

CytoSolver 3.0

Analysis of Unpaced iPSC-CM

Using CytoSolver 3.0 to analyze unpaced iPSC-CM calcium and contractility traces

Introduction

Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) are a valuable in vitro model for studying excitation contraction coupling, particularly when combined with advanced tools such as the IonOptix Calcium and Contractility Systems. Our systems allow real-time measurement of calcium transients and contractile behavior in paced or unpaced iPSC-CMs, providing valuable insights into their molecular and cellular mechanisms at different maturation levels, their response to different pharmacological treatments and genetic mutations. CytoSolver 3.0 software is a powerful tool that can be used to analyze the large amounts of data generated by IonOptix systems. It utilizes advanced algorithms to extract a broad range of parameters related to contractility and calcium handling, including contraction time, relaxation time, and peak calcium amplitude. In this article, we will detail how to use CytoSolver 3.0 to analyze paced cardiomyocytes, in both the desktop version (CSD) and cloud version (CSC) to analyze unpaced iPSC-CMs, while highlighting some of the challenges associated with analyzing calcium and contractility transients from slowly beating unpaced iPSC-CMs, and how to overcome them.

Key Definitions

Trace: Data collected during an experiment corresponding to a specific task in IonWizard. For example, the data obtained for the sarcomere length task would be referred to as the sarcomere length trace.

Segment: A specific section of time during which one or more tasks (traces) are recorded simultaneously. Therefore, a single segment may contain multiple traces, each representing data collected from different tasks during the same time interval.

Key definitions (cont)

Transient: A section within a segment that is usually delineated by pacing marks or, if the cells are unpaced, by the offset value specified in the import window.

Offset: The time duration in seconds by which the start of a transient is manually pushed backwards to create a baseline before a peak. This optimizes the transient(s) for data fitting and analysis. **While the default offset value of 0.02 seconds may be appropriate for certain traces, it is essential to choose suitable values for each uploaded file, keeping in mind that the duration of contractions can vary between experiments.**

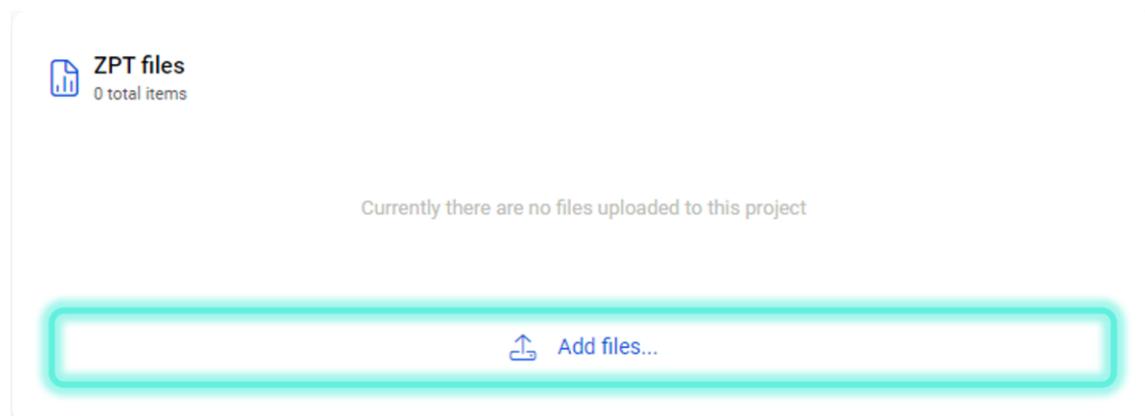
Time zero (T0): The initiation time of a transient as detected by software, measured in seconds.

Time zero (T0) shift: When the initiation time of the calcium transient differs from that of the contractility transient, it is referred to as T0 shift. T0 shift is especially significant in unpaced iPSC-CM, usually when the cells beat slowly.

Importing ZPT files with unpaced iPSC-CM

1. To begin, log in and navigate to the "Projects" tab found on the left-hand side of the screen.
2. Generate a new project and access it.
3. Within the ZPT files section, choose "Add files."

