TECHNISCHE UNIVERSITÄT DRESDEN

FAKULTÄT ELEKTROTECHNIK UND INFORMATIONSTECHNIK

MST Professur für Mess- und Sensorsystemtechnik

Faculty of Electrical and Computer Engineering, Laboratory for Measurement and Sensor System Techniques

Closed-Loop Optogenetics with Human Stem Cell-Derived Cardiomyocytes and Neuronal Networks

F. Schmieder[1,2], R. Wendland[1,2], MA. Sikandar[3], W.-H. Zimmermann[3], O. Bergmann[3], R. Habibey[4], V. Busskamp[4], L. Büttner[1,2], and J. Czarske[1,2]

1 Faculty of Electrical Engineering and Information Technology, Laboratory for Measurement and Sensor System Techniques, TU Dresden, 01062 Dresden, Germany

2 Competence Center **BIOLAS**, TUD Dresden University of Technology, Helmholtzstraße 18, 01062 Dresden, Germany

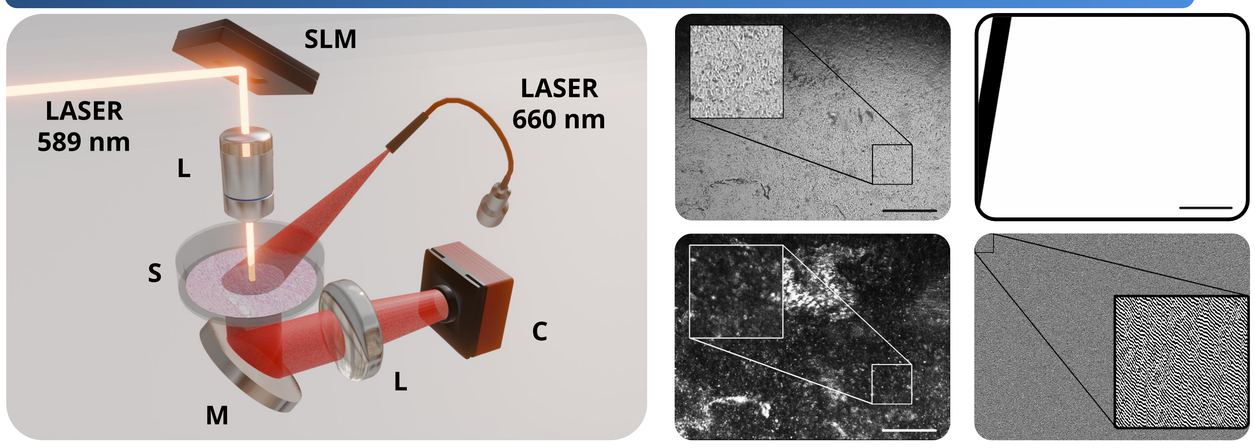
3 Department of Pharmacology and Toxicology, University Medical Center Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany

