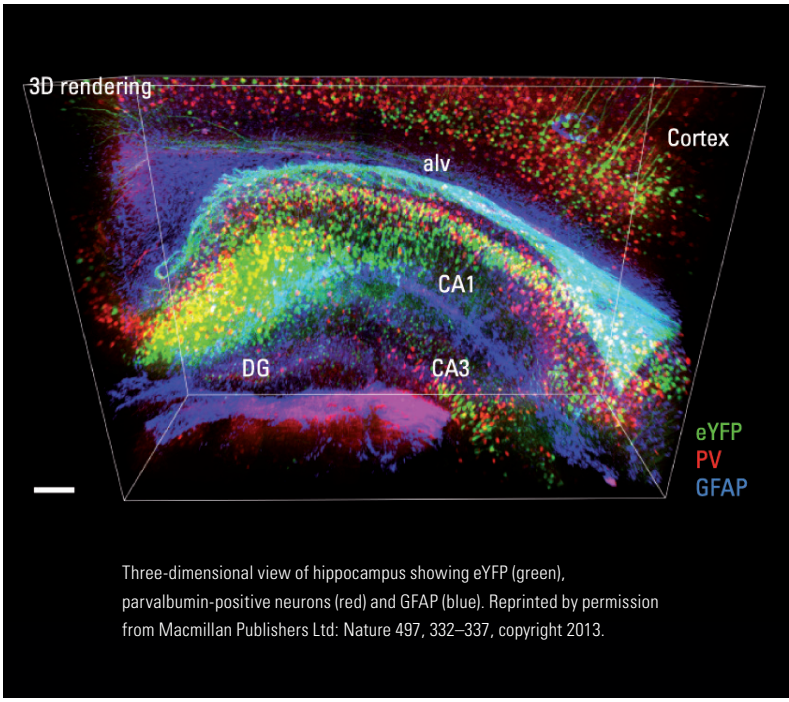
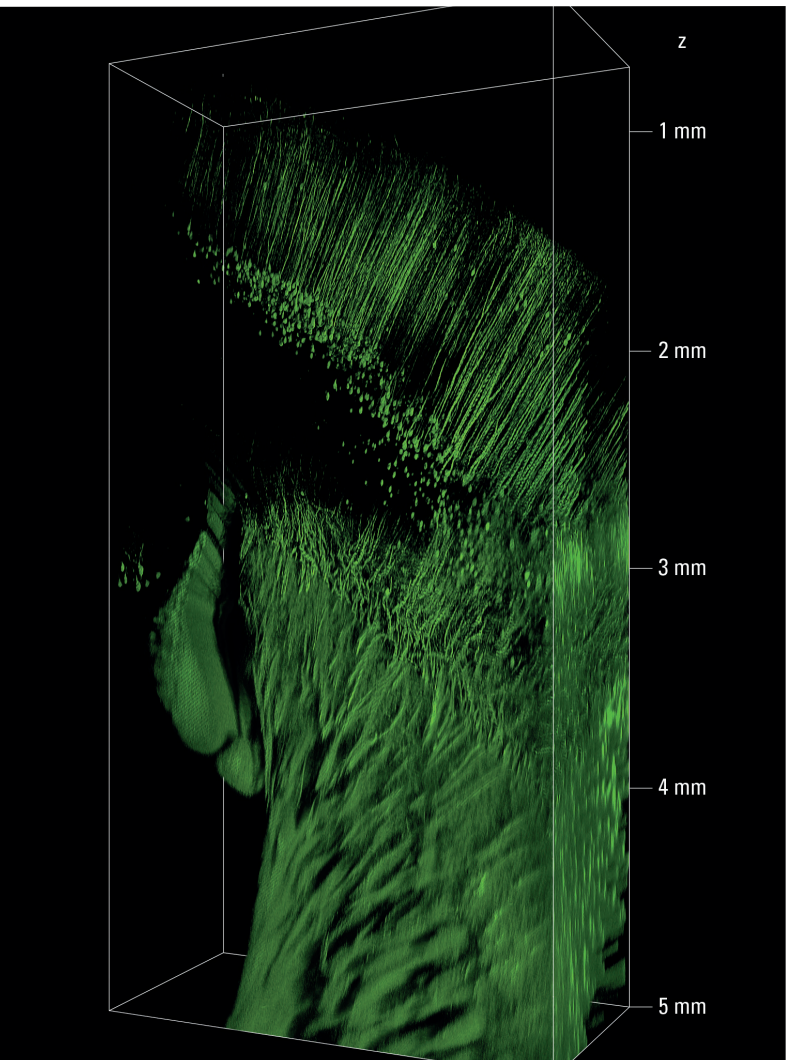
Living up to Life

