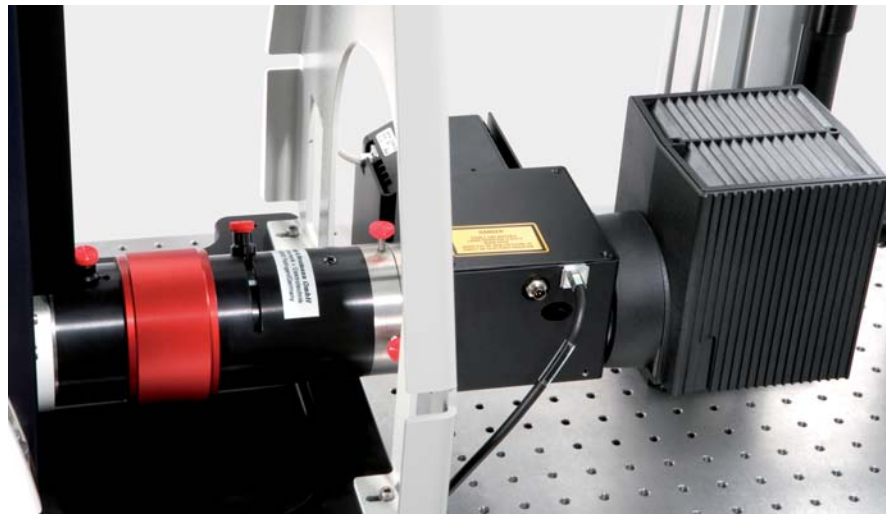
Tissue or brain slices up to a thickness of several hundreds of microns can be optimally imaged with infrared illumination. A specially designed infrared illumination filter in combination with an IR polarizer, IR analyzer and the infrared differential interference contrast (DIC) prisms give extremely good resolution even in the thickest specimens.

Used in combination or separately, fluorescence and DIC are great techniques for patch clamping. However, to avoid having any optical components in the fluorescent light path and ensure the highest photon collection efficiency for two-photon excitation fluorescence microscopy, the Dodt gradient contrast technique can also be used. This gradient contrast converts the phase information into an amplitude contrast. Images of neurons look similar to images obtained with DIC.

To study the fundamental properties of basal dendrites via patch-clamp recordings, it is now possible to combine two-photon excitation fluorescence microscopy with a scanning version of this technique, called infrared-scanning gradient contrast (IR-SGC). The infrared excitation laser light and the fluorescent light are separated by a dichroic mirror, underneath a high NA condenser. The fluorescence light is detected by Non-Descanned Detectors (NDD) and the IR-scanning gradient contrast images are detected by spatially filtering the forward scattered infrared laser light with a Dodt tube and subsequent detection by a photomultiplier tube. This allows the online-overlay of a highly contrasted IR image of a brain slice with the fluorescence image of the neuron system. This detection method is patented by a Leica patent: US 6,831,780 B2.



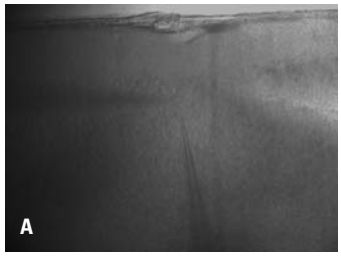
Optical light path of Dodt gradiant contrast

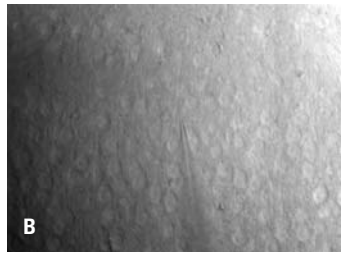


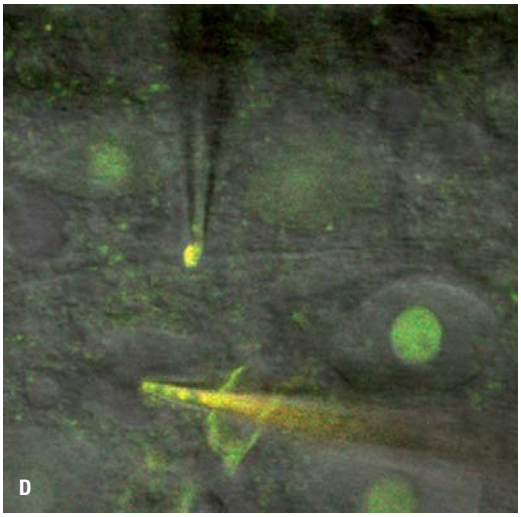
Detection efficiency

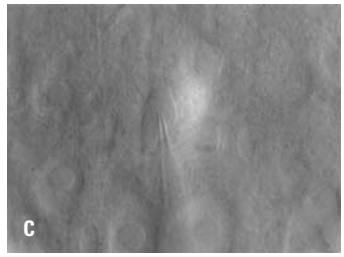
As with multi-photon excitation the fluorescence is only generated in the diffraction limited focal volume, the detectors can be placed directly behind the objective (reflected light detectors, RLD) as well as directly behind the condenser (transmitted light detectors, TLD) without losing spatial resolution. This close-coupling detection scheme results in the highest possible photon collection efficiency, as scattered fluorescent photons can also be collected over a large detection angle due to the high numerical aperture of the objective and the condenser. Two-channel detectors on both sides add a maximum of detection flexibility.

