PROSPECTIVE INSTRUMENTS

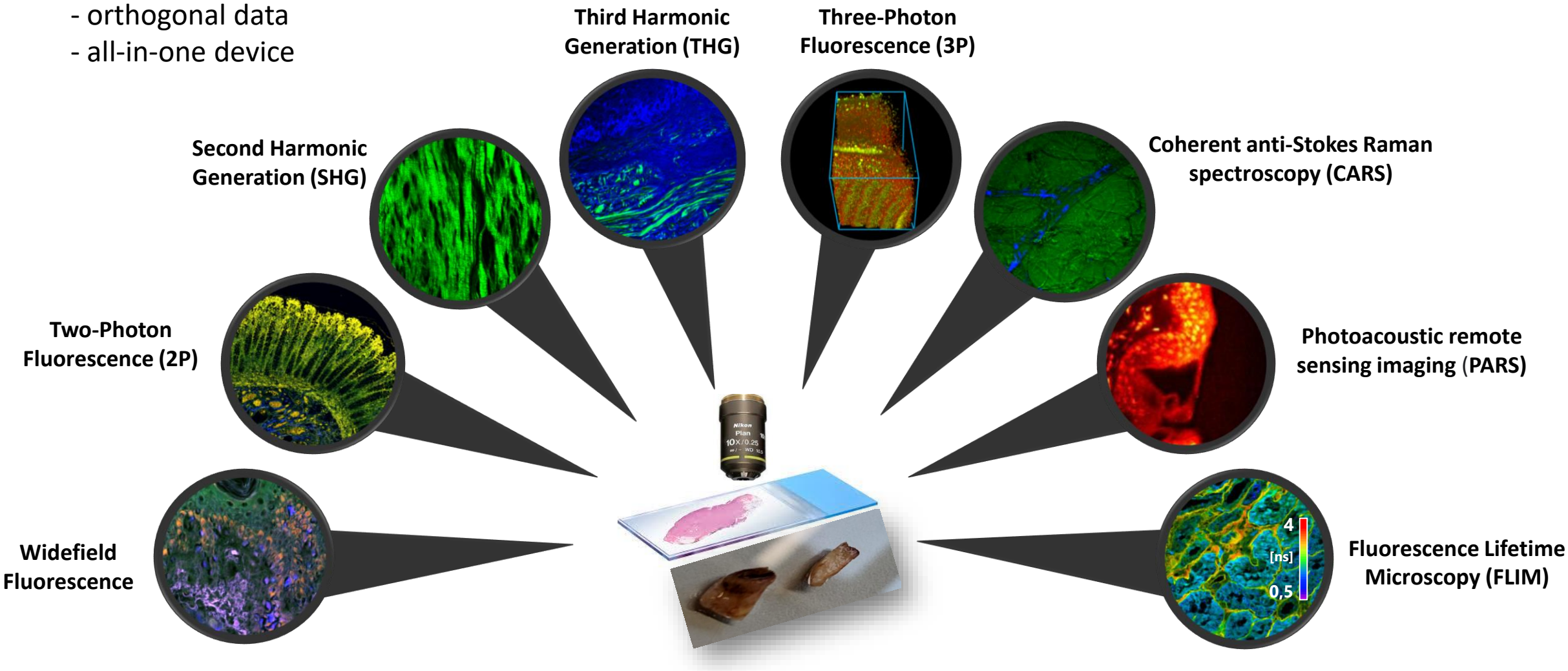
MULTIPHOTON MICROSCOPES
FEMTOSECOND LASERS
CONTRACT IMAGING SERVICE

WWW.P-INST.COM

MULTIMODAL IMAGING VISION

- non destructive
- orthogonal data
- all-in-one device

PROSPECTIVE

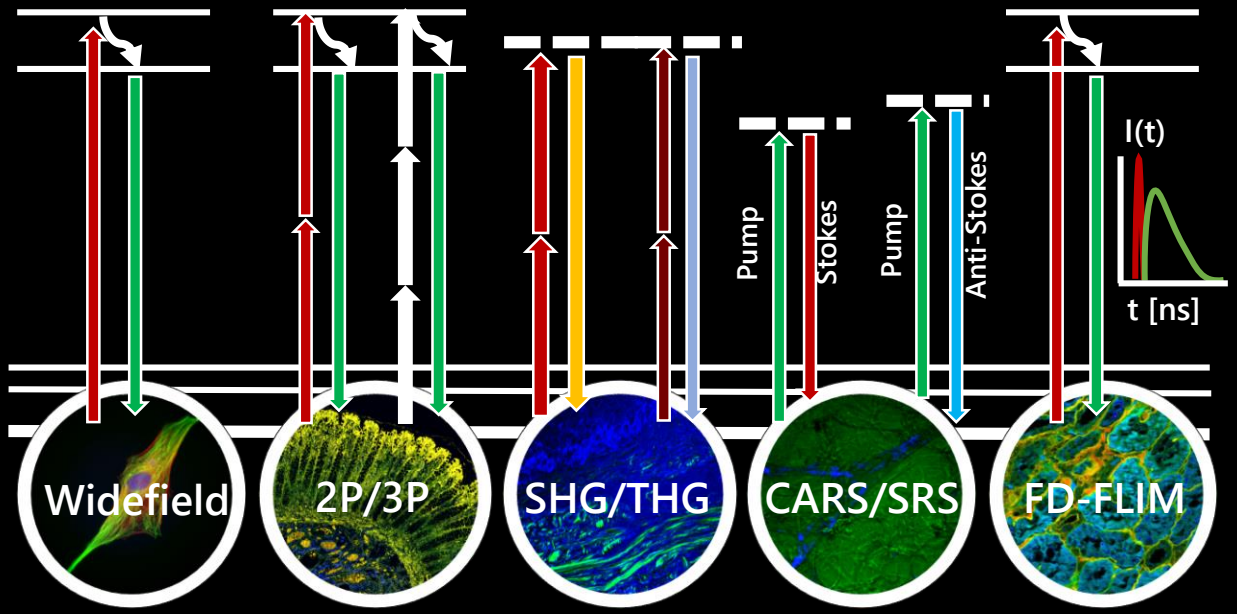


PROSPECTIVE
LIFE SCIENCE

TURN-KEY
EASY-TO-USE
COMPACT
MULTIMODAL

Multiphoton Microscopes

MULTIMODAL MICROSCOPY



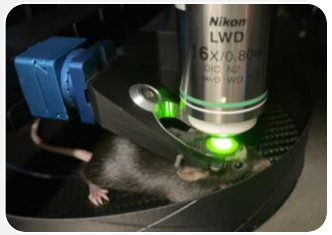
PROSPECTIVE
LASERS

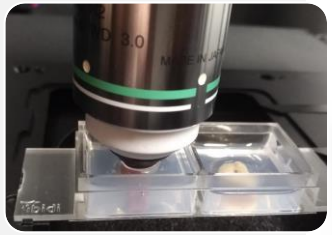
TWO-PHOTON, SHG & THG
OPTOGENETICS
FLIM
CARS & SRS

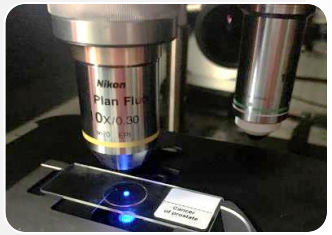
Femtosecond Lasers

MULTIPHOTON MICROSCOPES

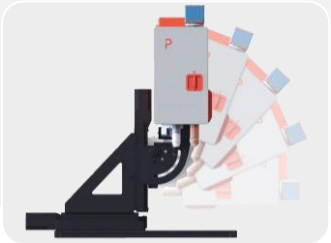
MP-SERIES

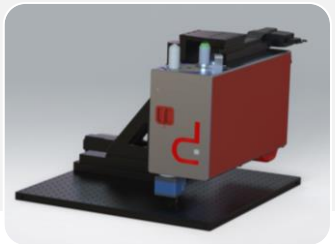






- Turn-key, Easy-to-use & rugged
- Compact & air-cooled
- Built in Laser (MPX) or 3P-ready (MPC)
- Multimodal (WF, 2P, SHG, CARS, FLIM)
- Large working distance, 360° access
- Upright & inverted
- Oblique angles



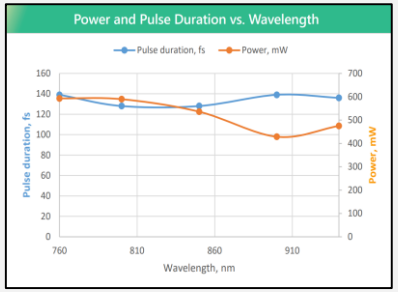


FEMTOSECOND LASERS

FSX-SERIES

PROSPECTIVE

- Turn-key & rugged
- Compact & air-cooled
- Dual-output
- Wavelength tuneable
- High power
- Low RIN & phase noise



