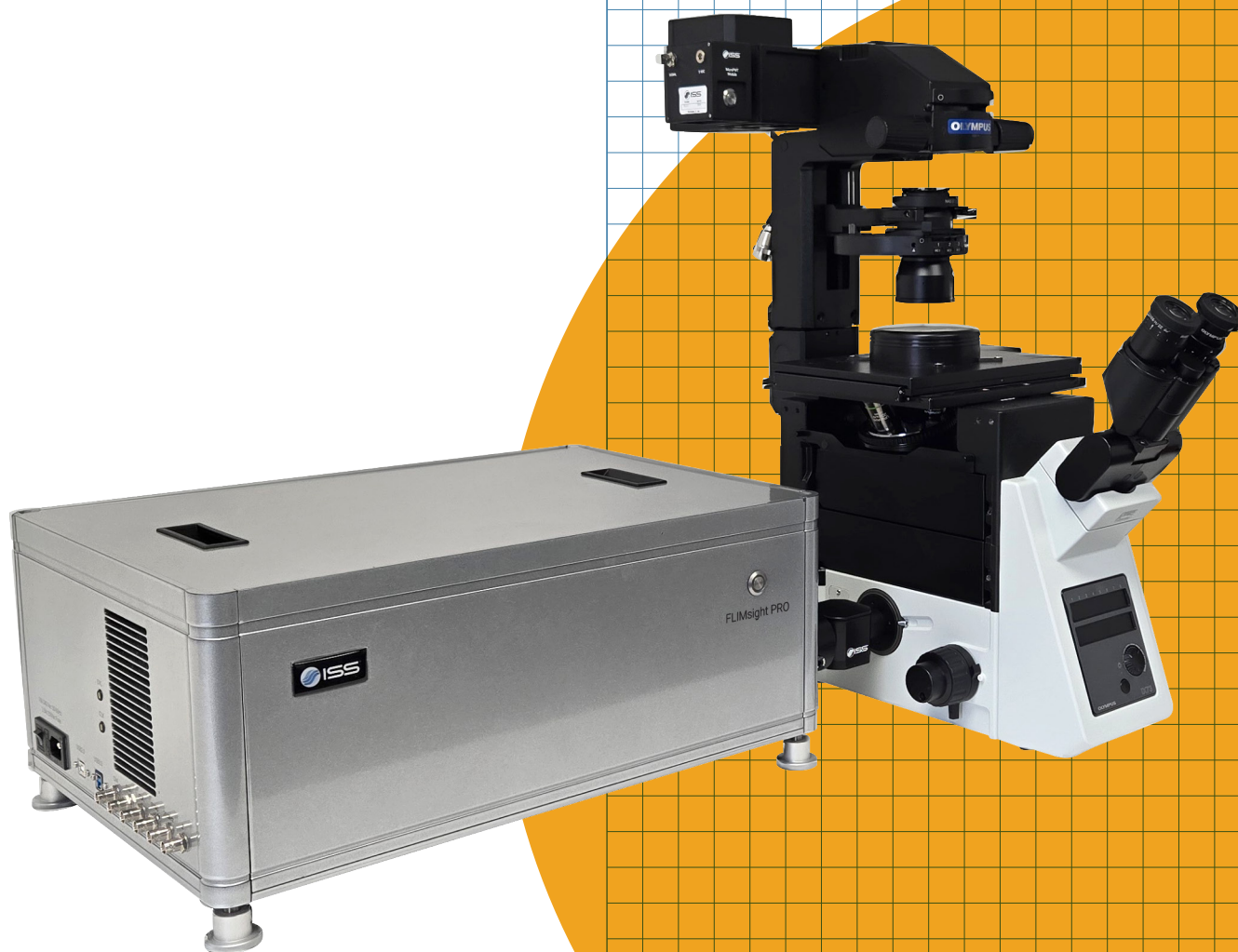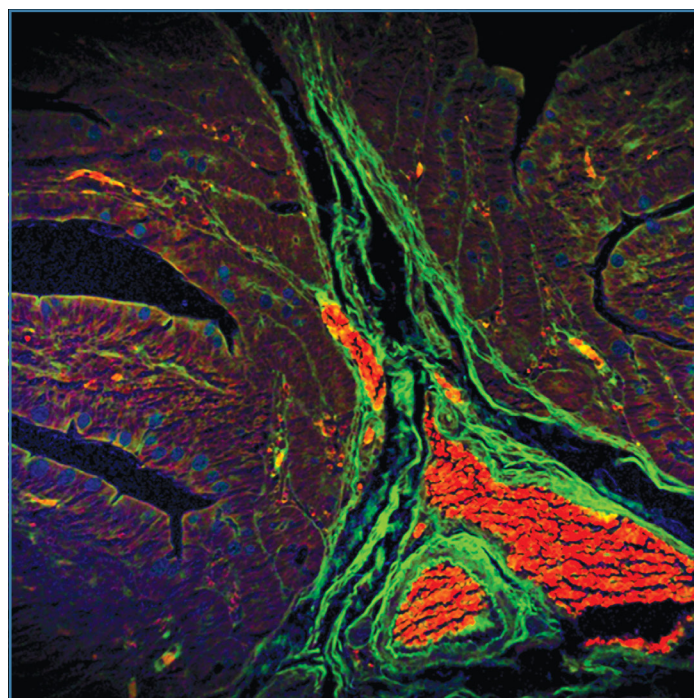