Compact clinical high-NA multiphoton endoscopy

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ABSTRACT

Multiphoton imaging methods are excellent for non-invasive imaging of living tissue without any need of additional contrast agents. The increasing demand for endoscopic techniques has forced the development of multiphoton endoscopes for imaging of areas with reduced accessibility like chronic wounds. Gradient index (GRIN) lenses can miniaturize the bulky distal focusing optics of conventional tomographs to a diameter of less than 1.4 mm and a numerical aperture (NA) of 0.8.

We combined a high NA clinical multiphoton endoscope with existing multiphoton tomographs like the DermaInspect® and the MPTflex® to enable the examination of wound healing processes.

Keywords: clinical multiphoton endoscopy, multiphoton tomography, scanning microscopy, nonlinear microendoscopy, clinical imaging, two-photon, small animal research, microendoscopy, molecular imaging

1 INTRODUCTION

High-resolution *in vivo* multiphoton tomography[1,2] has become a promising method for multiple applications. In dermatology this method allows for melanoma detection[3], the diagnostics of dermatological disorders or tissue engineering[4] . Especially significant is the increasing interest of the pharmaceutical and cosmetic industry for basic research[5], skin ageing measurements[6] as well as *in situ* drug monitoring[7] using *in vivo* multiphoton tomography.

Multiphoton imaging is achieved by focusing femtosecond laser radiation inside the specimen. Intrinsic fluorophores, such as elastin, melanin, flavines and reduced nicotinamide adenine dinucleotide (NADH)[2], are naturally part of the skin and can be used as biomarkers. The excitation of these molecules (with different excitation wavelengths) reveals the morphological structure of the skin and enables to distinguish between different kinds of fluorophores[8]. In addition to multiphoton excited fluorescence, second harmonic generation (SHG) can be induced by the excitation light interaction with the dermal collagen network[9]. The most important advantage of multiphoton imaging, is the ability to provide superior optical sectioning at depths of up to 200 µm [2]. There is no significant out-of-focus photostress and bleaching.

Studies for in vivo non-invasive imaging of human skin, especially skin cancer investigations[3], in vivo drug screening of cosmetic and pharmaceutical compounds[5], and diffusion of nanoparticles into the skin[10] have been carried out.

However the focusing optics of clinical high resolution multiphoton tomographs like the DermaInspect® or MPT*flex*®

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(JenLab GmbH) offers only limited accessibility from the outside of a specimen. The accessibility of hard-to-reach areas like wounds can be improved by the clinical multiphoton endoscopy which however may reduce the image quality[10]. Within this article a compromise between high resolution state-of-the-art imaging techniques and increased accessibility has been achieved by combining multiphoton endoscopy based on gradient index (GRIN) lenses with CE-marked clinical tomographs like the DermaInspect® and MPT*flex*®.

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2 EXPERIMENTAL DESCRIPTION AND SYSTEM SETUP

Multiphoton endoscopy is performed by the combination of a miniaturized rigid high-NA GRIN-lens with the clinical multiphoton tomograph MPT*flex*® (Fig. 1 a).

The tomograph contains a compact turn-key titanium:sapphire femtosecond excitation source (MaiTai XF-1, Spectra Physics) which is tunable in the wavelength range of (710 - 920) nm and generates < 100 fs pulses (~ 300 fs at specimen position) with a repetition rate of 80 MHz. Within the tomograph an optical attenuator regulates the output-power of the laser, a beam stabilization device corrects for internal misalignments of the laser beam and a safety unit prevents tissue damage within the specimen.

