FEMTO SMART Ram-I

HIGH-RESOLUTION, LABEL-FREE HISTOLOGICAL EXAMINATIONS

The FEMTOSmart Ram-I allows for immediate, label-free, high resolution microscopic morphological examination of *in vitro* histological specimens, without the need to fix or stain the samples in advance. It can be a fast and practical alternative of various dye-based imaging methods, e.g., classical hematoxylin-eosin staining, nucleus, membrane, and cytoskeletal staining, and can be used to follow processes involving the DNA / RNA, lipid metabolism, glucose uptake, or the uptake and intracellular distribution of drug molecules in pharmacological studies. The specimen of interest does not need to be prepared in any special way, such as freezing or fixation, so the structural elements of the sample remain intact.

- Transmission infrared detector for SRS measurements
- 4 detection channels (2 reflected and 2 transmitted) in the visible range, for CARS, SHG and fluorescence measurements
- Transmission Köhler illumination
- Robust design, waterproof sealing

FEMTOSMART RAM-I: SRS BASED IMAGING

The Ram-I is based on breakthrough results in the field of quantum mechanics: it performs imaging using non-linear scanning microscopy based on Stimulated Raman Scattering (SRS), and is supported by high-performance image processing algorithms using artificial intelligence. The FEMTOSmart Ram-I microscope uses SRS based imaging to detect different molecular bonds in the sample based on their spectral properties, thus omitting the need of fluorescent labeling. This allows us to identify the main structural elements and details of cells and tissues, and to recognize pathological changes. The microscope's optical system and special detectors provide high resolution and good signal, and the purpose-designed mechanics allow for long-term stable operation.

STIMULATED RAMAN SCATTERING (SRS) AND COHERENT ANTI-STOKES RAMAN SCATTERING (CARS)

During spontaneous Raman scattering, information on the chemical composition of samples is gathered by collecting the inelastic scattering of light that occurs when molecular vibration is induced by a visible CW laser. However, this spontaneous effect is weak: to overcome this disadvantage, boosted vibrational microscopy techniques have been developed. Vibrational frequencies characteristic of chemical bonds can be induced and detected using coherent Raman scattering techniques: SRS and CARS. During SRS, a fixed-frequency and a tunable laser beam, a pump and a Stokes laser are used for exciting and imaging specific molecules: using the tunable laser, the difference between their frequencies can be set to match a vibrational frequency characteristic of a molecular bond. When it is set, the absorption of a pump photon and the simultaneous emission of a Stokes photon leave the molecule vibrating: the loss of photons in the pump beam can be detected using a sensitive photodiode and a lock-In amplifier. Using this method, a high-quality structural image of a sample can be created by setting the frequency-difference to a bond present in our molecules of interest such as membrane lipids, nuclear components, etc. (Fig. 2A). In CARS, the emission of a blue-shifted anti-Stokes photon, which is the result of a further interaction with the pump beam, is recorded using a PMT.

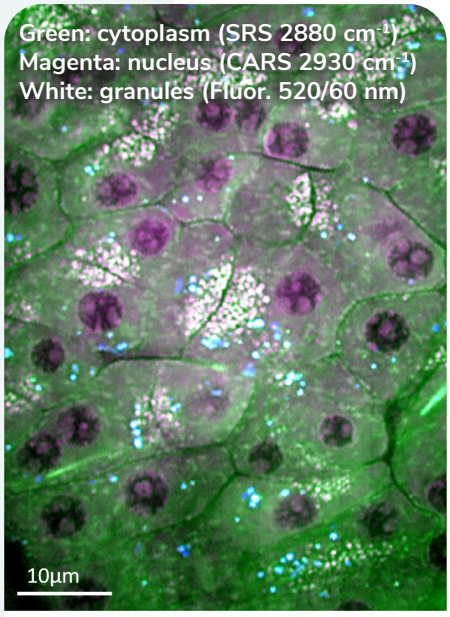

Figure 1: A 200 µm thick, squeezed pancreas slice from a Thy1 GCaMP mouse has been imaged with the FEMTOSmart Ram-I using SRS based imaging.