4 Dep. of Ophthalmology, Universitäts-Augenklinik Bonn, University of Bonn, Ernst-Abbe-Straße 2, D12 53127 Bonn, Germany

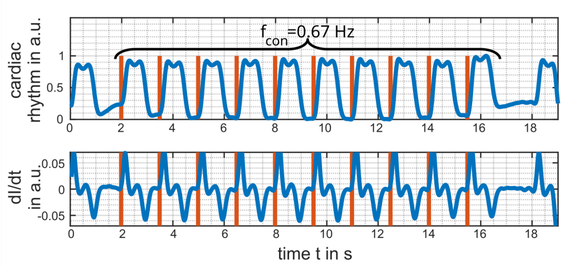
ABSTRACT

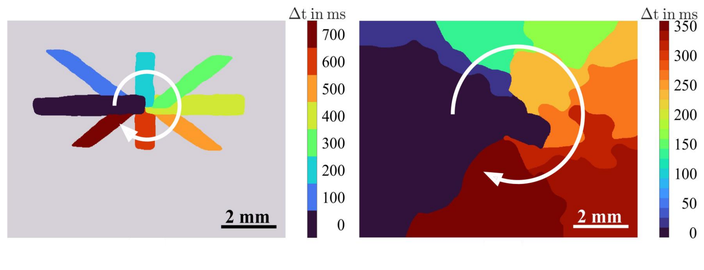
Optogenetics is a powerful tool to investigate and control cell activity on the cellular level. As part of PoL RA5, we have developed a holographic light stimulation platform at the competence center BIOLAS which is capable of addressing single cells with sub-cellular spatial resolution or cell groups simultaneously with arbitrary light patterns. Stimulation can be performed with two wavelengths to both activate and inhibit cell activity concurrently. Here, we present our latest optogenetic in-vitro experiments on human induced pluripotent stem cell-derived cardiomyocytes and neuronal networks. Our work aims on investigating physical disease dynamics on the tissue level in cardiac organoids by combining 3D holographic stimulation with a depth-resolved detection of contraction activity.

LABEL-FREE INVESTIGATION OF CARDIAC TISSUE DYNAMICS



Left: Simplified scheme of the optical setup for **holographic stimulation** (orange path) and **label-free imaging** (red path) of the hiPSC-CMs; components: SLM spatial light modulator, L lens, S sample, M mirror, C camera. Center: Widefield (top) and speckle image (bottom) of the same cardiac monolayer. Right: Stimulation pattern (top, illuminated area visualized in black) and respective hologram (bottom). Scale bars: 2 mm.



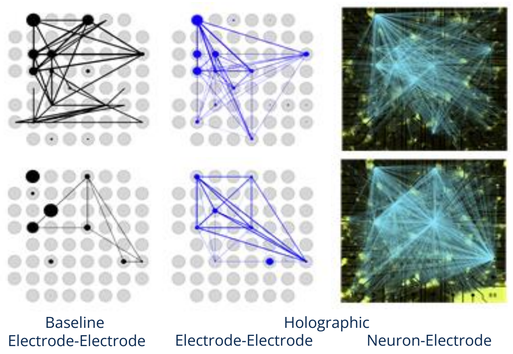


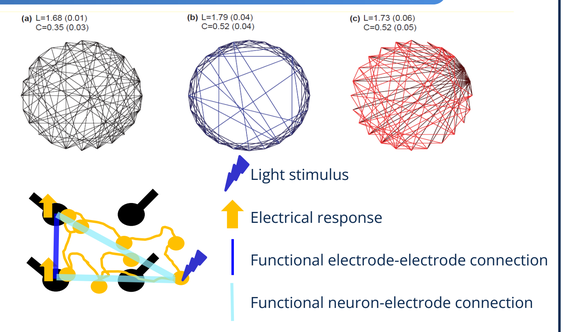
Left: Optical **pacing of hiPSC-CM** cultures (orange lines indicate stimulation times). Middle and right: **Optogenetically induced** rotating **contraction pattern** (middle: spatiotemporal stimulation pattern, right: response of the cardiomyocytes).

HUMAN NEURONAL NETWORK ANALYSIS

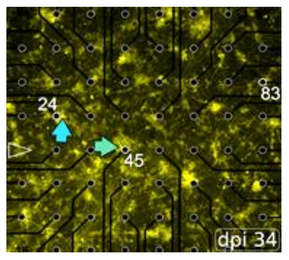
Aim is the extraction of **temporal evolution of connectivity graphs**, e.g. for disease modeling

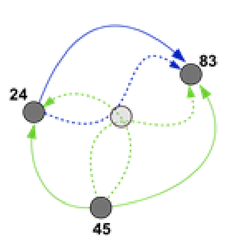
Exemplary maps from different evalutation modes

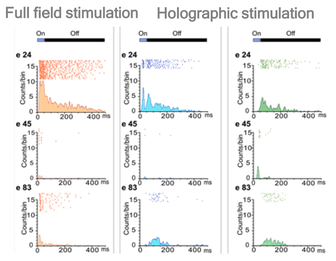




Extraction of functional **connectivity maps from single-cell stimulation trials**. Left: Connectivity from baseline recordings (no stimulation). Center: Electrode-electrode connectivity for comparison with baseline. Right: Neuron-electrode connections.



