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Development of a high-speed two-photon laser scanning microscope with an acquisition rate up to 180 frames per second

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The ability to observe fast biological phenomena such as turbulent cell flow and rapid micro-vasculature responses has been of increasing interest in rheological studies. Two-photon laser scanning microscopy is an asset to these studies as it enables deep tissue optical sectioning and good spatial resolution in various *in vivo* animal models. As such, we developed a high-speed laser scanning two-photon microscope capable of acquiring 512 x 512 pixel images at up to 90 fps (frames per second) or 512 x 256 pixels at up to 180 fps. The high scan rate of the fast-axis was implemented via a rapidly rotating polygonal scanning mirror with 128 facets. The slow-axis was scanned using a high-speed galvanometer. Image acquisition was achieved using a custom-built photo-multiplier tube amplifier with a broad frequency band. Verification of the developed two-photon microscope's acquisition speed was established by imaging a fluorescent resolution target being moved at a variety of constant speeds. Evaluation of the frame acquisition speeds were done by comparing successive frames. Finally, the system's applicability to biological studies was qualitatively demonstrated by observing the slight movements in a sedated mouse due to its heartbeats.

Figure1: Two-photon laser scanning microscope.

Figure2: Ti:Sa femtosecond two-photon laser source.

Biography

Ho Lee has research experience on the development of dermal drug delivery via miniaturized electro-osmotic pumps, to femto-second laser machining, to laser-scanning microscopy applications in dermatological studies. He completed his PhD in Mechanical Engineering from the University of Texas at Austin in 2003. He followed this with a Post-doctoral fellowship at the same university for a year, then another at Harvard Medical School for three years. Since 2006, he has been a Professor at Kyungpook National University in the Mechanical Engineering Department. During his tenure, he also spent an additional year on sabbatical at Harvard Medical School. Currently, he is an Associated Professor and Director of a research laboratory that specializes in bio-optical applications, laser machining and micro-welding, and, more recently, laser-based 3D printing.

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