Action Potential Measurements

iPSC-derived vs. Primary Cardiomyocytes

Experimental setup, dye loading / reloading procedures, and minimizing phototoxicity: critical differences between two common cell models

Introduction

Membrane potential dynamics are fundamental to excitation-contraction coupling in cardiomyocytes. Optical techniques using voltage-sensitive fluorescent dyes are frequently used in recording these electrical changes. While gold-standard methods like patch-clamp electrophysiology provide precise measurements, they are labor-intensive and limited in throughput. In contrast, dyes such as FluoVolt enable rapid recordings of action potentials, making them well-suited for functional assays and high-throughput screening applications.

Different cardiomyocyte models—such as induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) and primary adult cardiomyocytes—display distinct physiological traits. iPSC-CMs exhibit immature electrophysiological properties compared to adult cells, which can impact voltage dye loading and signal behavior. These differences are largely due to their developmental state and altered ion channel expression profiles. Additionally, we observed that dye uptake efficiency in iPSC-CMs is influenced by culture confluency, with higher confluency generally supporting more uniform labeling. In contrast, primary adult cardiomyocytes have more complex membrane structures and are more susceptible to chemical or mechanical handling. These model-specific characteristics require optimized FluoVolt loading protocols to guarantee efficient labeling and accurate capture of cell function.

This application note describes critical differences between iPSC-CMs and primary adult cardiomyocytes with respect to experimental setup, dye loading and reloading procedures, and phototoxicity.

Methods

Sample Preparation

iPSC-CMs: Human iPSC-CMs were derived using a standard differentiation protocol. Cells were cultured on 35-mm dishes at varying confluency (20% and 80%).

Primary Cardiomyocytes: Adult cardiomyocytes were isolated from 8 week old C57BL/6 mice left ventricles and were plated on 35-mm culture dishes for signal recording within 2–4 hours post-isolation.

System Setup

Fluorescence recordings were conducted using the IonOptix MultiCell Lite system. Excitation for FluoVolt was provided by a 470 nm LED (Cairn) and passed through a 470/40x bandpass filter. The filtered excitation light was reflected by a 505 nm dichroic longpass mirror (Chroma) within the microscope and focused onto the sample via a UPlanXApo 40× 0.95 NA objective (Olympus). Emission from FluoVolt was filtered through a 535/36 bandpass filter and detected using a photomultiplier tube. Primary cardiomyocytes were electrically stimulated using the MyoPacer (IonOptix) with biphasic pulses (4 ms duration, 1 Hz frequency, 20 V amplitude). iPSC-derived cardiomyocytes were recorded without pacing.

IonWizard Configuration

In IonWizard, the main setup difference between iPSC-derived cardiomyocytes (iPSC-CMs) and primary cardiomyocyte experiments is the sampling rate used to record fluorescence signals. This rate needs to exceed the speed of the action potential (AP) to avoid missing important details.

- iPSC-CMs often have slower APs, and therefore lower sampling rates—250 Hz, 500 Hz, or 1000 Hz—can be used. Lower rates also help reduce noise but may miss fast signal changes (Fig-1). *Users should choose sampling rates based on the speed of the action potential.*

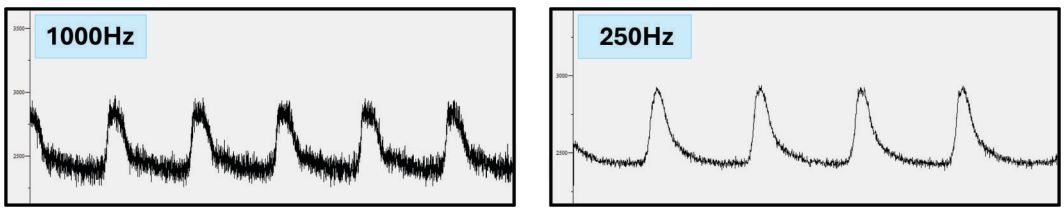

Fig 1. Lower data acquisition rates risk undersampling and reduced data information despite cleaner signal.

- Primary Cardiomyocytes fire much faster, therefore a sampling rate of at least 1000 Hz is needed. In some cases, 2000 Hz is recommended. One indication of under-sampling is if the fluorescence signal peaks before the TTL pacing signal on the trace. Increase the sampling rate if this occurs.

Methods (cont.)

To accurately capture voltage-sensitive dye signals in cardiomyocytes, two key adjustments must be made in IonWizard:

1. Hardware Timer Configuration (ZPT reader frequency) under the Hardware Manager.
2. Sampling Frequency Setup within the Experiment Epoch settings.