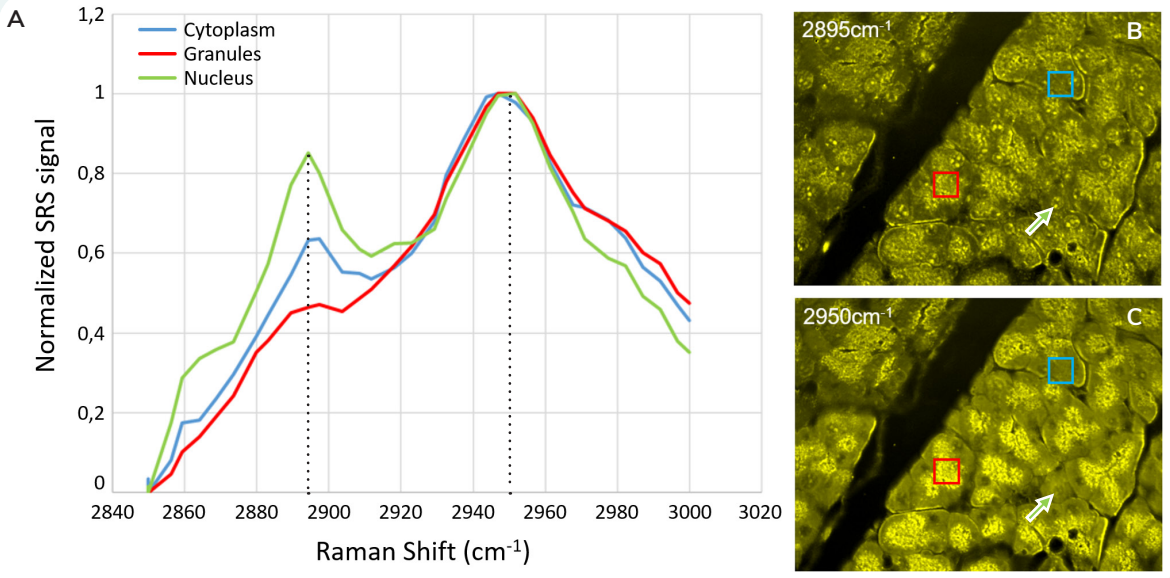

Figure 2: Mouse pancreas slice imaged with the FEMTOSmart Ram-I using SRS functionality.
A) SRS spectra of the cytoplasm, granules and nucleus (with blue, red, and green) were created using recordings on different wavenumbers. The SRS signal of the selected regions is plotted in the function of the wavenumber.
B, C) Example images with specific regions marked with blue and red squares and green arrows, respectively.

APPLICATIONS: FROM CELLS THROUGH TISSUES TO MODEL ORGANISMSAND ORGANOIDS

Both SRS and CARS enable dye-free, high-resolution imaging in living cells, tissues, and even organoids and live model organisms. In the research of neurodegenerative diseases, lipid-rich brain structures, such as myelin, and its degeneration can be followed, and even pathological amyloid-β deposits can be imaged label-free for Alzheimer's research with SRS. SRS can also detect protein secondary structure and misfolding, an important mechanism in many of these diseases. Combined with two-photon-excited autofluorescence and second-harmonic generation, SRS is perfect for studying physiological structures non-destructively, deep inside an intact organism: it is capable of the early diagnosis of many types of cancers, neurodegenerative diseases, and fibrosis. With SRS, organoids can also be probed from the macroscopic scale down to deep-sub-cellular details. SRS is increasingly used to study the uptake and distribution of pharmaceutical compounds by various cells and tissues: the metabolism of these molecules can also be followed based on their vibrational spectra. It is becoming a widely used tool for the quality control of pharmaceuticals, making high-resolution, label-free imaging of active ingredients possible, and can even replace some commonly used, but more outdated techniques, like the ones performed with electron microscopy. In food science, SRS is used for the label-free imaging of lipids, proteins, and water to determine their distribution in emulsions: by leaving a fingerprint in the CARS spectra, NaCl concentrations can be detected below a one percent level in a solution, making it a strong detection mechanism.

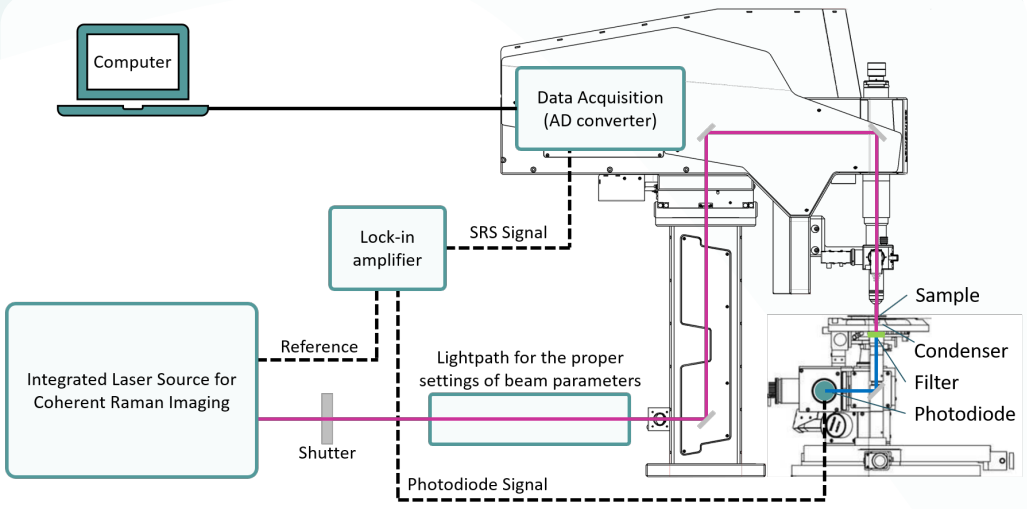

Figure 3: Schematic layout of the FEMTOSmart Ram-I