Leica MICROSYSTEMS



Three-dimensional view of hippocampus showing eYFP (green), parvalbumin-positive neurons (red) and GFAP (blue). Reprinted by permission from Macmillan Publishers Ltd: Nature 497, 332–337, copyright 2013.



A Clear View in Depth

CLARITY Objective with Motorized Correction Collar

The CLARITY clearing technique provides maximum imaging depth and highest resolution when combined with the appropriate optics. The Leica HC FLUOTAR L 25x/1.00 IMM (n_e=1.457) motCORR VISIR is specifically designed for optimized results from CLARITY-treated specimens.

- Matches the refractive index (RI) of CLARITY-treated specimens for bright, high-resolution images from deep within tissues
- Remote-controlled motorized correction collar for fast image optimization without disturbing the specimen
- Whole organ imaging possible up to a depth of 6 mm
- Broad range VISIR correction ideal for use with single- and two-photon excitation.

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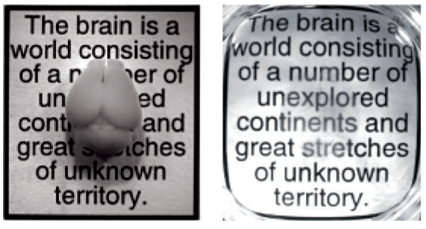
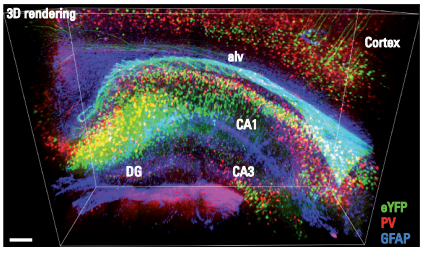
Thy1-YFP adult mouse brain treated with CLARITY. Confocal imaging with excitation at 514 nm. Courtesy of K. Deisseroth and R. Tomer, Stanford University, Palo Alto, CA, USA.

Tissue Clearing Improves Penetration Depth

Mapping of the mammalian brain with fluorescence microscopy remains a challenging task. Multiphoton excitation, which is the traditional method for imaging within light-scattering tissues, is still limited to a depth of few hundred micrometers. To investigate deeper lying structures, the specimen is typically sectioned into slices. Novel optical clearing methods use organic solvents to reduce light scattering and to maximize imaging depth in intact tissue.





Top: Thy1-eYFP line-H mouse brain before (left) and after CLARITY treatment (right)

Bottom: Three-dimensional view of hippocampus showing eYFP (green), parvalbumin-positive neurons (red) and GFAP (blue).

1 Structural and molecular interrogation of intact biological systems. Chung K, Wallace J, Kim SY, Kalyanasundaram S, Andalman AS, Davidson TJ, Mirzabekov JJ, Zalocusky KA, Mattis J, Denisin AJ, Pak S, Bernstein H, Ramakrishnan C, Grosenick L, Gradinaru V, and K Deisseroth. Nature 497, 332–337 (16 May 2013)

CLARITY REDUCES REFRACTIVE INDEX MISMATCHES FOR DEEP TISSUE IMAGING

Biological specimens consist of a variety of components with different refractive indices (RIs). These RI mismatches cause scattering of light, chromatic aberrations and distortion of the point-spread function.

