Supplemental Document

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Shot-noise limited tunable dual-vibrational frequency stimulated Raman scattering microscopy: supplement

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Supplementary information to “Shot-noise limited dual-vibrational frequency stimulated Raman scattering microscopy”

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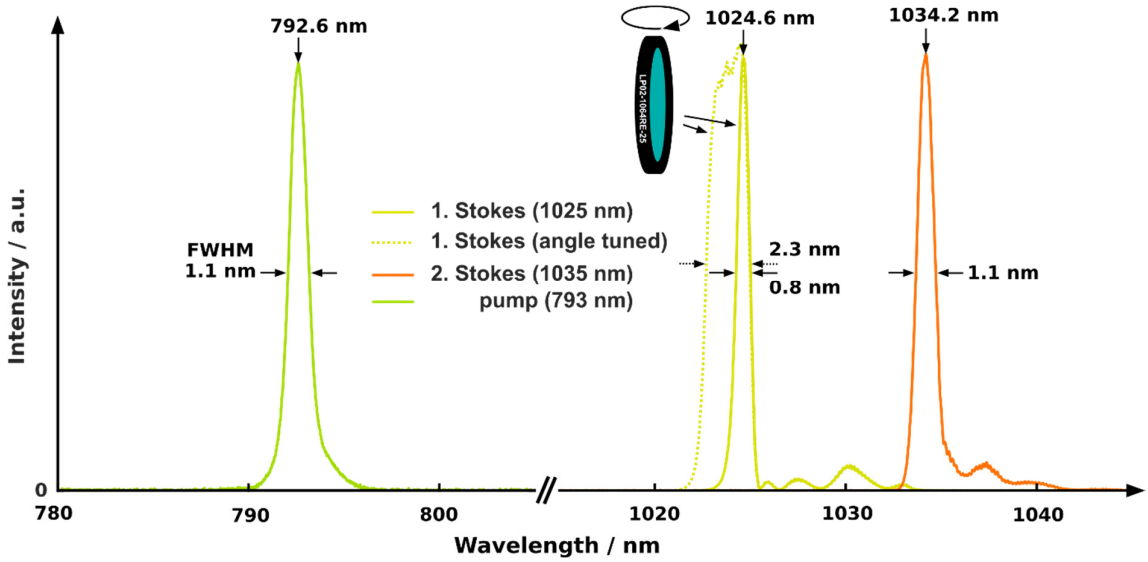
The following document provides information about the spectral and temporal width of the 3-colors laser system. Further, the raw SRS images are presented that correspond to the virtual histopathology images displayed in Fig. 2 of the main document.

Optical spectrum of the 3-color laser system

The spectrum was acquired using an optical spectrum analyzer (OSA, Yokogawa, AQ6370D). The results of the measurement are summarized in the sup. Fig. 1.



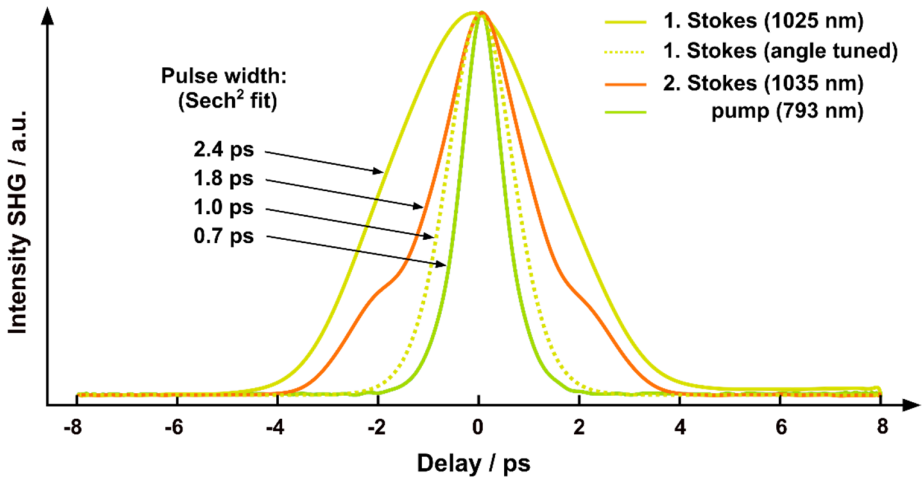
Sup. Fig. 1: Spectrum of the 3-color laser system.

As evident from SupFig. 1, tuning the transmission angle of the ultrasteep long-pass edge filter allows for tuning the bandwidth and output power of the Stokes beams. With the LP02-1064RE-25 (Semrock), the minimum achievable spectral width is 0.8 nm for a resulting power of 200 mW while already 600 mW are transmitted for a FWHM of 2.3 nm. Note that the spectral bandwidth of the fs-pump laser sets the accessible upper limit for the FWHM of each Stokes beam. Thus, our approach features the very unique advantage that the

temporal pulse width is readily adjusted by tuning the transmission angles of our long-pass edge filters. Changing the pulse width of other laser sources is usually either impossible, e.g. for fiber lasers, restricted to a narrow range or requires an elaborated cavity readjustment procedure.