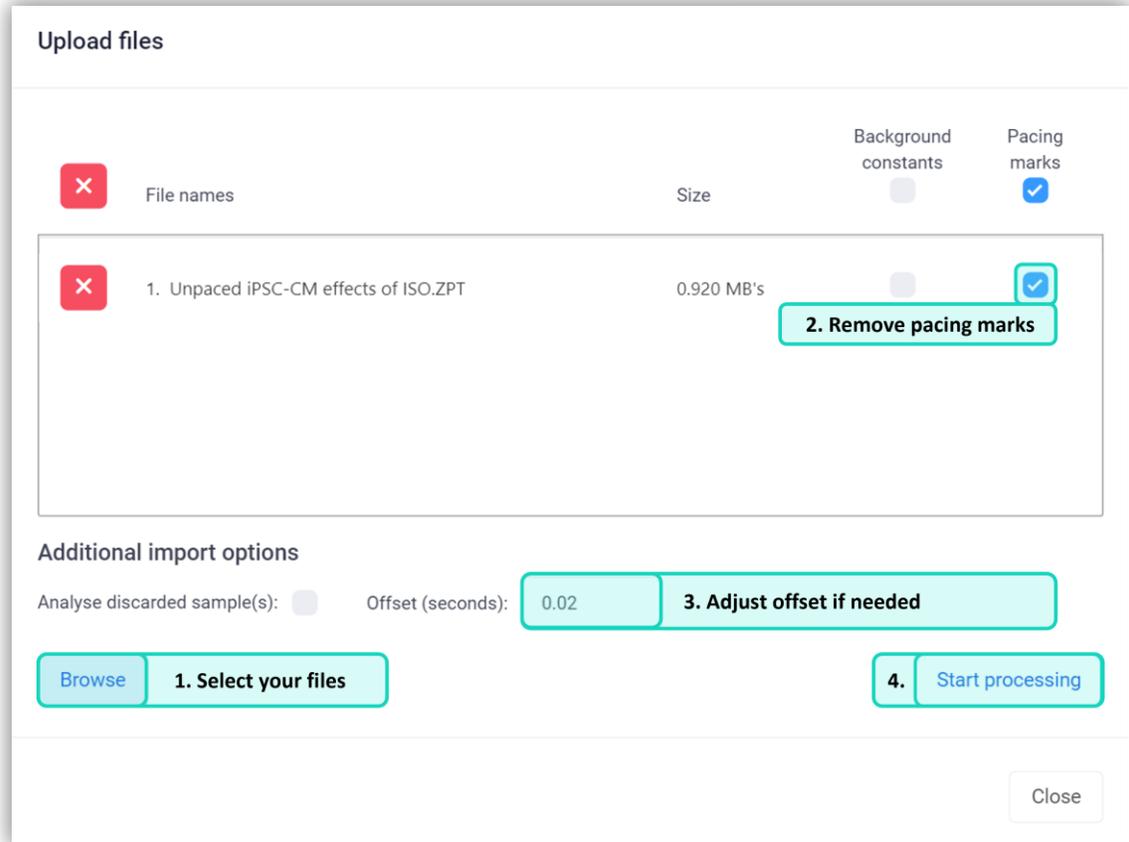
Figure 1: Adding data files to CytoSolver.



Importing ZPT files with unpaced iPSC-CM (cont)

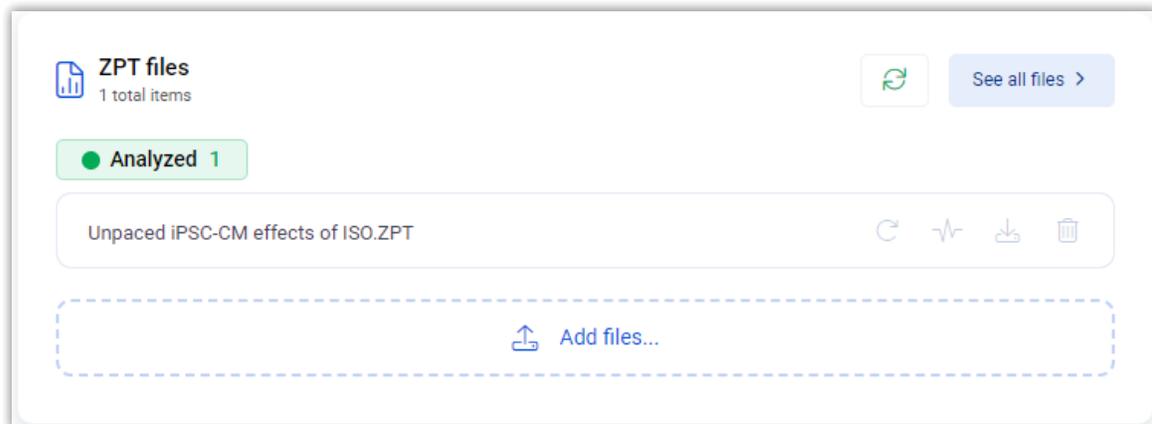
- After clicking on the “Add files”, a new window will appear, providing you with the option to upload your files. In the case of using CSC (as shown in the picture below), this window will be labeled as "Upload Files," whereas in CSD, it will be referred to as "CytoSolver Desktop - Advanced Import." From this window, you can select the "Browse" option to locate and upload saved zpt files from your computer.

