Project title: New second harmonic generation modalities in biological samples to separate between collagen and myosin

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Current state of the art

Myosin moves the muscles of eukaryotic organisms and is a tiny molecular motor [1]. It generates force and performs mechanical work by consuming adenosine triphosphate (ATP). As linear motors, it enables movement via the rail-like actin filaments or microtubules of the cytoskeleton inside a living cell. In this way, subcellular structures, but also larger units such as cells or organisms, can move in a directed manner [1, 2]. Using genetic engineering approaches, it has already been possible to produce a backward-moving myosin nanomotor [3]. Methods such as X-ray structure analysis and kinetic investigations serve to further elucidate the self-organization of ordered nanostructures from motor proteins, which are of technological interest. For molecular medicine, it is also important to understand structural relationships between the molecular linear motor and stabilizing structures in tissues.

Skeletal muscle consists of elongated fiber cells with myofibrils arranged in parallel along their entire length [1]. Myofibrils contain longitudinal sarcomeres whose high order of actin myofilaments and myosin proteins enable contraction. The well-known transverse striation of skeletal muscles results from the parallel arrangement of myofibrils in the muscle fiber (Figure 1). Several muscle fibers work together in bundles in the same direction. These are organized by structural proteins of the extracellular matrix, especially collagen fibers.

From the large and heterogeneous group of the collagen family, mostly fibrillar collagens are found. Specific microscopic imaging of both collagen and myosin is possible due to the non-centrosymmetric structures [4, 5, 6, 7, 8]. The use of ultrashort pulses of focused laser radiation results in transient high-power density and second order frequency doubling (second harmonic generation, SHG) [7, 8]. By using excitation wavelengths in the near infrared range, the second harmonics penetrate deep into the tissue and the muscle tissue can be mapped non-destructively in three dimensions (Figure 2). SHG polarimetry can be used to distinguish between myosin and collagen and furthermore the orientation of the collagen fibers [7, 8, 9]. Further contrast information can be obtained by evaluating forward to backward signal.

So far, there are hardly any approaches to modulate the wavelength for the generation of SHG to distinguish between myosin and collagen fibers [8, 9]. Yet this variation could have great potential. However, some contradictory results call for multimodal studies with evaluation of the spectroscopic information. Neither the assumption of complete Kleinman-symmetry nor the monotonic decrease of SHG efficiency with wavelength have been proven so far for second harmonics in biological samples. Rather, recent studies indicate a complex behavior, more pronounced with the backward-SHG signal rather than the forward one [8, 9].

Research goals and working program

To visualise collagen structures in the cornea of the eye, we have achieved the best SHG signals using a higher wavelength (1040 nm) than previously used (860 nm) [10, 11, 12]. The observed high resolution of structural differences cannot only be explained by the greater penetration depth into the tissue of the higher wavelength. Differences in the organisation of the collagen triple helix in the tissue layers of the cornea of the eye seem to be the cause [10]. In general, collagen fibres differ from myosin in muscles by their larger diameter (Figure 1). First analyses with the MPE-RS microscope (EVIDENT/Olympus) in combination with the Insight (Spectra Physics) laser of prepared skeletal muscles of the mouse show a separation possibility of both proteins in the SHG signal established for the eye at 1040 nm (Figure 2).

The aim of this project is to investigate the second order frequency doubling in biological samples when the excitation wavelength is changed. In particular, (1) complete spectral information of collagen and myosin SHG signal will be obtained from artificially produced samples. (2) In addition, the dependence of the signal intensity on the ion composition and concentration of the analysis solution is to be determined. (3) Step by

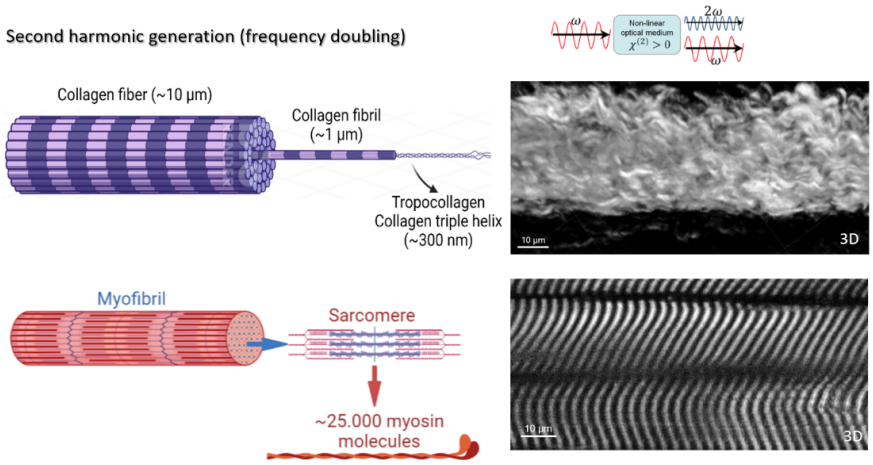

Figure 1: Second-harmonic generation (SHG, frequency doubling) in fibril-forming collagen (top) and myosin (bottom) (Schematic view created in BioRender.com).

step, the analysis will then be transferred to tissue samples of different thickness of the murine skeletal muscle. (4) Finally, the results will be verified using nonlinear microscopes other than the MPE-RS system.

(1) Collagen hydrogels and synthetically produced myosin are used to achieve stable sample quality. The efficiency and quality of the SHG signal is determined by stepwise varying the wavelength from 700 to 1200 nm. [Month 01-09 / Rostock]

(2) The SHG signal is not only influenced by the tissue properties, but also by the embedding medium used. Therefore, the influence of different solutions on the efficiency and quality of the SHG signal will be examined with the help of the in vitro samples. [Month 09-12 /Rostock]

(3) Mouse skeletal muscle preparations are prepared in different layer thicknesses, so that either only myosin or myosin and collagen are included. The preparations are examined either natively or after treatment in aqueous stabilisation media. [Month 12-30 /Rostock]

(4) The key results obtained on the MPE-RS system will be verified and validated on another nonlinear microscopy setup of the Canadian partner. [Month 31-36 /Canada]

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