Supplemental Document

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Label-free imaging of age-related cardiac structural changes in non-human primates using multiphoton nonlinear microscopy: supplement

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Animal data

Table S1: Data for the common marmoset monkeys (*Callithrix jacchus*) including age, weight, and gender

Age group	Age (Years / months or Days)	Weight (g)	Gender
Old	11y / 0m	306	m
	10y / 8m	332	m
	13y / 3m	336	m
Young Adult	1y / 0m	334	m
	2 y / 11m	468	m
	4y / 4m	560	m
Neonatal	24 days	78	f
	4 days	28.5	m
	6 days	30.5	m

Sample preparation

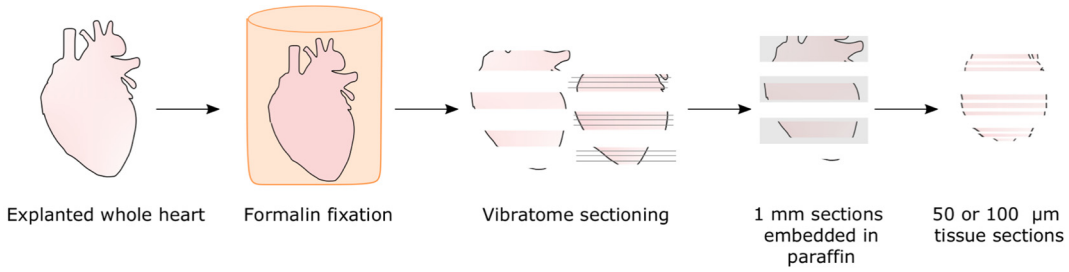


Fig. S1: Schematic for sample preparation. Excised hearts were fixed in formalin and sectioned using vibratome into 1 mm, 50 and 100 µm tissue slices. 1 mm tissue slices were embedded in paraffin and then section into 2 µm sections for histology. 50 and 100 µm sections were used for NLOM imaging.

Selection of the regions of intertest (ROI)

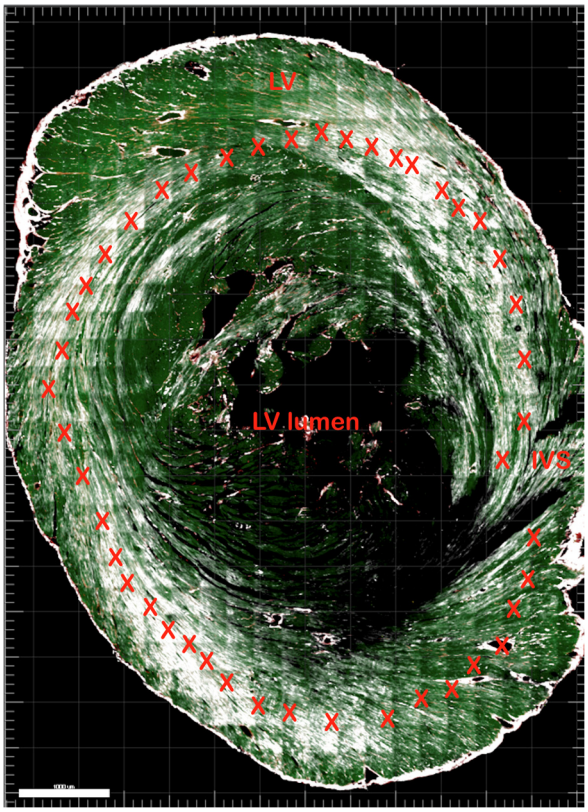


Fig. S2: Image of stitched cardiac tissue section obtained from a young adult heart. The red 'X' marks the ROI selection at the myocardial layer of the left ventricle (LV) and interventricular septum wall (IVS). All regions were selected randomly at the LV and IVS. Scale bar: 1000 µm.