Figure 2: File upload/ advanced import. User can choose which files to upload to CytoSolver Cloud or Desktop.



- After files have been prepared, they will appear as analyzed in the ZPT files section located under the overview tab.

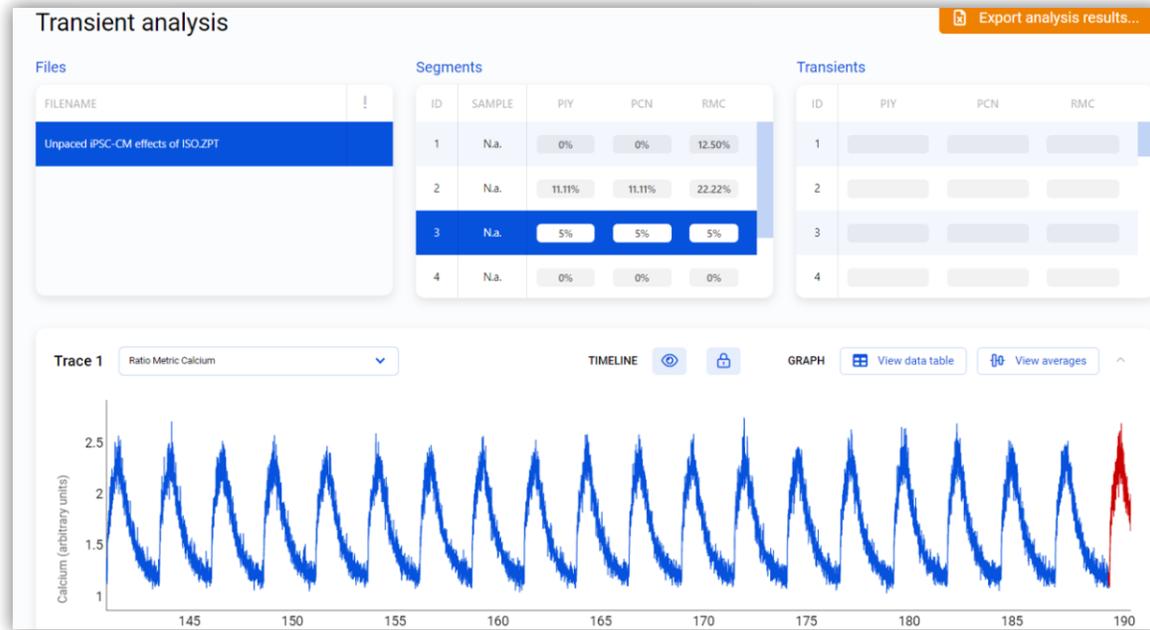
Figure 3: Upload/ import completion.



Checking analyzed transients

1. Access the "Transient Analysis" tab within your project.
2. The screen will display all analyzed files, segments, transients, and traces. The total number of traces will vary depending on the number of tasks in your experiment.
3. By selecting a **segment**, you can view all associated **transients**.
4. The complete segment can be viewed in the traces section.

Figure 4: Analysis results.
Transient results displayed within files and data segments.



5. To visualize a single transient, select the desired transient from the table. This will switch the trace section's view from a segment view to a transient view.

Figure 5: Single transient.
Selecting a transient from beneath "Transients" displays the individual transient.



Note: If transients are blue (as shown previously in figures 4 and 5), they have met the acceptance criteria of CytoSolver. At this point, no further adjustments are required, and you can proceed with “data visualization” or “export analysis results” using the designated buttons. To learn more about the criteria settings, please refer to the manual.

Addressing the issue of red transients

- **What are red transients?**

Red transients are rejected transients in CytoSolver. Rejected transients are marked as "incorrect" due to various factors discussed below in more detail.

- **Does the presence of red transients imply that data is unusable?**

The presence of red transients does not necessarily indicate that the data is unsuitable for use. The quantity of rejected transients within a segment determines whether further corrective measures are necessary.

- **What is acceptable vs alarming quantity of rejected transients?**

Acceptable	Concerning	Alarming
<25% Rejection	25% < Rejection < 50%	>50% Rejection

In many cases, the first or last transient, or both, within a segment may appear red due to the acquisition being initiated or terminated midway through the transient, which is not a cause for concern. However, if a significant number of transients are rejected, further investigation is required to identify the underlying cause and implement appropriate corrective measures.

- **What factors can cause transient rejection?**

Rejected transients can be due to poor data quality, overly strict criteria settings, or the presence of artifacts such as baseline drift. The following table is a list of the potential rejections and how to deal with them, for the full list please refer to the manual.

Table 1: Transient rejections and recommended solutions.

