

# CytoSolver 3.0 Batch Analysis

## CytoSolver 3.0 step-by-step guide to performing batch analysis

### Introduction

CytoSolver 3.0 (CS 3.0) is a powerful program package that includes both CytoSolver Desktop and CytoSolver Cloud. It has the capacity to automatically analyze calcium and contractility traces of primary cardiomyocytes or iPSC-CMs. It offers a comprehensive set of tools for analyzing fluorescence and motion data gathered with our systems that support automatic data annotation and repeated measures like MultiCell HTS or MultiCell Lite. **It can also examine data collected with our conventional Calcium and Contractility system** which doesn't support automatic data annotation and repeated measures. One of the key features of CytoSolver is its ability to conduct automated batch analysis with minimal manual intervention. This feature saves considerable time for researchers analyzing large datasets.

In this application note, we will provide a step-by-step guide to performing batch analysis using CS 3.0.

### 1. Data preparation

***Please note:*** Currently, CS 3.0 exclusively supports data obtained using our calcium and contractility systems in a ZPT file format. ZPT, or Zone Plot Trace, files are data files collected using IonWizard data acquisition software and are identifiable by their ".zpt" or ".ZPT" file extensions.

- If you have used our **MultiCell Lite or MultiCell HTS** systems to collect your data, no specific preparation of the saved ZPT files on IonWizard is required as data segmentation is automatically performed during acquisition. *You can proceed to point two.*
- If you have collected data using our **conventional Calcium and Contractility system**, please follow the guidelines provided below for preparing your data files before importing into CS 3.0.

## Data preparation (cont)

### Data segmentation

To obtain accurate and reliable results with CS 3.0, it is essential to properly segment the data. CS 3.0 analyzes traces segment by segment and reports the calculated parameters for each segment individually. CS 3.0 will consider the full trace as a single segment if data is not properly segmented in IonWizard, leading to inaccurate analysis. Data segmentation can be performed during acquisition or after acquisition is completed.

#### **a. Data Segmentation before acquisition:**

To segment data prior to acquisition, create an experiment with multiple epochs, each representing a segment within the recorded trace. Please, refer to the IonWizard manual for more information.

#### **b. Data Segmentation during acquisition:**

To segment data during acquisition, manually create a break (denoted as a red line on the data trace) by pausing the experiment and then resuming it, effectively separating the data into two segments. The number of segments can be determined by counting the red lines, plus one. Utilizing segmentation can aid in organizing data into distinct categories prior to importing it into CS 3.0.

#### **c. Data Segmentation for saved ZPT files:**

For saved ZPT files, data can be segmented using the text mark "Break".

1. Open the saved ZPT files in IonWizard and hover above the area where you would like to start a segment.
  2. Right-click and a pop-up window will show where you can type "Break".
  3. Next, hover to the end of the desired segment, right-click, and annotate this with "Break".
  4. Continue until all desired segments are created.
- Unwanted segments can be ignored by using the text mark "Delete". Deleted segments will be ignored by CS 3.0 during analysis.

**Note:** Exercise caution when using the "Delete" mark. In instances where a "Break" mark does not follow the "Delete" mark, CS 3.0 will disregard all information within that particular segment of the data until the end of the zpt file. However, when a "Break" mark precedes and succeeds the "Delete" mark, CS 3.0 will only overlook the trace located between the two "Break" marks.

