Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information for

Nonlinear and vibrational microscopy for label-free characterization of amyloid-β plaques in Alzheimer's disease model

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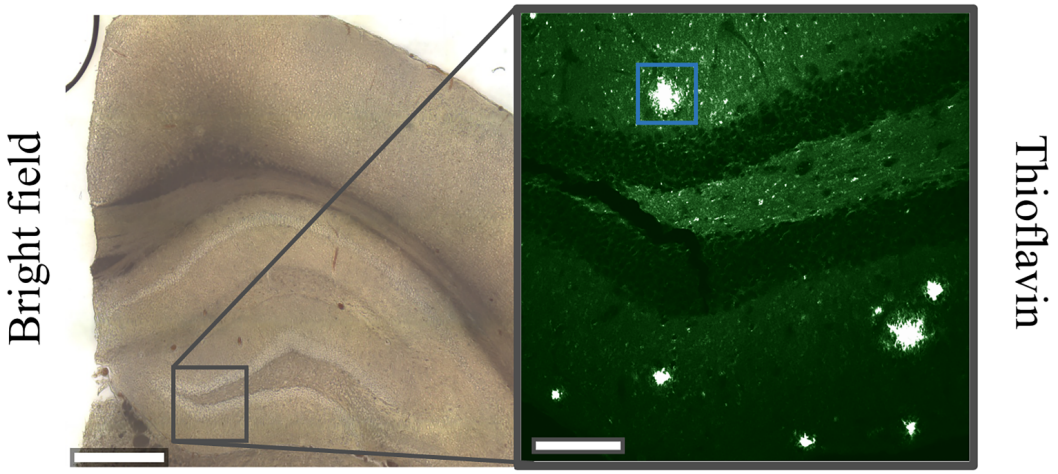

Fig. S1 Tissue identification in the ThioS stained TPEF image. Same thioS stained TPEF image present in Fig. 1C of the main article but with saturated Aβ plaques, favoring the identification of the hippocampus and showing other plaques in a 12-month-old animal. Left side: bright field image of the mouse brain slice (scale bar is 500 μm). Right side: ThioS stained TPEF image of the region shown by the grey square in the bright field image with several Aβ plaques (scale bar is 100 μm). The blue square also identifies the plaque studied in Figure 1 of the manuscript.

