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Label-free Electrophysiological Cytometry for Stem Cell-Derived Cardiomyocyte Clusters

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Abstract

Stem cell therapies hold great promise for repairing tissues damaged due to disease or injury. However, a major obstacle facing this field is the difficulty in identifying cells of a desired phenotype from the heterogeneous population that arises during stem cell differentiation. Conventional fluorescence flow cytometry and magnetic cell purification require exogenous labeling of cell surface markers which can interfere with the performance of the cells of interest. Here, we describe a non-genetic, label-free cell cytometry method based on electrophysiological response to stimulus. As many of the cell types relevant for regenerative medicine are electrically-excitable (e.g. cardiomyocytes, neurons, smooth muscle cells), this technology is well-suited for identifying cells from heterogeneous stem cell progeny without the risk and expense associated with molecular labeling or genetic modification. Our label-free cell cytometer is capable of distinguishing clusters of undifferentiated human induced pluripotent stem cells (iPSC) from iPSC-derived cardiomyocyte (iPSC-CM) clusters. The system utilizes a microfluidic device with integrated electrodes for both electrical stimulation and recording of extracellular field potential (FP) signals from suspended cells in flow. The unique electrode configuration provides excellent rejection of field stimulus artifact while enabling sensitive detection of FPs with a noise floor of 2 μV_{rms} . Cells are self-aligned to the recording electrodes via hydrodynamic flow focusing. Based on automated analysis of these extracellular signals, the system distinguishes cardiomyocytes from non-cardiomyocytes. This is an entirely new approach to cell cytometry, in which a cell's functionality is assessed rather than its expression profile or physical characteristics.

Introduction

Despite recent advances in stem cell differentiation, cell identification and purification remains a significant obstacle to clinical translation.¹ Conventional fluorescence² and magnetic³ cell cytometry, which have not fundamentally changed in the last thirty years, require exogenous labeling of cell surface markers. For many cell types, including cardiomyocytes, few or no reliable surface markers are available, though recent results with cardiomyocytes have identified SirpA and VCAM1 as promising candidates.^{4–6} Even as surface markers emerge, however, labeling molecules may interfere with the functionality of the cells of interest.^{7,8} Genetically-modified cells which express a fluorescent reporter gene⁹

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or confer antibiotic resistance for selected survival under a cell-type-specific promoter¹⁰ can also be used, but tumorigenesis is a major concern with genetic approaches. A non-genetic method that has recently been reported¹¹ gives highly enriched cardiomyocytes, but this method cannot distinguish among different populations of muscle cells. Finally, molecular labeling approaches to cell identification and purification provide no information about the stimulus-response characteristics of cells which is of paramount importance in assessing their functional utility.

Notably, many of the cell populations being explored for regenerative medicine are electrically excitable (e.g. cardiomyocytes, neurons, and smooth muscle cells).¹² Like all cells, they maintain concentration gradients of certain ions across their plasma membranes through the use of active ion transport proteins.¹³ Electrically excitable cells are unique in that they also feature voltage-gated ion channels which, upon activation by sufficient transmembrane electric fields, transiently open and allow ions to flow across the membrane down these concentration gradients. These ion currents lead to a voltage signal in the resistive medium surrounding the cell. This extracellular field potential (FP) signal can be detected with a nearby microelectrode. Each cell type has a characteristic expression profile including many different ion channels, each with unique gating kinetics.¹⁴ Therefore, each cell type has a unique FP signal which can provide rich phenotypic information (Fig. 1 and Fig. S5). Furthermore, electrophysiological signals change as a cell matures from an embryonic to an adult phenotype during stem cell differentiation.^{15,16} We propose the use of electrophysiology as a contrast signal for cell cytometry. In this paper, we show that Electrophysiology-Activated Cell Cytometry (EPACC) can distinguish differentiated human induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) clusters from undifferentiated iPSC clusters using these signals. As many cardiomyocyte differentiation protocols result in clusters of cells (100s–1000s of cells),^{15,17–22} we focus here on flow cytometric identification of such clusters, with the goal of achieving identification at single cell resolution and ultimately the ability to sort live single cells for downstream experiments. Furthermore, it has been observed that within a cardiomyocyte cluster, one electrophysiological phenotype predominates,²¹ suggesting that cluster identification may be a useful method of achieving a homogeneous phenotype while avoiding the technical challenges and limited throughput of single cell recordings. In principle, however, single cell cytometry and even sorting should be possible using this approach.

