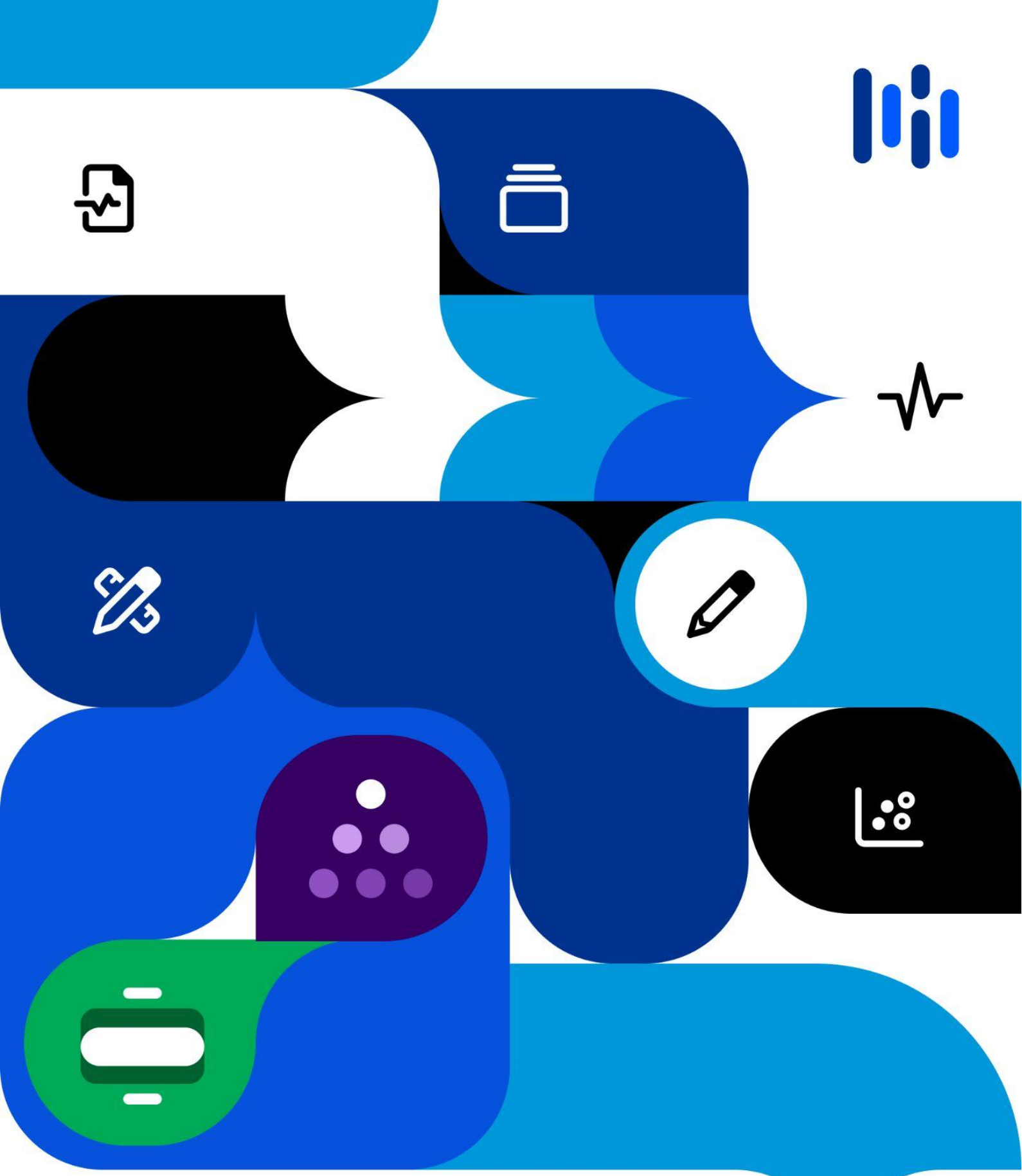


CytoSolver Desktop & Cloud 3.0

User manual

Updated: 2023-05-08

Table of contents

1 Preface

1.1 Document’s scope

This document’s primary objective is to serve as a comprehensive guide for users on how to properly use CytoSolver, as well as a reference to the processes available in the software. It is designed to be the go-to place for users when there are doubts about usage, concepts, troubleshooting, and any other questions related to the software.

Through the following sections, we aim to provide detailed and concise information that will assist users in navigating through CytoSolver. We cover all the essential topics related to the software, including the graphical user interface, project and experiment designs, data analysis tools, and more. We also provide helpful tips and tricks to ensure that users can make the most out of the software's features.

Our goal is to create a document that is accessible and easy to understand for all levels of users, whether they are beginners or advanced users. We strive to use clear and concise language that will guide users through the various features and processes of CytoSolver, ensuring that they get the best results from their data analysis.

Furthermore, we understand that questions may arise from time to time, and we want to make sure that our document is an authoritative source of information. Thus, we have included detailed troubleshooting tips, frequently asked questions, and other helpful resources to help users resolve any issues they may encounter.

We hope that this document will serve as a valuable resource for all users, from novices to experts, and help you achieve your research goals with confidence.

1.2 Document information

This section provides details about the document such as the version numbers, date of creation, and a change log that tracks updates and modifications made to the document over time and ensures users are working with the most up-to-date version.

Doc. version	Created at	Application	Document change log
1.0	2023-04-??	3.0	- First version of the user manual.

Table 1. CytoSolver 3.0 document information

1.2.1 Changelog Cloud

Application	Created at	Application change log
3.0	2023-05-15	- Application release

Table 2. Application changelog for CytoSolver Cloud

1.2.2 Changelog Desktop

Application	Created at	Application change log
3.0	2023-05-15	- Application 3.0 version release

Table 3. Application changelog for CytoSolver Desktop

1.3 Glossary of Terms

This section serves as a reference for users who might be unfamiliar with the technical language we use in this manual, helping to ensure the information is clear and easy to understand.

The glossary will help clarify misunderstandings or confusion about specific terms, allowing you to effectively use CytoSolver products.

Terms are in alphabetical order to ease the task of finding a term.

Term	Description
Annotation	In CytoSolver, this term refers to the tool that tags segments within the system. When a segment is tagged, it's considered "annotated". This tool collaborates with experiment designs to label the data accurately and improve viewing of results.
Artifact	Data anomaly that needs action during analysis or should be highlighted.
Background constant	Numerical value that is subtracted from the raw data to correct for background noise. By eliminating this noise, the resulting data is more precise and accurate.
Background measurement	A quantification of a signal from an empty area to be used as a reference point for correcting noise.
Epoch	A time segment where data are measured continuously without a pause. The end of an epoch is indicated by a red line in the data trace or the end of the file. One epoch usually contains the measurement of only one cell. Its equivalent in CytoSolver is “segment”.
Event	Change in a signal or parameter that is detected by IonWizard software and marked for further analysis.
Known trace	A "known trace" refers to a trace type that is recognized and understood by the CytoSolver system, which has a dedicated algorithm to analyze it.
Measurement	A quantification of a specific parameter or signal obtained from a biological preparation (sample) using IonOptix hardware. Examples of measurements include: calcium transients, force generation, sarcomere length among others.
Pacing frequency	The rate at which electrical stimuli are delivered to the sample to induce rhythmic contractions. It is typically measured in Hertz (Hz), which refers to the number of electrical stimuli delivered per second.
Repeated measurement	Acquiring the same parameter or signal multiple times from a single biological preparation (sample) under various experimental

Term	Description
	conditions, such as adding a drug or inducing hypoxia. This feature allows for paired statistical tests.
Sample	A sample stands for a location where a measurement takes place. This can contain one measurement or multiple measurements in an experiment (e.g. before and after a treatment), but always from the same location. For isolated cardiomyocytes, a sample usually equates to a myocyte. For iPSC-CM, a sample is usually a cluster.
Sampling frequency	The rate at which analog signals are acquired from the IonOptix hardware.
Segment	Equivalent to an epoch in IonWizard, except that it takes the text marks in IonWizard ('begin', 'end', and 'break') into account which can truncate an epoch or break an epoch into multiple segments.
T0 (T-zero)	The initiation time of a transient as detected by software, measured in seconds.
Tenant / Tenancy	Tenants represent a group of users, or in other words, an organization. Each organization has exclusive access to its own data that it can share within itself but not cross-organizations. This system provides greater control and customization for the users with the help of Organizational units, single-user permissions, and group-level permissions without the worry of leaking any data outside of itself.
Text mark	A tag or comment added manually using the IonWizard software that can be read by CytoSolver. It can contain only one of the following values: "Begin", "End", "Break", "Delete", "Background". See more information and usage in the dedicated section, 6.2.2 What are event marks and what are they used for.
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.*
Transient	A section within a segment that is usually delineated by pacing marks or, if the cells/samples are unpaced, by the CytoSolver application.
Unknown trace	This refers to a trace type that is unfamiliar to the CytoSolver system, causing the absence of a specialized algorithm for analyzing it.
ZPT File	"ZPT" stands for *Zone Plot Trace* file and it's the file format a data acquisition process results in when using IonWizard Software. We refer to these files simply as "ZPT files".

Table 4. Glossary of terms

2 Description of the software

2.1 Overview

CytoSolver is an efficient tool for automatic data analysis that streamlines the research process, both in a cloud-based and a (Windows) desktop version. With just a few clicks, you can upload batches of data and let CytoSolver do the rest.

CytoSolver is versatile and works well for analyzing large amounts of data in a variety of contexts. Whether you're analyzing hundreds of cells at once using MultiCell or MultiCell Lite, or examining data collected using traditional Calcium and Contractility methods, CytoSolver simplifies your research and accelerates your discoveries.

2.2 Purpose of the product

The software is ready to analyze your acquired data, automatically rejecting transients that don't meet your customized parameters, performing data averaging, and fitting curves for you, all in a matter of seconds. But CytoSolver is more than just a data analysis tool. It also allows you to easily organize your files and experiments using its project feature. This means that you can keep related data and experiments together in one place for easy access and management.
With the experiment design feature, you can easily create and attach experiments to your projects. This allows you to keep track of your experimental variables, and ensures that your data analysis is based on a well-designed experiment.

In addition to its powerful data analysis capabilities, CytoSolver also includes a tool to generate a summary of your results. This summary provides an overview of your data, which is especially helpful when presenting your findings to others or for quickly reviewing your research.

All of these features work together to make CytoSolver an efficient and effective tool for streamlining your research process, organizing your data and experiments, and generating clear and concise results summaries.

2.3 Intended uses

The intended uses of a product or service are critical to understanding its value and relevance for specific purposes.

Next we are defining what has CytoSolver been envisioned for:

1. **Automatic data analysis:** CytoSolver is intended to provide fast and efficient automatic data analysis for researchers. Its features include transient rejection, data averaging, and curve fitting.
2. **Customizable rejection parameters:** CytoSolver allows users to customize rejection parameters to suit their research. This enables researchers to have greater control over the quality of the data analyzed.
3. **Organizing files and experiments:** CytoSolver includes a project feature that allows users to organize their files and experiments. This makes it easy to keep related data and experiments together in one place for easy access and management.
4. **Generating results summaries:** CytoSolver includes a tool for generating summary results. This feature allows researchers to quickly review and present their findings to others.
5. **Versatility:** CytoSolver is versatile and works well for analyzing large amounts of data in a variety of contexts. Whether you're analyzing hundreds of cells at once using MultiCell or MultiCell Lite, or examining data collected using traditional Calcium and Contractility methods, CytoSolver simplifies your research and accelerates your discoveries.

However, we also have a list of **NOT** intended uses of CytoSolver. They are as follows:

1. **It's NOT a replacement for experimental design:** Although CytoSolver includes an experiment design feature, it is not intended to replace thoughtful experimental design. It is still essential to carefully plan and execute experiments in order to obtain reliable and relevant data.
2. **Does NOT serve as substitution for expert interpretation:** CytoSolver's automatic data analysis features are designed to assist in data interpretation, but they are not intended to replace expert interpretation. Researchers should always review and interpret their results in the context of their specific research question and domain knowledge.
3. **Does NOT provide primary data storage:** While CytoSolver allows you to organize and manage your data, it is not intended to be a primary data storage solution. It is important to securely store your data in a separate and reliable location to ensure its long-term accessibility and preservation. Nevertheless, we are committed to ensuring the security and privacy of your data, allowing you to confidently use our product for your daily research needs.
4. **NOT intending to provide conclusive results:** CytoSolver's analysis is based on the data and parameters provided by the user, and its results are dependent on the quality and suitability of the data. While it can assist in drawing conclusions from data, it is not intended to provide definitive or conclusive results. Researchers should always

exercise caution and consider the limitations and uncertainties associated with any data analysis tool.

As a provider of software for data analysis, it is important to clarify that we are not responsible for any errors, misinterpretations, or other issues that may arise from the use of our product in the context of research.

While we strive to provide the highest level of accuracy and reliability in our software, it is ultimately the responsibility of the user to carefully review and interpret the results in the context of their specific research question and domain knowledge. We strongly advise users to exercise caution and to carefully review their results before drawing conclusions or making decisions based on them. Furthermore, our product is not intended to replace thoughtful experimental design or expert interpretation, and we are not responsible for any issues that may arise from the use of our product in this context.

By using our software, users agree to release us from any liability arising from the use of our product, including but not limited to errors, misinterpretations, or other issues that may arise in the context of research.

3 Setup and updates

Before you begin using the software, it is important to ensure that your device meets the minimum system requirements.

In this section, you will find step-by-step instructions for downloading and installing the software, as well as information on how to configure any necessary settings. Whether you are a new user or upgrading from a previous version, this section will help you get up and running quickly and easily.

3.1 Installation and access

Properly installing software is a crucial step before using it on your device. However, the installation process can vary depending on the type of software and the operating system you're using. In this section, we will guide you through the installation process for desktop software and provide instructions on how to access the web version.

3.1.1 Installation of CytoSolver Desktop

First, we will cover the installation of CytoSolver Desktop software, which is typically downloaded and installed directly on your computer. We will provide step-by-step instructions on how to download and install the software, as well as any prerequisites or system requirements that may be necessary for installation. Additionally, we will offer tips on troubleshooting any issues that may arise during the installation process.

By following the instructions provided in this section, you can ensure a successful installation of Cytosolver.
The installer for CytoSolver Desktop can be acquired in two ways, if you have access to the CytoSolver Cloud platform you can download the installer yourself from the user sidebar menu. Refer to chapter 4.2.5 Download CytoSolver for Desktop for the exact location of the download. If you do not have access to the platform you can always request the installer from the IonOptix Sales team at info@ionoptix.com.

After acquiring the installer for CytoSolver Desktop please follow the steps:

1. Run the “CytoSolver Desktop Installer 3.0” that you’ve downloaded. Agree to the terms of service, and click the Install button;

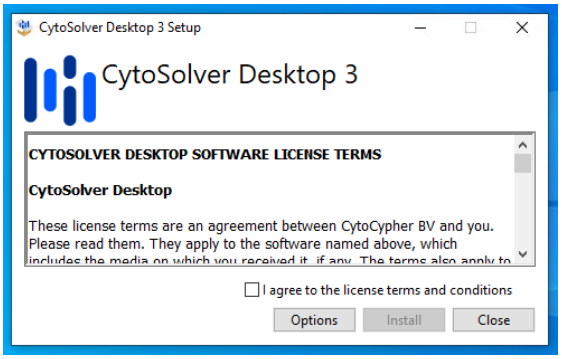


Figure 1. CytoSolver Desktop 3 Setup

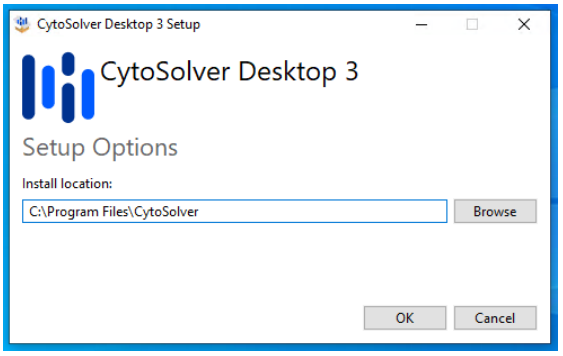


Figure 2. CytoSolver Desktop 3 Setup Options

2. The installation process will begin with preparation of any files that need to be copied or installed for the application to work. After that’s done the installer will ask you about your Registered email and product key that you can acquire from the IonOptix Sales team;

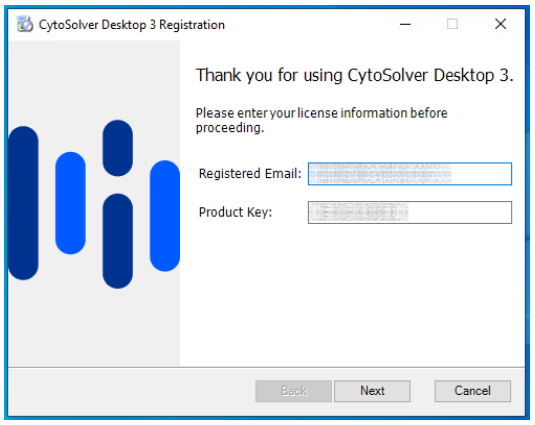


Figure 3. CytoSolver Desktop 3 product key validation.

3. After filling in your information and clicking on the next button, the installation process will continue and finish the installation. From here you can close the installer and start using CytoSolver Desktop right from your computers desktop.

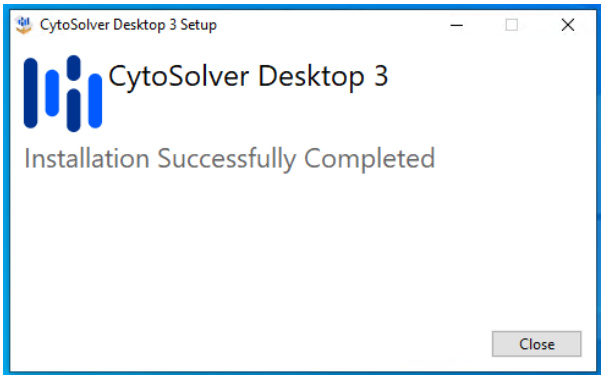


Figure 4. CytoSolver Desktop 3 successful installation.

3.1.2 Access to CytoSolver Cloud

Secondly, we will discuss how to access the web version of the software. Unlike the desktop software, the web version doesn't require installation, but rather access through a web browser. We will provide information on which web browsers and operating systems are compatible with the software, and we will offer instructions on how to access and use the software via your browser.
By following the instructions provided in this section, you can ensure easy access to the web version CytoSolver Cloud software through your browser.

3.1.2.1 Minimum system's requirements Desktop

The following section outlines the minimum system requirements to run CytoSolver on a Windows operating system. It's important to ensure your system meets these requirements to avoid any potential performance issues or software errors. Please note that while we have tested CytoSolver extensively, there may be other factors unique to your system that could affect its performance.

Here are the minimum system requirements for Desktop:

	Minimum	Recommended
Operating system	Windows 10 x64 (1709 and later)	
Processor	AMD A8 / Intel® Core™ i3	AMD Ryzen™ 3 / Intel® Core™ i5
Memory	4 GB RAM	8 GB RAM
Graphics	Integrated	Dedicated
Storage	5 GB	10 GB

Table 5. CytoSolver Desktop minimum system requirements

Please ensure that your computer meets the minimum system requirements before installing and running CytoSolver. Additionally, please note that other applications running in the background may affect CytoSolver's performance. We recommend running CytoSolver in a clean environment to have an optimal experience.

In addition to the requirements for browsers and operating systems, there is also a list of recommended software to use in conjunction with CytoSolver. Having these recommended tools installed is NOT mandatory, but they can help you get the most out of CytoSolver, streamline your workflow, and facilitate data analysis.

Here's the list of recommended software:

1. **IonWizard 6 or higher**, contact IonOptix sales representative for more information, info@ionoptix.com

3.1.2.2 Browser's requirements Cloud

The CytoSolver user interface is a web application that can be accessed through most modern web browsers. Our team has tested and optimized the application for most of the major browsers. This is the information about the browsers we work with and their respective versions:

Browser	Version
Google Chrome	94+
Mozilla Firefox	92+
Microsoft Edge	94+

Table 6. CytoSolver Cloud browser's requirements

While other web browsers may work, we recommend using one of the aforementioned browsers for the best experience. It's also recommended that you keep your browser up-to-date with the latest version available to ensure compatibility and security.

3.2 Updates

As software is continually evolving, updates and upgrades are frequently released to improve functionality, fix bugs, and add new features. Keeping your software up to date is essential to ensure optimal performance and security. Updates of CytoSolver Desktop are distributed through new installers and follow the same process as the installation of a new application. Updating your application also requires your email and product key so make sure that you have them ready before starting off the installer.

4 Account Cloud

For any information on the CytoSolver products, account types, acquiring licenses, and any other questions please contact our sales team at ***info@ionoptix.com***.

4.1 Account types

The application provides two account types out of the box. Those are the admin account and the user account. When creating a tenancy, the provided user account information will be turned into an admin account. The admin account has all the available permissions for their tenancy and can create new admins and users. More about what an admin account can do and more information on the topic of permissions can be read in the "6.2 Understanding "Administration" (admins) Cloud" section.

In the following sections, we will go over the user account-related settings and preferences that all accounts have access to.

4.2 User menu

To access the user menu you can click on your name or portrait in the top right corner of the application. Doing so you will see a sidebar open up from the right side of your window.

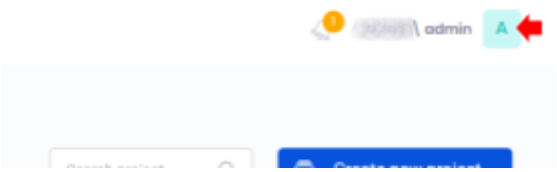


Figure 5. User menu.

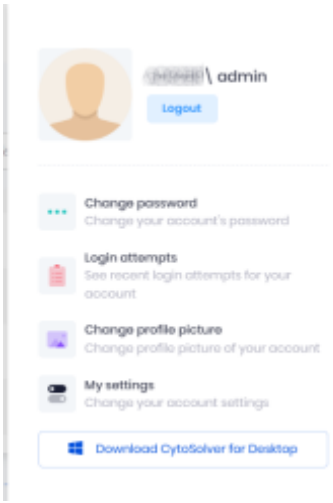


Figure 6. User sidebar

In this sidebar you will be able to find options for changing your password, insight into recent logging attempts, changing your profile picture (portrait), and a number of personal preferences.

4.2.1 Change password

First option on your User menu will be the option of changing your current password. This can be simply done by entering your current password, and the new password, and as a verification repeating your new password again. The password complexity settings are configured to by default require the following characters:

- At least a single digit
- Lowercase
- Non-alphanumeric
- Uppercase characters

The password's length is also required to be at least eight characters long. All of those settings can be configured by your Tenancies administrator. More on the configuration of password complexity settings can be found in the "6.2 Understanding "Administration" (admins) Cloud" section.

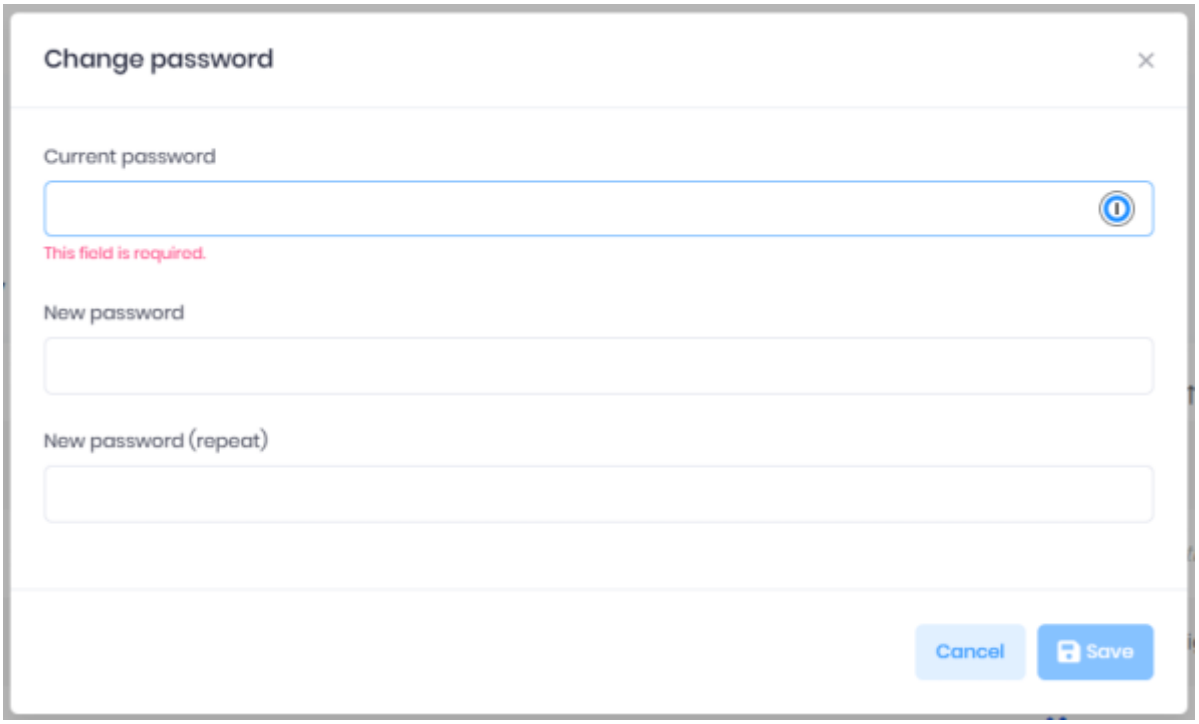


Figure 7. Change password.

4.2.2 Login attempts

In the following menu item you will find the Login attempts screen. On this screen, you can review any past login attempts that have been done by your account. With this screen, users and admins can audit their accounts' login attempts.

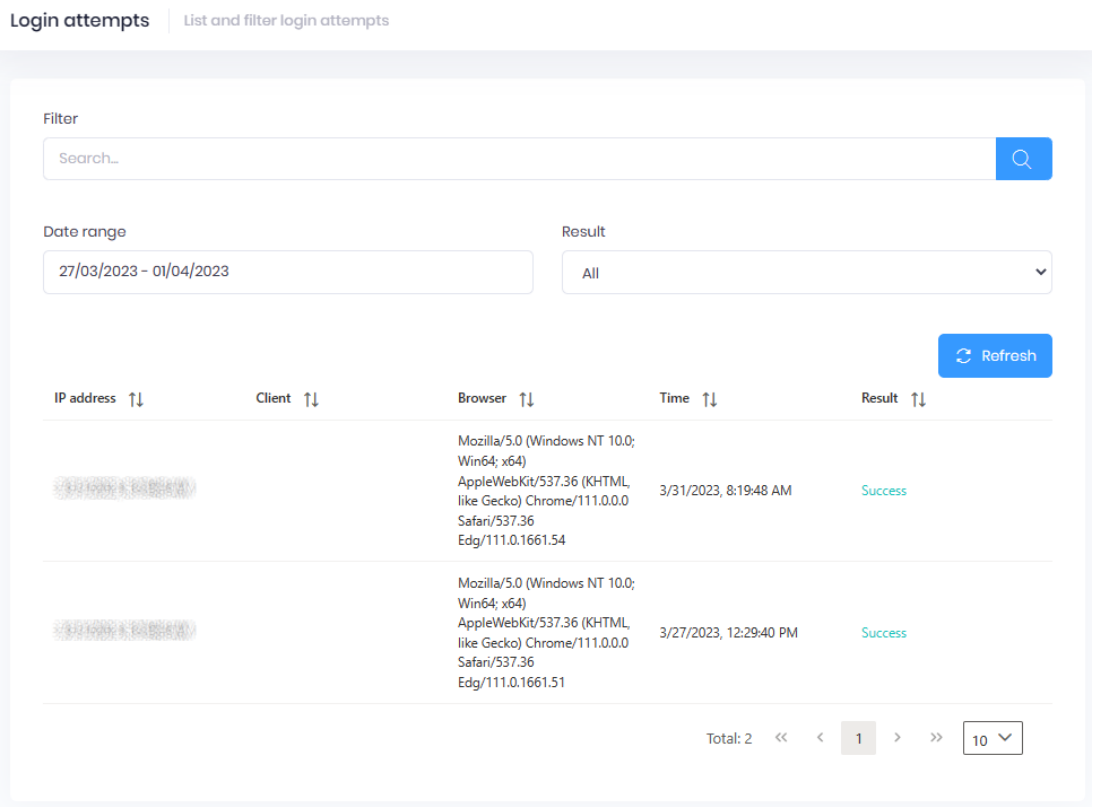


Figure 8. Login attempts.

4.2.3 Change profile picture

Users also have the ability to change their profile picture (also called portrait). Profile pictures are used in a number of places, e.g., the User menu sidebar, and user lists. What's important to remember is that the profile pictures can be seen by everyone within the tenancy.

Figure 9. change profile picture.

4.2.4 My settings

User settings enable the user to change their previously filled-in personal information (except for the initial administrator not being able to change their username). This is where all users can change their first name, surname, email, user name, and also importantly, their preferred time zone.

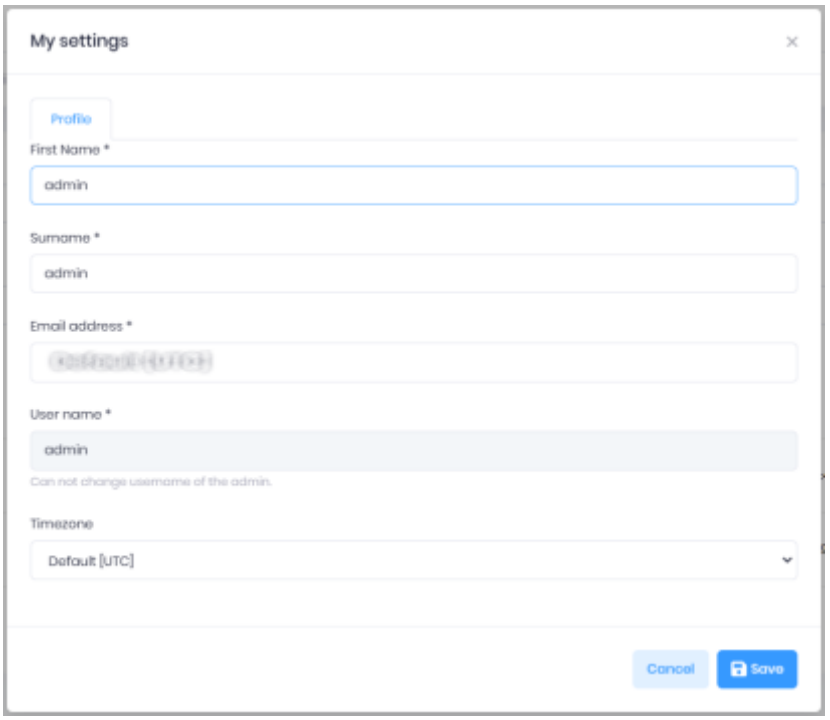


Figure 10. CytoSolver Cloud - My settings

4.2.5 Download CytoSolver for Desktop

Last option in the menu is for the user to download CytoSolver for Desktop. For returning users this version of the application will feel more familiar as it is built on top of our previous CytoSolver Desktop 2.0 version. In the latest version, 3.0, you can use all of the standard features of the CytoSolver product, i.e., Projects, Experiment designs, Transient analysis, and even the Data visualization tools. **The main feature that is missing from the Desktop application is the collaborative aspect of the CytoSolver Cloud.**

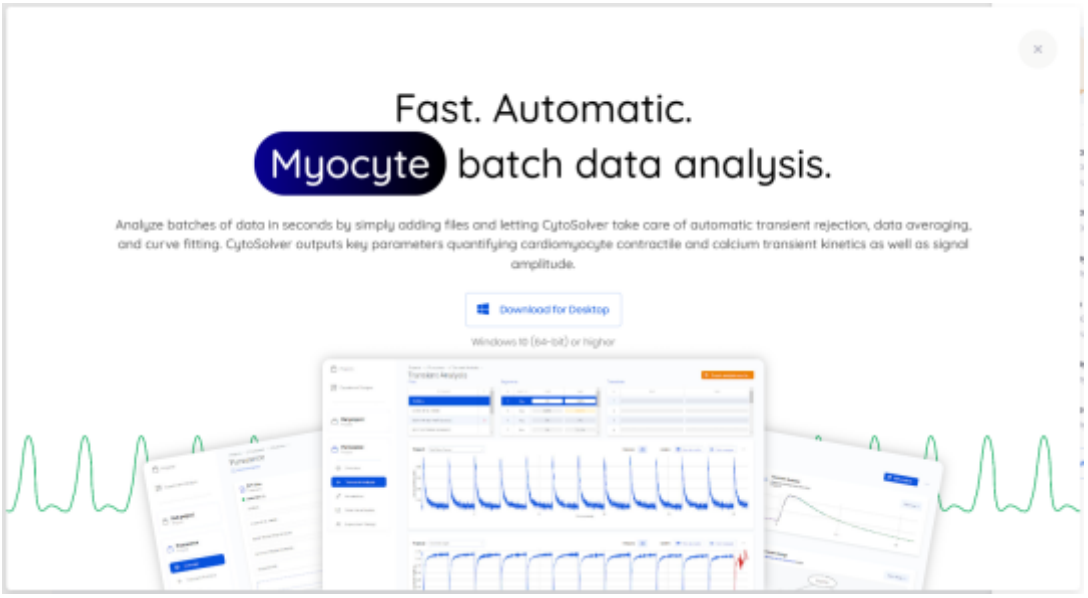


Figure 11. CytoSolver Cloud - Download CytoSolver for Desktop

5 Instructions before use

This section provides information and guidelines for preparing the data acquired with IonWizard software before using the CytoSolver software to perform the analysis. You will find information on the supported ZPT-formatted files, and all you need to know about them to correctly perform your analysis. Additionally, you will find instructions on how to perform custom modifications to your files which are later reflected on the CytoSolver tool.

5.1 Understanding the Graphical User Interface (GUI)

The graphical user interface (GUI) of a software application is crucial for a user-friendly and intuitive experience. In the case of CytoSolver, the GUI provides easy access to the main features and functions of the software. This section will provide an overview of the CytoSolver GUI, including the layout of the interface, data management options, and customization settings.

The purpose of this section is not to address issues with the intuitiveness or user-friendliness of the CytoSolver software. Rather, the focus is on providing an explanation of the updated graphical user interface (GUI) in version 3.0. This is because there are significant differences between the previous GUI and the current update, and it is important for users to understand these changes in order to make the most of the software's capabilities.

5.1.1 Layout

The layout described below consists of simple, familiar elements that are commonly found across various platforms.

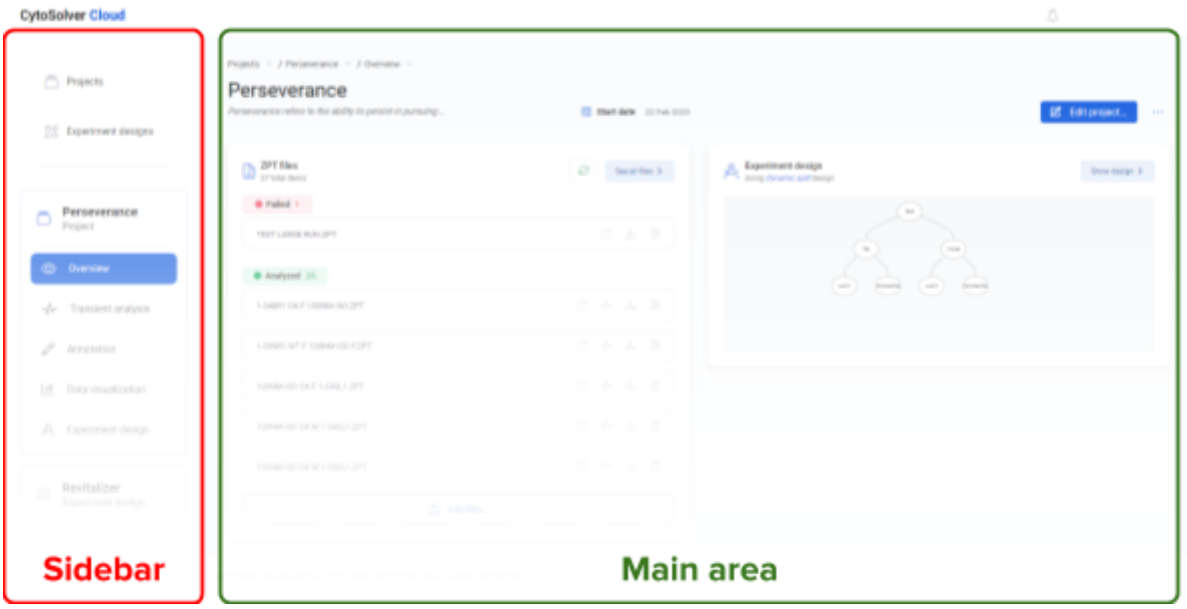


Figure 12. CytoSolver 3.0 General layout

5.1.1.1 Sidebar

In the GUI the sidebar is located on the left-hand side of the screen. It is an essential component of the GUI, as it provides users with access to various tools and features. The sidebar alone **enables you to navigate through all available pages** on the CytoSolver platform.

At the moment, the sidebar cannot be minimized, meaning it remains open and accessible at all times, providing constant access to the project and experiment design explorers.

The sidebar contains two main sections:

1. Explorers
2. Recently opened items

When a tool, project, or experiment design is selected and its contents are displayed or actions are performed on it, it becomes "active". The active state is denoted by a marine blue hue which indicates the current focus of the application. For instance, when a tool is selected, the background of the tool's name in the sidebar turns blue, providing a clear indication of the current location within the UI.

5.1.1.1.1 Explorers

CytoSolver's explorers are basic menus that provide a list of specific items for easy access. The software features two explorers: the project explorer and the experiment design explorer. The *project explorer* displays a list of all projects that have been created, allowing users to quickly navigate to the desired project. By clicking on a project in the project explorer, you can view the files and data associated with that project.
The *experiment design explorer* displays a list of all experiment designs that have been created, allowing users to quickly navigate to the desired design. By clicking on an experiment design in the explorer, you can view the structure of the experiment.

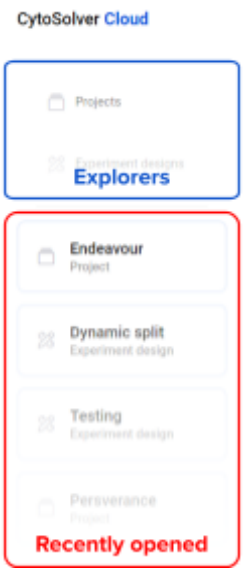


Figure 13. CytoSolver 3.0 Sidebar layout including explorers and recently opened items.

5.1.1.1.2 Recently opened

The "Recently used" section displays all the projects and experiment designs that have been opened and interacted with. This is a dynamic area that constantly updates to show the most recently used items at the top. You can also remove items from this section if you no longer need them. In Cytosolver Cloud, you can even reorder this list to better suit your needs, while for CytoSolver Desktop the order is fixed.

Projects and experiment designs are placed together in this section, without any specific grouping. To differentiate between them, you can either:

1. Read the label right underneath each item on the list, which specifies whether it is a "Project" or an "Experiment design", or
2. Look at the iconography. Projects are represented by a rectangle with two horizontal lines on top, while Experiment designs are represented by a pencil and a ruler forming a cross together. Refer to the table below to become familiar with the icons.

Figure 14. Icon for a Project.

Figure 15. Icon for an Experiment design

When clicking on an item from the sidebar it will immediately show content on the designated “Main area”. However, the behavior of each sidebar item is different, depending on if it’s a project or an Experiment design.

Project items on the sidebar have submenus or “Tools”. When clicking on the project’s name the item expands/collapses to show/hide the tools you work with. When opening a new project, by default, the item selects the project’s “Overview” tool.

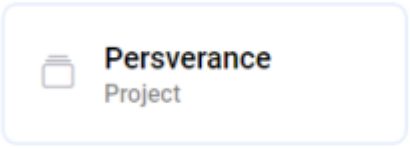


Figure 16. Example of collapsed project item on the sidebar.

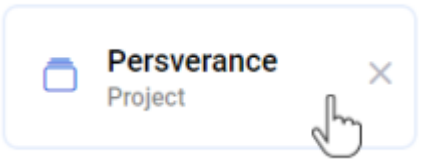


Figure 17. Example of collapsed project item on the sidebar. Hovered on to show closing action.

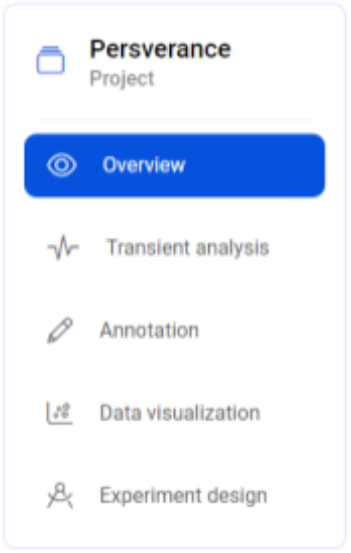


Figure 18. Example of expanded Project item on the sidebar.

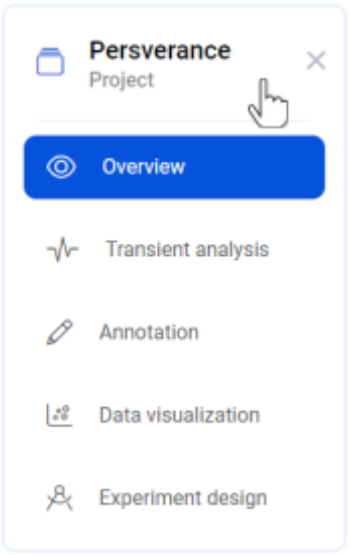


Figure 19. Example of expanded Project item on the sidebar. Hovered on to show closing action.

Experiment design sidebar items are a bit more simple. They do not contain subtools, hence clicking on them will directly navigate to the design editor.

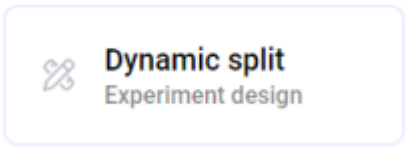


Figure 20. Example of regular (not selected) Experiment design item on the sidebar.

Figure 21. Example of viewed (selected) Experiment design item on the sidebar.

5.1.1.2 Main area

The main area of the CytoSolver interface is designed to be a versatile and dynamic space where you can:

1. View results
2. Conduct analysis
3. Create visualizations, and
4. Organize your projects.

Its layout is carefully designed to allow users to easily navigate through different views and tools, while also maximizing the available screen space to display relevant data and visualizations.