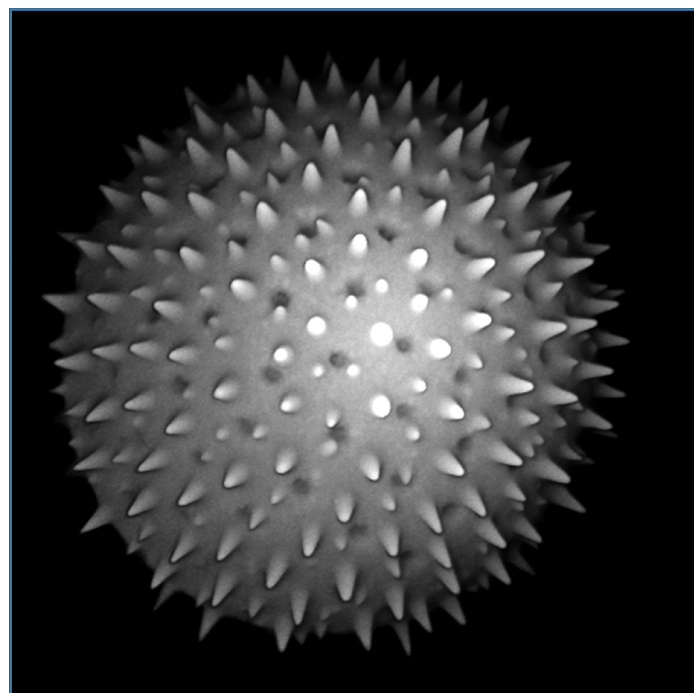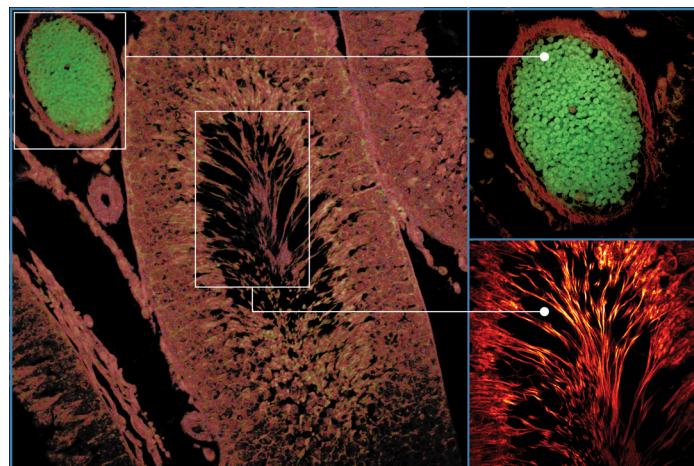# FLIMsight PRO