To date, all work exploring the relationship of electrophysiology to cell phenotype has been done with adherent cultures, tissue slice preparations, or *in vivo*. Even with cells which are adhered on sensing electrodes, FP signals are notoriously weak. Furthermore, field stimulation produces dramatic artifacts in the recording which can obscure these signals.²³ Our system addresses these problems in several ways. First, since cells are confined in a microchannel, the ohmic voltage drop in the vicinity of the cells increases since current is confined to the cross-section of the channel.²⁴ Second, we employ a differential detection scheme, placing a pair of sensing electrodes on an equipotential line in the stimulus field. This dramatically reduces the stimulus artifact seen by the sensing amplifier as compared with a single-ended recording. The spacing of the electrodes is designed to minimize thermal noise ($<2 \mu\text{V}_{\text{rms}}$) and maximize the recorded FP (50–200 μV). Third, we employ an artifact suppression algorithm which eliminates artifact through a combination of template subtraction, linear filtering, and least squares exponential curve fitting/subtraction.

Currently there is no way to perform cell cytometry based on cell electrophysiology. This work demonstrates for the first time that electrical signals can be detected from suspended cells in flow, and that differentiated iPSC-derived cardiomyocyte clusters and undifferentiated iPSC clusters can be distinguished using these signals. Although we focus specifically on electrophysiology and first proof-of-concept here, we ultimately envision a

new paradigm of cell cytometry in which a cell's dynamic, functional response to stimulus—be it electrical, optical, chemical, or mechanical—is assessed on a cell-by-cell basis.²⁵

Materials and Methods

System Operation

Fig. 2a–b depicts the operation of our electrophysiology-activated cell cytometer. Cell clusters (~200 μm in diameter) are introduced into the device as a dilute suspension through a central channel and hydrodynamically focused over a detection region via flanking sheath flows. Two detection electrodes on the floor of the channel, one which is positioned directly under the cells and one which is positioned several cluster radii away (transverse to the flow), measure the differential voltage signal generated by the cluster using a low-noise instrumentation amplifier. When a cluster passes into the detection region, it causes a spike in impedance between these two electrodes, in accordance with the Coulter principle.²⁶ When this spike is detected, a short electrical pulse is delivered through two large stimulus electrodes positioned directly upstream and downstream of the detection electrodes. If longer recordings are desired (for example, to detect spontaneous beating or to examine the cells' response under multiple stimulus conditions), the flow can be stopped so that the cluster is stationary. Due to their geometry in the channel, the stimulus and detection electrodes form a balanced bridge circuit, with the detection electrodes on an equipotential line in the stimulus field. The stimulus artifact seen by the amplifier is common-mode and thus rejected. Capacitive coupling of the stimulus and detection electrodes still leads to some artifact, which is removed in software. After automated analysis of the FP, the outlet flow is switched to an output reservoir using external electromechanical valves (Fig. 2c–e). Fig. 2f–h shows the physical system.

Instrumentation

The instrumentation employed here has been previously described.²⁷ A custom printed circuit board (PCB) containing an instrumentation amplifier and an optoisolated, battery-powered stimulator is interfaced to the microfluidic chip via spring-loaded gold pins. A glass slide coated with a thin film of indium tin oxide (ITO) is positioned underneath the device and DC current through the ITO warms the device to 37°C uniformly over the area of the chip. Temperature on the slide is monitored using a thermistor. The device is positioned under an upright microscope equipped with a video camera for visual inspection of cell positioning and contractions. The entire system is enclosed in a Faraday cage to minimize power line and radio frequency (RF) interference. Custom LabVIEW controller software in conjunction with a 16-bit data acquisition module (National Instruments, Austin, TX) is used to generate stimulus pulses and digitize signals from the device at a sampling rate of 100 kHz. An LCR meter (Model 4284A, Agilent, Santa Clara, CA) is used to monitor the impedance between the detection electrodes, and this information is continuously relayed to the LabVIEW controller via a GPIB bus. When a cell is detected, the LabVIEW controller turns off the LCR meter's interrogation signal and disconnects it from the detection electrodes via two analog switches. At that point, the stimulus pulse is delivered and the recorded signal from the instrumentation amplifier is processed. The LabVIEW controller also automates a syringe pump (PHD Ultra, Harvard Apparatus, Holliston, MA) for cell suspension and sheath flow delivery, controls the electromechanical valves for outlet flow switching (Pneumadyne, Plymouth, MN), and maintains the temperature by modulating the current through the ITO heater using a closed-loop proportional-integral-derivative (PID) controller. Figs. S2 and S3 depict the instrumentation.

