Supplementary information

Correlative nonlinear optical microscopy and infrared nanoscopy reveals collagen degradation in altered parchments

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Supplementary material

Circular dichroism

Circular dichroism (CD) spectra of the collagen solution were recorded on a JASCO J-810 spectropolarimeter to assess the efficiency of the denaturation procedure. Quartz cells of 1mm optical path-length (QS, Hellma) were used and the temperature was set at 20 °C during the experiments. The thermal treatment was performed in the collagen stock solution and this solution was then quickly diluted to a concentration of 0.043 mg/mL in order to avoid saturation of the detector during CD measurements.

Figure S3 shows that the native collagen CD spectrum is mainly composed of a positive peak at 220 nm ($n\pi^*$ amide transition) and a negative peak at 198 nm ($\pi\pi^*$ amide transition), as expected from the litterature.[1] Those bands are specific for the helicoidal structure of the polyproline II (PPII), a structure stabilized by the triple helix conformation.[1] This CD spectrum changes dramatically upon

the thermal treatment. The $n\pi^*$ peak totally disappears and the intensity of the $\pi\pi^*$ peak strongly decreases. It reveals a substantial disruption of the PPII helix, which corresponds to an effective collagen denaturation. It means that our thermal treatment is an efficient mild procedure that totally unfolds the collagen triple helices while minimizing chain hydrolysis.

Reference

[1] Fasman, G. D. *Circular dichroism and the conformational analysis of biomolecules*. Springer, New York, (2010).

Supplementary figures

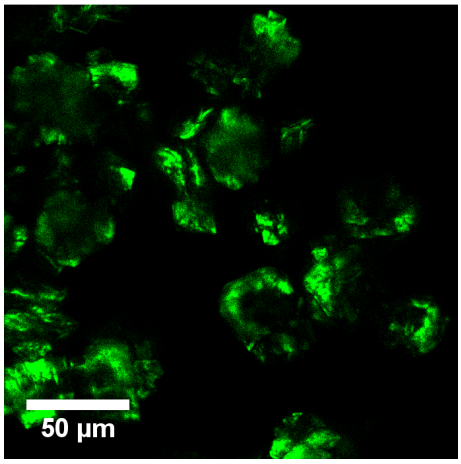


Fig. S1. SHG imaging from carbonate calcium particles. Carbonate calcium particles deposited on a microscope slab are image by NLO microscopy. They exhibit strong SHG signals due to surface anisotropy.

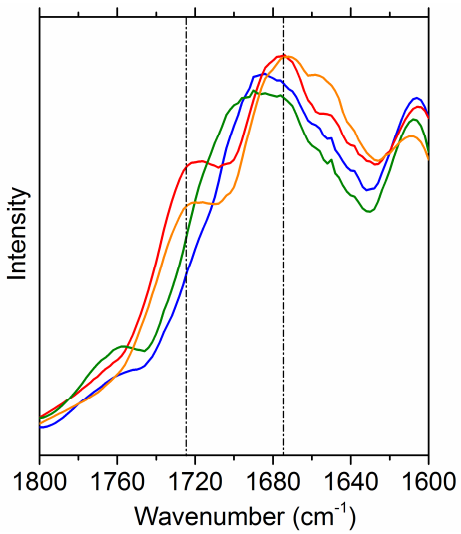


Fig. S2. IR nanoscopy spectra of Maritime map sample scrap. NanoIR spectra recorded between 1600 and 1800 cm^{-1} from local areas of different particles (spectra in green and blue correspond to local points from the correlative imaging in Fig. 6). The gel-carbonyl band around 1720 cm^{-1} is clearly visible.

