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## Determination of the origin of third harmonic generation in bone for intravital nonlinear optical microscopy of bone tissue

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Osteocytes, the most abundant cells present in bone, regulate bone mass by chemical signaling through the lacunar-canalicular network (LCN). Bone diseases such as Hypophosphatemic Rickets cause painful bone twisting in children, and progress on understanding this disease is slow. The structural and mechanical properties of the LCN are difficult to understand since the LCN is embedded in bone, complicating in vivo studies. Currently, serial sectioning is most commonly used to study the LCN however, it usually produces morphological distortion because soft tissue embedded in bone does not cleave well.

Alternatively, nonlinear optical microscopy can be used to interrogate the LCN noninvasively with high spatial resolution in vivo without requiring physical sectioning. Nonlinear optical microscopy with ultrafast femtosecond fibre lasers in the telecom wavelength range have several advantages including improved penetration through bone and emission of third harmonic generation (THG) signal in the visible wavelength range. The LCN produces THG while the collagen in bone produces second harmonic generation (SHG).

To determine the origin of the THG signal from the LCN, an investigation was performed. Laser ablation was performed in bone in order to study the THG signal directionality. Forward- and epi-detected THG imaging was performed in live, dissected and ablated bone. It was found that THG signal is directed mostly in the forward direction, and originates from the bone-interstitial fluid boundaries. Interestingly, the THG intensity of canaliculi varied depending on their angle to the optical plane, having significantly higher amplitudes at higher angles.

Subsequently, a study of mice with transgenic hypophosphatemic rickets was performed to demonstrate the technique. THG imaging revealed statistically significant differences in the lacunar volume and density of canaliculi, offering the opportunity to study dynamical structural changes to the LCN during disease progression.

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