Microfluidic Device Fabrication

Fig. S4 shows the fabrication procedure and a detailed description of our fabrication process is provided in the supplementary information. Briefly, 20nm of titanium followed by 100 nm platinum was patterned onto 50 × 50 mm piranha-cleaned glass slides using a standard metal evaporation and lift-off process. An insulating layer of silicon nitride was then deposited to a thickness of 400 nm using plasma-enhanced chemical vapor deposition (PECVD) and subsequently patterned using SF₆ reactive ion etching to reveal the electrode areas which were to be in contact with the fluid and contact pads around the perimeter of the chip for off-chip connections. Polydimethylsiloxane (PDMS) microfluidic devices, which were molded using standard soft lithography techniques from SU8/silicon molds,²⁸ were then bonded to the electrode slides following surface treatment with O₂ plasma. The PDMS device was carefully aligned to the electrodes under a stereoscope. After bonding, the detection electrodes were individually platinized to reduce their impedance.²⁹

Experimental Procedure for iPSC-CM Cytometry

All stem cell experiments, methods, and protocols for this study were approved by the Stanford University Stem Cell Research Oversight (SCRO) committee. Details of our stem cell maintenance and cardiomyocyte differentiation protocols can be found in the supplementary information. Our protocol begins with a monolayer of iPSCs, and through the course of differentiation these cells tend to form dense, rounded clusters in the range of 100–300 μm. Cardiomyocyte differentiation almost exclusively occurs within these clusters. Although our cytometer is capable of working with a range of cluster diameters (see Fig. S12), we should also point out that other differentiation techniques result in clusters of a more uniform diameter,^{30,31} and may ultimately be more appropriate for cluster cytometry. For this study, clusters which had undergone differentiation were manually scraped from their culture well using a finely drawn sterile Pasteur pipette. These were allowed to incubate for 1 hr, causing them to round up prior to experiments. Both iPSC-CM and undifferentiated iPSC clusters were drawn into a syringe, along with a small volume of culture medium. The syringe was connected to the inlet of the device and pushed using a syringe pump automated with the LabVIEW controller software. Cells were flown at a constant velocity while the electrode impedance was monitored continuously. When the impedance of these electrodes increased by more than 10%, the flow was immediately stopped, resulting in the cluster being positioned directly over the electrodes, and a stimulus pulse train was delivered. For cytometry experiments, we administered a sequence of twenty (20) 0.4 ms wide pulses at 0.1 s interval. Stimulus parameters (current, pulse width, frequency) can either be fixed or can be swept through different values depending on the parameter that is to be measured (refractory interval, stimulus threshold, etc.). Most iPSC-CM clusters visibly contracted spontaneously in the channel. All clusters contracted during stimulation (n=25). Cells could be repeatedly stimulated with no apparent degradation in signal strength or cell viability for over an hour.

Results

Stimulus Artifact Elimination

FP signals are notoriously weak. Furthermore, field stimulation produces dramatic artifacts in the recording which can obscure these signals.²³ EPACC addresses these problems in three ways.²⁷ First, cells are confined in a microfluidic flow cell which effectively boosts the FP amplitude since current is confined to the cross-section of the channel, and thus the ohmic voltage drop is increased.²⁴ Second, we employ a differential detection scheme, placing a pair of sensing electrodes on an equipotential line in the stimulus field. This dramatically reduces the stimulus artifact seen by the sensing amplifier as compared with a single-ended recording. The integrated microelectrodes are coated with Pt black to minimize

impedance and further reduce artifact. The spacing of the microelectrodes is designed to minimize thermal noise ($<2 \mu V_{\text{rms}}$) and maximize the recorded FP (50–200 μV). Third, we employ a software artifact suppression algorithm to remove the remaining artifact which, thanks to the hardware suppression techniques, falls within the dynamic range of the amplifier. The algorithm eliminates artifact through a combination of template subtraction, whereby a template artifact signal uncontaminated by a FP is subtracted from the signal, followed by least squares exponential curve fitting and subtraction of the remaining artifact. Fig. 2 illustrates the operation of the device (detailed in the methods section) and Fig. S3 illustrates the results of the various suppression mechanisms.