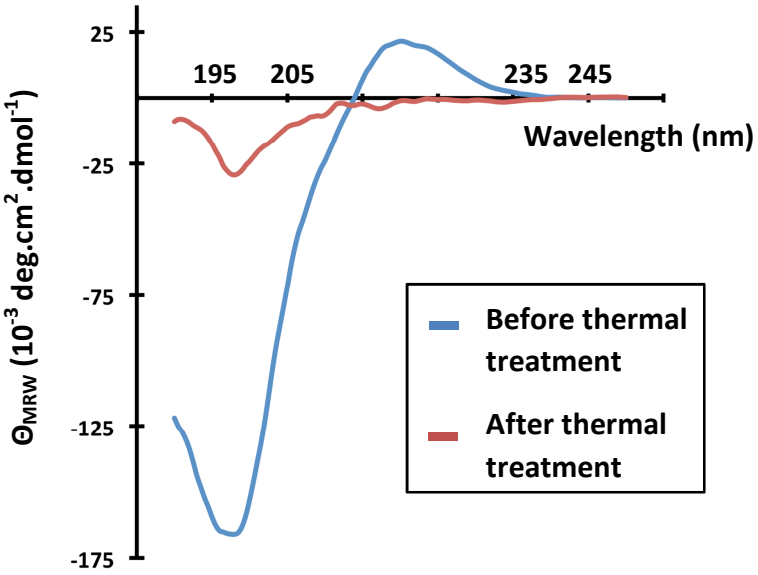
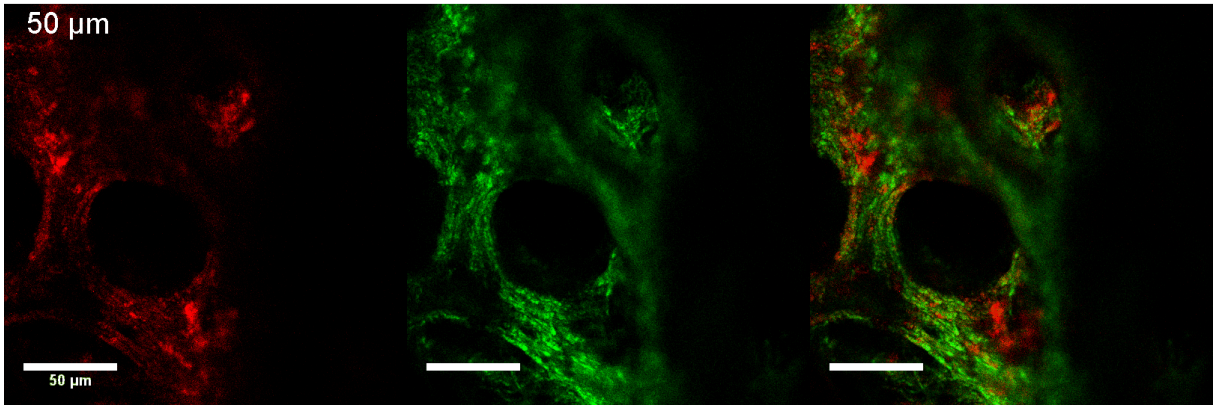
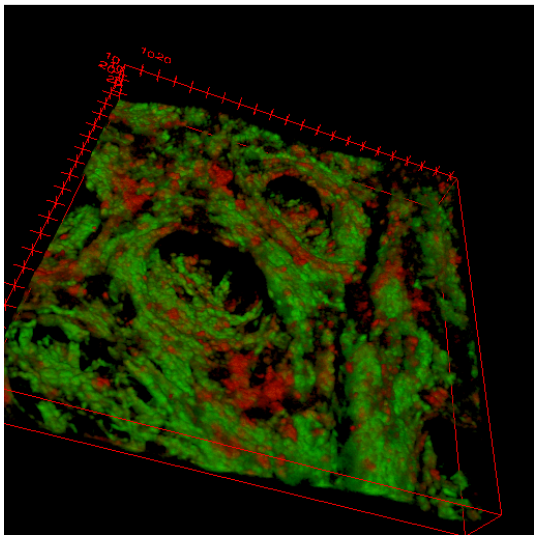


Fig. S3. CD spectra of collagen solution before (blue) and after (red) thermal treatment applied for the denaturation. Spectra are recorded at 20 °C from 250 nm to 190 nm. The collagen concentration is set to 0.043 mg/ml, and a quartz cell of 1 mm optical path is used.