## Data preparation (cont)

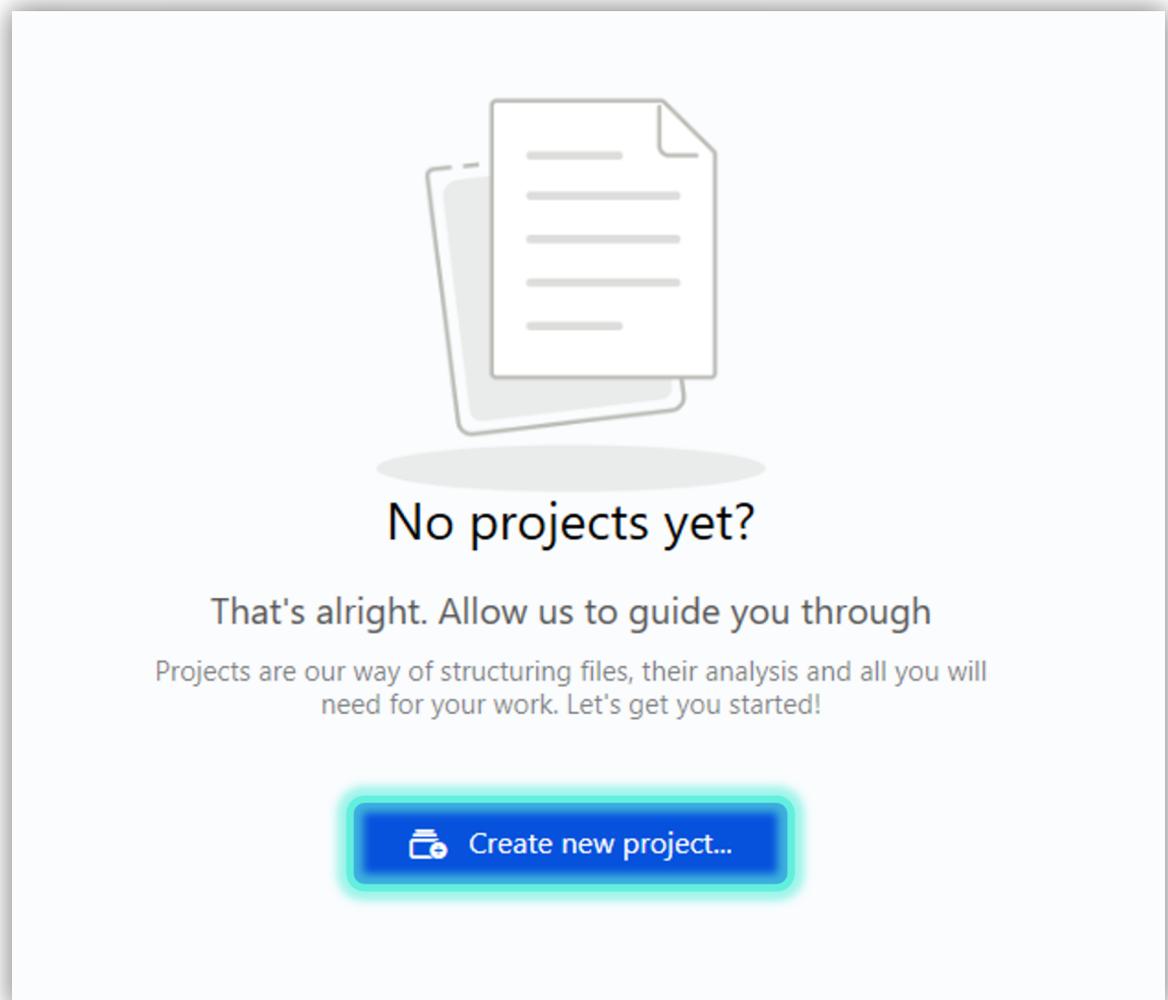
- If a background measurement for calcium has been performed (recommended), the text mark "Background" should be used to annotate the calcium background segment. CS 3.0 will subtract background values before calculating the ratio of ratiometric fluorescent dyes such as Fura-2.

**Note:** Unless the last measurement was the background, a "Break" text mark should follow it to prevent using inaccurate traces as background. For a detailed video tutorial on data segmentation during acquisition and for saved ZPT files, please refer to [video pending]. By properly segmenting the data, reliable and accurate results can be obtained with CS 3.0.

## 2. Importing data

For first-time users of CS 3.0, the first step is to create a **project**, which acts as an independent folder for organizing and managing data, analyses, and files, with each project being self-contained for easy accessibility. To create a new project, select the "Create new project" tab.

**Figure 1: New project creation.** Projects allows users to organize and manage data, analyses, and files.



## Importing data (cont)

The project creation process involves providing a name. The “Experimental Design” field is optional at this stage and can be assigned later. For detailed information on project organization, editing, and deletion, please refer to the CS 3.0 manual.

**Figure 2: Project naming.** Users can choose project names and descriptions.

**Create new project**

Name \*  Start date  Set by default to today

Allowed: A-Za-z0-9^!#\$%&'()\*+,-./:;<=>?@[^`{|}~ &

Description

**Can be added later!**

Experiment design  500 characters left

Cancel

After creating the project, you can import multiple files (batch import) for analysis from the "Add files" option on the overview page of the project.

**Important note:** CS 3.0 has batch import limitations. Both the CytoSolver Desktop and CytoSolver Cloud versions allow only up to 20 files to be imported per batch. Nonetheless, it's not necessary to wait for the analysis of the first batch to complete before importing the next one.