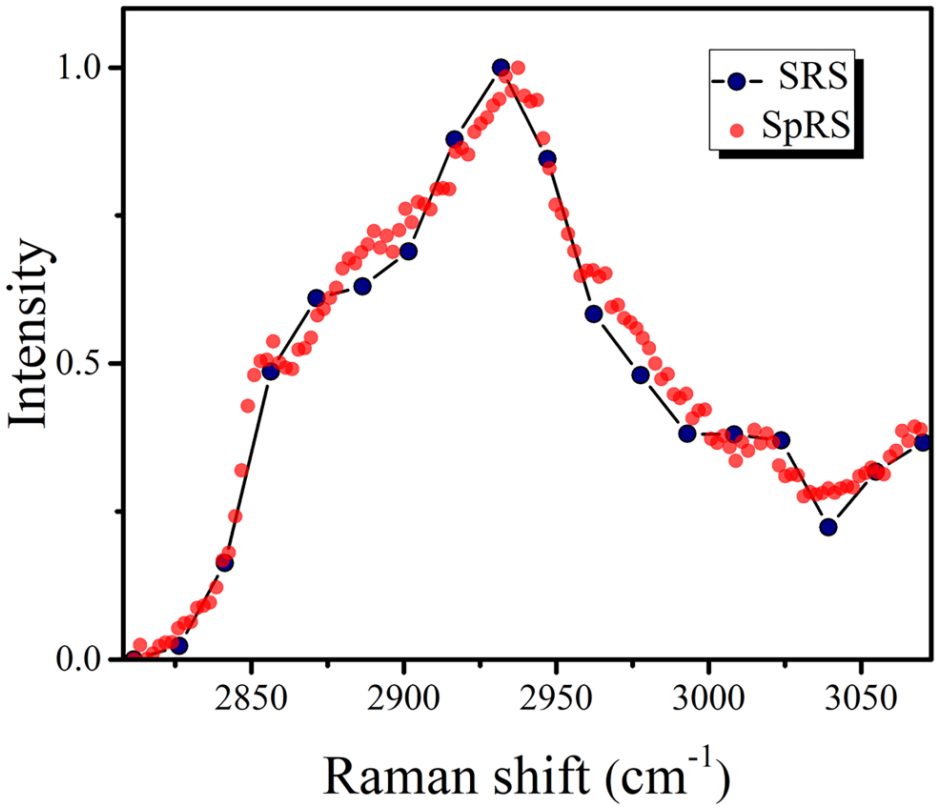

Fig. S2 Stimulated Raman microscopy *versus* spontaneous Raman spectra. Comparison between stimulated Raman scattering (SRS, blue) and spontaneous Raman scattering (SpRS, red) spectra obtained from mouse brain tissue images. The images were acquired from different scattering geometries (transmission for the SRS and reflection for the SpRS), but demonstrate a high spectral correspondence.

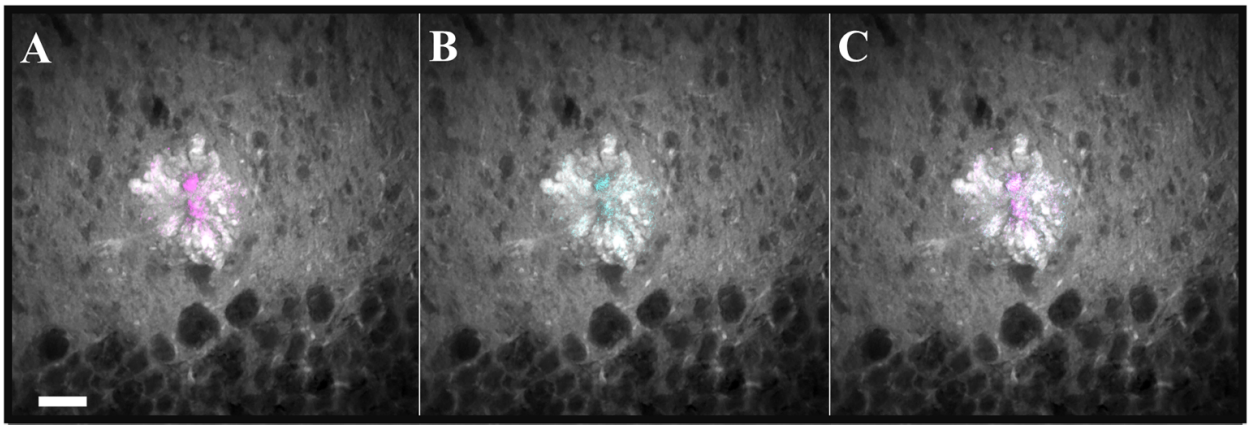

Fig. S3 Comparison between high-resolution SRS images of amide I and phenylalanine and spatial distribution of both core biomarkers. (A,B) merged image of the halo and core, demonstrating the spatial distribution of the response based on the frequency of amide I (A) and phenylalanine (B). There is a spatial correlation in the distribution of both biomarkers in the core, although they present appreciable differences. (C) the merged image of amide I (A) and phenylalanine (B), as well as the halo, also demonstrate this distribution. In all figures, the halo image is based on the frequency attributed to lipids, 2850 cm^{-1}. Images without a scale bar share the scale bar of the leftmost image in the same line, which is 20 μm.

