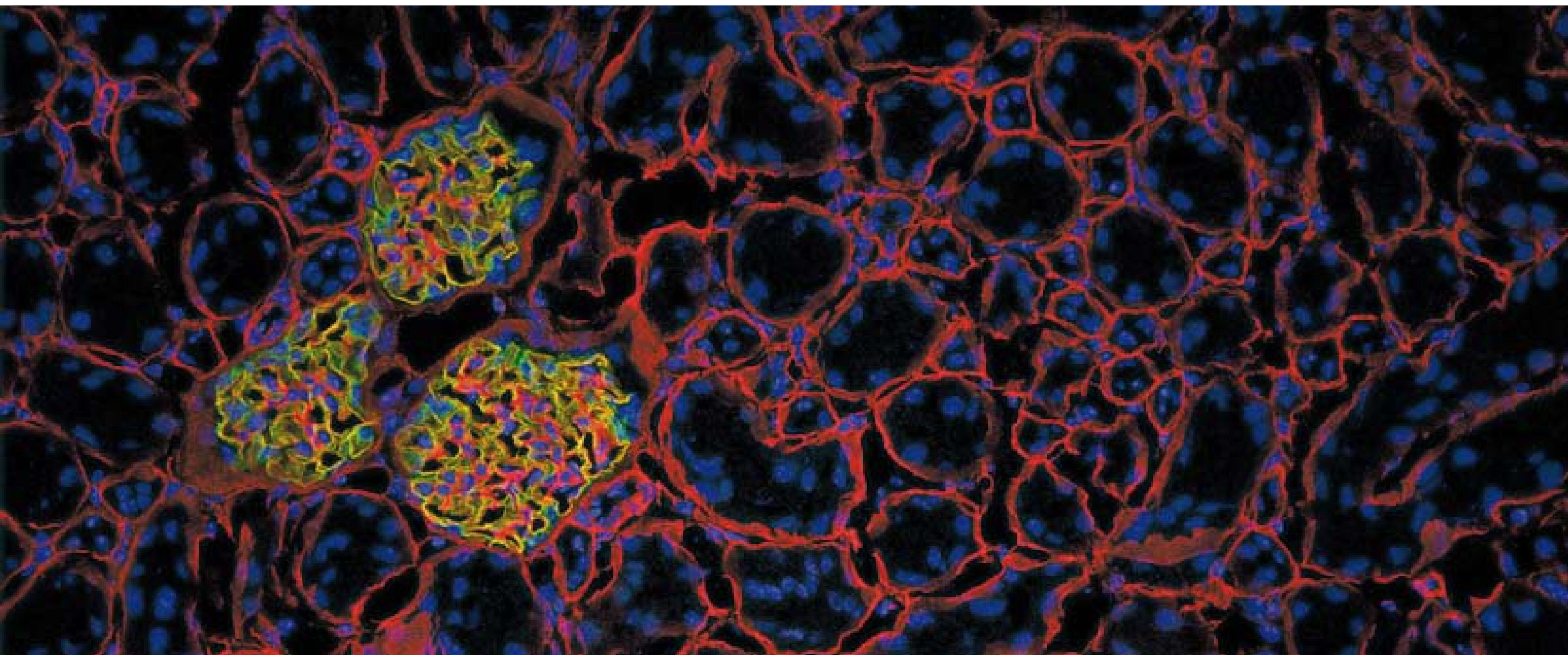
LSM 710

In *Tune* with Your Application

Enjoy new freedom in selecting fluorescent dyes with In *Tune*, the new laser system for the LSM 710. Whatever the wavelength, you can match the excitation of any dye perfectly, from blue to far red. Open new possibilities for designing your experiment, whether for intensity or lifetime imaging.