Apart from its high NA, the new DM6000 CFS patented turret condenser for brightfield and interference contrast provides a number of other advantages. The system allows the exchange between dry and oil condensers. The condenser base with condenser head 1.4 NA oil S1 stands for highest collection efficiency, while the patented condenser base provides a watertight seal with an outlet pipe for liquid leaking from the sample.





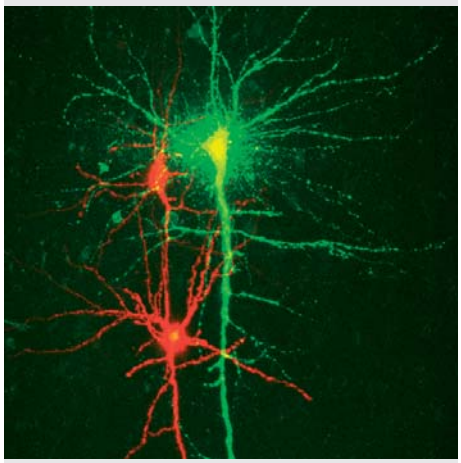




Gradient contrast with camera, magnification 0.35x (A), 1x (B), 4x (C). Online overlay of IR-SGC and fluorescence image (D).

Features and Benefits

- Close-coupled two-channel NDDs both in reflected and transmitted light paths
- Electrophysiology condenser system with outlet pipe for draining
- Condenser base with condenser head 0.9 NA S1
- Condenser base with condenser head 1.4 NA oil S1 for highest collection efficiency



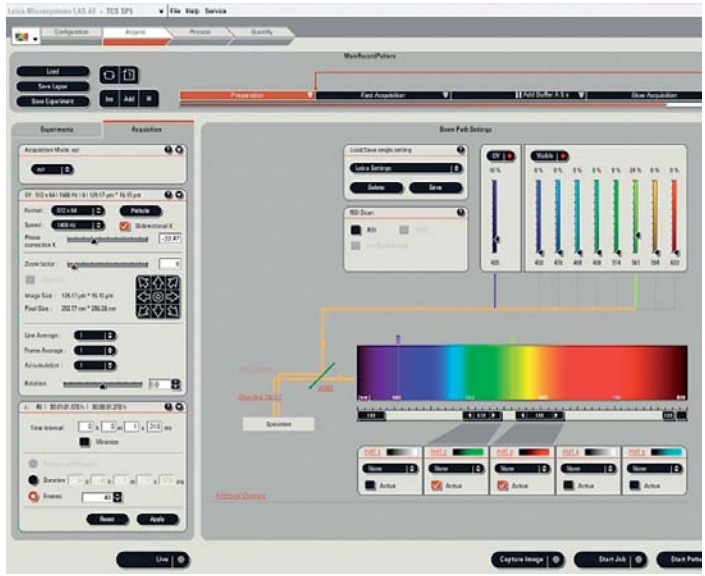
Double labeled neurons of brain slices. Courtesy of Thomas Nevian, Institute of Physiology, Bern, Switzerland

Two worlds in one

Two completely different experimental requirements can be satisfied with one single system. The Leica TCS SP5 with the Tandem Scanner provides classical morphology on large samples, where high spatial resolution is required, e.g. research on structures of cytoskeleton, organelles or tissues, as well as physiology and biophysics imaging, where temporal resolution becomes very important.

Calcium is an important second messenger to trigger many signalling cascades in neurons. Functional imaging of calcium influx is possible with calcium ion sensitive indicator dyes, but calcium transients in neurons are very fast. Therefore, they are typically recorded in line scan mode, also known as xt-scan mode. The laser beam is continuously scanned back and forth along the same line and fluorescence over time is recorded. The resulting image consists of one spatial and one time axis. The resonant scanning system of the Leica TCS SP5 oscillates at 8000 Hz, enabling a line rate of 16,000 lines per second. Fast dynamics of initial calcium inflow can now be investigated.

Even in the xy-scan mode, the scan rate can be as high as 180 frames per second in the frame size 512 x 64. This frame rate enables imaging of extended regions of the dendritic tree and multiple spines at the same time.