- 2P polymerization
- Supercontinuum generation
- Terahertz generation
- Quantum optics
- Amplifier seeding
- 2P/SHG/THG microscopy
- FLIM microscopy
- CARS/SRS microscopy
- Spectroscopy
- Neuroscience & Optogenetics

2PEF/3PEF VS. WIDEFIELD

PROSPECTIVE

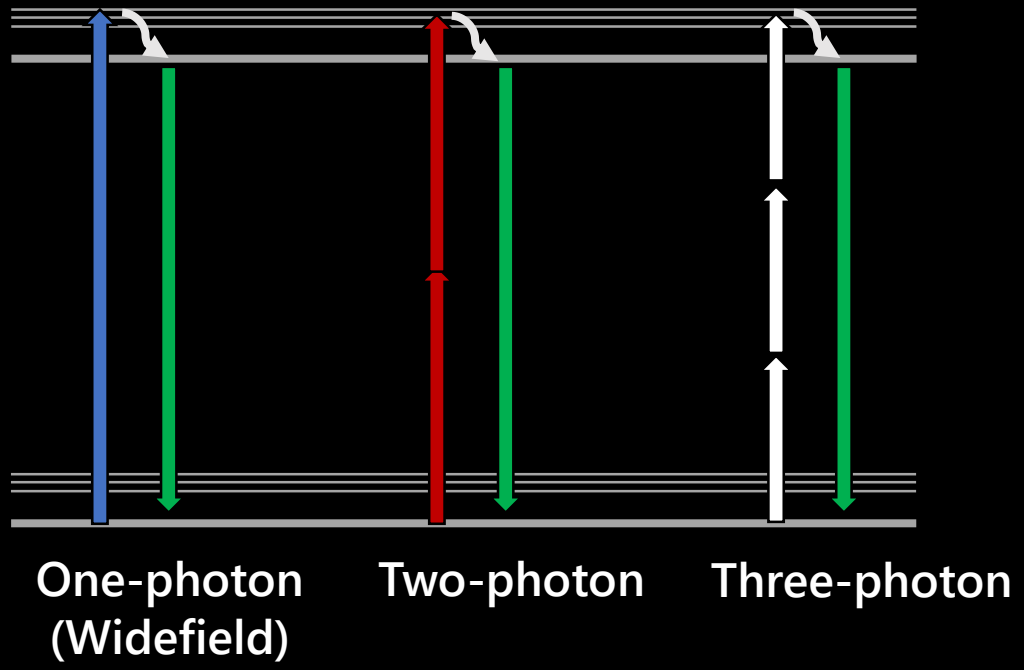
1. 2P/3P excitation is intrinsic confocal due to a confined excitation volume in 3D space

2. 2P/3P excitation is sample saving: only fluorescence in focal plane and point scanning

3. 2PEF/3PEF uses NIR light for higher penetration depth

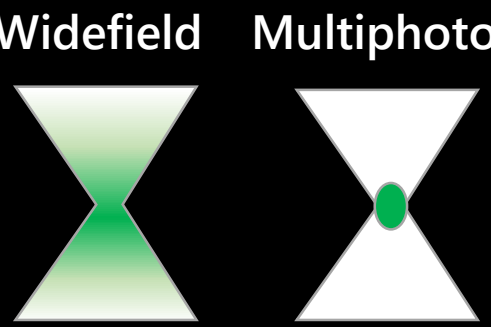
MULTIPHOTON EXCITATION FLUORESCENCE

EXCITATION & ABSORPTION



For non-linear microscopy techniques: signal ∝ Power^n

→ multiphoton absorption is spatially confined to the perifocal region. The absence of multiphoton absorption in out-of-focus planes makes this technique ***intrinsic confocal!***



**Drobizhev, M. et al. Two-photon absorption properties of fluorescent proteins. Nat Methods 8, 393–399 (2011)*

***Helmchen F, Denk W. Deep tissue two-photon microscopy. Nat Methods. 2005 Dec;2(12)*

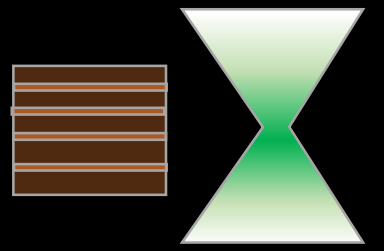
MULTIPHOTON EXCITATION FLUORESCENCE

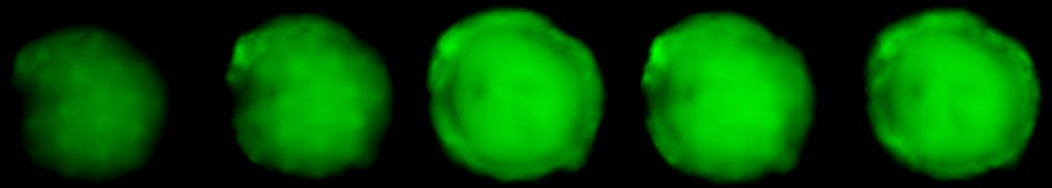
OPTICAL SECTIONING

PROSPECTIVE

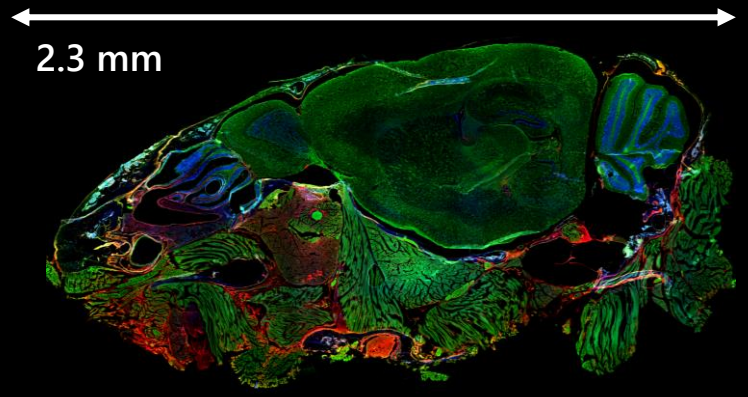
Widefield

2D Sample (<20 µm)





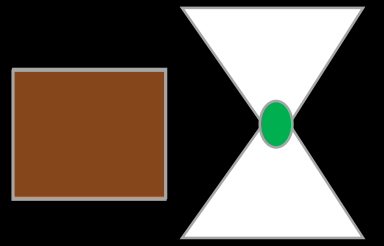
1 µm 7 µm 21 µm 28 µm 35 µm

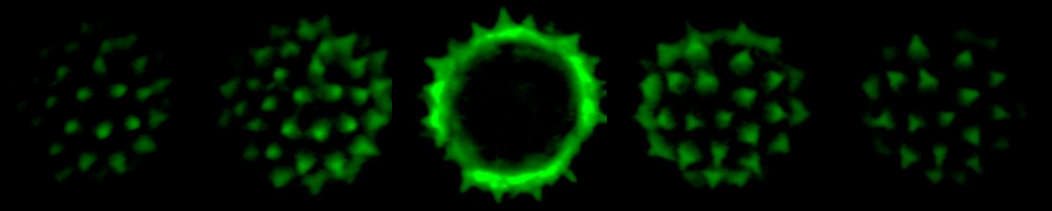


Widefield

Multiphoton

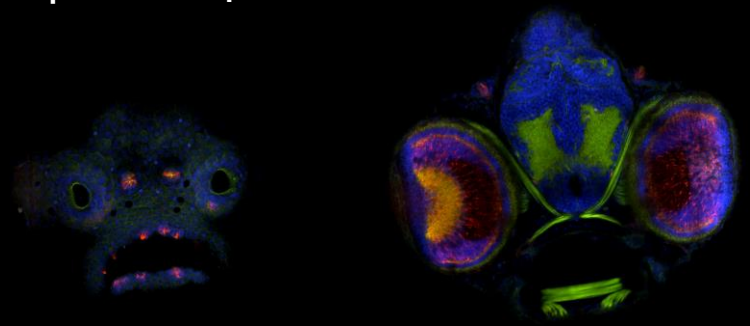
3D Sample (>20 µm)