Cardiac morphology in all age groups

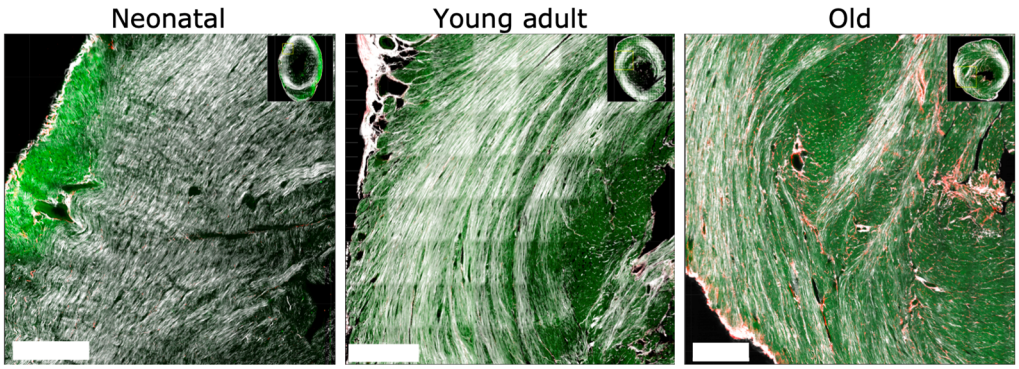
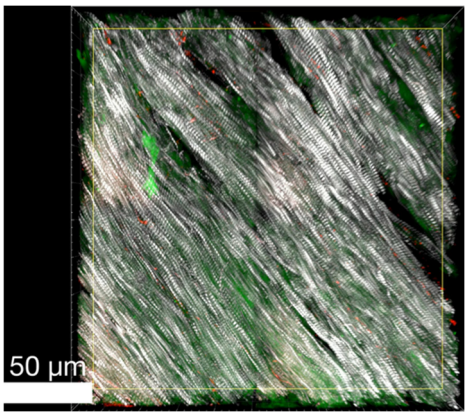
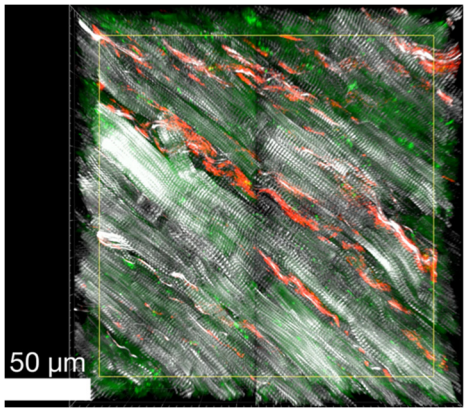


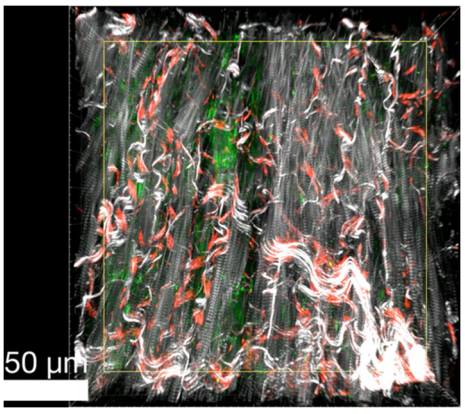
Fig. S3: Overview of NLOM label-free images in different age groups. Representative overview showing cardiac morphology in neonatal, young adult and old hearts for autofluorescence (green), backward SHG (red) and forward SHG (white). Scale bar: 200 µm.



Visualization S1: SHG and TPEF signals showing cardiac structural composition neonatal heart. Supporting data for Figure 3.



Visualization S2: SHG and TPEF signals showing cardiac structural composition young adult heart. Supporting data for Figure 3.



Visualization S3: SHG and TPEF signals showing cardiac structural composition old/geriatric heart. Supporting data for Figure 3.

3D rendering of a myofibril segment

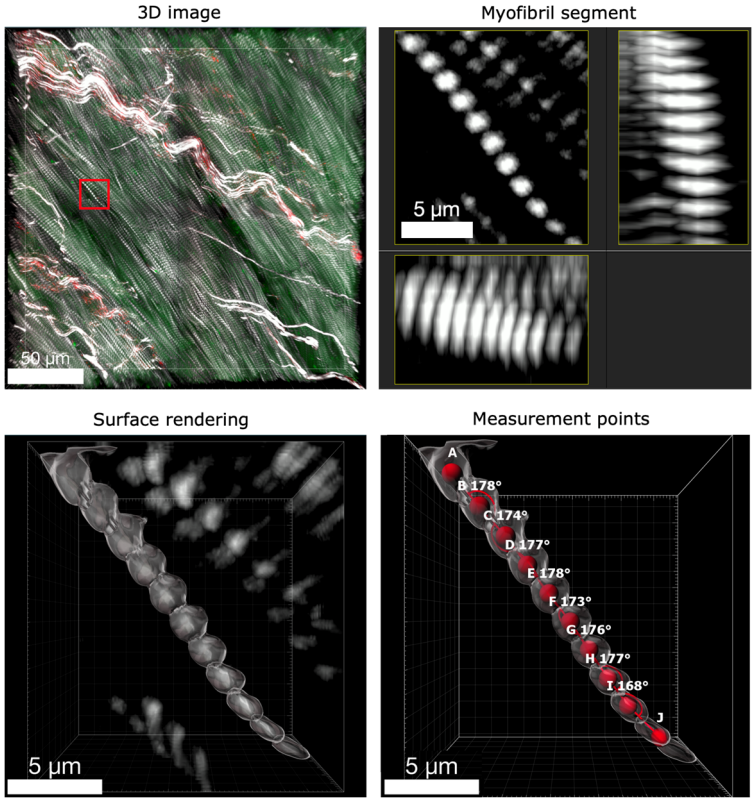


Fig. S4: Workflow for 3D surface rendering and angular distribution analysis along single myofibril segments. A segment of single myofibril was cropped from a 3D volume in xy, yz and xz plane to extract ten SH signal emitting A bands in an adult heart. 3D Surface rendering was performed on the isolated fragment of the myofibril. A measurement point was set at the center of mass for each A band. Angular distribution was determined between the measurement points.