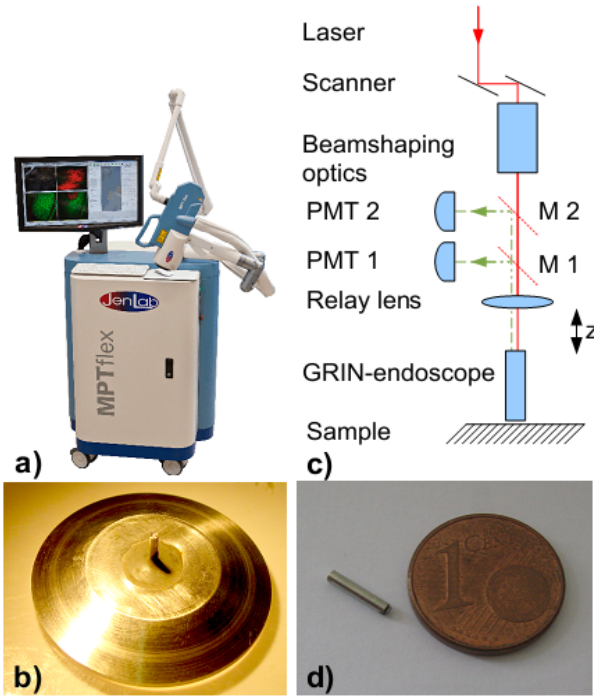

Figure 1: a) MPTflex® multiphoton tomograph; b) microendoscope with adaptor-plate; c) schematic setup of scan-detector head;PMT 1,2: photo multiplier modules; M1,2 dichroic mirrors ; d) single endoscope in comparison.

A flexible articulated mirror-arm, optimized for near infrared (NIR) transmission, guides the laser beam to the probe head. Within the probe head the laser passes a fast scanning device for 2D (XY) scanning and beam shaping optics for beam expansion (fig. 1 c).

The expanded beam is directed to a 5x 0.25 NA focusing relay lens which is required for maximum optical coupling efficiency (NA adaptation) into the high-NA focusing GRIN-endoscope (NA = 0.8; diameter = 1.4 mm; length = 7 mm) (fig. 1 b and c). The working distance of the microendoscope allows performing two-dimensional scans up to a tissue depth of approximately 200 µm.

To enable optical sectioning, the *z-position* of the focusing optics can be adjusted manually by changing the distance

between the entrance surface of the endoscope and the 5x 0.25 NA relay lens (see fig. 1 c).

Autofluorescence- and SHG- signals that are generated inside the specimen and collected with the microendoscope are deflected by dichroic beamsplitters onto a large active area dual-channel single photon detection device PMT-JL-SPC (JenLab GmbH, Jena, Germany). The detection bands of the two spatially separated detectors are 390-400 nm and 409-680 nm (FWHM) to separate SHG- from autofluorescence-signals, respectively. Such a dual-channel detector arrangement enables for instance to distinguish between intratissue elastic fibers and collagenous structures simultaneously. A color-glass filter (BG 39, Schott, Mainz) blocks reflected light of the excitation laser.

The dual channel detection device is supported by a controller box (JenLab GmbH, Jena), which additionally controls the 2D-scanning process and the image generation. The intensity per pixel is stored as 16-bit value and processed by the JenLab control software.

The overall field-of-view of the optical system covers 130 x 130 μm^2. For the *in vivo* measurements which are presented here a maximum power of 35 mW was used.

3 RESULTS AND DISCUSSION

The lateral and axial resolutions were determined by measuring the point spread functions (PSF) of 0.2 µm microspheres (Flouresbrite™ plain YG , Polyscience, Inc., Warrington). For the measurement of lateral resolution fixed microspheres were imaged with a mean power of 2 mW and a laser wavelength of 785 nm (fig. 2 a and b). The lateral resolution determined by the full width at half maximum (FWHM) of the measured intensity distribution was 0.87 µm (PSF in figure 2 c).