**Figure 3: Batch import.** Users can upload/import multiple files for analysis

Projects / Batch Analysis / Overview

**Batch Analysis**

[+ Add description](#) Start date 21 Mar 2023

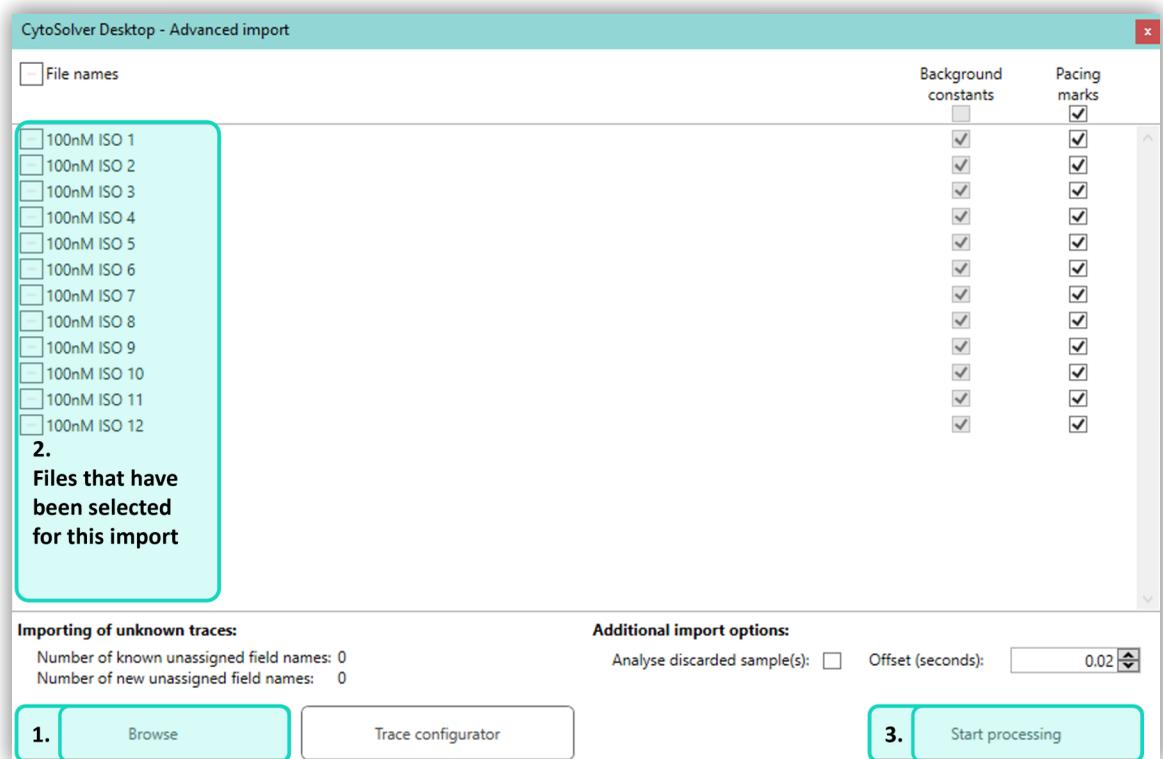
ZPT files  
0 total items

Currently there are no files uploaded to this project

## Importing data (cont)

Upon selecting the "Add files" option in CS 3.0, the "Advanced Import" window will be displayed. To import files for analysis, click on "Browse" and select the desired files, bearing in mind the file import limits mentioned earlier. In this example, we will be analyzing 12 ZPT files, each containing two repeated measures.

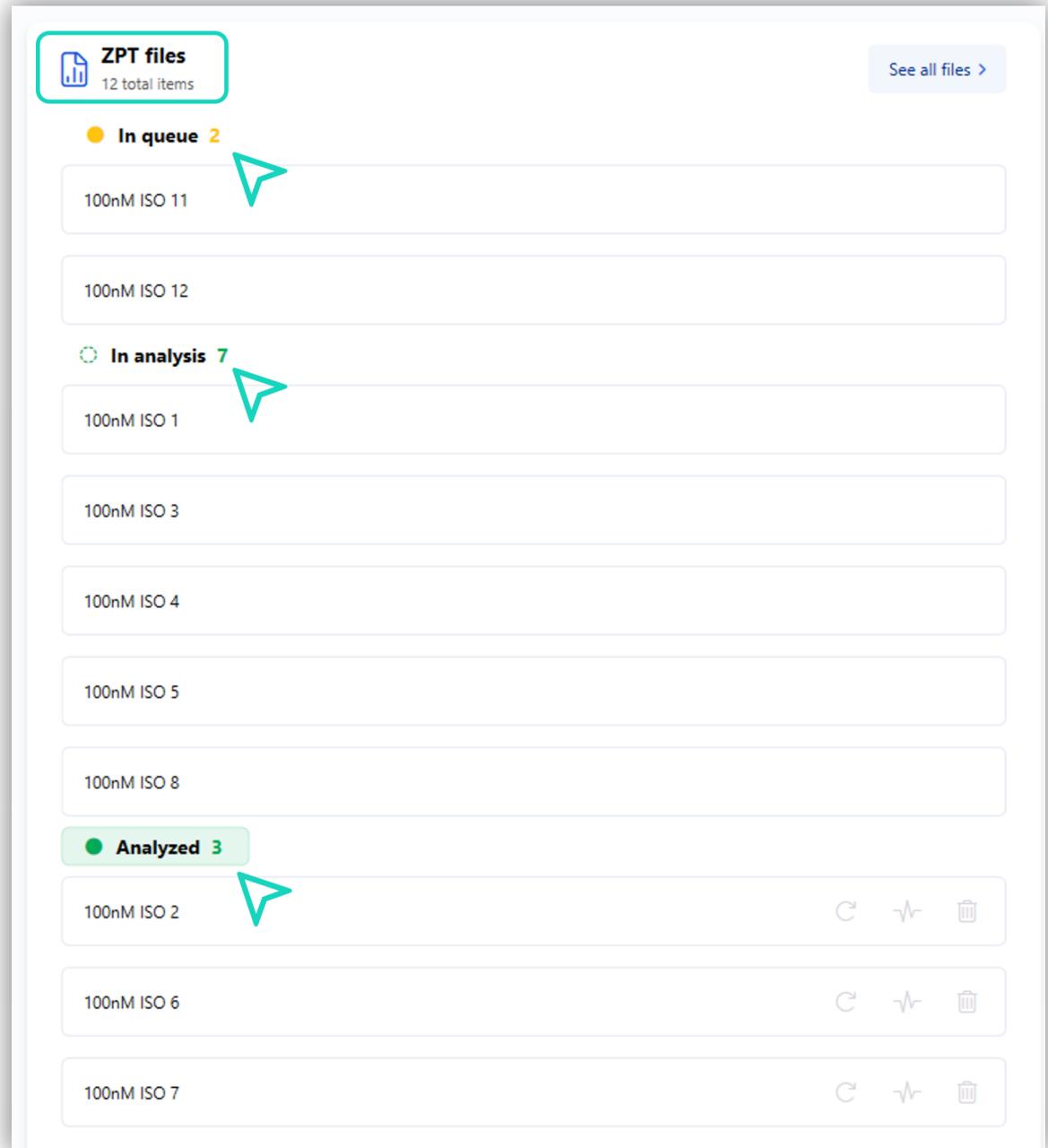
**Figure 4: File selection.** Users can choose files and begin processing data.