5.1.1.2.1 Header

In a user interface, the header typically refers to the topmost section of the application window or webpage. In our case, it contains important elements such as an additional navigation menu, search bars, contextual menus, etc.. The header serves as a consistent visual reference point for the user and can provide quick access to essential features and functions of the application.

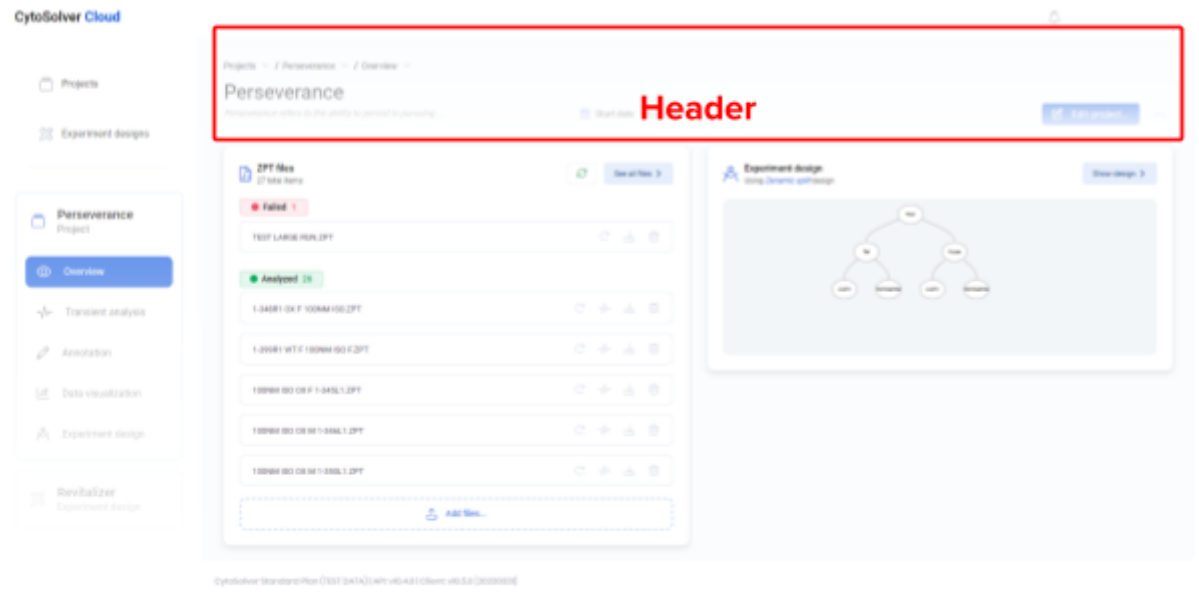


Figure 22. CytoSolver 3.0 Header.

Next we are describing the most important UI elements for this CytoSolver 3.0 version and how to use them.

5.1.1.2.1.1 Contextual menu

Following its definition, "*A contextual menu is a UI element that appears upon some interaction with the interface, and offers a limited set of choices that are available in the current state, or context, of the operating system or application to which the menu belongs.*"

Put in other words, it's a menu that offers you a shortcut to tools or actions that are related to the specific context you're in. Examples of these menus are the right-click buttons you find in many interfaces, but also buttons that are specific to a screen.

In CytoSolver's case, a contextual menu is a menu that appears when clicking on buttons marked with three dots spread across the interface.

Figure 23. Example of contextual menu icon.

Some **examples** to help you understand where are they placed and why:

1. In the Projects explorer you find a list of Projects that you can access. At the end of each line there's a contextual menu offering quick actions that might be useful when using the list, such as editing the project, exporting its results, deleting it, etc.
2. In the Project's Overview tool you find a contextual menu at its top-right corner. Again, it contains project-related actions, a bit more enriched than the previous menu described earlier, but they are all meant to perform actions solely for project.

5.1.1.2.1.2 Pathway

In the Cytosolver context, "pathway" (a.k.a *breadcrumbs* or *path trails*) refers to a navigational element that shows the user's current location within the app. It typically appears as a horizontal trail of clickable links, with each link representing a higher level of the hierarchy of the current page.

For example, a pathway for a project called "Endurance" might look like "Projects > Endurance > Overview" with each link leading to the corresponding page at that level of the hierarchy. Pathways provide users with a way to easily understand their location within a site, navigate back to higher-level pages and access page-specific actions.

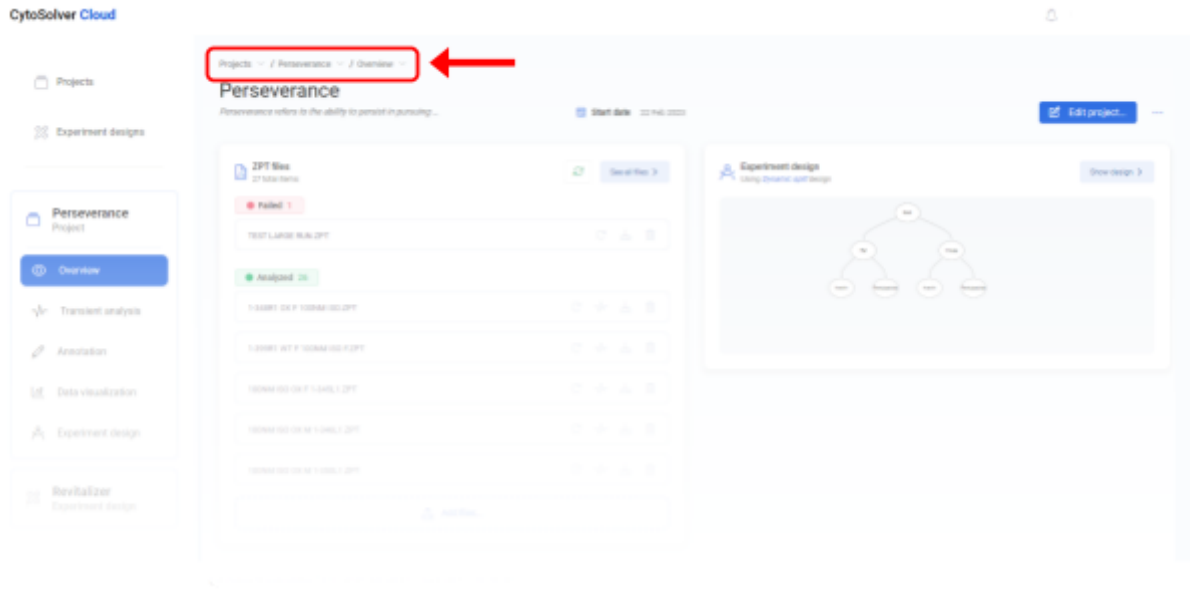


Figure 24. CytoSolver 3.0 pathway location. Both for Projects and for Experiment designs.

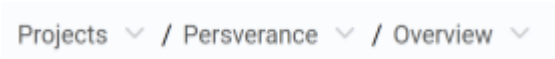


Figure 25. Cytosolver 3.0 pathway example for Projects.

Figure 26. Cytosolver 3.0 pathway example for Experiment designs.

The pathway will have a structure of 2 or 3 levels:

1. **Level 1:** Contains the Projects or the Experiment designs explorer. By clicking on the downward-pointing arrow you can access any of them.
2. **Level 2:** Contains the selected Project or Experiment design. By clicking on the downward-pointing arrow you can switch between selected Projects or Experiment designs while remaining within the same category, such as from one Project to another or from one Experiment design to another.
3. **Level 3:** Only shown for Projects, as they contain additional tools in comparison to Experiment designs. Here you can switch in between:
    a. Overview of the project
    b. Transient analysis
    c. Annotation
    d. Data visualization
    e. Experiment design

Level 3 also features a collection of tools that are present in other locations of the system, but have been placed here for convenience:

1. Add files: Allows you to add more files to CytoSolver.
2. Criteria settings: Opens the Criteria settings modal.
3. Export analysis results: Opens the export modal.

The pathway will remain fixed at the upper left corner of the main area, irrespective of the number of levels it contains

TIP

Switching between tools for different projects is easy using the second level of the navigation. For instance, if you are currently viewing the analysis results of the "Endurance" project under "Projects > *Endurance* > Transient Analysis", you can switch to another project like "Persistence" by expanding the second level of the pathway, selecting "Persistence", and navigating to "Projects > *Persistence* > Transient Analysis". This is where the results for the "Persistence" project are displayed.

5.1.1.2.2 Content/Action area

The main area of operation is where you will view results and use tools to add new data, export calculations, tag your files, and more. This area is different for every page.

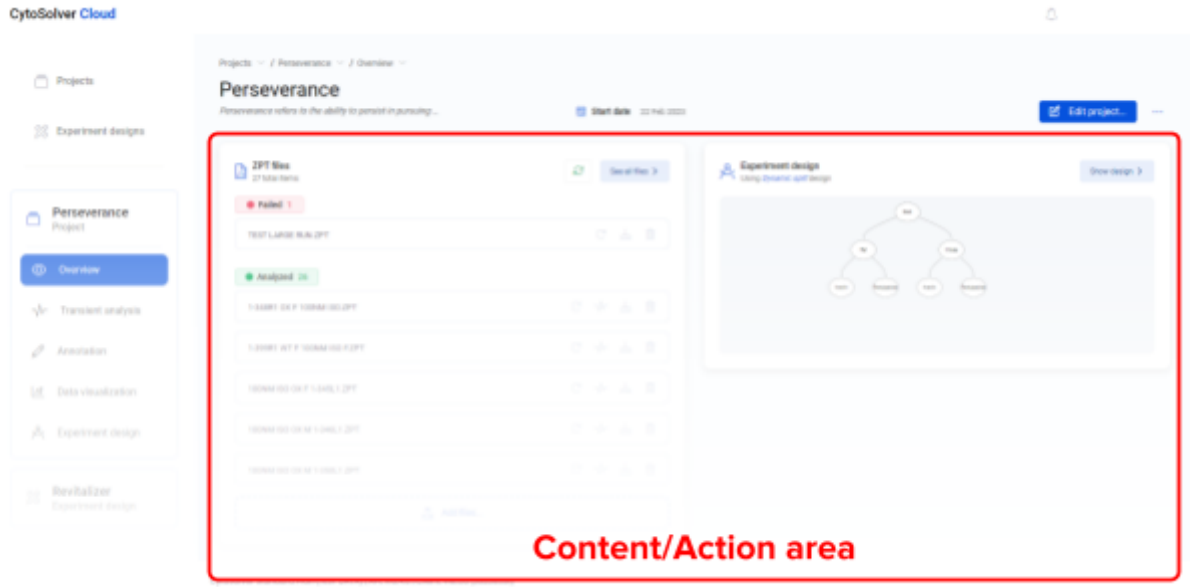


Figure 27. Cytosolver 3.0 content/action area.

5.1.2 Notifications

5.1.2.1 Pop-up updates Cloud

The notification system provides feedback to the user about the status of tasks that are being performed by the system. This is done by a small pop-up notification that appears on the bottom-right corner of the screen to inform the user of the progress of any ongoing tasks and to provide updates when a task has been completed.

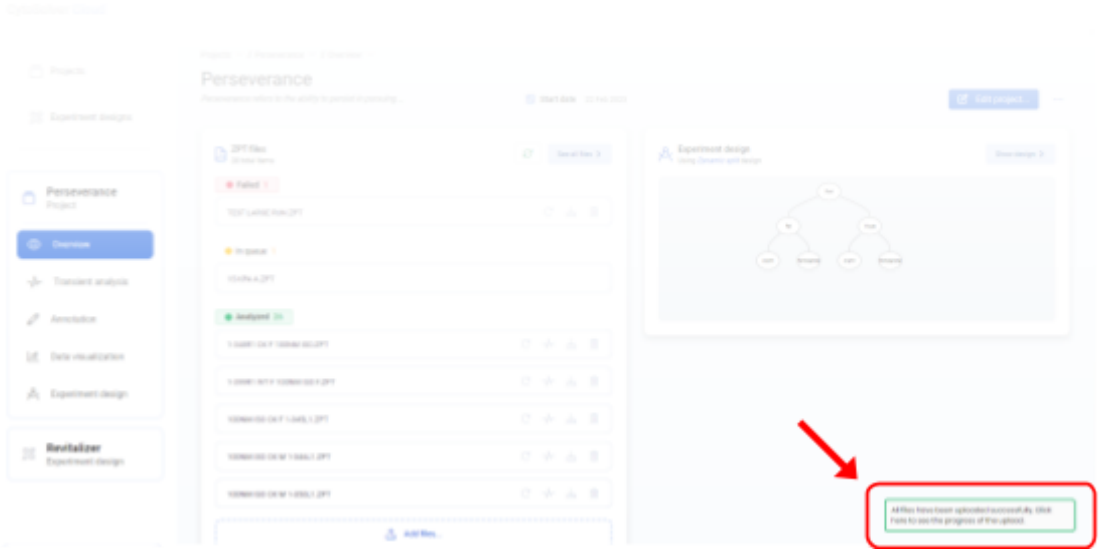


Figure 28. Pop-up notification location.

Examples of pop-up notifications in CytoSolver 3.0 include:

1. When files are being uploaded
2. When files have finished uploading
3. When the Experiment design has been updated

5.1.2.2 Confirmation prompts

Confirmation prompts are a type of pop-up window in a graphical user interface that require a user to confirm an action before it is executed. They are often used to prevent accidental deletion of data or other irreversible actions, as well as to provide feedback and ensure that the user understands the implications of the action they are about to take.

These confirmation prompts usually appear as a separate window that blocks interaction with the main window until the user responds. These modals usually present a question that needs to be answered by choosing between two options, such as "Yes" or "No". However, instead of "Yes" and "No", they may also provide options such as “Confirm” or “Accept”, or "Cancel" or "Discard", respectively.

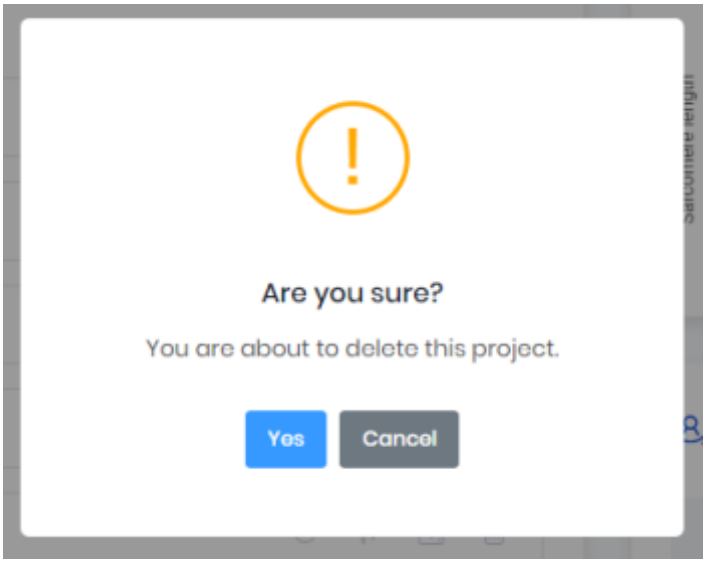


Figure 29. Pop-up notification example.

Examples of confirmation prompts in CytoSolver 3.0 include:

1. Deleting a Project from the system.
2. Switching the Experiment design that a Project has attached.
3. Modifying the Criteria Settings of a Project.

Confirmation prompts typically contain a brief message informing the user of the action that is about to be taken and providing a choice of buttons to either confirm or cancel the action.

In general, these prompts can help to improve the usability and safety of an application by reducing the risk of user error and providing clear feedback about the consequences of user actions.

5.1.2.3 Warnings

Warnings are used to alert users about potential issues or errors that may arise when performing an action. They are designed to prevent unintended consequences and to encourage users to review their choices before proceeding with a potentially risky or irreversible action.

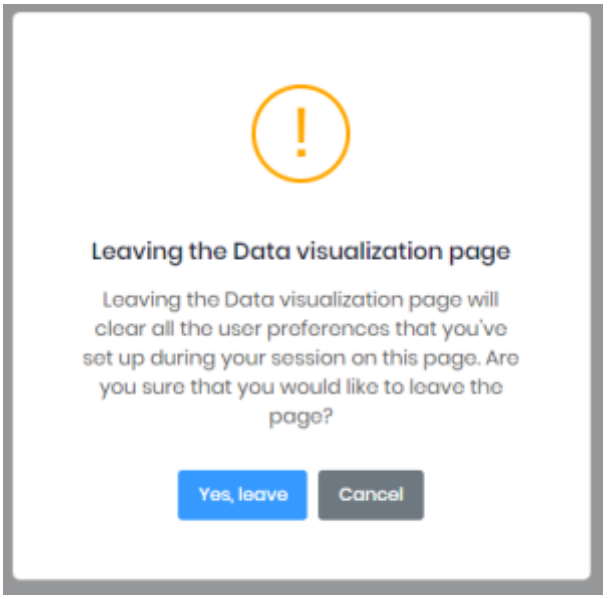


Figure 30. Warning prompt example.

Warnings can also provide immediate notification of a problem in a less intrusive way, such as informing the user when a character is not accepted right at the spot where it happens (contextual).

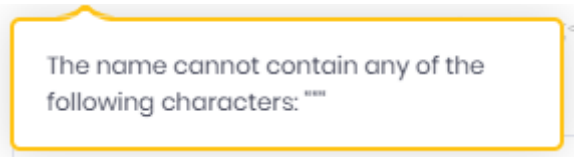


Figure 31. Contextual warning example.

5.2 Preparing your data

5.2.1 What are ZPT files

ZPT or Zone Plot Trace files are files collected with IonWizard data acquisition software. You will see these files have a ".zpt" or a ".ZPT" format (both valid), and look like the following image:

Figure 32. Visual example of ZPT file icon.

We refer to these files simply as "ZPT files". To make best use of CytoSolver, data should be collected with the use of the 'epoch' concept in IonWizard (for more information consult the IonWizard Core & Analysis Functions manual).
CytoSolver considers separate epochs based on breaks, denoted by the red line (seen when opening epoch-separated files in IonWizard), as different measurements or treatments of the sample. **Using epochs to structure files during acquisition is imperative for automated analysis.** Files containing background epochs can also improve the quality of the CytoSolver analysis process.

In the following figure we can see an example of a trace taken from IonWizard software with tags denoting what each part of the trace represents.

Creating epochs/breaks in a continuously acquired ZPT-formatted file to prepare it for analysis in CytoSolver is described in "Creating extra/new segments in zpt-files".

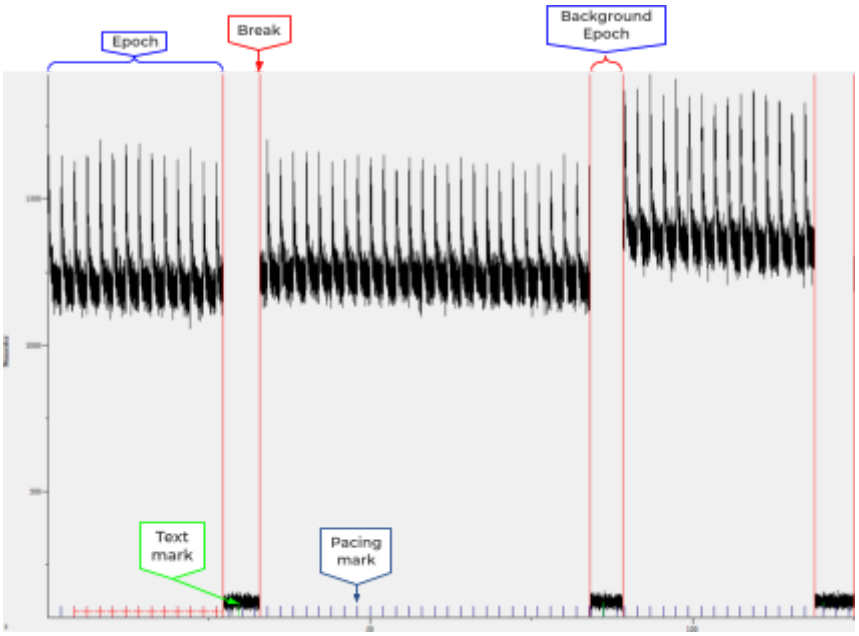


Figure 33. Example of epochs and other concepts in IonWizard.

5.2.2 What are event marks and what are they used for

An "event mark" is a visual cue that is placed on the data timeline to divide the data into “chunks” or segments based on time and data content. By “visual” we mean it can be explicitly seen using IonWizard (colored lines divide the data), however Cytosolver does not show such divisions in its interface, as it merely reads them and splits and/or tags the data accordingly.

Typically, an event mark represents a specific point in time that is significant for the data being analyzed, such as the start or end of a particular segment. For example, an event mark indicates when a segment starts and ends. It can also indicate which segments should be discarded.

By placing event marks on the data timeline, it becomes possible to analyze data in smaller, more manageable segments.

NOTE

We refer to *epochs* while we’re talking about data acquisition or IonWizard-related procedures to prepare data. Later, when explaining Cytosolver procedures, we will refer to them as *segments*.

Event marks may be added automatically by the software during acquisition or manually by the User, depending on the specific application and User’s desires.

Once the event marks have been added, the data can be easily segmented and analyzed to gain insight into the underlying patterns and trends. CytoSolver software is able to read these event marks and perform analyses based on them.

In the following table you can find a summary of supported text marks by CytoSolver.

Event mark	Description	Kind	For files acquired with
Background	Identifies a segment as background (fluorescence).	Full-width	Any
Delete	Marks the segments as to be ignored for the analysis.	Full-width	Any
R(N)	Corrects the repeated measure number of a segment to N. E.g. If a segment's repeated measure is 2, R(3) will overwrite it and set the repeated measure value to 3.	Full-width	MultiCell
Break	Divides un-paused data traces into multiple segments.[1]	Single-point	Any except MultiCell
Begin	Defines and replaces the begin of the segment interval [2]	Single-point	Any
End	Defines and replaces the end of the segment interval.	Single-point	Any

Table 7. Supported text marks by CytoSolver

Please mind the marks are **NOT case sensitive**. You can write marks like "Delete", "deleTe", "DELETE", "DeLeTe", and so on, they will all be valid. However, **it will take spelling into account**, therefore "delte" or "deletel" won't be accepted terms.

5.2.2.1 Kinds of text marks and how to use them

In this section, we will explore the different kinds of text marks and how to use them effectively. By understanding the proper usage of each kind of text mark, you can better categorize and manually adjust your data if necessary.

5.2.2.1.1 Full-width text marks

Full-width text marks are tags that, once placed within a segment , affect the full segment. This is the case of the "Delete" and the "Background" marks:

1. Adding a "Delete" mark anywhere within an segment will show the segment in gray color when visualizing the file on Cytosolver.
2. Adding a "Background" mark anywhere[3] within a segment will tag that segment as a background measurement.

---

[1] When dealing with Non-MultiCell files, our recommendation is to use "Break" to segment data rather than using "Begin" and "End".

[2] "Begin" and "End" must be used together.

[3] For MultiCell files, if background measurements are identified in a single repeat measure, all segments in that sample will be automatically assigned as background.

5.2.2.1.2 Single-point text marks

Single-point text marks are tags that are placed in a specific point in the file's timeline, and serve as indicators of events in time. This is the case of the "Begin", "End" and the "Break" marks:

1. By adding "Begin" and "End" marks in a part of a segment, you can make a smaller section within that segment that starts and ends at those marks, essentially **creating a new segment** which is a subset of the original one. It's like cutting a piece out of a larger object to make a smaller piece.
   Please note **only when both marks are placed is the segment sliced**. Placing only a "Begin" mark or only an "End" mark will result in the same segment.
2. Adding a "Break" mark at a specific point within a segment **will split** said segment in two. Mind this is a split of the original segment, not a duplication. E.g. Imagine a segment starts at t=0 and ends at t=10. Placing a "Break" text mark at t=3 will result in two segments:
   a. 1st segment goes from t=0 to t=3
   b. 2nd segment goes from t=3 to t=10
   After the process, the segments will appear to have been split from the beginning and will each have their own unique ID.

TIP

To make things easier you can create 'shortcut keys' for each text mark. Shortcut keys can only be used while acquiring data and can be assigned in IonWizard under the 'Collect tab -> Mark text'.

5.2.2.2 How to create extra/new segments

If data is acquired without the use of *segments* it is possible to create extra/new segments manually in IonWizard to prepare the file for analysis with CytoSolver.

1. Open a ZPT file in IonWizard.
2. Right-click on a specific point in the trace visualizers to add an event mark. The event mark will be created at the exact point in time where you click, so mind the position on the X-axis where you click.
3. Add the “Break” text mark at locations in your file where you want to create a segment.
4. Optionally, add the “Delete” text marks on the newly created slices that you want to remove from the analysis.
5. Optionally, add the “Background” text marks on the newly created slices to indicate background measurements.

For more information about the tool, you can always reference the “Help” menu item in IonWizard.

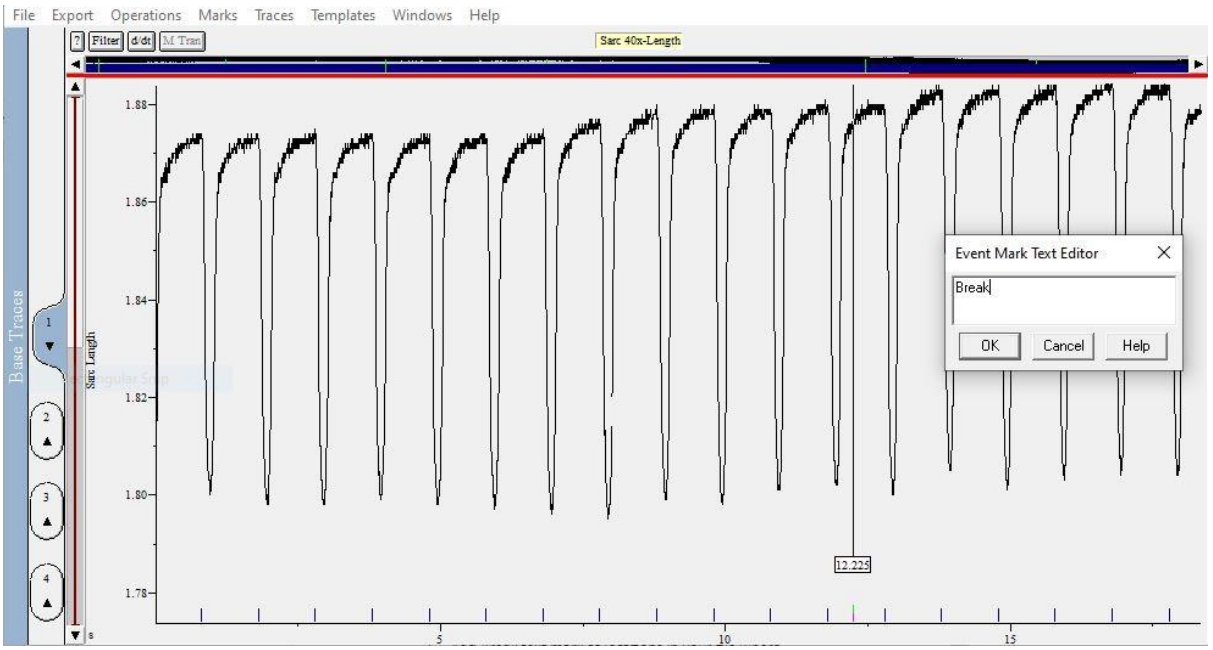


Figure 34. Adding an event mark on IonWizard through right-click at any point of the rendered trace data.

5.3 Understanding new concepts

This section provides a comprehensive understanding of the fundamental concepts and principles in the CytoSolver environment. These concepts and principles are essential for comprehending the analysis of data and how the user interface displays the necessary insight to interpret your data and analysis results. Reading through this section will establish a strong foundation of knowledge, providing the necessary understanding to effectively use the CytoSolver environment.

5.3.1 Data composition

5.3.1.1 Traces

In our context, the term "**Trace**" (also in Glossary) encompasses all data within a file pertaining to a single trace type. For instance, if a file has 3 distinct data types (Pixel correlation, Pixel intensity, and Sarcomere length), it is considered to have 3 trace types. Although each trace type contains all the data within the file, the data is measured using a different approach for each trace type.

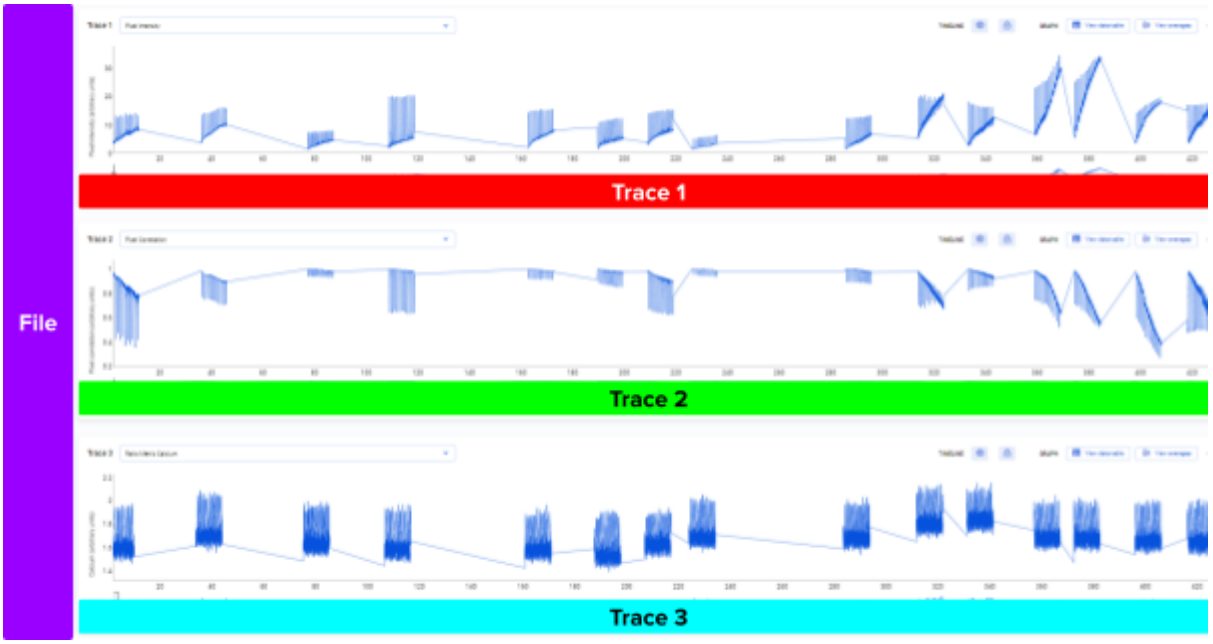


Figure 35. Example of a file containing 3 different trace types.

5.3.1.1.1 Trace types

Cytosolver is able to analyze the following types of traces:

They can be grouped in categories, depending on their measurement type:

1. Length-based traces:
    a. Sarcomere length
    b. Edge length
2. Photometry-based traces
    a. Ratiometric Calcium
    b. Single Wave Length
3. CytoMotion traces
    a. Pixel intensity
    b. Pixel correlation
4. Custom traces
    a. Cs1
    b. Cs2
    c. Cs3
    d. Cs4

5.3.1.1.2 Constructed traces

It should be noted that certain traces are "constructed," which implies that they have been generated using a formula that utilizes raw data obtained from our systems. The following traces are the constructed traces:

1. **Ratiometric calcium,** constructed using the following formula**:**
$$\frac{(numerator\ amplitude - numerator\ background\ value)}{(denominator\ amplitude - denominator\ background\ value)}$$
2. **Sarcomere Length,** constructed using the following formula:
$amplitude\ *\ sarcomere\ constant$
3. **Single wave length,** constructed using the following formula:
$amplitude\ *\ background\ value$
4. **Edge length,** constructed using the following formula:
$(Right\ Amplitude\ *\ edge\ constant)\ -\ (Left\ Amplitude\ *\ edge\ constant)$

5.3.1.2 Segments

In the CytoSolver environment, a trace is composed of one or more "**Segments**" (also defined in the Glossary). These segments represent different measurements recorded in the same file during various time ranges. Typically, segments are separated into visible "chunks" that form clusters of discontinuous data. Although the rendering libraries of the user interface link the last point of one segment to the first point of the next segment, there is no actual data between them. The user interface will display this linked representation between segments, despite the absence of any data.

NOTE

Perhaps you are more accustomed to the term "Epoch" when referring to this type of data. This term originates from IonWizard, but it shares the same meaning as a "Segment," which is a concept within the CytoSolver environment. In other words, each new segment in CytoSolver is equivalent to an epoch, and these segments are separated from one another by “epoch breaks” (See more in ZPT files organization).

In the image below, there’s an example of a trace containing 15 segments.

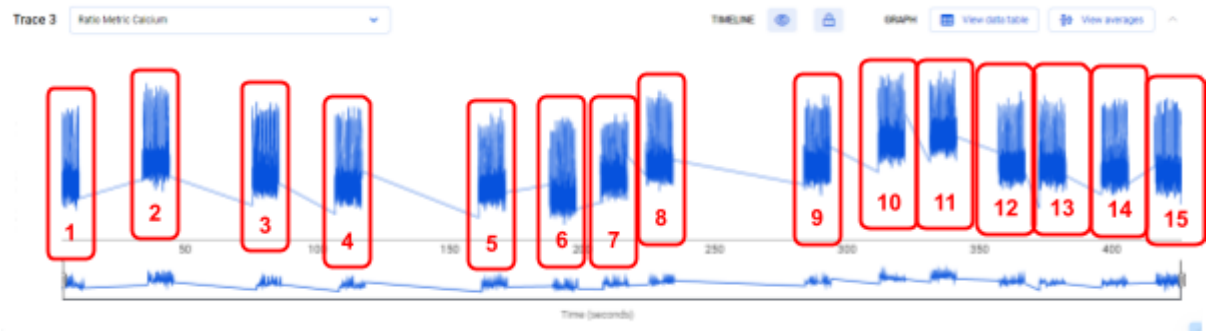


Figure 36. Example of a trace containing 15 Segments.

5.3.1.3 Transients

Within the CytoSolver environment, a segment can be further broken down into one or more "Transients." These transients are a fundamental concept of CytoSolver, as they isolate each cell contraction into a single, minimal unit. This decomposition of a segment is based on the contained data, and the purpose of the transients is to enable detailed analysis of each contraction.

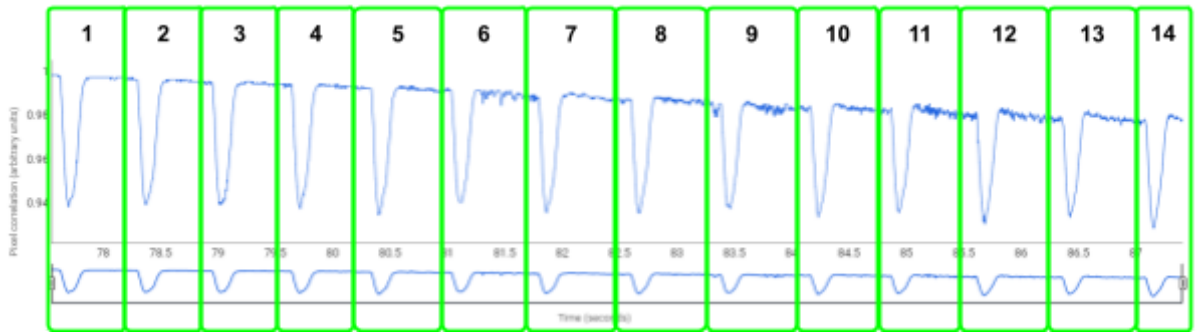


Figure 37. Example of a segment containing 14 Transients.

In contrast to segments, each transient is connected to one another, as they originate from continuous points in time. The split of transients is carried out by CytoSolver's algorithm, which applies its own proprietary algorithms to extract the final units of data and provide additional insights.

Through the use of these transients, the analysis of cell contractions can be significantly improved, providing a more detailed understanding of the data. This approach enables the isolation of individual contractions, allowing for the identification of potential issues or areas of interest in the data. Additionally, the use of CytoSolver's algorithms ensures that the resulting transients are accurately and consistently defined, providing reliable insights into the data.

5.3.2 Settings

To ensure accurate analysis, it is important that each transient event (contraction), is represented by a sufficient number of data points. While we recommend a minimum of 50 data points for each transient, it may be necessary to adjust this requirement depending on the sampling frequency used. In other words, the number of data points required for each transient may vary based on the rate at which data is collected.

NOTE

Stimulation frequency may be modulated during experimentation only between epochs, changing frequency mid-epoch will distort analysis.

Adequate representation of transient events is crucial for accurate analysis of the underlying physiological processes.

Sampling frequency	Pacing frequency
2000 Hz	¼ Hz - 40Hz
1000 Hz	¼ Hz - 20 Hz
500 Hz	¼ Hz - 10 Hz
250 Hz	¼ Hz - 5 Hz
100 Hz	¼ Hz - 2 Hz

Table 8. Transient event data point requirements and sampling frequency

5.3.3 Limitations

To ensure adequate analysis, each transient should have a minimum of 50 data points, which may be adjusted depending on the sampling frequency.

5.3.4 Performing fluorescence background corrections

Background corrections for fluorescence measurements (typically calcium signals) are recommended as they lead to improved signal-to-noise ratio, which increases measurement sensitivity and accuracy.

These **corrections are made in IonWizard**, there are practically 2 ways to do this:

1. Record the background separate from the sample measurements. Enter the average of your background signal(s) into IonWizard under `Operations` and `Constants`. CytoSolver will automatically subtract the background constant(s) from the corresponding raw fluorescence trace(s) .
2. Record the background in a separate segment and label the segment with a text mark named 'background' (See Full-width event marks for more indications). CytoSolver will automatically subtract the average background signal from fluorescence data within the ZPT file.

CytoSolver imports the corrections along with the files. However, for the corrections to be processed, they must satisfy **one of the following conditions**:

1. There is at least one background segment.
2. Background constant(s) have been defined.

When **both conditions are met**, the checkbox under "Background constants" in the user interface (UI) for adding files to the project will be **available** for each file that meets the conditions (***Case A***, below in the image). This means you can choose between:

1. Checking the box, which will lead to using the background constants found in the file to apply the fluorescence signal correction.
2. Leaving empty or unchecking the box, which will lead to the algorithms using the file's segments marked as a "Background" to calculate constants and apply those in the fluorescence signal correction,

When **at least one of the conditions is NOT met**, the checkbox under "Background constants" in the user interface (UI) for adding files to the project will be **unavailable** for each file that does not meet the conditions. However, you might be in one of these cases:

1. If the checkbox is **unavailable** and **unchecked** it can mean:
    a. There are no background segments nor constants (***Case B*** below): In this case, no correction is applied.
    b. There are only background segments but no constants **(Case C)**: In this case, the correction is applied using the background segment .
2. If the checkbox is **unavailable** and **checked** (***Case D*** below), it means there are background constants present in the file which will be used for the correction, but there are no background segments.

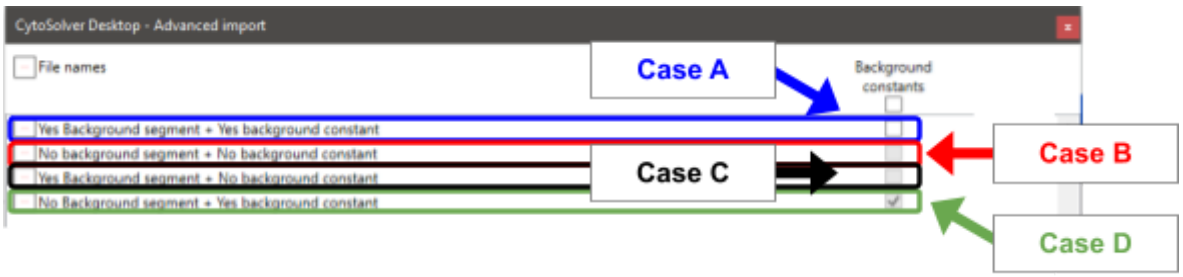


Figure 38. Example of available/unavailable "Background constants" additional option.

Here is a summarized table that helps comparison of all the possible cases:

Case	Background segment	Background constant	Checkbox available	Checkbox value	Correction applied
A	✅ Yes	✅ Yes	Available	Up to user	Yes
B	❌ No	❌ No	Unavailable	Unchecked	No
C	✅ Yes	❌ No	Unavailable	Unchecked	Yes
D	❌ No	✅ Yes	Unavailable	Checked	Yes

Table 9. Summary of potential background correction options and conditions in CytoSolver

5.3.5 Learning about rejection criteria

In this section, we will explore the concept of rejection criteria in greater detail, examining how they are established and what factors influence their selection.

5.3.5.1 What is a rejection criteria

The *Criteria Settings* are a set of configurations for each trace type. CytoSolver makes use of rejection criteria to automatically reject transients/segments that contain artifacts, i.e. an unintended or undesirable feature or characteristic in the trace data that can affect the quality and accuracy of the data analysis results. If a transient/segment is rejected the rejection reason is presented in the export results table under the 'Remark' column.

IMPORTANT

The **criteria settings are specific to each project**, so any changes made to them will only affect that particular project.

5.3.5.1.1 User-adjustable rejection criteria

The terms are in alphabetical order to ease the task of finding a term.

Criteria	Description
Baseline Threshold	Checks the baseline of the transient/segment. The transient/segment is rejected when the baseline falls outside the threshold range.
D/dt max	Checks the maximum of the first derivative. The transient/segment is rejected when d/dt max is larger than the threshold.
D/dt2 max	Checks the maximum of the 2nd derivative. The transient/segment is rejected when d/dt2 max is larger than the threshold.
Minimum number of datapoints	Checks the number of datapoints in each transient. If the number of datapoints is lower than the specified minimum, the transient is rejected. Minimum requirement is 2 and this criterion cannot be switched off.
Offset from Transient Limit	Checks the number of data points available preceding the Transient Limit. If the offset is larger than the number of data points available, the transient/segment is rejected. It has a minimum value of 2 and a maximum of 15.
PSNR	Checks the peak signal to noise ratio (PSNR) of the transient/segment. The transient/segment is rejected if the PSNR is lower than the threshold.

