CytoSolver 3.0 Analysis of Paced CMs

Using CytoSolver 3.0 to analyze paced primary cardiomyocytes and iPSC-CM calcium and contractility traces

Introduction

IonOptix calcium and contractility systems are widely utilized in cardiovascular research for precise measurement of calcium transients that initiate cardiomyocyte contraction, while also allowing assessment of their contractile properties. The functional properties of primary cardiomyocytes can also be evaluated with these systems, enabling researchers to investigate the mechanisms involved in regulating cardiac function via the effects of pharmacological agents.

In particular, the IonOptix system can be advantageous for analyzing paced iPSC-CM and primary cardiomyocytes, accurately measuring the timing and duration of contractions in response to specific electrical stimulation rhythms. CytoSolver 3.0 software is a powerful tool that can be used to analyze the extensive data generated by IonOptix systems. The software extracts a wide range of parameters related to contractility and calcium handling, such as contraction time, relaxation time, and peak calcium amplitude. This article will provide detailed instructions on using CytoSolver 3.0 to analyze paced cardiomyocytes, in both the desktop version (CSD) and cloud version (CSC).

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 <u>section within a segment</u> that is usually delineated by pacing marks or, if the cells are unpaced, by a significant change in data representing a spontaneous event.

Background measurement: A recording of fluorescence signal in a region of interest (ROI) in the sample that contains no cells or other fluorescent structures. This signal is then subtracted from the fluorescence signal recorded in the ROI containing the cells of interest to correct for background fluorescence and obtain more accurate calcium signals.

Background constants: Numerical values obtained from the calcium background measurement that are used to correct for background fluorescence in the sample being analyzed for calcium signals. These values are plugged in the constants window under operations tab in IonWizard.

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

Pacing marks: Markers on a recorded signal that indicate the timing of electrical stimulation events used to pace the sample. <u>They are used to establish TO.</u>

Trace abbreviations

SAR: Sarcomere length
RMC: Ratiometric Calcium
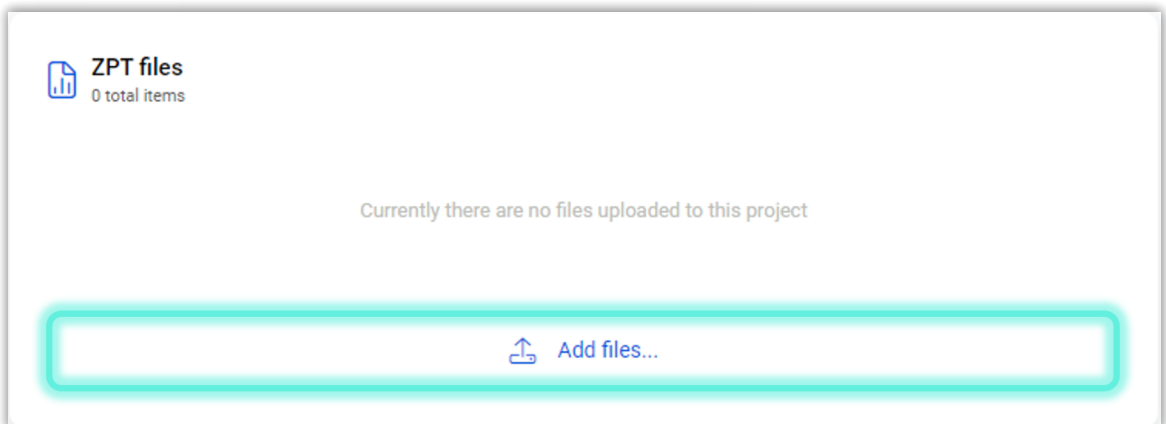PIY: Pixel Intensity
PNC: Pixel Correlation

Importing ZPT files

To begin, log into CSC or open CSD and navigate to the "Projects" tab on the screen.

1. Generate a new project and access it.
2. Within the ZPT files section, choose "Add files."

Figure 1: Adding data files to CytoSolver.

Importing ZPT files (cont)

3. 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.
4. Once zpt files have been imported, any paced cells will have pacing marks automatically selected. Similarly, if background constants were entered into the zpt files, they will also be checked.
5. Click on "Start Processing" to analyze your data.

