TECHNISCHE UNIVERSITÄT DRESDEN
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Optogenetics Research with Human Stem Cell-Derived Cardiomyocytes and Neural Networks

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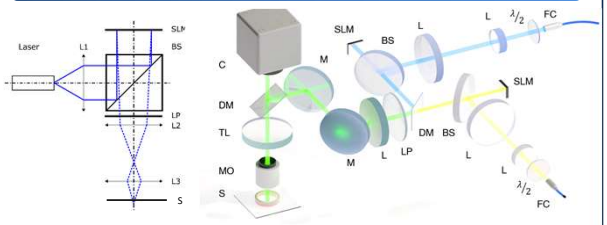
ABSTRACT

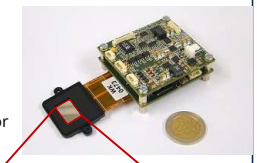
Optogenetics is a powerful tool to investigate and control cell activity on the cellular level. We have developed a holographic light stimulation platform which is capable of addressing single cells with sub-cellular spatial resolution or cell groups with arbitrary light patterns. Stimulation can be 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. Future work will focus on closed-loop control of the light stimulation as well as 3D stimulation and read-out towards all-optical optogenetic experiments on functional organoids.

OPTOGENETIC STIMULATION PLATFORM

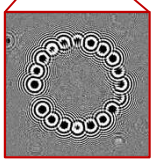


Computer-generated hologram-based **optogenetic stimulation platform**. Left: Schematic setup using Fresnel holograms. Right: Two-wavelength setup for **simultaneous stimulation and inhibition** using Fourier holograms. FC- fiber coupler; $\lambda/2$- half-wave plate; L- lens; BS- non-polarizing beam splitter; SLM- spatial light modulator; DM- dichroic mirror; LP–linear polarizer; M- mirror; TL- tube lens; MO- microscope objective; S- sample; C- camera.

Numerical aperture: $NA = 0.16$
Working distance: $z_w = 31$ mm
Lateral FWHM: $d_T = 8$ µm
Pitch: $\Delta d = 1.4$ µm
Single cell size: ≈ 10 µm
SLM: 4DD ferroelectric LC Modulator
3.1 M Pixel, 8.2 µm pixel pitch

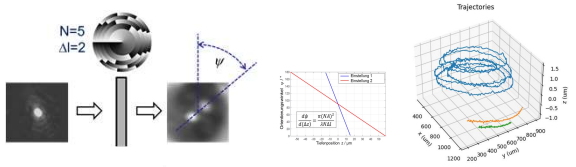


Stimulation pattern



Digital hologram

3D LOCALIZATION MICROSCOPY

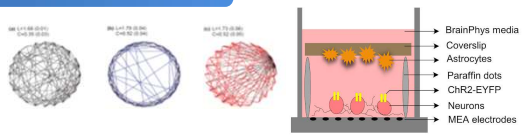


object point (e.g. cell nucleus) | double-helical phase mask | depth-coded image in camera | depth calibration | 3D localization and tracking

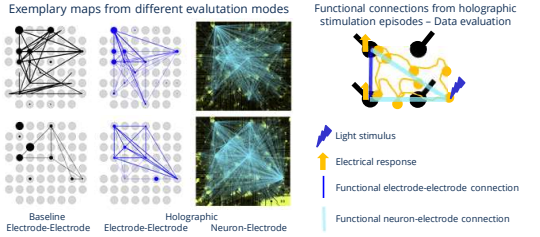
- Single-shot localization of cell nuclei, high frame rate
- Analysis of excitation wavefront in cardiomyocyte samples

HUMAN NEURAL NETWORK ANALYSIS

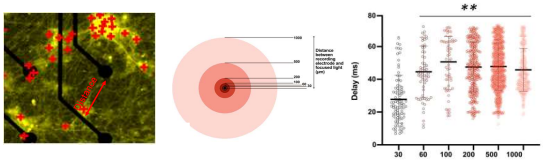
Left: Aim is the extraction of **temporal evolution of connectivity graphs**, e.g. for disease modeling. Right: Banker culture of induced neurons on Multielectrode array supplemented by rat astrocytes



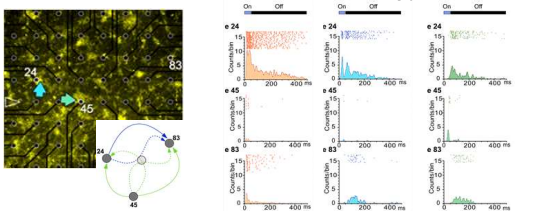
Extraction of functional **connectivity maps from single-cell stimulation trials**. Left: Connectivity from baseline recordings (no stimulation). Center: Electrode-electrode connectivity for comparison with baseline. Right: Neuron-electrode connections.



Stimulus response delay as a function of Euclidean neuron-electrode-distance. Surprisingly, larger distances show no significant differences in response delay ($p<0.01$)



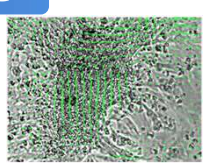
Extraction of connectivity motifs from **Peristimulus Time Histograms (PSTH)**. Full Field stimulation (left) masks individual neuron responses. Single-Neuron stimulation allows discrimination of direct (e.g. e45, right) and indirect responses (e.g. e83, right).

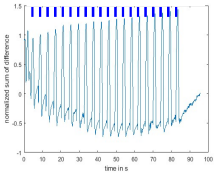


Schmieder, F., Habibey, R., Striebel, J., Büttner, L., Czarske, J. & Busskamp, V. "*Tracking connectivity maps in human stem cell–derived neuronal networks by holographic optogenetics*", Life Science Alliance 5, (2022)

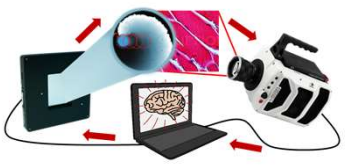
OPTOGENETIC PACEMAKING

Optogenetic pacemaking of a monolayer of ChR2-expressing human induced human cardiomyocytes. Left: Widefield image. Arrows indicate direction of cell deformation. Right: 100 % correlated reaction to 20 stimuli.

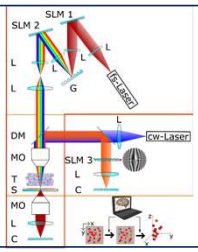




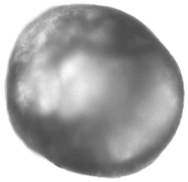
OUR VISION



Closed-loop control of the excitation and termination of cardiac excitation wavefronts with optogenetic pacemaking for disease modeling as well as trial-by-trial investigation of neuronal networks.



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
BIOLAS BIOMEDICAL COMPUTATIONAL LASER SYSTEMS

Collaborations:
- W. Derks, O. Bergmann, Center for Regenerative Therapies Dresden (CRTD), TU Dresden
- R. Habibey,V. Busskamp, Department of Ophthalmology, Medical Faculty, University of Bonn

DRESDEN concept Exzellenz aus Wissenschaft und Kultur