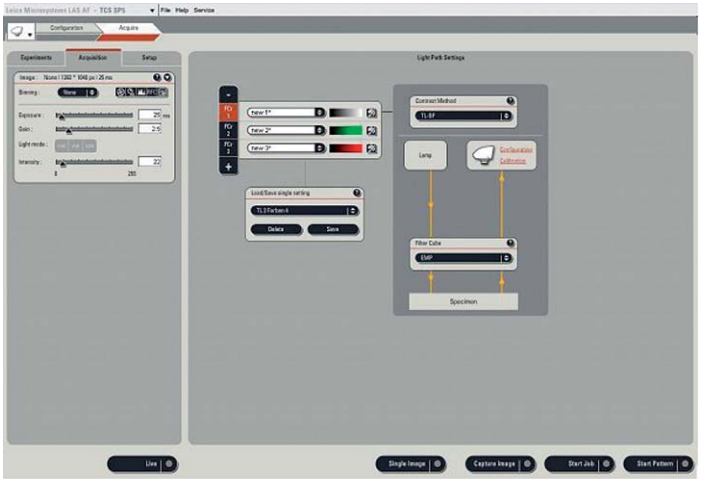
Confocal interface

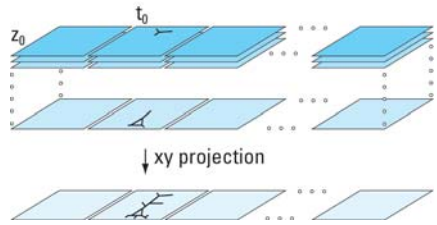
New XYTZ-Scan Mode for 3D visualization of calcium transients
The dendritic tree has a complex three dimensional structure. Repeatedly acquiring complete 3D stacks gives a temporal resolution much too low for imaging the fast calcium transients in dendrites. To circumvent this problem, optical sectioning, the fast scanning of the resonant system and the triggering capability are combined for the new XYTZ-Scan Mode.

Individual time series are taken at different focal depths and combined into a 4D image stack. A stimulus is always delivered before the same frame of each time series, synchronized by a trigger out event. After a complete focal series, the image data is projected into a 3D data stack over time. For each structure in the sampled 3D volume the fluorescence transients can be analyzed. The time course of fluorescence in all parts of the dendritic tree can be seen clearly.

Scanner patching and optimized workflow
In combination with the IR-SGC, which makes electrophysiology needles visible in the scanning mode, the extreme speed of the resonant scanner allows electrode patching while imaging at video rates, without ever switching to the camera mode. Together with the one-for-all electrophysiology 20x 1.0 objective, scanner patching minimizes the number of steps between experiment setup and data collection, optimizing your workflow.

So even though you might never need it, the workflow oriented user interface of the Leica TCS SP5 with Leica DM6000 CFS includes the camera as well. Whether you are doing CCD-camera or confocal / multiphoton imaging, the other option is always just one fast click away. Integrating both operation modes in the same software allows you to concentrate on what's in your images, not on where they came from.

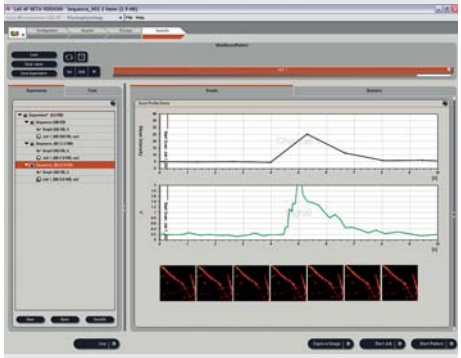

Camera interface



XYTZ-Scan Module:Time series at different focal positions, stimulus (t_0) is always delivered before the same frame of each time series

Features and Benefits

- Software-integration of CCD-camera and confocal scanner
- Single click switching between camera and confocal
- Workflow oriented user interface



Data Correlation (ψ/F)

Recording of electrical (e.g. patch-clamp) data is typically related to stimulation (electrical or chemical sensory stimulation of the animal). Current or voltage is recorded briefly before, during and after stimulation. The time frame for recording data after stimulation depends on effect-relaxation.

