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SUPPLEMENTARY INFORMATION

Visualizing intra-medulla lipids in human hair using ultra-multiplex CARS, SHG, and THG microscopy

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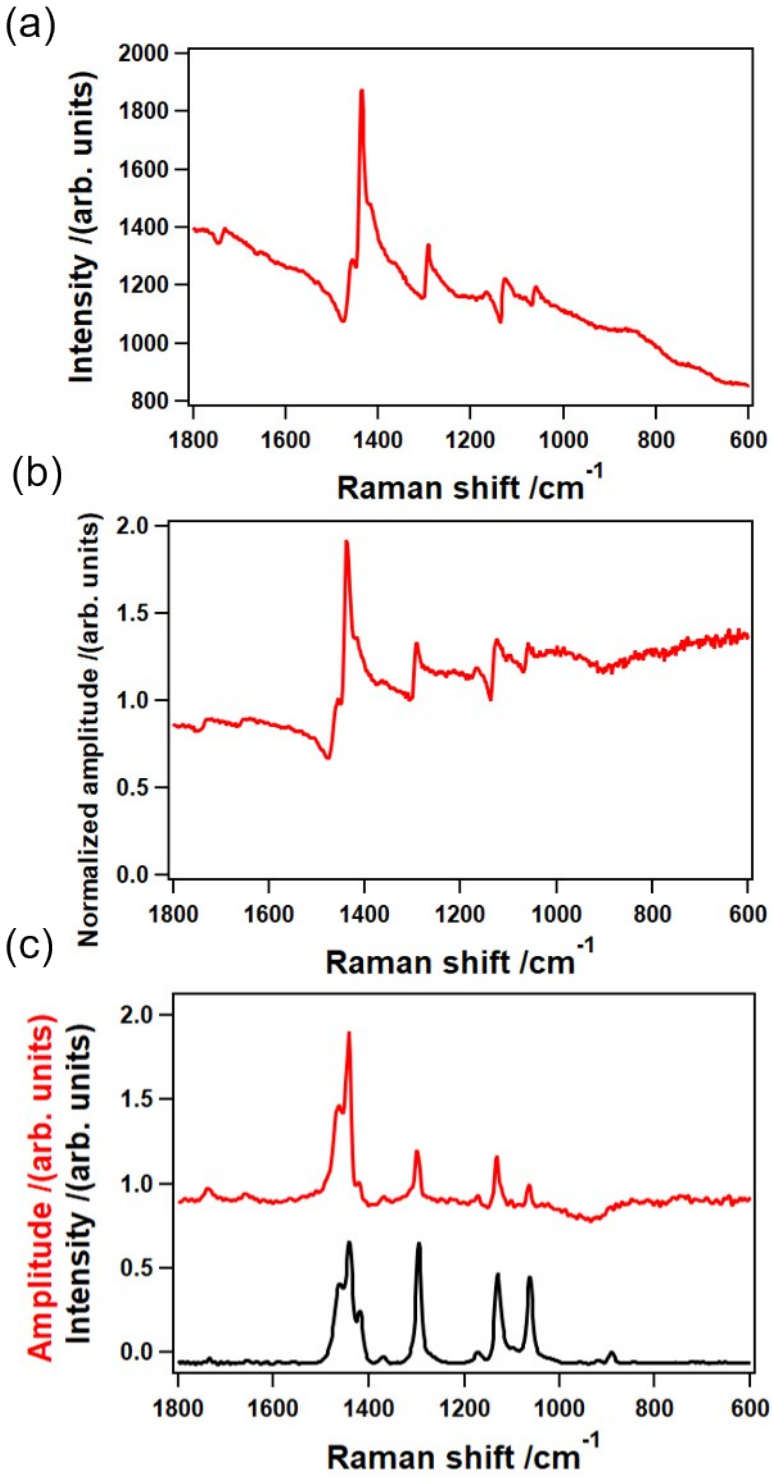
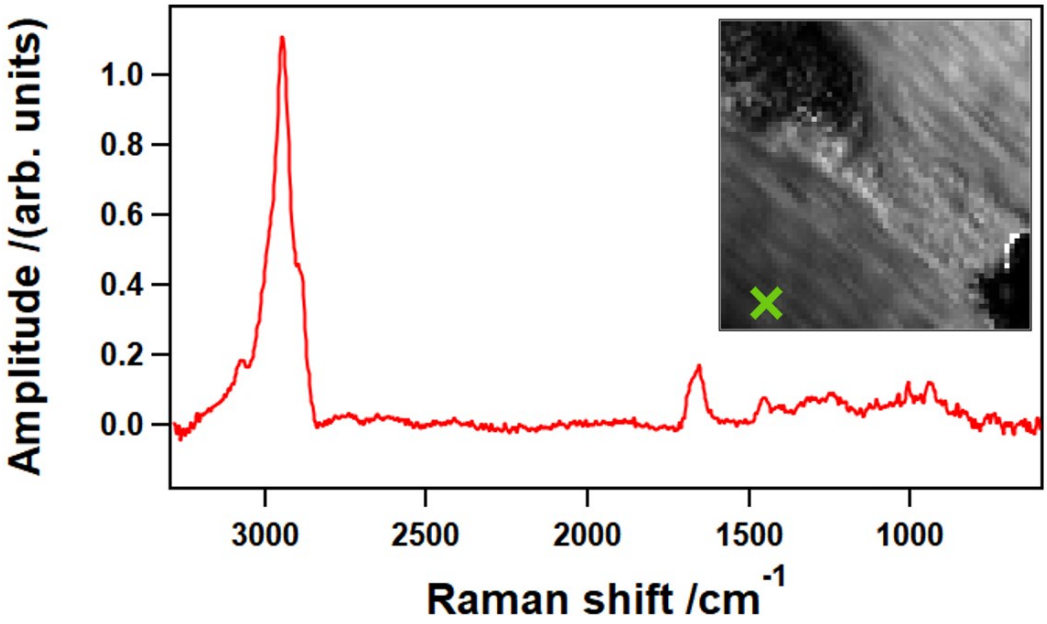
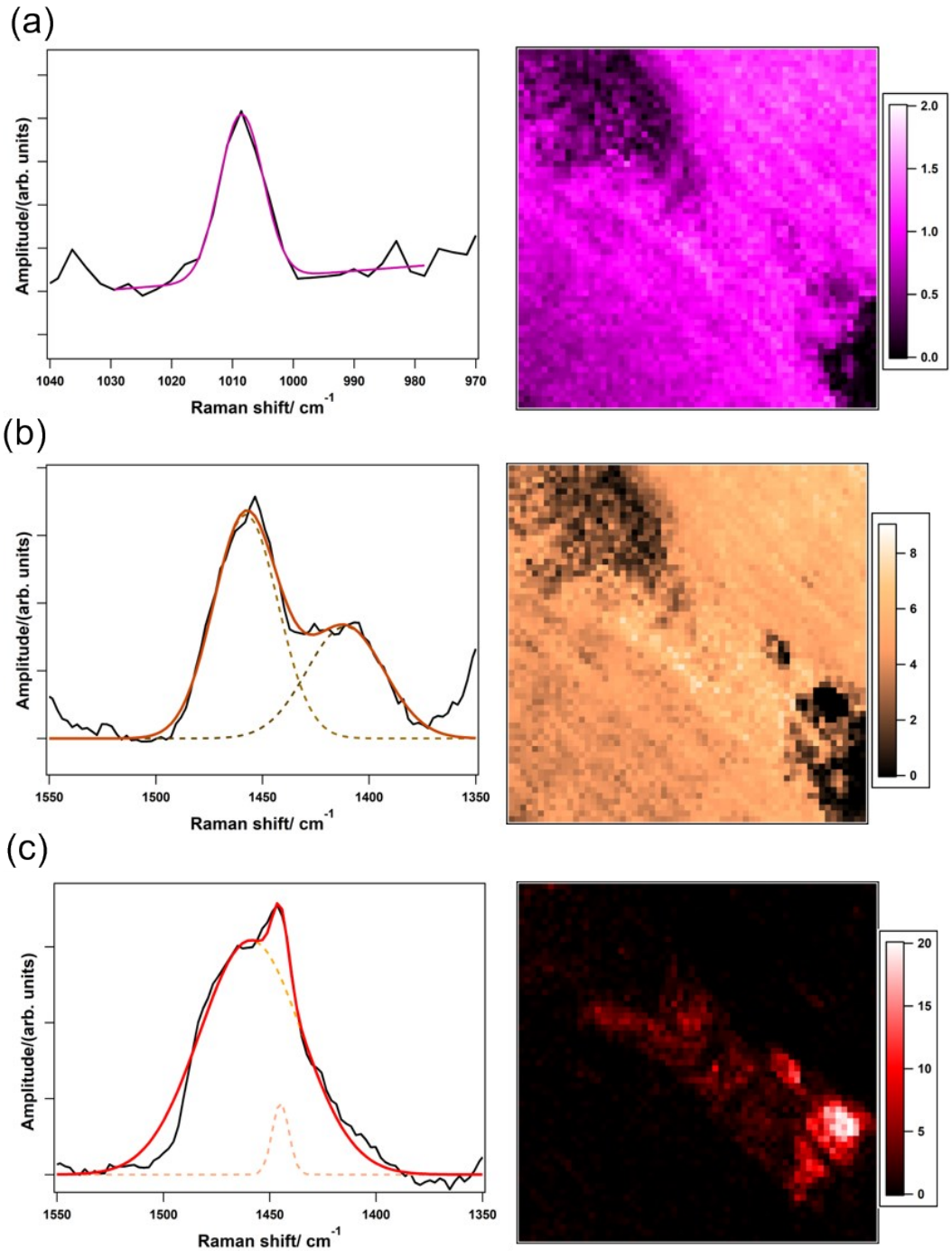


