

CytoSolver 3.2 Analysis of Unpaced & Irregular iPSC-CM

Improved analysis of unpaced iPSC-CM calcium and contractility traces.

Introduction

CytoSolver 3.2 introduces several critical upgrades over version 3.0 that improve the analysis of unpaced and irregular iPSC-CMs. The previous version lacked the configuration flexibility required for variable-frequency data, whereas version 3.2 allows for granular, per-file tuning directly at the import stage.

Key analysis differences between CytoSolver 3.0 and 3.2 include:

- **Configurable File Properties:** Version 3.2 introduces fully adjustable parameters for Threshold, Skip Time, Offset, Contraction Escape, and Irregular Return Threshold.
- **Irregular Beats Detection:** A new irregular beats detection system has entirely replaced the older arrhythmia detection model.
- **Selectable Detection Traces:** You can now manually assign the main transient detection trace directly in the project configuration.
- **Relaxation parameters can be calculated relative to T0 or Peak Time:** Adjustable exclusively from the Edit Project tab, the Return to baseline % option determines whether recovery times are calculated relative to peak time or t0, which solely affects the relaxation parameters within your transient analysis results.

Purpose & Scope

Standardize the batch analysis of iPSC-CM clusters to eliminate false rejections caused by T0 shifts, baseline noise, or mismatched pacing syncs.

Pre-Analysis Setup

- Ensure all files are in the supported **ZPT** format.
- Navigate to the **Overview** window (left side of the screen) on either CSD or CSC, and open the **Edit Project** tab to assign Transient Detection Trace. The default is Ratiometric Fluorescence trace, which can be adjusted using the Switch trace tab.

Fig. 1: Assigning transient detection trace.

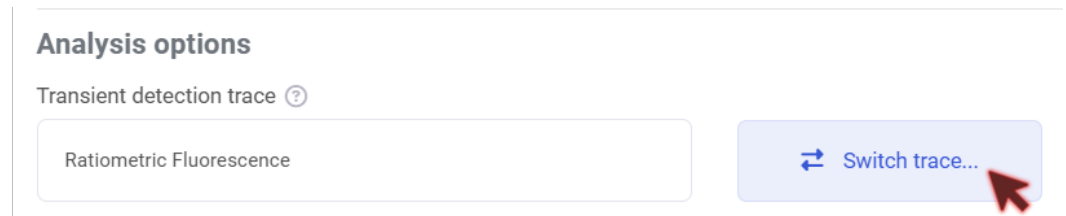
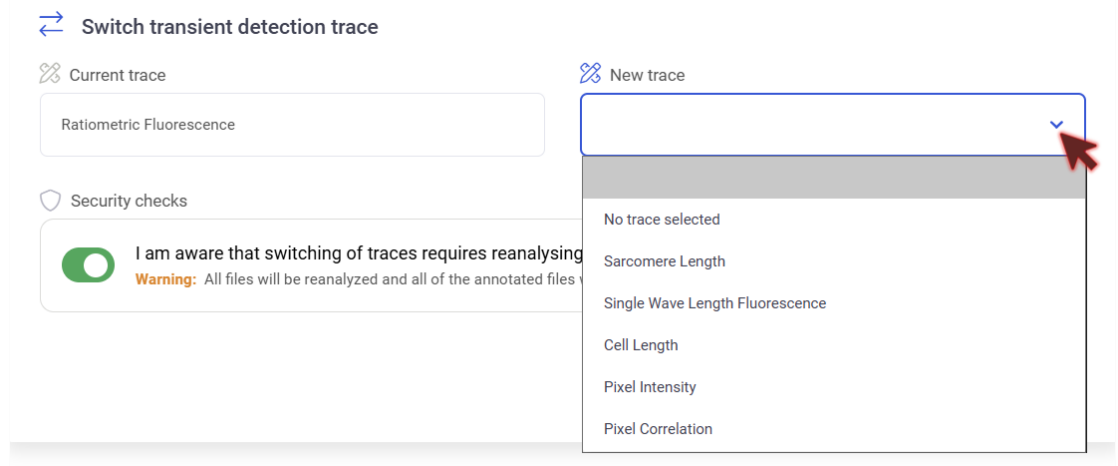


Fig. 2: Assigning transient detection trace.



How to pick the Transient Detection Trace?

Default to the signal that captures the fastest biological response in your sample. In most cases, this means using calcium data or action potential data (fluorescence signal) as the transient detection trace.