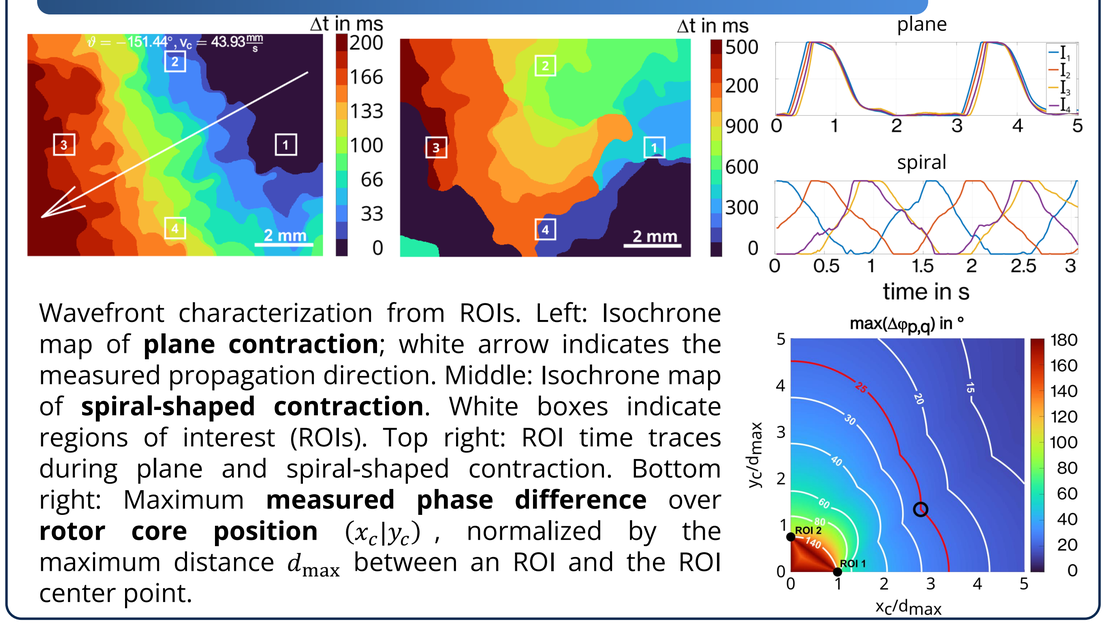




Extraction of connectivity motifs from **Peristimulus Time Histograms (PSTH)**. Full Field stimulation (left) masks individual neuron responses. Single-Neuron stimulation allows discrimination of direct (e.g. e45, right) and indirect responses (e.g. e83, right).

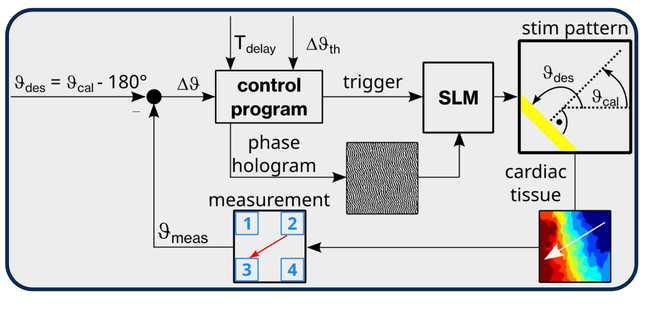
Schmieder, F., Habibey, R., Striebel, J., Büttner, L., Czarske, J. & Busskamp, V. "*Tracking connectivity maps in human stem cell–derived neuronal networks by holographic optogenetics*", Life Science Alliance 5, (2022)