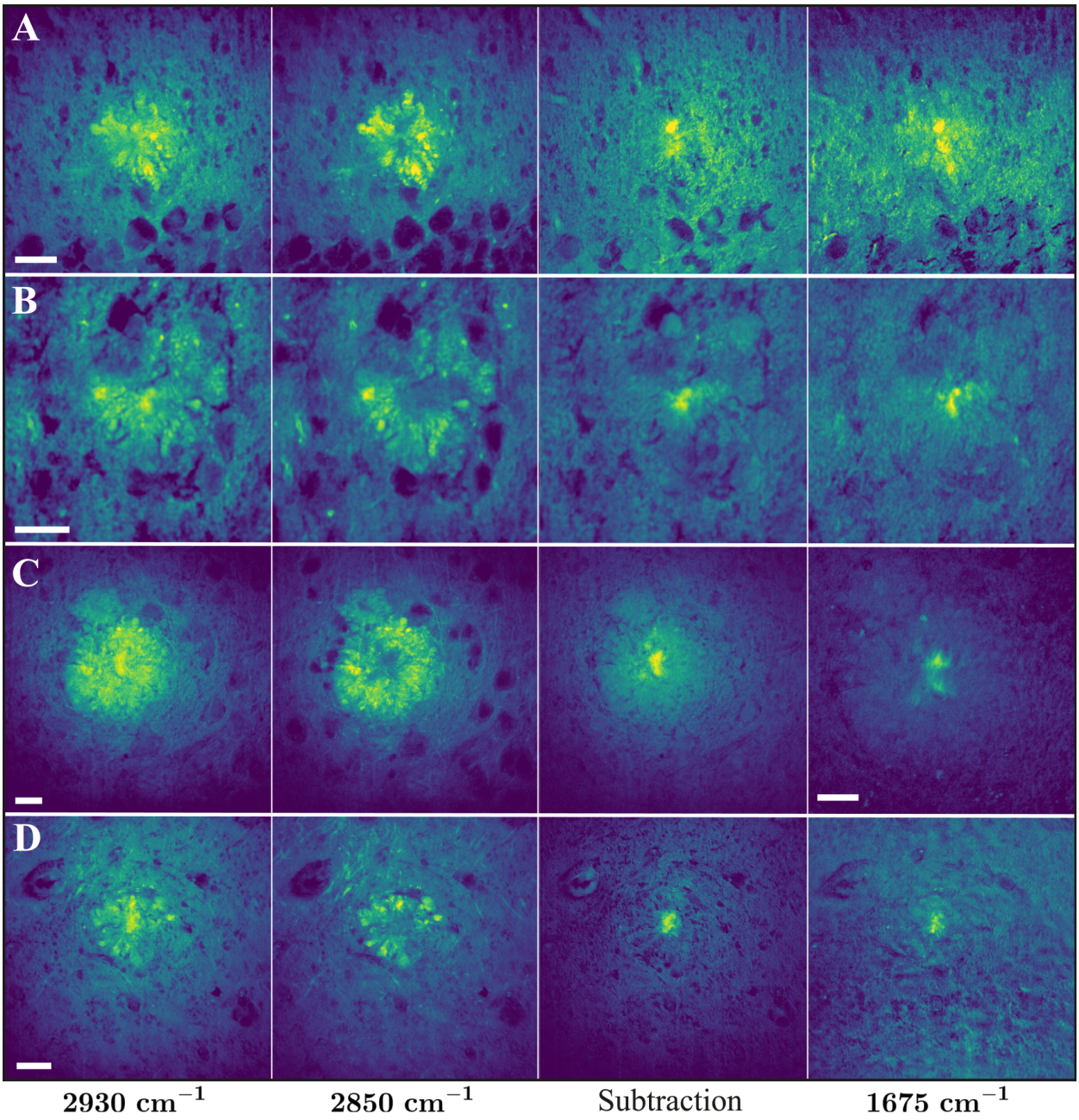

Fig. S4 Subtraction of SRS images in the high-frequency region and comparison with SRS images based on the vibrational mode of amide I. (A-D) different Aβ plaques distributed in three vibrations: 2930 (proteins/lipids), revealing both the core and the halo; 2850 (lipids), with only the halo in evidence; and 1675 (amide I), with only the core in evidence. This last column should be compared to the subtraction column obtained by subtracting images at frequencies 2930 and 2850 cm^{-1}. The images based on amide I and those obtained by subtraction show a significant spatial correlation.