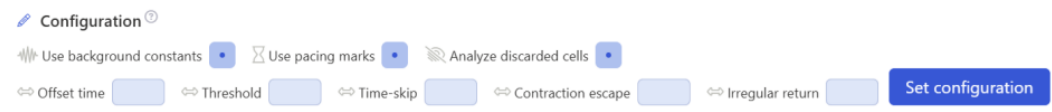
- **If fluorescence signal is clean:** Keep it as the transient detection trace but raise the Threshold (%) higher.
(Threshold % explained in the adjust bulk configuration section below).
- **If fluorescence signal is noisy:** Do NOT simply drop the Threshold (%), as this will cause the algorithm to falsely detect multiple intervals back-to-back. **Instead, switch the transient detection trace to a cleaner signal (e.g., Pixel Intensity).**

Fig. 3: Assigning transient detection trace.

Import and Configuration Procedure

- Navigate to **Add files** from the Overview window.
- **Select Files:** Click "Add files" to open your system's file explorer and upload your batch of ZPT files.

• Adjust Bulk Configurations:

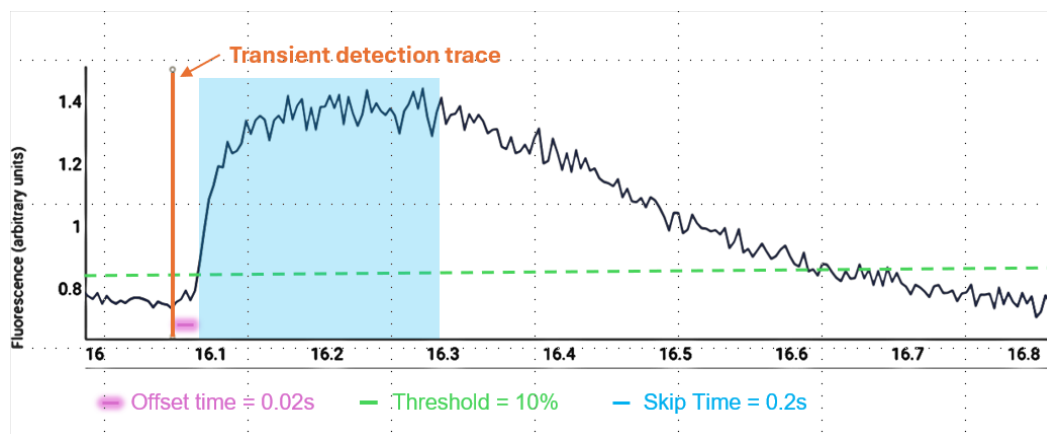


- **Offset Time:** The offset time manually shifts the detected start of a transient backward to ensure a flat baseline is captured just before the peak. It will be completely overridden if pacing marks are enabled.
 - **How the offset applies:** The offset you set is applied to the **transient detection trace** to establish the transient marks. These marks are then used for all other traces in that file, meaning all subsequent traces are calculated based on this initial offset.
 - **Offset Math Requirement:** If you switch to a contractility trace in a file where there is noisy fluorescence data, you must manually calculate the time delay between the faster calcium signal and the slower contractility signal. Add this time difference to your **Offset Time** to ensure the entire calcium transient is captured: change offset from 0.01 to 0.05 to establish a flat baseline area prior to the peak.
- **Threshold:** The threshold defines the exact percentage a signal must deviate from its mean to be recognized as a valid beat.

Tip: Increase this value to prevent high baseline noise from triggering false transients, or lower it to ensure weak, subtle contractions are not completely missed.
- **Time Skip:** Time skip defines the minimum interval in seconds during which CytoSolver will intentionally ignore any new transient events.

Tip: Decrease this setting for fast-beating cells to accurately capture rapid fluctuations, or increase it for slower frequencies to prevent minor signal variations from being falsely detected as separate beats.

Fig. 4: Data trace illustrating detection trace, offset time, threshold %, and time skip.



Import and Configuration Procedure (continued)

- **Contraction Escape:** The contraction escape parameter sets the minimum percentage threshold a signal must achieve to be counted as a valid contraction. Any signal that falls below this designated level—which defaults to 10% and should be adjusted based on your data's peak height and noise—is filtered out and flagged as a contraction escape.
- **Irregular Return:** Irregular return parameter establishes a minimum percentage threshold used to detect additional peaks during the signal's return phase, which indicate irregular contractions. After smoothing the data with a Savitzky-Golay filter, the software uses this setting—which defaults to 15% and should be adjusted based on peak height and signal-to-noise ratio—to accurately flag these secondary beats
- **Evaluate Pacing Constraints:** Uncheck the Use Pacing Marks checkbox if any of the following conditions apply:
 - Part of the file is unpaced.
 - The Calcium and Contractility data are not synced (TO mismatch).
 - The iPSC-CMs are failing to respond to the pacing stimulus.

Pacing signal warning: When pacing marks are selected, the software strictly uses them as the absolute TO and ignores the actual trace data.

