Multiphoton imaging of ultrashort pulse laser ablation in the intracellular parasite Theileria -
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Theileria annulata is an intracellular parasite that infects and transforms bovine leukocytes, inducing continuous proliferation of its host cell both in vivo and in vitro. Theileria-infected cells can easily be propagated in the laboratory and serve as a good model for laser ablation studies. Using single pulses from an amplified ultrashort pulse laser system, we developed a technique to introduce submicrometer holes in the plasma membrane of the intracellular schizont stage of Theileria annulata. This was achieved without compromising either the viability of the organisms or that of the host cell that harbors the parasite in its cytoplasm. Multiphoton microscopy was used to generate image stacks of the parasite before and after ablation. The high axial resolution allowed precise selection of the region of the membrane that was ablated. It also allowed observation of the size of the holes generated (in fixed, stained cells) and determination of the structural changes in the parasite resulting from the laser pulses (in living cells in vitro). This technique opens a new possibility for the transfection of Theileria or delivery of molecules to the schizont that may prove useful in the study of this special host-parasite relationship. © 2008 Society of Photo-Optical Instrumentation Engineers.