sunflower pollen

Depth = 230 µm



MPE Microscopy

BROAD RANGE OF APPLICATIONS

PROSPECTIVE

- 2D/3D/4D imaging
- In-vitro & in-vivo
- Whole slide imaging
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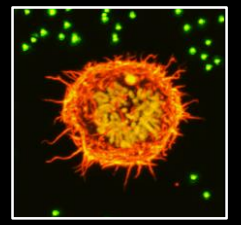
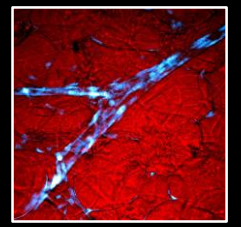
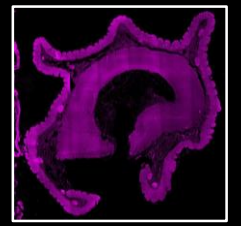
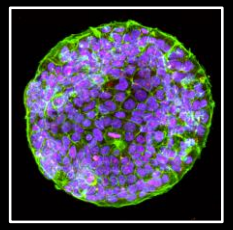
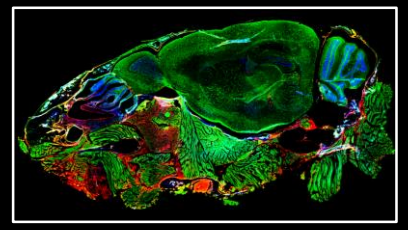
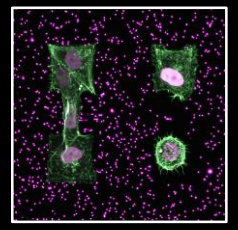
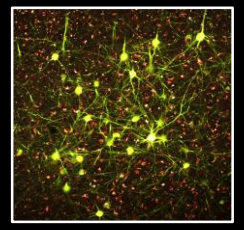
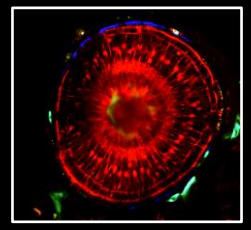
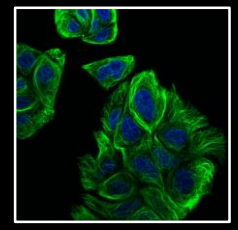
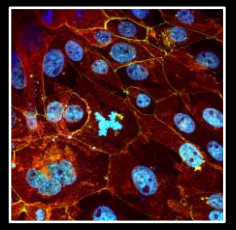
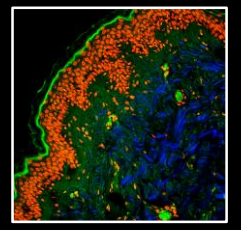
- Non-destructive
- Deep tissue imaging
- Pathology & Cancer
- Neuroscience
- Optogenetics
- Tissue Engineering & Bioprinting
- Spheroids / Organoids

BROAD RANGE OF APPLICATIONS

PROSPECTIVE

- 2D/3D/4D imaging
- **In-vitro & in-vivo**
- Whole slide imaging
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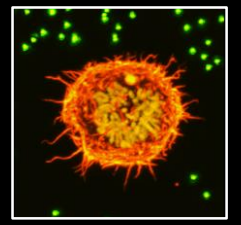
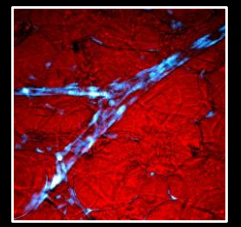
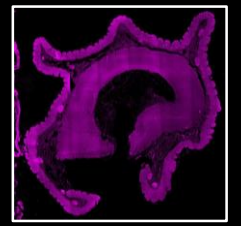
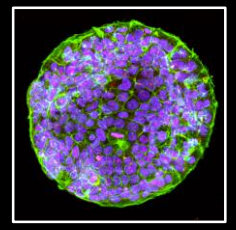
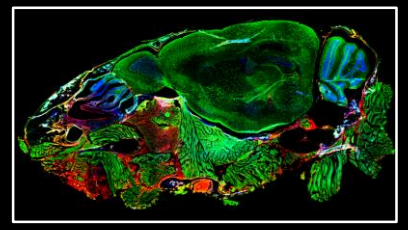
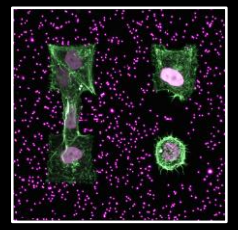
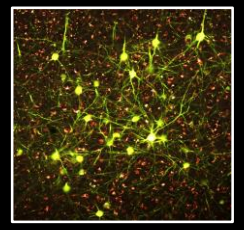
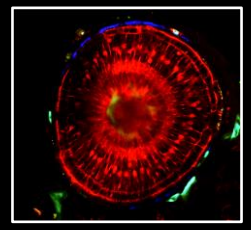
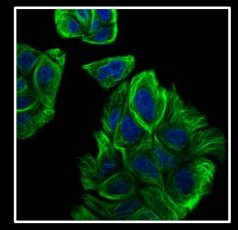
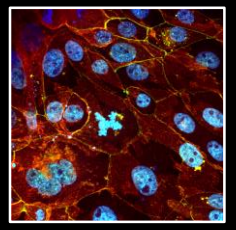
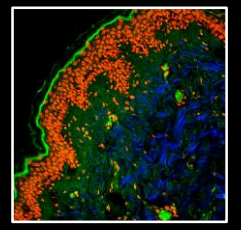
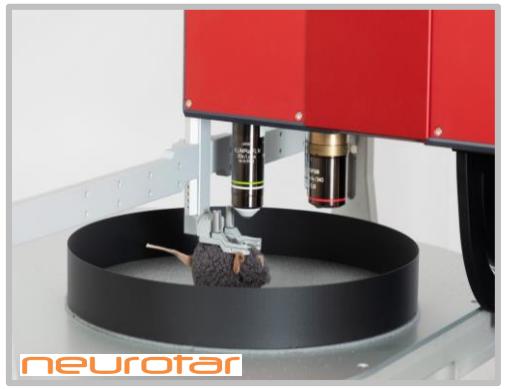
- Non-destructive
- **Deep tissue imaging**
- Pathology & Cancer
- **Neuroscience**
- Optogenetics
- Tissue Engineering & Bioprinting
- Spheroids / Organoids