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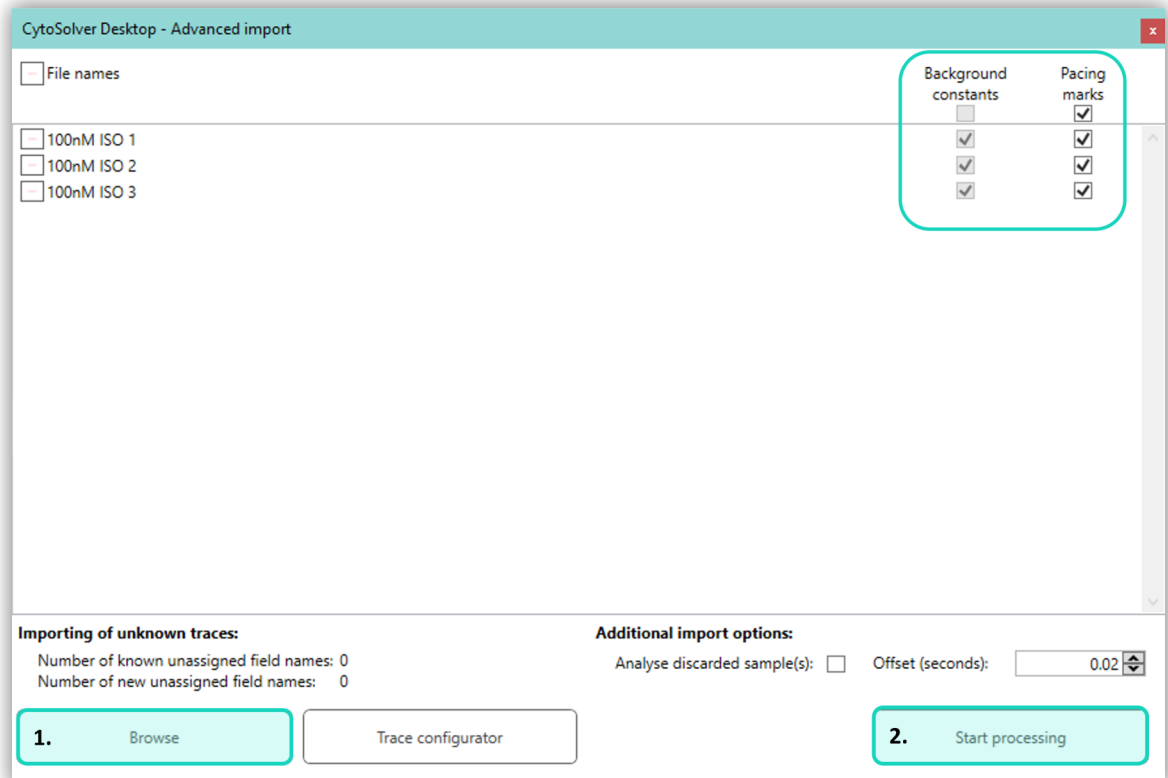

Checking analyzed transients

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. You can view all associated transients by selecting a segment.
4. The complete segment can be viewed in the traces section.