## 3. Data analysis

Please note, analysis speed is influenced by both the size of each file and the total number of imported files. CS 3.0 allows for real-time tracking of the analysis progress of imported files on the project's overview page. Users can view a display of the total number of imported files, as well as which files are in queue, under analysis, and have been fully analyzed.

**Figure 5: Tracking analysis progress.** CytoSolver 3.0 allows real-time tracking of analysis progress.



**Figure 4: Single transient.** Selecting a transient from beneath “Transients” displays the individual transient.

After Data has been processed on the overview page, you may proceed to examine data closely on the “Transient Analysis” page.

### Transient analysis page

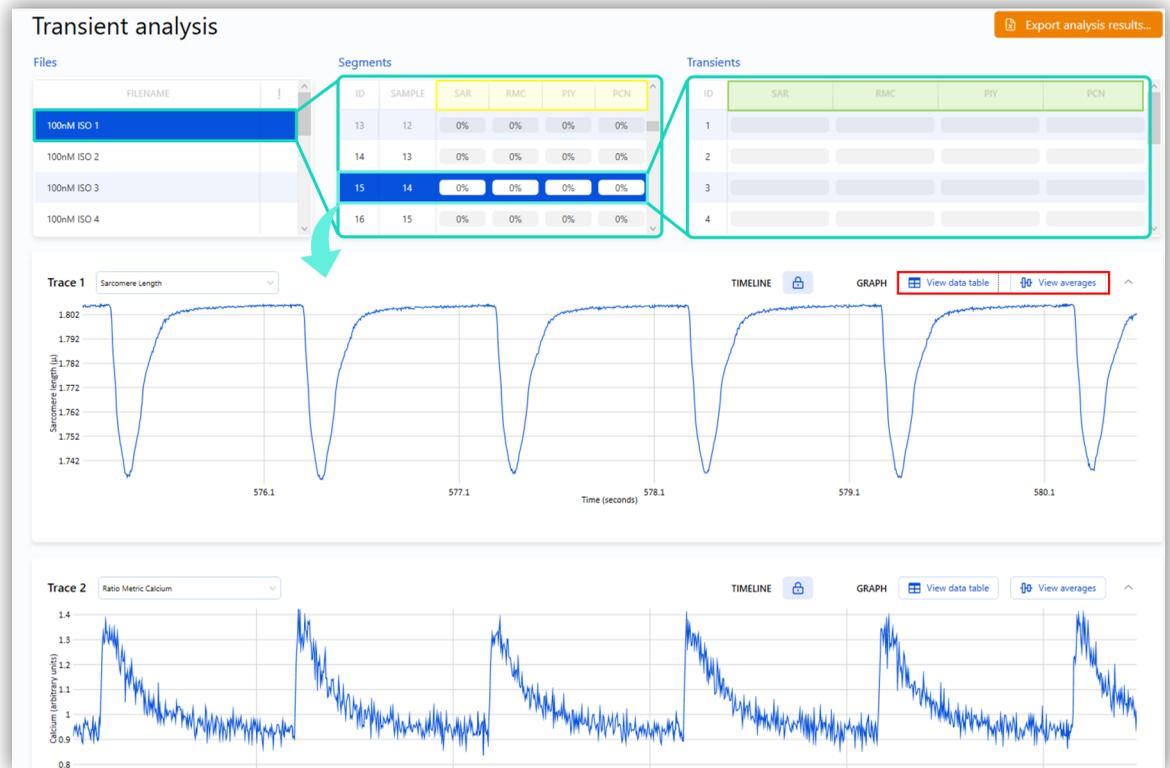
The Transient Analysis page provides a comprehensive and detailed presentation of the analyzed data. At the top of the page, imported files, segments, and transients are prominently displayed. Analyzed traces graphs can be found below the tables, while % accepted vs. rejected traces/segments are reported in the segment table below the yellow box