Below is a summary of recommended timer values and sampling rates for iPSC-CMs and primary cardiomyocytes:

Table 1. Recommended IonWizard settings adjustments.

Parameter	iPSC-CMs	Primary CMs	How to modify
Timer pacing frequency	1000Hz	2000Hz	Navigate to: **Collect → Hardware → Hardware Manager** → Bottom-right corner, select **"Timers"** → Click **"Configure Timers"** (For 2000Hz) → Set **Countdown Value = 5000** → Click OK.
Single wavelength frequency	250, 500 or 1000Hz	1000 or 2000Hz	Navigate to: **Collect → Experiment** → Select experiment → **Edit** → **Epoch tab** → Adjust frequency on the right side of the screen.

FluoVolt loading protocol

Table 2. FluoVolt loading in primary adult and iPSC-derived cardiomyocytes, the latter with alternate protocols for relative confluency levels.

Step	Primary Adult Cardiomyocytes	iPSC-CMs (single cells or <25% confluence)	iPSC-CMs (monolayers)
1. Reagent Mix	20 µL of 100× PowerLoad™ (Component B) + **1 µL** of 1,000× FluoVolt™ dye (Component A)		20 µL of 100× PowerLoad™ (Component B) + **2 µL** of 1,000× FluoVolt™ dye (Component A)
2. Dilution	Add 2 mL HBSS* to tube, vortex for 10 sec	Add 2 mL HBSS* to tube, vortex for 10 sec	
3. Pre-Wash	NA	Quick wash of adherent cells twice with HBSS*	
4. Dye Loading	Add 2 mL of FluoVolt™ Loading Solution, **incubate 15 min at RT**	Add 2 mL of FluoVolt™ Loading Solution, **incubate 30 min at RT**	Add 2 mL of FluoVolt™ Loading Solution, **incubate 20 min at RT**
5. Post-Wash	Remove dye, wash cells twice with HBSS*		
6.De-Esterification	10 min at 37°C		
7. Final step	Start perfusion system with HBSS*	Add 3 mL of fresh HBSS*	

** HBSS contains 1.26mM of $CaCl_2$, for all steps HBSS was at 37°C*

Observations

Dye Reloading

Reloading FluoVolt in iPSC-CMs is possible after 12 hours, using identical loading conditions as initial application.

Phototoxicity Evaluation

A common issue when measuring action potential with FluoVolt is the potential for phototoxicity and artificial prolongation of the APD.

iPSC-CMs:

Spontaneous activity recorded for 30 seconds showed no detectable signal degradation (Fig-2, A), or APD90 prolongation across multiple regions (Fig-2, B).

