

## INVESTIGATION OF EARLY BONE MINERALIZATION BY CARS MICROSCOPY

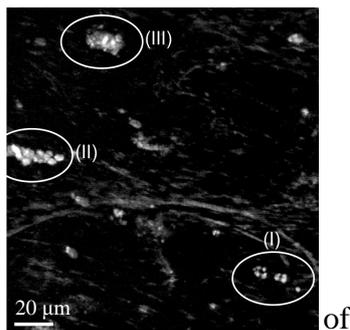
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The non-invasive and label-free approach of CARS microscopy makes it a very attractive imaging tool for studying cells and tissue. Here we present a 60-day study of early bone mineralization, where the cells are imaged at fixed times. We image the hydroxyapatite at  $950\text{ cm}^{-1}$  (see figure 1) and lipids droplets at  $2845\text{ cm}^{-1}$ . Collagen fibrils are simultaneously imaged using second harmonic generation microscopy. Spontaneous Raman spectra are recorded separately. We recorded the differentiation of multipotent adult human bone marrow stromal cells towards osteogenic lineage, followed by the initiation and growth of hydroxyapatite mineralization, resulting in the formation of a bone layer that appears to be guided by the collagen fibrils. The bone formed after 60 days is similar to in vivo bone. The combination of several non-linear imaging techniques gives a unique insight of how cells grow bone.

We also present an extension to the CARS technique where we are able to separate different molecules based on the amplitude and phase of the CARS signal. This “vibrational phase contrast CARS microscopy” (VPC-CARS) [1] provides background free imaging (see figure 2) and chemically specific detection of substances with partially overlapping resonances, from a measurement at only a single vibrational frequency.



of early bone mineralization.

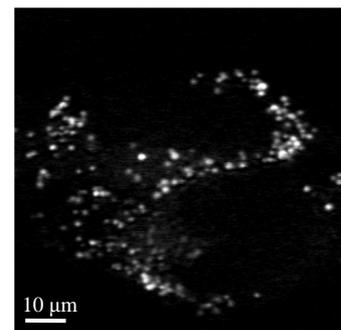
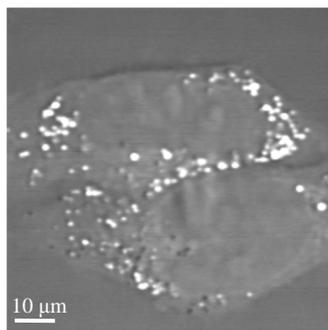


Figure 2: Image of the lipid droplet distribution in two HeLa cells imaged at  $2845\text{ cm}^{-1}$ . Left: Forward CARS, right: VPC-CARS (background free).

[1] M. Jurna, J. P. Korterik, C. Otto, J. L. Herek, and H. L. Offerhaus, “Vibrational Phase Contrast Microscopy by Use of Coherent Anti-Stokes Raman Scattering,” *Physical Review Letters*, **103**, 043905 (2009).