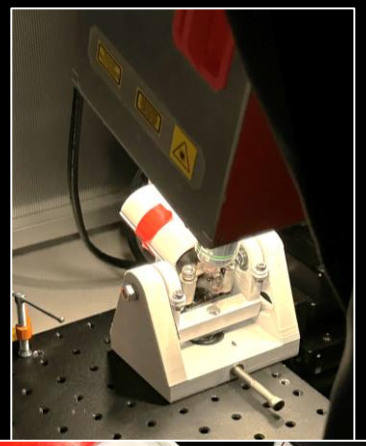





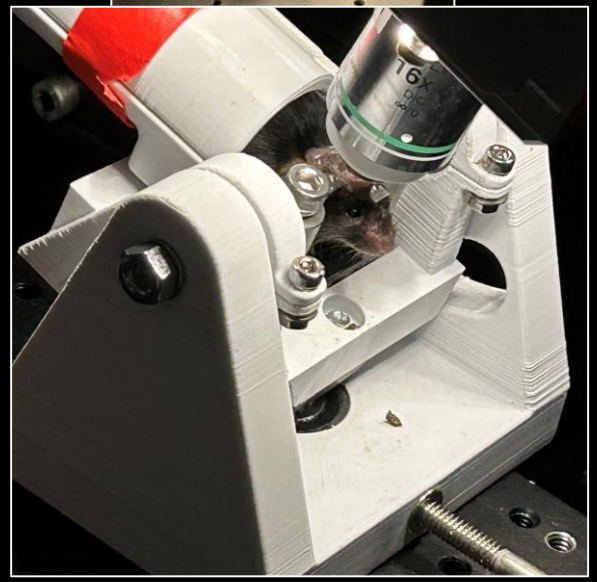
MPX NEUROEXPLORER

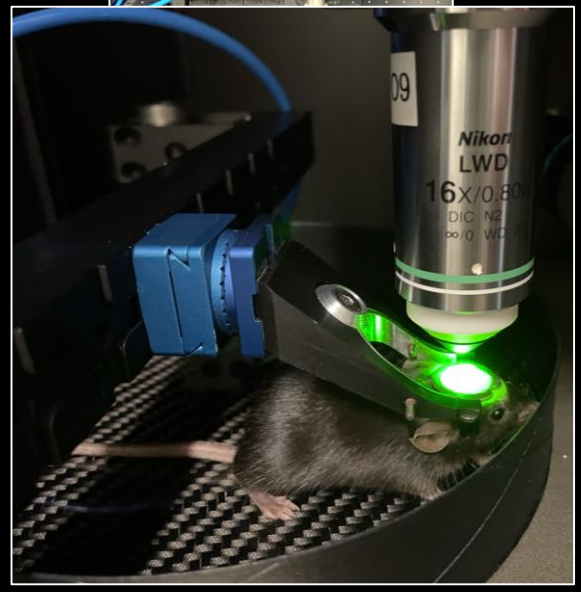


MPX NEUROEXPLORER

PROSPECTIVE

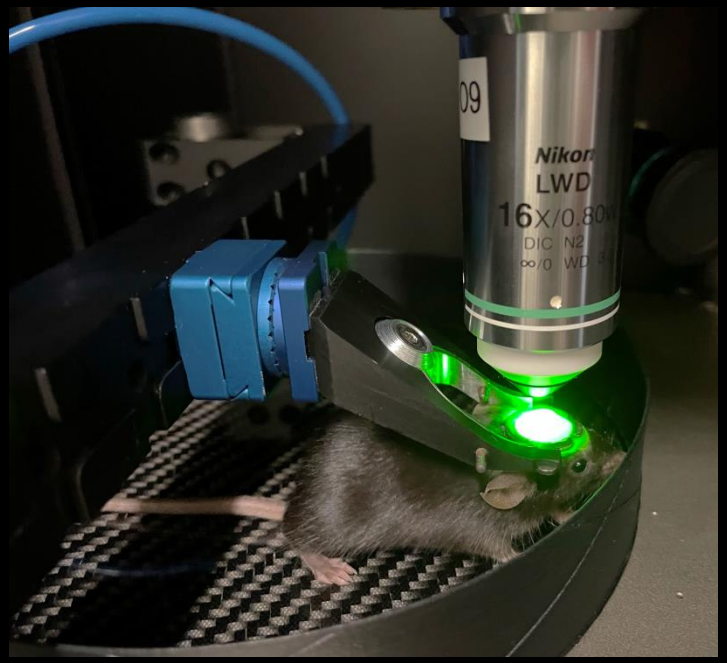




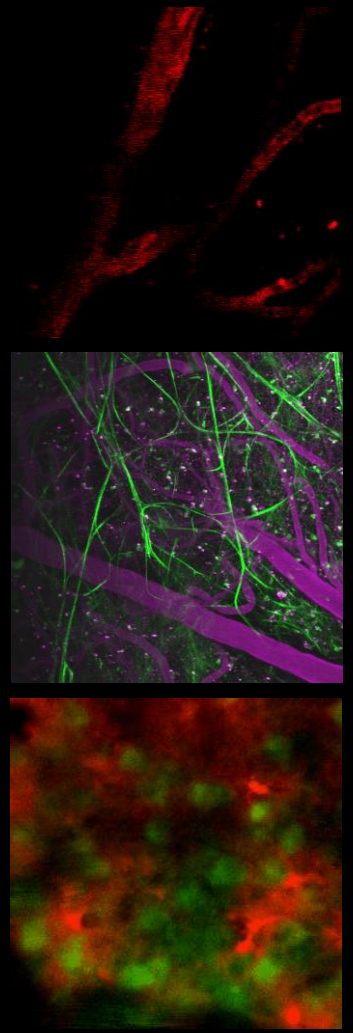


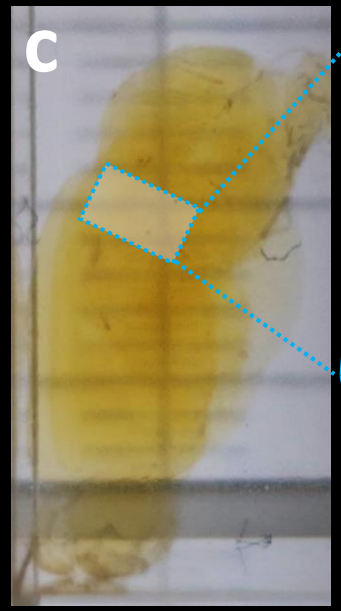
MPX NEUROEXPLORER: IN-VIVO & WHOLE BRAIN IMAGING

PROSPECTIVE

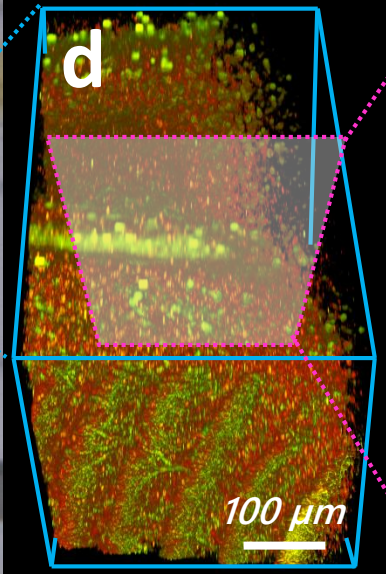


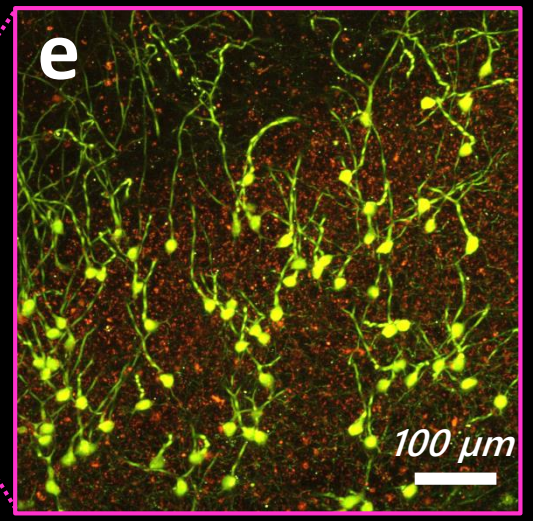
Microscope setup of live mouse in-vivo brain imaging



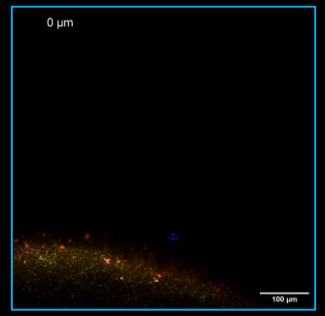


Whole cleared mouse brain





Exemplary z-plane showing stained neurons



Volume scan of the cleared mouse brain with a total depth of~3.5 mm in depth

Kiderlen & Krainer, Wiley Analytical Science, April 2023

BROAD RANGE OF APPLICATIONS

PROSPECTIVE

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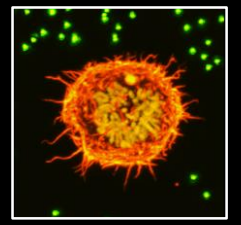
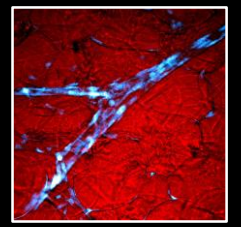
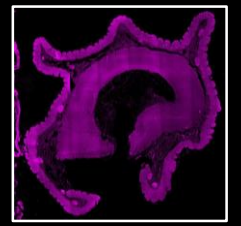
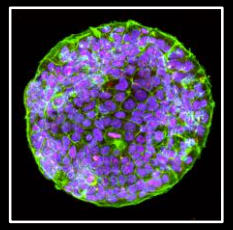
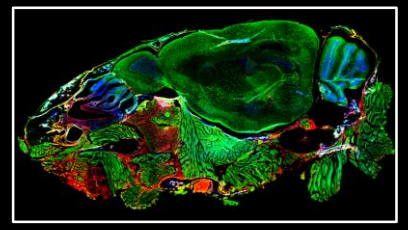
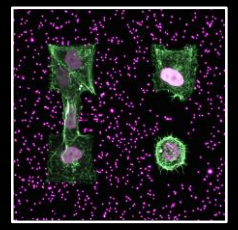
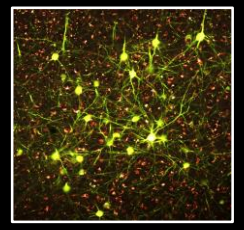
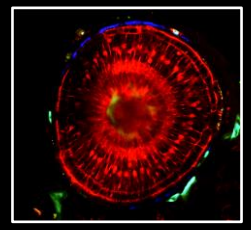


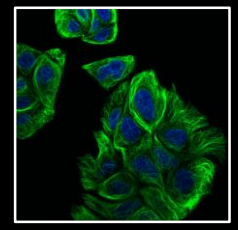
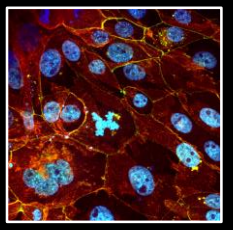
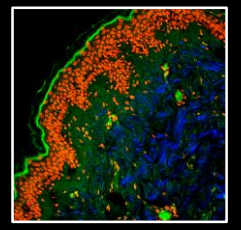

EXAMPLE: LABEL-FREE BIOMARKERS

PROSPECTIVE

BIOMARKER: „measurable indicator of some biological state or condition" (Wikipedia)

Optical properties:	e.g. fluorescence, absorption
Molecular properties:	e.g. metabolic state
Mechanical properties:	e.g. stiffness
Morphological properties:	e.g. cell nucleus
Structural properties:	e.g. wall thickness

Biomarkers in medicine:
Predictive, diagnostic, prognostic indicator

Biomarkers in Research:
"Contact-free, non-destructive"