Criteria	Description
R^2 double exponential rejection	Checks the R^2 Double Exp fit of a transient/segment. The transient/segment is rejected if the R^2 Double Exp fit is lower than the threshold.
R^2 double exponential threshold	Checks the R^2 Double Exp fit of a transient/segment. If it is below the threshold, Tau 1 and Tau 2 are set to NaN in the results table.
R^2 peak rejection	Checks the R^2 Peak fit of a transient/segment. The transient/segment is rejected if the R^2 peak fit is lower than the threshold.
R^2 peak threshold	Checks the R^2 Peak fit of a transient/segment. If it is below the threshold Time to Peak, Time to Peak 10 - Time to Peak 90 are set to NaN in the results table.
R^2 return (recovery) rejection	Checks the R^2 Return fit of a transient/segment. The transient/segment is rejected if the R^2 return fit is lower than the threshold.
R^2 return (recovery) threshold	Checks the R^2 Return fit of a transient/segment. If it is below the threshold, Time to Baseline 10 - Time to Baseline 90 are set to NaN in the results table.
R^2 single exponential rejection	Checks the R^2 Single Exp fit of a transient/segment. The transient/segment is rejected if the R^2 Single Exp fit is lower than the threshold.
R^2 single exponential threshold	Checks the R^2 Single Exp fit of a transient/segment. If it is below the threshold, Tau is set to NaN in the results table.
Y Lower Limit	Checks the lowest data point. Transient/segment is rejected if the lowest data point is lower than the threshold.
Y Upper Limit	Checks the highest data point. Transient/segment is rejected if the highest data point is above the threshold.
$Y_{max} - Y_{min}$	Checks the deviation (or peak height) of the transient/segment from the baseline. The transient/segment is rejected when Ymax – Ymin is lower than the threshold.

Table 10. Glossary of user adjustable rejection criteria in CytoSolver

Criteria settings can be split in two categories: User-adjustable criteria and Fixed criteria, which we will detail in the following subsections.

5.3.5.2 How to adjust rejection criteria

The thresholds of the rejection criteria can be modified or switched off completely at any point in time.

Any alteration of the criteria settings will change the output of the Transient Analysis result. Reanalyzing your ZPT files after the *criteria* modifications is not strictly required, but highly advised, as they are used to perform the analysis. Hence we suggest performing changes before any file import as, once the files are added to a project, they are immediately put in the analysis queue and you will need to wait until the analysis is done to modify the criteria again.

NOTE

Any alteration of the criteria settings might change the output result of the Transient Analysis, hence, reanalyzing after any criteria modification is important.

The default values each criteria setting for each trace type has are set to be quite permissive, i.e. their tolerance margin is high and they avoid having too many rejections. Be cautious when editing the rejection criteria, as this may add false positive transients to the results.

To edit the *criteria settings* you need to access the Criteria settings modal. You have 2 ways to access it:

1. Through the pathways or top navigation:
    a. Upon opening a project, the pathway is displayed at the top of the page's content, regardless of the project tool being used.
    b. The navigation has 3 levels, corresponding to the following format: “Projects’ home / Selected project / Tools and actions”
    c. Expanding the 3rd level (“Tools and actions”) and navigating to the *Actions* section, you will find “Criteria settings…”
    d. Clicking on the “Criteria settings…” menu will pop up the modal to start editing criterias.

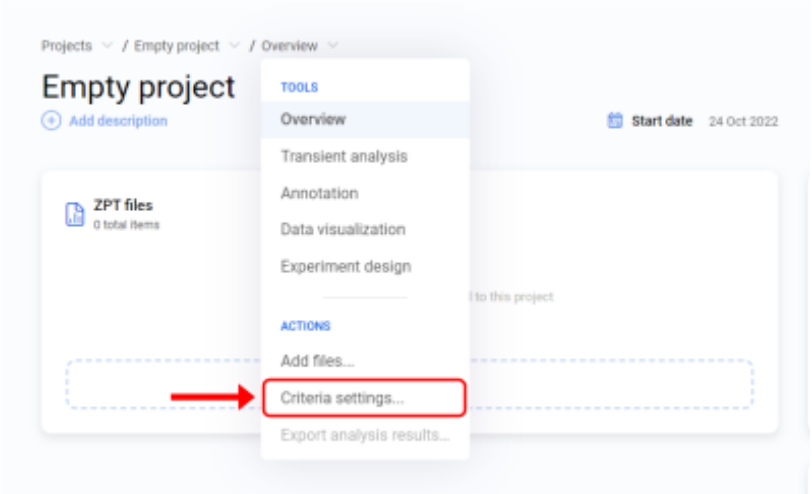


Figure 39. Opening a project’s Criteria Settings menu through top navigation.

2. Through the contextual menu (···):
    a. Upon opening a project, the contextual menu is displayed at the top-right corner of the screen on the Overview page.
    b. Clicking on it will open the menu, and navigating to the *Analysis actions* section, you will find “Criteria settings...”
    c. Clicking on the “Criteria settings...” menu will pop up the modal to start editing criterias.

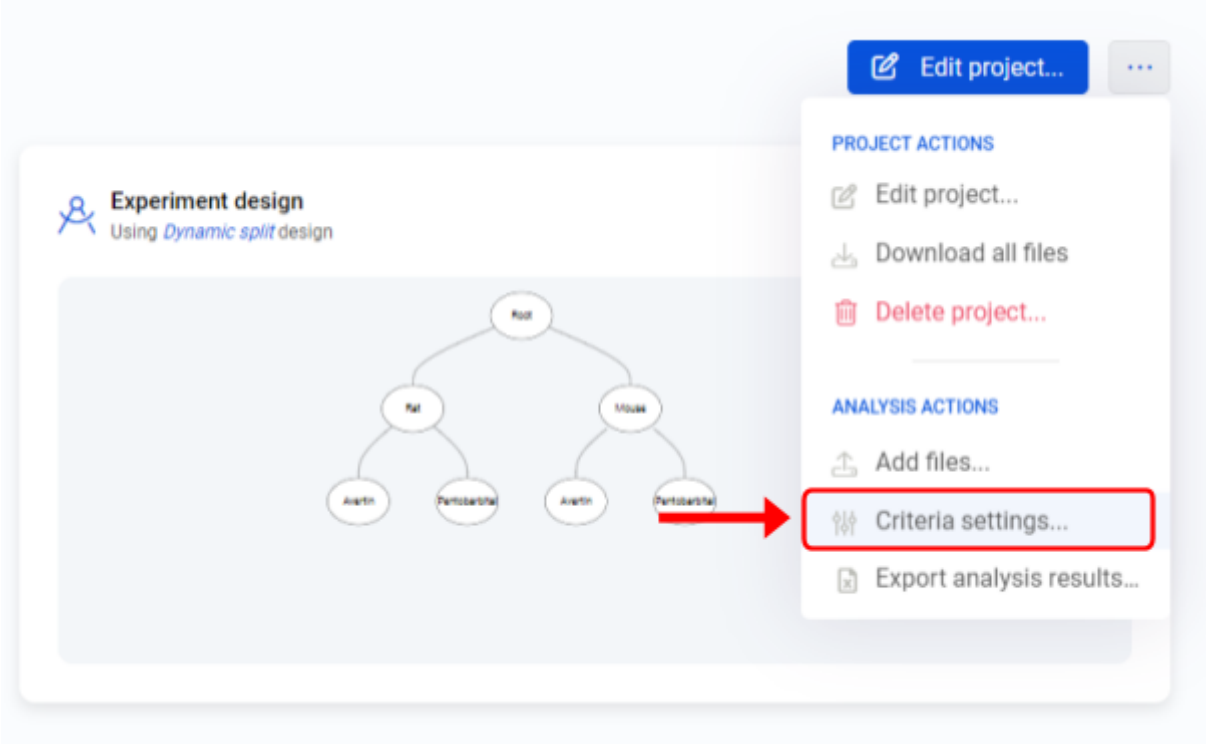


Figure 40. Opening a project’s Criteria Settings menu through project’s contextual menu (ellipsis button).

Regardless of where you have opened it from, the modal window will have a consistent appearance as shown below:

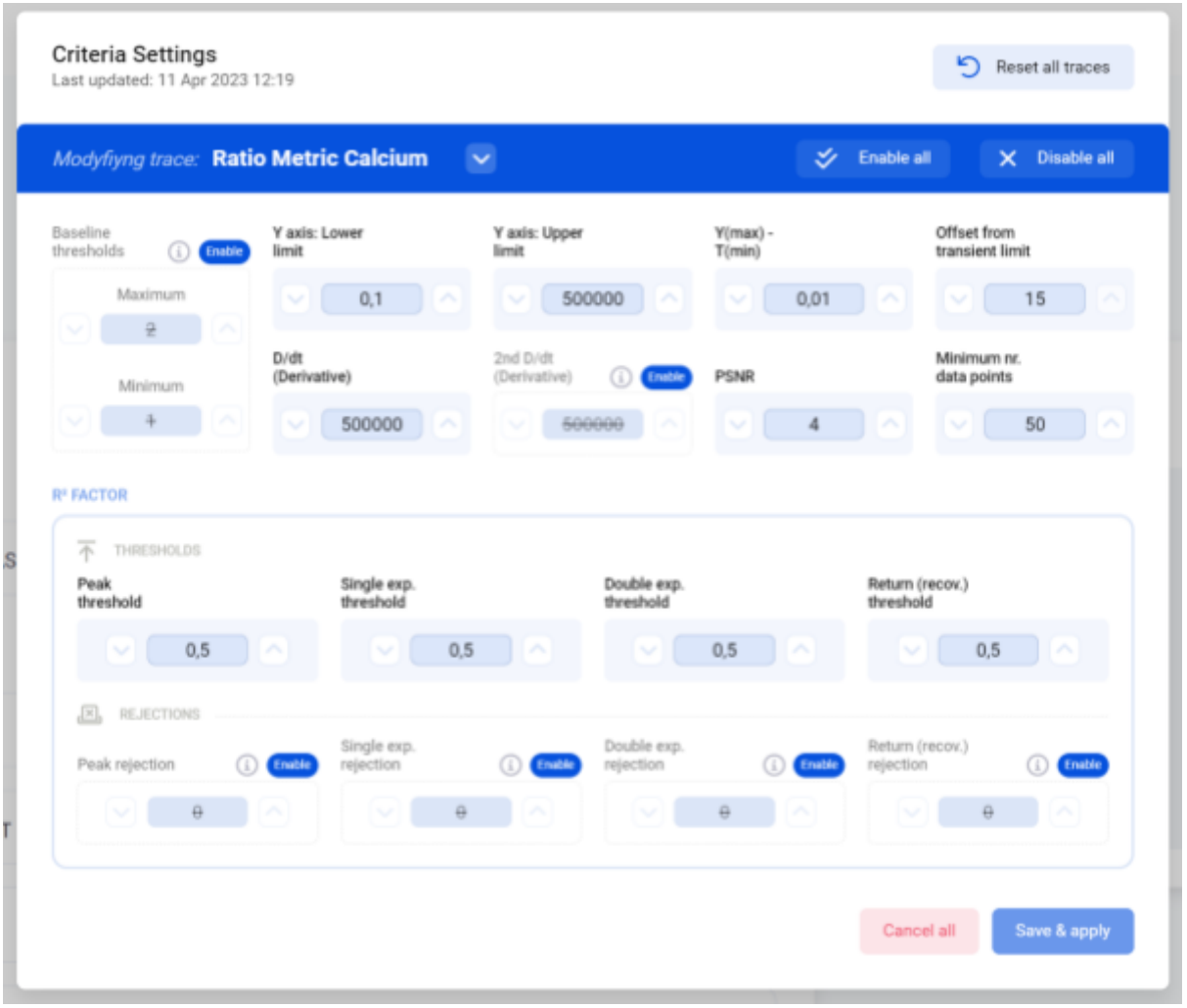


Figure 41. Criteria Settings modal window.

Once the Criteria Settings window appears you can start modifying the values. Please keep in mind the changes are NOT applied until you click “Save and apply” to properly store the altered values.

Again, these changes do not modify the analysis results you have already obtained in old analyses. You will need to reanalyze the files again to see the changes happen.

TIP

If you have modified the criteria settings of one or more trace types, and you know which files contain those trace types, you can individually reanalyze only those files. The files that do not contain the traces affected by the criteria modifications will not see their results modified.

5.3.5.2.1 Steps to correctly perform changes in your criteria settings

By following the steps provided in this section, you can be confident that you are making the correct changes to the criteria settings and that the project's analysis will function as expected:

1. **Choose the trace type to modify:** The drop-down seen below allows you to alter the criteria settings of the selected trace type. No other trace types will see their criteria settings modified.

Figure 42. Trace selector in Criteria Settings modal window.

2. **Enable/disable criteria settings:** Make sure the criteria are enabled in order to be able to apply them. Disabled criteria settings will not be taken into account for the analysis. You can enable/disable all the criterias at once by clicking "Enable all" or "Disable all", respectively. This will only affect the selected trace type.

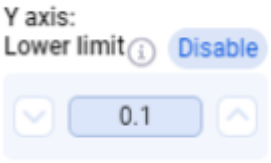


Figure 43. Enabled criteria setting.

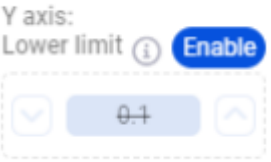


Figure 44. Disabled criteria setting.

3. **Input a sensible value:** The default value of each criteria setting provides an indication of the expected range of values for that field. For example, the Sarcomere Length trace type lower Y-value and upper Y-value default values are set to 1 and 3.2, respectively, suggesting that the appropriate values would fall within that range. However, it's important to note that the arrow buttons for changing values increment/decrement by 1 step, which may not be helpful for criteria that use a scale of thousands (like some trace types have).
4. **Save and apply changes:**
    a. Click on "Save and apply" to properly store the altered values.
        i. Please note the button will be disabled if no changes have been made.
    b. You can also choose to "Cancel all" your changes, in which case a confirmation modal will ask you if you want your modifications to be saved or not:
        i. Clicking "Yes" will store the values, just like in option (a) above
        ii. Clicking "No" will remove all changes

5.3.5.2.2 Restore all criterias to default

If you would like to restore to default all the values of all trace types, you can click “Reset all traces”. **All trace types will be affected by this change, not just the one that was selected from the drop-down menu.**

5.3.5.3 What are fixed rejection criteria

The following table describes all the existing Non-adjustable rejection criteria. These values are hard-coded inside our algorithms, and cannot be changed via the interface or any other configuration file.

Criteria	Description
Large derivative limit	Establishes a threshold beyond which the derivative of a function or data set is considered large. It has a specific value for each trace type: ● > 8 (greater than eight) for Sarcomere Length ● > 200 for Cs1, Cs2, Cs3 ● > 2000 for EdgeLength ● > 200000 for Ratiometric Calcium, Single Wave Length, Pixel Correlation and Pixel Intensity.
Number of data points before transient limit	At least 2 data points preceding the electrical stimulation mark are required to calculate the Baseline. Transients that have less data points preceding the electrical stimulation mark are rejected.
Number of large derivatives	Number of large derivatives must be lower than 5.
Transient direction	Checks the transient/segment direction of the trace. For example, sarcomere shortening should contain negative going transients/segments.

Table 11. Glossary of fixed rejection criteria in CytoSolver

6 Operation/Use

6.1 Understanding “Administration” (admins) Cloud

The Administration sidebar menu time of the application is exclusive to CytoSolver Cloud and specifically to the users that have the Administration permissions. More on the permissions topic can be found in the “6.1.2 Roles” section.
This menu item contains various menus regarding the Tenancy settings. This is where the administrators of the Tenancy can manage Organization Units, Roles, Users, and Settings, and review the systems Audit logs.

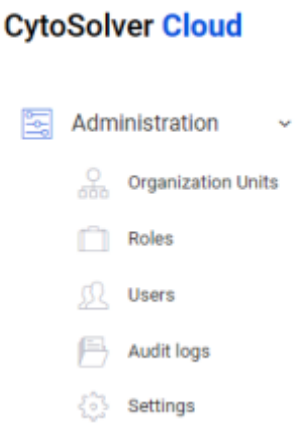


Figure 45. Cytosolver Cloud - Administration.

In the following sections we will go over each Administration option and its usage.

6.1.1 Organization Units

The Organization Units are one of the ways we can manage the permissions of our users. By default in the system we provide our tenants with two roles, Admin, and User, while Admins have all the permissions in the system, the Users have none. An option would be to grant our Users role the permissions that they need or manage their permissions through Organization Units. More on role-based permissions can be read in the following section, "6.2.2 Roles".

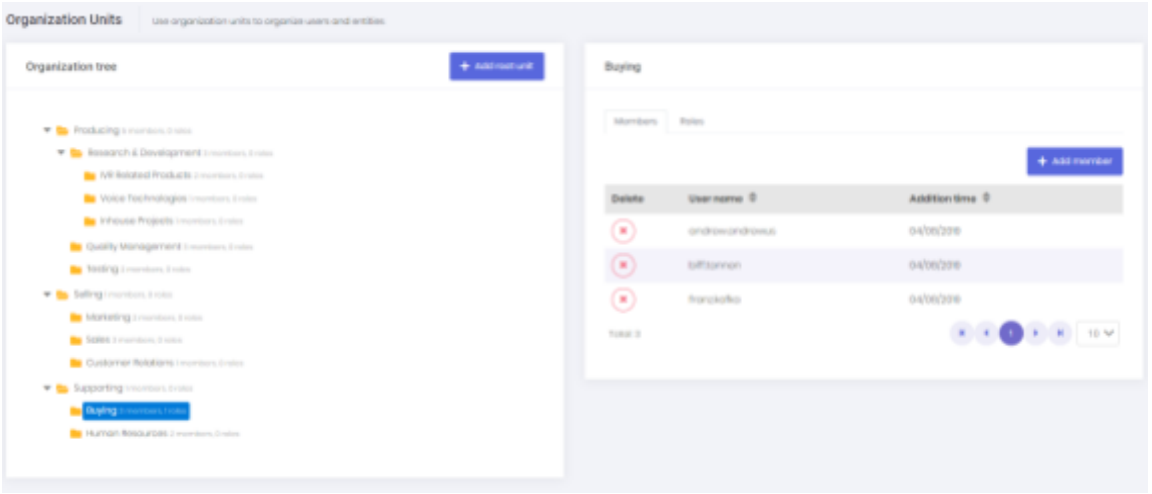


Figure 46. Cytosolver Cloud - Administration - Organization Units.

While the Organization Units system makes it easier to work with a large user base that should be divided into many groups and roles, the Roles-based system makes it easier to work with a handful of users that are all sharing the same permissions.

To optimally make use of the Organization Units system we need to create our roles first and give them the appropriate permissions, more about this can be read in the "6.1.2 Roles" section.
The workflow for the Organization Units requires the creation of root units that can be created with the "Add root unit" button in the Organization tree section on the left, followed by adding the unit members with the "Add member" button in the Organization unit name section on the right. We can also optionally add sub-units by right-clicking on the Organization unit that we have created. After adding our members to our Organization unit we need to assign the role(s) to that unit for the members to inherit the permissions. This can be done by switching to the "Roles" tab on the Organizations unit name section on the right and clicking on the "Add role" button.
After adding our Organization Units, the members, and the roles, our users should now have the additional permissions of all of the associated roles of their units on top of the main role that is assigned to their account. As you can see the Organization Units allow us to manage the permissions of a group of users instead of having to manage each user individually. As mentioned before, this is a useful system when dealing with a larger group of users with varying permissions.

6.1.2 Roles

The Roles are the underlying system behind user-specific and Organization Units settings. With the Roles, we can manage groups of permissions that we can then assign to users and/or associate with units.
On the Roles screen, you're able to manage, create and delete (only custom roles) the roles in your tenancy. Managing and deleting can be done through the "Actions" button on the Role item, and creation can be done with the "Create new role" button in the top right corner.

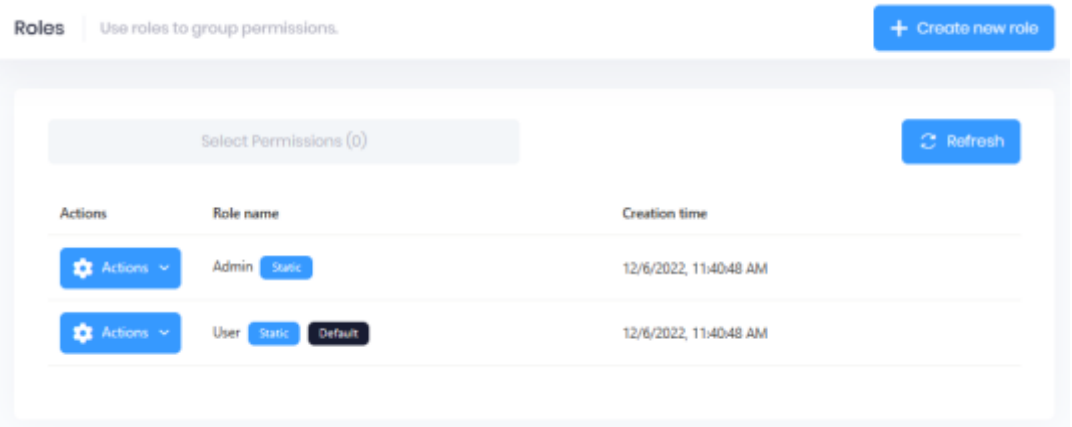


Figure 47. Cytosolver Cloud - Administration - Roles.

6.1.2.1 Edit roles

By clicking on the "Edit" button in the "Actions" we can manage a number of settings related to our roles. Those settings are, the roles name, if the role is the default role that is assigned to new users, and the roles specific permissions.

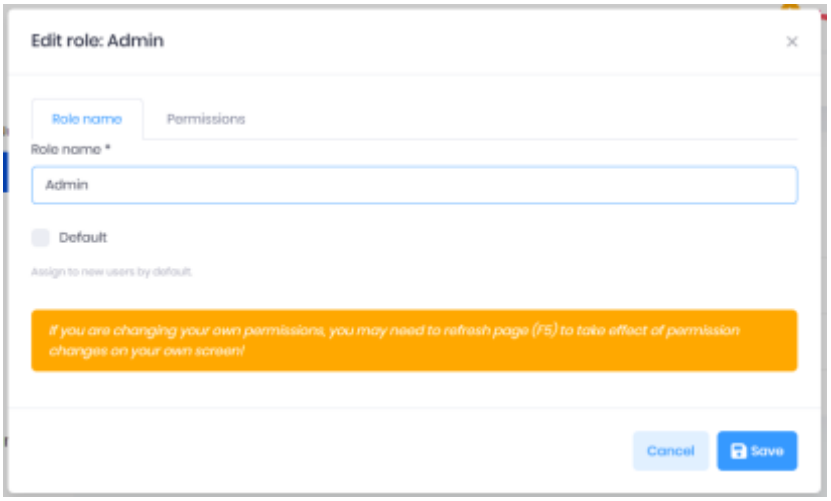


Figure 48. Cytosolver Cloud - Administration - Roles - Edit role - Name.

On the Roles Permissions tab we can see all the permissions that are available to the role in the system. Each page, feature, component, or widget, can have its own permissions. When

your users are not able to perform some specific actions within the system it is always a good idea to review their roles (or Organization Units) permissions.
Insufficient permissions to some components can result in users seeing a permission-denied window or just the lack of buttons or pages on their views.

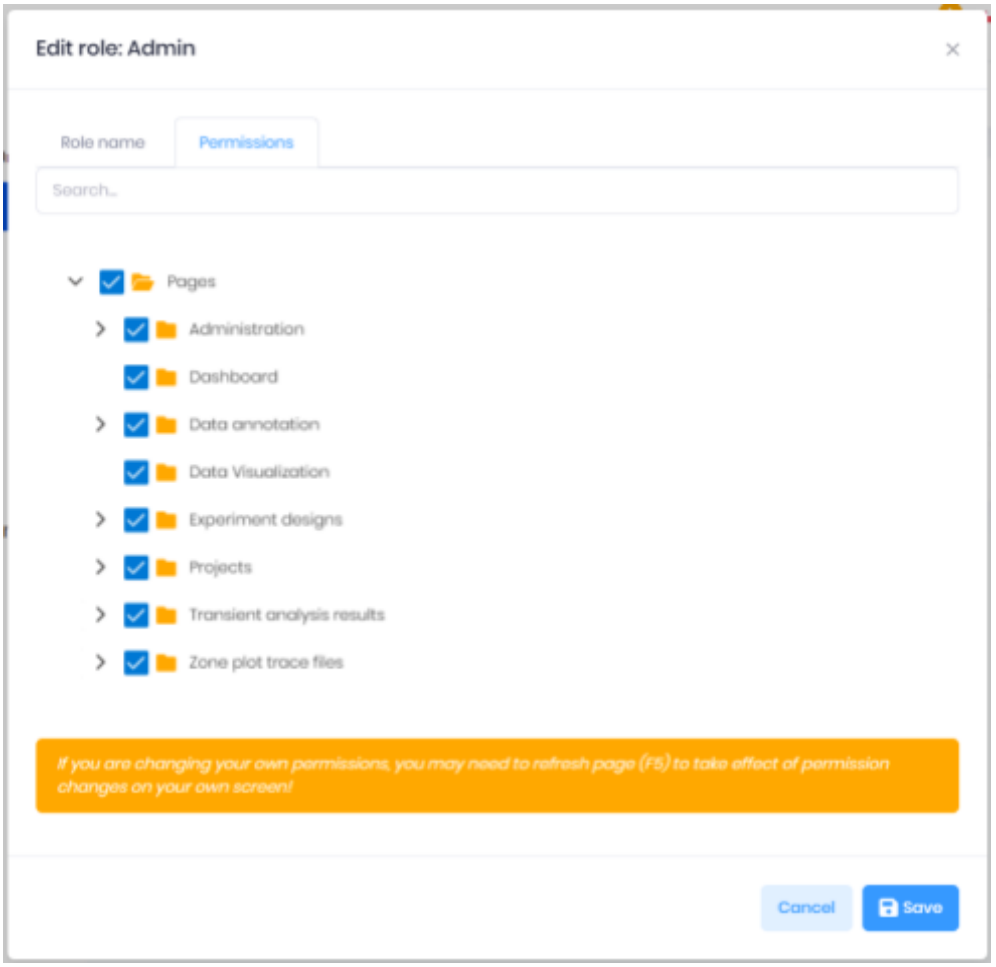


Figure 49. Cytosolver Cloud - Administration - Roles - Edit role - Permissions.

6.1.2.2 Create roles

The Create new role screen enables you to create new roles with your chosen permissions. Those roles just as the standard roles can be assigned to your users (6.1.3 Users) and/or your Organization Units (6.1.1 Organization Units).

6.1.3 Users

The Users screen gives us the ability to create and manage our tenancies users. From this screen we can create, edit, update user-specific permissions, lock or unlock, and delete our users. All of the actions can be performed from the “Actions” button on each individual user, except for the “Create new user” button which is located in the top right corner of the screen.

Figure 50. Cytosolver Cloud - Administration - Users.

6.1.3.1 Create new users

The user creation window will present us with three tabs, User information, Roles, and Organization Units.

The user information screen lets us provide some basic user information, as well as some basic password and activation rules. We recommend leaving those rules as they are and only changing them when it is necessary.

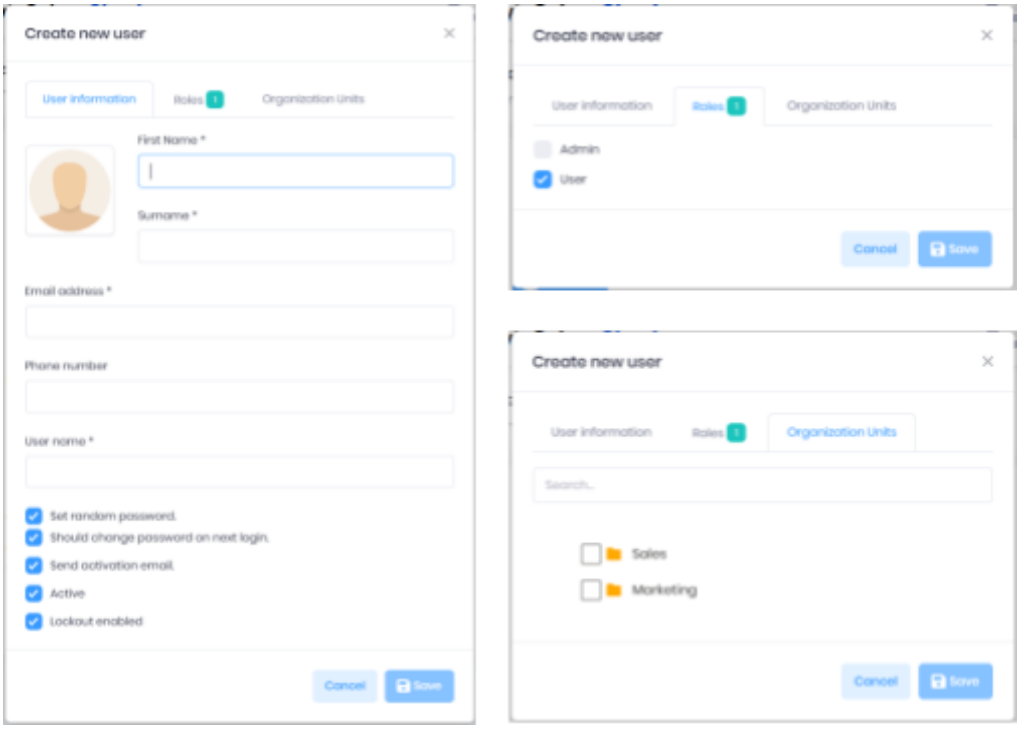


Figure 51. Cytosolver Cloud - Administration - Users - Create new user.

On the roles tab we can give the user a specific role if the by default assigned User role is not applicable. For more information on the Roles, please refer to “6.1.2 Roles”.
As last, the Organization Units tab lets us access a convenient shortcut to adding the user to a specific Organization unit that we have created before the creation of the user for more fine-grained permissions. For more information on the Organization units, please refer to “6.1.1 Organization Units”.

6.1.3.2 Edit users

On the Edit user screen we can edit all of the users’ information that we or the user input during the creation of the account. This screen is similar to the Create new user screen that we can see in the “6.1.3.1 Create new users” section. The main differences between both screens are the three disabled user creation rules, set a random password, change the password on the first login, and send a confirmation email at the bottom of the screen. Those options can be enabled if needed when the user forgets their password or maybe hasn’t received the confirmation email before.
The other two tabs, Roles and Organization Units, can be reviewed in the prior create new users section.

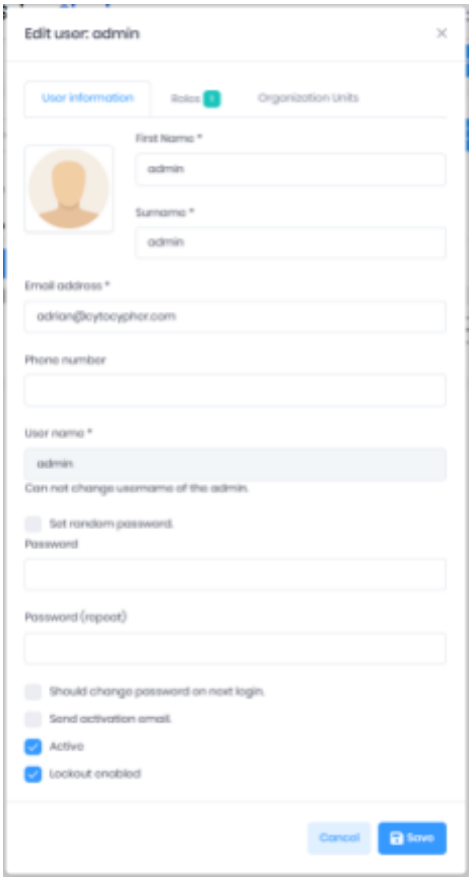


Figure 52. Cytosolver Cloud - Administration - Users - Edit.

6.1.3.3 Edit user-specific permissions

In prior sections we've discussed the Roles, "6.1.2 Roles", and the Organization Units, "6.1.1 Organization Units". There is a third option for managing user-specific permissions. That is done through the Users Permissions screen that we can see here (accessible through the Users screen "Actions" button). On this screen, we can override any permissions that have been configured through the Roles or Organization Units options. Overriding means that no changes to the assigned Role or assigned Organization Units will change anything for those users. We can always reset the overridden rules by using the "Reset special permissions" button at the bottom of the screen.

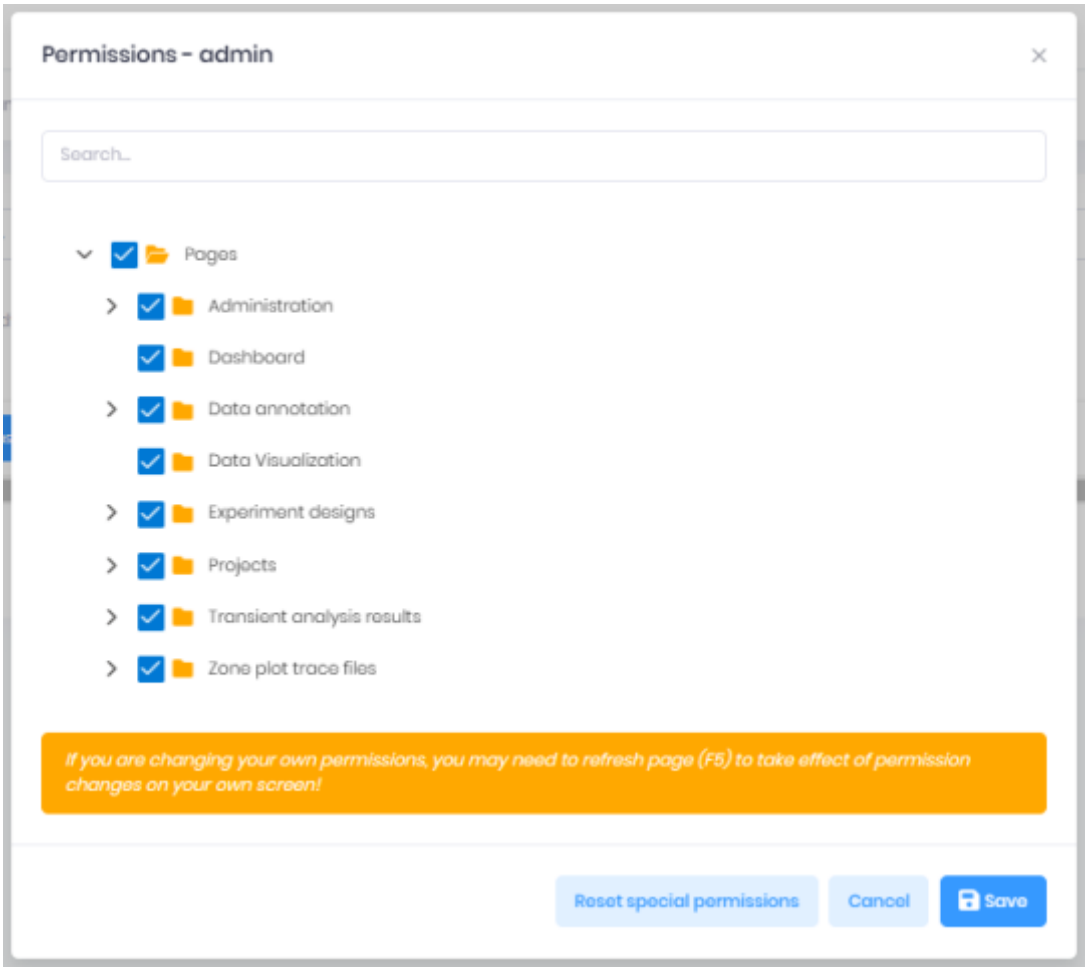


Figure 53. Cytosolver Cloud - Administration - Users - Permissions.

6.1.4 Audit logs

The Audit logs provide us with logs of specific actions and requests that have been performed by all the tenancy users over a period of time. They can give us insights into operations that have been performed successfully or unsuccessfully.
Click on the magnifying glass can give us more information on each operation.

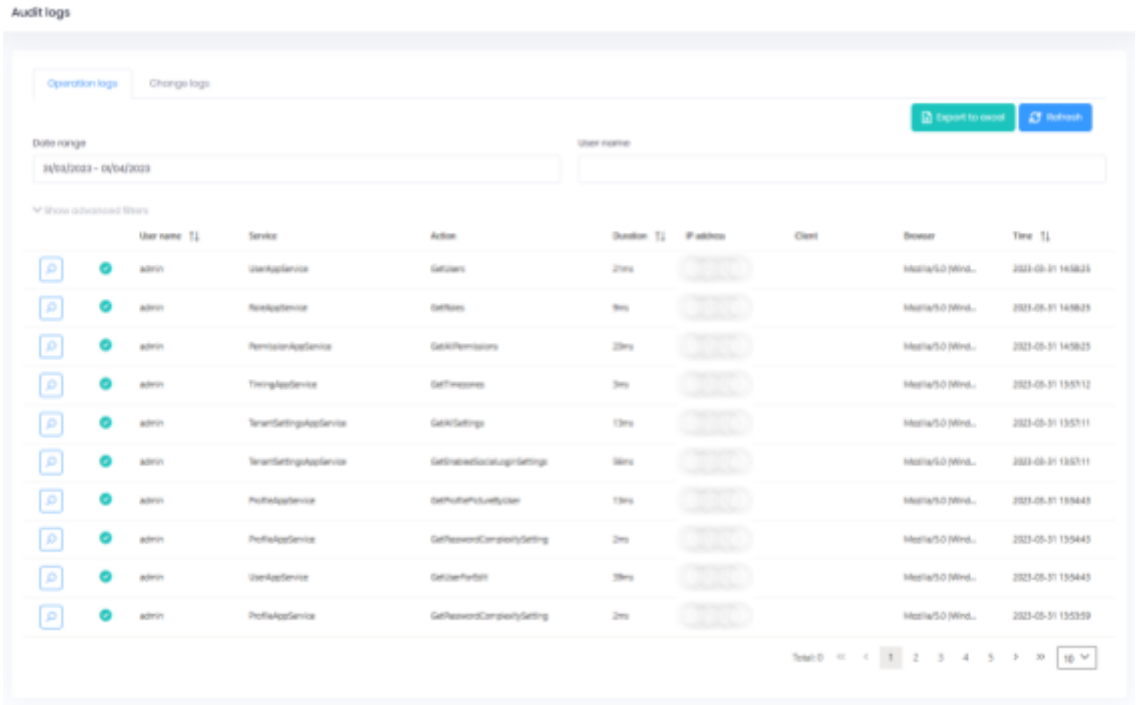


Figure 54. Cytosolver Cloud - Administration - Audit logs.

6.1.5 Settings

The Administration Settings screen presents us with three tabs, each responsible for a different aspect of the Tenancy settings. We'll go over each tab one by one.

Starting with the General tab, we can see a single setting being present there, that being the default tenancy Timezone. The default Tenancy timezone configures how date- and time-related information is presented to the viewer. This setting does not modify the time that is saved to the database and only configures the formats, and the offset that the application needs to consider when showing that information.

The User management tab lets us modify the default behavior related to user registration, user sessions, and profiles.

As last on the Security tab, we can change the default settings for password complexity and user lockout. By default, password complexity is set up to require it to be at least eight characters, contain digits, lowercase, and uppercase characters, and also at least a single non-alphanumeric character. As for the lockout settings, the accounts are by default locked after five failed login attempts and will stay locked for five minutes. After the lockout duration has passed the user will be able to try to log in again.

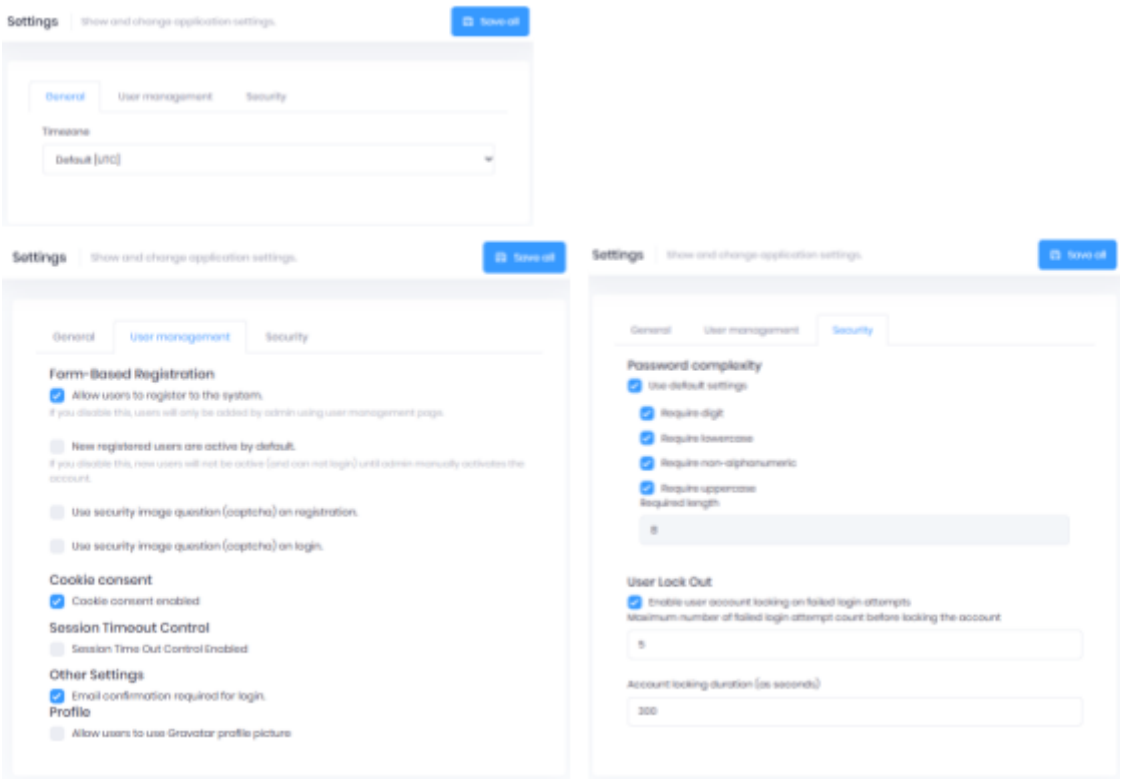


Figure 55. Cytosolver Cloud - Administration - Settings.

6.2 Understanding “Projects”

In version 3.0 of CytoSolver, one key feature that requires clarification is the concept of “**Projects”**. Projects act as organizational "folders" where users can store and manage files, analyses, and other data. It is essential to understand that each project is entirely independent, with no sharing of files or other information between projects. This ensures that each project is self-contained, allowing users to keep their data organized and easily accessible.

KEY CONCEPT

Projects act as organizational "folders" where users can store and manage files, analyses, and other data.

When using CytoSolver 3.0, you are not required to align your projects with those of your organization. You may create as many projects as you need to efficiently organize your work, but it is essential to name them appropriately to avoid confusion.

Within a project, you will have access to all the necessary tools for data analysis, visualization of results, and exporting them for your convenience. These results can then be used in your reports or publications.

You have the flexibility to modify the content of a project, including its metadata, such as the name or description, to ensure its suitability for any stage of your research.