Finding the right combination of dyes for an experiment with multiple labels is often a tricky business. On top of that the absence of an optimal excitation source limits the number of useful dyes. Then, even greater challenges arise when performing Förster Resonance Energy Transfer (FRET) experiments based on fluorescence lifetime imaging microscopy (FLIM). For quite a long time pulsed lasers have been available only for a small set of wavelengths in the visible spectrum, especially in the green range, and this has compromised the choice of suitable FRET pairs dramatically.
With In *Tune* such restrictions no longer exist. This pulsed laser provides any wavelength ranging from 488 to 640 nm.

In *Tune* is proving its value all round. It's versatile when exciting any kind of dye or combination of dyes that might be optimal for your experiment. With the fast and flexible detection technology of the LSM 710 and In *Tune*, the fluorescence signal can be detected very close to the excitation wavelength. It's stable and reliable and features a low noise level comparable to gas or diode lasers.
Indeed, because suitable excitation sources were never available before, this new laser system allows you to easily measure fluorescence lifetimes of dyes that simply could not be evaluated until now.



The New Tunable Laser for Laser Scanning Microsope LSM 710

We make it visible.

Set the Optimal Excitation Wavelength

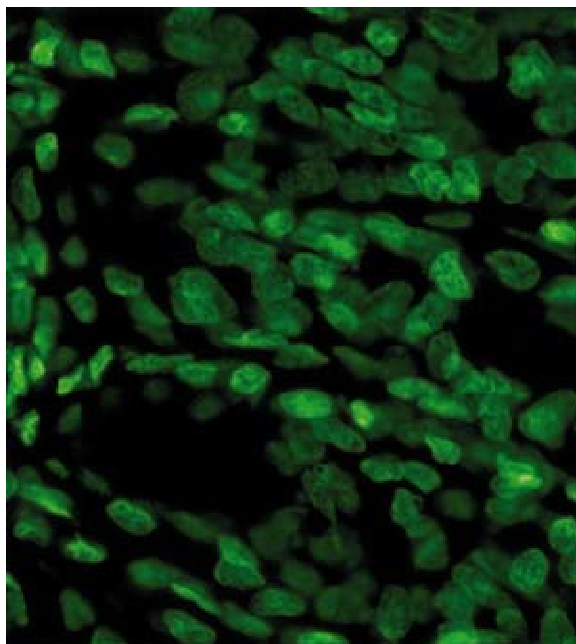
The adaptable, fast detection scheme of the LSM 710 is state of the art and In *Tune* is its perfect complement – a laser system that provides any wavelength in the visible range.

In *Tune* enables you to set the optimal excitation wavelength for a given dye or combination of dyes, minimizing cross excitation and optimizing the detection window. Because of its narrow bandwidth (less than 3 nm) and its low noise characteristics, it can substitute lasers in the visible range, especially any green, orange or red laser sources. Combine and use it simultaneously with any additional laser available in the system, from near UV to far red. The image quality you experience will stand full comparison with the high quality you are accustomed to when working with the LSM 710 using fixed wavelength lasers.

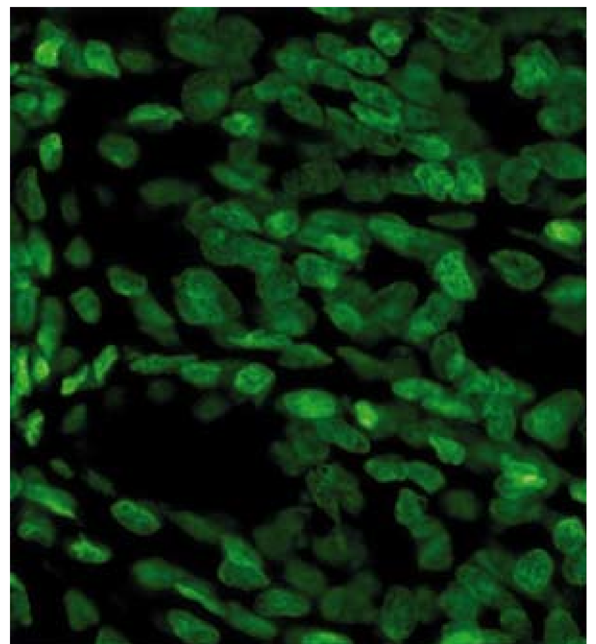
Wavelength range	488 – 640 nm
Power	> 1,5 mW
Repetition rate	40 MHz
Pulse length	< 5 ps
Tuning speed	< 50 nm / s
Bandwidth	< 3 nm

Specification In *Tune*

Mouse kidney section.
Nuclei stained with Sytox green.
Excitation using In Tune at
490 nm with 3 µW at the sample plane.