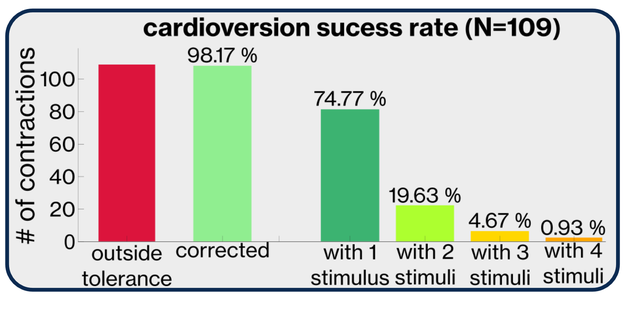
REAL-TIME WAVEFRONT MONITORING



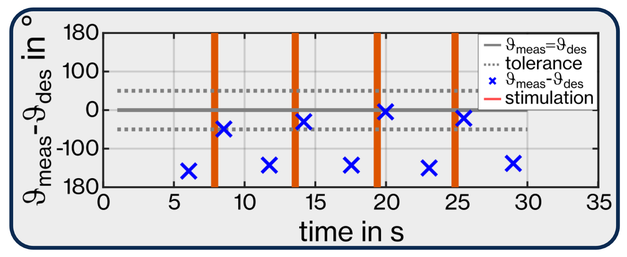
Wavefront characterization from ROIs. Left: Isochrone map of **plane contraction**; white arrow indicates the measured propagation direction. Middle: Isochrone map of **spiral-shaped contraction**. White boxes indicate regions of interest (ROIs). Top right: ROI time traces during plane and spiral-shaped contraction. Bottom right: Maximum **measured phase difference** over **rotor core position** $(x_c|y_c)$, normalized by the maximum distance $d_{\max}$ between an ROI and the ROI center point.

ALL-OPTICAL CLOSED-LOOP CONTROL

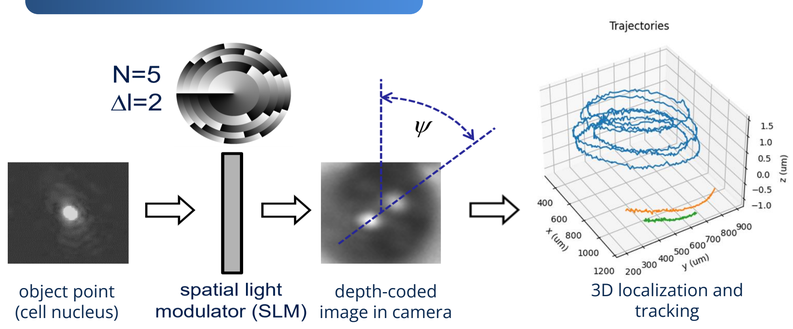




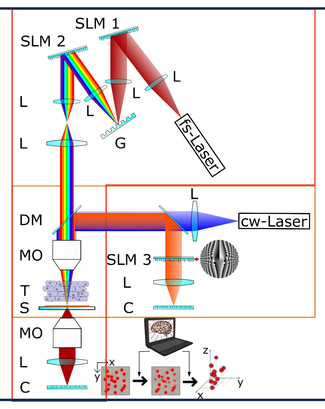
Left: Schematic of the **closed-loop control of contraction wave** propagation. Top right: Number of **successful propagation angle corrections** over all tries and numbers of successful correction after 1,2,3 and 4 stimulations. Bottom Left: Wavefront propagation angle errors during an experiment.



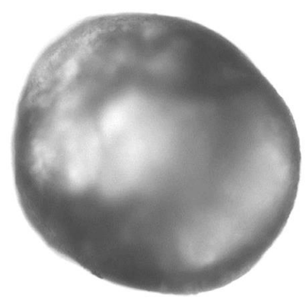
OUR VISION



Depth-resolved analysis of cardiac contraction wave-fronts with high spatiotemporal resolution based on **3D single-shot microscopy** and particle position tracking.



Simultaneous **three-dimensional** two-photon optogenetic cellular **excitation with temporal focusing** and AI-supported three-dimensional particle tracking with engineered point spread functions.



Investigation of **self-organizing cardiac organoids** with perpetual excitation wavefronts using three-dimensional cellular excitation and movement tracking.

PHYSICS OF LIFE

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