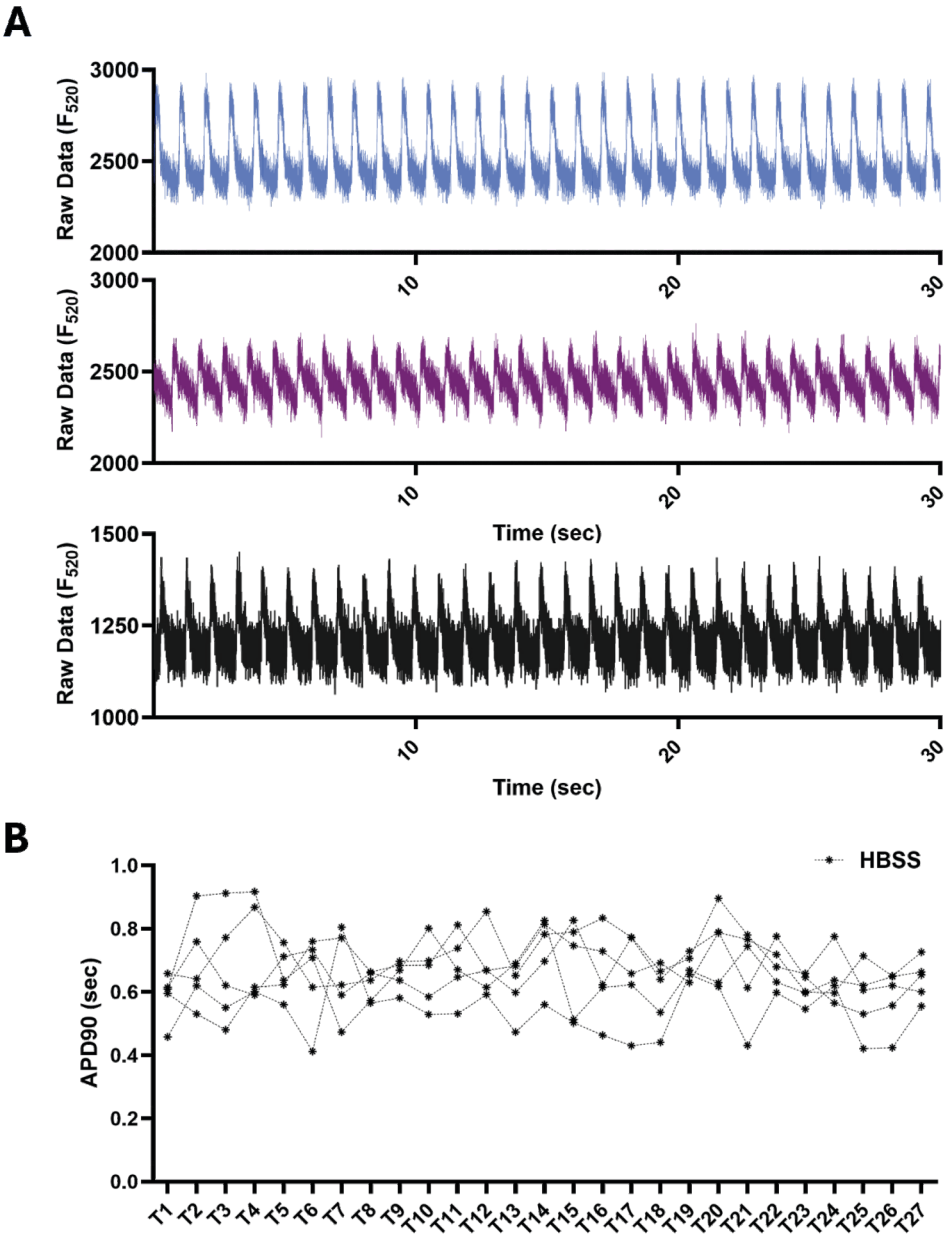

Fig 2. (A) Representative action potential recordings captured at 1000 Hz in iPSC-derived cardiomyocytes (iPSC-CMs) 10 days post-differentiation, recorded in HBSS at room temperature. (B) APD90 values, were tracked across 27 transients per cluster, revealing no significant prolongation indicative of phototoxicity, n=5, N=1.

Observations (cont.)

Primary CMs – Acute Excitation Testing:

FluoVolt signals were recorded for 10 seconds in paced adult primary CMs (1 Hz). In the control condition (HBSS), no significant plateau following the action potential peak (Fig-3, A) or prolongation of APD90 (Fig-3, B) was observed across multiple cells, indicating that FluoVolt excitation did not induce phototoxic effects or artificial APD90 prolongation within this timeframe.

To address whether cells could undergo repeated excitation without developing artificial AP prolongation within the 10-second window, and whether they respond appropriately to β-adrenergic stimulation, we performed a second round of measurements adding 10 µM Isoproterenol (ISO). In the same cells previously recorded under HBSS, APD90 remained stable from the first to the tenth transient (Fig-3, C). ISO treatment resulted in the expected shortening of APD90 compared to HBSS-treated controls across multiple cells (Fig-3, D and E), consistent with its known physiological effects.

Our results demonstrate that 10 sec excitations do not induce phototoxicity, as action potential responses remain stable with repeated stimulations - *when recordings are spaced a few minutes apart* - , and cells retain normal responsiveness to β-adrenergic stimulation.