Mouse kidney section.
Nuclei stained with Sytox green.
Excitation using the Argon laser at
488 nm with 3 µW at the sample plane.

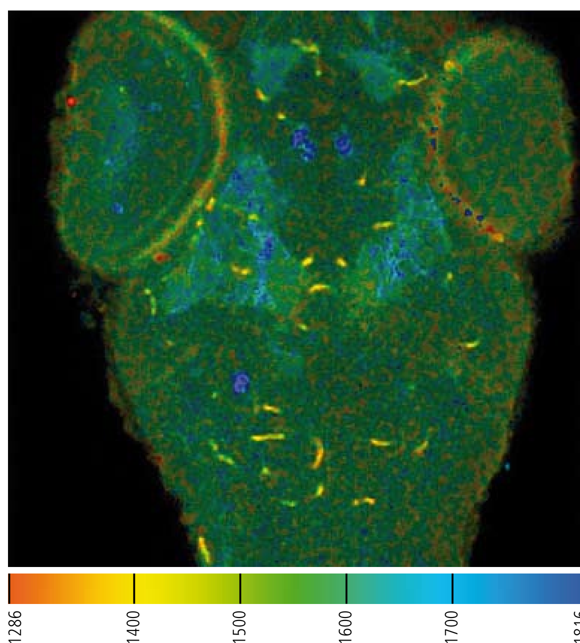


Capture Images Showing Fluorescent Lifetimes

Acquire lifetime data as never before.
Let the experiment, not the laser, decide which FRET pair to use.

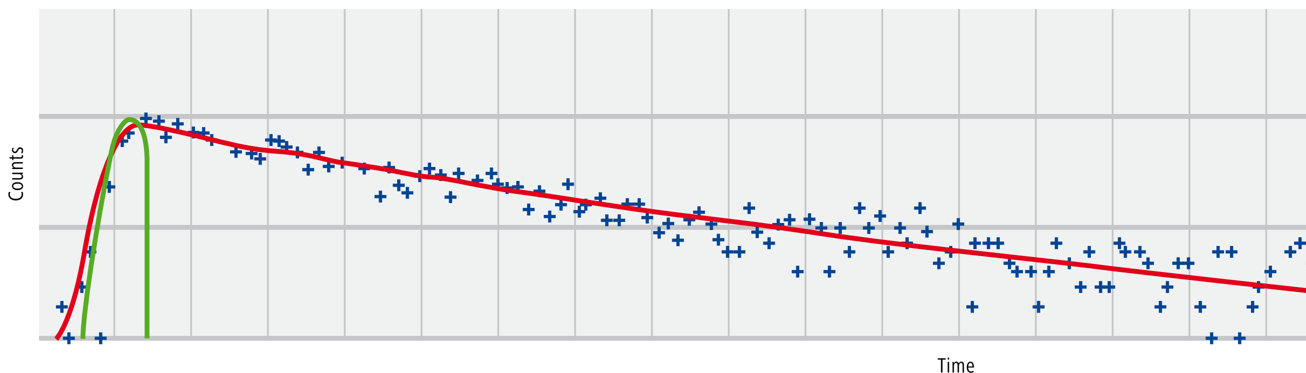
The fluorescence lifetime of dyes or proteins is an excellent indicator for the local environment. It reveals important information – for example, about pH, temperature or the oxygen level. As such, measuring lifetimes of fluorescent molecules will reveal changes in their environmental parameters over time. Another widespread use of FLIM is the study of protein-protein interactions in intact cells by FRET. A close proximity between two molecules, each linked to a fluorescent dye or fluorescent protein, allows energy to be transferred from the donor to the acceptor molecule, shortening the lifetime of the former. The change in lifetime is a direct measure of the distance between the two partner molecules.