6.2.1 How are Projects organized

As explained in the GUI (Graphical User Interface) area, a list of Projects can be found under the Projects explorer. This feature allows you to easily access and manage your projects. Projects can be created, filtered, and deleted through the Projects explorer.

The Projects explorer allows you to efficiently organize your work by grouping related projects together and keeping them easily accessible. By utilizing this organizational feature, you can streamline your workflow and optimize your research process.

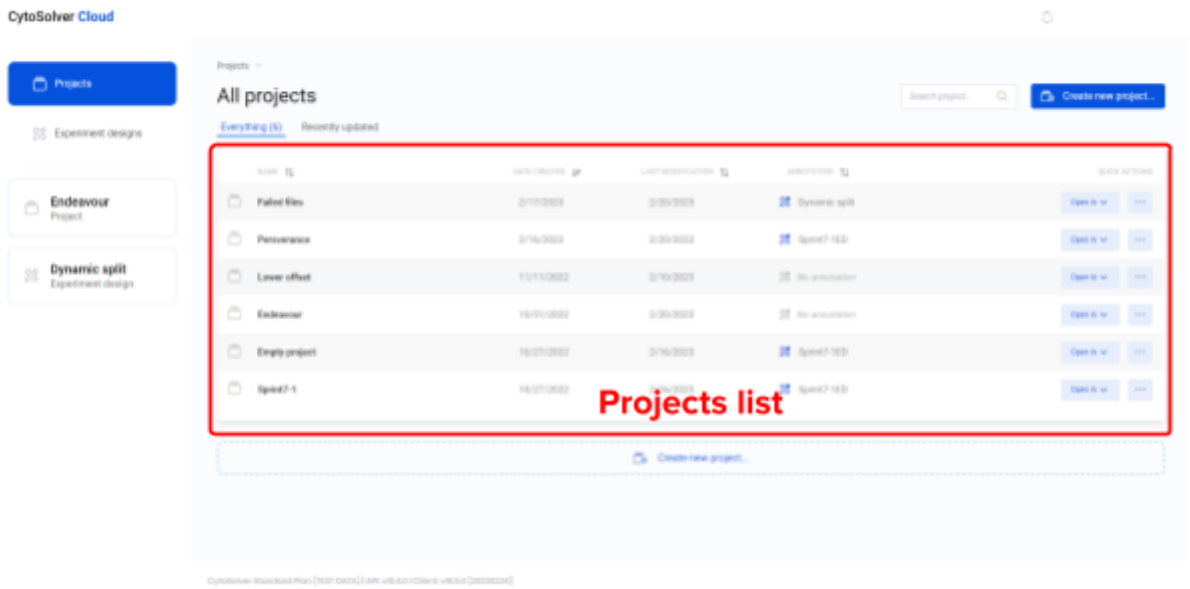


Figure 56. Project's explorer containing all the existent projects in your account.

6.2.1.1 Displayed information

When viewing the list of projects, some information about each project is displayed for quick reference. This information includes:

1. **Name:** The name you assigned to the project.
2. **Creation date:** The date it has been created.
3. **Last modification date:** The date on which the last modification has been made. This includes modifying the project's metadata, such as name, description, etc.
4. **Experiment design used to annotate:** If a project has an Experiment design attached, it will show its name here.

This feature enables users to easily identify and locate the desired project, and also helps to keep projects organized and distinguishable from each other. Additionally, having access to this information at a glance can save time and make the research process more efficient.

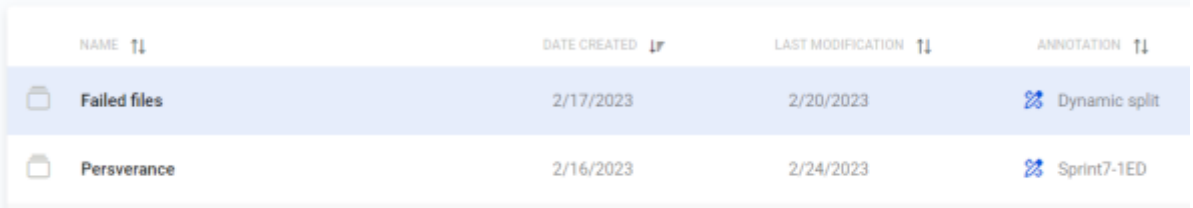


Figure 57. Example of Project information in Project explorer.

6.2.1.2 Quick actions

In the list of projects, each project provides access to its available tools through the Quick Actions menu. By clicking on this menu, you can quickly access a project's data within the selected tool. Essentially, it functions as a shortcut for easy navigation.

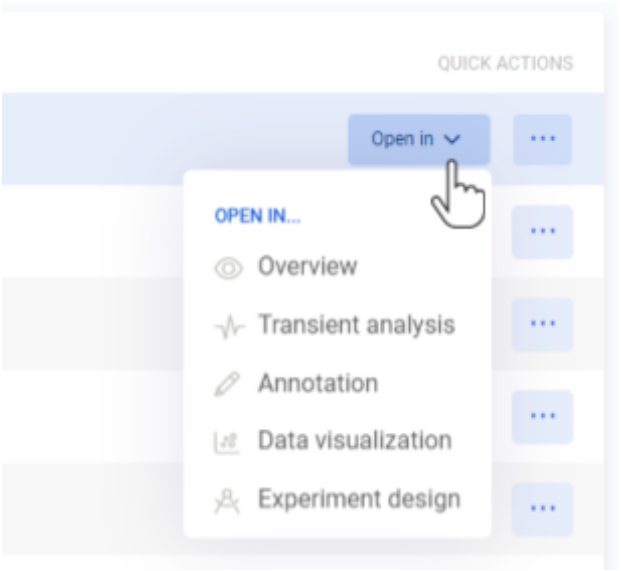


Figure 58. Project's explorer quick actions for every project.

Furthermore, the Contextual menu also provides shortcuts for project-related actions, such as editing, exporting results, and deleting the project. These options allow for efficient management of projects and data within the software.

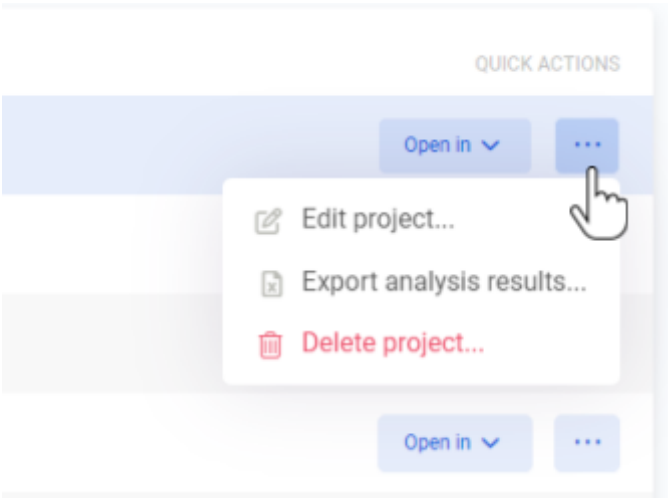


Figure 59. Project's explorer contextual menu actions for every project.

6.2.1.3 Categories and search bar

Projects can be filtered by the last modification date by selecting the "Recently Updated" tab. A project is considered updated only when it's modified (not created). This tab displays only the projects that have been modified recently. On the other hand, the "Everything" tab shows all the projects and the total number of projects found. This filtering feature allows for quick and easy navigation through the projects, based on your needs and priorities.

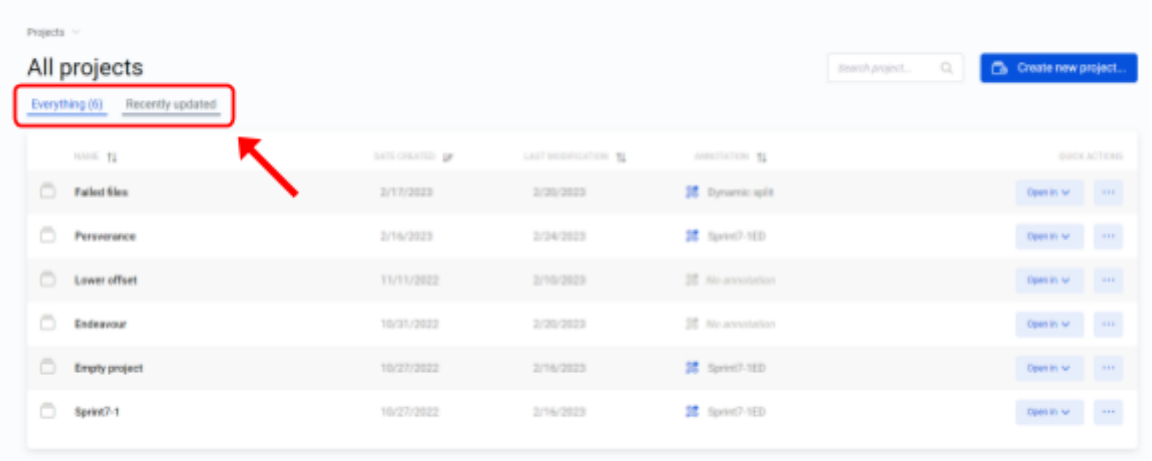


Figure 60. Project's explorer categories location.

The search bar in the project explorer enables users to efficiently locate a specific project by name or keyword. It can match both full names and partial names. For example, when searching for "Pers" it can match words such as "**Pers**everance" and "dis**pers**er". Additionally, the search filter is not case-sensitive and will match regardless of upper-case or lower-case letters. This feature allows for greater flexibility and ease of use when navigating through the software.

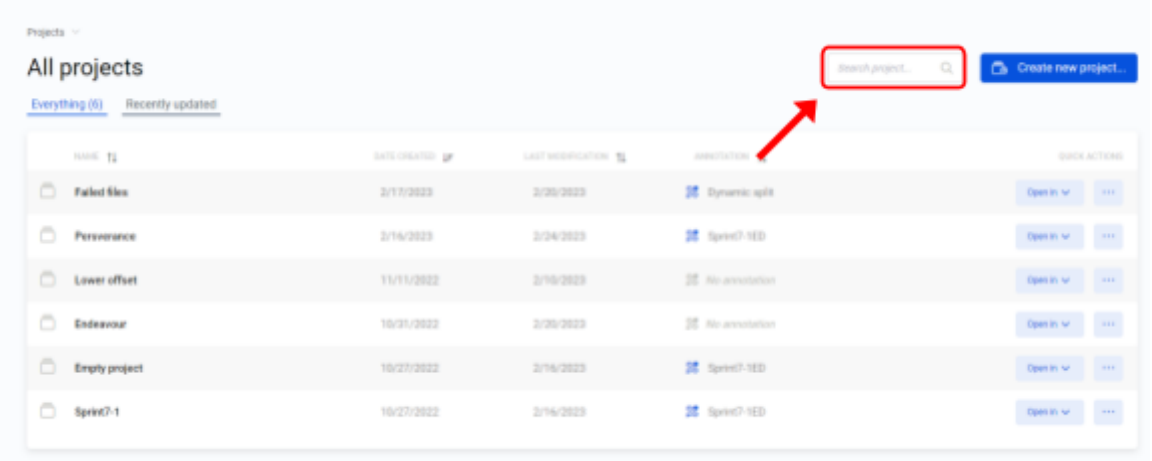


Figure 61. Project's explorer search bar location.

To use the search bar within the project explorer:

1. Locate the search bar at the top of the project explorer window.
2. Type in the search term(s) for the project you want to find. No need to press “Enter “ or any other UI element, the filter is immediate.
3. The project explorer will display a list of projects that match the search term(s).
4. If needed, use the filtering options such as the "Recently Updated" tab or "Everything" tab to narrow down the search results.
5. Click on the desired project to open it.

If there is no match for the search term entered in the project explorer search bar, the software will display a message indicating that no projects were found.

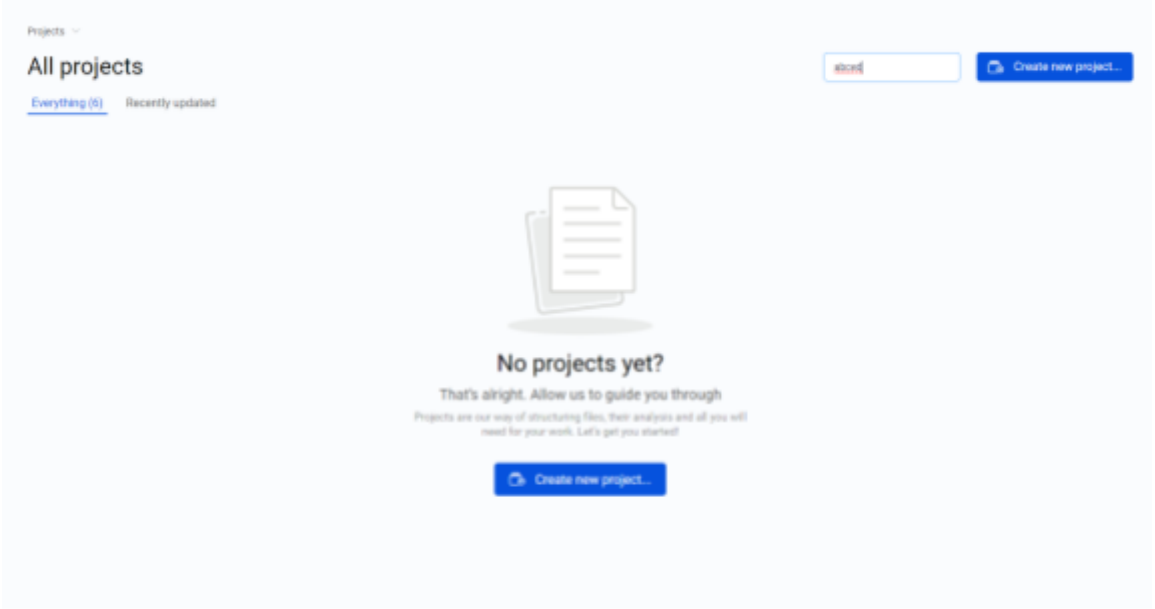


Figure 62. Project’s explorer search has no matches.

6.2.2 How to create, edit and delete a Project

Creating, editing, and deleting a project in the CytoSolver 3.0 software is a simple and straightforward process. The following steps outline how to perform these actions:

6.2.2.1 Create a new project

In order to create a project:

1. Click on the "Create new project..." button in the project explorer.
2. In the pop-up window, enter:
    a. **Name:** The identifier of your project. We recommend using different names for every project.
    b. **Start date:** (Optional) This is automatically set to today, but can be modified to any date.
    c. **Description:** (Optional) A short description of the project.
    d. Experiment design: (Optional) The Experiment design you want to use in the project.

3. Click the "Create project" button to create the project.
4. The new project will appear in the project explorer, and you can begin working on it.

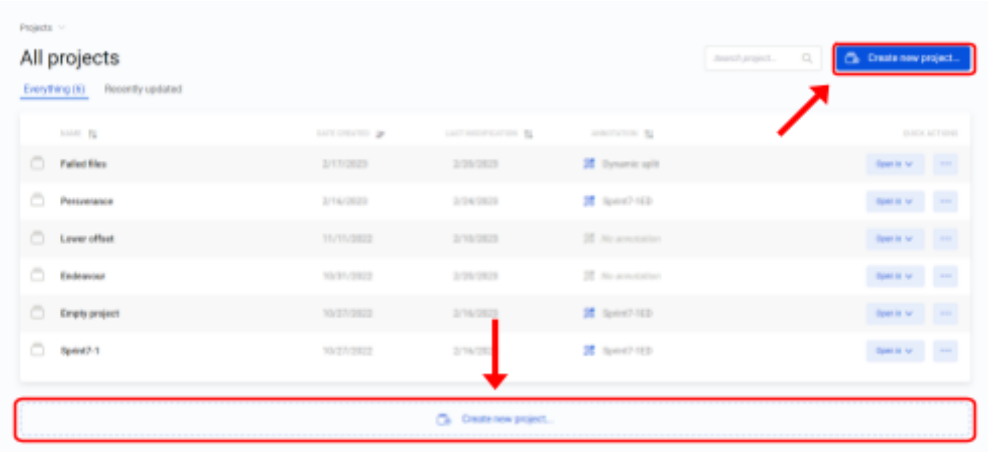


Figure 63. Project's explorer locations to create a new Project.

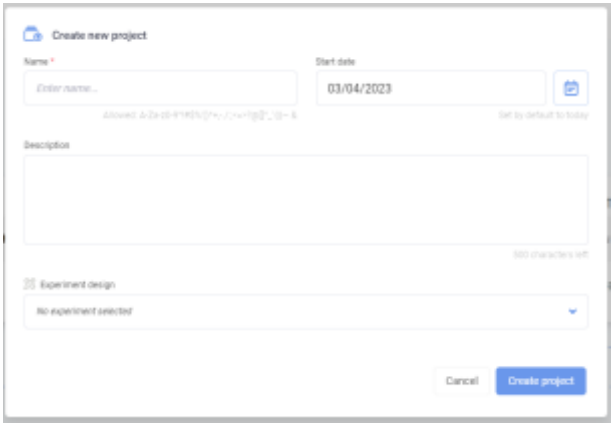


Figure 64. Modal window to create a new Project.

6.2.2.2 Editing an existing project

To edit a project you have several locations:

1. Location 1: Through the Project's explorer, by opening the contextual menu of the project to modify.

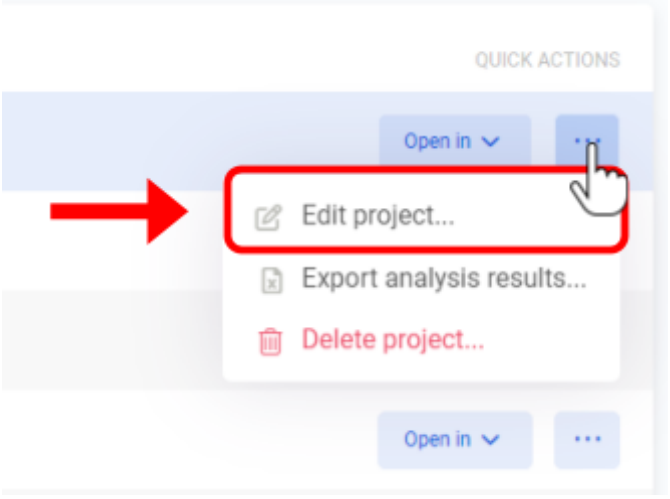


Figure 65. Location 1 - Edit project through explorer's contextual menu.

2. Option 2: Through the Project's Overview, by clicking the dedicated button.

Figure 66. Location 2 - Project's explorer contextual menu actions for every project.

3. Option 3: Through the Project's contextual menu.

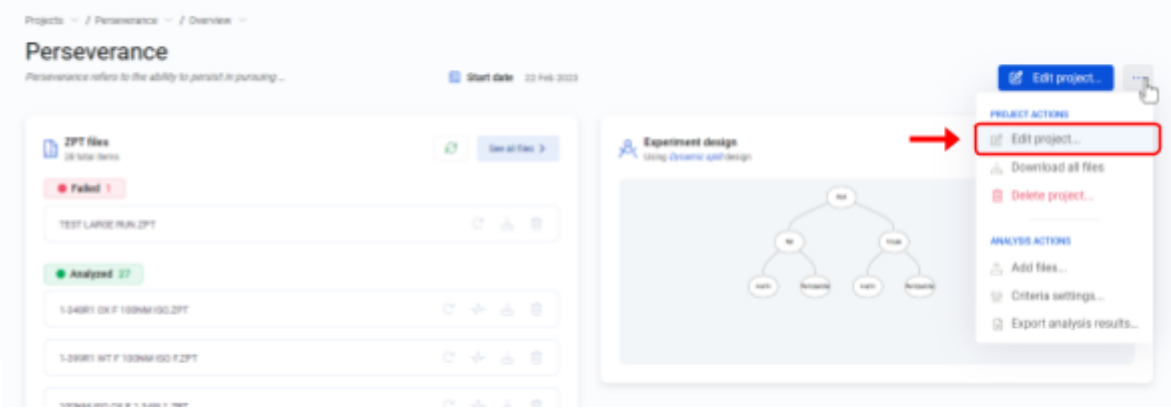


Figure 67. Location 3 - Project's overview contextual menu.

In any of the three options the "Edit project" modal will appear. Additionally, by clicking the "Add description" button (only visible if the project description is empty) under the project's name in the Project's Overview page at the header, you can also open the modal.

Steps to edit a Project:

1. Open the pop-up window from any of the 3 locations mentioned above.
2. In the pop-up window, modify the project's name, description, start date and experiment design as desired.
3. Click the "Save changes" button to save the changes.
4. The edited project will now reflect the changes made.

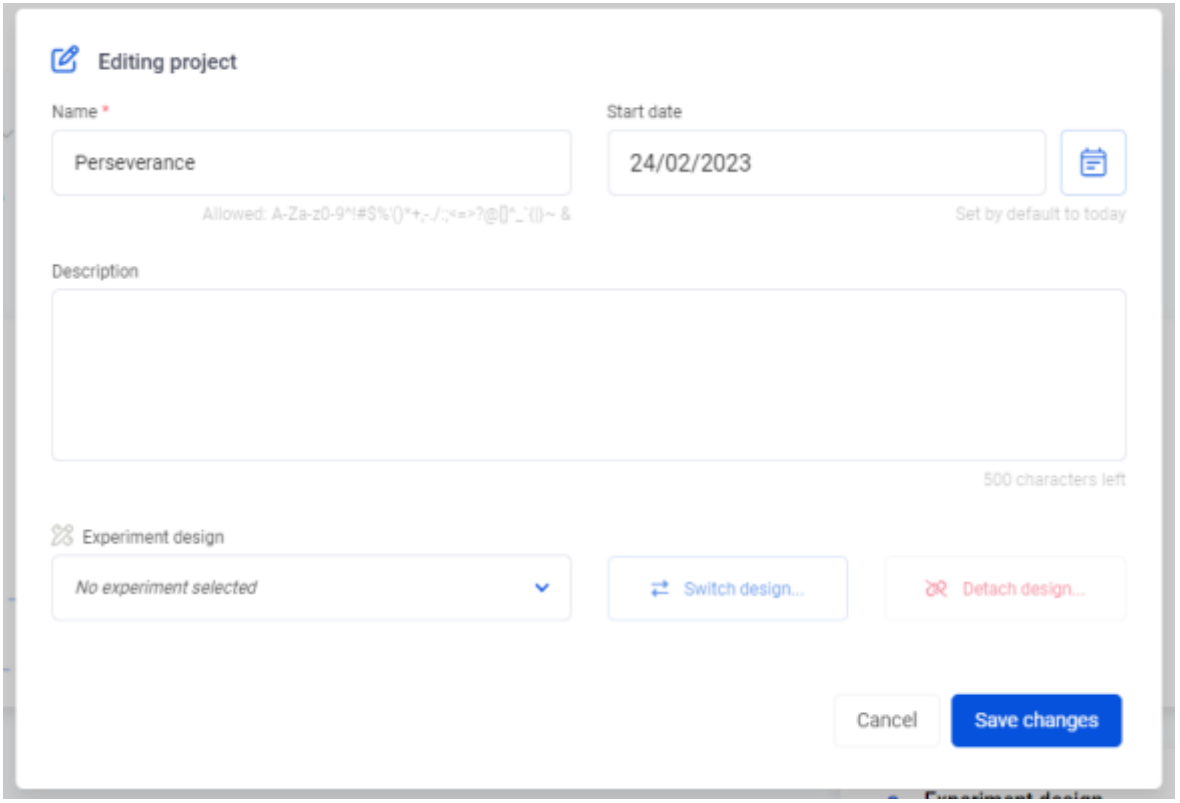


Figure 68. Edit project modal window.

6.2.2.3 Deleting a project

Finally, for deleting a project you can follow the steps below. Please be aware **this process is irreversible**, so once deleted the project and the contained files cannot be recovered:

1. Click on the contextual menu of the project you wish to delete in the project explorer.
2. Select "Delete" from the contextual menu.
3. Confirm the deletion by clicking "Yes" in the pop-up window.
4. The project will be deleted, and it will no longer appear in the project explorer.

These simple steps enable users to efficiently create, edit, and delete projects in the CytoSolver 3.0 software. By keeping projects organized and easily manageable, this feature helps streamline the research process and enhances productivity.

6.2.3 How to add new files

To begin the analysis process, you must have at least one file available in your project. These files contain data acquired through our systems using IonWizard software. Note that CytoSolver only accepts files in ZPT format, which stands for "Zone Plot Trace" files.

In the CytoSolver system, adding files can be accessed from three different locations:

1. On the Project's Overview contextual menu
2. The pathway's third level actions, or
3. the "ZPT files" widget.

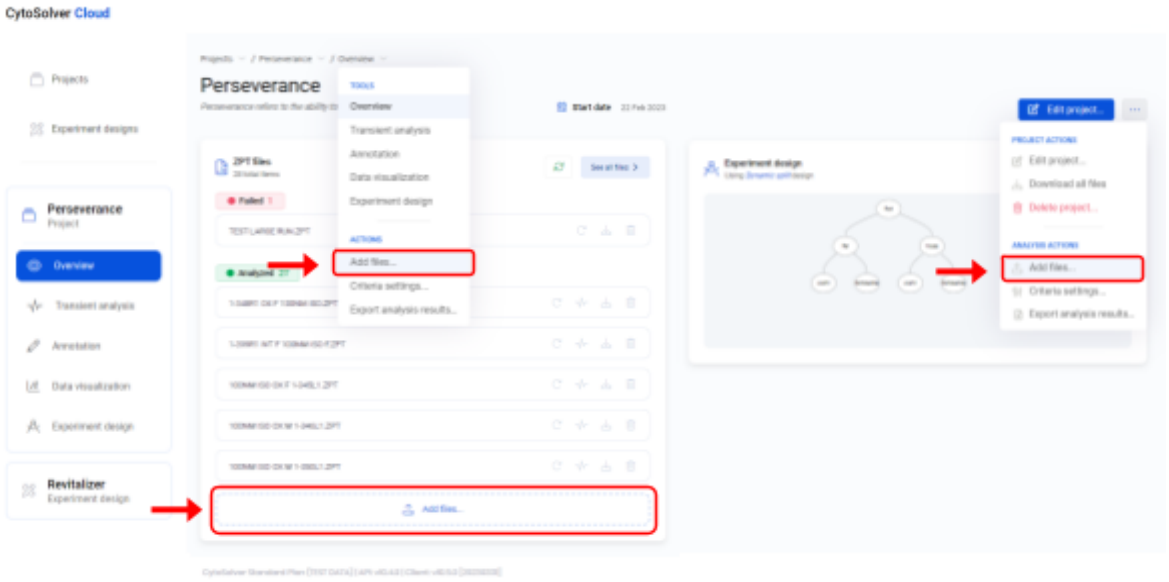


Figure 69. Locations to add new files.

Regardless of the access point, a modal window used to upload files will appear for both systems. However, the process for uploading files in CytoSolver Cloud slightly differs from that of CytoSolver Desktop.

IMPORTANT

CytoSolver Desktop allows you to add up to 20 files, **but once the analysis process has begun, you cannot add any additional files until the current batch has finished processing**.
In contrast, CytoSolver Cloud enables you to add files in batches of up to 20 at a time, **without needing to wait for the current batch to complete processing.** This means you can continuously add new batches of 20 files to be analyzed, even while previous batches are still being processed.

The following instructions explain how to add a new file to both CytoSolver Cloud and CytoSolver Desktop.

6.2.3.1 Adjusting additional import options

6.2.3.1.1 Background constants

Background constants refer to numerical values that are subtracted from the raw data in order to correct for background noise. By eliminating this noise, the resulting analysis is more precise and accurate.

NOTE

Background constants only apply to files containing fluorescence measurements such as, Ratiometric Calcium or Single Wavelength trace types (see Trace types).

This correction is **always** performed, however, it can choose between two approaches:

- ✅ By **enabling** the "Background Constants" checkbox, the analysis will utilize the background constants present in the file.
- ❌ Conversely, by **disabling** it, the analysis will instead calculate the *background constant* based on the *background measurements*. Background measurements are the traces marked with an event mark that contains the word “BACKGROUND” (See Text marks).

IMPORTANT

Enabling the “Background constants" option requires the file to contain both background measurements and constants. However, **it is not possible for CytoSolver Cloud to determine beforehand whether a file contains this information until after it is uploaded** and the analysis begins. Therefore, this option is always available in CytoSolver Cloud, but will only be utilized if the necessary information is present in the file. If you prefer not to use the background constants when both constants and measurements are present, simply uncheck the checkbox.

6.2.3.1.2 Pacing marks

Pacing marks indicate the pace at which the contraction-relaxation rhythm happens. More specifically, within a Segment, it will determine the start(s) and end(s) of the Transients.
Sometimes a ZPT file has pacing marks, sometimes it doesn't, depending on whether they were measured when data was acquired.

When analyzing ZPT files, you have two options to choose from:

- ✅ **Enabling** the pacing marks option will utilize any existing marks within the file. In cases where no marks can be found, an auto-detection algorithm will be applied to analyze the data.
- ❌ **Disabling** pacing marks will initiate the auto-detection algorithm without attempting to locate existing pacing marks. This means that even if pacing marks exist within the file, they will be ignored.

By default, the "Pacing Marks" option is enabled for all files. However, you can choose to disable it for one or more specific files, or for all files in general.

6.2.3.1.3 Analyze discarded samples

During data acquisition, it is possible that some samples/segments may be discarded from certain files. By default, CytoSolver will not analyze these discarded samples/segments, although they remain visible. However, if you wish to include them in the analysis, you can enable this option by checking the corresponding checkbox.

6.2.3.1.4 Offset (seconds)

When using the transient auto-detection algorithm, the start of a transient is indicated with a delay because the algorithm relies on detecting peaks or valleys in the data.
In order to establish a straight line (or baseline area) before the peak/valley and accomplish an ideal transient, an offset is manually applied to move the start of the transient backwards.

IMPORTANT

If there are **any pacing marks in the file and those are used** (by enabling the checkbox when adding the ZPT file to CytoSolver), **they will override the offset value**.

You have the option to modify the default offset value of 0.02 seconds to any value you require, keeping in mind that the units are seconds. It is important to choose appropriate values, as contractions' duration can vary significantly from one trace to another, and even from one file to another.

For instance, if the start of the transient is detected at a time point of 3.75 seconds, it will be shifted to 3.73 seconds.

Figure 70. Example of applying an offset.

NOTE

The offset is only applied if a file holds contractility AND fluorescence trace types (see Trace types).

6.2.3.2 Uploading files to Cytosolver Cloud

Once you land on the pop-up window that you see below, you can start adding files following the next steps:

1. **Step 1:** Click on “Browse” to open your system’s file explorer. Select all the ZPT files you want to upload through the system’s pop-up window. If you have accidentally imported more files than you intended to, you can remove them individually or remove all of them at once. It’s also important to note that the current system only allows **batch upload of up to 20 files**, with each individual file not being larger than 25 megabytes.

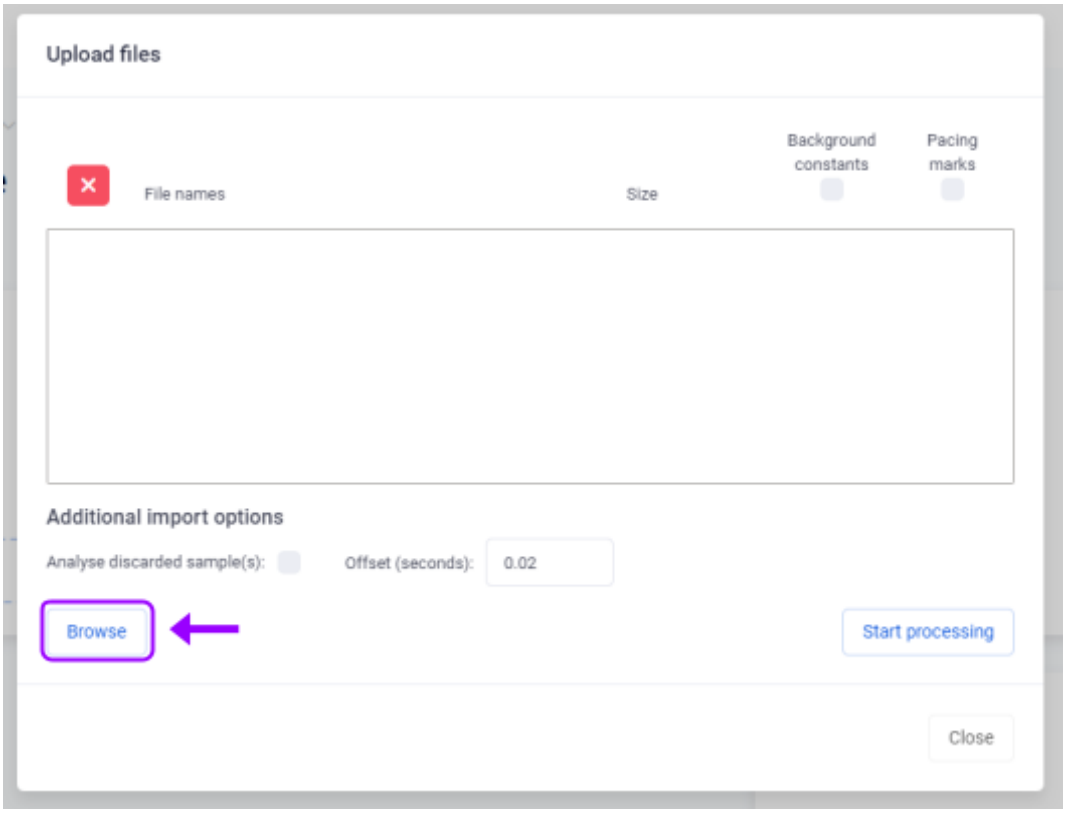


Figure 71. Upload files on CytoSolver Cloud - Step 1: Locating your files in the system.

2. **Step 2:** Customize the “Additional import options” based on your preferences, as these will impact the final analysis results. Please keep in mind that once the files are uploaded, the analysis will commence immediately using the adjusted options. CytoSolver Cloud always has checkboxes for “Background constants” and “pacing marks” enabled.

IMPORTANT

Once files have been processed, **the Additional import options cannot be modified, even after the analysis is completed**. To apply a new combination of analysis options, you will need to delete and upload the files again.

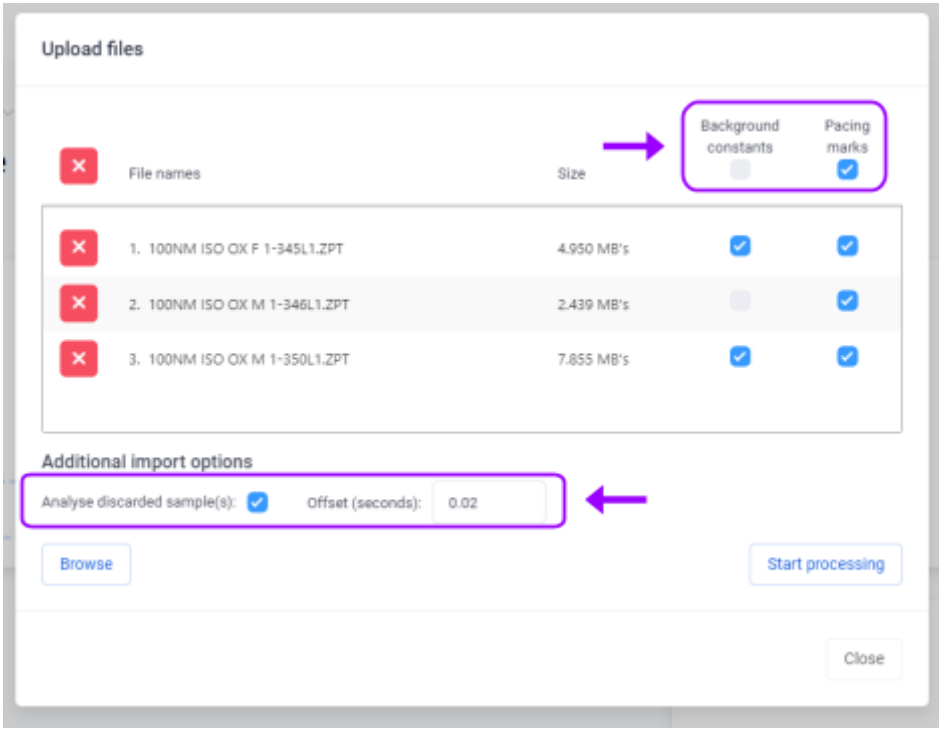


Figure 72. Upload files on CytoSolver Cloud - Step 2: Adjusting additional import options.

3. **Step 3:** To start the analysis process, simply click on the ”Start processing” button. This will add all the files to the analysis queue and make them ready for analysis.

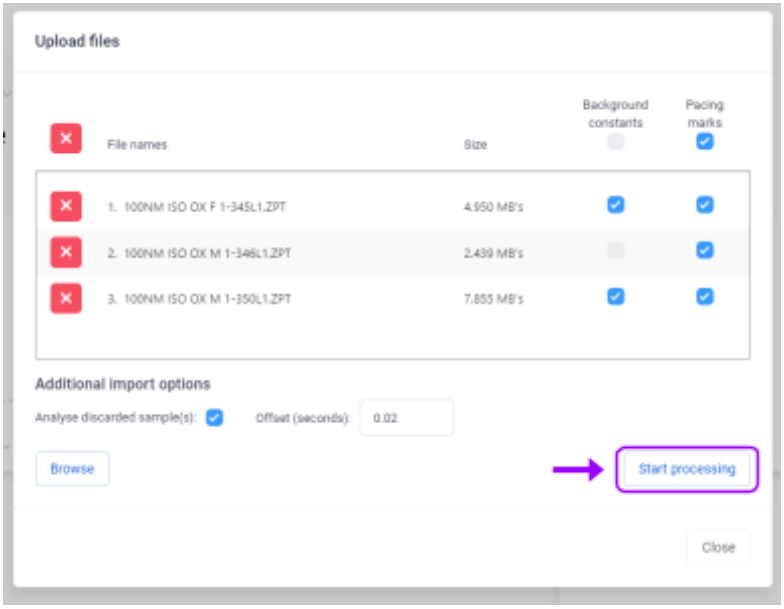


Figure 73. Upload files on CytoSolver Cloud - Step 3: Process the files.

If you attempt to select files that are not supported by CytoSolver Cloud, you will see an indicator within the pop-up modal pointing out that the file type is not valid. In the event that the file is still uploaded, it will be rejected during analysis and the analysis will be marked as “Failed”.

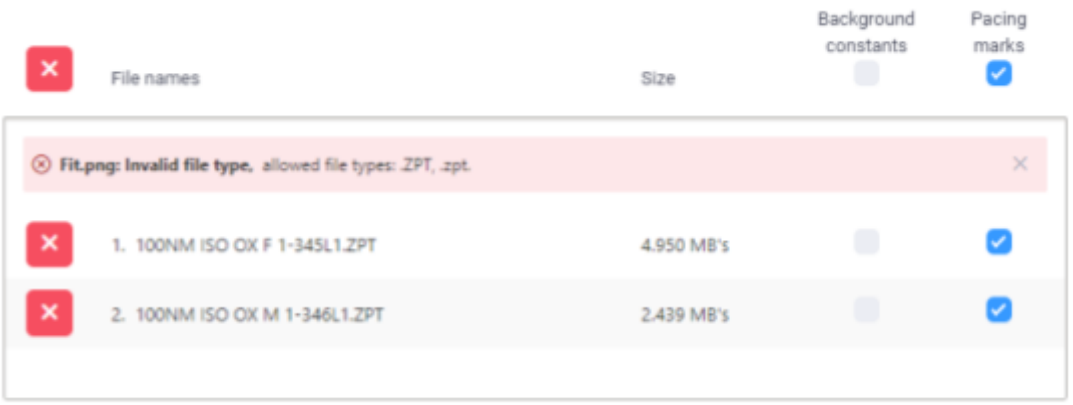


Figure 74. Example of error while attempting to upload a file in an unsupported format.

6.2.3.3 Importing files to Cytosolver Desktop

While the basic steps for using CytoSolver Desktop are similar to those for CytoSolver Cloud, the interface may appear different and the location of items may vary.
Additionally, the CSD software provides a trace conversion feature that enables users to map acquired data onto existing traces. This feature is accessed through the Trace Configurator tool.

6.2.3.3.1 Steps to import files

After reaching the pop-up window shown below, you can begin adding files by following the steps outlined below:

1. **Step 1:** Click on “Browse” to open your system’s file explorer. Select all the ZPT files you want to upload through the system’s pop-up window. Please note that only files in ZPT format will be shown/displayed. If you have accidentally imported more files than you intended to, you can remove them individually or remove all of them at once.

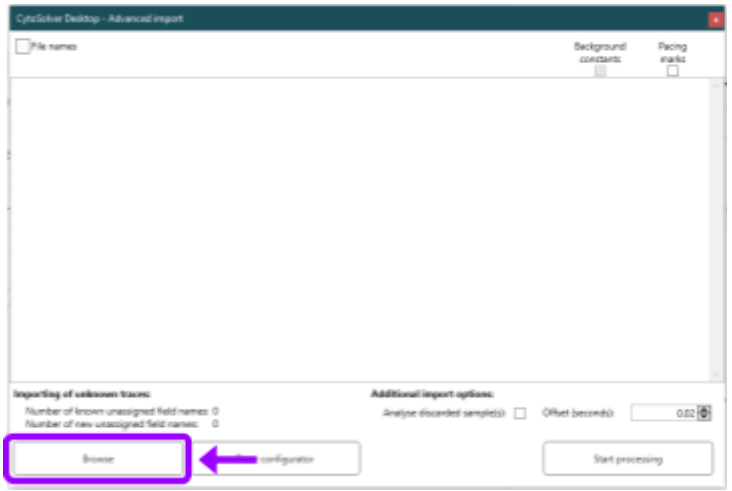


Figure 75. Upload files on CytoSolver Desktop - Step 1: Locating your files in the system.

2. **Step 2:** Customize the “Additional import options” based on your preferences, as these will impact the final analysis results. Please keep in mind that once the files are uploaded, the analysis will commence immediately using the adjusted options. Availability of the "Background constants" checkboxes in CytoSolver Desktop is based

on the presence of background segments and constants. “Pacing marks” checkboxes are always available.

