Nonlinear optics for in vivo experimental biology: cellular imaging and manipulation

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Nonlinear optics has become an important tool for experimental biology because it enables the dynamic imaging of cellular structures within a whole, living organism. Multiphoton microscopy, in which the nonlinear absorption of two or more photons excites fluorescence, enables visualization of not just the structure, but also the motion and function of individual cells in an animal. Two-photon microscopy is rapidly becoming a mainstream biological tool. Nonlinear photodisruption has also been used for manipulation of cells, including cutting and ablating, with sub-cellular resolution. We used these capabilities in the investigation of the stem cell niche in the mouse intestine. Stem cells and Paneth cells are arranged in a precisely alternating pattern at the end of tubular structures called crypts (Fig. a). To study how this pattern is determined, we implant windows in the abdomen of transgenic mice that express green fluorescent protein in stem cells. The same cells could be visualized repeatedly using multiphoton microcopy (Fig. b). We then use femtosecond laser ablation to remove several cells and studied the resulting dynamics. We found that the alternating pattern of cells recovers quickly and involves cell rearrangement rather than proliferation. Recent advances in lasers increased the maximum depth of imaging by shifting to longer wavelengths. Using a combination of novel surgical stabilization techniques, higher-order nonlinear excitation, fast acquisition, and image reconstruction techniques, we also demonstrate deep imaging of the beating, live heart. We use 1,700 nm excitation light generated by soliton self-frequency shifting of 1,550-nm femtosecond pulses (tunable from 120 kHz -10 MHz, max pulse energy of 1.5 μJ) in a photonic crystal rod. To image the heart, the chest of an anesthetized mouse is opened. To stabilize a region of the heart for observation, a 3D-printed titanium probe with a coverglass is attached to the heart surface (Fig. c). Vasculature is labeled with a retro-orbital injection of Texas-Red conjugated dextran, enabling blood flow visualization by the motion of red blood cells. The mouse heart contracts at a rate of ~10 Hz, causing in-frame motion artifacts when using galvonometric scanners (~1 Hz frame rate) commonly used in laser scanning microscopy (Fig. d). Resonant scanners acquire data at a higher rate (~30 Hz frame rate) and eliminate motion artifact to acquire a complete image at high temporal resolution. Second and third harmonic generation (SHG and THG) are also visible in the tissue (Fig. e).

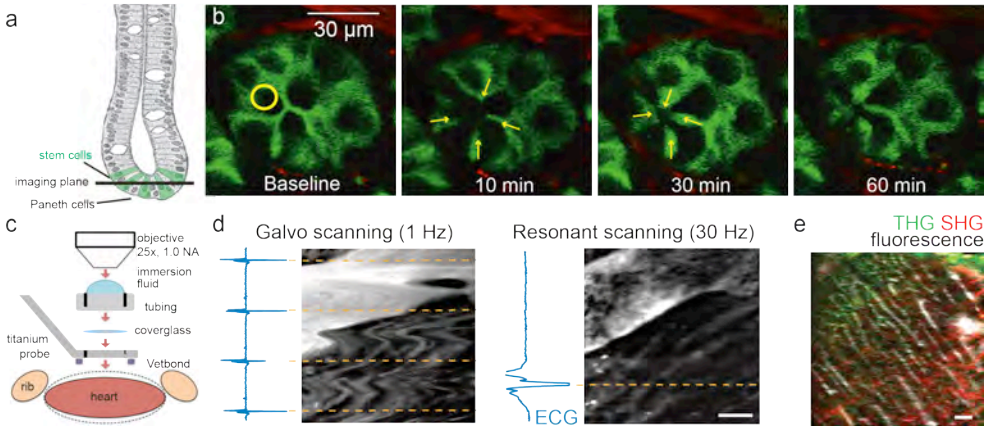

Figure. Multiphoton microscopy in mouse models. (a) Schematic of the stem cell niche in intestine [adapted from Henry Gray (1918) Anatomy of the Human Body]. (b) Stem cells (green) and Paneth cells (black) are arranged in a distinct checkerboard-like pattern in the mouse intestine. Yellow circle show site of ablation with femtosecond laser pulses (~100 nJ). Within minutes, stem cells around ablation site push into ablated region. (c) Setup for imaging beating heart in mouse. (d) Comparison of scanning modes shows heart vessels (labeled with intravenous dye injection) higher frame rate avoids motion artifact. (e) Heart capillaries are visualized with a fluorescent dye injection and third and second harmonic generation is visible in the surrounding tissue.