Enhanced Field Potentials of Cells in Microchannels

In traditional FP recording using microelectrode arrays, cell adhesion is an important factor in obtaining good signal-to-noise ratios (SNR)^{32,33} and making recordings from nonadhered cells in suspension is challenging. However, by confining cell clusters to a microchannel with a cross-sectional area approaching that of the clusters, we show that the FP amplitude increases such that adhesion is not necessary and cells can be analyzed in suspension (Fig. S9). A quantitative description of FP enhancement in microchannels is provided in the supplementary information. Through a combination of hydrodynamic focusing, impedance-based cell detection, and microchannel confinement, FPs can be reliably detected from iPSC-CM clusters in suspension.

FP Signals Recorded from iPSC-CMs

Both spontaneous and evoked FPs are clearly observed in iPSC-CM clusters whereas no such signals exist in undifferentiated iPSC clusters (Fig. 3a). Some differentiated clusters produce both spontaneous and evoked FPs while some only produce evoked FPs. Furthermore, among iPSC-CMs, substantial variability exists in the FPs, suggesting, as has been previously reported²¹, a mixture of phenotypes (Fig. 3b). By applying different stimulus patterns, the electrophysiological phenotype can be quantitatively assessed (Fig. 3c–e). Different regions of the heart are known to display distinct stimulus-response characteristics (refractory interval, stimulus threshold, etc.), and these parameters can be measured with our system. iPSC-CM clusters can be repeatedly stimulated and clusters produce consistent FPs. This allows averaging over multiple FPs to increase SNR by N , revealing more subtle details of the FP signal. Fig. S5 illustrates this technique by averaging 10 successive FPs and highlights the important features¹⁵ of the FPs which can be quantitatively assessed from these recordings. The durations of the various phases of the cardiac action potential: depolarization (t_{dp}), plateau (t_{slow}), and repolarization (t_{rp}) are particularly important when assessing phenotype, as well as whether or not the cell spontaneously beats, and if so, its intrinsic spike interval (t_{si}). Signals can be detected from cell clusters in motion or clusters which are held stationary over the electrodes. Fig. S6 shows FPs from an iPSC-CM cluster which is being moved back and forth over the detection region: first quickly, then slowly, and then held stationary. Finally, clusters can be flown through the cell cytometer device repeatedly over a period of several hours without any apparent loss in viability, based on their ability to produce evoked FPs and visible mechanical contractions.

Automated Cytometry of iPSC-CMs

The cell cytometer is capable of automatically detecting, stimulating, and identifying clusters based on their response to stimulus. Fig. 4 and Movie 1 show an unresponsive, undifferentiated iPSC cluster, followed by a responsive iPSC-CM cluster being analyzed in the device. In this case, responsiveness is defined as having at least one evoked FP during the stimulus pulse train, although clusters can also be distinguished based on refractory interval, stimulus threshold, or response time.

Discussion

Our results indicate that EPACC is a promising approach for addressing cell identification and analysis for regenerative medicine applications. Evaluating cells based on their functional response to stimulus, electrophysiology in this case, can provide valuable insight into their therapeutic potential. Electrophysiology is the gold standard for subtyping neurons and cardiomyocytes, with different cell types producing dramatically different signals.^{21,34,35} Neurons, for example, are characterized by rapid Na^+/K^+ depolarization/repolarization currents and produce sharp FP “spikes”. The refractory period for neurons is <10 ms. Cardiomyocytes, on the other hand, have relatively slow repolarization currents which can be accompanied by an additional Ca^{2+} inward current which causes the cell membrane to remain depolarized longer. This prolongs the FP duration to about 100 ms, with refractory periods over 100 ms. Certain cardiomyocytes also undergo spontaneous depolarization (i.e. nodal pacemaker cells), and this too can be quantitatively assessed using EPACC. The heart is a mosaic of different myocyte phenotypes, including atrial and ventricular cardiomyocytes, nodal pacemaker cells, and vascular smooth muscle cells.³⁶ During development, the heart undergoes extensive remodeling, and so the electrophysiology of cardiomyocytes and smooth muscle is also an indicator of maturity.^{15,37,38} FP rise time, duration, and frequency of spontaneous contraction have all been shown to correlate with stem cell-derived cardiomyocyte maturation from an embryonic to an adult phenotype. Cardiomyocyte maturity is thought to be critical for regenerative medicine applications. Interestingly, it has been shown that within a given stem cell derived population, cardiomyocyte maturity is heterogeneous and does not necessarily correlate with age in culture.³⁹ Therefore, *ex vivo* maturation may not be sufficient to produce suitable populations, and technologies which can identify, and ultimately purify cells based on maturity will be advantageous.