EXAMPLE: LABEL-FREE BIOMARKERS

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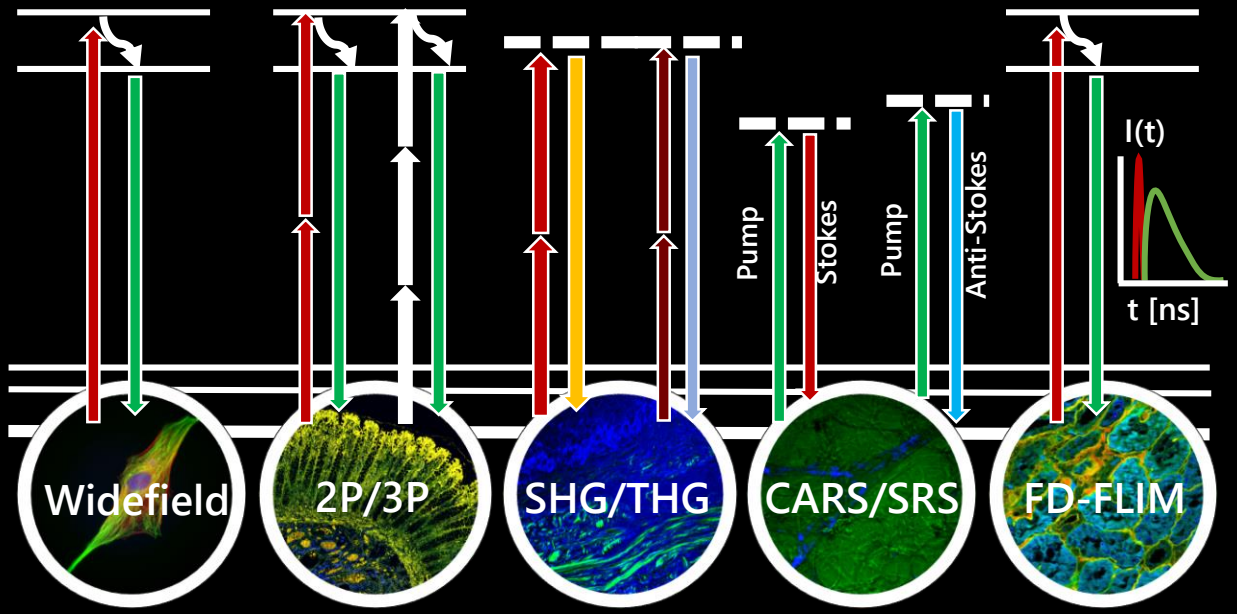
PROSPECTIVE
LIFE SCIENCE

TURN-KEY
EASY-TO-USE
COMPACT
MULTIMODAL

Multiphoton Microscopes

MULTIMODAL MICROSCOPY



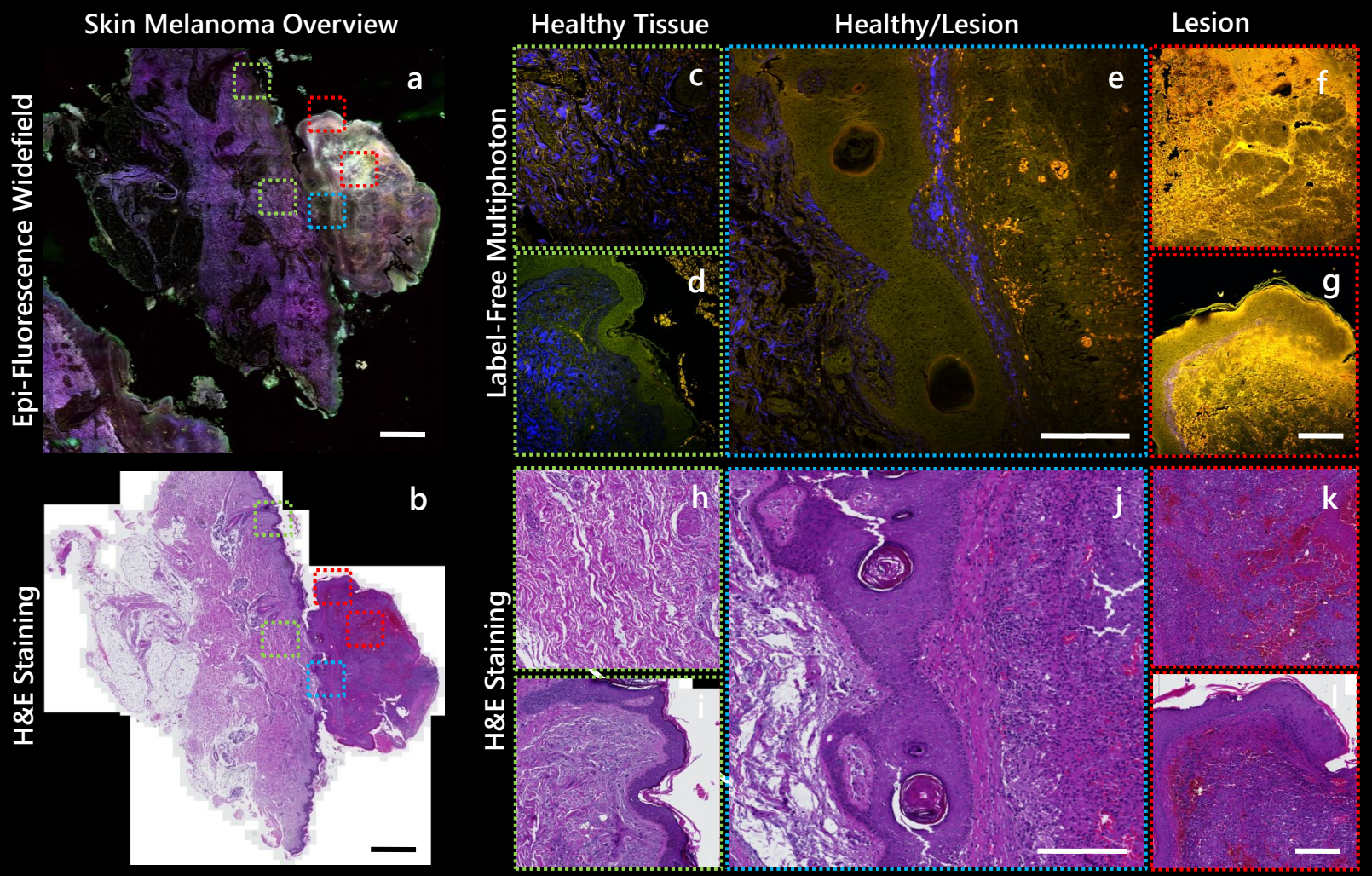
PROSPECTIVE
LASERS

TWO-PHOTON, SHG & THG
OPTOGENETICS
FLIM
CARS & SRS

Femtosecond Lasers

EXAMPLE: WIDEFIELD GUIDED MULTIPHOTON IMAGING

PROSPECTIVE

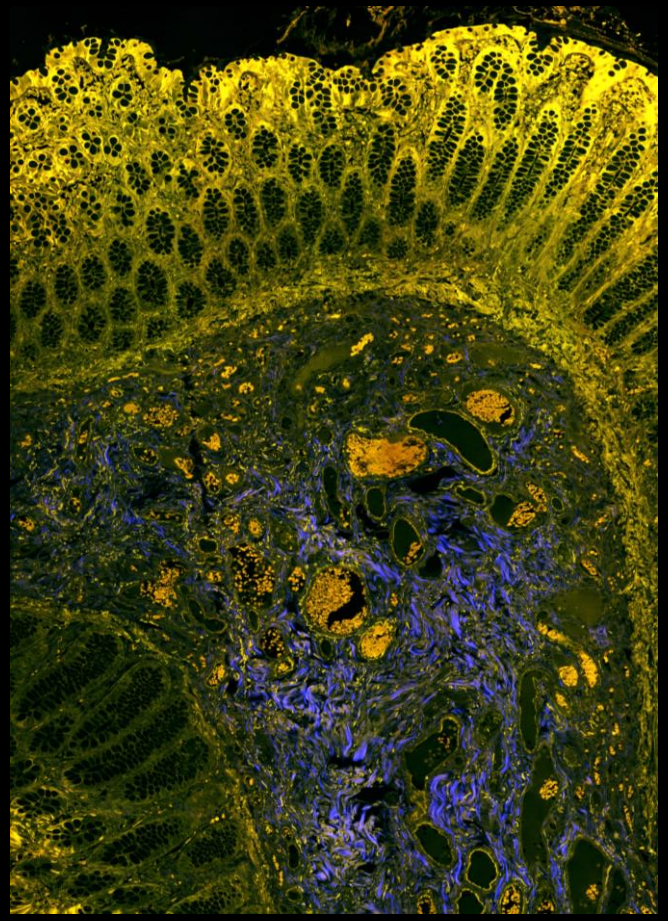


Kiderlen & Krainer, Wiley Analytical Science, April 2023

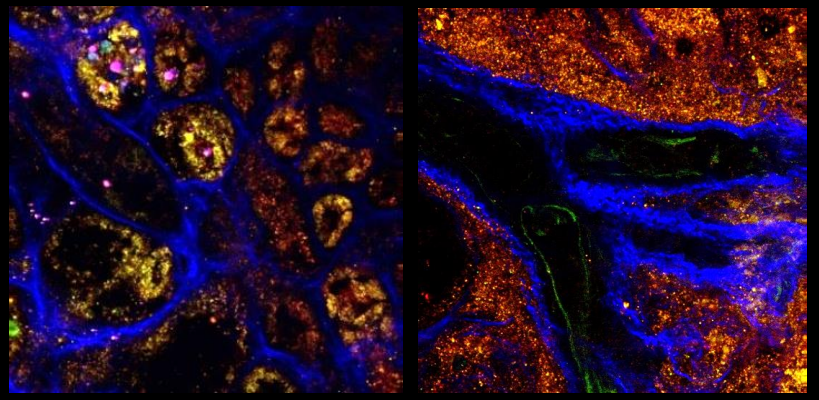
EXAMPLE: BIOMARKERS AND LABEL-FREE IMAGING: COLLAGEN