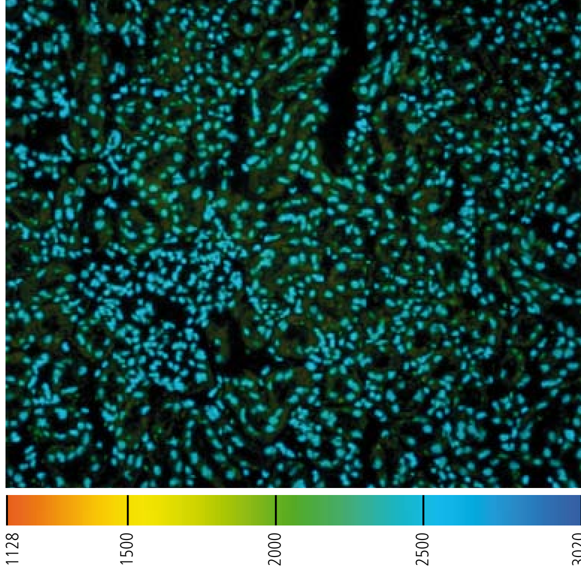
Up to now the choice of available FRET pairs has been hampered by the limited number of pulsed lasers providing lines in the visible range that would best excite the donor molecule. Yet the pairing of the two fluorescent molecules is crucial, as the emission of the donor molecule needs to match the spectral range for the excitation of the acceptor molecule.



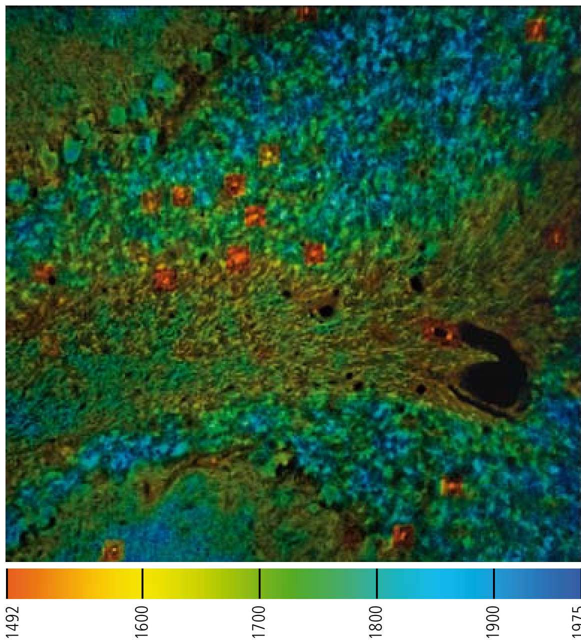
Color coded FLIM image (ps) of a 3-day old zebrafish embryo. Whole mount stained for GFP transgene (Alexa Fluor 488), catecholaminergic systems (Alexa Fluor 555) and serotonergic cells (Alexa Fluor 594). Lifetime image acquired using 562 nm for excitation. Specimen: T. L. Tay, R. Nitschke; University of Freiburg, Department of Developmental Biology and Life Imaging Center, Germany

Typical distribution curve of photon counts per time when measuring fluorescence lifetime.