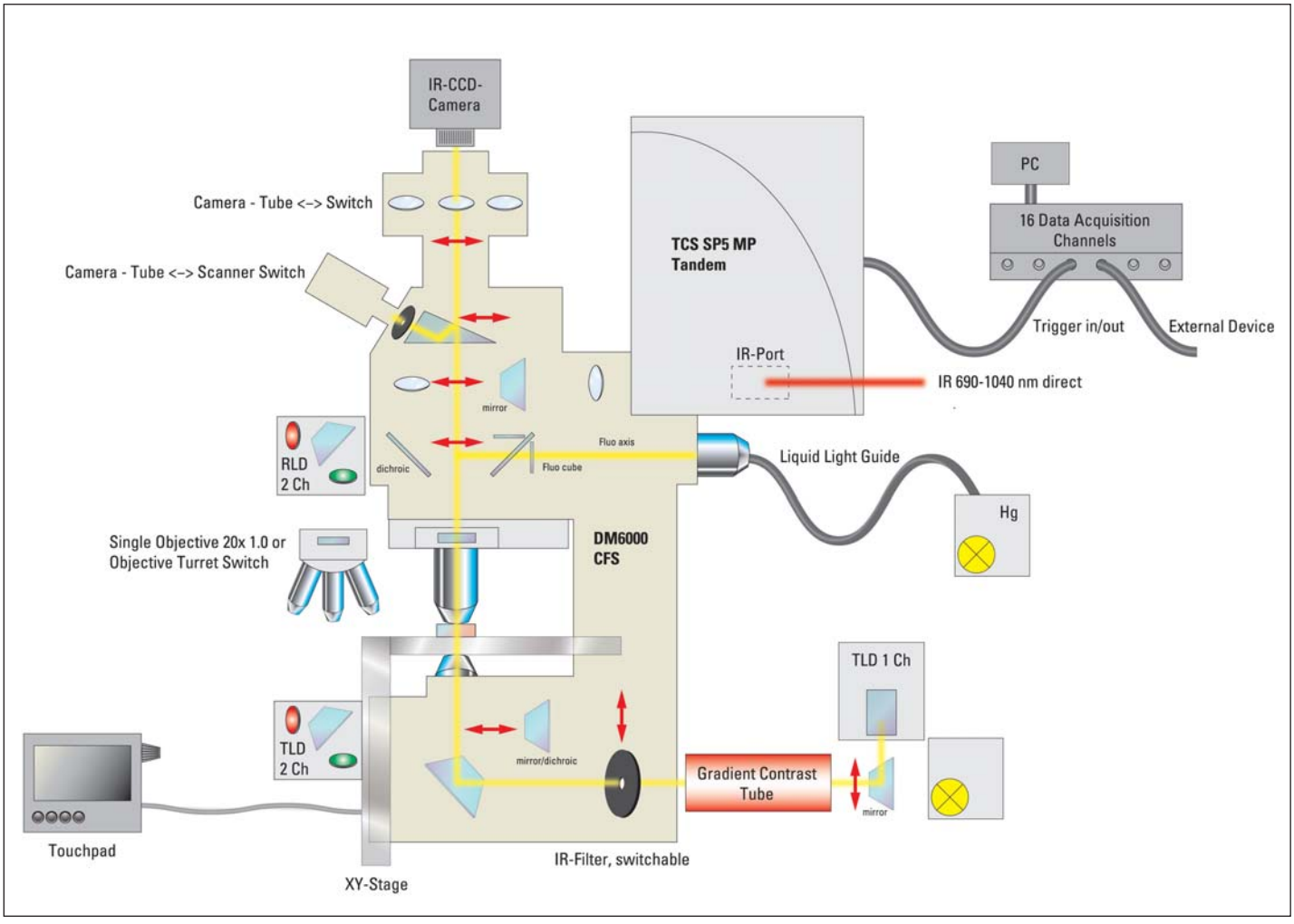
To synchronize the image acquisition and the electrophysiological recordings, precise triggers are necessary. The Leica TCS SP5 hardware provides different types of outbound triggers, such as frame, line and pixel triggers. They can be used to synchronize the application of stimuli or external recording devices. On the other hand, input triggers can be used to start or continue image scanning in response to arbitrary external events, thus increasing the flexibility in data acquisition. For example, the synchronization of heart beat and image acquisition could be performed by a special input trigger to minimize the influence of heart activity on the image data.

With the Leica software LAS AF, data evaluation of the electrophysiological signals can be performed online providing precise correlation with imaging data. For the analysis of electrical and optical data, the relevant basic functions are implemented. Online data evaluation enables the validation of the recorded data and is important in helping the researcher quickly decide how the external parameters should be modified for the best results.

Options for data correlation

- Correlation of optical and electrical data
- Input triggering to start image acquisition
- Output triggering to control stimuli
- Synchronization of scanning with external devices
- Control of environmental conditions by online data analysis

System Components



Leica Confocal Fixed Stage System DM60000 CFS

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Leica Microsystems' mission is to be the world's first-choice provider of innovative solutions to our customers' needs for vision, measurement and analysis of microstructures.

Leica, the leading brand for microscopes and scientific instruments, developed from five brand names, all with a long tradition: Wild, Leitz, Reichert, Jung and Cambridge Instruments. Yet Leica symbolizes innovation as well as tradition.

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• Specimen Preparation

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