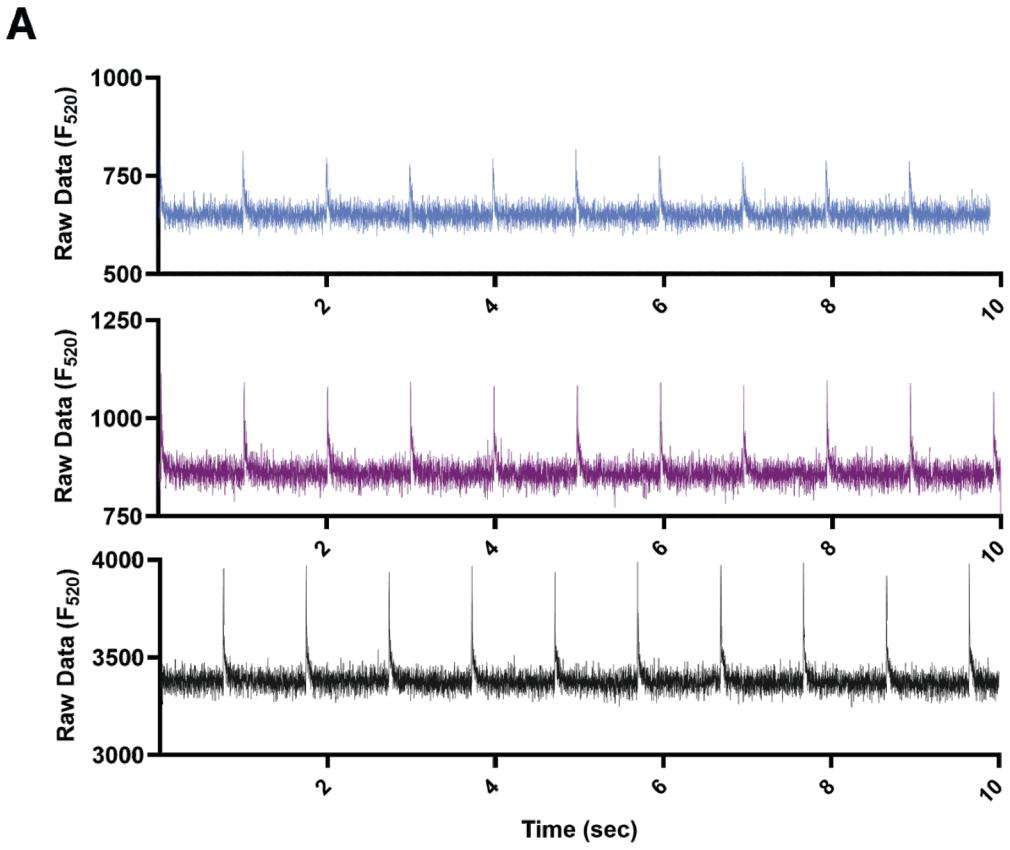

Fig 3. (A) Representative action potential traces obtained at 1000 Hz from murine primary adult cardiomyocytes, recorded in HBSS at 37°C.

Observations (cont.)

Primary CMs – Acute Excitation Testing (cont.):