PROSPECTIVE

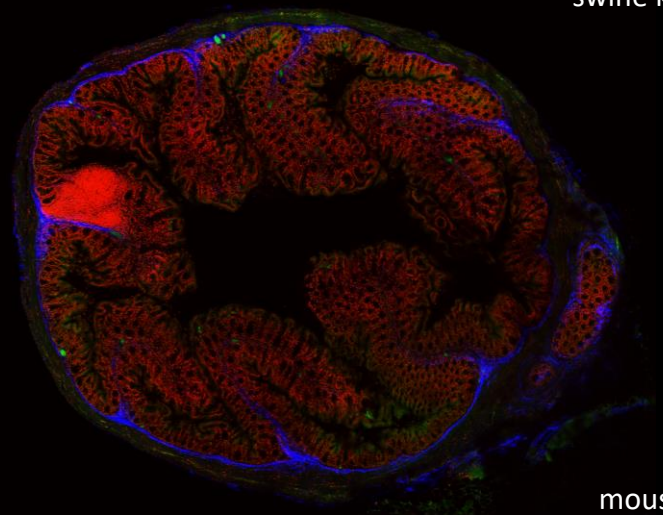
Collagen in 2D Tissue Sections



Collagen in 3D Tissue

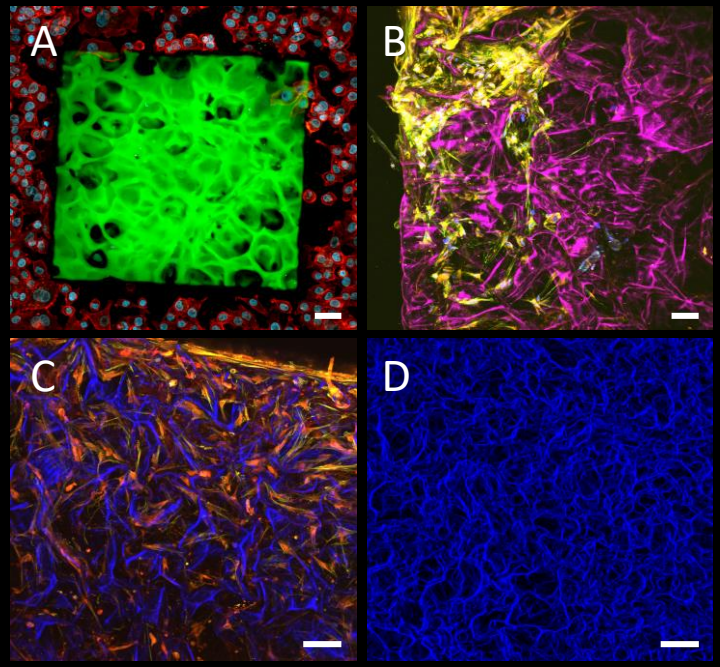


swine kidney



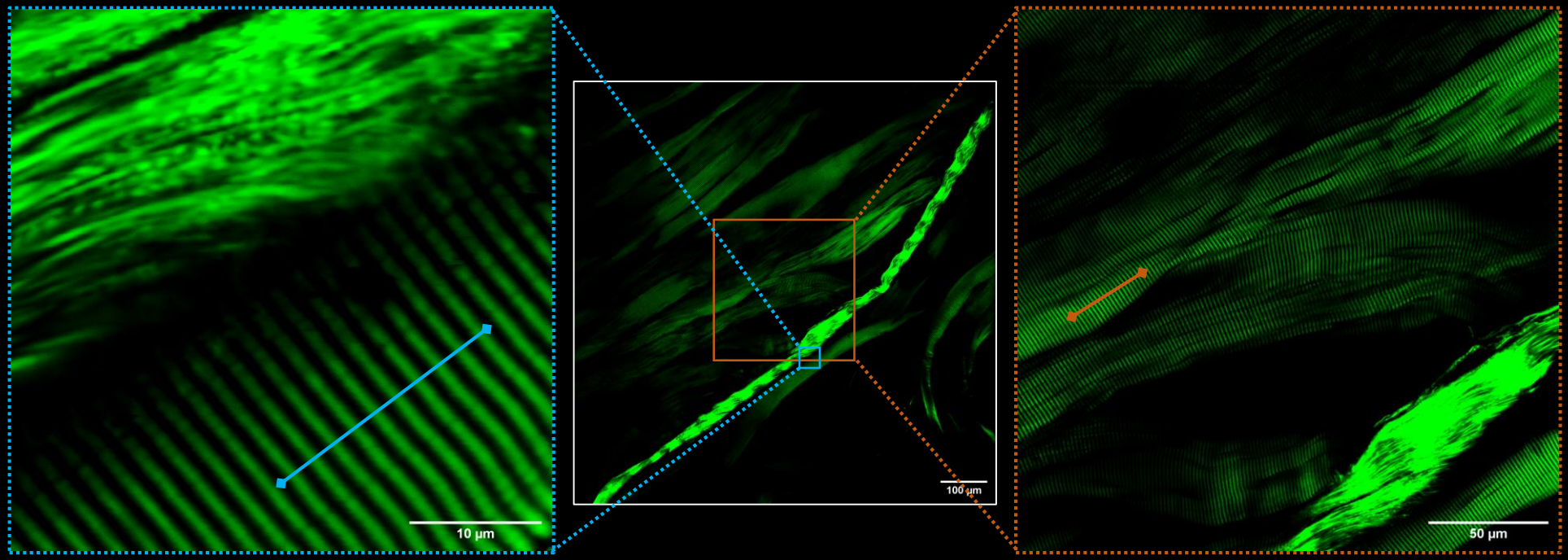
mouse colon

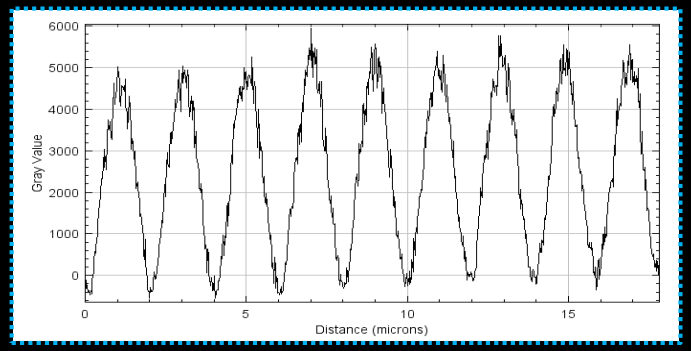
Collagen-Scaffolds



EXAMPLE: BIOMARKERS AND LABEL-FREE IMAGING - MUSCLE

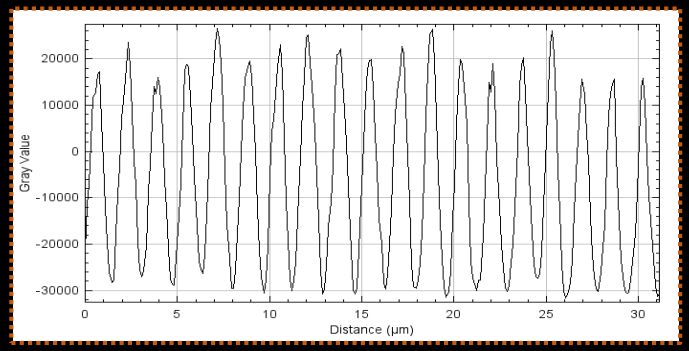
PROSPECTIVE





Label-free mouse muscle longitudinal section

Prospective Instruments:
MPX-1040 – SHG @ 520 nm



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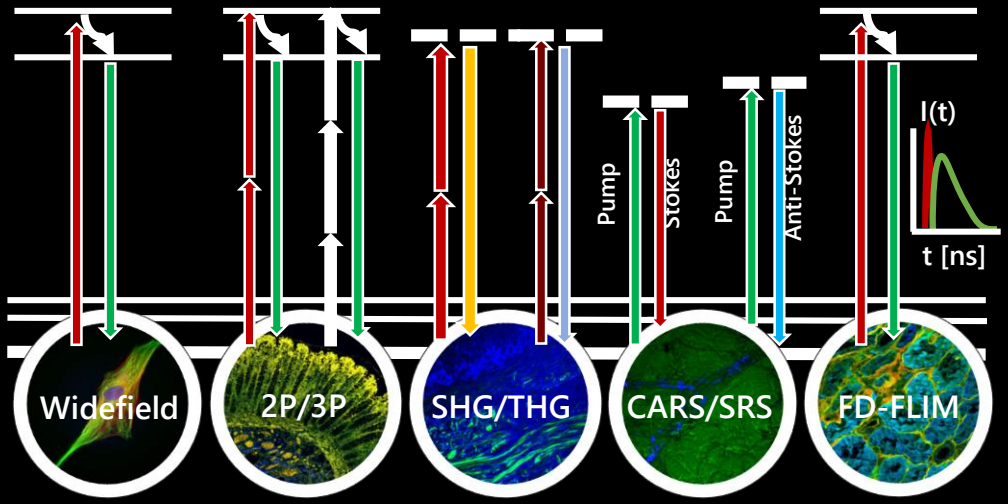
Multiphoton Microscopes

MULTIMODAL MICROSCOPY



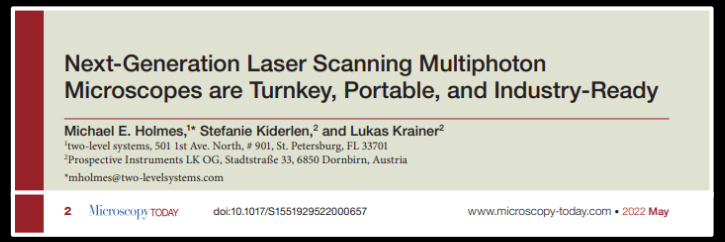
- ✓ Intrinsic confocal
- ✓ Allows optical sectioning → 3D volume scans
- ✓ NIR fs-Lasers allows higher penetration depth
- ✓ NIR fs-Lasers: less photodamage
- ✓ Multiplexing up to 4 channels simultaneously
- ✓ Multimodality: orthogonal data

PROSPECTIVE
LASERS

TWO-PHOTON, SHG & THG
OPTOGENETICS
FLIM
CARS & SRS

Femtosecond Lasers

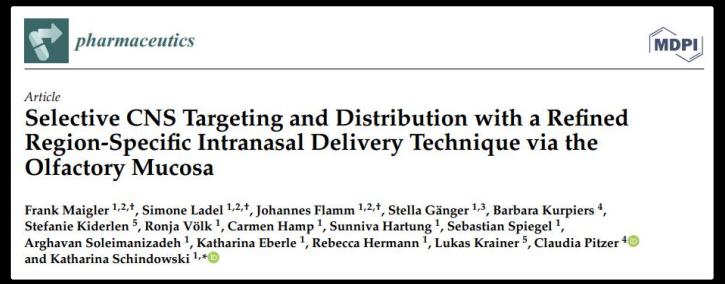
REFERENCES



Next-Generation Laser Scanning Multiphoton Microscopes are Turnkey, Portable, and Industry-Ready

Michael E. Holmes,[1]* Stefanie Kiderlen,[2] and Lukas Krainer[2]
[1]two-level systems, 501 1st Ave. North, # 901, St. Petersburg, FL 33701
[2]Prospective Instruments LK OG, Stadtstraße 33, 6850 Dornbirn, Austria
*mholmes@two-levelsystems.com

2 Microscopy TODAY doi:10.1017/S1551929522000657 www.microscopy-today.com • 2022 May

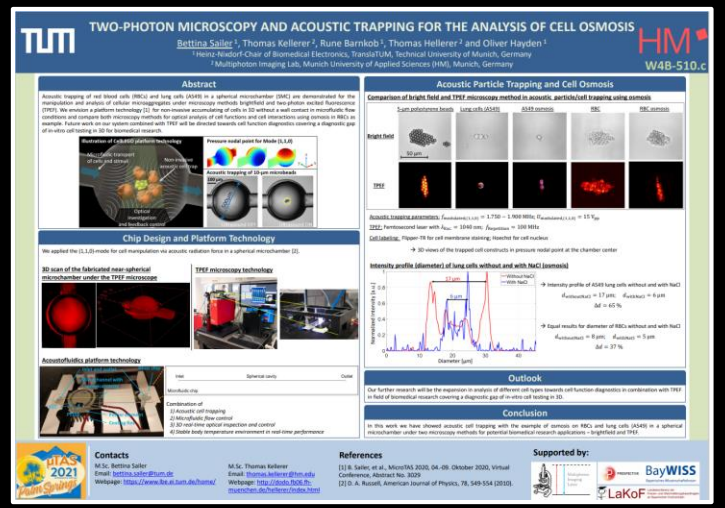


pharmaceutics MDPI

Article

Selective CNS Targeting and Distribution with a Refined Region-Specific Intranasal Delivery Technique via the Olfactory Mucosa

Frank Maigler [1,2,†], Simone Ladel [1,2,†], Johannes Flamm [1,2,†], Stella Gänger [1,3], Barbara Kurpiers [4], Stefanie Kiderlen [5], Ronja Völk [1], Carmen Hamp [1], Sunniva Hartung [1], Sebastian Spiegel [1], Arghavan Soleimanizadeh [1], Katharina Eberle [1], Rebecca Hermann [1], Lukas Krainer [5], Claudia Pitzer [4] and Katharina Schindowski [1,*]



TWO-PHOTON MICROSCOPY AND ACOUSTIC TRAPPING FOR THE ANALYSIS OF CELL OSMOSIS

Bettina Saller [1], Thomas Kellerer [2], Rune Barnkob [1], Thomas Hellerer [2] and Oliver Hayden [1]
