Multiphoton Microscopy (MPM) as an Upgrade for the TissueSurgeon

Emanuel Särchen, Fabian Will, Diego Ramírez, ROWIAK GmbH, Hanover

Multiphoton or nonlinear microscopy is a well known technique to perform deep tissue imaging with high contrast and high resolution. Low bleaching effects occur and less or no photo damage is induced compared to other techniques.

We use an infrared ultrafast laser emitting short pulses at 1030 nm wavelength (the same laser as for cutting). Infrared laser light is less scattered in tissue compared to UV- or VIS- laser light. Therefore deep imaging is possible.

Imaging is achieved by exciting capable fluorescent dyes, autofluorescence, second harmonic generation (SHG) or third harmonic generation (THG) of the incident laser wavelength. The laser light is focused to a small spot using an objective lens with a high numerical aperture but long working distance (400 µm with 1 mm microscope slide, up to 1 mm using thinner slides). Only in the center of the focal volume, the light intensity is high enough to generate fluorescence signals by two or three photon absorption and SHG or THG signals. Therefore, the excited volume can be smaller than the focal size (Fig. 1). That leads to high resolution in the micrometer or sub micrometer range. Hence, the emitted SHG or THG light emits in wavelength of 515 nm or 343 nm. We use chromatic filters to separate the different signals.

The MPM can easily be adapted in the TissueSurgeon without replacement of the key components by changing of some optics, adding a proper detection system and integrating the data acquisition and display in the main software. The same laser, scanner and optical path will be used. MPM imaging can also be used for defining regions of interest to cut with the TissueSurgeon.

First measurements at porcine cornea revealed strong SHG signals from the corneal stroma (Fig. 2) and THG signals from epithelial and endothelial cells (Fig. 3). Furthermore, SHG signals from collagen in bone and teeth could be detected properly (Fig. 4). SHG-Imaging of necrotic lung specimen differentiated healthy alveoli from necrotic area (Fig. 5). Existing TissueSurgeon systems can be equipped with MPM module, too.

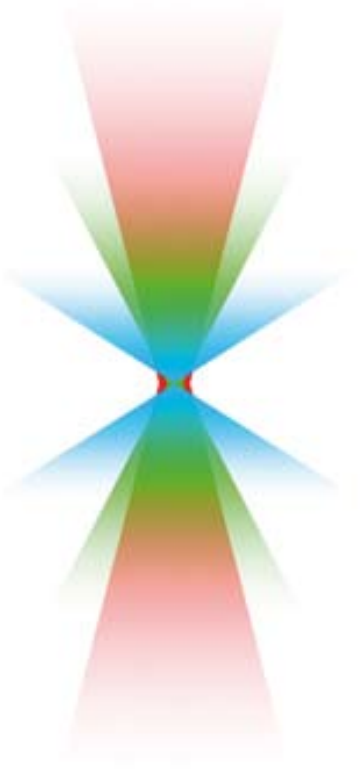

Fig. 1: Excitation with 1030 nm (red), SHG (green) and THG (blue) emission.