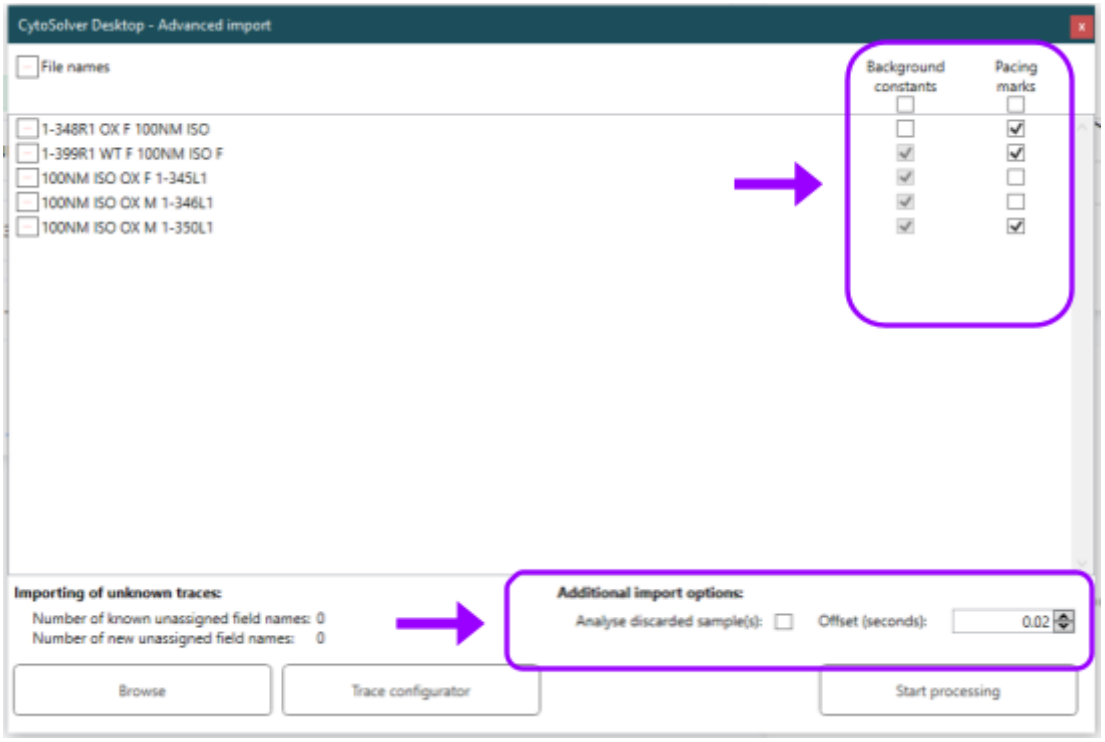


Figure 76. Upload files on CytoSolver Desktop - Step 2: Adjusting additional import options.

3. **Step 3:** Check whether there are any unknown traces (See Glossary for definition) in your files. This can be done by checking the “Number of unassigned field names” field. There are two cases possible:
    a. The number is 0: Then there are no unknown traces. You can proceed to the next step (4).
    b. The number is N, larger than 0:
        i. Then there are N unknown traces that you need to assign to a specific known trace that resembles it in shape or content.
        ii. To do that, you can click on “Trace configurator” (further explained in Converting traces through Trace Configurator).
        iii. On the “Fieldnames(s)” column you will see the names of the unknown traces, and on the “Raw Traces” the list of known traces we have an algorithm for.
        iv. By assigning a *Raw trace* (known) to a *Fieldname* (unknown trace), CytoSolver knows what specific operations should perform on it and how to analyze the unknown trace, doing it in the same manner as it would do with the known trace.
        v. Click “OK” to save the changes, or “Cancel” to discard the assignments.

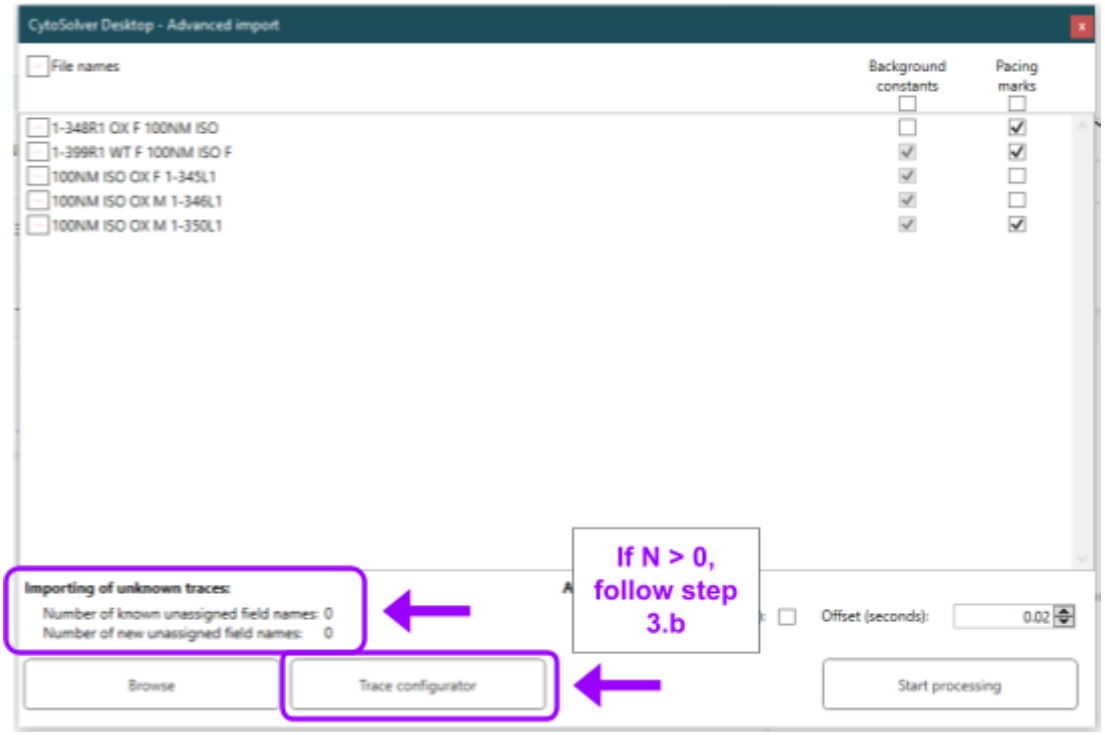


Figure 77. Upload files on CytoSolver Desktop - Step 3: (1) Assigning known traces.

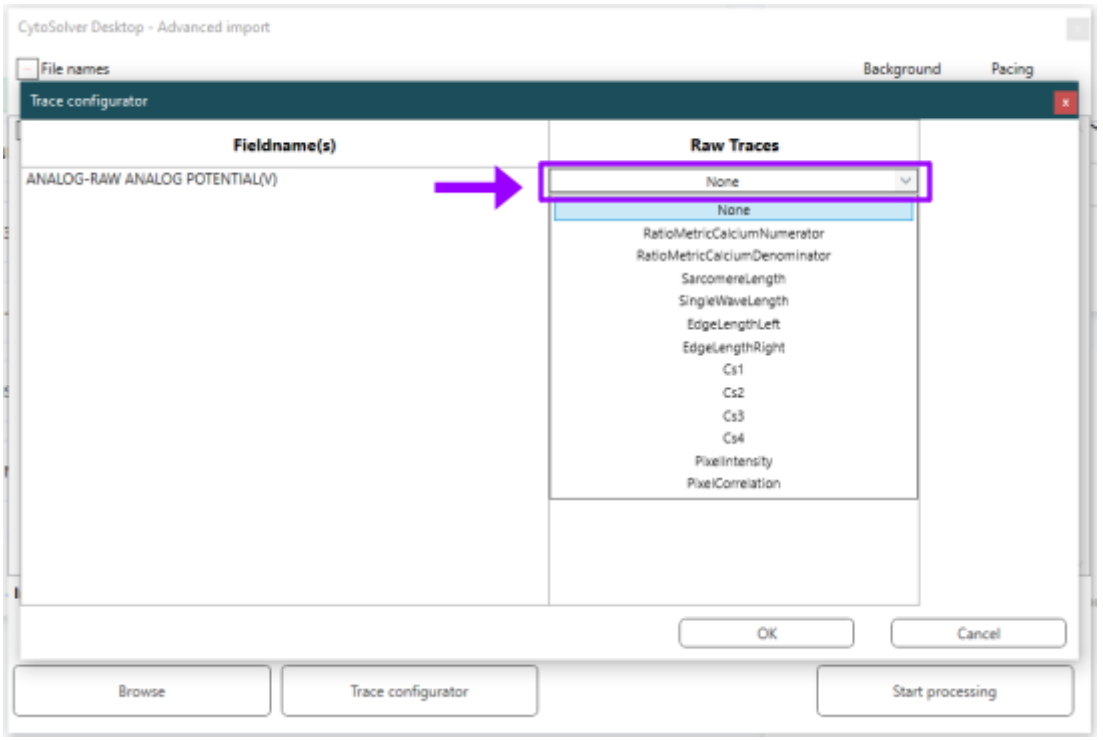


Figure 78. Upload files on CytoSolver Desktop - Step 3: (2) Assigning known traces.

4. **Step 4:** To start the analysis process, simply click on the ”Start processing” button. This will add all the files to the analysis queue and make them ready for analysis.

Keep in mind that while files are being processed, the UI will not allow you to add additional files or use other tools.

6.2.3.3.2 Converting traces through Trace Configurator

Raw traces often require conversions to extract their biological meaning. This is due to the fact that our measurements are taken through cameras, which capture images and convert them into pixels. Transforming these pixels into biological information is crucial for analysis and interpretation.

To achieve this, the raw data (pixels) must undergo a conversion process that transforms them into a constructed trace, which is a known trace that can be used to analyze and interpret the biological information contained within the raw data.

NOTE

Keep in mind a “Pixel unit” on the tables refers to the general description of a pixel: the smallest unit of a digital image, represented as a single point of color or brightness on a screen or digital sensor.

In the next table, you can see how the conversions are performed.

Known trace type	How do we convert it
Sarcomere length	Pixel unit is converted into sarcomere length via multiplication with the calibration constant, stored in the ZPT file. The sarcomere constant can be edited in IonWizard (File > Constants).
Edge length	The pixel unit is converted into length and the left edge is subtracted from the right edge.
Ratiometric calcium	During acquisition, each ratiometric trace is obtained as a pair of traces: a numerator trace and a denominator trace. The final known trace is constructed by dividing the numerator trace by the denominator trace.
Single wavelength	*No construction.*
Ratiometric calcium with interpolated numerator	Is constructed by dividing the interpolated numerator trace by the denominator trace.
Pixel correlation / Pixel intensity	These are dimensionless contrast-based methods not requiring conversion.

Table 12. How are raw trace converted

6.2.4 How to delete files

Removing files from the CytoSolver system will erase their analysis results. Such files will not appear in exports, nor will they be considered in the calculation of averages.

You can remove a file from two different locations:

- The Project's Overview, within the ZPT files summary.
- The list of all the files contained in the project.

In both cases interacting with a list of files will allow you to delete them.

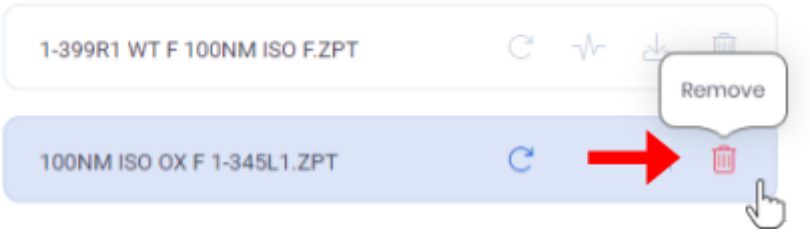


Figure 79. Location of the remove button to delete files.

6.2.5 Understanding the Project overview

Various pieces of information make up a project. Essentially, a project is unique due to its:

1. **Name:** Indicates the name identifier you have provided.
2. **Description** *(optional)*: Shows a brief summary of the project.
3. **Starting date** *(optional)*: Denotes when the project has been initiated.
4. **Experiment design** *(optional)*: Specifies the organization and labeling of the experiment conducted in the project.

These fields are also the ones shown when creating a project.

Figure 80. Example of the Project's Overview.

6.2.5.1 ZPT Files box (widget)

The ZPT files are a crucial component of CytoSolver and are necessary for starting a project's analysis. It is important to note that once you add a file to CytoSolver, it will be automatically analyzed. CytoSolver uses information from these files to display vital analysis information and offers tools for file management."

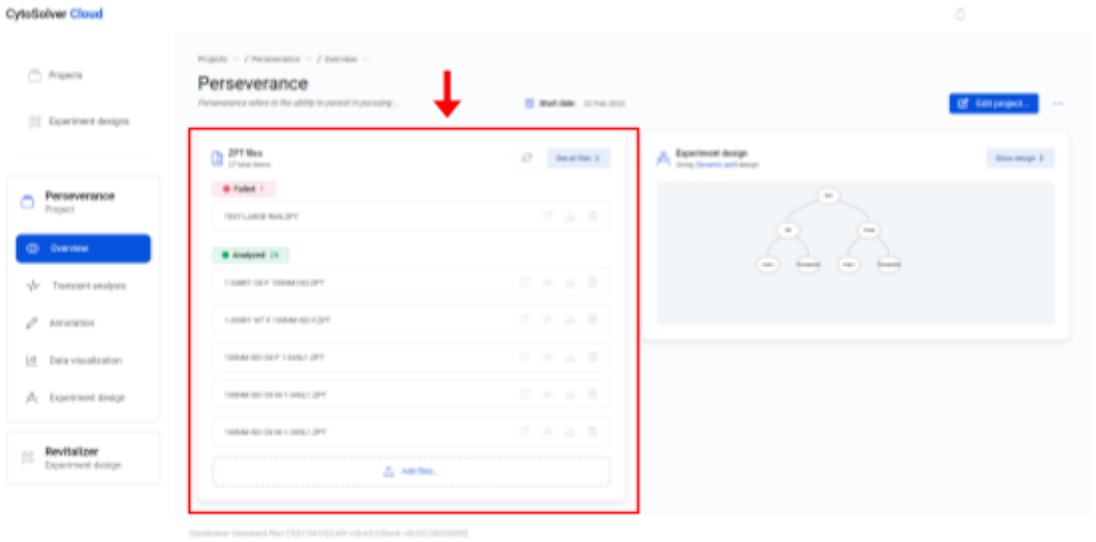


Figure 81. Location of the ZPT files controller (widget).

The Project Overview provides a widget that gives a brief overview of the files, highlighting key events. This widget displays the total number of files, their statuses, and several available actions that can be performed.

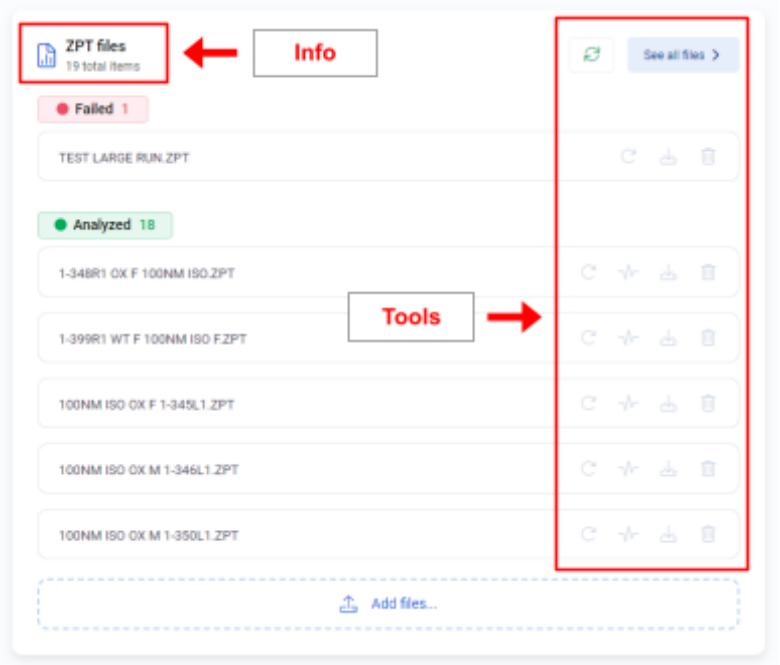


Figure 82. Example of the ZPT file summary on Project Overview page.

6.2.5.1.1 Actions

Each file has a particular set of actions that can be performed on it. The availability of these actions may depend on the file's status (see File status below), but primarily, there are four actions available:

1. **Reanalyze:** Reprocesses the file using the Criteria settings set at the time the action is triggered. This action is only available for files that have been successfully analyzed.
2. **View:** Navigates to the file's results in the Transient Analysis tool.
3. **Download (only in CytoSolver Cloud):** Downloads the uploaded file to your local computer.
4. **Remove:** Deletes the file from CytoSolver (see Deleting files)

KEY CONCEPT

When you add files to a project, **they are immediately processed** and sent to a queue for analysis to generate results.

6.2.5.1.2 Files' status

The status of a file provides information on its current position in the processing pipeline. A ZPT file can be exclusively in one of the following statuses, regarding its position towards the analysis:

1. 🟡 **In queue:** Right after uploading, the file is waiting to be analyzed.
2. 🟢 **In analysis:** It's being analyzed by the Transient Analysis tool.
3. 🟢 **Analyzed:** Transient Analysis tool finished processing the file and the results are ready to visualize.
4. 🔴 **Failed:** Something went wrong with the analysis and they could not be processed. There are no results to show.

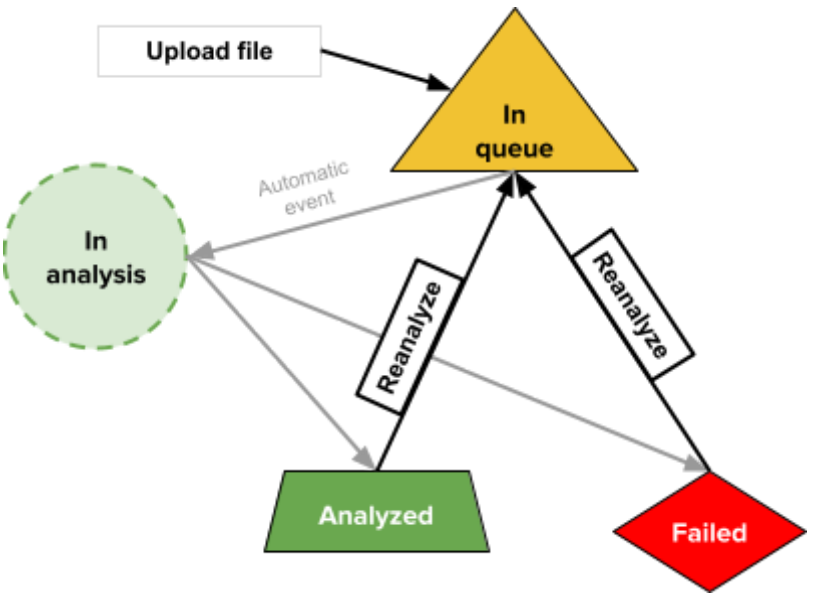


Figure 83. Relation between states.

Modifying a file's state to any other state cannot be done by the User via a direct interaction. For example, you cannot choose to set a file's status from "Stored" to "Failed".
However, there are actions that change a file's status. If you choose to reanalyze a file, this will change from "Analyzed" to "In queue" and later to "In analysis". These changes happen automatically, there's no manual action that a User can do to change it.

The Project Overview displays a summary of files, grouped by status with the number of files indicated in each category. The widget shows up to five items per status.

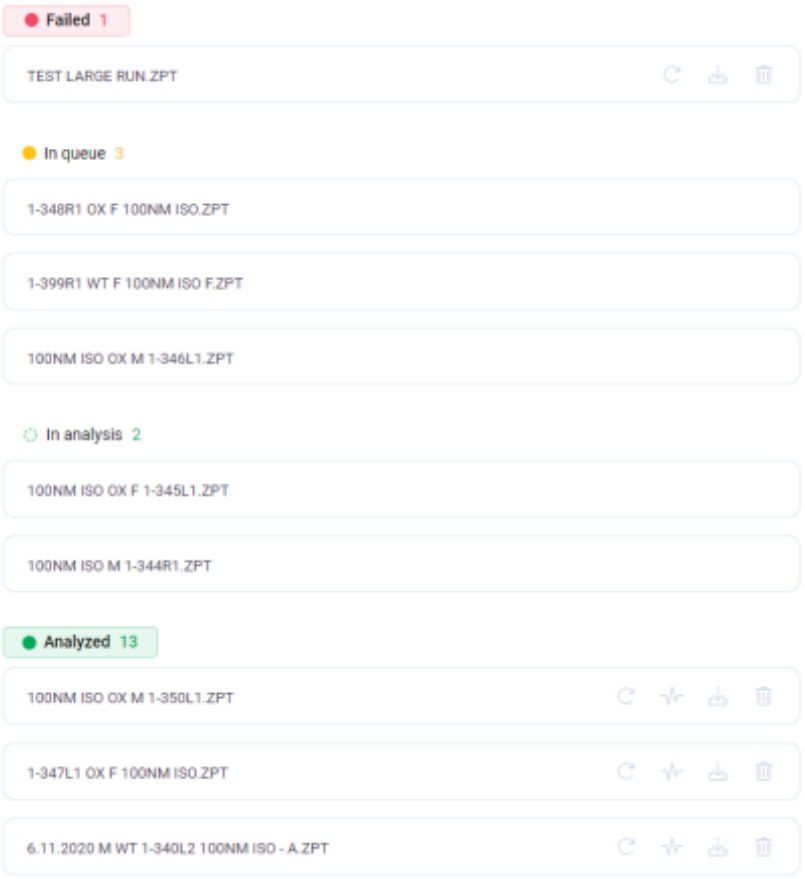


Figure 84. Example of file statuses.

If a status category contains more than five items, you can access a pop-up window to view all the files in that category by clicking "See all files".

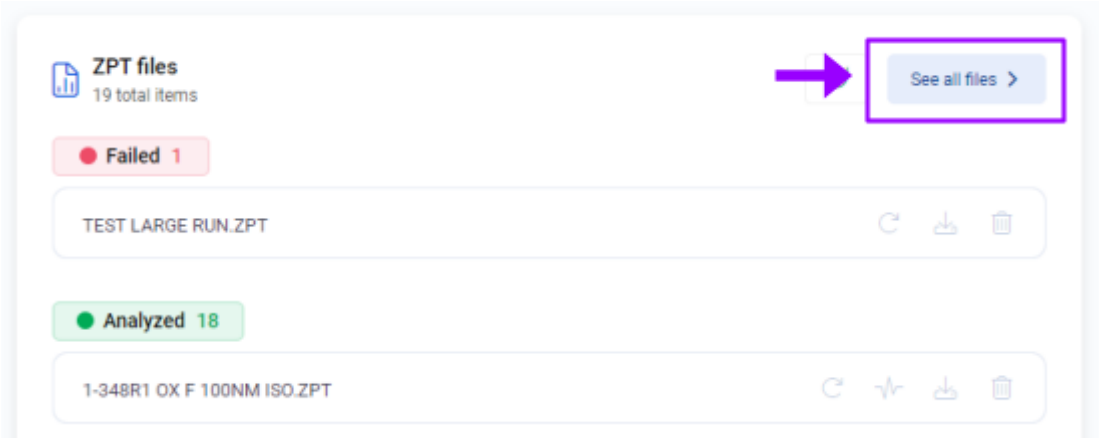


Figure 85. Location of "See all files" to check all the files available under a status.

CytoSolver Cloud has a refresh period of 7.5 seconds for the status of each file. If you want to update the information manually, you can also do so by using the refresh button on the upper-right corner of the widget. CytoSolver Desktop displays updates in real-time, eliminating the need for manual refreshing. Therefore, the refresh button is not shown.

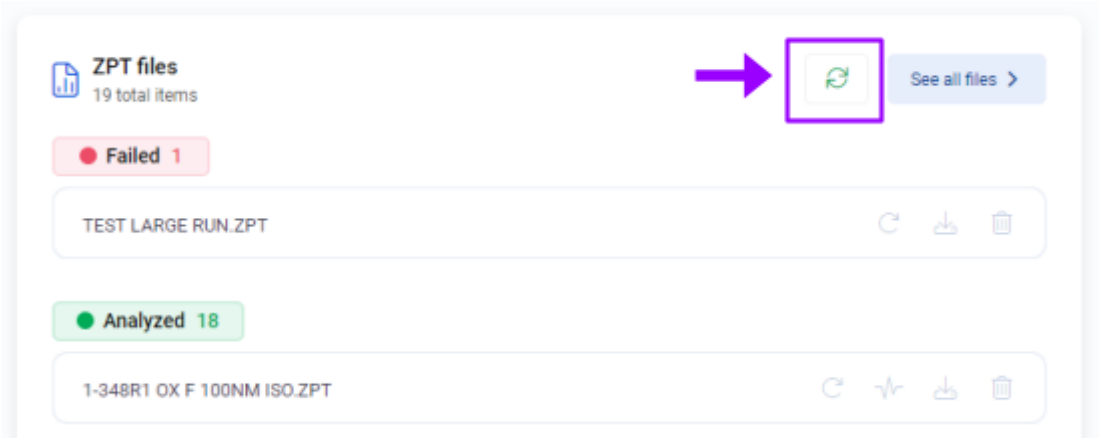


Figure 86. Location of refresh button to update statuses in CytoSolver Cloud.

6.2.5.2 Experiment design box (widget)

Finally, the Experiment design box displays the design that has been attached to the Project. The graph inside the box is interactive, allowing you to zoom in or out to explore the details, but it cannot be modified. Its purpose is to provide an informative reflection of the current state.

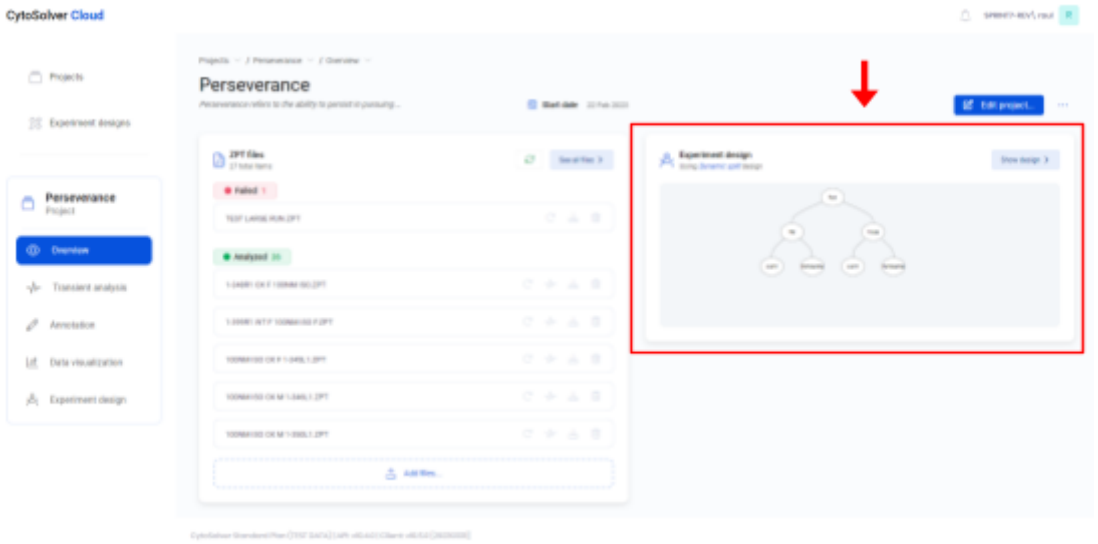


Figure 87. Location of the Experiment design controller (widget).

You can also access the Experiment design tool within a Project by clicking on the "Show design" button.

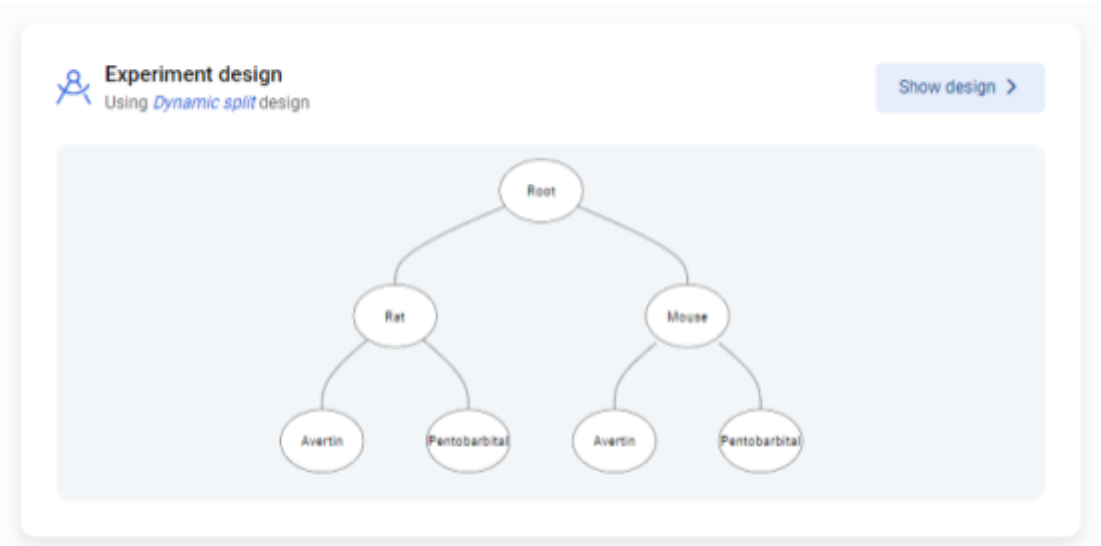


Figure 88. Example of the Experiment design box (widget).

6.2.6 What tools can a project use

Within a project, you'll find a suite of five submenus or tools that enable you to efficiently access, read, label, and structure your data and results.

The following are the tools within a Project:

1. Overview
2. Transient analysis
3. Annotation
4. Data visualization
5. Experiment design

When a project has an Experiment design attached, it will show all 5 tools as available, in every UI element that grants access to them (Project sidebar item, pathways, etcetera).

However, if there is no Experiment design attached, the "Annotation" tool and the "Experiment design" tool will not be accessible or displayed, resulting in a modified menu:

1. Overview
2. Transient analysis
3. ~~Annotation~~
4. Data visualization
5. ~~Experiment design~~

6.3 Understanding “Experiment designs”

In addition to projects, CytoSolver features a second organizational unit called "**Experiment Design**". This structure allows you to create a tree-like hierarchy that represents the different branches of your experiments.
For instance, if an experiment involves testing two species of animals with two different compounds, there will be four different branches within the experiment design. More specifically, if you choose “Rat” and “Mouse” as species and “Avertin” and “Pentobarbital” as compounds, the experiment design will look like this:

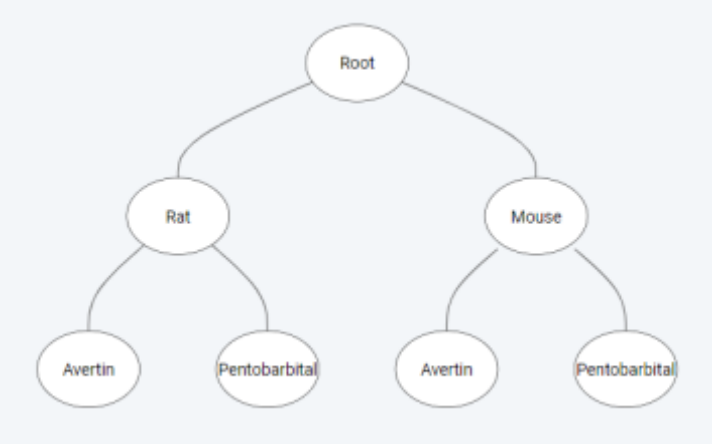


Figure 89. Example an experiment design structure.

This structure becomes particularly useful when dealing with multiple projects. If any change needs to be performed (like for example a new species needs to be added) instead of modifying each project individually, you can modify the experiment design only once. The experiment design can then be attached to the project, allowing for a seamless organization of the research data. By utilizing experiment designs and projects, you can better manage your research and maintain consistency across multiple experiments.

6.3.1 How are Experiment designs organized

Experiments follow the same organizational structure as Projects (as explained above in 6.3.1 How are Projects organized?). The Experiment designs explorer contains a list of all Experiment designs.

This feature allows you to easily access and manage your projects. Experiment designs can be created, and filtered through the Experiment designs explorer.

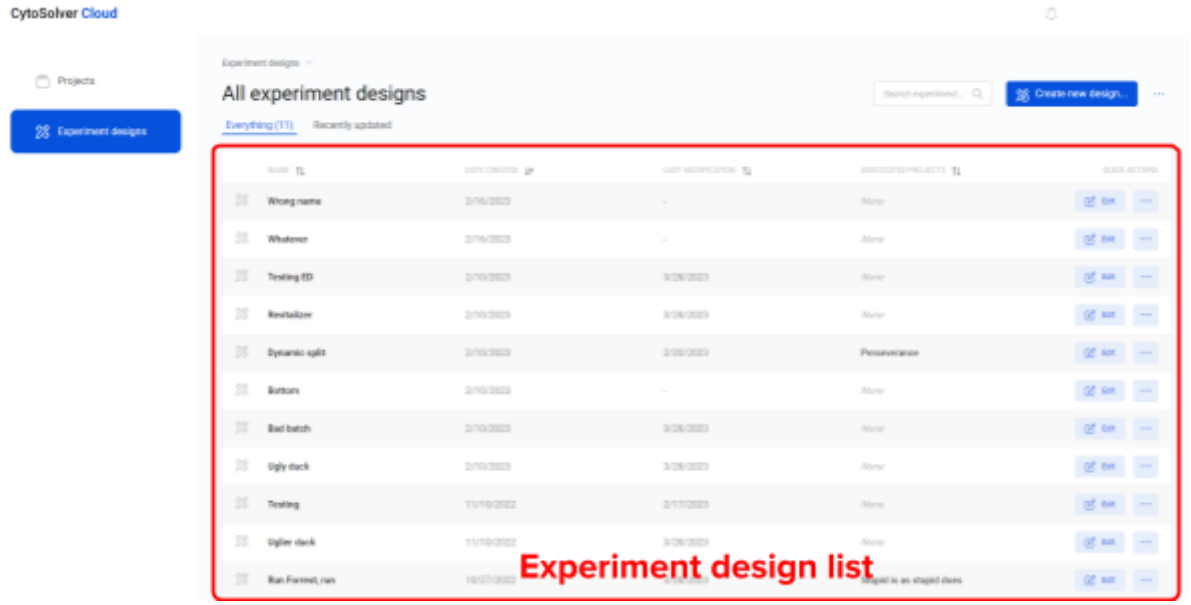


Figure 90. Project's explorer containing all the existent projects in your account.

6.3.1.1 Displayed information

When viewing the list of Experiment designs, some information about each Experiment design is displayed for quick reference. This information includes:

1. **Name:** The name you assigned to the Experiment design.
2. **Creation date:** The date it has been created.
3. **Last modification date:** The date on which the last modification has been made. This includes modifying the Experiment design's metadata, such as name or description.
4. **Associated projects:** List of Projects that have this Experiment design attached.

Figure 91. Example of Project information in Project explorer.

This feature enables users to easily identify and locate the desired Experiment design, and also helps to keep Experiment designs organized and distinguishable from each other. Additionally, having access to this information at a glance can save time and make the research process more efficient.

6.3.1.2 Quick actions

In contrast to the list of Projects, the list of Experiment designs enables Quick actions that directly affect the Experiment design. The contextual menu offers shortcuts for experiment-related actions, such as editing and exporting results.

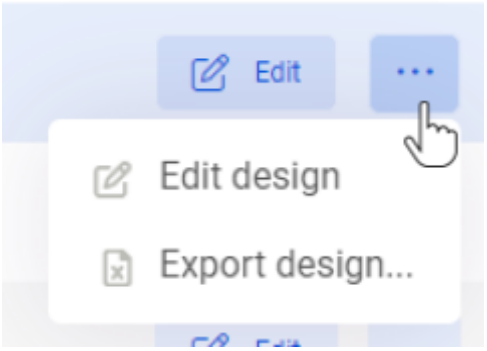


Figure 92. Experiment designs' explorer contextual menu actions for every item.

6.3.1.3 Categories and search bar

The categories and the search bar for Experiment designs work exactly the same as for Projects. You can check that under 6.3.1.3 Categories and search bar.

6.3.2 How to create, edit, and delete an Experiment design

6.3.2.1 Create an Experiment design

In order to create an Experiment design:

1. Click on the "Create new design..." button in the Experiment design explorer.
2. In the pop-up window, enter the name and description of the new Experiment design.
3. Click the "Create" button to create the Experiment design.
4. The new project will appear in the Experiment design explorer, and you can begin working on it.

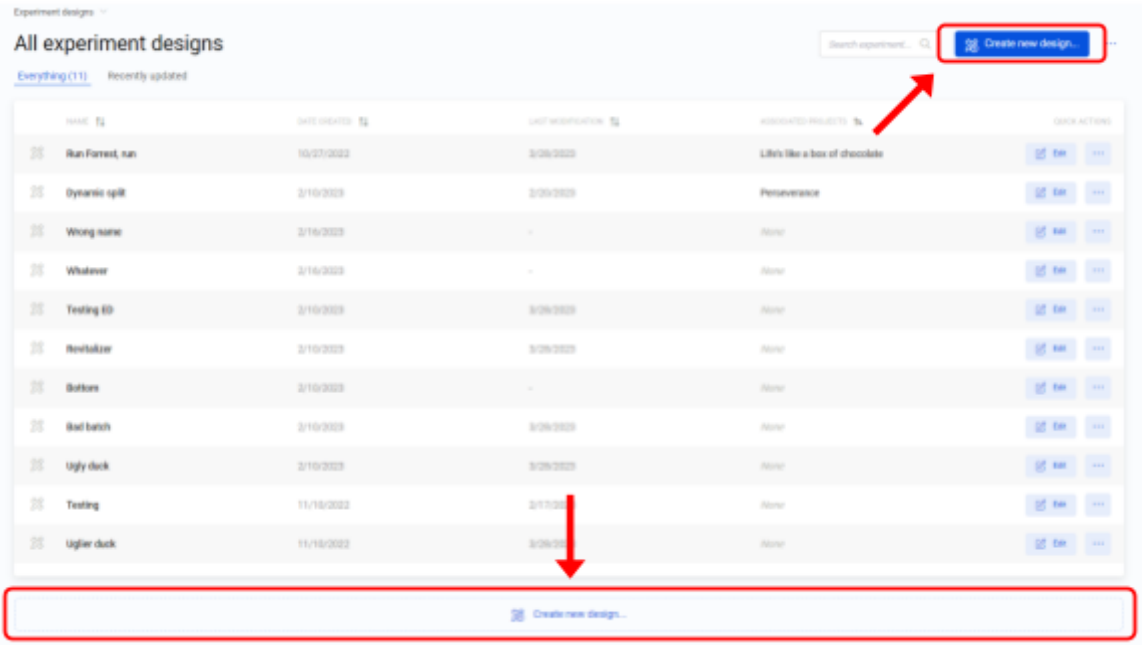


Figure 93. Experiment designs' explorer locations to create a new Experiment design.

6.3.2.2 Edit an Experiment design

To edit a project you have one single location: By opening the experiment design in the editor and clicking the "Edit design..." button, a modal will appear.

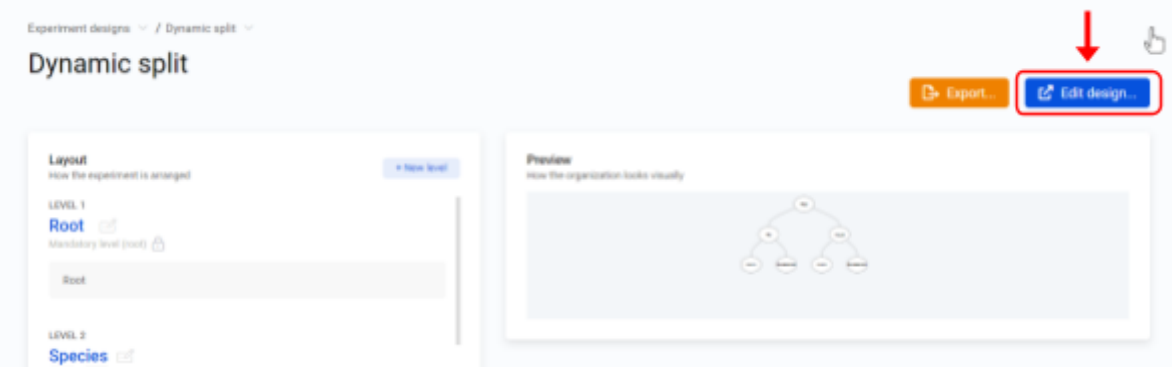


Figure 94. Experiment design editor "edit design.." button location.

Steps to edit an Experiment design:

1. Open the pop-up window from the location mentioned above.
2. In the pop-up window, modify the Experiment design's name or description as desired.
3. Click the "Save changes" button to save the changes.
4. The edited Experiment design will now reflect the changes made.

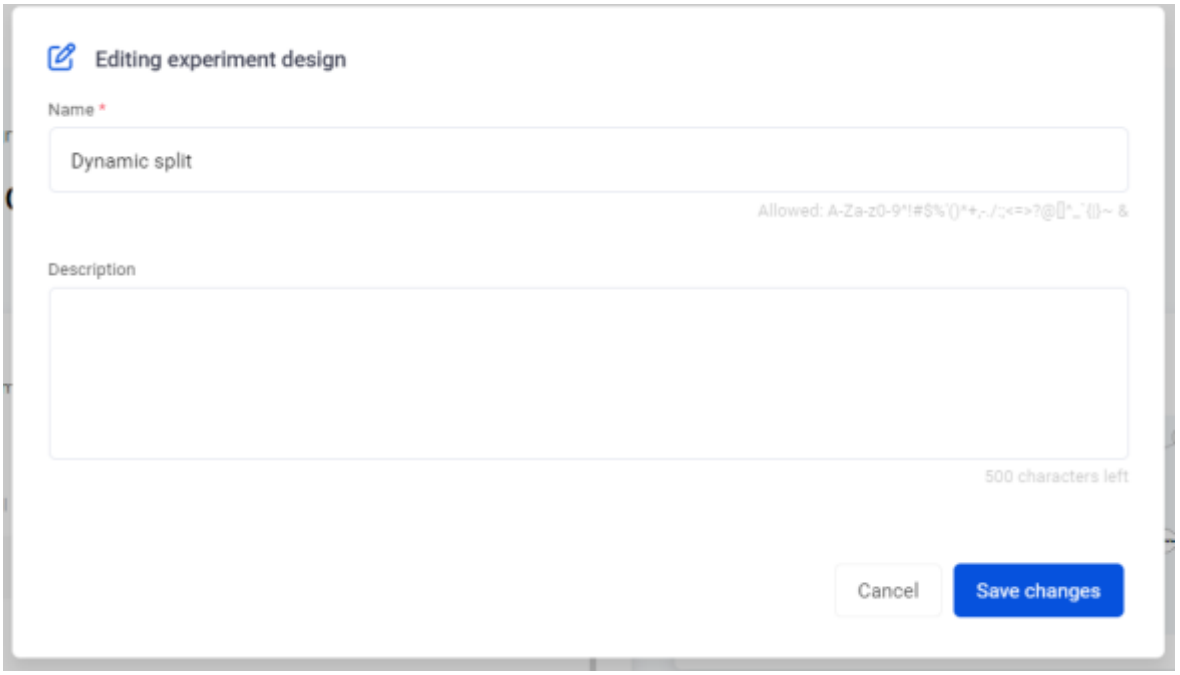


Figure 95. Edit Experiment design modal window.

