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Simultaneous two-photon fluorescence and reflected light confocal imaging of in vivo human skin

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Journal

BIOPHYSICAL JOURNAL, 74(2)

ISSN

0006-3495

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Publication Date

1998

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Peer reviewed

Barry R Masters, William W Mantulin, Enrico Gratton, and Peter T C So.

Simultaneous two-photon fluorescence and reflected light confocal imaging of in vivo human skin.

32nd Annual Meeting of the Biophysical Society, Kansas City, Missouri, 1998.

Biophys J. 1998; 74(2 Pt 2): A185, Tu-Pos229.

Abstract

In vivo human skin has been imaged with both two-photon reflected light (Raijadhyaaksha et. al., 1995, *J. Investigative Dermatology*, 6, 946-954; Masters et. al., 1997, *J. Microscopy*, 185, 329-338), and two-photon fluorescence microscopy (Masters et. al., 1997, *Biophys. J.*, 72, 2405-2412). The two forms of microscopy, two-photon fluorescence and confocal reflected light imaging, provide complementary information. Two-photon fluorescence imaging can be used to probe cellular morphology and functional states in deep tissue. The endogenous chromophore, NAD(P)H, can be relied upon to provide sufficient cytoplasmic contrast to yield information on cell shape. Further, cellular redox activity can be monitored by measuring NAD(P)H concentration. On the other hand, confocal reflected light imaging can detect certain cellular structures invisible to two-photon microscopy. Reflected light confocal microscopy maps the changes in tissue refractive index and no endogenous chromophores are required. This mechanism allows the imaging of cellular membrane boundaries as well as intracellular nucleic structures in skin and corneal systems independent of the tissue's biochemical state. A combined two-photon and confocal microscope has been developed to imaging human skin in vivo. This work is supported by the American Cancer Society, the DuPont Educational Aid Program, and NIH RR03155.