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# Real-time, Parallelized and Non-contact Read-Out of 3D Cardiac Tissue Models

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**Summary:**
We present a read-out technology for cardio high throughput screening of cardiac 3D tissue models. It is based on optical diffraction and allows non-contact, label-free and parallel operation on any 96 well plate formats.

**Keywords:** high throughput screening, cardiac tissue models, spheroid, Speckle, well plates

## Background, Motivation an Objective

Pharmaceutical drug and toxicology screening is facing a paradigm shift today. For decades, compounds were tested on adherent, two-dimensional cell cultures. As endpoint, the cellular response to the respective candidate compound was determined using tailored assays. In future, three-dimensional tissue and organ models will increasingly replace the simple cell cultures [1]. These three dimensional models are far more physiological and render not only cellular functions, but also organ function beyond the cellular level [2]. This opens up the potential, to rule out much more candidates (false leads) from further examination at an early stage, saving time, resources and subsequent animal tests. The most prevalent pharmaceutical screen is evaluating compounds regarding their effect on the cardiac system. To this end, beating cardiomyocyte cell aggregates are exposed to drug candidates and the effect on the beating behavior is recorded. At present, confluent adherent cardiomyocyte cell cultures are grown on multi electrode arrays (MEA)]. But modern three-dimensional cardiac models are not suited to contact methods like MEA. Hence, a sensitive, contact-less read-out technology is needed, capable of parallel operation on multi-wall-plates and preferably automatable.

## Description of the New Method or System

Here we present an optical non-contact and label-free technology based on Speckle diffraction. In contrast to imaging modes, diffraction methods reduce the 3D information to a 2D pattern, eliminating the need for delicate focusing and adjustments. Together with a signal analysis algorithm without any image rendering, the technology meets all requirements for high throughput screening (HTS) of cardiac 3D models [3].

The samples in the 96-well plate are illuminated by 96 laser diodes at a power of < 1 mW. Beam shaping is performed by 12×8-aperture array and a 12×8-lens array. Laser power may be adjusted individually for each well to ensure uniform signal amplitude over the well plate, alternatively to create power gradients or power patterns.

Since diffraction obeys different optical rules as imaging, a camera only may record the speckle pattern of the well on its optical axis. Instead of arranging 96 cameras under the well plate, we use a diffusive screen for Speckle pattern projection, transforming diffraction optic into imaging optics. The projection screen with 96 Speckle patterns may now be recorded by a single video camera. The video image is then segmented to 96 Speckle patterns, which are then analyses pixel-wise before summation:

$$\Delta D(t) = \sum_{n=1}^{N} |D_n(t) - D_n(t-1)| \quad (1)$$

The simple data processing allows to record the ΔD(t) of all 96 wells in parallel at a rate up to 50 Hz using a standard notebook computer.

## Results

The technology was tested using spheroids of cardiomyocytes derived from induces pluripotent stem cells (hiPSC). Such spheroids are very basic heart muscle tissue models, exhibiting cardiac contractions at rates around 1 Hz. They consist of approximately 10.000 cells and are about 400 µm µm in diameter.

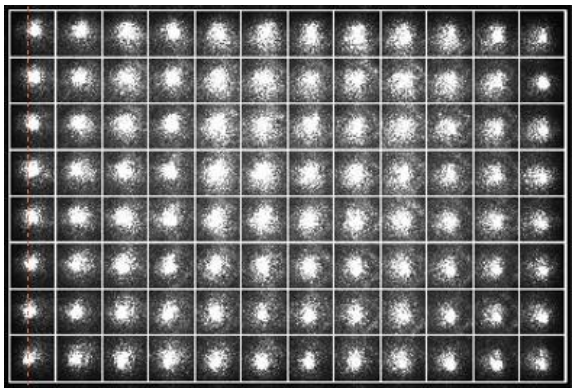

*Fig. 1. 12×8 segmentation of the video signal into 96 individual Speckle patterns. The centered patterns appear brighter due to the angular dependence of diffuser scattering properties.*

As a first result, the camera image displays appropriate Speckle patterns from the entire well plate. Slight dislocations of the well plate do not compromise the dynamic signal. The segmentation of the video signal works well and the contractions of the cardiac tissue models lead reliably to nicely modulated ΔD(t) readings.

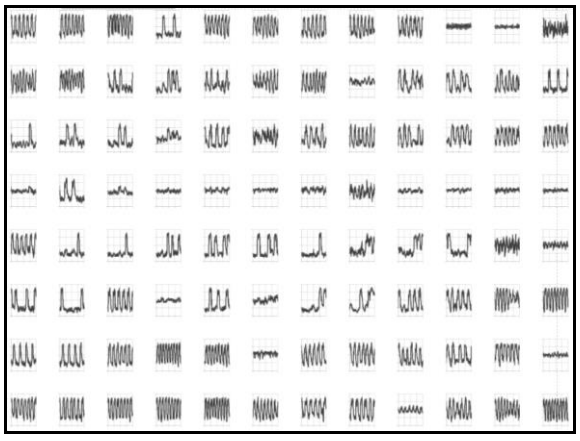

*Fig. 2. Parallel in-line recording of ΔD(t) from all samples of the well plate. Some samples are inactive in this example, the beating frequencies vary significantly between the channels.*

In order to extract the beating frequency of cardiac tissue model we calculate a frequency spectrum using Fast Fourier Transformation (FFT) and fit an error function to the data.

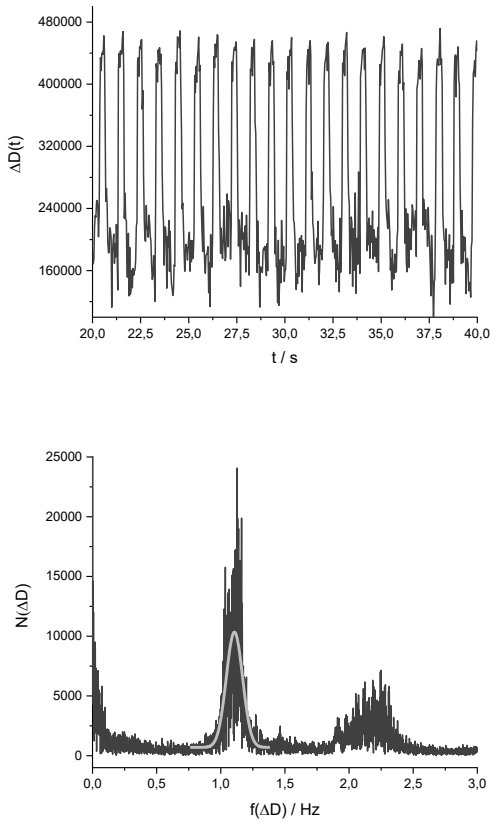


*Fig. 2. A section of a ΔD(t) data set and the respective frequency spectrum.*

The technology proved to be capable of sensitive HTS on modern cardiac 3D tissue models. The parallel operation and the insusceptibility to spatial dislocations, as well as the low requirements to data processing resources make the technology an ideal candidate for automated screening workflows.

## Acknowledgement

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