› RI mismatches reduce intensity, resolution, contrast and penetration depth

The CLARITY method[1] covalently links proteins and nucleic acids to a network of polymers while removing light-scattering lipids. The hydrogel-hybrid maintains the native structure and volume of the tissue.

By mounting the CLARITY-treated ("clarified") brain in a refractive-index-matched medium like FocusClear® it becomes fully transparent.

MULTI-ROUND MOLECULAR PHENOTYPING IN INTACT SYSTEMS

Many clearing methods leave lipid bilayers impermeable, limiting penetration of macromolecules for whole mount staining methods and reducing stability of the tissue.

› CLARITY renders tissues firm and easy to handle

The nanoporous hydrogel mesh created by CLARITY is permeable for macromolecules and allows rapid diffusion of antibodies and nuclear acid probes.

Therefore, investigation of many molecular phenotypes – like protein complexes, gene expression profiles, and neuronal circuits – are possible in a single brain.

› CLARITY allows for multiple rounds of molecular phenotyping

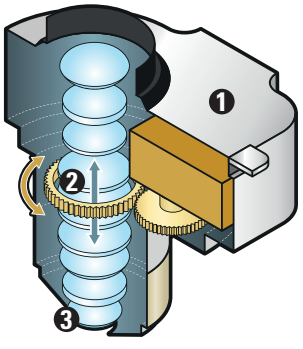
Leica motCORR™: Motorized Correction Collar for Best Optical Performance in Thick Tissues

The ability of confocal laser scanning and multiphoton microscopes to highly resolve objects in xy and z is exploited to the maximum when the refractive indices of the objective, the specimen and all intermediate optical media match. The Leica motCORR™ provides fast and precise adjustment of the optics to restore optimal imaging conditions.

EFFECTS OF REFRACTIVE INDEX MISMATCH INCREASE WITH DEPTH

Refractive index (RI) inhomogeneities in clarified tissues are largely removed. Still, mismatches can occur between the immersion medium and the specimen. The longer the distance the light has to travel through a medium with mismatched RI, the stronger are the negative effects (see figure on the right).

With a free working distance of 6 mm, optimal adjustment of the objective's correction collar at each z-position is crucial for bright and contrast-rich images from deep within tissues.



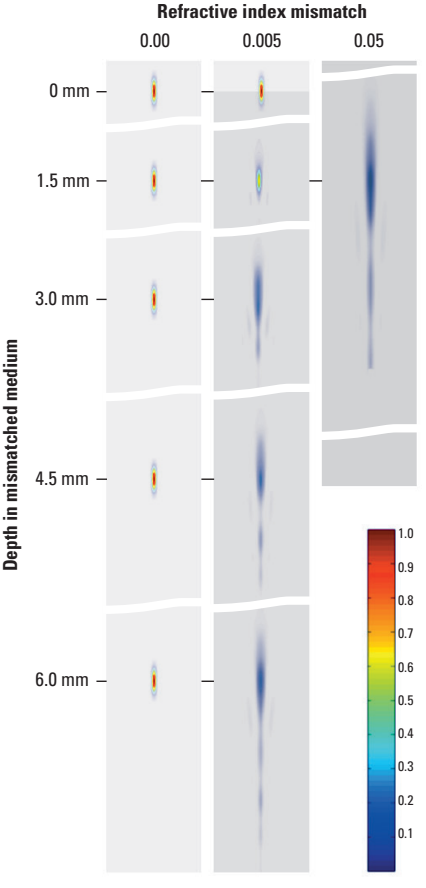
1 motor
2 lens group correcting for changes in refractive index
3 front lens

SOFTWARE-CONTROLLED CORRECTION FOR REFRACTIVE INDEX MISMATCH

The motorized correction collar of the Leica HC FLUOTAR L 25x/1.00 IMM motCORR VISIR is driven by a precise and robust motor. Remote control by LAS AF (Leica Application Suite Advanced Fluorescence) microscope software or the control panel is easy.

