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Session Title: Platform AZ: Muscle Mechanics & Ultrastructure

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Abstract Title: Quantitative Analysis of Function and Structure of Mouse Skeletal Muscle, *in vivo*, using Multi-Photon Excitation Microscopy

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We have adapted an inverted 2-photon excitation Zeiss 510 system to perform micron scale microscopic studies on an *in vivo* mouse muscle preparation that permits control of length and muscle excitation while following force generation. A periscope was added that extends the objective from the stage and creates degrees of freedom to couple to the muscle. A kHz piezo-electric objective focusing unit was used to provide accurate tracking along the Z axis, while a filtered PMT was placed directly above the objective for optimal SNR (constructed by LSM Technologies). The mice were anesthetized and the tibialis anterior (TA) muscle exposed. The muscle was restrained using dual pin system (Aurora Scientific) pinching the bone above the TA while either the foot or tendon was directly attached to a force transducer (Aurora Scientific). The objective (10x or 20x) was coupled to the tissue using a isotonic optical gel with no coverglass. Exogenous dyes ANEPPS-8 (endothelial cells), fluorescein-dextran (RBC velocity) and Syto-Orange (nuclei reference) were used. Using this system, the geometry of the muscle cells and vascular structures could be directly accessed at different muscle lengths or after contractile activity. Within the muscles the sarcomere lengths and mitochondria energy state could be directly accessed for both slow and fast twitch muscle fibers in the same field, along with the regional capillary flow. Using the sarcomere spacing, the in-plane resolution was <2 microns at depth >150 microns. In plane tissue motion correction was performed using an algorithm that accounted for the complex deformation occurring within this spatial scale. This quantitative muscle model coupled to the spatial and temporal resolution of the 2-photon microscope will provide a unique insight into the cell biology of muscle contraction, *in vivo*.

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