Figure 3: Analysis results.
Transient results displayed within files and data segments.
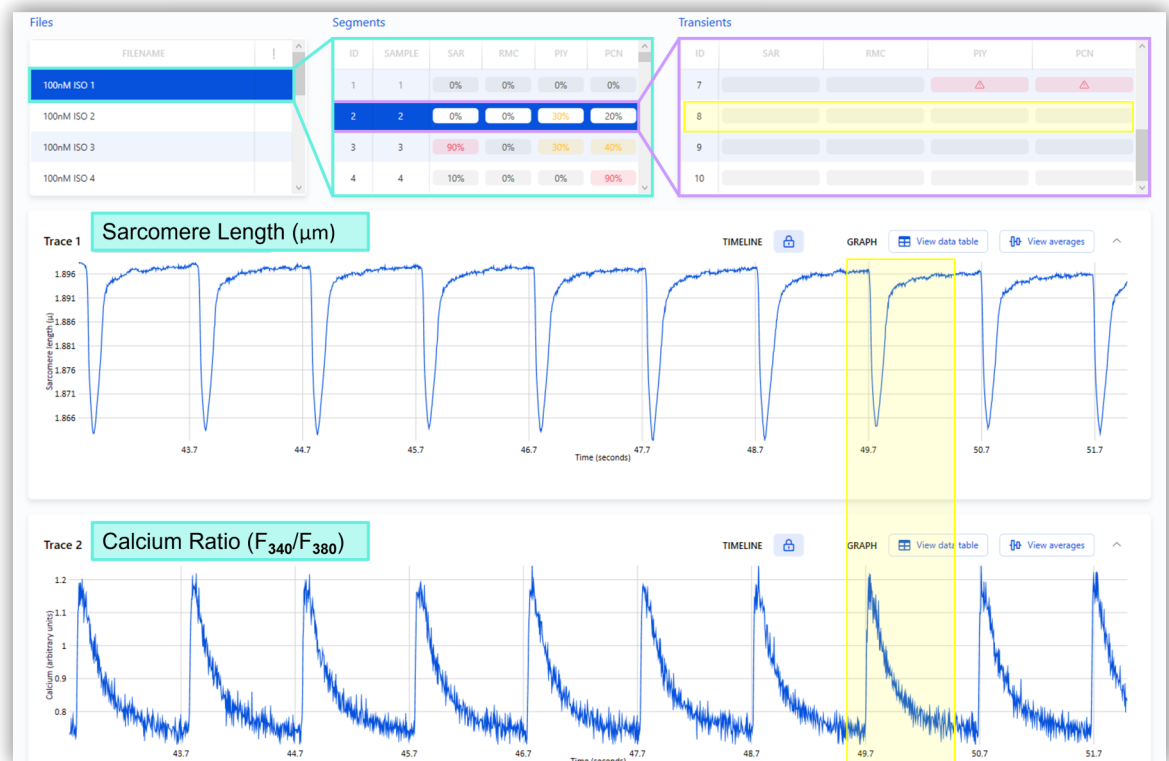
5. To visualize a single transient, select the desired transient from the table. This will switch the trace section's view from a segment view to a transient view. *Here we are visualizing transient 8 from segment 2 in the first file.*

Figure 4: Single transient.
Selecting a transient from beneath "Transients" displays the individual transient.
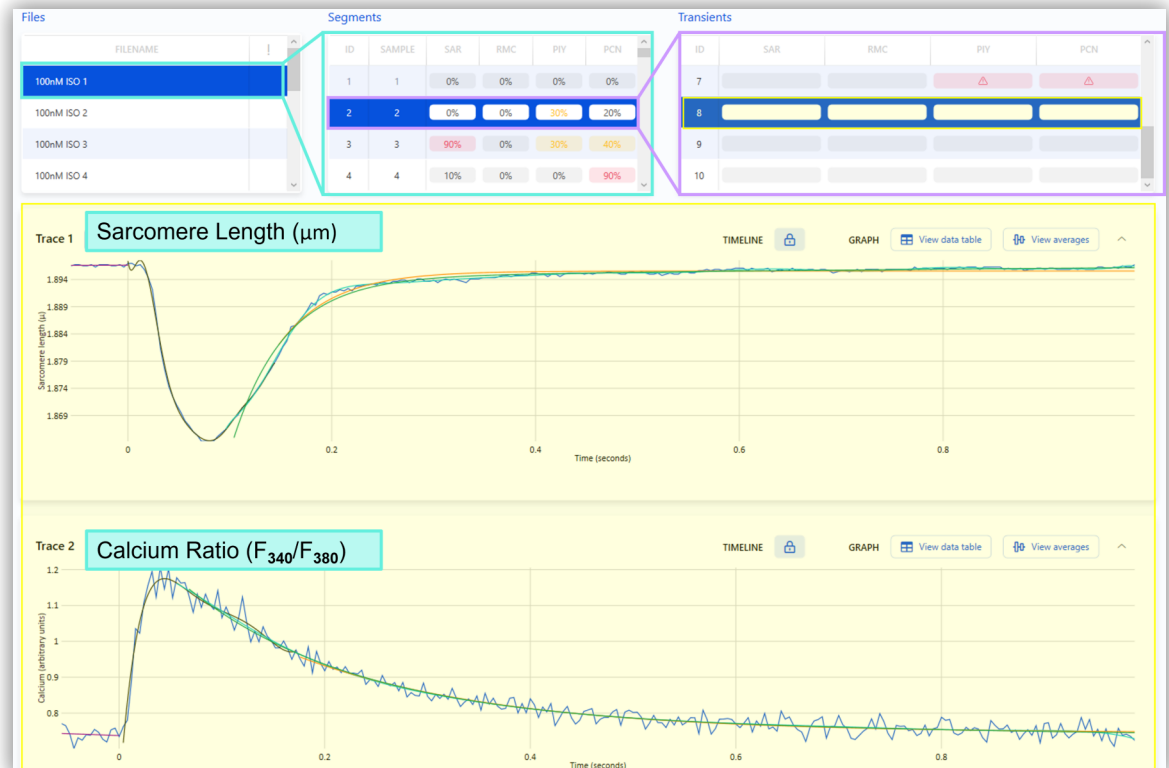
Note: If transients are blue (as shown previously in figures 4 and 5), they have met the acceptance criteria of CytoSolver. At this point, no further adjustments are required, and you can proceed with "data visualization" or "export analysis results" using the designated buttons. To learn more about 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.

