

# Fully Automated Skeletal Muscle-on-chip Platform for Contraction Analysis

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## Summary:

Lab-on-chip technology enables the in vitro analysis of biological processes with simultaneous monitoring of various physiological aspects. In this context, muscle-on-chip systems allow for the study not only of classical parameters but also of contractile function, thanks to the integration of electrodes for electrical stimulation that simulate nerve impulses. In this work, we present an automated platform based on time-lapse microscopy, which enables an in-depth analysis of contraction of hiPSC-derived myotubes, using machine learning algorithms for the extraction of fundamental contraction parameters.

**Keywords:** Lab-on-chip, Skeletal Muscle Contraction, Time-Lapse Microscopy, Machine Learning

## Background, Motivation and Objective

In recent years, biomedical research has shifted toward advanced in vitro models that more accurately reproduce human pathophysiology, overcoming the limitations of animal models. In this context, the transition from conventional cultures to more complex systems has improved the structural representation of tissues, highlighting the need to integrate dynamic functionalities and continuous monitoring systems. Lab-on-chip platforms represent a promising solution, combining microfluidics, sensors, and data-acquisition systems into miniaturized devices that enable precise, real-time analysis with reduced resource consumption. The integration of time-lapse microscopy (TLM) techniques and advanced data analysis algorithms also allows for the extraction of complex quantitative information from dynamic biological systems. Among the various applications, muscle-on-chip systems play a key role, enabling not only structural but also functional analysis of muscle tissue through electrical stimulation and contraction monitoring [1]. These systems enable quantitative study of physiological and pathological responses, offering new perspectives for disease modeling, drug testing, and the development of personalized medicine. Currently, most advanced skeletal muscle models rely on three-dimensional systems, where cells are embedded within a matrix and form contractile tissues anchored to flexible pillars. The deflection of these structures, combined with their known

mechanical properties, allows for the estimation of contractile force. While these models provide a physiologically relevant environment, force alone represents a limited descriptor of muscle functionality. In this work, we propose an automated platform based on a two-dimensional muscle-on-chip system integrated with TLM and machine learning algorithms. Particle image velocimetry [2] (PIV) based algorithms are employed to extract key parameters of contractile dynamics, enabling a more comprehensive characterization of muscle function beyond force measurement alone.

## Description of the New Method or System

The muscle-on-chip device is based on the platform presented in our work [3]. Briefly, each layer was fabricated using laser cutting techniques on sheets of Polystyrene (PS) from Petri dishes, Polymethyl Methacrylate (PMMA) and Cyclic Olefin Polymer (COP). The electrodes made of laser induced graphene [4] (LIG) were synthesized through the CO<sub>2</sub> laser on commercial PI tape, which was directly bonded onto PS substrates. After the laser processing, the non-functionalized residual portions of the tape were carefully peeled off, leaving only the patterned conductive regions adhered to the substrate. Micro-grooves approximately 70 µm wide were created on the substrate to aid myocyte alignment, forming compact myofibrils oriented along the distance between the electrodes (see Fig. 1). Human Induced Pluripotent Stem Cells (hiPSCs)

were seeded onto the device and differentiated into skeletal myotubes over ~15 days. After maturation, the chips were electrically interfaced via a dedicated connector and placed in a microscope-compatible incubator, ensuring controlled temperature, CO<sub>2</sub>, and humidity. Electrical stimulation was applied using biphasic pulse trains (6 V peak-to-peak, 0.5 Hz, ~5% duty cycle), while contraction dynamics were recorded using 20× TLM. Videos were processed using a multi-step PIV-based pipeline. A fast PIV-based pre-screening was first applied on downsampled data to identify regions of interest (ROIs) exhibiting significant contractile activity. Local velocity fields were computed using FFT-based cross-correlation, and temporal signals were extracted by averaging velocity magnitude within spatial windows. From these signals, amplitude, variance, and slope were extracted, normalized, and combined into an aggregated activity score. ROIs were selected based on a 95th percentile threshold of the extracted features. A high-resolution PIV analysis was then performed on selected ROIs to extract accurate velocity fields and local motion signals. These were used to compute representative contraction profiles and cumulative displacement dynamics. Finally, spatial mapping of contraction signals enabled identification of contraction centers, both in instantaneous and cumulative conditions.



Fig. 1. Schematic view of the platform

## Results

The microfluidic device supported the maturation and differentiation of iPSC-derived cells into mature skeletal muscle fibers, characterized by elongated, aligned morphology. Electrical stimulation successfully induced repeatable contractile responses synchronized with each applied pulse. A database of TLM videos was analyzed using the fully automated pipeline based on PIV. The proposed framework enables the identification of contraction centers directly from raw video data and the extraction of their local dynamics

(see Fig. 2). For each identified contraction center, the system reconstructs the temporal evolution of motion, including instantaneous velocity signals and cumulative displacement profiles. This allows the spatiotemporal distribution of contractile activity to be quantified, providing a detailed characterization of how mechanical signals propagate across the tissue over time. The combined analysis of local and cumulative descriptors enables a robust functional fingerprint of the tissue, capturing both the intensity and temporal organization of contraction events. This automated approach provides a scalable tool for quantitative assessment of muscle functionality, with potential applications in drug screening, disease modeling, and personalized medicine. In particular, the ability to resolve contraction dynamics at the level of individual functional regions may enable discrimination between different cellular populations and pathological states, as well as systematic evaluation of pharmacological responses.

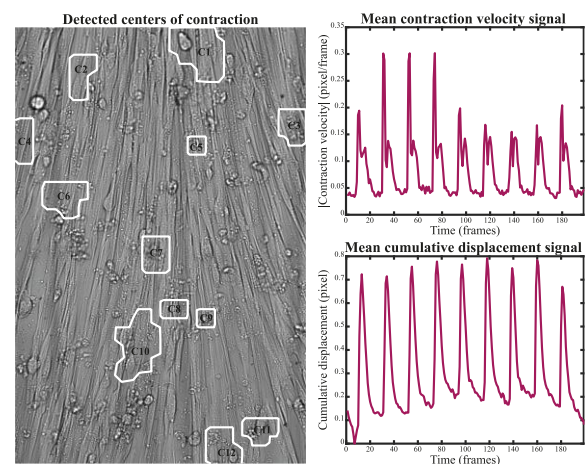


Fig. 2. Example of information extracted from the tool: detected centers of contraction and corresponding mean velocity and cumulative displacement

## References

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