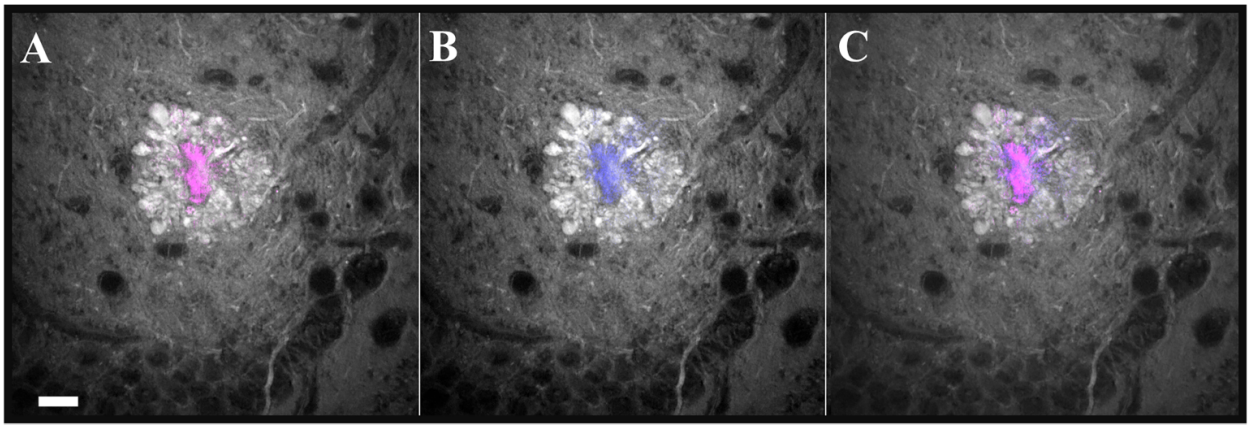

Fig. S5 Comparison between high-resolution SRS images of amide I and amide B and spatial distribution of both core biomarkers. (A,B) merged image of the halo and core, demonstrating the spatial distribution of the response based on the frequency of amide I (A) and amide B (B). There is a spatial correlation in the distribution of both biomarkers in the core; however, no appreciable difference can be ensured. (C) the merged image of amide I (A) and amide B (B), as well as the halo, also demonstrate this distribution. In all figures, the halo image is based on the frequency attributed to lipids, 2850 cm^{-1}. Images without a scale bar share the scale bar of the leftmost image in the same line, which is 20 μm.

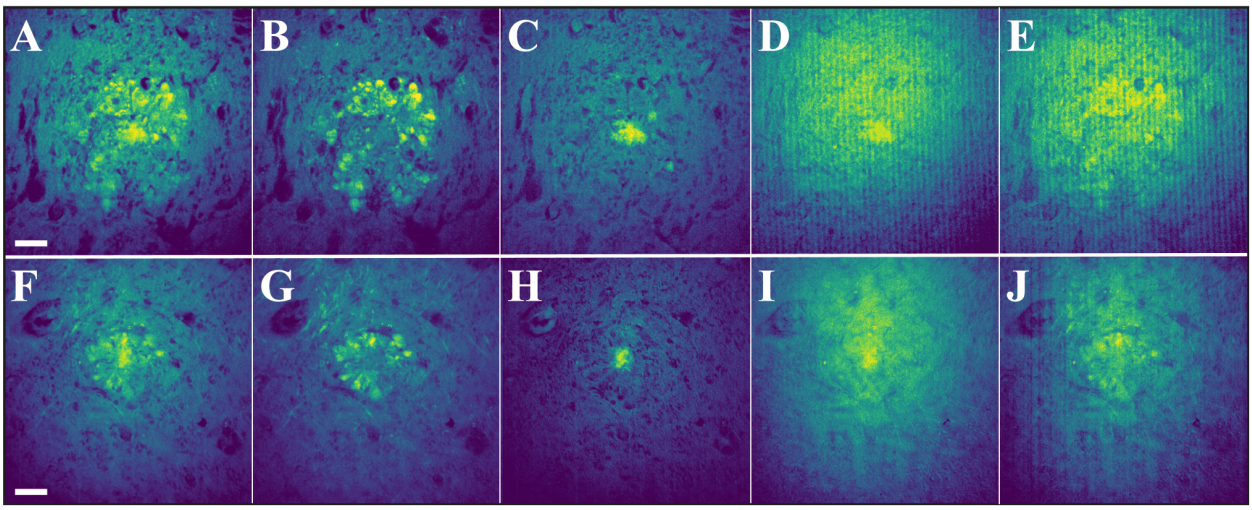

Fig. S6 High-resolution SRS image with core and halo biomarkers in the high-frequency spectral region for two different Aβ plaques.(A,F) and (B,G) are images based on the known 2930 cm^{-1} (proteins/lipids) and 2850 cm^{-1} (lipids) frequencies, respectively. (C,H) is the subtraction of these images, showing the core in the high-frequency region. (D,I) is the image based on the 3070 cm^{-1} (amide B) frequency, which shows the core in the high-frequency region, correlating with the image obtained by subtraction (C,H). (E,J) is the halo image based on the 3019 cm^{-1} frequency attributed to unsaturated lipids. Images without a scale bar share the scale bar of the leftmost image in the same line, which is 20 μm.