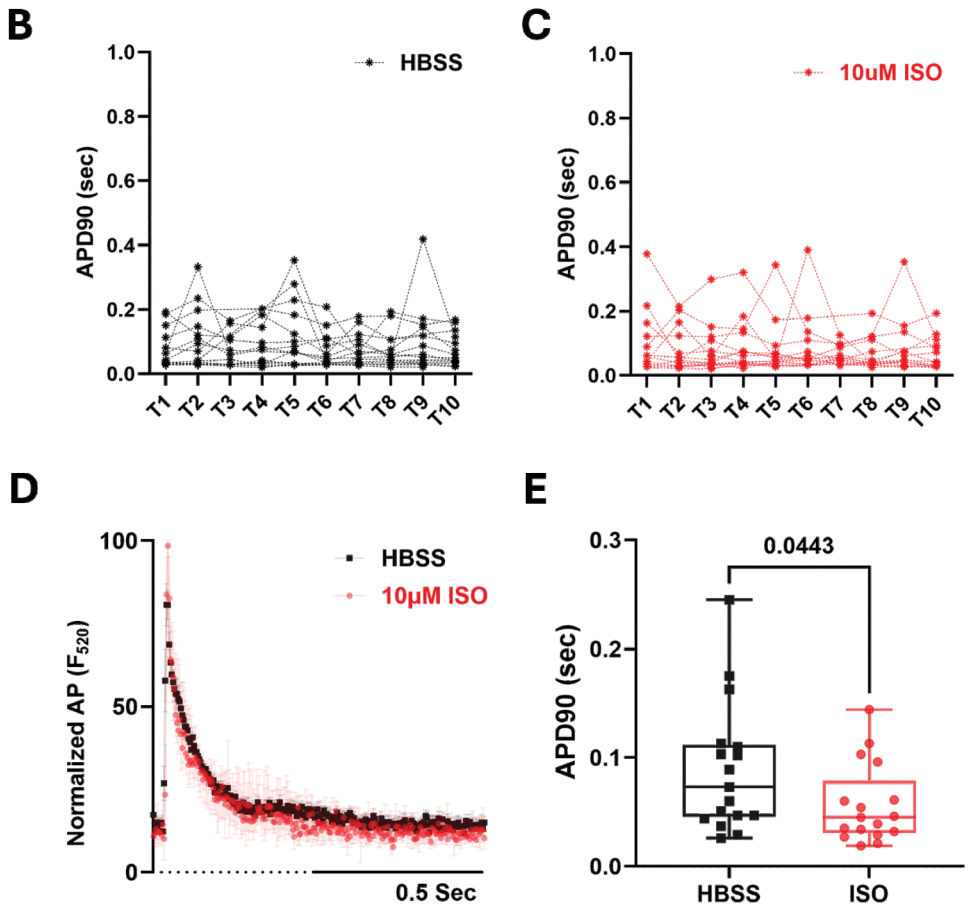

Fig 3 (cont.). ((B) APD90 measurements across 10 transients per cell in HBSS, with no significant prolongation observed, suggesting no phototoxic effects from the experimental conditions. (C) APD90 measurements following the addition of 10 µM isoproterenol, across 10 transients per cell, showing no significant prolongation, further confirming the absence of phototoxicity after repeated measurements. (D) Averaged normalized action potential signals from murine primary adult cardiomyocytes before and after isoproterenol treatment.(E) APD90 before and after isoproterenol treatment, showing a significant reduction in APD90 after isoproterenol compared to HBSS, indicating expected response to isoproterenol, data analyzed using paired 2-tailed Student t test. Panels D and E, data presented as mean ± SEM. Panels B-E, n=17, N=2.

Primary CMs – Prolonged Excitation Testing:

To assess whether prolonged FluoVolt excitation induces phototoxicity, primary cardiomyocytes were continuously paced (1 Hz), and fluorescence signals were recorded for 80 seconds. Phototoxic effects became evident after approximately 25 seconds, with a progressive decline in signal quality observed thereafter (Fig-4).

This is particularly important for users of IonOptix standard calcium and contractility systems, where the probability of extended light exposure is higher. To minimize phototoxic effects during FluoVolt-based recordings, we recommend the following practices:

- Limit fluorescence signal acquisition to 20 seconds per cell.
- Pause excitation when locating new cells by clicking the Pause button in the IonWizard experiment window to avoid unnecessary LED light exposure.
- Use the aperture to restrict excitation to a single cell at a time.

Observations (cont.)

Primary CMs – Prolonged Excitation Testing (cont.):

For Multicell Lite and MultiCell/MultiWell HTS users, where the illumination is apertured and not full-field, and LEDs are triggered only during active measurement, it is still recommended to:

- Limit acquisition to 20 seconds per cell
- Avoid *immediate repeated recordings* from the same region of interest (ROI).

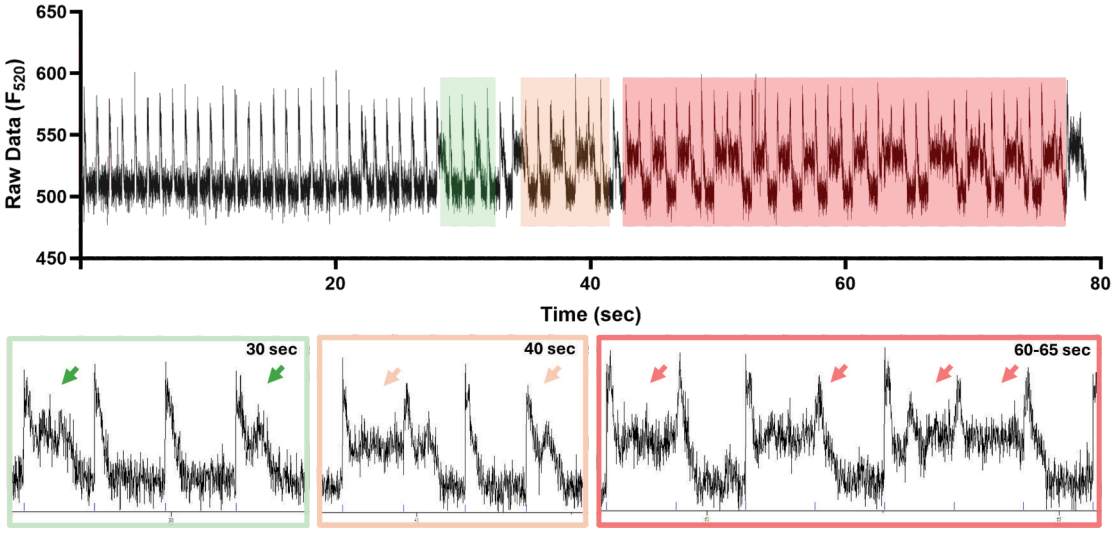

Fig 4. Representative action potential trace recorded at 1000 Hz from a left ventricular murine primary adult cardiomyocyte, with extended optical excitation to illustrate the onset of phototoxic effects and its progression.

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