The FLIMsight PRO is an all-in-one benchtop solution that seamlessly integrates 2-photon excitation, fluorescence lifetime imaging, and phasor plot analysis for real-time, label-free diagnostics. Its compact design and user-friendly workflow simplify advanced imaging for researchers and clinicians alike.

The FLIMsight PRO is a state-of-the-art time-resolved confocal microscope that seamlessly integrates two-photon excitation, fluorescence lifetime imaging (FLIM), and phasor plot analysis into a single, compact platform. Designed for high-resolution, label-free imaging, this system provides researchers and clinicians with a powerful tool for real-time molecular and metabolic analysis.

Optimized for detecting key metabolic coenzymes such as NADH and FAD, the FLIM system features dual-channel fluorescence lifetime acquisition, enabling precise differentiation of metabolic states in biological tissues. The system's integrated two-photon laser, operating at 780 nm with a 40 MHz repetition rate, ensures deep tissue penetration while maintaining high spatial resolution. Detection is achieved through fast photomultiplier tubes (PMTs) with a 150 ps instrument response function (IRF), allowing for highly sensitive and accurate lifetime measurements.

Utilizing FastFLIM technology, the system captures lifetime decay dynamics with exceptional speed, while advanced analysis tools, including multi-exponential fitting and phasor plot analysis, enable intuitive and quantitative data interpretation. With its streamlined workflow, compact design, and fully integrated hardware and software, the FLIMsight PRO simplifies advanced fluorescence imaging, making cutting-edge FLIM applications more accessible to both biomedical researchers and clinical professionals.

TOP RIGHT: An example of Tissue Imaging.

MIDDLE RIGHT: An example of 3D volume rendering with FLIMsight PRO. A stack of z-sectioning images acquired from a pollen grain.

BOTTOM RIGHT: Image acquired from a hematoxylin and eosin-stained tissue. Together with ISS FastFLIM technology, it is straightforward to collect the fluorescent decay data at each image pixel. The time resolved data is quantitatively unmixed by the ISS VistaVision phasor plots module, producing three separate images which represent the fluorescence intensity contributed exclusively by distinct fluorescent species. The merged image is constructed utilizing false red, green and blue colors for shortest, intermediate and longest lifetime species, respectively.

FLIMsight PRO Configuration
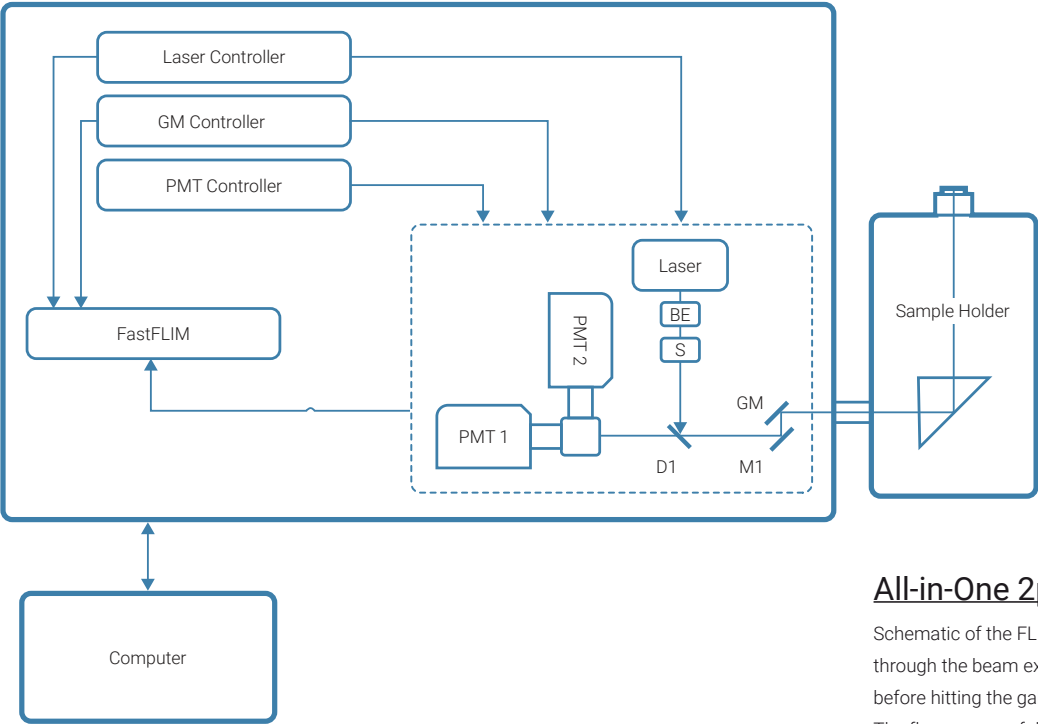
All-in-One 2p FastFLIM System

