**Dual Mode Reflectance and Fluorescence Confocal Laser Scanning Microscopy for In Vivo Imaging Melanoma Progression in Murine Skin**


**ABSTRACT**

A system was designed and developed for simultaneous fluorescence and reflectance contrast in vivo confocal imaging of murine skin using 488 nm (fluorescence mode) and 830 nm (reflectance mode) laser light sources. B16 melanoma cells and B16-enhanced green fluorescent protein (EGFP) cells were inoculated intradermally into transgenic C57BL/6-TgN (ACTbEGFP) 10sb and non-transgenic C57BL/6 mice, respectively. The inoculation sites were imaged sequentially over a 20 d period. The in vivo confocal images were correlated with ex vivo conventional microscopy. The combined modality system provided single-cell resolution and adequate image registration. In fluorescence mode, B16 melanoma cells appeared as dark objects in the bright background of the GFP expressing murine cells of the C57BL/6 transgenic mouse, and the B16-EGFP melanoma cells had a bright signal within a dark background in C57BL/6 mice. In the C57BL/6 transgenic mouse, a population of fluorescent dendritic cells was observed in the vicinity of the tumor cells. The reflectance images provide a useful reference for those areas in the dermal tissues lacking a fluorescent signal. Combined reflectance/fluorescence in vivo confocal laser scanning microscopy holds significant promise for studies of tumor progression in murine skin.