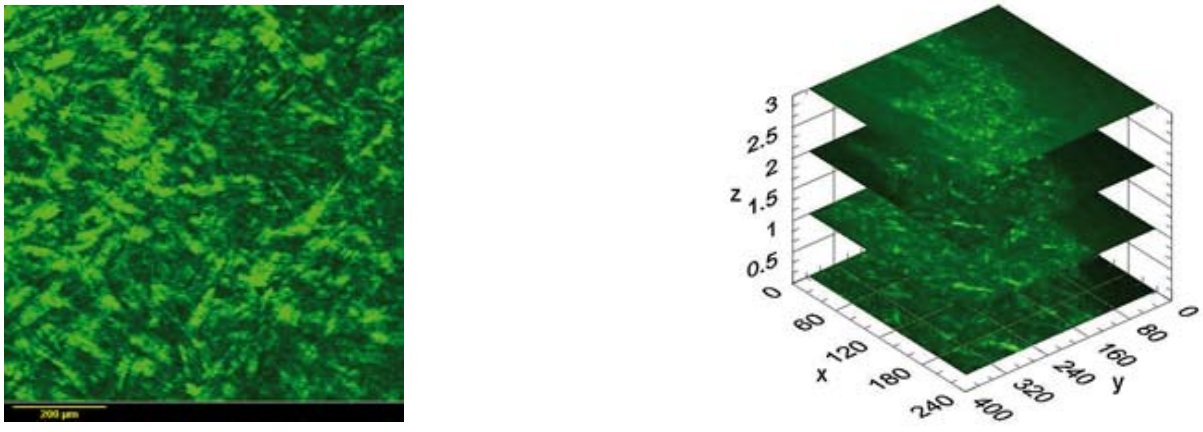

Fig. 2: SHG signals from corneal stroma, fibrillar collagen can be seen, right: z-scan of 4 layers in the stroma with 5 µm distance, picture size: 370 x 200 pixels correspond to 1000 x 850 µm. Maximum pixel number is 500 x 1600 pixels at present.

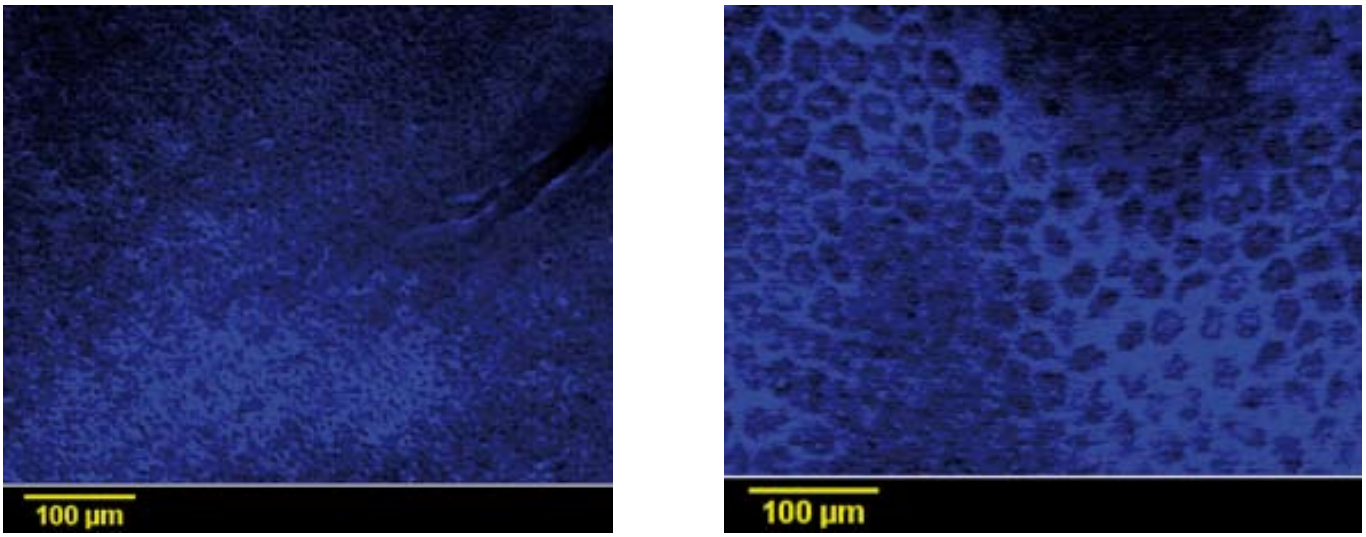

Fig. 3: THG signals from cell membranes of epithelial (left) and endothelial cells (right) of porcine cornea.