Schematic of the FLIMsight PRO. The excitation laser beam goes through the beam expander (BE) and it is reflected by the dichroic (D1) before hitting the galvo-mirrors (GM) and entering into the microscope. The fluorescence follows in reverse the same path, traverses the dichroic (D1) and it is detected by the light detectors. The signal is processed by the FastFLIM unit; the images are displayed on the computer screen connected to the unit via the USB port.

Label-Free NADH FLIM
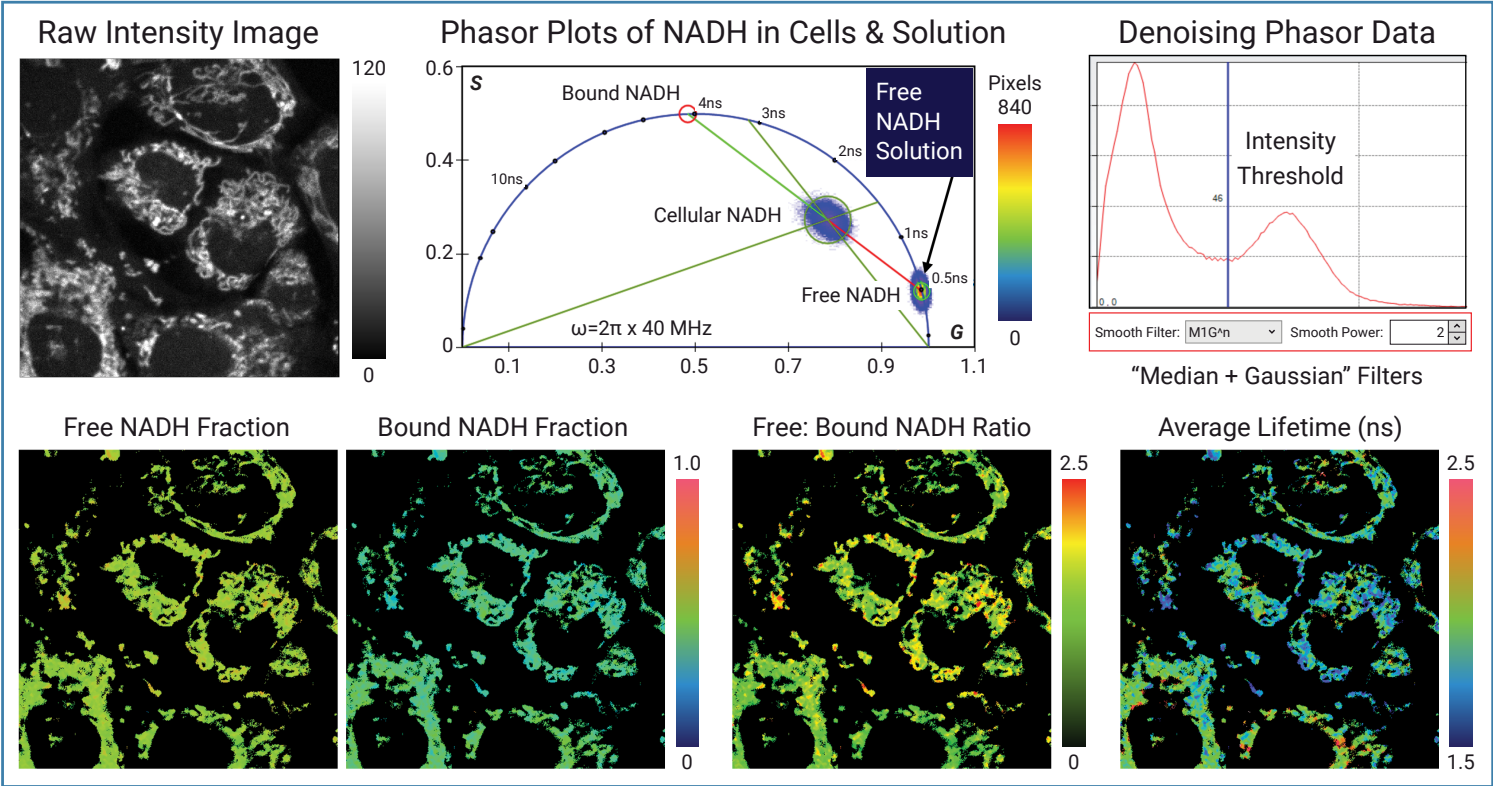
Two-Component Lifetime Unmixing. Using the phasor plots of the FLIM images acquired from NADH in live cells and solution which only contains the free form, multi-image phasor analysis (MiPA) derives the phasor coordinate of the bound form of the cellular NADH. MiPA computes the fractions of both free and bound NADH for each pixel in the cellular NADH image, producing a cellular map depicting the ratios of free-to-bound NADH. Given the free and bound NADH lifetimes, it also generates the cellular NADH average lifetime map.