## Data analysis (cont)

Furthermore, the reason for rejection for each transient within a segment can be viewed in the transients table below the green box. This allows for a more in-depth analysis of the data, enabling users to better understand the nature of the rejected transients and make informed decisions about the subsequent analysis.

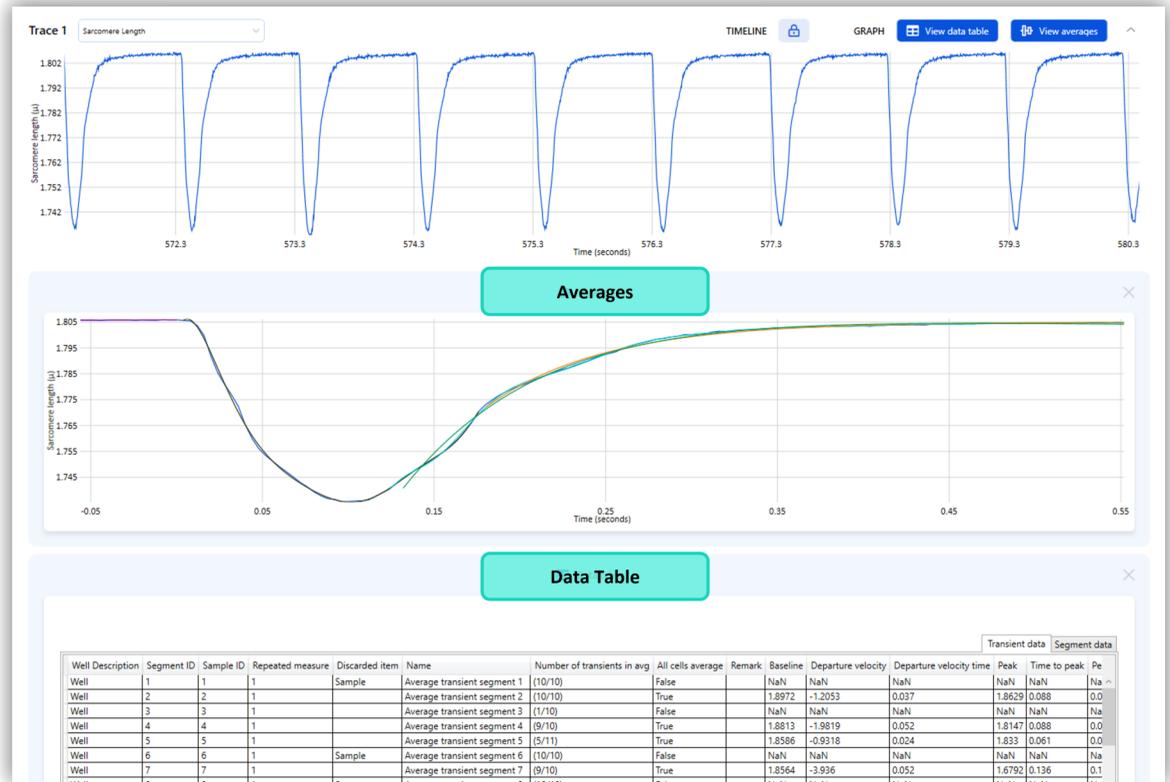
By default, CS 3.0 displays data for the selected file, segment, or transient graphically. In this particular example, segment 15 is being viewed, as indicated below. Notably, the options to "View data table" and "View averages" are not currently selected, as shown in the red box.

**Figure 6: Data display.** Results from selected file, file segment, and segment transients are shown. Users can select which data to show.



To view the averaged data graphs and/or data tables, please select the corresponding button(s). **Once selected, the button will appear solid blue with white lettering.** The data will then be displayed as shown as follows.

**Figure 7: Data averaging and table display.** Data averages and data table can be selected and displayed.



It is worth noting that the Averages and Data tables are displayed for each trace below the graph section of that particular trace. This provides users with a comprehensive and detailed view of the analyzed data, allowing for in-depth analysis and interpretation of the results.