Characterization of the temporal width of the 3-color system

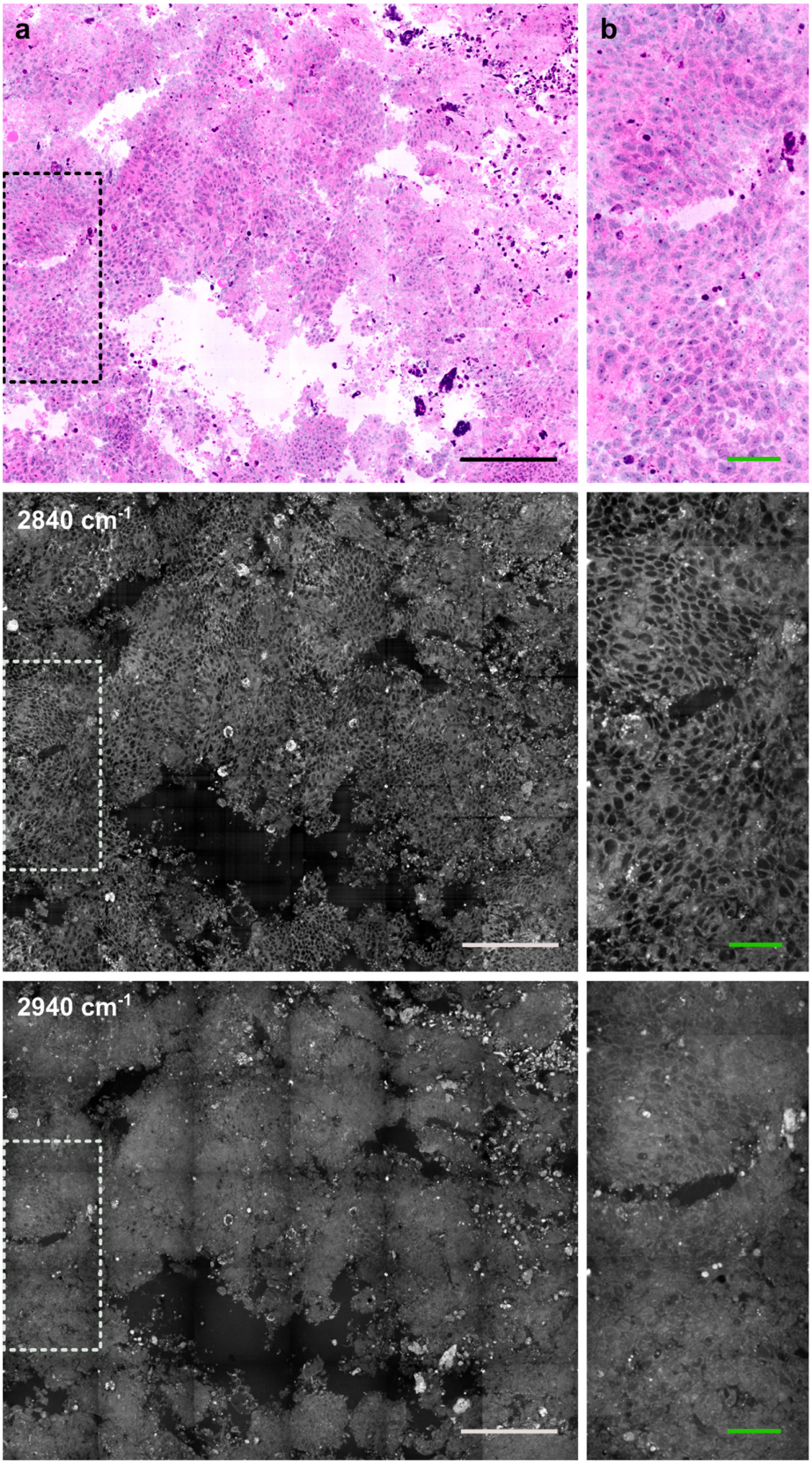
For determination of the temporal pulse width, we used an autocorrelator (APE, pulseCheck). Note that the laser pulses were measured just before the laser scanning microscope, i.e. after passing several lenses, mirrors, beam splitters and EOMs. The results of the autocorrelation measurement are displayed in the SupFig. 2.



Sup. Fig. 2: Autocorrelation of the 3-color laser system.

Native SRS-images for the generation of Fig. 2 of the main document

The SRS-images acquired at 2840 cm^{-1} and 2940 cm^{-1} are displayed in the SupFig. 3. These images represent the flat-field corrected raw data. To minimize the impact of the uneven illumination, we removed low spatial frequencies that cause image vignetting. In general, this procedure does not lead to artifacts that would compromise the diagnostic value of the virtual HE images since the diagnostically relevant features are smaller than one tenth of our field of view (≈200 µm). Quite the contrary, the flat-field correction enables for a smooth visual transition from one image tile to another improving the match of our virtual HE-images with classical dye-based HE-images.



Sup. Fig. 3: illumination corrected SRS data for the virtual HE-image generation.