Color coded FLIM image (ps) of mouse kidney section stained for the podocyte specific protein Podocin (Alexa Fluor 488, green), the basement membrane marker Nidogen (Alexa Fluor 555, red) and Nuclei (Topro-3, blue). Lifetime image acquired using 640 nm for excitation. Specimen: B. Hartleben, R. Nitschke; University of Freiburg, Medical Clinic IV and Life Imaging Center, Germany

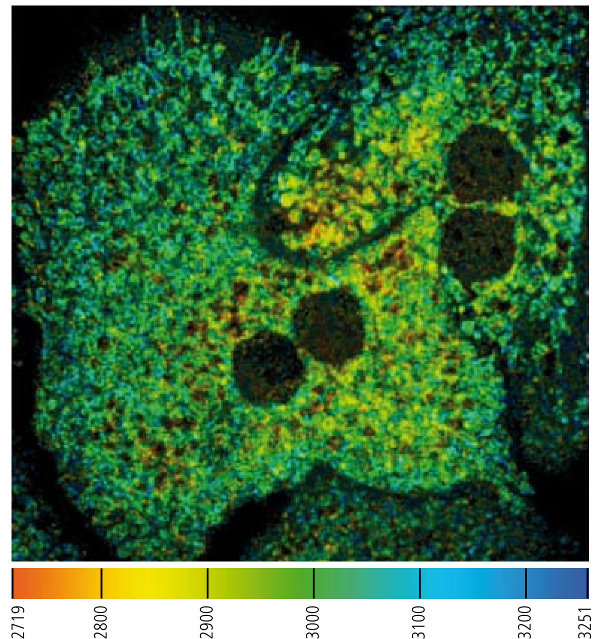


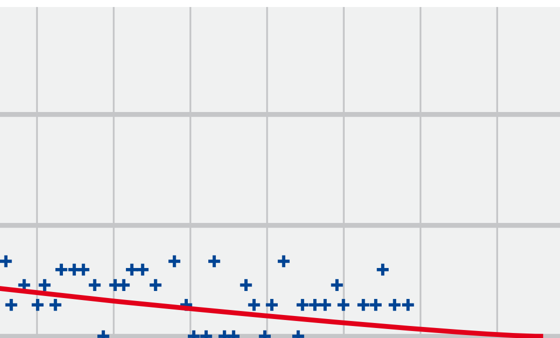
Color coded FLIM image (ps) of mouse cerebellar section stained for KCC2 (potassium-chloride cotransporter 2, primary antibody kindly provided by C. Rivera, University of Helsinki, Institut for Biotechnology) (Alexa Fluor 488) and parvalbumin (Alexa Fluor 568). Lifetime image acquired using 491 nm for excitation. Specimen: O. Kántor, R. Nitschke; University of Freiburg, Institut of Anatomy and Cell Biology, Department of Molecular Embryology and Life Imaging Center, Germany

Also, with In *Tune,* when searching for a FRET pair you no longer need to consider the excitation wavelength. The wavelength range of the laser lets you measure the lifetime of any dye excited within the spectral range of 488 to 640 nm.

Typical lifetimes of fluorescent dyes are on the order of a few nanoseconds. Therefore the optimal pulse repetition rate of lasers for lifetime measurements lies between 10 and 50 MHz. In *Tune,* with its repetition rate of 40 MHz, fits nicely into this range, providing a pulse every 25 ns. This leaves enough time to reliably determine the lifetime of most of the fluorescent molecules in use today.