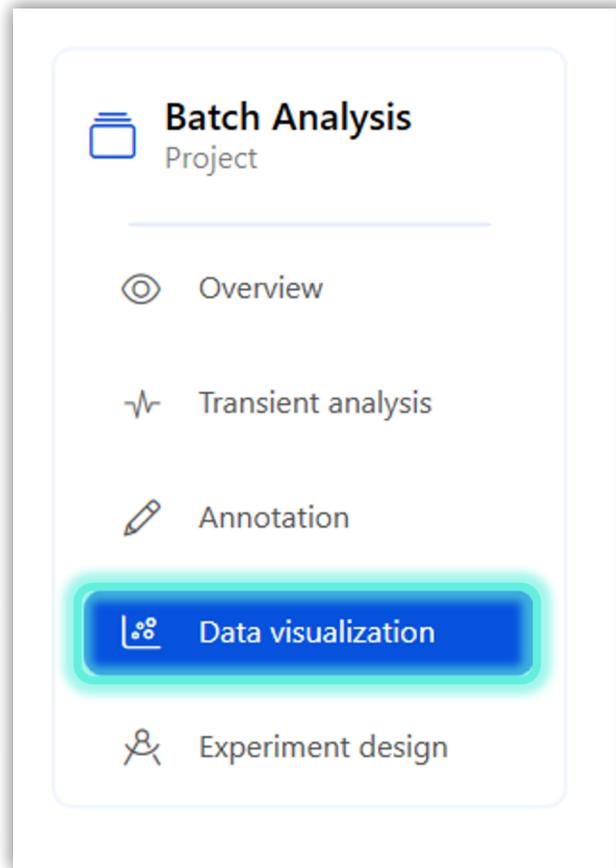
#### 4. Data visualization page (DataVis)

With CS 3.0, users can visualize data in a wide variety of graphs such as bar graphs, plot boxes, normalized data graphs, correlation plots, distribution plots, and many more.

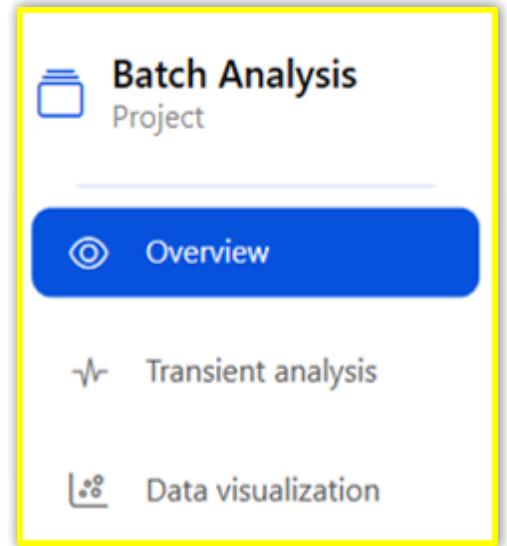
**While data annotation is not required to analyze your data, properly annotating data prevents data misinterpretation within DataVis.** Experiment design is a key component in achieving proper data annotation. We highly recommend referring to the CS 3.0 manual to obtain a more comprehensive understanding of the process.

Once the data has been properly annotated, please proceed to click on data visualization located on the left side of the screen.

**Figures 8 (left) and 9 (right): Selecting data visualization.** DataVis can be used to create visual interpretations of data if an experiment design has been assigned to the project (left). This option will not shown otherwise (right).