6.3.3 Understanding the Experiment design editor

The Experiment Designer Editor has a list-like format similar to a text editor that enables you to create an experiment structure. The page is divided into two parts:

1. **Layout** (left side): The input area where you can add the terms for your experiment design.
2. **Preview** (right side): A live preview of the tree-like graph being built as you set up the structure on the left.

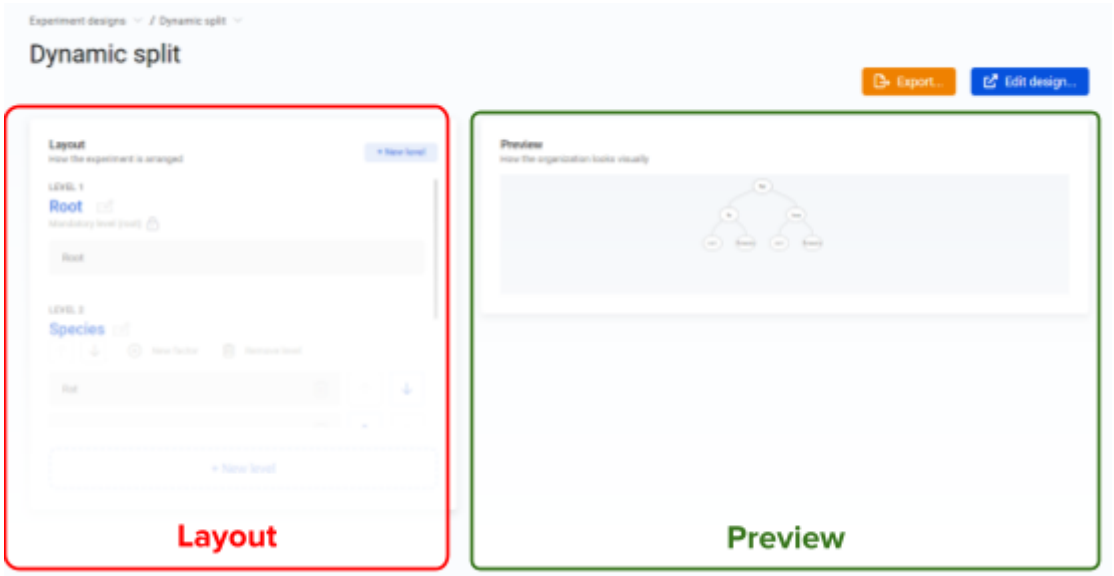


Figure 96. Preview of the Experiment designer editor sections.

The Layout has a list-like structure consisting of two divisions: Level and Factor(s). Level identifies the condition or item (e.g., "Species," "Component," or "Intensity"). Factors are subcategories within a level that specify defining characteristics. There is no limit on the amount of levels and factors you can create.

Figure 97. Preview of the Experiment designer "Layout" item

6.3.3.1 How to add a level

To add a new level, you can use any of the “+ New level” buttons located at:

1. The top-right corner of the Layout.
2. The bottom of the layout.

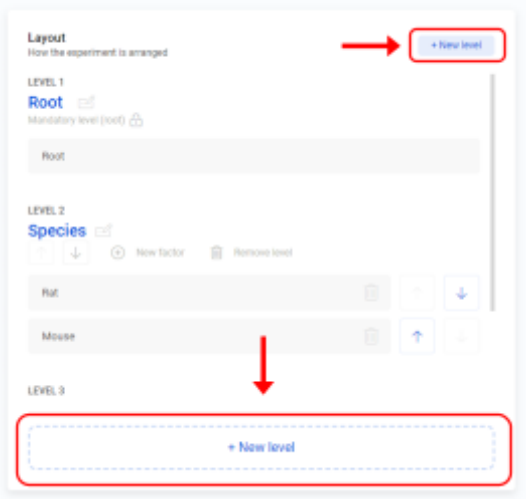


Figure 98. Location of the “+ New level” buttons.

When creating a new level, each of them will be assigned a unique numeric identifier to indicate its position in the hierarchy, such as Level 1, Level 2, and so on.

To avoid confusion in experiments or during annotation, we recommend using distinct names when naming levels. In case of duplicate level names, the system will automatically rename the duplicate level with a unique name. For instance, if you name two levels as "Species," the second one will be automatically renamed to "Species 1".

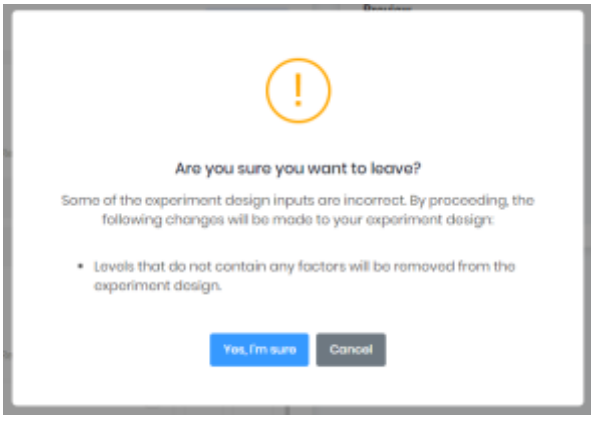


Figure 99. Modal informing about empty levels removal.

When adding new levels without adding any factors underneath, please note that they will be removed from the experiment design once you move to another page and save the changes. In case this situation arises, a notification will appear before leaving the page to inform you about the removal.

TIP

Upon creating a new level, the focus will automatically shift to the level's name. **Once you've finished typing the level's name, pressing Enter will add the first factor automatically**, enabling you to start typing in its name. Subsequently, by pressing Enter again, a new factor will be created, and the process can be repeated as many times as necessary. The workflow is designed to resemble an ordered list, much like any text editor. Finally, after you've added all the required factors, you can leave the last one blank and press Enter again to complete the process.

How to delete a level

To delete a level, you can use the "Remove level" option within the level. This will remove the level along with its factors, reflecting the changes in the Preview graph. Please note that this action does not require confirmation and it's irreversible.

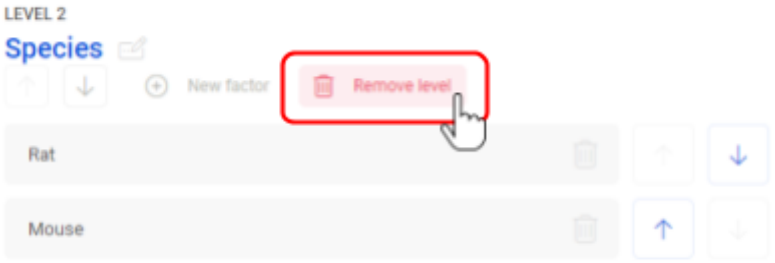


Figure 100. Location of the "Remove level" button.

When removing all factors within a level, the level is automatically deleted.

How to update and reorder a level

You can also rename the level by clicking on the dedicated button and inputting a new name. The same rule applies for renaming levels as when you initially set the name: duplicates will be automatically corrected.

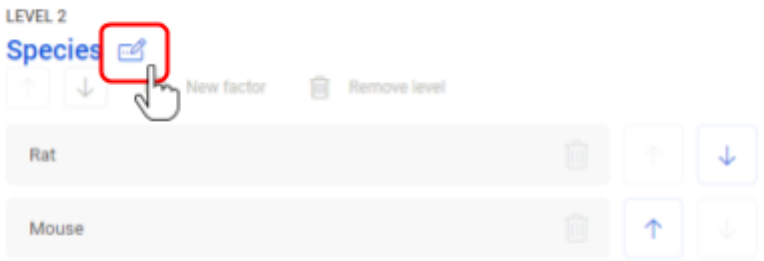


Figure 101. Location of the "Edit level" button.

Additionally, you have the option to reorder the levels according to your preferences by moving them up or down the list. This can be done using the buttons located directly below the level's name.

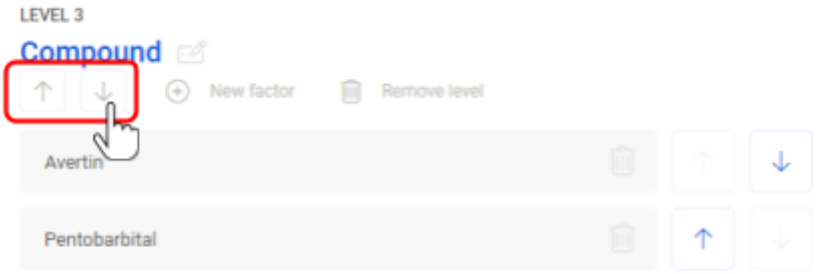


Figure 102. Location of the “Reorder level” buttons.

The arrows will become disabled when you reach the top or bottom of the list, indicating that no further movements are possible. It is important to note that Level 1 cannot be reordered.

6.3.3.2 How to add a factor

To add a new factor within a level, simply click the "New factor" button, which will shift the focus to the input field for the factor's name. Upon typing the desired name, pressing Enter will create a new factor below. Additional factors can be created in the same manner by repeating the process.

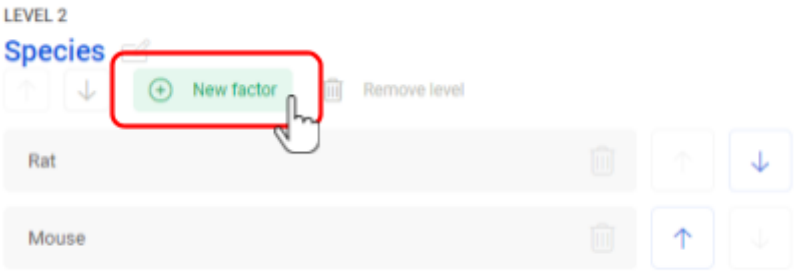


Figure 103. Location of the “New factor” button.

To avoid confusion in experiments or during annotation, we recommend using distinct names when naming factors too. In case of duplicate factor names within a level, the system will automatically rename the duplicate factor with a unique name. For instance, if you name two factors under Level 2 as "Rat," the second one will be automatically renamed to "Rat 1".

6.3.3.3 How to delete a factor

To delete a factor, simply select the "Remove factor" option located next to each individual factor. This action will result in the removal of the selected factor, which will be reflected in the Preview graph. It is important to note that this action is irreversible and does not require confirmation.

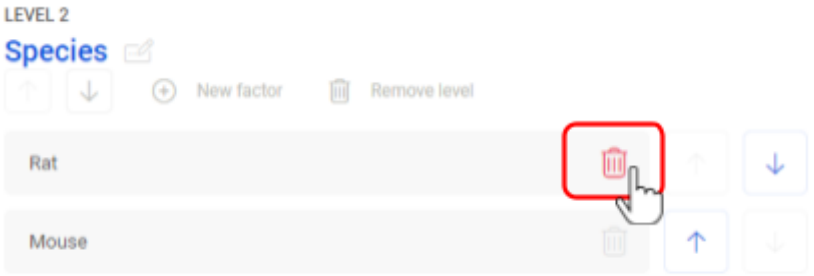


Figure 104. Location of the “Delete factor” button.

6.3.3.4 How to update and reorder a factor

You can also rename the factor by clicking the input field and inputting a new name. The same rule applies for renaming factors as when you initially set the name: duplicates will be automatically corrected.

Additionally, you have the option to reorder the factors according to your preferences by moving them up or down the list within the level. This can be done using the buttons located at the end of each factor.

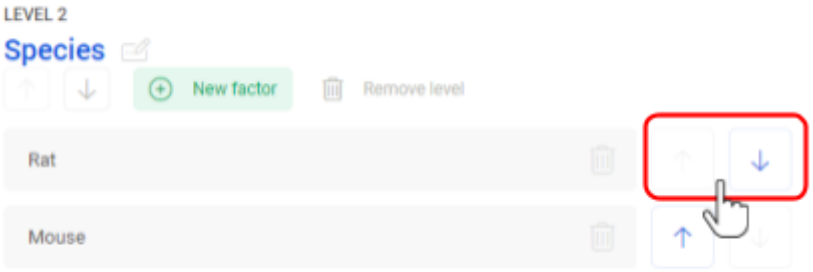


Figure 105. Location of the “Reorder factor” buttons.

The arrows will become disabled when you reach the top or bottom of the list, indicating that no further movements are possible.

6.3.3.5 How does the experiment design affect the annotation

When utilizing an Experiment design to annotate files or segments, it is important to note that you will always be using the latest version of the design.

IMPORTANT

Once you have updated your Experiment design, **it is important to reannotate your files or segments.** This is because the Experiment design is not synced with your annotations, but rather serves as a source of data.

However, it is important to keep in mind that annotations are NOT synced with updates to the design. This means that if any changes are made to the design (such as renaming, reordering, or deletion), annotations made prior to these changes will not be updated. Instead, the file or segment in question will need to be reannotated using the latest version of the Experiment design.

6.3.4 Exporting and importing

6.3.4.1 Export an Experiment design

You have the option to export your Experiment design as a JSON file, which can be shared or imported into another system. This ensures that you can reproduce the structure you created in the Experiment design editor accurately. This action can be performed from three locations:

1. From the Experiment designs explorer, where you see the entire list of Experiment designs. Each individual Experiment design has its own export button under the contextual menu.

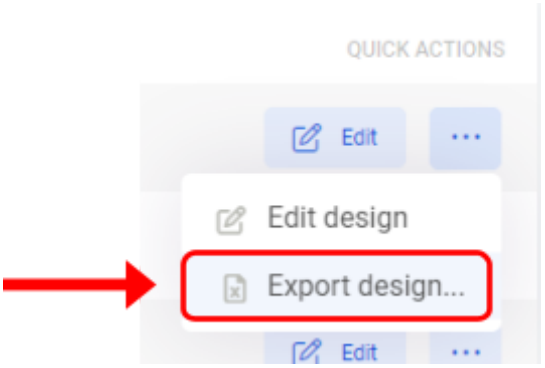


Figure 106. Location of the "Export design" button in the Experiment design list.

2. Within the Experiment design editor, on the top-right corner, under “Export...”.

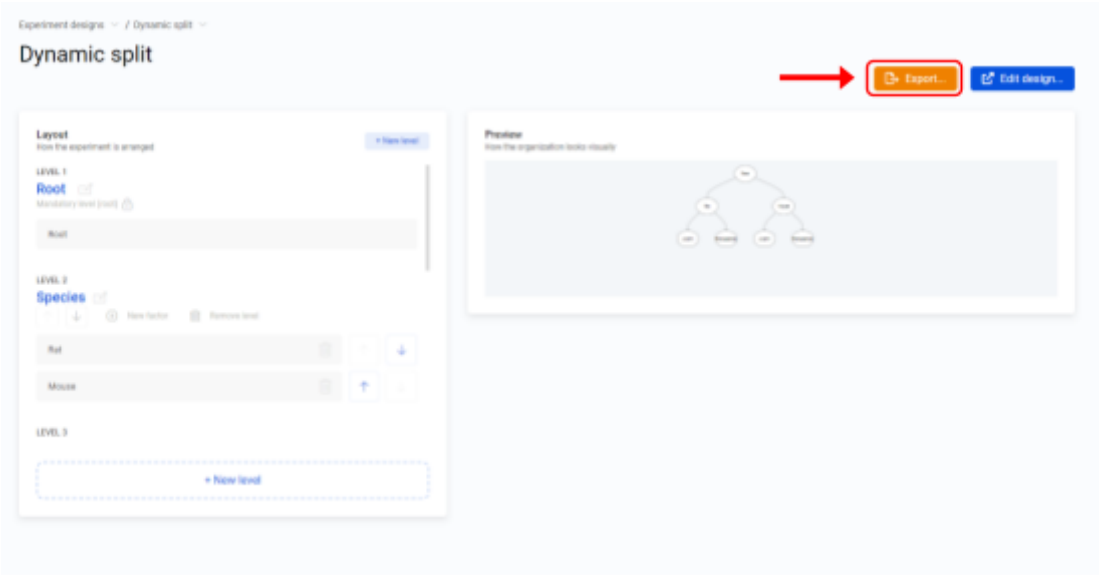


Figure 107. Location of the “Export...” button in the Experiment design editor page.

3. Within the Project > Experiment design preview, on the top-right corner, under “Export...”.

Figure 108. Location of the “Export...” button in the Experiment design preview page.

6.3.4.2 Import an Experiment design

If you have an existing Experiment design that you want to import, you can do so by accessing the contextual menu of the Experiment designs explorer and selecting "Import existing experiment design." This will load the data from a previously exported design, creating an identical replica.

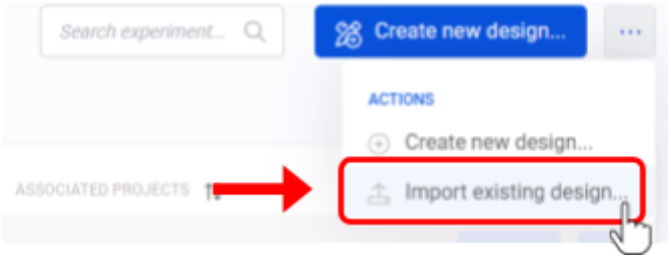


Figure 109. Location of the “Import existing design...” button.

6.4 Viewing analysis results with Transient analysis tool

The Transient Analysis feature has undergone some modifications since the release of CytoSolver 2.0. Despite these changes, the fundamental functionalities have remained intact. Rest assured, we will provide a comprehensive explanation of the updates, obviating the need to recall any prior information.

This is how the new Transient Analysis tool looks in CytoSolver 3.0 (Cloud):

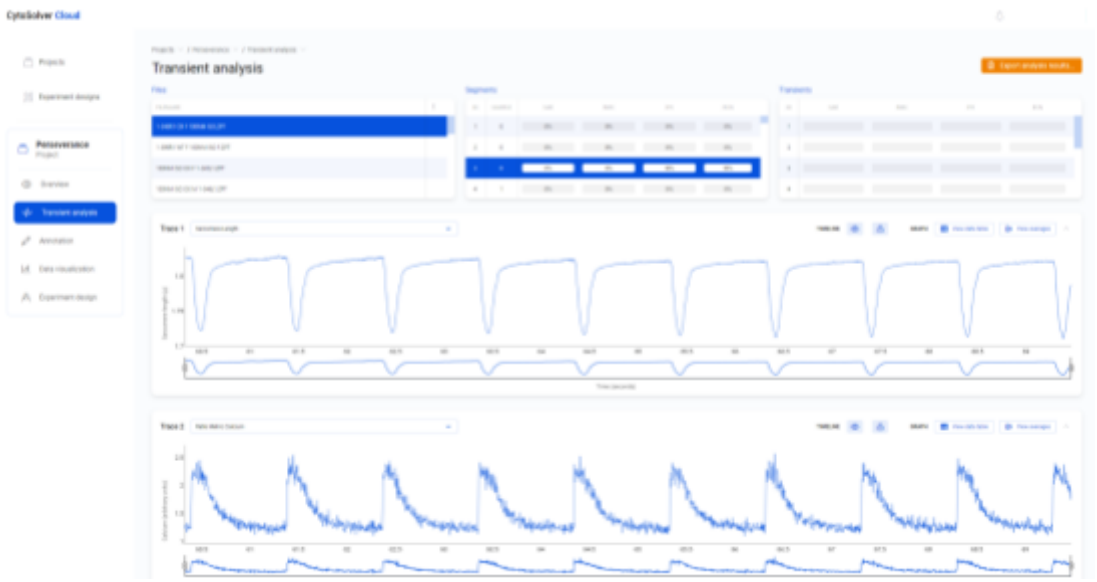


Figure 110. Example of the Transient Analysis tool.

6.4.1 Understanding the scope of the Transient analysis tool

The purpose of the Transient analysis tool is twofold:

- to present acquired data and
- to display the results of the analysis.

Each ZPT file provides a clear differentiation of trace types based on their acquisition methodology, with each type separated and presented distinctly. Additionally, segments within each trace represent periods during which the data was collected, with breaks demarcating each interval.

The next step is to examine the segment data and ascertain whether it can be further segmented into smaller chunks, named *transients*, each of which represents a sequence of contraction and relaxation, or a "beat," of the muscular item being analyzed.

Once the analysis is complete, you view the average transients per segment per trace and compare them with each other. The results are displayed in an interactive graph that shows the acquired data, the outcome of the algorithm (e.g., analyzed, rejected, discarded), and fitting lines that describe the shape of the data. There's also a table that gives you numerical results about the analysis.

6.4.2 Understanding the Transient Analysis algorithm

CytoSolver's automated algorithmic approach enables efficient and accurate identification of transients within each segment of the ZPT file. While the file is well-organized and demarcates trace types and segments, further analysis requires determination of the start and end points of each transient event. In the absence of pacing marks, our algorithm employs techniques to analyze the shape of the data and identify significant peaks and subsequent returns to normalcy, splitting segments into transients.

Although there is significant variability in the data shapes, our algorithm has been designed to optimally detect transients and extract relevant statistics.

6.4.3 Understanding the analysis cycle

Once files are added to a project, they are **automatically analyzed** using the Criteria settings in place at that time, and the Transient Analysis tool displays the results. Any newly added files will also be analyzed automatically, even if a previous batch has not yet finished analysis.

If changes are made to the Criteria Settings, you will need to reanalyze the files to see the updated results. This will create a new version of the results that reflect the changes made to the Criteria Settings.

IMPORTANT

If you reanalyze the files containing annotated segments, **the annotations will be lost** as the number of segments may vary due to rejected ones, based on the Criteria settings.

Note that if you use the results of the Transient Analysis tool elsewhere, either inside or outside of CytoSolver, you should be aware that after reanalysis, any calculations that depend on those results may need to be redone. This is because the updated analysis results may have changed the underlying data used in those calculations.

KEY CONCEPT

We analyze a reference trace type and extract the segment splits from it. Then, we utilize that information to split the remaining trace types.

In our data analysis process, we utilize a single trace type as a reference to split the data into transients. Specifically, we analyze this reference trace type and then utilize the split to analyze the remaining trace types. To achieve this, we employ a prioritized list of trace types, using the type at the top of the list to guide the analysis. If the data for the first trace type is not available, we move down the list to the second one, and so on, until we can identify a reference trace type for splitting the data into transients.

The **Transient analysis priority order for trace types** is the following:

1. Pixel Correlation
2. Pixel Intensity
3. Sarcomere Length
4. Edge Length
5. RatioMetric Calcium
6. Single Wave Length
7. Cs1
8. Cs2
9. Cs3
10. Cs4

It is important to note that the list of trace types provided is not mandatory. In other words, the presence of a Pixel Correlation trace type is not required in the ZPT file. The algorithm selects the trace type with the highest priority from the available trace types. For instance, in a file with four traces - RatioMetric Calcium, Pixel Intensity, Cs1, and Sarcomere Length - the algorithm will analyze the Pixel Intensity trace (trace type B) first, as it has the highest priority in the list. It will then utilize the split generated from this trace to analyze all other remaining trace types in the file.

6.4.4 Navigation through Files - Segments - Transients

The interface directly reflects the division of files, segments, and transients described earlier. You will see a list of files that the project contains, including all the traces. The list of segments shows the segments that each trace contains. The list of transients consists of the items detected by our algorithm.

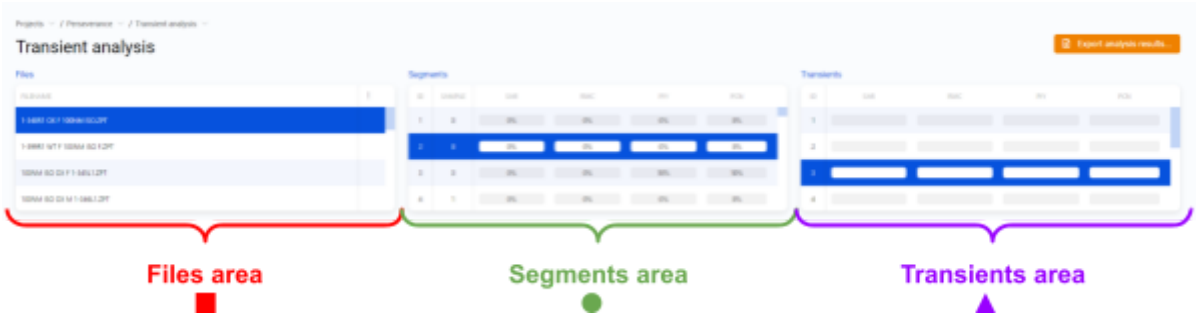


Figure 111. Example of the Transient Analysis tool.

6.4.4.1 Navigation

To navigate efficiently, it is best to follow a left-to-right approach. Begin by selecting the file you wish to explore, then choose the desired segment to preview a slice of the trace. If you want to delve deeper, you can select a transient.

Whenever you select an item from any of the three lists or boxes, it will be highlighted by a blue background. This highlighting serves to indicate your current location and keep you informed of your progress throughout the process. In essence, by using the navigation from left to right, you are “zooming” into fixed portions of the data.

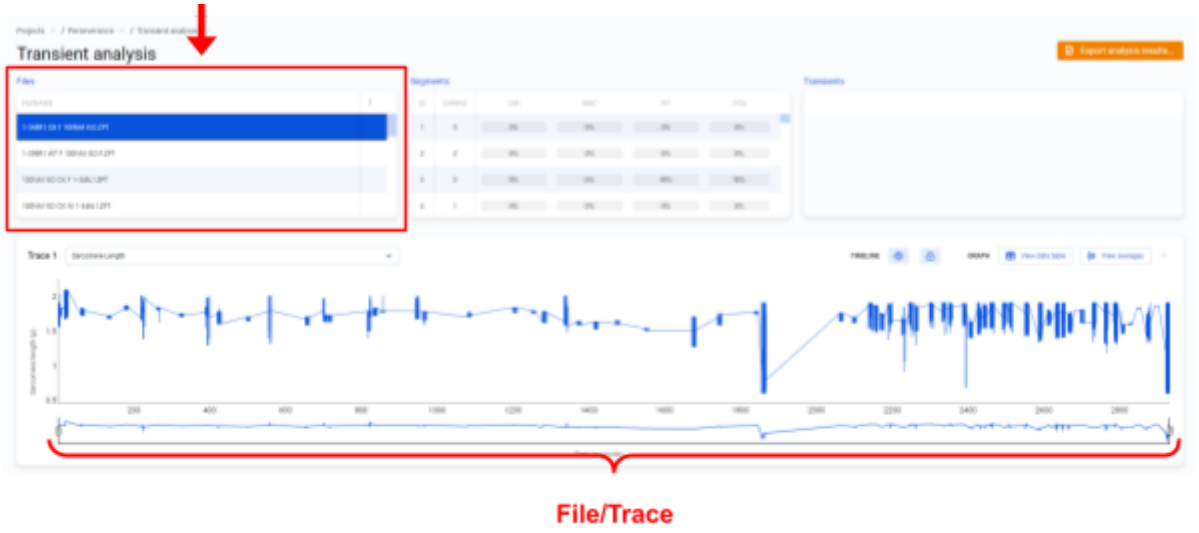


Figure 112. Viewing a file in Transient Analysis.

When you select a file from the list, you are presented with a visualization of the data for all the traces across the entire file. The data is displayed as distinct chunks that are connected by a thin line. Each of these chunks represents a segment, and the line connecting them is included solely due to the plotting library.

Upon selecting a segment, its data will be promptly displayed. The complete traces of the file will no longer be visible, as they have been replaced by the data of the chosen segment. It is essential to note that only one type of item (file, segment, or transient) can be plotted simultaneously.

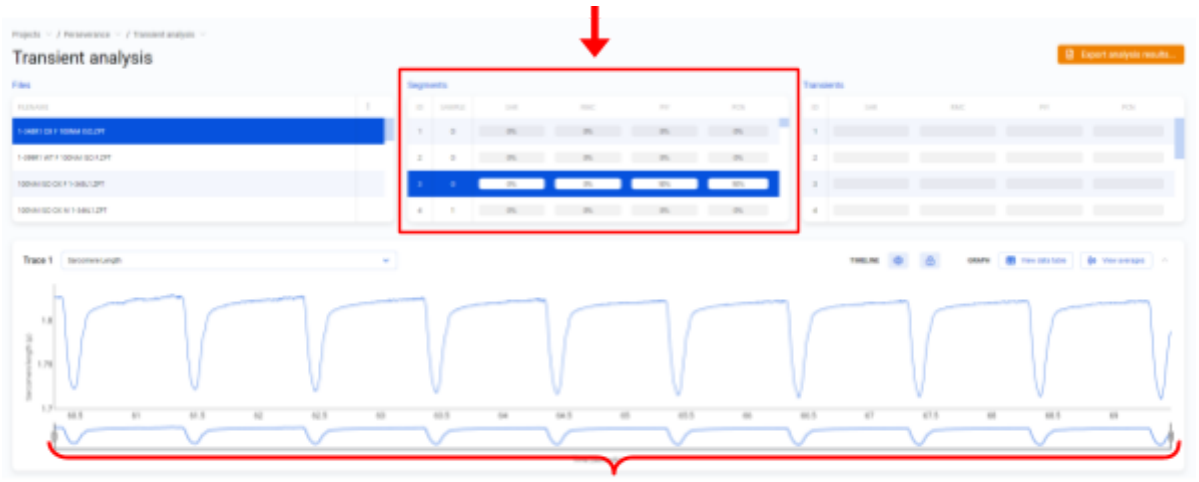


Figure 113. Viewing a segment in Transient Analysis.

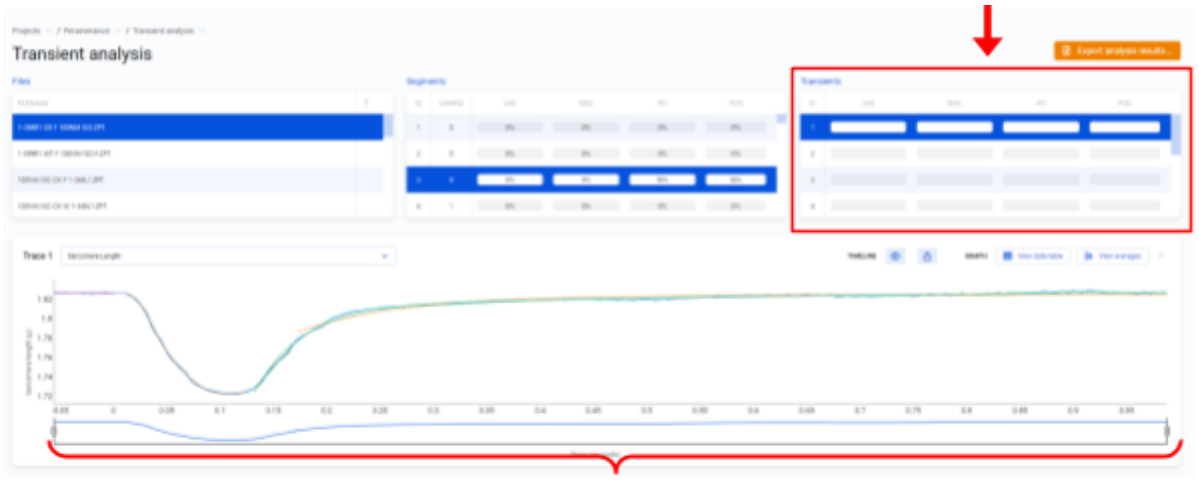


Figure 114. Viewing a transient in Transient Analysis.

6.4.4.2 Displayed information

Each of the boxes present in the Files - Segment - Transient navigation have a set of columns identifying and adding more information about the contained items.

The box containing **files** has 2 columns:

1. **File name:** refers to the identifying name of the added file.
2. **Alerts:** displays a colored geometric shape to indicate whether the total number of rejected transients (all transients from all segments within the file) has exceeded a certain threshold:
    a. A yellow square (🟨) says the number of rejected transients is more than 50%.
    b. A red triangle (🔺) says the number of rejected transients is more than 75%.

    Hovering over the figures will indicate the exact percentage.

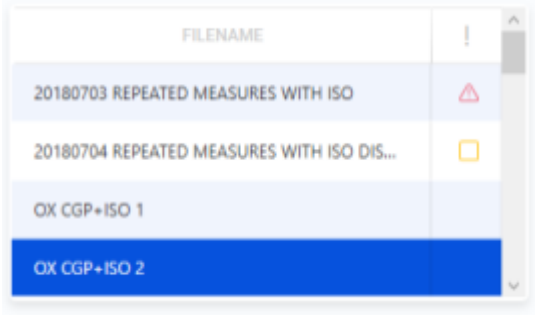


Figure 115. Example of alerts.

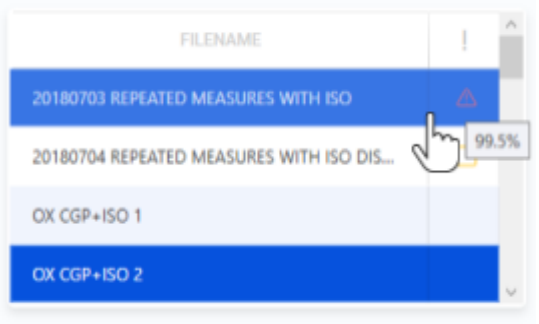


Figure 116. Example of alerts hovered.

The box containing **segments** has 2 main columns, plus rejection information:

1. **ID:** It serves as an identifier for each segment.
2. **Sample ID:** A Sample ID is a unique identifier that is assigned to a group of one or more segments that belong to the same sample. A sample represents a set of measurements that were taken from a single source, such as a biological sample or a physical sample. Each sample is assigned a unique ID, and all segments that are associated with that sample are tagged with the same ID. This allows for easy grouping and analysis of related data, such as when comparing measurements from different samples or when tracking changes in a single sample over time. In the given example, Sample ID 1 corresponds to measurements taken from one cell, while Sample ID 2 corresponds to measurements taken from another cell, and each repeat measure is assigned a unique identifier to distinguish it from the other measurements within the same sample.
3. Rejection columns: There are as many as trace types the file contains. Each column in the table represents a specific trace type and includes a color-coded percentage value that indicates the percentage of rejected transients:
    a. If **more than 25%** of the transients have been rejected, the color is **yellow**.
    b. If **more than 50%** of the transients have been rejected, the color is **red**.

The box containing **transients** has 1 main column, plus rejection information:

1. **ID:** It serves as a unique identifier for each transient within a segment and helps locating and referencing specific transients efficiently.
2. Rejection columns: There are as many as trace types the file contains. Each column in the table represents a specific trace type and includes the kind(s) of rejection the transient has (see Rejection types right below).

6.4.4.3 Rejection types

Rejections refer to transients that have been marked as "incorrect" due to various factors, including poor data quality, strict criteria settings, or the presence of artifacts such as baseline drift or arrhythmias. Understanding the reasons for rejections is crucial for effectively filtering and analyzing data and ensuring accurate research results. A transient may be rejected for multiple reasons, and any number of rejections above one marks it as rejected.

Rejected transients **are excluded from the transient average calculation**, except when the rejection is based on Peak-to-Noise Ratio (PSNR), which checks data quality. In such cases, the rejected transient is included in the average transient calculation.

IMPORTANT

Rejected transients are those found to be faulty during analysis due to their data content, while discarded transients are those marked at acquisition time to be excluded from analysis altogether. This distinction is important in data analysis and can impact the accuracy of research findings.

This section provides a comprehensive list of the various types of rejections that may occur during transient analysis, allowing to identify and investigate the reasons behind rejected transients and adjusting accordingly:

Rejection	Description	Avg.
AbsAmplitudeLowerLimit	It's the absolute value of the amplitude's lower limit. It means the Y-axis minimum value is lower than the criteria setting "Y Lower Limit".	No
AbsAmplitudeUpperLimit	It's the absolute value of the amplitude's upper limit. It means the Y-axis maximum value is higher than the criteria setting "Y Upper Limit".	No
BaselineThreshold	The automatically calculated baseline value is not in between the thresholds set in the criteria setting "Baseline Threshold".	No
MaxDerivativeLimit	The data contains points whose first derivative is larger than the criteria setting "D/dt max". Read here more about the derivative calculation.	No

Rejection	Description	Avg.
MinimalDeviation	The difference between the Y-axis maximum value and the Y-axis minimum value (a.k.a deviation) is higher than the criteria setting "$Y_{max} - Y_{min}$". Probably the data is too flat, there's no peaks.	No
NoOfLargeDerivatives	The number of peaks detected in data (large derivatives) exceeds the limit.	No
NumOfDataPointsBeforePacing	The number of data points before the contraction (peak) is not sufficient. The sufficient value is set by criteria setting "Minimum number of datapoints"	No
PeakSignalToNoiseRatio	The PSNR (peak signal-to-noise ratio) is too low. **Note:** It's only checked if the transient is not rejected due to *TransientDirection* (see below).	Yes
SecondOrderDerivativeLimit	The data contains points whose second derivative is larger than the criteria setting "D/dt2 max". Read here more about the second derivative calculation.	No
TransientDirection	The direction of the transient is not correct according to the check analysis. Each trace has a direction specified in its metadata. By reading the transient's data it is ensured: • 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. A rejection of this kind would mean: 1. **max < min** in traces marked as **Positive** or **Both** 2. **min < max** in traces marked as **Negative** or **Both**	No

Table 13. Rejection types

6.4.5 Trace inspectors

The trace inspectors are graphical representations that display the user-selected data within the Files-Segments-Transients navigation. In addition to presenting the data, they offer a range of tools and features that enhance data analysis and facilitate a deeper understanding of the research. The following section provides a detailed explanation of these features.

6.4.5.1 Data viewer

6.4.5.1.1 Displayed data and units

The viewer comprises two axes which serve as the primary means of orientation and interpretation of the data. The X-axis, also known as the horizontal axis, provides a consistent representation of time. The Y-axis, on the other hand, is the vertical axis, and its units may vary based on the type of data being displayed.

Here's the axes unit specification:

1. The **X-axis** consistently represents time in seconds.
2. The **Y-axis** units may vary based on the type of data being displayed. The following units are displayed for specific trace types (seen indented under them):
    a. **Micrometers (µm)**
        i. Sarcomere length
        ii. Edge length
        iii. Cs1
        iv. Cs2
        v. Cs3
        vi. Cs4
    b. **Arbitrary units**
        i. Pixel Correlation
        ii. Pixel Intensity
        iii. Ratiometric Calcium
        iv. Single wave length

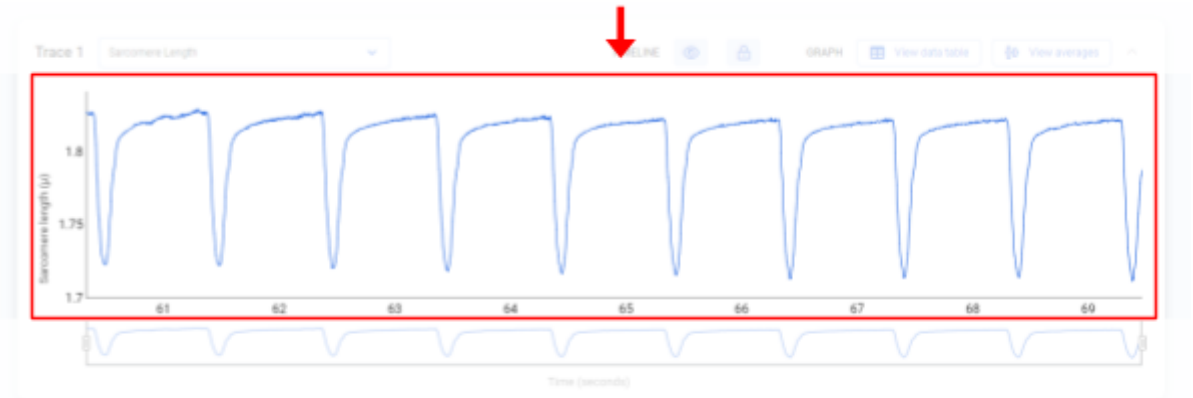


Figure 117. Viewing a transient in Transient Analysis.

6.4.5.1.2 Coloring

To assist in easily distinguishing between different types of data, the data viewer incorporates a color-coded system that represents the status of the data.

The following list outlines the colors used to represent each data status in both CytoSolver Desktop and Cloud:

- **Normal data:** displayed in blue.
- **Rejected data:** displayed in red, indicating that it cannot be fitted according to the set transient requirements.
- **Discarded data:** displayed in gray, indicating that it has been excluded from analysis.

The following list outlines the data visible only on CytoSolver Desktop

- **Background data:** displayed in gray, indicating it is not part of the analyzed data.

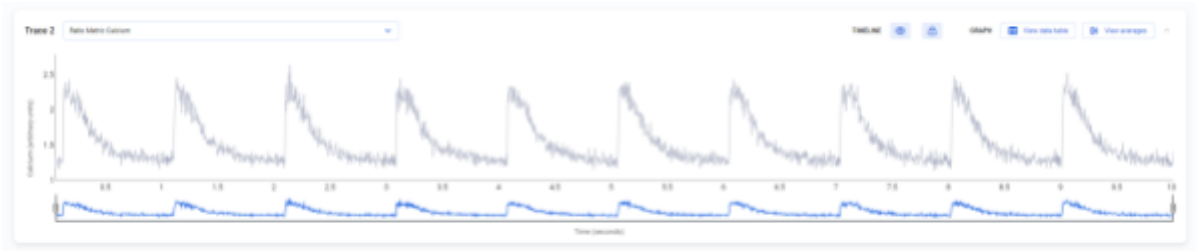


Figure 118. Example of discarded segment.

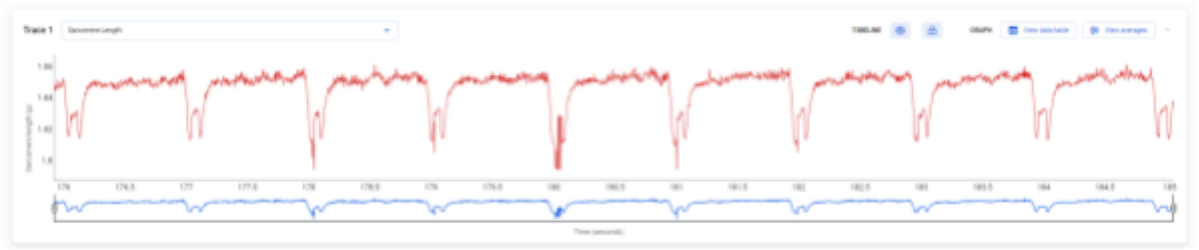


Figure 119. Example of rejected segment.