[1] Heinz-Nixdorf-Chair of Biomedical Electronics, TranslaTUM, Technical University of Munich, Germany
[2] Multiphoton Imaging Lab, Munich University of Applied Sciences (HM), Munich, Germany

HM W4B-510.c

Abstract

Acoustic trapping of red blood cells (RBCs) and lung cells (A549) in a spherical microchamber (SMC) are demonstrated for the manipulation and analysis of cellular microaggregates under microscopy methods brightfield and two-photon excited fluorescence (TPEF). We envision a platform technology [1] for non-invasive accumulating of cells in 3D without a wall contact in microfluidic flow conditions and compare both microscopy methods for optical analysis of cell functions and cell interactions using osmosis in RBCs as example. Future work on our system combined with TPEF will be directed towards cell function diagnostics covering a diagnostic gap of in-vitro cell testing in 3D for biomedical research.

Acoustic Particle Trapping and Cell Osmosis

Comparison of bright field and TPEF microscopy method in acoustic particle/cell trapping using osmosis

Chip Design and Platform Technology

We applied the (1,1,0)-mode for cell manipulation via acoustic radiation force in a spherical microchamber [2].

3D scan of the fabricated near-spherical microchamber under the TPEF microscope

TPEF microscopy technology

Acoustofluidics platform technology

Intensity profile (diameter) of lung cells without and with NaCl (osmosis)

Outlook

Our further research will be the expansion in analysis of different cell types towards cell function diagnostics in combination with TPEF in field of biomedical research covering a diagnostic gap of in-vitro cell testing in 3D.

Conclusion

In this work we have showed acoustic cell trapping with the example of osmosis on RBCs and lung cells (A549) in a spherical microchamber under two microscopy methods for potential biomedical research applications – brightfield and TPEF.

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Webpage: http://doubs.fk06.fh-muenchen.de/hellerer/index.html

References

[1] B. Saller, et al., MicroTAS 2020, 04.-09. Oktober 2020, Virtual Conference, Abstract No. 3029
[2] O. A. Russell, American Journal of Physics, 78, 549-554 (2010).

