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SRS: 3D Mapping and Hyperspectral Imaging (HSI)

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We use this protocol and it's working

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Abstract

We have developed a protocol for utilizing stimulated Raman scattering (SRS) to acquire label-free measurements of specific macromolecules. When performing SRS imaging we can acquire total protein, total lipid, unsaturated lipid, and saturated lipid concentration from distinct Raman shift wavenumbers. We can also acquire a hyperspectral sweep across multiple different Raman wavenumbers to acquire a hyperspectral image (HSI) of the CH region of the Raman spectrum.

This protocol presents the acquisition of images via our SRS modality.

Attachments



[SRS_protocol.docx](#)

19KB

Section 1: Tissue Preparation

- 1 Obtain fresh and fixed (4% PFA) tissue block samples and keep stored in 50mL tubes of 1x phosphate buffered saline (PBS) in a 4°C refrigerator.
- 2 Perform tissue sectioning for imaging utilizing Precisionary Compressstome.
- 3
 - a. Fill Compressstome buffer tray with 1x PBS.
- 4
 - b. Embed tissue block onto specimen disk using 4% agarose solution.
- 5
 - c. Secure specimen disk into slicing chamber.
- 6
 - d. Use dial to select for 120 µm thick sections.
- 7
 - e. As slicing occurs, utilize a fine brush to collect 120 µm thick slices and transfer to petri dish/50mL tubes filled with 1x PBS for storage.
- 8 Mount tissue sections on glass slides for imaging.
- 9
 - a. Clean glass slide with 70% ethanol and lint-free wipes
- 10
 - b. Create well on glass slide with Invitrogen 120 µm thick spacer.
- 11
 - c. Use fine brush or sterile tweezer to transfer tissue into well and then fill with 1x PBS using pipette
- 12
 - d. Lower coverslip onto spacer carefully, avoiding the formation of bubbles
- 13 Store glass slides in a 4°C refrigerator before imaging.

Section 2: SRS Imaging

- 14 Start-up SRS microscope system.
- 15
 - a. Power on picoEmerald dual laser system, Si photodiode detector, and Zurich lock-in amplifier.
- 16
 - b. Configure picoEmerald laser system: tunable pump from 780–990 nm, 5–6 ps pulse width, and 80 MHz repetition rate and Stokes beam with fixed 1031 nm wavelength, 6 ps pulse width, and 80 MHz repetition rate.
- 17
 - c. Adjust average excitation power of both lasers to 500 mW for 3D imaging.
- 18 Mount microscope slide onto microscope stage.
- 19
 - a. Apply immersion oil to the high-NA (1.4 NA) oil condenser.
- 20
 - b. Mount microscope slide onto the condenser.
- 21
 - c. Apply a large water droplet onto microscope slide for the water-immersion objective lens.
- 22 SRS acquisition
- 23
 - a. Only acquire SRS following multiphoton (MPF) and/or second harmonic generation (SHG) to avoid photobleaching.
- 24
 - b. Olympus FV3000 Software: Select image resolution: 256×256 for 3D mapping, 512×512 for single region of interest and HSI.
- 25
 - c. Olympus FV3000 Software: Select pixel dwell time and time constant: 40 μ s and 30 μ s, respectively.

- 26 d. 3D Mapping:
- 27 i. Olympus FV3000 Software: Select "MATL" for mosaic imaging and "Range: ON" to initiate 3D mapping.
- 28 ii. Olympus FV3000 Software: Under "Range" window of Fluoview software: Select step size, number of z steps, and top of tissue surface
- 29 iii. Olympus FV3000 Software: Under "Map" tab: Use rectangular fitting to create a mosaic map over the x-y region of interest/whole tissue.
- 30 iv. Tune pump laser for each macromolecule wavelength between each acquisition:
- 31 1. 791.3 nm for CH₃ asymmetric stretching/total protein (2938 cm⁻¹)
- 32 2. 797nm for CH₂ asymmetric stretching/total lipid (2848 cm⁻¹)
- 33 3. 794.6 nm for saturated lipids (2885 cm⁻¹)
- 34 4. 786.5 nm for unsaturated lipids (3015 cm⁻¹)
- 35 v. Acquire 3D SRS mapping and stitch (.oir file)
- 36 e. HSI Imaging:
- 37 i. On laser control panel select hyperspectral sweep and select range of wavelengths: 781.5-806.5nm (3100-2700 cm⁻¹) and choose a step number of at least 60.
- 38 ii. Olympus FV3000 Software: Select region of interest and acquire multiple sequential images to generate hyperspectral stack (.oir files)