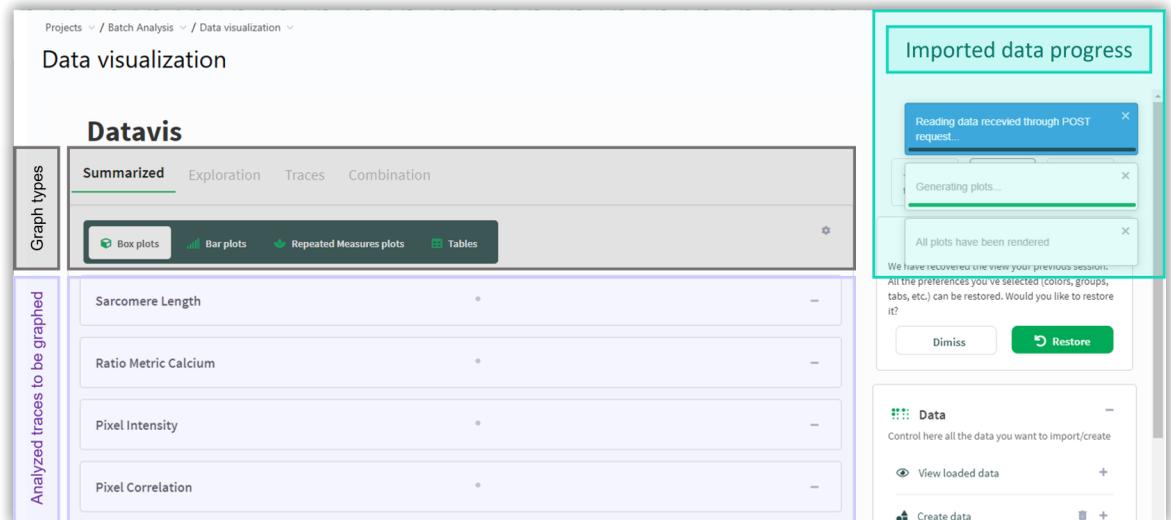


Please note that in the event no experiment design has been assigned to the project, the “Annotation” and “Experiment design” tabs will not be available. This can be observed in the yellow box below.



Upon accessing the Data Visualization page, data processing will be initiated and may require several moments to complete. The data processing progress will be displayed in the top right corner of the (highlighted below for illustration) page, allowing for real-time monitoring of the progress.

**Figure 10: DataVis processing.** Real-time monitoring of data processing.



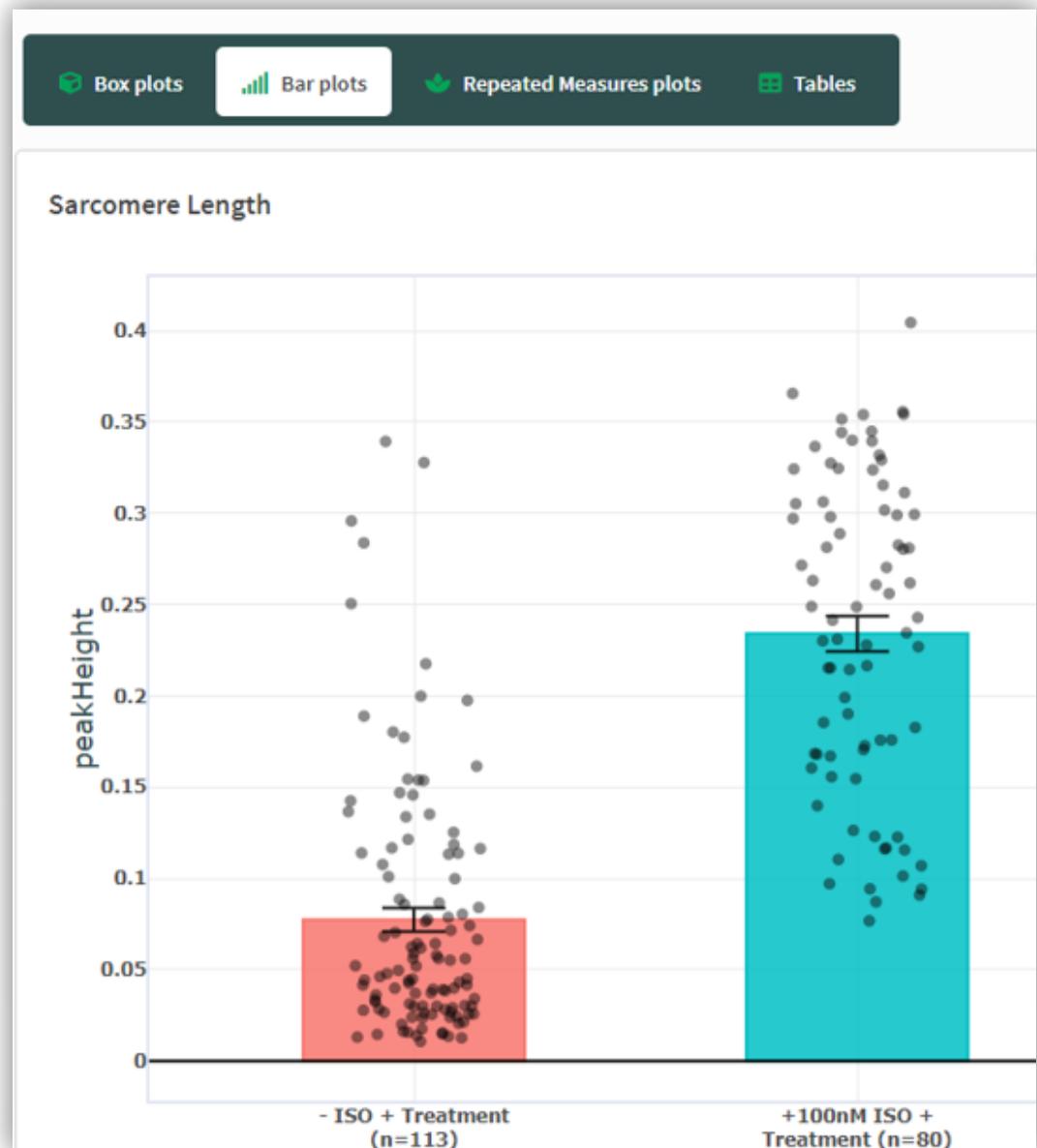
## Data visualization (cont)

In this application note, we show examples of some graphs that can be generated in CS 3.0's data visualization page. However, for a thorough exploration of the page and its complete array of capabilities, we strongly suggest referring to the CS 3.0 manual and accompanying video tutorial.