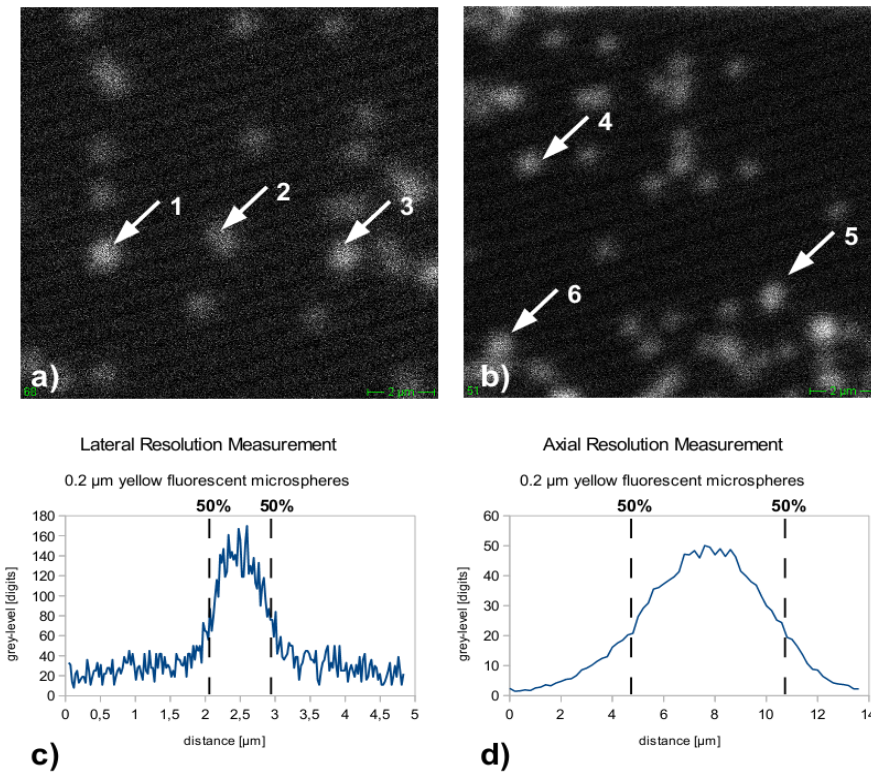

Figure 2: a) and b) Measurement of the point spread function of 0.2 µm spheres; c) Lateral point spread function; d) Axial point spread function.

To determine the axial resolution optical z-sectioning of microspheres with steps of 0.2 µm has been performed. Axial displacement has been realized with a piezo-driven actuator (MIPOS 500, Piezosystems, Jena). The axial resolution at FWHM of the axial intensity distribution was measured to be 5.5 µm (PSF in figure 2 d).

In vivo Measurements

High resolution *in vivo* measurements on human skin (forearm) were performed with a maximum laser power of 35 mW and 760 nm excitation wavelength. To match the indices of refraction of the skin and of the microendoscope material water was used in between.