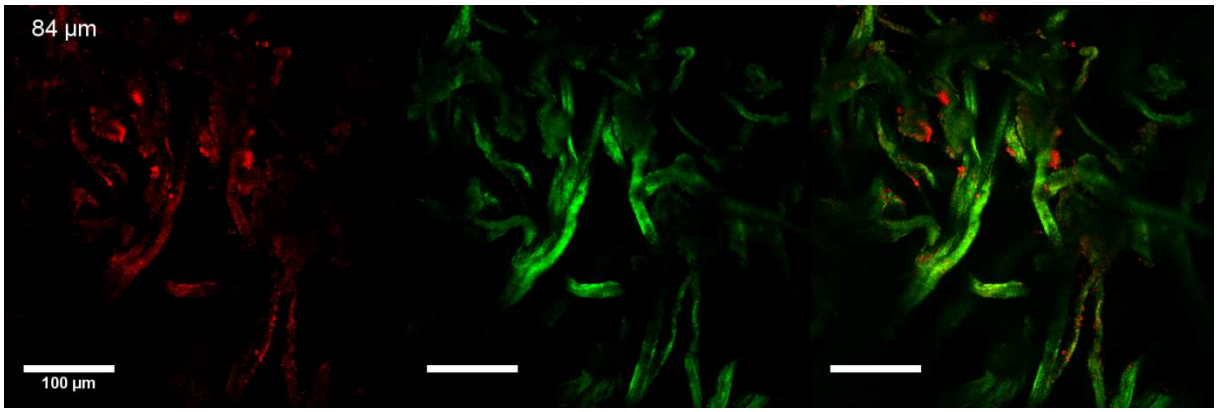
Supplementary movies



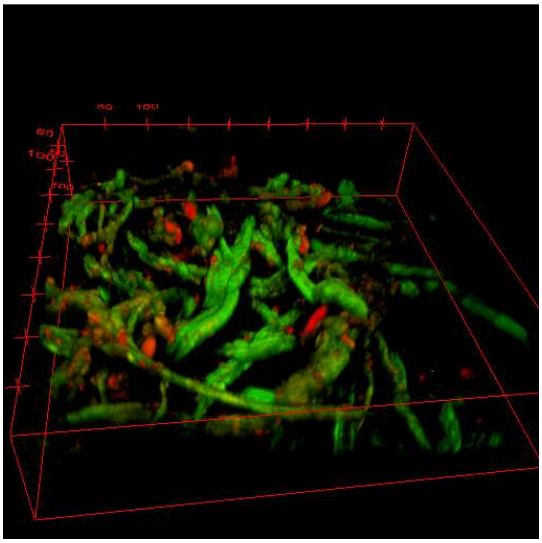
Supplementary Video S1. NLO microscopy imaging with depth of untanned dry skin on grain side. 2PEF signals (in red, left part) probably stem from keratin residues left after removing the hair during manufacturing. SHG signals (in green, center part) are emitted by collagen fibrils. Merge 2PEF/SHG signals (right part). Same sample as Fig. 1B-C, z-step = 1 µm.



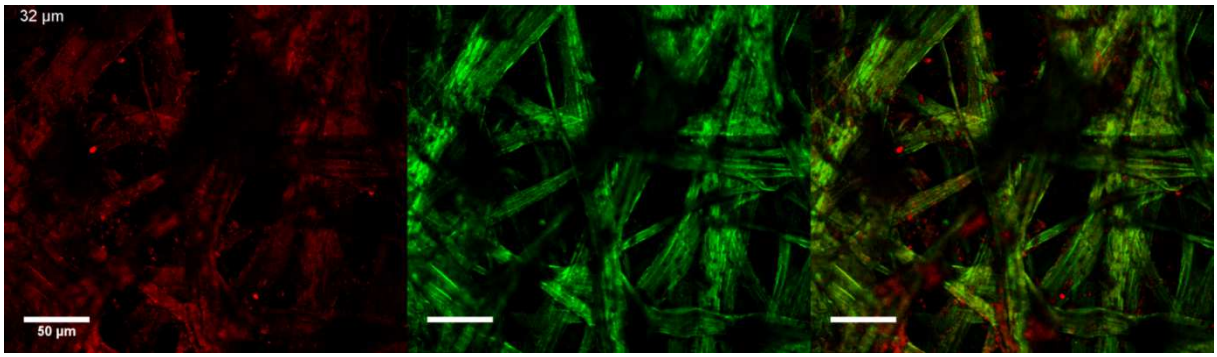
Supplementary Video S2. NLO microscopy 3D imaging of untanned dry skin on grain side. 3D imaging of 2PEF (in red, from keratin) and SHG (in green, from collagen fibrils) signals. Same sample as Fig. 1B-C, 3D views : 215 x 215 x 70 μm^3.



Supplementary Video S3. NLO microscopy imaging with depth of untanned dry skin on flesh side. 2PEF signals (in red, left art) probably stem from residues of fat or elastin. SHG signals (in green, center part) are emitted by collagen fibrils. Merge 2PEF/SHG signals (right part). Same sample as Fig. 1E-F, z-step = 1 µm.



Supplementary Video S4. NLO microscopy 3D imaging of untanned dry skin on flesh side. 3D imaging of 2PEF (in red, from fat or elastin) and SHG (in green, from collagen fibrils) signals. Same sample as Fig. 1E-F, 3D views : 440 x 440 x 140 μm^3.



Supplementary Video S5. NLO microscopy imaging with depth of preserved parchment on flesh side. 2PEF signals (in red, left part) probably stem from residues of fat or elastin. SHG signals (in green, center part) are emitted by collagen fibrils. Merge 2PEF/SHG signals (right part). Same sample as Fig. 2D-E, z-step = 1 µm.