Supplementary Figure 1 | Comparison of Im[$\chi^{(3)}$] spectrum calculated by maximum entropy method (MEM) with spontaneous Raman spectrum. (a) The raw CARS spectrum of wax ester (bees wax). **(b)** intensity-corrected CARS spectrum of (a). **(c)** Retrieved Raman-equivalent Im[$\chi^{(3)}$] spectrum from (b) using MEM (red) and spontaneous Raman spectrum.

Note that the sample preparation was different, but was prepared from the same reagent bottle.



Supplementary Figure 2 | Ultra-multiplex CARS spectrum at cortex.
Im[$\chi^{(3)}$] spectrum at green cross in the inset is, which is not saturated in CH stretching region. As shown in the spectral profile, we can evaluate full spectral information of almost all vibrational fundamental modes.



Supplementary Figure 3 | Reconstruction of the CARS images. (a) Phenyl-ring breathing vibrational mode at 1008 cm^{-1} was fitted with a Gaussian function (left), and the amplitude was mapped out (right); (b) and (c) Spectral profile in CH bending vibrational mode was complicated, and were clearly different between the protein-rich and lipid-rich areas. Therefore, we first fitted representative protein-rich(b) and lipid-rich(c) spectral profiles using

the sum of two Gaussian functions, and defined $f(\tilde{\nu})$ and $g(\tilde{\nu})$ as the protein-rich and lipid-rich representative spectral profiles, respectively. Here $\tilde{\nu}$ corresponds to Raman shift. Then, we fitted spectral profiles in all spatial points using the sum of $f(\tilde{\nu})$ and $g(\tilde{\nu})$ with two coefficients, namely $c_f f(\tilde{\nu}) + c_g g(\tilde{\nu})$. The representative protein-rich and lipid-rich spectral profiles ($f(\tilde{\nu})$ and $g(\tilde{\nu})$) as well as the decomposed each Gaussian function are indicated in (b) and (c) (left). Two coefficients are then mapped out (right).