### Examples of data visualization plots:

#### a. Bar plots:

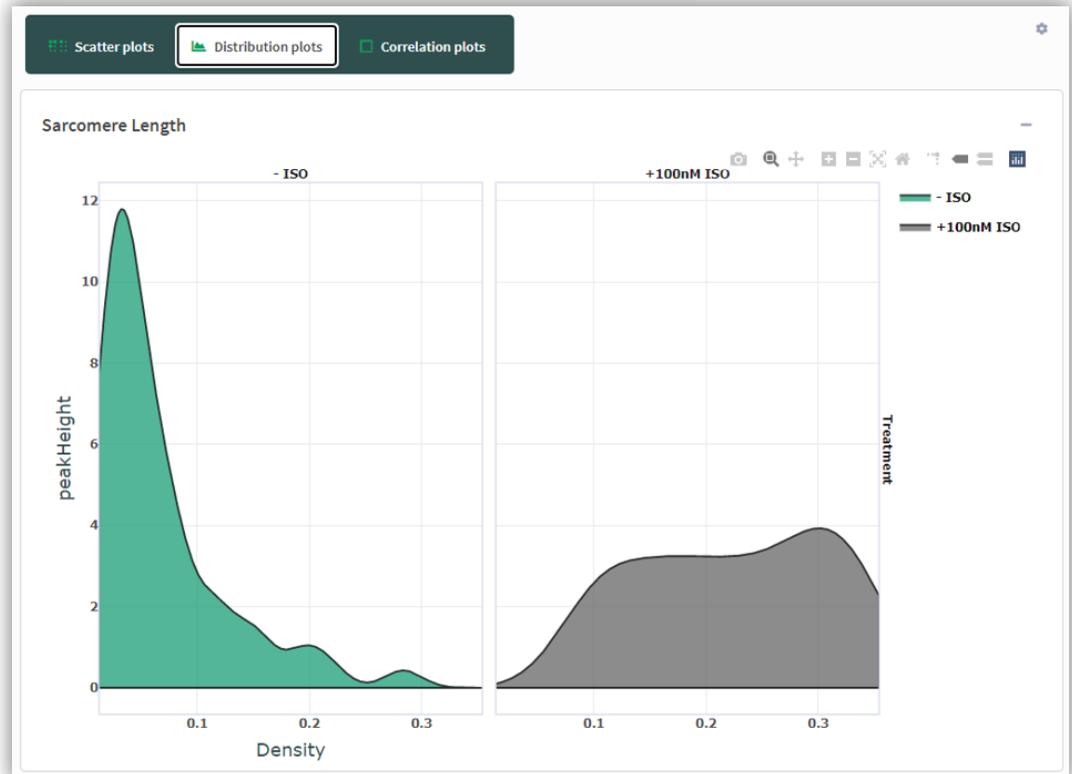
**Figure 11: Example bar plot.**  
DataVis allows users to display bar plots of annotated data.



## Data visualization (cont)

### a. Distribution plots:

Figure 12: Example distribution plot.



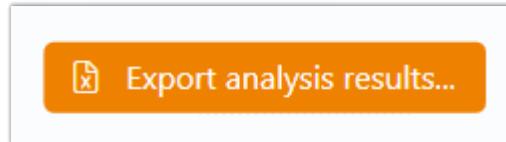
### a. Combination plots:

Figure 13: Example combination plot.

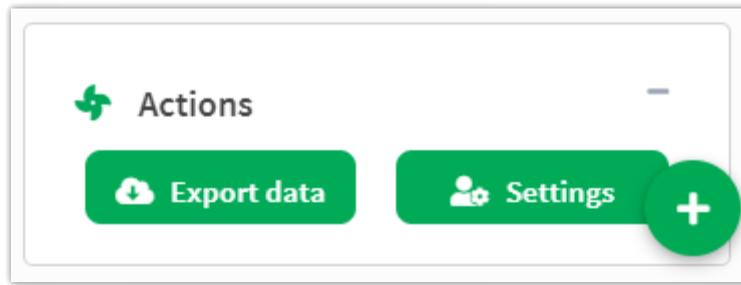


## 5. Data export

CS 3.0 offers two distinct locations from which data can be exported, each serving a specific purpose. The first is the Transient Analysis page, where the user can simply select the "export analysis results" orange box located in the upper right corner.



The second is the "export data" tab situated at the bottom right corner of the Data Visualization page.



For further clarification on the differences between the two export methods, refer to the CS 3.0 manual pages.



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