If data is unsynced or cells are unresponsive, leaving this checked will force alignment failures.

Verification

- Click **Start processing** to send the configured batch to the analysis queue.
- Open the **Transient analysis** tab to visually verify that proper transient limit lines are drawn at the correct intervals without TO shifting.
- Enable the transient limit lines in the viewer (disabled by default):
 - **CytoSolver Desktop (CSD):** Navigate to the **View** menu in the top-left toolbar, select **Show plot**, and enable **Transient limit**.

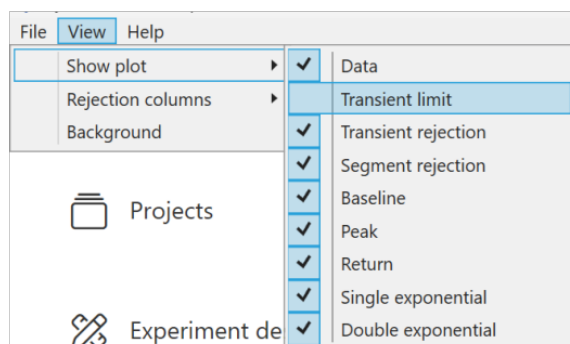
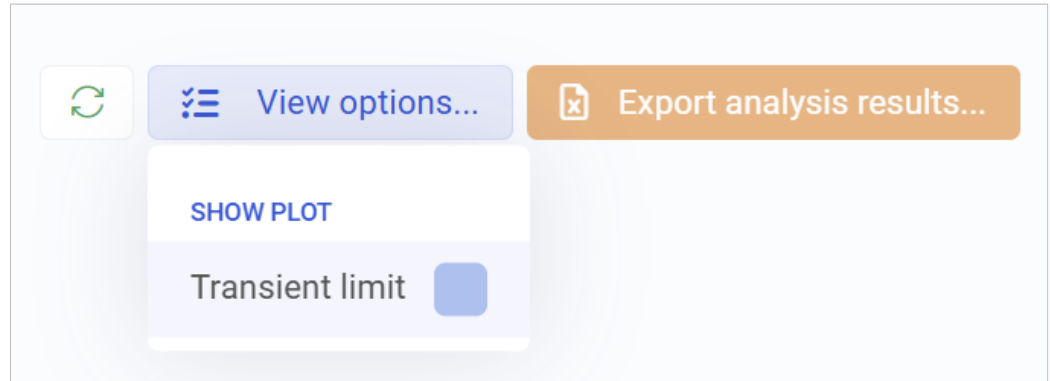


Fig. 5. Enabling Transient limit in CytoSolver Desktop.

Fig. 6. Enabling Transient limit in CytoSolver Cloud.

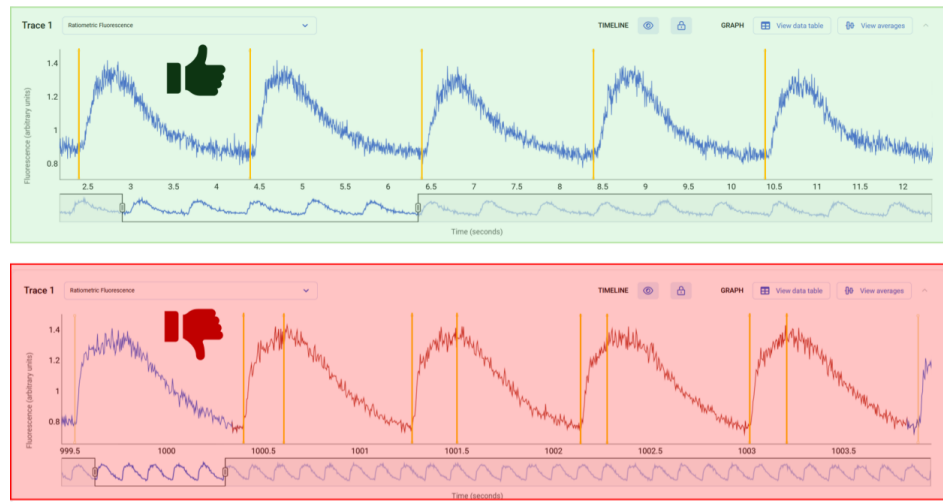
Verification (continued)

- **CytoSolver Cloud (CSC):** Click the **View options** button in the upper-right corner and turn on **Transient limit** under the "Show plot" section.



- **Verify Alignment:** There should be exactly one line before the peak of the transient. If there are multiple lines back-to-back, the detection has failed, and you must return to the file configurations to adjust the **Threshold (%)** or **Offset Time**.

Fig. 7. Verifying correct or incorrect peak detection.





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