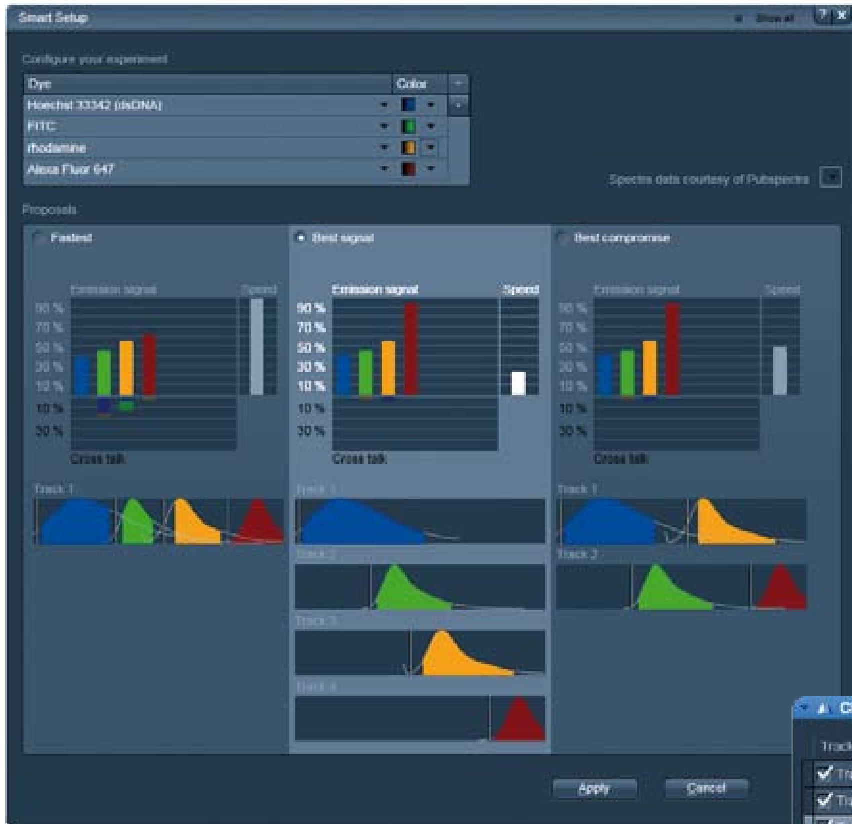
Color coded FLIM image (ps) of hepatocytes stained for Cytochrome C (Alexa Fluor 488) and Mitochondria (Alexa Fluor 564). Lifetime image acquired using 568 nm for excitation. Specimen: R. Pick and R. Nitschke; University of Freiburg, Molecular Medicine and Life Imaging Center, Germany





Easy to Use, Easy to Upgrade

The new flexibility available with In *Tune* is set and controlled by the LSM software ZEN. Smart Setup chooses the optimal excitation wavelength and detection window for the selected dye.



Smart Setup suggests hardware settings to image a sample with four labels.

Greater flexibility often comes at the cost of greater complexity. Using In *Tune* with the LSM 710, the new freedom of choice for the excitation wavelength creates a number of possibilities for setting the hardware parameters for the experiment. To keep it simple and easy to use, ZEN with its Smart Setup function takes care of this step. Only the fluorescent labels or proteins present in the specimen need to be indicated. ZEN then translates this choice into the hardware settings, which are optimized to get the best detection efficiency for the dyes.

The control and operation of the laser itself is completely integrated into the ZEN software. The wavelength is set either by selecting a number from a drop down menu or by typing this number into the editing field. You can upgrade any LSM 710 and the laser can be added to your system on site.

Choose a wavelength from the drop down menu to use In Tune for imaging.

LSM 710 set up with Axio Observer.
In *Tune* can be upgraded to and used with LSM 710 and LSM 710 NLO systems set up with Axio Observer, Axio Imager or Axio Examiner.

Dr. Roland Nitschke,
Life Imaging Center,
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"In Tune, the new tunable laser will extend our experimental options in two major directions. The excitation wavelengths from 488 – 640 nm are now freely accessible, opening doors to the use of a multitude of new dyes for FLIM experiments.
For our conventional confocal imaging In Tune ideally extends the LSM 710 usage for multi-labeled samples on the excitation side, matching the flexibility of the QUASAR detector on the emission side. By tuning the laser to intermediate wavelengths, we will now be able to detect the last 10–20 % of the emission signal, which are otherwise often lost due to the small Stoke's shift of most commercial fluorescent dyes. I was impressed by the ease of use of this laser, which is mandatory for our multi-user imaging facility."

Figure coverpage:
Mouse kidney section stained for the podocyte specific protein Podocin (Alexa Fluor 488, green), the basement membrane marker Nidogen (Alexa Fluor 555, red) and Nuclei (Topro-3, blue). Specimen: B. Hartleben, R. Nitschke; University of Freiburg, Medical Clinic IV and Life Imaging Center, Germany

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