Stem cells give rise to cardiomyocytes with action potential waveforms characteristic of nodal, atrial, and ventricular tissues²¹. Although ventricular-like cardiomyocytes are desirable for many regenerative medicine applications, there is currently no way to specifically identify this fraction. Many stem cell differentiation protocols involve the production of cell clusters (i.e. via embryoid bodies),^{15,17–22} and it has been shown that within a given cluster, a particular action potential type predominates.²¹ Therefore, even identifying intact cardiomyocyte clusters (rather than individual cells) would be very useful, as recently reported in a study in which engineered cardiac tissue was effectively formed from human pluripotent stem cell-derived cardiomyocyte clusters,⁴⁰ and so we have focused on that goal here. In that study, the clusters were selected via genetically-encoded antibiotic resistance; moving forward, our cytometer would potentially allow for future identification of these types of clusters, but without the need for transgene introduction. Additionally, several recent techniques which involve forced aggregation result in monodisperse cardiomyocyte clusters whose size can be tailored in the range of 200–400 μm .^{30,31} Such techniques would be ideal for generating clusters for a cytometer such as this one. We have optimized this cell cytometer to work with clusters of about 200 μm , although we have observed signals from clusters as small as 90 μm in diameter. We analyzed the relationship between cluster diameter and FP amplitude and found a very weak correlation, suggesting that this technique is much more sensitive to cells in the immediate vicinity of the electrode than it is to the overall cluster (Fig. S12). In principle, single cell recordings should also be possible, although cell trapping may be necessary to ensure cells are accurately positioned over recording electrodes. Some differentiated clusters have pacemaker-like activity and spontaneously contract (at frequencies of 1–5 Hz). Other clusters in the same iPSC cultures do not spontaneously contract, but they do contract when stimulated. In cardiac tissue engineering applications aimed at replacing damaged ventricular tissue, pacemaker-like cells

are undesirable because they could lead to spontaneously-arising ectopic arrhythmias. Electrophysiological cytometry can identify these pacemaker-like clusters.

This study shows for the first time the use of extracellular FP recordings from suspended cells as a contrast signal for label-free cell cytometry. When applied to neural or cardiovascular regenerative medicine applications, this cytometry technology promises a low false positive rate, because undifferentiated stem cells and most other differentiated cells do not express the voltage-gated ion channels required to produce a FP signal. We have shown that stimulus artifact can be completely eliminated to within 100 μ s of the end of the stimulus pulse, and thus it is unlikely that the artifact would be mistaken for a FP, which generally occurs > 1 ms after stimulation. Furthermore, we have recently developed optogenetic means of stimulation, which, in principle, should completely eliminate stimulus artifact due to its orthogonality to the evoked electrical response.⁴¹ Although our evoked electrical signals are weaker than more traditional patch clamp signals, in which the transmembrane action potential is directly measured using an invasive pipette which breaks the cell membrane, our results indicate that they are nevertheless sufficient to distinguish differentiated and undifferentiated cell clusters. Unlike patch clamping, extracellular FP recordings are completely non-invasive and preserve the viability of cells. All clusters analyzed in our experiments (n=25) showed no reduction in FP amplitude following repeated stimulation in the device. Furthermore, FPs showed no discernible changes in shape over this time period. We therefore conclude that stimulation and recording does not adversely affect cell viability or electrophysiological phenotype. Furthermore, the microelectrodes and the microfluidic channel can be used repeatedly for larger numbers of cells, so this technology is suitable for cell cytometry. Although these measurements are slow (100–1000 ms per cluster) compared to those of conventional flow cytometry or FACS, we see no upper limit on the degree to which our system could be parallelized to provide the necessary throughput for downstream applications.

A cardiomyocyte's electrophysiological phenotype is intimately tied to the task which it must perform once implanted in the host organ, namely: produce an organized contraction in response to electrical excitation. We propose that electrophysiological homogeneity of implanted cardiomyocytes would therefore lead to improved tissue organization and systolic output, improved electromechanical coupling within the host myocardium, and a reduced incidence of arrhythmias. Future sorting technologies based on electrophysiological cytometry may substantially reduce the incidence of teratoma formation, because it is unlikely that undifferentiated cells will produce signals which could be mistaken as depolarization currents. This type of sorting technology would also be useful in quantitatively assessing the effects of pharmacological agents on cardiomyocyte populations, which is an important requirement for drug toxicity screening. Finally, electrophysiological signals are powerful indicators of cell differentiation and maturity, and EPACC could serve as a medium- throughput platform for providing insight into fundamental questions in developmental and stem cell biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Stem cells and their electrically-excitable progeny. Extracellular field potential (FP) signals are unique to electrically-excitable cells such as cardiomyocytes, smooth muscle cells, and neurons. Undifferentiated stem cells do not produce FPs, nor do most other somatic cell types.