6.4.5.1.3 Hover-over information

The data viewer incorporates a hover window that appears when hovering over the plot. The hover window provides users with additional information and details related to the data point currently under the cursor.

The information presented in the hover-over menu varies based on the data displayed in the viewer:

1. When a **file** or a **segment** is shown, the time (X-axis) is displayed in black at the top of the pop-up, providing users with an accurate time reference. Additionally, the exact value of the data (Y-axis) is displayed next to its label. The label and the color of the lines will depend on the status of the specific portion of the segment, as described in Coloring.

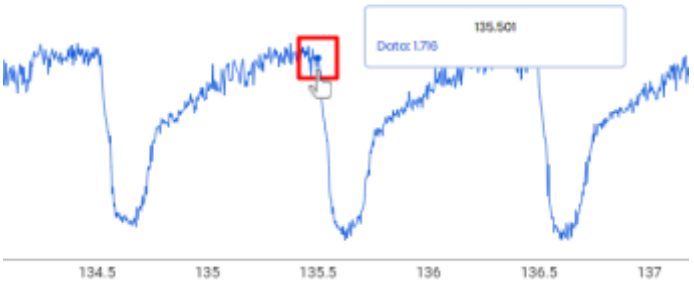


Figure 120. Example of hover-over information on a segment.

2. When a **transient** is shown, the time (X-axis) is still displayed in black at the top of the pop-up, providing users with an accurate time reference. However, there are now more lines to be displayed, in accordance with the fitting lines that have been calculated for the transient:
    - **Data:** The transient's data, displayed in ocean blue.
    - **Baseline fit:** Fit of the first section of the transient, before contraction (before the start of the peak), displayed in fuchsia.
    - **Peak fit:** Descent into the peak, the contraction, displayed in dark gray.
    - **Return fit:** Recovery from the peak, the relaxation, displayed in aquamarine blue.
    - **Single Exponential fit:** Displayed in orange.
    - **Double exponential fit:** Displayed in green.

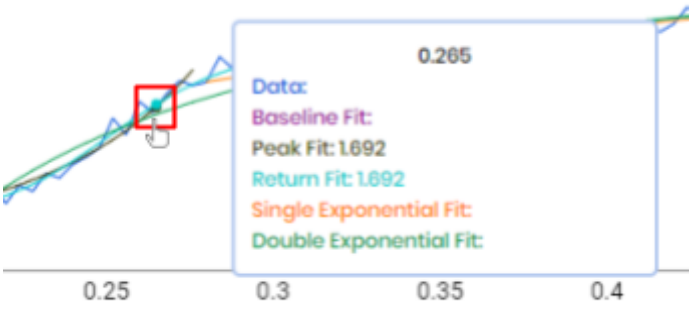


Figure 121. Example of hover-over information on a transient.

IMPORTANT

In the **CytoSolver Cloud version, the hover-over menu is automatically displayed** when users hover over the graph. This menu provides users with additional information related to the selected data points, displaying all available labels, even if not all are available.
Conversely, for the **CytoSolver Desktop version, users need to hold the left mouse button down on the graph** to activate the hover-over menu. However, this menu will only show the labels for the data that is available.

6.4.5.1.4 Hide/show data Desktop

CytoSolver Desktop allows you to selectively display data in the graphs. You can choose which data to show or hide, enabling you to focus on the information that matters to you.

To access this feature, navigate to the "Transient Analysis" tool of your project and click on the "View" menu in the Windows toolbar located in the upper-left corner. All enabled menu items are marked with a check, and disabled ones are not.
Under the “Show plot” menu, you can view all data being rendered in the data viewer, with labels that match the Hover-over section. By default, all data types are enabled, except for “Transient limit”.

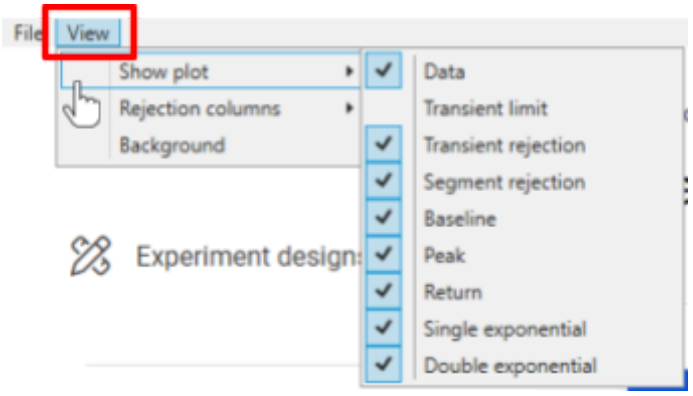


Figure 122. View > Show plot” menu.

The “Rejection columns” option hides/shows columns in the Segments and Transients boxes, allowing you to remove any unneeded columns from view.

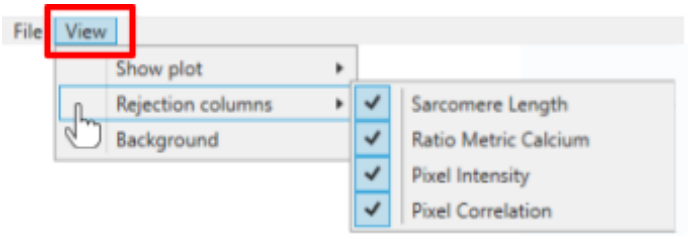


Figure 123. View > Rejection columns” menu.

Finally, the “Background” option displays backgrounds if they have been measured. They will appear in both the graphs and the segment list on the Transient analysis page. This menu

item is simple to use, just click on it to enable or disable it as needed since it does not have any subitems.

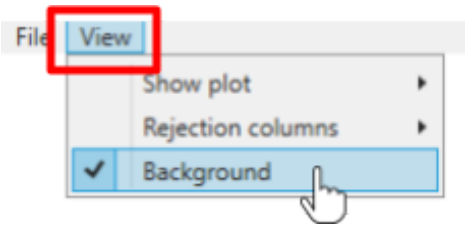


Figure 124. View > Background" menu.

CytoSolver Cloud does **NOT** include this feature.

6.4.5.1.5 Transient dividers or T0 (T-zero) Desktop

The trace viewers display a type of data called transient dividers, also known as "T0" or "T-zero," which marks the initial point of the transient. T0 is represented by a vertical orange bar that clearly indicates where the transient begins in time.

In the image below you can see the transient dividers marked by red arrows.

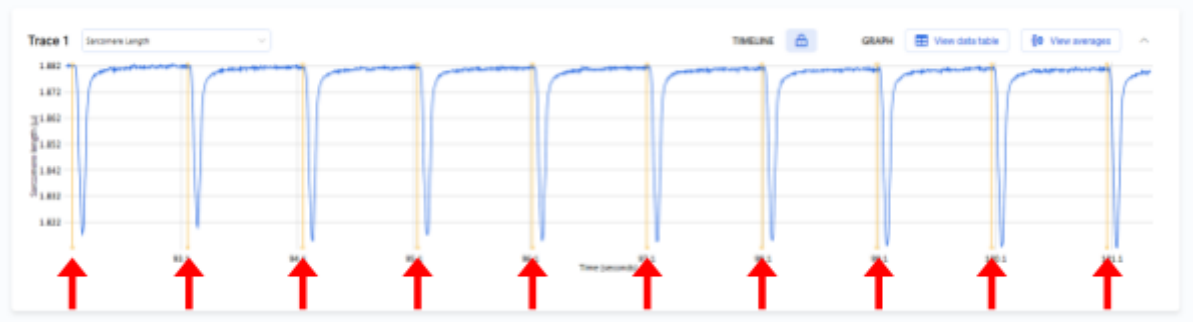


Figure 125. Example of transient dividers.

TIP

It's best to use the T0 rendering feature only when necessary as it can be demanding for your computer. It is easier to render when visualizing segments, but takes longer when viewing full traces. We suggest not enabling T0 when viewing a file as the data is too zoomed out for it to be useful.

CytoSolver Cloud does not include this feature.

6.4.5.2 Manipulating data viewer: Using zoom to refine the view

This section will show you how to trim displayed data by zooming in and out using the data view. It is important to note beforehand that CytoSolver Desktop and CytoSolver Cloud differ in their approach to this functionality.

CytoSolver Cloud features a visible UI element that allows you to interact with the timeline. The timeline serves as the X-axis data, controlling the time of the data. Additionally, the data viewer contains two buttons at the top, enabling you to show/hide the timeline and to sync/unsync all the traces' timelines.

Figure 126. Timeline in CytoSolver Cloud.

6.4.5.2.1 Show/hide timelines Cloud

The Transient Analysis page displays multiple traces, each with its own timeline. You can choose to show or hide each timeline individually, giving you control over the display of the data.

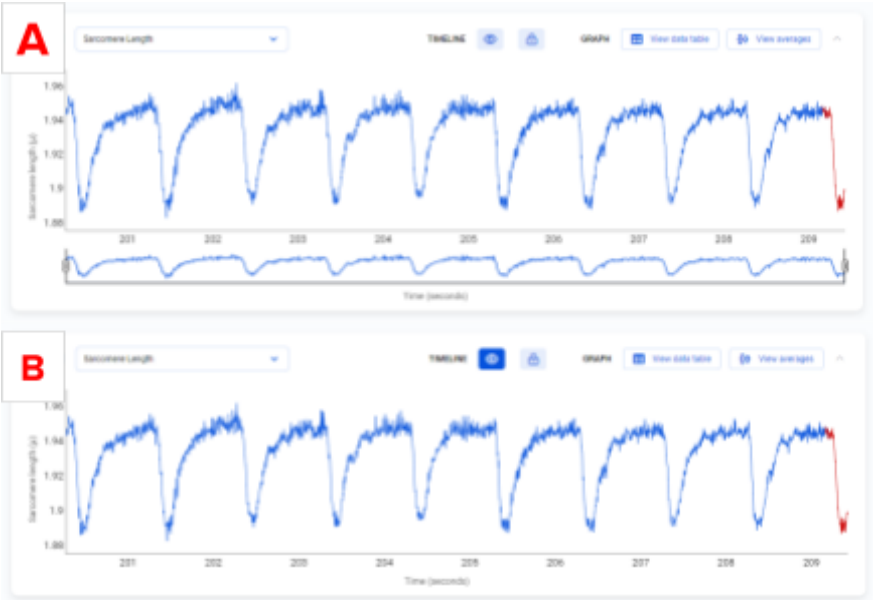


Figure 127. Shown and (B) hidden modes of the timeline in CytoSolver Cloud.

6.4.5.2.2 Sync/unsync timelines Cloud

When zooming in or out, you'll observe not only the trace where you're performing the action changes, but all the traces do. This is because they are all synced, hence all trace types will zoom in or out at the same time.

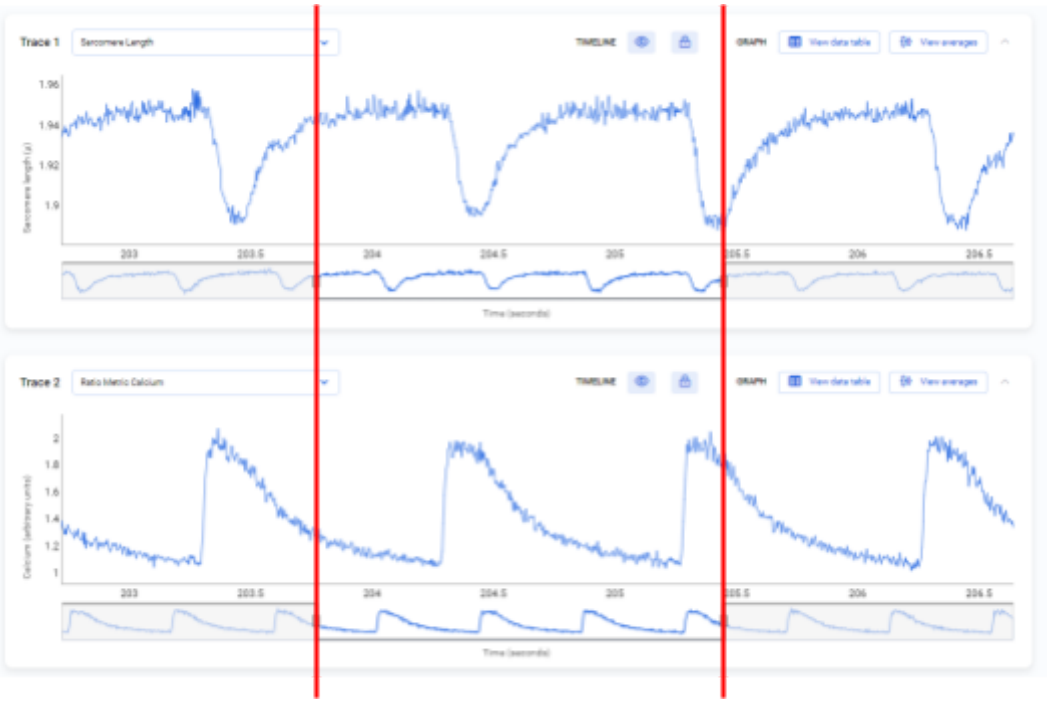


Figure 128. Synced timelines in CytoSolver Cloud.

Figure 129. Unsynced timelines in CytoSolver Cloud.

6.4.5.2.3 Zooming in and out in CytoSolver Desktop

The data viewer in CytoSolver Desktop offers the flexibility to zoom in and out of the data by utilizing the scroll function of your mouse within the graph areas. You can easily return to the original zoom level by double-clicking (left click) twice.

To **zoom in or out of the X-axis (time)**, you will need to scroll up or down within the data view. Keep in mind that the X-axis zoom-in has no limit, allowing you to scroll infinitely. However, it is important to note that excessive zooming can result in the data becoming incomprehensible and lose its meaning. Conversely, there is a limit to the zoom-out function, which is determined by the selected item in the navigation. For instance, if you have selected a segment, you are restricted to that specific data, and you cannot zoom out beyond that point.

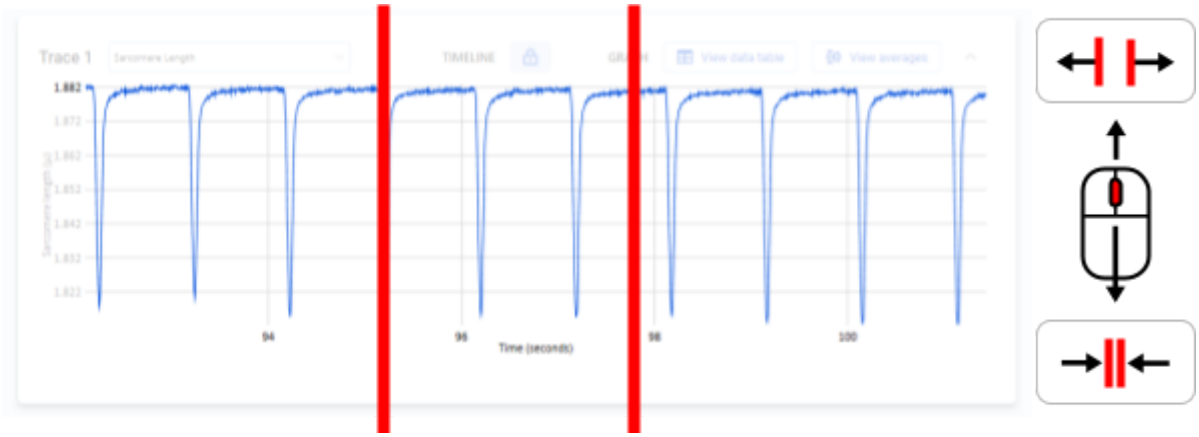


Figure 130. X-axis zoom in/out by scrolling up/down.

To **zoom in or out of the Y-axis (analyzed data)**, you will need to hold the Ctrl (Control button) key on your keyboard and simultaneously scroll up or down within the data view. Similar to the X-axis, there is no limit to the zoom-in function for the Y-axis. However, the zoom-out function maintains the shape of the selected item to ensure that it remains sensible and comprehensible.

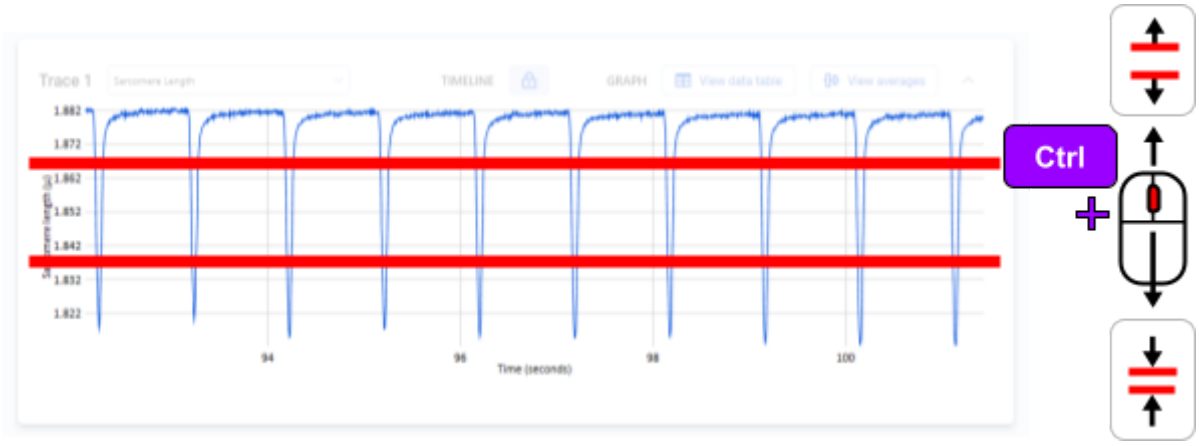


Figure 131. Y-axis zoom in/out by holding Ctrl key + scrolling up/down.

6.4.5.2.4 Zooming in and out in CytoSolver Cloud

CytoSolver Cloud provides a more intuitive and interactive approach to zooming in and out of graphs. To zoom in, simply click and drag either horizontally or vertically to select the desired portion of the graph. The selected area will turn gray to indicate it's being selected. To zoom out, double-click on the graph to reset the zoom level.

To **zoom in on the X-axis (time)**, you will need to left-click and drag **horizontally** within the data view. The data viewer features a supplementary timeline that displays a miniature version of the data. This aids in zooming along the X-axis. To achieve this, simply drag the timeline handles available on both sides of the data viewer.

To **zoom out**, simply double-click on any location of the graph, and the zoom will reset to its initial state - the same state you see when you first select a file, segment, or transient.

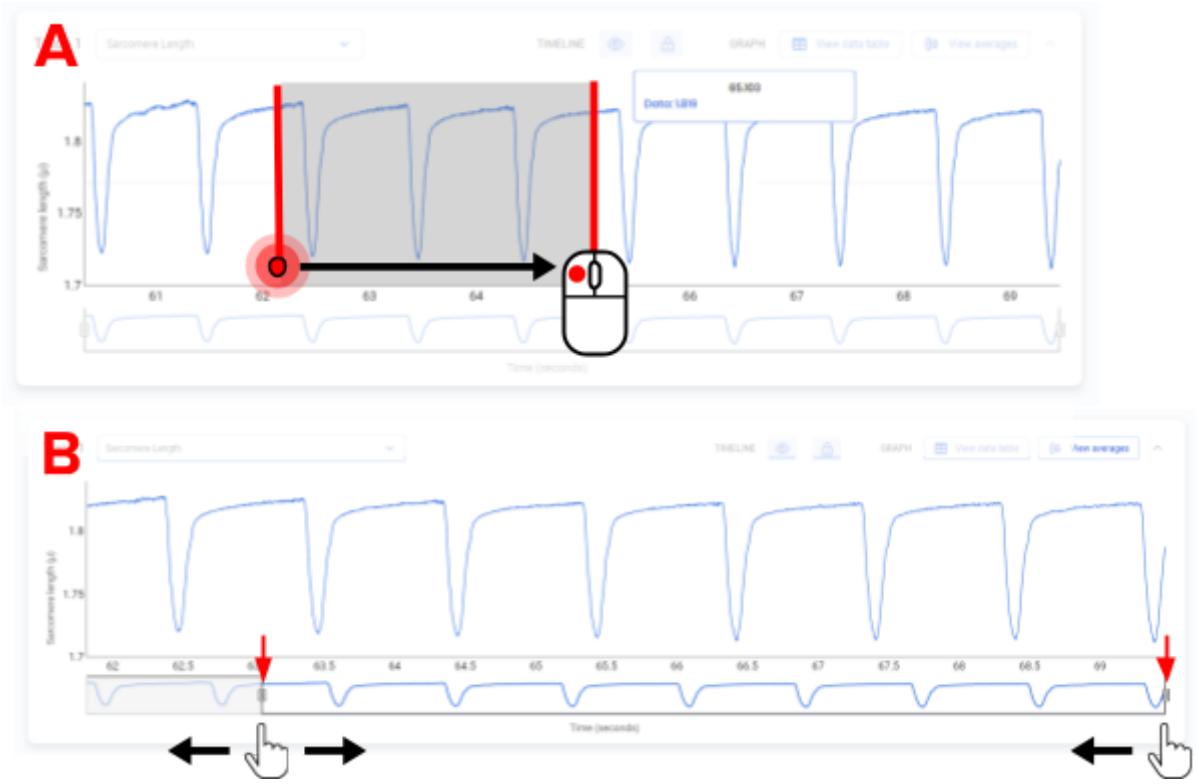


Figure 132. X-axis zoom by (A) horizontally selecting the zoom area and (B) pulling the handles.

To **zoom in on the Y-axis (analyzed data)**, you will need to left-click and drag **vertically** within the data view. To **zoom out**, simply double-click on any location of the graph.

Figure 133. Y-axis zoom by vertically selecting the zoom area.

6.4.5.2.5 Panning in CytoSolver Desktop

The data viewer in CytoSolver Desktop provides a flexible way to analyze data in graph format. With the ability to zoom in on specific regions of the graph, you can get a closer look at areas of interest. However, when zoomed in, it can be difficult to see the entire graph.

To explore other areas of interest while zoomed in on a graph in CytoSolver Desktop, you can use the panning options. Simply **hold down the Shift key and then drag the right mouse button in the graph area** in any direction to move around. However, please note that the view will not go beyond the end of the data. If you're not zoomed in enough, the panning action will not have any effect.

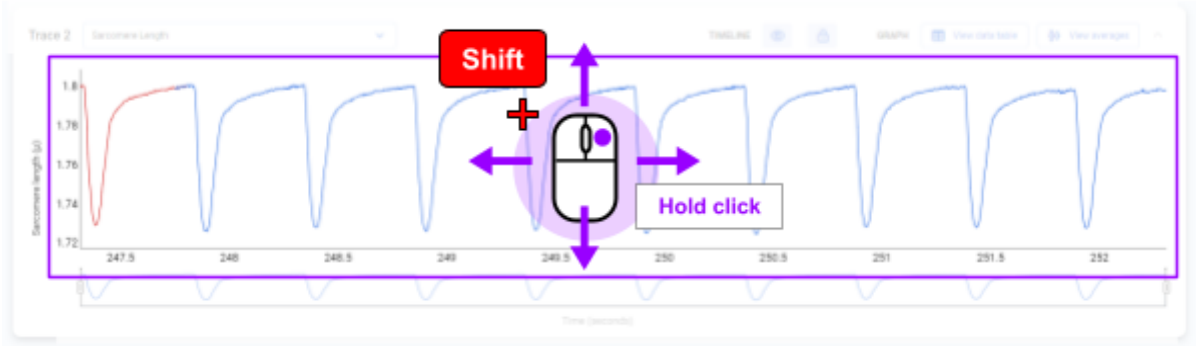


Figure 134. Example of how to pan content in CytoSolver Desktop.

By using the panning options in combination with the zoom feature, you can gain a comprehensive understanding of the data and make informed decisions based on your analysis.

6.4.5.3 Averages

The data viewer allows displaying the average of the selected item right underneath it. This average always shows an average transient. For a full trace, it will show the average transient of that trace, while for a segment will show the average transient of that segment. There's no average for a transient, because that is just the transient itself.

Each trace type has its own average trace viewer, which can be individually enabled or disabled.

GOOD TO KNOW

When comparing an average to a selected file or segment, the visual representation may appear stretched because both data viewers have the same width. It is important to take note of this visual distortion and to refer to the x-axis time scale when analyzing the data.

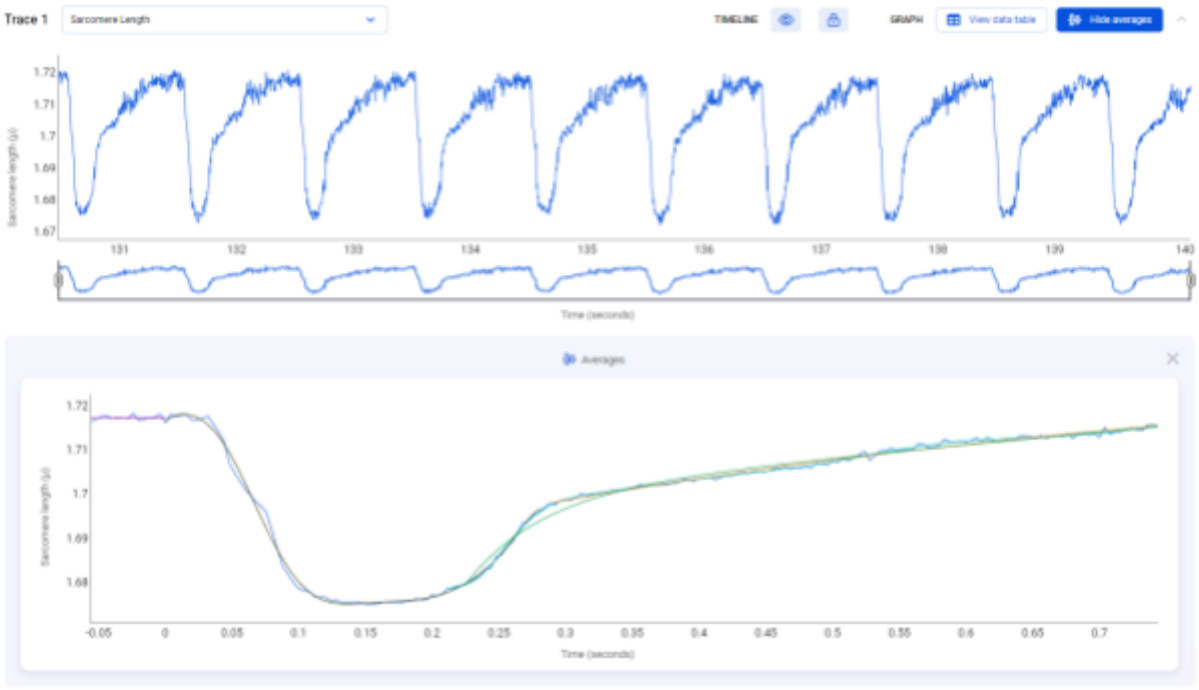


Figure 135. Example of the average transient seen under the selected segment.

6.4.5.3.1 Criteria for including/excluding data from averages

The averages are calculated from all the transients available in the trace/segment. However, not all the transients are eligible for being part of the average.

Transients **INCLUDED** in the averages:

- Transients that have been correctly analyzed and their shape passes all the checks for a correct transient.
- Transients that have been rejected **exclusively** due to *low PSNR (PeakSignalToNoiseRatio)*. If there is any other rejection (additionally or not to the PSNR) found in the transient, it will **not** be included in the averages calculation.

Transients **NOT INCLUDED** in the averages:

- Discarded transients that have been marked for deletion.
- Background data, as it's not considered as a transient, but rather as calibration data.
- Transients that have been rejected due to **any rejection except** *low PSNR (PeakSignalToNoiseRatio)*.

NOTE

You can check what the *PeakSignalToNoiseRatio* rejection means in User-adjustable rejection criteria. Besides, you can find here a summary of the Rejection types.

Figure 136. Example of the average transient not showing for discarded elements.

6.4.5.3.2 Zooming in and out in average trace viewers

The second data viewer has the same zoom-in and zoom-out capabilities as the original trace viewer. Refer to the previous section on "Zooming in and out for CytoSolver Desktop and CytoSolver Cloud.

6.4.5.3.3 Units in average trace viewers

Even though the new view presents a condensed version of the data, the units remain consistent. The Y-axis retains the same units as the original trace, meaning that if the original trace was in micrometers, the average will also be in micrometers for Sarcomere Length trace types. The X-axis continues to be measured in seconds.

6.4.5.4 Data table

CytoSolver provides not only visual representations of the data, but also additional crucial information that may be difficult to identify through interaction with the data viewers or may be absent. This data includes crucial information about segments, transients, and experiments:

1. On one hand, there is data that is necessary to identify the segment/transient that you are interested in, in order to match it with the data viewer. This includes segment/transient IDs, samples, and other relevant information.
2. On the other hand, there is information such as well description, whether a segment/transient has been discarded or rejected, and other essential details.
3. Lastly, there is calculated data, which includes information about the measurements such as beat frequency, time it takes to reach the peak of the transient, and other important information.

IMPORTANT

The data table changes depending on the data you are viewing:

1. When the average **data viewer is hidden** and only the trace data viewer is shown, the data table shows **information for the regular trace viewer**.
2. When the average **data viewer is shown**, the data table shows **information for the average** you are visualizing.

Next you can find a comprehensive explanation of all the values that can be found in these tables, sorted in the same order as the tables themselves.

Segment table	
Parameter	Description
Well description	Name of the well used in the experiment.
Discarded item	Specifies whether any part of the segment or sample has been discarded. If the segment has been deleted, the word "Segment" will be used. Similarly, if the sample has been discarded, the word "Sample" will be used. If nothing has been discarded, this section will be left empty.
Sample ID	Displays the identification number assigned to the sample. The numbering begins at 1 and increases with each subsequent sample.

Segment table	
Parameter	Description
Repeated measure	Displays the identification number of the repeated measurement. The numbering begins at 1 and increases with each repetition.
Segment ID	Displays the identification number assigned to the segment. The numbering begins at 1 and increases with each subsequent segment.
Beat frequency average	Average beat frequency of the preparation for this segment.
Beat frequency variance	Variance of the beat frequency for this segment.
Beat frequency std	Standard deviation of the beat frequency for this segment.
Number of beats	Number of beats detected in the segment. This equals the number of transients.

Table 14. Content of the segments data table when visualizing results in Transient Analysis tool

Transient table		
Parameter	Description	Units
Collected at	The date and time of the first acquired data point after starting the experiment in IonWizard software. This date and time is based on the configuration of the acquisition system where the data is being acquired. All ZPT files that have been collected with IonWizard 7.3.0 or newer are saved with their UTC representation, for older files they're saved in their local time.	*NA*
Analyzed at (UTC)	Date time on Analysis PC at start of analysis (UTC).	*NA*
Well description	Name of the well used in the experiment.	*NA*
Segment ID	Displays the identification number assigned to the segment. The numbering begins at 1 and increases with each subsequent segment.	*NA*
Sample ID	Displays the identification number assigned to the sample. The numbering begins at 1 and increases with each subsequent sample.	*NA*
Repeated measure	Displays the identification number of the repeated measurement. The numbering begins at 1 and increases with each repetition.	*NA*

Transient table		
Parameter	Description	Units
Discarded item	Specifies whether any part of the segment or sample has been discarded. If the segment has been deleted, the word "Segment" will be used. Similarly, if the sample has been discarded, the word "Sample" will be used. If nothing has been discarded, this section will be left empty.	*NA*
Segment start	Start time of the segment measurement.	(sec)
Segment end	End time of the segment measurement.	(sec)
Break duration between segments	The duration of the break between the previous and current transient measurements.	(sec)
Transient number	Displays the identification number assigned to the transient, adding the word “Transient” at the beginning. The numbering begins at 1 and increases with each subsequent transient within the segment.	*NA*
Transient start	Start time of the transient measurement, determined by the transient detection algorithm or a pacing mark.	(sec)
Transient end	End time of the transient measurement.	(sec)
Notes	Indicates the notes written in the text marks that have been inserted in IonWizard.	*NA*
Number of transients in avg.	**Only contains information when the average trace is shown.** Indicates the number of transients that have been included in the calculation of the average transient. It has the following format: (A/B), where A is the number of transients included and B is the total number of transients available.	*NA*
All cells average	Indicates whether the averaged transient is included in the trace average.	*NA*
Remark	Includes a list of the rejections assigned to a transient.	*NA*
Baseline	Pre-stimulation baseline value of the recorded signal.	(a.u)[4] or (µm)

[4] (a.u) stands for “Arbitrary unit”. They are relative units of measurement used in science and technology to indicate the ratio of a quantity, such as amount of substance or intensity, to a predetermined reference measurement.

Transient table		
Parameter	Description	Units
Departure velocity	The maximum velocity of the deflection phase of a transient refers to how quickly a system's output changes away from its normal, steady-state value after a sudden change in input. It is the fastest speed at which the output is moving away from its steady-state value.	(a.u)/sec
Shortening velocity (Sarcomere Length)		(µm/sec)
Departure velocity time	The time it takes from t_0 to reach maximum departure/shortening velocity.	(sec)
Shortening velocity time (Sarcomere length)		(sec)
Peak	Value of the transient at its maximal deflection from baseline (positive or negative), this is, the exact value of the output at the point of maximum deviation. In contractility traces like *Sarcomere Length* we name this “peak shortening”, while in the rest of trace types we name it just “peak”.	(a.u)
Peak shortening (Sarcomere length)		(µm)
Time to Peak	The time of peak occurrence in a transient response is the amount of time that has elapsed between the beginning of the transient (t_0) and the point at which the output reaches its highest value (Peak).	(sec)
Time to Peak shortening (Sarcomere length)		(sec)
Shortening amplitude	The distance from the Baseline to the Peak, calculated as: *peak - baseline*.	(µm)
Fluorescence amplitude		(a.u)
Peak Height		(a.u)
Percent shortening	It compares the height of the Peak value (above or below the baseline) to the baseline itself, expressing the difference as a percentage of the baseline.	%

Transient table		
Parameter	Description	Units
Percent change	Calculated following the next formula: 100 x Peak height / Baseline.	%
Percent motion		%
R^2 (peakfit)	Goodness of the fit of the *Peak* phase. Refers to how well the data within the *Peak* phase matches a line with a specific shape. It is a value that indicates how well the line approximates the data within the phase.	*NA*
Time to N% Peak shortening	The time of peak occurrence in a transient response is the amount of time that has elapsed between the beginning of the transient (t_0) and the point at which the output reaches its highest value (Peak).	(sec)
Time to N% Fluorescence peak		(sec)
Time to N% Peak		(sec)
R^2 (recoveryfit)	Goodness of the fit of the *Recovery* phase. Refers to how well the data within the *Recovery* phase matches a line with a specific shape. It is a value that indicates how well the line approximates the data within the phase.	*NA*
Time to N% Relaxation	The recovery phase of a transient is characterized by the time it takes for the transient to return to a percentage of its peak value. N can be 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% of its peak value.	(sec)
Time to N% Fluorescence Baseline		(sec)
Time to N% Baseline		(sec)
Relaxation velocity	The maximum velocity refers to the highest speed achieved during the recovery phase of the transient.	(µm/sec)
Return velocity		(a.u/sec)
Relaxation velocity time	Duration between reaching 95% of the peak value and achieving the maximum return velocity during the recovery phase.	(sec)
Return velocity time		(sec)

Transient table		
Parameter	Description	Units
Time constant (τ)	The time constant, represented by the single exponential tau value from the exponential decay function, describes the speed of recovery after stimulation, such as calcium uptake or muscle relaxation. A higher tau value indicates a longer time for the system to return to its original state or baseline.	(sec)
R^2 (Single exp fit)	Goodness of the fit of the *Single Exponential*.. It is a value that indicates how well the line approximates the data within the phase.	*NA*
Time constant 1 (τ1)	τ1 is the fast time constant in a double exponential fit model that characterizes the rate of decay on a short timescale. T1 represents the fast component of the decay of calcium concentration or contractile force in a myocyte.	(sec)
Time constant 2 (τ2)	τ2 is the slow time constant in a double exponential fit model that characterizes the rate of decay on a longer timescale. τ2 represents the slow component of the decay of calcium concentration or contractile force in a myocyte.	(sec)
R^2 (Double exp fit)	Goodness of the fit of the *Double Exponential*. It is a value that indicates how well the line approximates the data within the phase.	*NA*
PSNR	PSNR or Peak Signal-to-Noise Ratio indicates the quality of the acquired data. A higher PSNR value indicates better quality and lower distortion, while a lower PSNR value indicates lower quality or greater distortion.	*NA*

Table 15. Content of the transient data table when visualizing results in Transient Analysis tool

6.4.6 Exporting analysis results

Once you have completed your data analysis, the next step is to use the results to extract conclusions and potentially to communicate your findings to others. One way to share your results is by exporting them in a format that can be easily understood and shared. Exporting analysis results allows you to present your findings to a wider audience, including colleagues, stakeholders, and decision-makers.

In this section, we will discuss the various ways in which you can export your analysis results, including exporting to common file formats like TSV (tab-separated values) and XLSX (Microsoft Office Excel).

We will also cover some basics for exporting data, including choosing the right data for you, the right file format and any additional information you might want to add, ensuring that your results are clear and readable.

Finally, we will explore how this data can be forwarded to CytoSolver's Data visualization tool (more instructions below).

Whether you are conducting data analysis for research, business, or personal projects, this section will provide you with the knowledge and skills you need to effectively communicate your results to others.

6.4.6.1 Where can you export the analysis

Once all the ZPT files have been analyzed, the results are ready to be exported into a file you can have available on your PC.
You can access the "Export results..." menu from 4 different locations:

1. Through the top navigation:

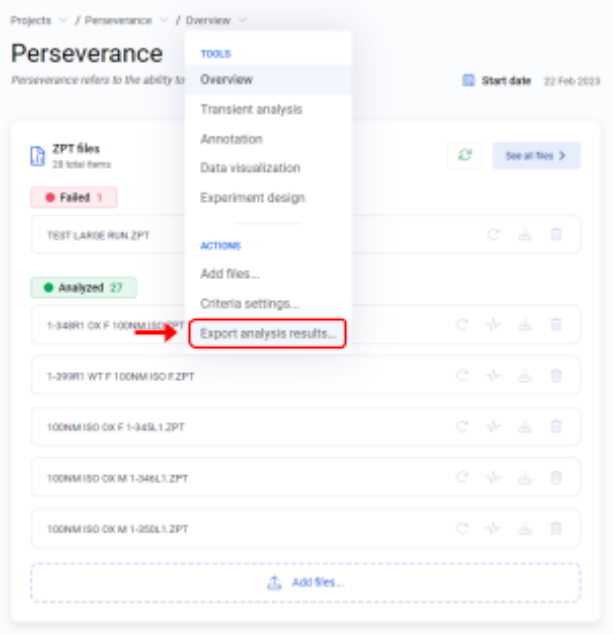


Figure 137. Export analysis results from breadcrumbs navigation.

2. Through the contextual menu of the Project Overview:

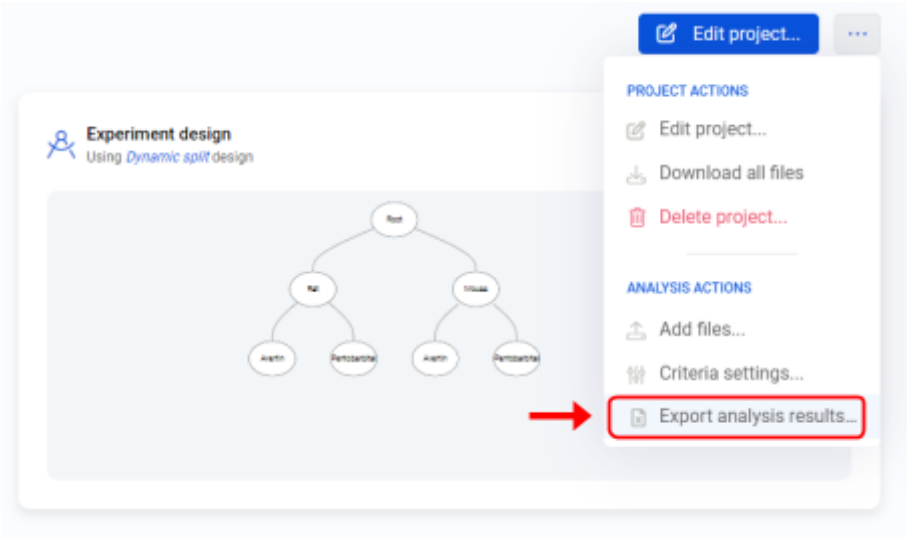


Figure 138. Export analysis results from contextual menu.

3. Through the contextual menu of the Projects list:

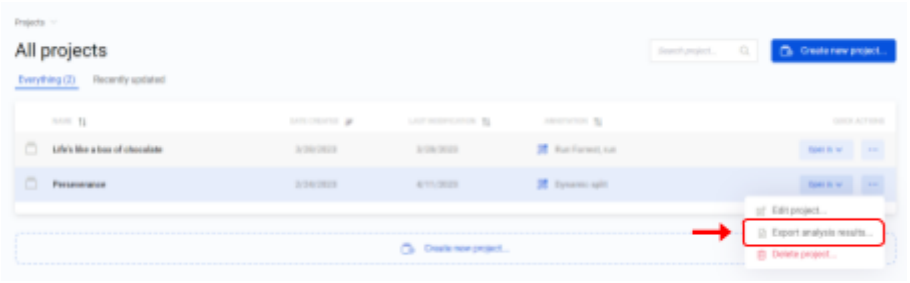


Figure 139. Export analysis results from Projects contextual menu.

4. Through the Transient Analysis tool:

Figure 140. Export analysis results from Transient analysis.

To properly export them

1. Click on `Export` in the menu bar
2. Make selection of what data you want to export
    a. Transient export:
        i. Only the averaged transients;
        ii. Only the individual transients;
        iii. Or, both.
    b. Multi- or Single- sheet/file export:
        i. In TSV (tab separated) format;
        ii. In XLSX format;
        iii. Or, both.
    c. Export the raw transient data (be careful, greatly increases the size of the file)

6.4.6.2 Export as TSV file(s)

When exporting data as TSV, the resulting files will contain different content based on the type of data being exported. Here are the possible options:

1. **Averaged data:** This type of export will only include the averages that were calculated during the analysis. Includes the average data of a trace and the average per segment.
2. **Individual data:** If you choose to export individual data, the resulting file will include the analysis results for each individual transient.
3. **Both:** If you want to export both averaged and individual data, you can choose to include both in the same file.