Instrument Specifications

Software Overview

VistaVision – Laser Scanning Confocal Imaging & FLIM/PLIM Software. FLIMsight PRO features VistaVision, a comprehensive, user-friendly software package for the acquisition and analysis of multiphoton imaging, FLIM and FLIM-FRET data in multiple dimensions (x, y, z, t, P – macro position for tiling , λ - emission spectrum, τ - lifetime).

Computer

High performance computer loaded with OS Windows 11 64 bit
64 GB RAM & 2 TB SSD
34", 3440 x 1440 resolution, flat color monitor

Laser

780 nm, 40 MHz Rep Rate

Galvano-Mirrors

Set of two mirrors for XY scanning

Detectors

Two PMTs, IRF<150 ps, 280 - 620 nm

Acquisition

FastFLIM

Automation Control

USB port

Electrical

110-240 V, 50-60 Hz

Dimensions

57 x 39 x 22 cm

Weight

28.5 Kg

Scan Modes:
- Single spot
- 1D Line (X, Y or Z)
- 2D Image (XY, XZ, YZ)
- 3D Image Stack (XYZ)
- Each can be combined with time (t)

Scan Settings:
- Pixels Number: 1 x 1 to 4096 x 4096
- Dwell Time: 0.5 µs to infinity
- Optical Zoom: 1 to 40
- Field Diameter: 18 mm

Data Acquisition by Photon Counting:
- Photon Mode
- Time Tagged Mode
- Time Tagged Time Resolved Mode

Data Display and Analysis:
- Image enhancement by thresholding, contrast adjustment & smoothing
- Image false coloring with more than 50 LUTs & overlay of multiple channels
- Image quantification by advanced mathematical operations
- FLIM data analysis includes both fitting (up to 5 components) & phasor plots

Image Storage Formats:
- ISS proprietary formats with meta information
- Export to TIFF, GIF, JPEG, PNG, BMP, AVI, TXT
- Plots can be saved & exported to GIF, TIFF, JPEG, PNG, Bitmap & Metafile formats

For more information please contact ISS at iss@iss.com or visit us at iss.com

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