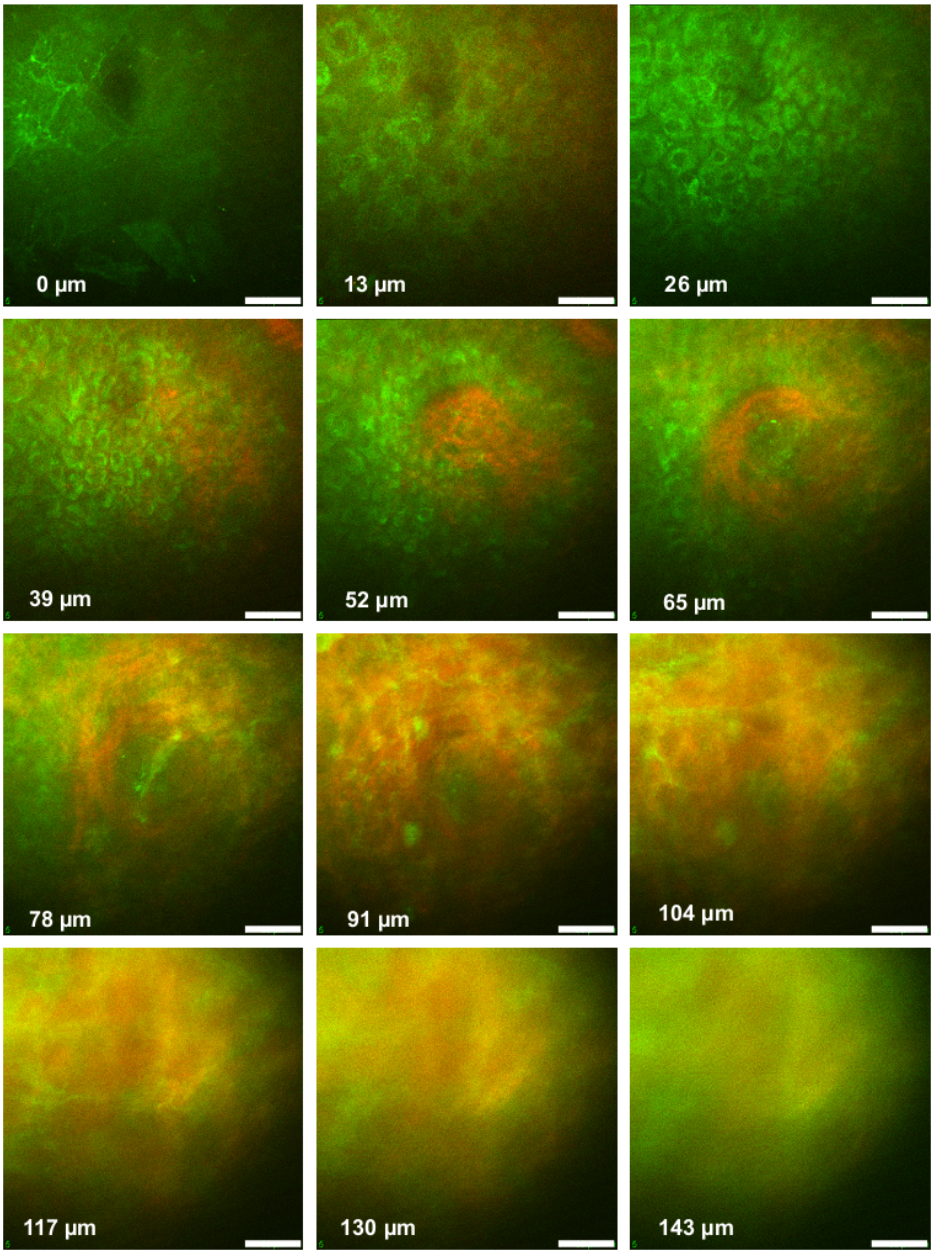

Figure 3: In vivo optical sectioning on human skin (P_{max} =35 mW at 760 nm; micron bar = 30 µm). The numbers indicate the imaging depth inside the skin.

Figure 3 shows a stack of 12 optical sections at different z-positions with steps of 13 µm. Each optical section (color online) is an overlay of intensity signals from two spectral detection channels which were simultaneously acquired.

The stack of optical sections demonstrates that high contrast features can be imaged up to a depth of about 117 µm. Within the first three sections (0 µm – 26 µm) most of the image contrast stems from autofluorescence signals. All sections deeper than 26 µm reveal an additional contrast which is caused by SHG-signals coming from the laser light interaction with collageneous structures inside the skin. For clarification of the dual-channel imaging mode the images of

fig. 3 at depths of 65 µm and 91 µm are illustrated in fig. 4 as two simultaneously acquired images showing autofluorescence- and SHG-signals separated from each other.

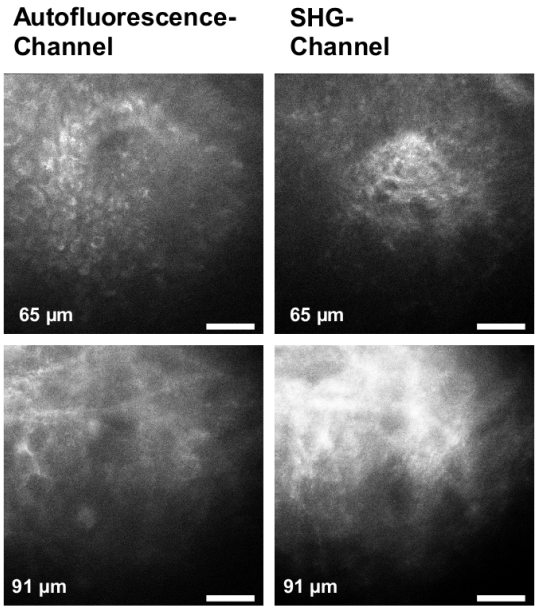

Figure 4: Illustration of dual channel image acquisition. Autofluorescence-images left and SHG-images right (micron bar = 30 µm).

Further studies will show if increasing the excitation laser power up to 50 mW can positively influence image quality of deeper regions. Especially for intra-corporal measurements the image quality may be increased, due to the lack of scattering in the stratum corneum. Within the shown *in vivo* images movement-artefacts are hardly visible.

4 CONCLUSION

This article summerized the capability for two-photon high-resolution imaging of human skin based on the combination of microendoscopic focusing optics and commercially available medical certified tomographs.

Especially useful is the application of these multiphoton endoscopes in combination with the tomograph MPTflex® and its articulated mechanical arm. The arm provides stabilized endoscopic diagnostics with the advantage of an increased flexibility and accessibility especially for clinical and cosmetic examinations.

The presented *in vivo* images on human skin show that high-resolution images can be acquired from depths of significantly more than 100 µm. The lateral resolution of 0.87 µm of these miniaturized endoscopes (NA 0.8) is a compromise between the high resolution of bulky focusing optics (NA 1.3; resolution < 0.36 µm) and its in vivo accessibility.

Such a compromise simplifies the multifunctional application of multiphoton imaging on the detection of human skin diseases or animal research. Especially promising seems the introduction of these endoscopes in clinics for wound healing investigations and studies on Ulcus cruris.

5 ACKNOWLEDGEMENT

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