Figure 2.

Our electrophysiology-activated cell cytometry (EPACC) system employs a microfluidic flow chamber with a unique integrated microelectrode configuration which maximizes SNR while minimizing stimulus artifact in recorded signals. (a–b) Conceptual operation showing impedimetric cell cluster detection, electrical field stimulation and FP recording (c) The large stimulus electrodes and small detection electrodes form a balanced bridge circuit, where current flows equally between the stimulus electrodes and the cell through resistances R_{sc} or between the stimulus electrodes and the reference detection electrode through resistances R_{sd} . R_b represents the bulk resistance while R_d represents the resistance between the detection electrodes, which impacts SNR. (d) Longitudinal cross-sectional view and FEM model illustrating field stimulation. Current is injected into the device through the

double-layer capacitance C_s . A small fraction of this current flows through R_{sc} and charges up the membrane capacitance C_m . This leads to an increase in transmembrane voltage, ΔV_m . If $\Delta V_m > \sim 30$ mV, voltage-gated Na channels in the cell membrane open, initiating a transmembrane action potential which leads to an extracellular FP signal. **(e)** Transverse cross-sectional view and FEM model illustrating a depolarization current and resulting FP. Excitation causes voltage-gated Na^+ channels in the cell membrane to open, which allow Na^+ ions to rapidly diffuse into the cell. This leads to a high current density and an associated ohmic voltage drop in the surrounding resistive medium, represented by R_d , which is measured by a pair of electrodes (with capacitance C_d). Details of these models are provided in the supplementary methods. **(f)** Assembled microdevice comprising a custom instrumentation amplifier PCB, and microfluidic device. **(g)** PDMS microfluidic channels bonded to a glass slide containing Pt electrodes. **(h)** Microphotograph of integrated stimulus/detection electrodes within the microfluidic channel.

Figure 3. EPACC provides label-free classification of pluripotent stem cells and their cardiomyocyte derivatives. Many important physiological parameters can be quantitatively assessed using different stimulation protocols. **(a)** Using a common stimulus protocol (0.6 mA, 400 us pulses for 2 s), iPSC cardiomyocytes and non-cardiomyocytes can be distinguished. Both spontaneous and evoked activity can be detected, allowing cardiomyocytes without spontaneous activity (and thus posing a lower risk of ectopic arrhythmia upon implantation) to be specifically selected. **(b)** Example FPs: three iPSC-CM clusters with both spontaneous and evoked FPs, one iPSC-CM cluster with only evoked FPs, and one undifferentiated iPSC cluster with no activity. Three waveforms (green, blue, and black) are overlaid to show the consistency of the measurement. Interestingly, in the case of clusters without spontaneous activity (e.g., the “evoked only” case presented here), the response time is substantially slower. **(c)** Stimuli-response characteristics of one iPSC-CM cluster. Various parameters can be ascertained by applying different pulse protocols including: cluster response time, refractory period, and stimulus current threshold. These parameters are known to indicate cardiomyocyte phenotypes. **(d)** Distribution of spontaneous contraction intervals in iPSC-

CMs. Note the substantial variability. Different cardiac phenotypes (atrial, ventricular, nodal, etc.) show different spontaneous contraction intervals, so this could also be used as a phenotypic indicator. (e) Distribution of average FP amplitudes in iPSC-CM clusters. For a detailed view of an FP, see Fig. S5.

Figure 4.

Automated identification of stem cell derived clusters. The first cluster does not produce any FP signals, while the second produces both evoked and spontaneous FPs. While the system is pumping and waiting for a new cluster, the waste fluid goes to the waste outlet. Flow rate is set to 1500 $\mu\text{L/hr}$, impedance is in the range of 10 $\text{k}\Omega$ with approximately a 10 % increase when clusters flow over electrodes. A 0.6mA, 0.4ms, 10Hz pulse train is administered, the stimulus artifact is eliminated, and the resulting FP signal is analyzed. The full video of these runs is provided in Movie 1