- **Is there a way to view the percentage of transient acceptance or rejection?**

CytoSolver solely displays the percentage of rejection, which can be viewed on the transient analysis result page in the segment window. The exported files also show this information, but instead of reporting the percentage of rejection, they display the number of accepted transients per segment as "Number of transients in avg."

Addressing the issue of red transients (cont)

- **Is it possible for me to find out why a transient was rejected?**

Certainly, with CytoSolver you can view the reasons for rejection for each transient in a segment. This information is available on either CSD or CSC in the Transients window, where rejected transients are highlighted in red. By hovering over a rejected transient, you can see the specific reason for its rejection.

Figure 5: Transient rejection. Reasons for rejections are displayed by hovering over exclamation symbol under "Transients".
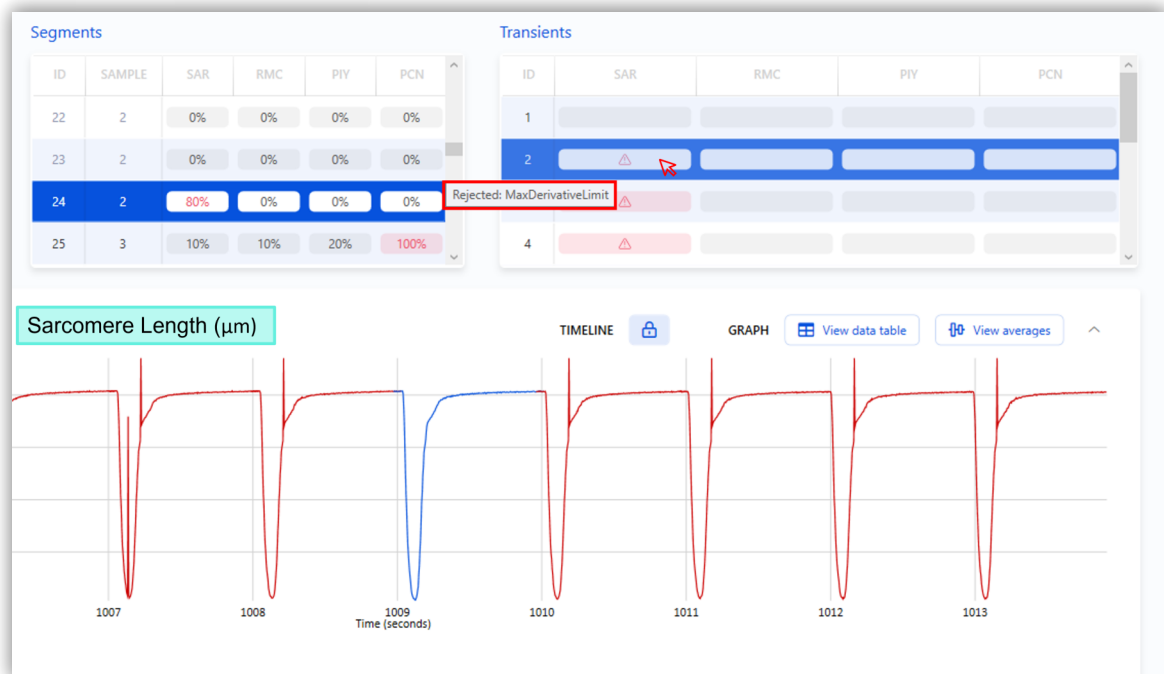
In the exported files, the reasons for rejection can be found under the "Remarks" column. A rejected transient will be labeled as such, followed by an explanation of why it was rejected. For example, the rejected transient above would be reported in exported files as "Rejected: Max Derivative Limit".

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

The rejection of transients can be attributed to factors such as inadequate data quality, excessively stringent criteria settings, or the existence of artifacts such as baseline drift. For a comprehensive list of these reasons, please consult the manual, as well as our application note titled "Using CytoSolver 3.0 to analyze unpaced iPSC-CM calcium and contractility traces," which offers suggestions for resolving some of these problems.
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