IN VIVO MULTIPHOTON MICROSCOPY USING A HANDHELD SCANNER WITH LATERAL AND AXIAL MOTION COMPENSATION.

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In this work we present a handheld multiphoton fluorescence microscope designed for clinical imaging that incorporates axial motion compensation [1] and lateral image stabilization [2]. Optical coherence tomography (OCT) based range finding is used to track the axial position of the skin surface, and lateral motion compensation is realised by imaging the speckle pattern arising from the OCT beam illuminating the sample. Our system is able to correct lateral sample velocities of up to ~65 μm s^{-1}. Combined with the use of a negative-curvature microstructured optical fibre [3] to deliver tunable ultrafast pulses from a mode-locked laser to the handheld multiphoton scanner without the need of a dispersion compensation unit, this instrument has potential for a range of clinical applications. The system is used to compensate for both lateral and axial motion of the sample when imaging human skin in vivo.

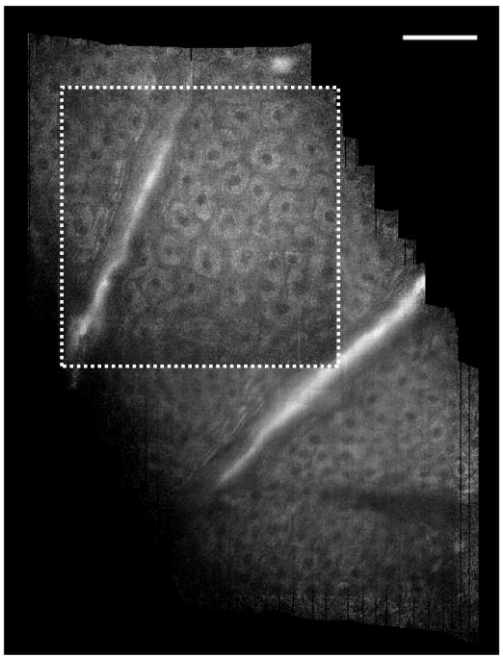

Figure 1. Multiphoton fluorescence imaging of human dorsal forearm skin. During the acquisition the forearm was positioned on a flat surface and the scanner was handheld and slowly sliding across the surface of the skin. Image data were acquired over a period of 15 s, at a depth of approximately 30 μm below the skin surface. The figure consists of the final corrected image (composed of 14 frames), with the region within the white dashed rectangle showing a single frame. All image data was acquired with lateral and axial motion compensation switched on. Scale bar is 50 μm. Figure adapted from [2].

[1] B. Sherlock, S. Warren, J. Stone, M. Neil, C. Paterson, J. Knight, P. French, and C. Dunsby, "Fiber-coupled multiphoton microscope with adaptive motion compensation," *Biomed. Opt. Express* **6**, 1876 (2015).

[2] B. Sherlock, S. C. Warren, Y. Alexandrov, F. Yu, J. M. Stone, J. C. Knight, M. A. A. Neil, C. Peterson, P. M. W. French, and C. W. Dunsby, "In vivo multiphoton microscopy using a handheld scanner with lateral and axial motion compensation," *J. Biophotonics* **11**, e201700131, (2018).

[3] B. Sherlock, F. Yu, J. Stone, S. Warren, C. Paterson, M. A. A. Neil, P. M. W. French, J. Knight, and C. Dunsby, "Tunable fibre-coupled multiphoton microscopy with a negative curvature fibre," *J. Biophotonics*, **9**, 715-720 (2016).