› Fast adjustment of Leica motCORR™ without disturbing the specimen
› Minimized training effort by easy, precise control of the correction collar using motCORR™

Distortion of point-spread function (PSF) increases dramatically with depth.
Calculated PSFs for multiphoton excitation at 850 nm with Leica HC FLUOTAR L 25x/1.00 IMM motCORR VISIR for different penetration depths into the specimen (for 0.05 RI mismatch only at 1.5 mm depth). RI mismatch between correction collar and specimen is 0 (left), 0.005 (middle) and 0.05 (right). Correction collar position remains unchanged.

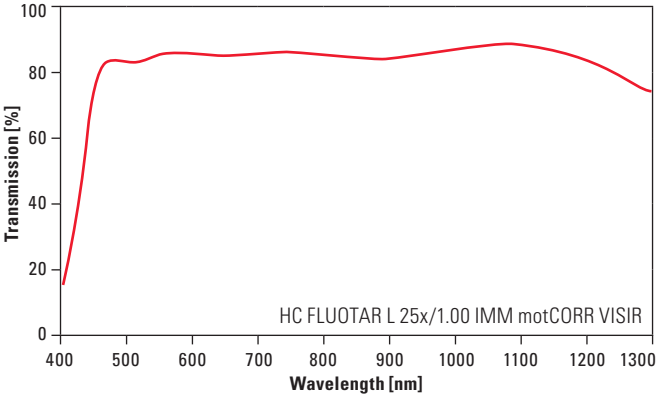


VISIR Correction for Confocal and Multiphoton Microscopy

The broad spectral correction of the objective ensures outstanding imaging performance with single- and two-photon excitation. Color correction of this objective is complemented by the range of refractive index correction available from the motorized correction collar, to produce high axial and lateral colocalization in specimen cleared with the CLARITY method and mounted in FocusClear®.

Maximum Transmission in VIS and IR for Brighter Images

Superior anti-reflective coatings make the Leica HC FLUOTAR L 25x/1.00 IMM motCORR VISIR highly transmissive (>85%) from 470 to 1200 nm. This maximizes the number of photons for excitation and results in brighter images from deeper tissue sections. Efficient collection of emitted photons reduces the need for high laser powers and protects the specimen from photobleaching.



DEEP TISSUE IMAGING OF CLARIFIED SPECIMENS WITH SINGLE-PHOTON AND TWO-PHOTON MICROSCOPY

Imaging Technique	General Principle	Advantages	Limitations
Single-photon confocal microscopy	Confocality achieved by removing out-of-focus light with confocal pinhole	Broad range of fluorescent labels suitable for multi-color imaging.	Photobleaching of the whole tissue by out-of-focus excitation light. Performance dependent on dispersion of specimen.
Two-photon microscopy	Excitation by two photons of approximately twice the wavelength – and half the energy – necessary for one-photon excitation. No pinhole required.	Photobleaching restricted to the focal plane. Highly efficient detection with non-descanned detectors. Increased depth by reduced scattering of infrared excitation light.	Not all fluorescent labels appropriate. Pulsed infrared laser required.

SPECIFICATIONS OF LEICA HC FLUOTAR L 25X/1.00 IMM (N_E=1.457) MOTCORR VISIR

Magnification	25x
Numerical Aperture	1.0 in n_e=1.457
Free Working Distance	6 mm
Correction Collar	Yes, motorized with full control via LAS AF software
Objective Thread	M32 for use with single objective holder on Leica DM6000 FS and CFS
Field of View	22 mm diagonal in intermediate image plane with Leica TCS SP8 1800 Hz scanner

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