Supported by: BayWISS LaKoF

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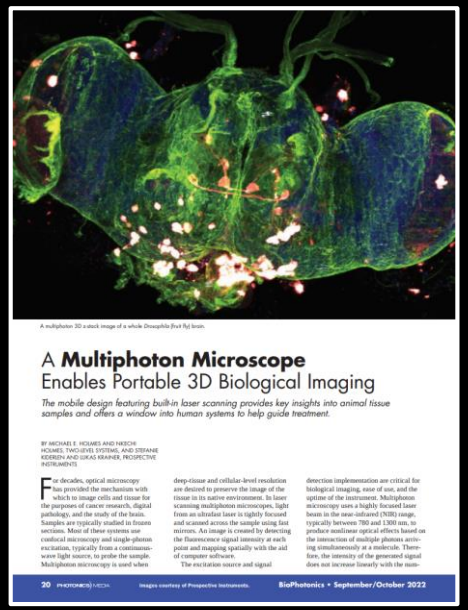


A Multiphoton Microscope Enables Portable 3D Biological Imaging

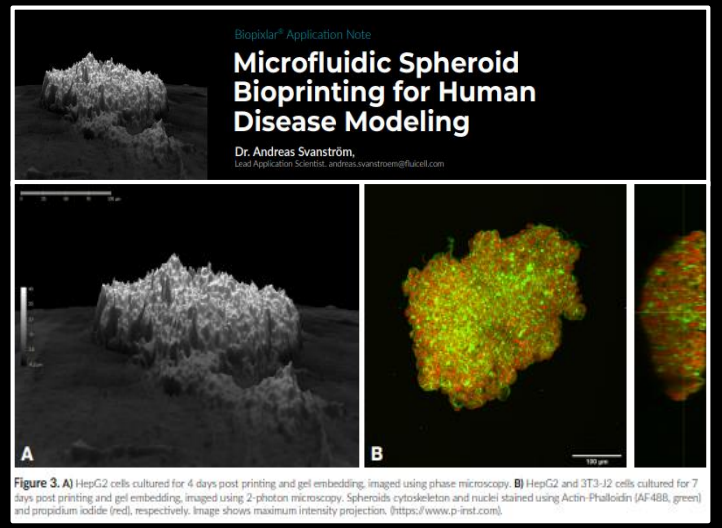
The mobile design featuring built-in laser scanning provides key insights into animal tissue samples and offers a window into human systems to help guide treatment.

BY MICHAEL E. HOLMES AND NIKECHI HOLMES, TWO-LEVEL SYSTEMS, AND STEFANIE KIDERLEN AND LUKAS KRAINER, PROSPECTIVE INSTRUMENTS

For decades, optical microscopy has provided the mechanism with which to image cells and tissue for the purposes of cancer research, digital pathology, and the study of the brain. Samples are typically studied in frozen sections. Most of these systems use confocal microscopy and single-photon excitation, typically from a continuous-wave light source, to probe the sample. Multiphoton microscopy is used when deep-tissue and cellular-level resolution are desired to preserve the image of the tissue in its native environment. In laser scanning multiphoton microscopes, light from an ultrafast laser is tightly focused and scanned across the sample using fast mirrors. An image is created by detecting the fluorescence signal intensity at each point and mapping spatially with the aid of computer software. The excitation source and signal direction implementation are critical for biological imaging, ease of use, and the uptime of the instrument. Multiphoton microscopy uses a highly focused laser beam in the near-infrared (NIR) range, typically between 780 and 1300 nm, to produce nonlinear optical effects based on the interaction of multiple photons arriving simultaneously at a molecule. Therefore, the intensity of the generated signal does not increase linearly with the num-

20 PHOTONICS MEDIA Images courtesy of Prospective Instruments. BioPhotonics • September/October 2022

PROSPECTIVE



Biopixlar® Application Note

Microfluidic Spheroid Bioprinting for Human Disease Modeling

Dr. Andreas Svanström,
Lead Application Scientist, andreas.svanstroem@fluicell.com

A B

Figure 3. **A)** HepG2 cells cultured for 4 days post printing and gel embedding, imaged using phase microscopy. **B)** HepG2 and 3T3-J2 cells cultured for 7 days post printing and gel embedding, imaged using 2-photon microscopy. Spheroids cytoskeleton and nuclei stained using Actin-Phalloidin (AF488, green) and propidium iodide (red), respectively. Image shows maximum intensity projection. (https://www.p-inst.com).

Artikel | Mikroskopie Lichtmikroskopie 3 April 2023

Turnkey Multiphoton Microscopy for 3D samples

Label-free and 4D imaging in Life Science and Medicine

Stefanie Kiderlen, Lukas Krainer

Wiley Analytical Science Award Winner

ACKNOWLEDGEMENTS

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Monika Puchalska - Nencki Institute (Warsaw, Poland)
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Dr. Rainer Schindl & Dr. Linda Waldherr – MedUni Graz (Graz, Austria)
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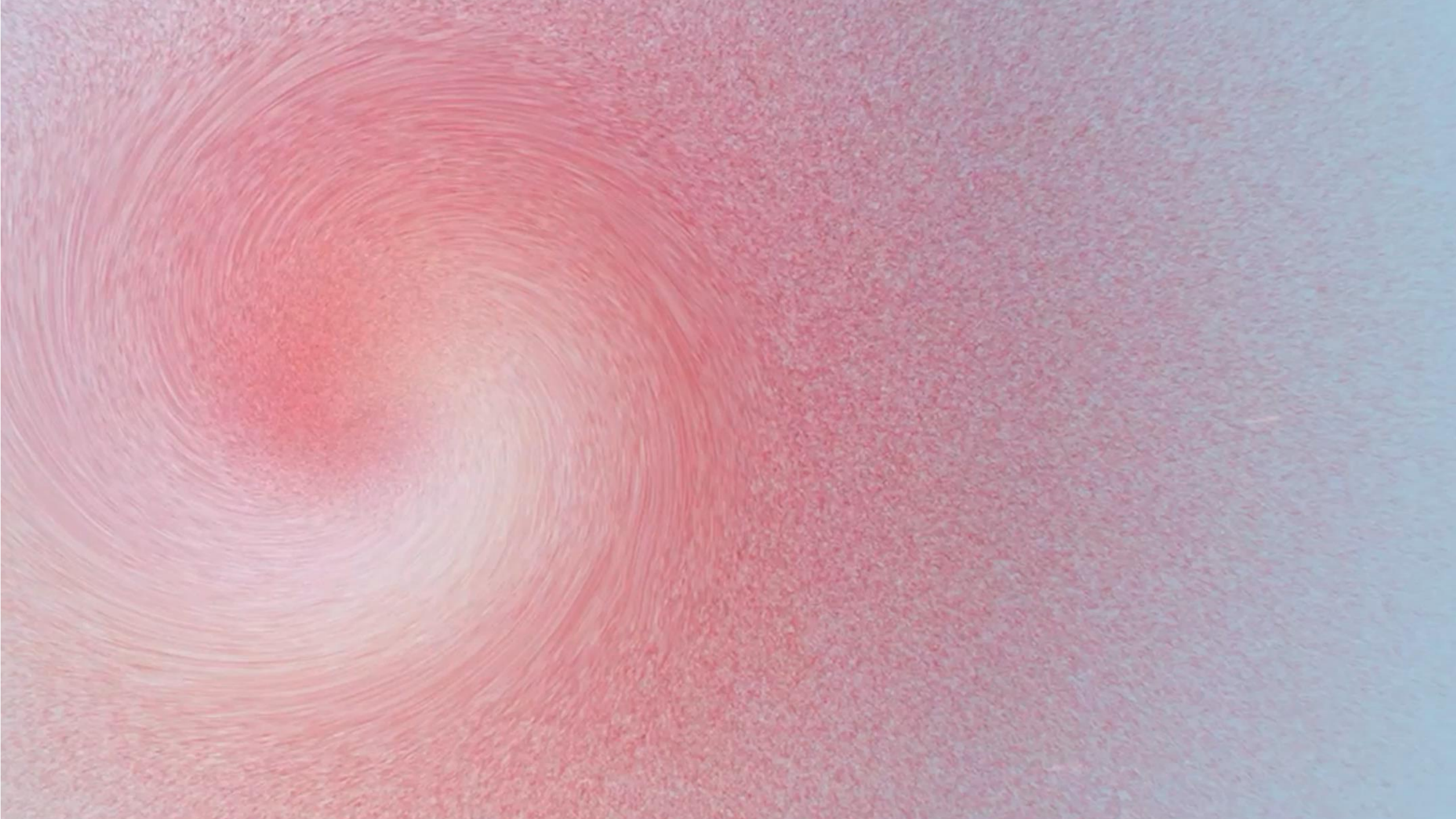
Dr. Andreas Svanstroem – Fluicell (Mölndal, Sweden)
Prof. Katharina Zimmermann – HCB (Biberach, Germany)
Prim. Felix Offner & Dr. Brano Zagrapan – Pathologie LKH (Feldkirch, Austria)
Dr. Luc Reymond – Spirochrome (Lausanne, Switzerland)
Dr. Lucas Onder – Kantonspital (St. Gallen, Switzerland)
Dr. Pouya Rajaeipour – Phaseform (Freiburg, Germany)
Dr. Marc Zuend (Zurich, Switzerland)
Katja Karelina – Neurotar (Helsinki, Finnland)
Dr. Hans Fried - DZNE (Bonn, Germany)



THANK YOU!

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