Transient rejections can occur due to a number of factors. This table describes the rejection message and potential resolutions.

	REJECTION	DESCRIPTION	RECOMMENDATION
OVERLY STRICT CRITERIA SETTINGS	Abs Amplitude Lower Limit	Y-axis minimum value is lower than the <u>criteria setting</u> "Y Lower Limit".	Adjust criteria settings
	Abs Amplitude Upper Limit	Y-axis maximum value is higher than the <u>criteria setting</u> "Y Upper Limit".	<i>It is crucial to maintain consistent criteria settings for all files in the project to avoid any data misinterpretation.</i>
	Baseline Threshold	The automatically calculated baseline value is not in between the thresholds set in the <u>criteria setting</u> "Baseline Threshold".	** Revise experimental procedures and recollect data. Refer to app note "Best practices for measuring intracellular calcium using Fura-2 and contractility in primary cardiomyocytes and iPSC-CM"
	Max Derivative Limit	The data contains points whose derivative is larger than the <u>criteria setting</u> "D/dt max". This means adjacent data points are very far from each other causing non-physiologic steep slopes**.	
POOR DATA	Minimal Deviation	The difference between the Y-axis maximum value and the Y-axis minimum value (a.k.a deviation) is higher than the <u>criteria setting</u> " $Y_{max} - Y_{min}$ ". The data may be too flat, there are no peaks.	
	Peak Signal To Noise Ratio (PSNR)	The PSNR (peak signal-to-noise ratio) is too low.	Revise experimental procedures and recollect data. Refer to app note "Best practices for measuring intracellular calcium using Fura-2 and contractility in primary cardiomyocytes and iPSC-CM"

Table 1 (cont): Transient rejections and recommended solutions. Transient rejections can occur due to a number of factors. This table describes the rejection message and potential resolutions.

	REJECTION	DESCRIPTION	RECOMMENDATION
TO SHIFT	Transient Direction	<p>The direction of the transient is not correct according to analysis algorithm.</p> <p>Each trace has a direction specified in its metadata. By reading the transient's data it is ensured that:</p> <ul style="list-style-type: none"> The maximum value of the transient is higher than the minimum value for transients whose direction (specified in the properties) has been set to Positive or Both. The minimum value of the transient is higher than the maximum value for transients whose direction (specified in the properties) has been set to Negative or Both. <p>A rejection of this kind would mean:</p> <ol style="list-style-type: none"> max < min in traces marked as Positive or Both min < max in traces marked as Negative or Both 	<p>Adjust the offset value</p> <p><i>Sometime this can be challenging when cells are treated with different drugs that affect their frequency of contraction.</i></p>
TO SHIFT	Num Of Data Points Before Pacing	<p>The number of data points before the contraction (peak) is not sufficient. The sufficient value is set by <u>criteria setting "Minimum number of datapoints"</u></p> <p><i>The minimum number of data points recommended is 50, if for some reason that was changed in the criteria settings to <50, Please adjust it to 50.</i></p> <p>The total number of points could potentially drop below 50 if there is a shift in T0 between traces due to an incorrect offset value leading to this error.</p>	<p>Make sure that minimum number of datapoints in criteria settings is >50 and the offset value allows detection of a baseline.</p>

Choosing the appropriate offset value

- **Is one offset value suitable for multiple files?**

No, a single offset value is not applicable for various files since the duration of contractions may differ significantly from one trace to another, or from one file to another. Consequently, offset values must be adjusted accordingly.

For example, if cell contractility is too slow ($<$ full transient/second), the start point of the calcium signal transient (TO) that defines the baseline, will typically appear on the upward side of the transient. In this case, adjusting the offset value will push back TO to incorporate the baseline and then the transient is accepted.

- **What range can be used for the offset value?**

For cells with fast beating rates, a range of (0.01-0.05 sec) is generally accepted. However, for cells with slower beating rates, the offset may be as high as 0.2 sec. ***Please note that these are general guidelines, and each case is unique.***

- **How can one determine the optimal offset value?**

To find the best offset value, it's important to determine the TO shift between the calcium and contractility traces.

a. If all segments have similar beating speeds, a single offset value can be used to analyze all transients. To calculate the shift, locate the point where the peak begins on the contractility trace, take note of the time displayed, and then do the same for the calcium trace. Subtract the two values to get the offset value (sec).

b. If different segments have different speeds, such as when slow beating iPSC-CM are treated with ISO which can increase beat frequency, two offset values may need to be calculated and the analysis may need to be repeated twice.

Special Case:

In some experimental protocols, cells may be paced for only one part of the recording and then allowed to beat spontaneously. In these cases, it is recommended to disable the marks and use the offset value.



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