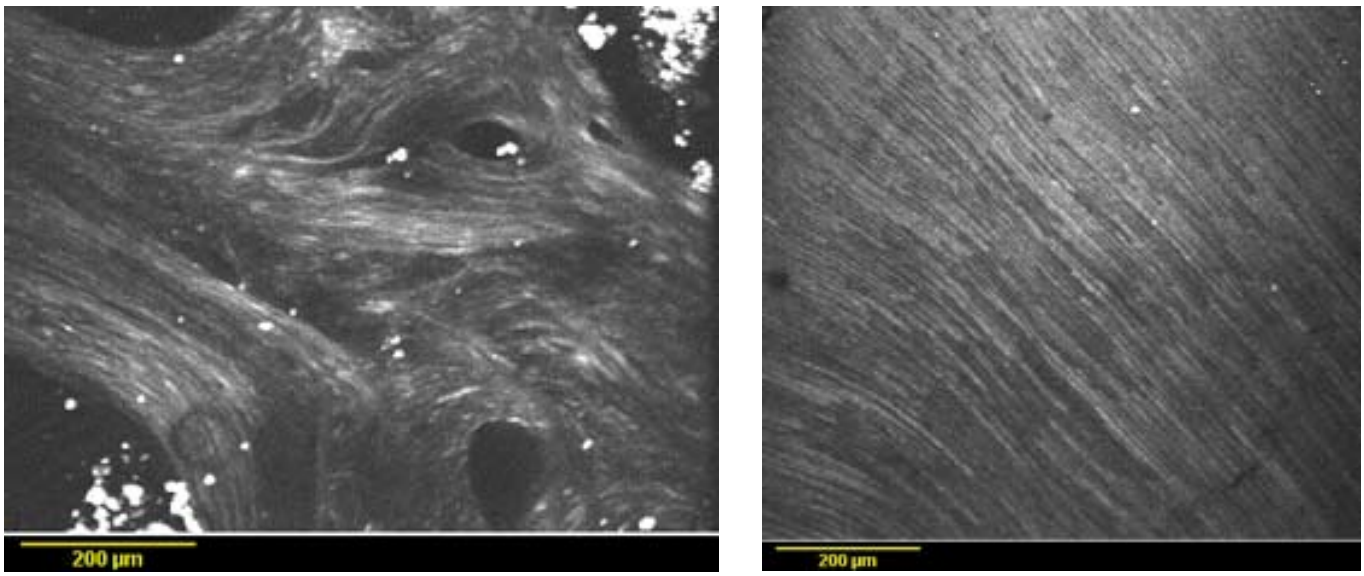

Fig. 4: SHG signals from collagen in porcine bone (left) and dentin of deer teeth (right).

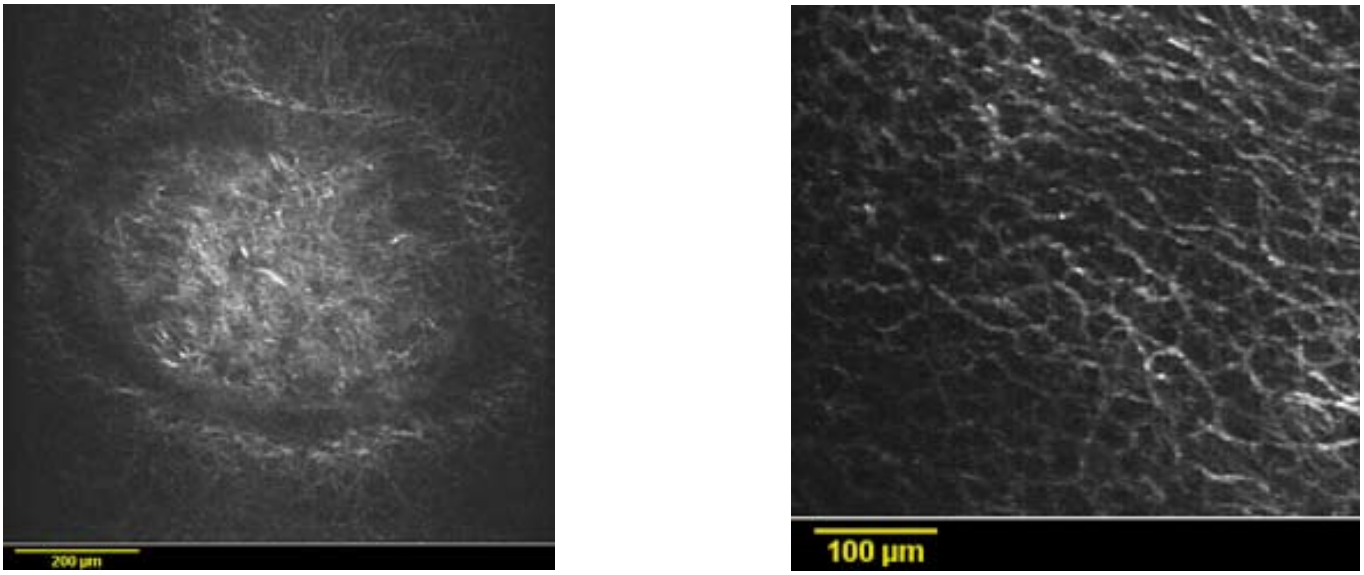

Fig. 5: SHG signals of necrotic lung specimen, left: Necrotic area in the center of the image is surrounded by healthy alveoli structures, right: SHG from collagen in lung reveals alveoli.