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Correlative SHG/TEM imaging of collagen fibrils

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Type I collagen is a major structural protein in mammals and shows highly structured macromolecular organizations specific to each tissue. This biopolymer is synthesized by cells as triple helices, which self-assemble outside the cells into fibrils (10-300 nm) that further form fibers, lamellae or other 3D networks. This assembly mechanism depends critically on the collagen concentration, as well as on the temperature, pH and ionic strength of the solution in vitro. Thorough characterization of collagen fibrillogenesis is crucial to understand the biological mechanisms of tissue formation and remodeling and to design new collagen-based biomaterials.

In this work, we continuously monitored the formation of collagen fibrils using time-lapse *in situ* Second Harmonic Generation (SHG) microscopy. Fibrillogenesis was triggered by increasing the pH in a dilute solution of collagen I and the increase of the fibril density was quantified in the SHG image stacks acquired sequentially overnight. Our results showed reproducible dynamics of fibrillogenesis that could be changed by tuning the pH. In addition we investigated surface-mediated fibrillogenesis by adding silica nanoparticles to the solution. We used Two-Photon excited fluorescence microscopy to visualize the stained nanoparticles and quantify the self-assembly of collagen around these nanoparticles to study the influence of inorganic materials on collagen matrices structuration.
This study shows that SHG microscopy is a valuable technique for in situ 3D imaging of fibrillar collagen with sub-micrometer resolution. However, this optical technique cannot resolve most of the fibrils, which impedes quantitative measurements of the fibril diameter. Moreover, SHG is a coherent multiphoton signal, which scales in a complex way with the total collagen density.

Hence, we correlated SHG microscopy to TEM to determine the sensitivity of SHG microscopy and to calibrate SHG signals as a function of the fibril diameter. To that end, we synthetized isolated fibrils with various diameters and successfully imaged the same fibrils with both techniques, down to 30 nm. The SHG signal scaled as the fourth power of the fibril diameter, as expected from analytical and numerical calculations. These results represent a major step towards quantitative SHG imaging of nm-sized collagen fibrils.

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