



CytoSolver 3.0 (CS 3.0)

FAQs: CytoMotion Contractility Module

Frequently asked questions related to the analysis of contractility data acquired with CytoMotion.

1. Why does CytoSolver 3.0 (CS 3.0) reject the majority of contractility segments recorded using CytoMotion module?

CS 3.0 is highly recommended when using the Cytomotion module to record contractility in iPSC-CM. CS 3.0 might reject the majority of the transients due to one or more of the following:

- Direction of pixel intensity and pixel correlation is inverted. The correct direction for pixel intensity is to increase with contraction while pixel correlation decreases. If recorded data displays the opposite, CS 3.0 will reject all data. This is common with incorrect reference frame selection; pay attention when recording data that you select the reference frame when cells are relaxing. For more info about CytoMotion Lite best practices, check the following resource:
<https://www.ionoptix.com/resource/ionoptix-video-primary-and-cultured-cardiomyocyte-contractility-algorithms/>

- In some cases when experiments are set to record for 1000 seconds continuously and users move from one ROI to another without pausing data recording in IonWizard, data may have areas with high and low values for pixel correlation and pixel intensity. This makes CS 3.0 confuse different areas with one large noisy signal. In those cases, data will be rejected, and also you will notice that segmentation by CS 3.0 is not correct.
 - **To prevent this**, it is recommended during acquisition to pause before moving to a new area of interest. This will create a new segment automatically (horizontal red line in the zpt file).
 - **To resolve the issue post-acquisition**, open the zpt file in IonWizard and add "break" marks. To add a mark, simply right-click and add the word "break". This will segment the data and you will see a short colored line where the mark is added. Save your file and upload it to CS 3.0.

2. Is it good practice to consider pixel intensity and pixel correlation peak height, baseline, and peak values as absolute indicators of contractility?

No, the only values from pixel intensity and pixel correlation that can be used as absolute values are kinetic data, number of beats and beat frequency.

Parameters such as baseline, peak, peak height and any other parameter labeled with arbitrary units should not be used as absolute value. One acceptable way of using these parameters is creating delta data.



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