Besides, the content can also be held in to one single file or be split into multiple files:

1. **Single files:** All the information is held in one single file. There will be 2 files, since there are 2 different types of data:
    a. Beat frequency analysis data: A beat frequency file contains the average beat frequency data for segments, determined through peak detection in experiments on spontaneous beating constructs. It also includes information on beat frequency variance, standard deviation, and the number of beats detected per segment.
    b. Transient data: A file that contains extracted parameters from each analyzed transient that was recorded during acquisition, such as: Peak height, percent change, time to peak 50%, etc.
2. **Multiple files:** the information is split into different files, generating one file per group unit. A group unit can be:
    a. A ZPT file
    b. A trace type
    c. A segment

    Like this, you can split your data in many files, each containing a very specific part of the analysis. Be aware this might heavily increase the number of files you get in the end.
3. **Both:** It will generate both the content of the “single files” and the “multiple files selection”.

Number of files (right →) / Transient export (down ▼)	**Single files**	**Multiple files**
Averaged data	**2 files** • File #1 with Beat frequency analysis data • File #2 with **averaged** transient analysis data	**2 files** x **N ZPT file(s)** x **M Trace type(s)** = **2** x **N x M** files Per trace type within each ZPT file: • File #1 with Beat frequency analysis • File #2 with **averaged** transient analysis data
Individual data	**2 files** • File #1 with Beat frequency analysis data • File #2 with **individual** transient analysis data	**1 file** x **N ZPT file(s)** x **M Trace type(s)** = **1** x **N** x **M** files • File #1 with Beat frequency analysis data
		1 file x **N ZPT file** x **M Trace type(s)** x **L Segment(s)** = **1** x **N** x **M** x **L** files • File #1 with **individual** transient analysis data
		Grand total = **(1** x **N** x **M)** + **(1** x **N** x **M** x **L)** files
Both	Both averaged and individual data specified above.	Both averaged and individual data specified above

Table 16. Expected number of files and their content when exporting transient results into Tab-separated values (TSV) format

6.4.6.3 Export as XLSX (Excel) sheets/files

When exporting data as TSV, the resulting files will contain different content based on the type of data being exported. The options are the same as for the TSV file above:

1. Averaged data
2. Individual data
3. Both

However, since Excel-formatted files allow pagination, the end result when using XLSX differs a bit in terms of file.

Number of files (right →) / Transient export (down ▼)	**Single sheets**	**Multiple sheets**
Averaged data	**1 file, 2 sheets** • Sheet #1 with Beat frequency analysis data • Sheet #2 with **averaged** transient analysis data	**1 file** x **N ZPT file(s)** = **1** x **N** files Per Trace type: • Sheet #1 with Beat frequency analysis data • Sheet #2 with **averaged** transient analysis data
Individual data	**1 file, 2 sheets** • Sheet #1 with Beat frequency analysis data • Sheet #2 with **individual** transient analysis data	**1 file** x **N ZPT file(s)** = **1** x **N** files Per ZPT file: • 1 sheet per trace type with Beat frequency analysis data • 1 sheet for each segment within a trace type with **individual** transient analysis data
Both	Both averaged and individual data specified above.	Both averaged and individual data specified above

Table 17. Expected number of files and their content when exporting transient results into Excel (XLSX) format

6.4.6.4 Additional options

You have the option to enable additional settings in addition to selecting the export mode and content of each file/sheet:

- **Export raw data:** Exports the exact data points of all the transients. The columns will be named as following:
  - y - N: Data points occurring **before** the start of the transient (T0) that correspond to the offset and are denoted by the order number (N).
  - y N: Data points occurring **after** the start of the transient (T0) that correspond to the offset and are denoted by the order number (N).
- **Prepend current date and time to filename:** Adds current date and time to the *beginning* of the file name. Available on CytoSolver Desktop only.

- **Append current date and time to filename:** Adds current date and time to the *end* of the file name. Available on CytoSolver Desktop only.

6.4.6.5 Exported parameters

The exported parameters are practically the same as the ones found in the Transient data table, although it has at least 4 more columns (highlighted in blue in the table below):

1. Sarcomere length
2. Filename
3. Annotation (one or more columns if the Project has an Experiment design attached)
4. Trace name

Export table		
Parameter	Description	Units
Collected at	The date and time of the first acquired data point after starting the experiment in IonWizard software. This date and time is based on the configuration of the acquisition system where the data is being acquired. All ZPT files that have been collected with IonWizard 7.3.0 or newer are saved with their UTC representation, for older files they're saved in their local time.	*NA*
Analyzed at (UTC)	Date time on Analysis PC at start of analysis (UTC).	*NA*
Sarcomere constant	Pixel to micron calibration constant used to calculate the sarcomere length trace.	*NA*
Filename	Name of the exported ZPT file.	*NA*
Well description	Name of the well used in the experiment.	*NA*
Segment ID	Displays the identification number assigned to the segment. The numbering begins at 1 and increases with each subsequent segment.	*NA*
Sample ID	Displays the identification number assigned to the sample. The numbering begins at 1 and increases with each subsequent sample.	*NA*
Repeated measure	Displays the identification number of the repeated measurement. The numbering begins at 1 and increases with each repetition.	*NA*
"GLX Annotation"	Annotation columns. There is one column per each level in the Experiment design. They are tagged as GLX, where the X is an order number. Their value specifies the factor used to annotate a specific item.	*NA*

Export table		
Parameter	Description	Units
Discarded item	Specifies whether any part of the segment or sample has been discarded. If the segment has been deleted, the word "Segment" will be used. Similarly, if the sample has been discarded, the word "Sample" will be used. If nothing has been discarded, this section will be left empty.	*NA*
Segment start	Start time of the segment measurement.	(sec)
Segment end	End time of the segment measurement.	(sec)
Break duration between segments	The duration of the break between the previous and current transient measurements.	(sec)
Trace name	Name of the trace type (See trace types for more info)	*NA*
Transient number	Displays the identification number assigned to the transient, adding the word “Transient” at the beginning. The numbering begins at 1 and increases with each subsequent transient within the segment.	*NA*
Transient start	Start time of the transient measurement, determined by the transient detection algorithm or a pacing mark.	(sec)
Transient end	End time of the transient measurement.	(sec)
Notes	Indicates the notes written in the text marks that have been inserted in IonWizard.	*NA*
Number of transients in avg.	**Only contains information when the average trace is shown.** Indicates the number of transients that have been included in the calculation of the average transient. It has the following format: (A/B), where A is the number of transients included and B is the total number of transients available.	*NA*
All cells average	Indicates whether the averaged transient is included in the trace average.	*NA*
Remark	Includes a list of the rejections assigned to a transient.	*NA*

Export table		
Parameter	Description	Units
Baseline	Pre-stimulation baseline value of the recorded signal.	(a.u)[5] or (µm)
Departure velocity	The maximum velocity of the deflection phase of a transient refers to how quickly a system's output changes away from its normal, steady-state value after a sudden change in input. It is the fastest speed at which the output is moving away from its steady-state value.	(a.u)/sec
Shortening velocity (Sarcomere Length)		(µm/sec)
Departure velocity time	The time it takes from t_0 to reach maximum departure/shortening velocity.	(sec)
Shortening velocity time (Sarcomere length)		(sec)
Peak	Value of the transient at its maximal deflection from baseline (positive or negative), this is, the exact value of the output at the point of maximum deviation. In contractility traces like *Sarcomere Length* we name this "peak shortening", while in the rest of trace types we name it just "peak".	(a.u)
Peak shortening (Sarcomere length)		(µm)
Time to Peak	The time of peak occurrence in a transient response is the amount of time that has elapsed between the beginning of the transient (t_0) and the point at which the output reaches its highest value (Peak).	(sec)
Time to Peak shortening (Sarcomere length)		(sec)
Shortening amplitude	The distance from the Baseline to the Peak, calculated as: *peak - baseline*.	(µm)
Fluorescence amplitude		(a.u)

[5] (a.u) stands for "Arbitrary unit". They are relative units of measurement used in science and technology to indicate the ratio of a quantity, such as amount of substance or intensity, to a predetermined reference measurement.

Export table		
Parameter	Description	Units
Peak Height		(a.u)
Percent shortening	It compares the height of the Peak value (above or below the baseline) to the baseline itself, expressing the difference as a percentage of the baseline. Calculated following the next formula: 100 x Peak height / Baseline.	%
Percent change		%
Percent motion		%
R^2 (peakfit)	Goodness of the fit of the *Peak* phase. Refers to how well the data within the *Peak* phase matches a line with a specific shape. It is a value that indicates how well the line approximates the data within the phase.	*NA*
Time to N% Peak shortening	The time of peak occurrence in a transient response is the amount of time that has elapsed between the beginning of the transient (t_0) and the point at which the output reaches its highest value (Peak).	(sec)
Time to N% Fluorescence peak		(sec)
Time to N% Peak		(sec)
R^2 (recoveryfit)	Goodness of the fit of the *Recovery* phase. Refers to how well the data within the *Recovery* phase matches a line with a specific shape. It is a value that indicates how well the line approximates the data within the phase.	*NA*
Time to N% Relaxation	The recovery phase of a transient is characterized by the time it takes for the transient to return to a percentage of its peak value. N can be 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% of its peak value.	(sec)
Time to N% Fluorescence Baseline		(sec)
Time to N% Baseline		(sec)
Relaxation velocity	The maximum velocity refers to the highest speed achieved during the recovery phase of the transient.	(µm/sec)
Return velocity		(a.u/sec)

Export table		
Parameter	Description	Units
Relaxation velocity time	Duration between reaching 95% of the peak value and achieving the maximum return velocity during the recovery phase.	(sec)
Return velocity time		(sec)
Time constant (τ)	The time constant, represented by the single exponential tau value from the exponential decay function, describes the speed of recovery after stimulation, such as calcium uptake or muscle relaxation. A higher tau value indicates a longer time for the system to return to its original state or baseline.	(sec)
R^2 (Single exp fit)	Goodness of the fit of the *Single Exponential*. It is a value that indicates how well the line approximates the data within the phase.	*NA*
Time constant 1 (τ1)	τ1 is the fast time constant in a double exponential fit model that characterizes the rate of decay on a short timescale. T1 represents the fast component of the decay of calcium concentration or contractile force in a myocyte.	(sec)
Time constant 2 (τ2)	τ2 is the slow time constant in a double exponential fit model that characterizes the rate of decay on a longer timescale. τ2 represents the slow component of the decay of calcium concentration or contractile force in a myocyte.	(sec)
R^2 (Double exp fit)	Goodness of the fit of the *Double Exponential*. It is a value that indicates how well the line approximates the data within the phase.	*NA*
PSNR	PSNR or Peak Signal-to-Noise Ratio indicates the quality of the acquired data. A higher PSNR value indicates better quality and lower distortion, while a lower PSNR value indicates lower quality or greater distortion.	*NA*
y - N or **y N**	Transient raw data point. Columns named as y.N (one period symbol only) are the data points before the transient start (T0), and y..N (two periods) are the ones after transient start (T0).	*NA*

Table 18. Content of the export table when exporting transient analysis results

6.5 Labeling your ZPT files with Annotation tool

6.5.1 Understanding the scope of the Annotation tool

The Annotation tool in CytoSolver enables you to categorize your files and segments based on your experimental design. This categorization is similar to labeling and is crucial for effective data analysis using the Data Visualization tool. While the term "category" is not explicitly used in the CytoSolver ecosystem, it is used in its intended sense.
By assigning labels to your data, **you can compare the results of different experiments using the Data Visualization tool.** For instance, if you conducted an experiment using two compounds, "A" and "B," you can label the files containing the data with these two distinct labels ("A" and "B") to analyze the differences between their effects.

You have the flexibility to label an entire file under a single category, which will apply the same label to all segments within the file. Alternatively, you can label individual segments or assign multiple labels within a single file.

In the upcoming sections, we will explore how to handle the experiment design associated with your project that requires annotation. We will also cover the Annotation tool's unique features and functionalities.

6.5.2 Managing the attached Experiment design

While using the Annotation tool, you will have access to information about the Experiment design you are working with, as well as options for managing the design.

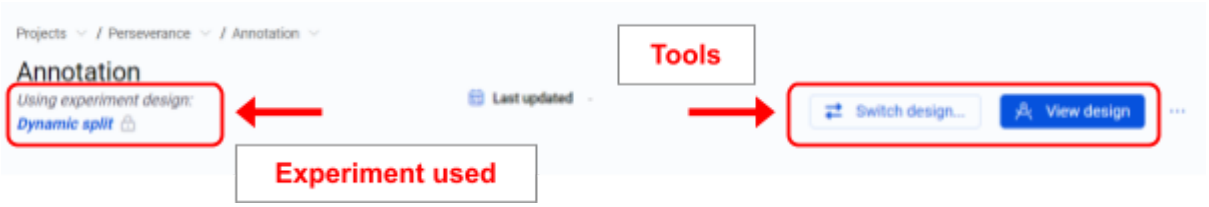


Figure 141. Location of Experiment design and tools related to it within the Annotation page.

6.5.2.1 Viewing Experiment design

By clicking on the "View design" button, you will be taken to the Experiment design preview page, where you can only view the design. To modify the design, you must navigate to the editor by clicking on the "Edit design" button. Although you can detach the Experiment design from the project or export it, you cannot modify it directly from the preview page.

Figure 142. Location of Experiment design and tools related to it within the Annotation page.

It is important to note that, even though the term "Experiment design" is mentioned on the preview page, you are still within the project's tools. Therefore, you have not navigated to the Experiment design editor. You can confirm this by paying attention to the sidebar items.

6.5.2.2 Detaching Experiment design

CytoSolver offers a crucial feature that allows you to detach your Experiment design from your project. Once detached, they will no longer be linked, and you will not be able to annotate the project using that specific design.

Figure 143. Location of Experiment design and tools related to it within the Annotation page.

It is important to note that this action **is irreversible and will result in the deletion of all annotations made using that particular Experiment design in the selected project**. To prevent accidental detachment, CytoSolver has implemented multiple security measures.

IMPORTANT

Detaching an Experiment design from a project or switching from one Experiment design to another, **permanently erases any annotation performed on the affected project**. Even if reattaching the same Experiment design back, the annotation will be lost and not recovered.

6.5.2.3 Switch Experiment design

You can switch from one Experiment design to another by clicking on the "Switch design" button, which is a shortcut that eliminates the need to detach the current design before attaching a new one.

However, it is important to note that this action does not attempt to match any data from the old Experiment design to the new one, and any previous annotations made using the old design will not be transferred or matched with the new design.

Switching design is available from two different places in the Annotation page:

1. The visible action button that is tagged as “Switch design...”
2. The contextual menu.

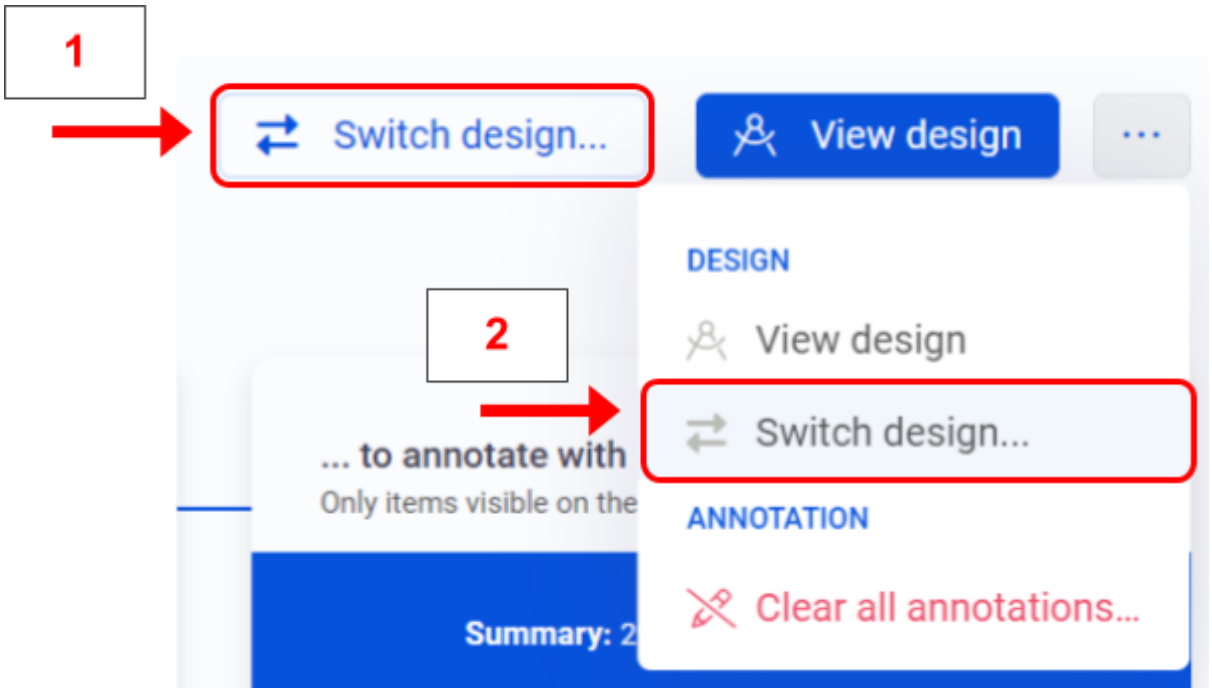


Figure 144. Location of the “Switch design” button within the Annotation page.

6.5.3 Displayed data per file/segment

The tool for annotations presents a list of files added to your project, displaying each file's name, number of segments, creation date, and annotation status.
To reveal the segments included in each file, expand its corresponding row. This screen does not feature any graphs but is instead an organizational tool.
The segments also provide information on the acquired data, aiding in filtering information, and display the annotation status.

6.5.3.1 Annotation-specific file information

Every file on the annotation screen contains the following information:

- Filename: The name of the ZPT file being worked on.
- Visible/Total: Shows the number of segments available after filtering (visible) and the total number of segments in the file after being detected in analysis (total).
- Date created: The time the file was acquired.
- Status: Indicates the level of annotation, which can have one of three different statuses:
    - None: Indicates that there are no annotated segments in the file.
    - Partly: Indicates that at least one segment in the file has been annotated in some form.
    - Full: Indicates that all segments in the file have been annotated, filling all existing levels of annotation.

We'd like to emphasize that partially annotating all the segments of a file still leaves that file partially annotated. Take the following examples:

1. In **Case A** (below) all segments are annotated, but they're only partially annotated, which leaves the file partially annotated.
2. In **Case B** only some segments are fully annotated, which leaves the file partially annotated.

Figure 145. Annotation status cases of partial annotation.

6.5.3.2 Annotation-specific segment information

Every segment on the annotation screen contains the following information:

- Segment ID: The numerical identifier of the segment.
- Repeated measure: Indicates the order number of the repeated measurement.
- Well number: The number of the well from which the data was acquired.
- Text marks: Event marks included in the segment (See Event marks for more information).
- Annotation: Displays a preview of the tags used to annotate the files. Hovering over it reveals the actual value of the annotation. Annotations can appear in two colors:
    - Yellow: Indicates partial annotation of the segment.
    - Green: Indicates full annotation of the segment.

While annotating segments, the status operates slightly differently. Fully annotating a segment requires selecting a value other than "None" for each level described in the Experiment design. All levels must be completed for a full annotation. If any level is incomplete (regardless of its position or level number), the annotation becomes partial.

The following are examples of a partial annotation:

Level 1	Level 2	Level 3
“Root”	“Rat”	*None*
“Root”	*None*	“Avertin”
None	“Rat”	“Avertin”

Table 19. Example of partial annotation

A full annotation will look like this:

Level 1	Level 2	Level 3
“Root”	“Rat”	“Avertin”

Table 20. Example of a full annotation

6.5.4 Annotation procedure

The annotation page comprises two primary operational areas, each corresponding to a distinct action. The first is the selection area, where you choose files and/or segments you wish to annotate. The second is the annotation area, where you choose values for the levels of the Experiment design to annotate your selection.

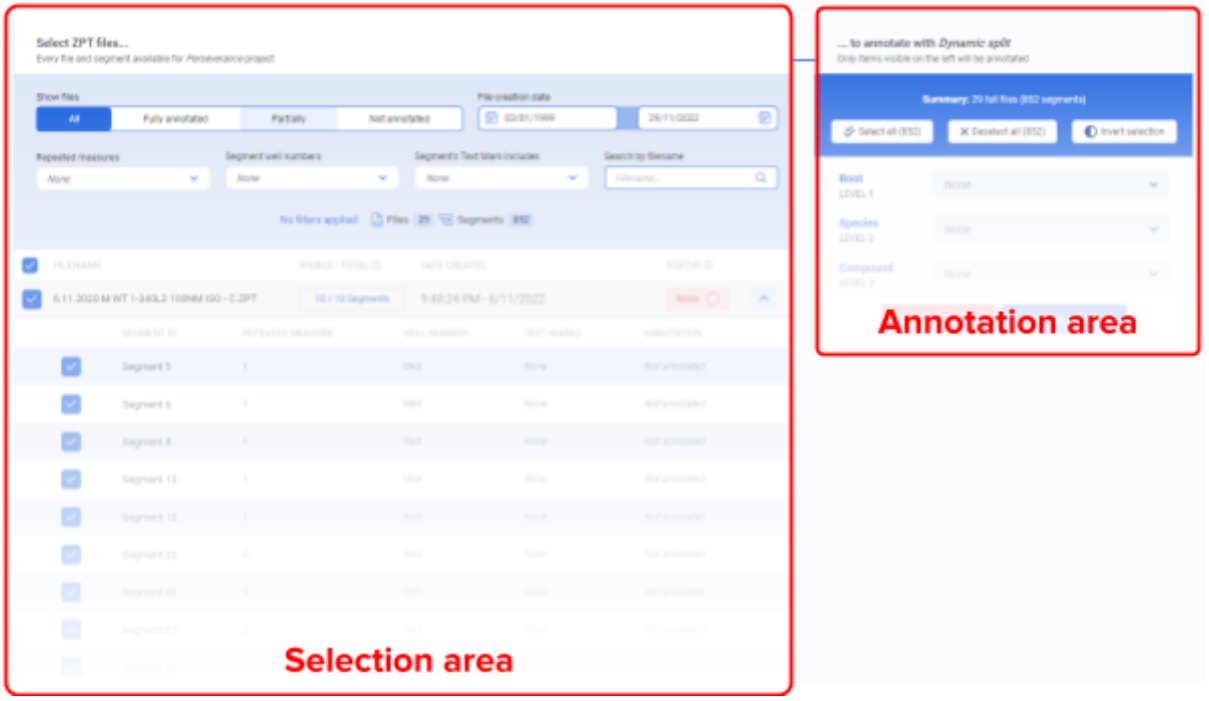


Figure 146. Annotation page areas.

KEY CONCEPT

The "Selection area" on the left side part of the page **selects files and segments to annotate. Then,** on the right side you find **the Annotation area, which performs the annotation.**

6.5.4.1 Selection area

6.5.4.1.1 How to filter files/segments in the view

The selection area enables users to choose files and segments individually or in batches to commence annotating. To facilitate this task, the Annotation tool incorporates a filter feature that enables the selection of specific characteristics of the files/segments.

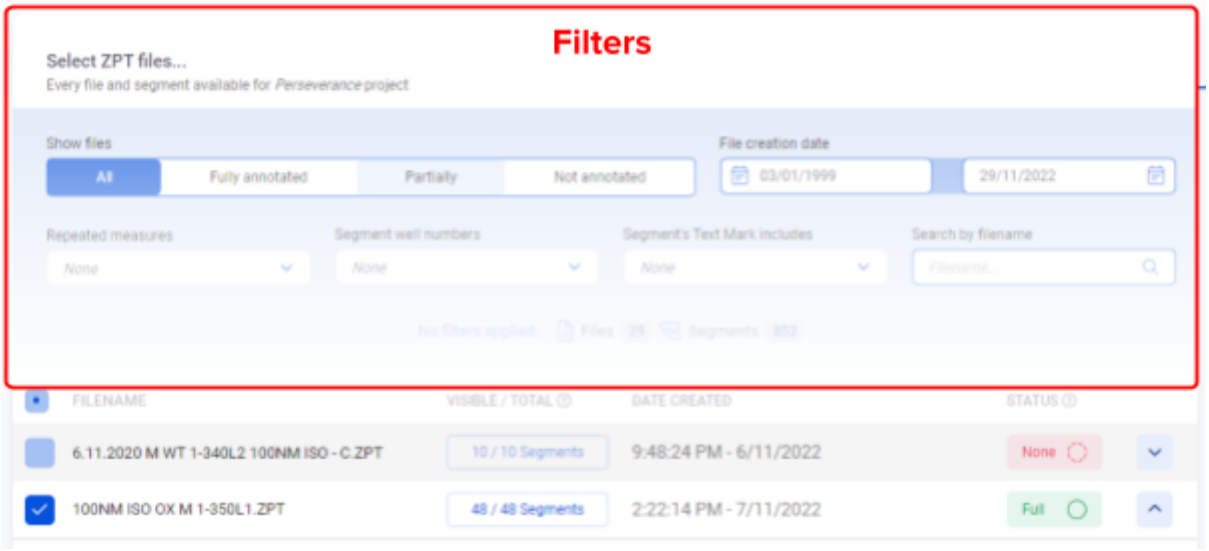


Figure 147. Filters in the Selection area within the Annotation page.

IMPORTANT

When annotating files/segments, only the marked (blue checkbox) items visible on the UI will be annotated. This means that if you have marked a file/segment but it has been filtered out by the filters, it will NOT be annotated.

The filter feature narrows down the search by selecting only the items of interest. It visually hides elements that are not relevant while annotating. This also means that, even if items are selected (marked with a blue checkbox), they won't be annotated if they're filtered out. Only visible items marked with a blue checkbox will be annotated.

KEY CONCEPT

Filtering files or segments in CytoSolver Desktop removes them from the current view. If an element is filtered out, it becomes hidden and no action performed on the screen will affect those hidden elements.

The top part of the Selection area includes 6 filters:

1. Show files: Allows you to filter based on annotation state. The options include:
    a. All: Includes all existing files.
    b. Fully annotated: Includes only files whose state is fully annotated (“Full”).
    c. Partially: Includes only files whose state is “Partly” annotated.
    d. Not annotated: Includes only files that are not annotated.
2. File creation date: Allows filtering files by their creation data in a range, including both the start date (from 00:00) and the end date (until 23:59).
3. Repeated measures: Filters based on the segment’s attribute “Repeated measures”
4. Segment well numbers: Filters based on the segment’s attribute “Well number”
5. Segment’s Text mark includes: Filters based on the segment marks that have been added to a segment.
6. Search by filename: Filters by the name of the ZPT file.

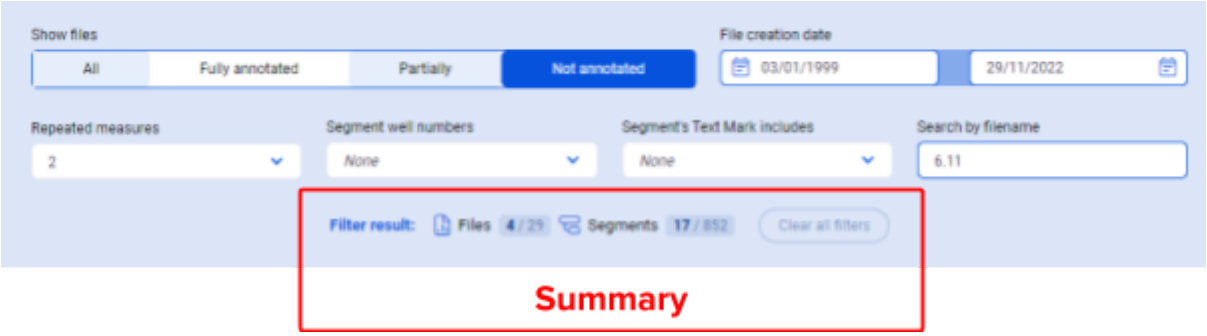


Figure 148. Filters in the Selection area within the Annotation page.

If you have applied one or more filters, a summary of the filtered result will be displayed beneath the Filter area. The summary provides information on the number of visible files/segments after the filters have been applied, in the format of [Number of visible] / [Number of total]. The filtered items, including both files and segments, are displayed individually.

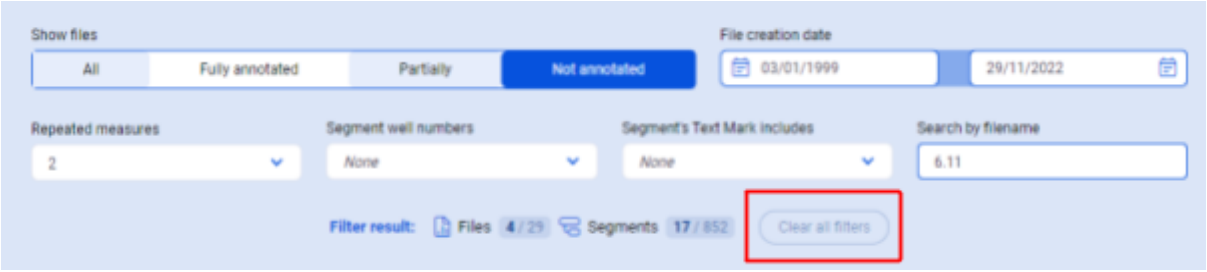


Figure 149. Clear all filters in the Selection area within the Annotation page.

To remove all active filters, simply click the “Clear all filters” button. If there are no filters applied, the summary will display the total number of files and segments available for annotation.

6.5.4.1.2 How to select the files/segments that need to be annotated

To select files and segments for annotation, check the blue checkbox next to the desired items. You can select and deselect multiple items at once to streamline your task.
To select all files in the list, check the checkbox at the top of the list, which is not associated with any specific file. This will select all files in the list for annotation.

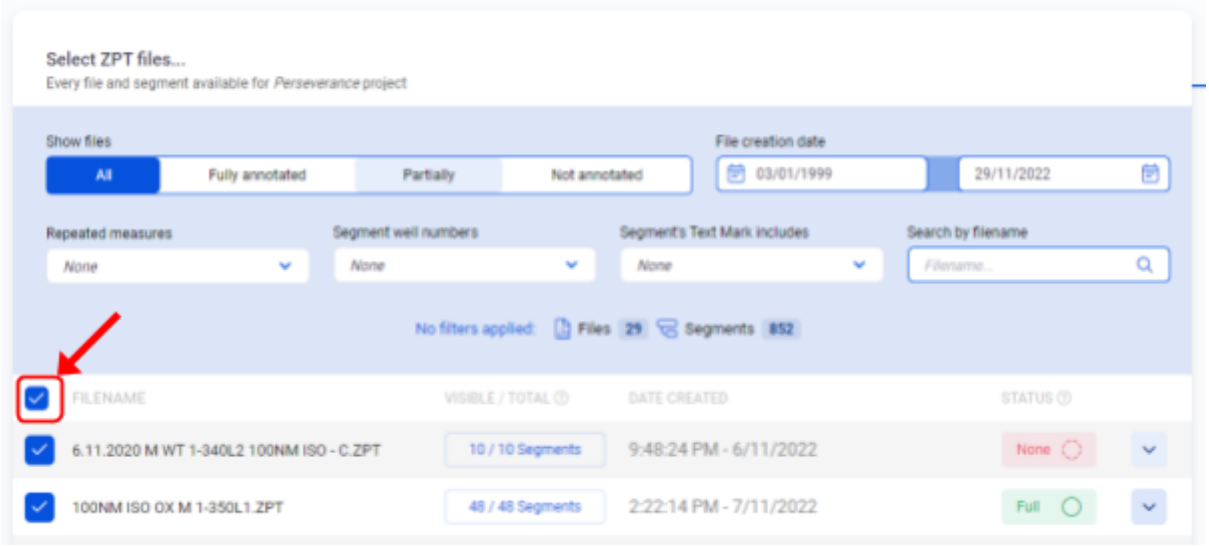


Figure 150. Location of "Select/Deselect all files".

You will see the status of this checkbox will change to a blue dot if the list of files is partially selected (i.e. not all the items are selected). By clicking on the checkbox you can change the state from partial to fully select the list or to deselect the whole list.
The same principle applies to segments as it does to files. However, the "master" checkbox for a set of segments is the checkbox of the file that contains them.

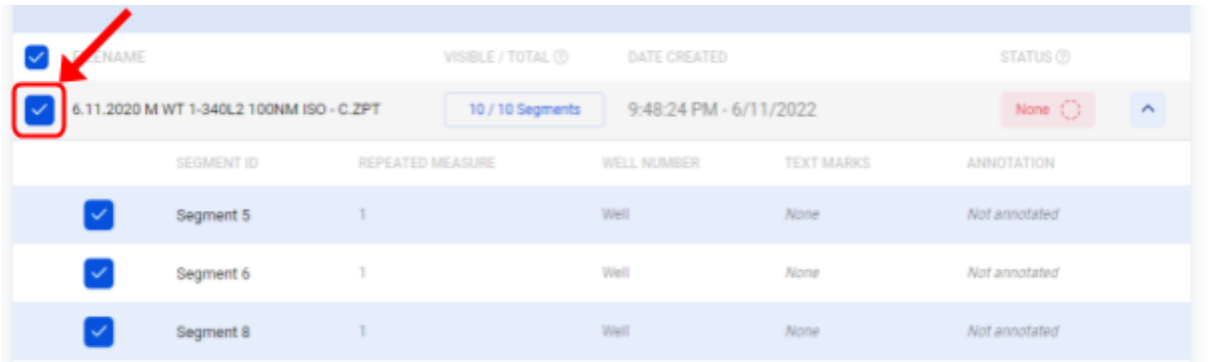


Figure 151. Location of "Select/Deselect all segments".

In addition to selecting files and segments individually, you can also use the buttons on the right-hand side of the Annotation area to select or deselect all possible[6] files and segments. There is also an option to invert the selection, which allows you to select what was previously deselected and vice versa.

[6] Applies only to items that have NOT been filtered out and are still visible.

6.5.4.2 Annotation area

The Annotation area provides essential information required for accurate annotation. It is important to note that the segments are the items that are being annotated, while the files serve as a mere organization item. Hence, a file can contain segments with varied annotations.

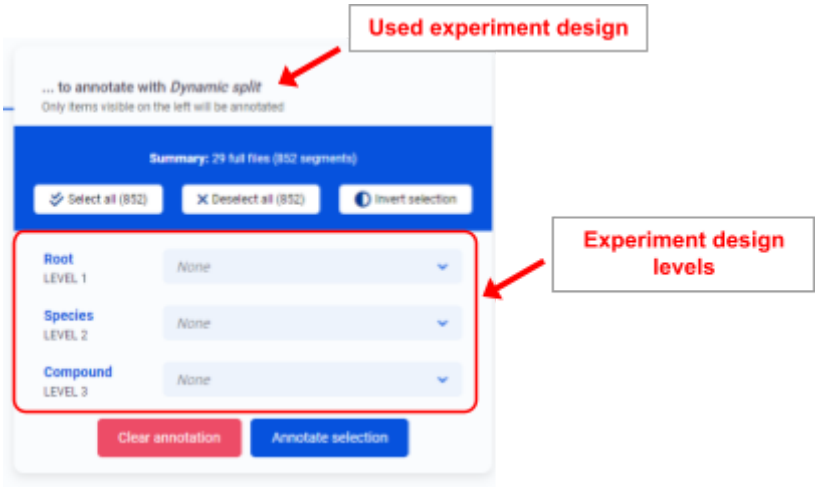


Figure 152. Location of "Select/Deselect all segments".

6.5.4.2.1 How to annotate files/segments

The top of the Annotation area displays the Experiment design name followed by a summary of the number of selected files and segments.

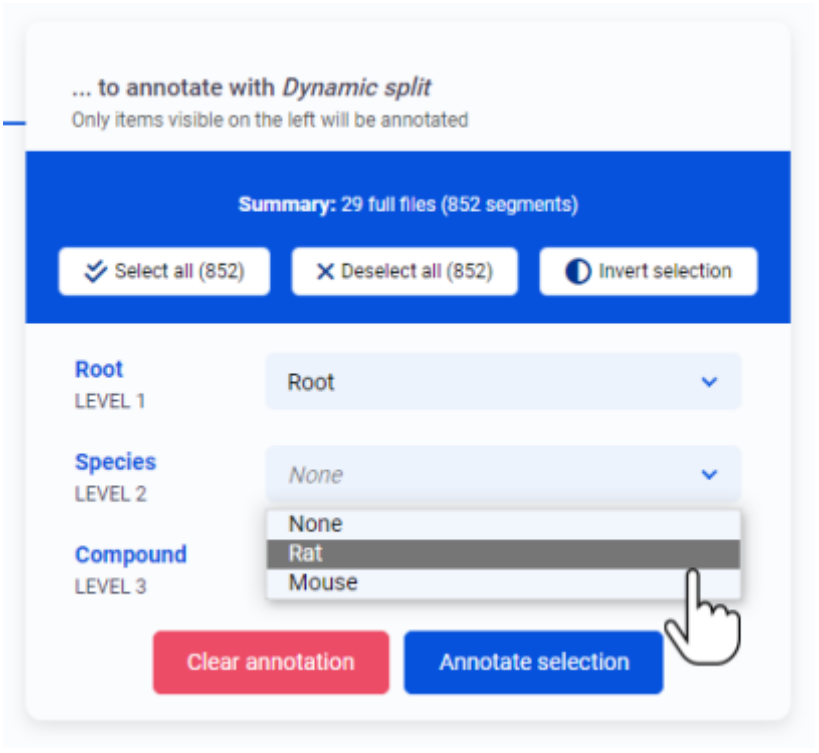


Figure 153. Choosing values for annotation.

To annotate you need to:

1. **Choose the desired factors for each level** specified in the Experiment design by selecting from the drop-down menus. The drop-down menus correspond to each level of the design and include the level number and name. The factors specified for each level in the Experiment design are listed in the drop-down menus. If you select "None" the corresponding level of annotation will be left blank, resulting in a partial annotation.
2. Once you have finished selecting all the desired values, **click "Annotate selection"** to finalize the annotation. The selected items will then appear as annotated in the Selection area.

6.5.4.2.2 How to clear the annotation of files/segments

In order to clear the annotation of selected files or segments you only need to select the desired items to erase, just like when annotating, and clicking “Clear annotation” instead.

IMPORTANT

If you annotate a file or segment and choose "None" as the value for any or all of the annotation levels, it will NOT delete the annotation. The annotation will still be retained and no changes will take effect.

6.6 Extracting project's averages and creating plots with Data visualization tool

Data visualization tool in CytoSolver 3.0 is integral to its all-in-one package, as it enables statistical analysis and graphing of experimental data using analyzed data from previous steps. This tool generates visually informative graphs in various formats including bar graphs, correlation plots, and normalized data plots. It provides researchers with the flexibility to choose the best representation for their data. Additionally, researchers can easily export data that has been statistically analyzed, including metrics such as standard deviation (SD) and standard mean error (SEM), to be imported into commonly used software tools to perform statistical hypothesis testing calculations.

6.6.1 Understanding the scope of the Data Visualization (DataVis) tool

The purpose of this section is to familiarize you with datavis interface layout.

When you are on the DataVis page, the tool's name "DataVis" will appear at the center of the screen.

A horizontal line will be visible underneath it containing the names of different plot groups such as "Summarized", "Exploration", "Traces", and "Combination". The selected group will be highlighted with black font and a green line underneath, while the unselected groups will have gray font and no line.

Specific plot types within the selected group will be displayed on another banner below the general plots banner. The selected plot will have a white box surrounding it, while remaining plots will have a banner's background. Each group will have a different set of plots.

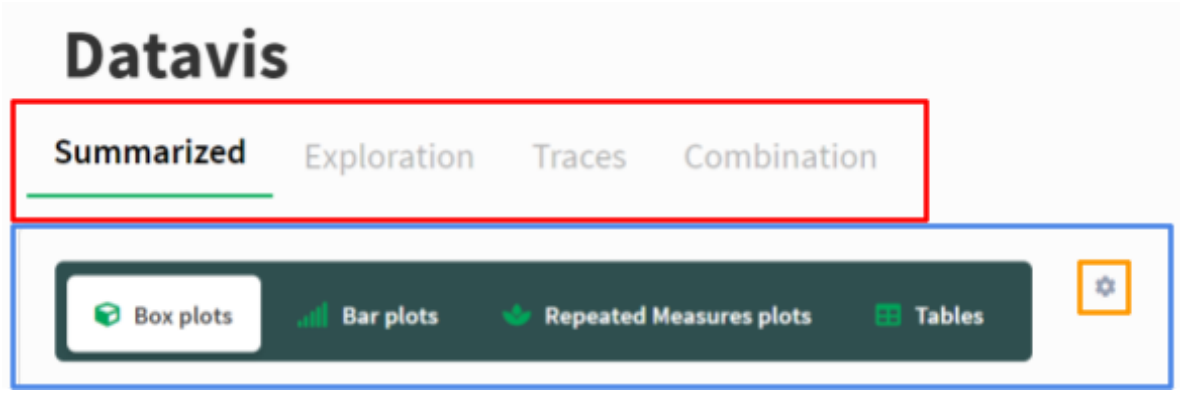


Figure 154. DataVisualization (DataVis) page banners.

By clicking on the settings button, a pop-up window will appear that allows you to customize various features within the primary interface.

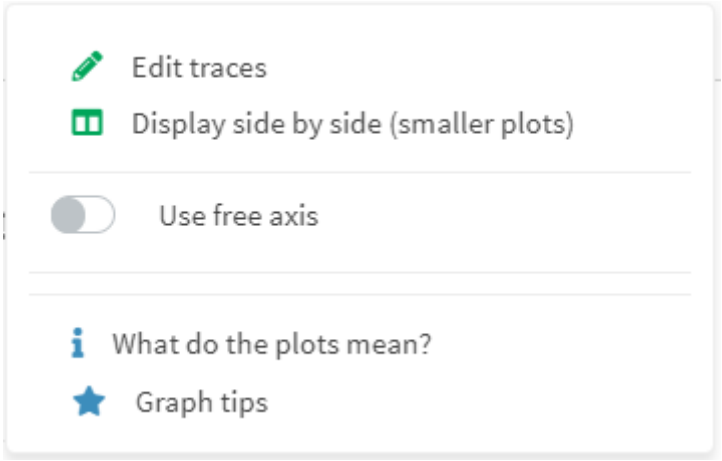


Figure 155. DataVisualization (DataVis) settings.

When you choose "Edit Traces", a "*Plot Traces Management*" pop-up window will open, allowing you to hide certain displayed plots, reveal previously hidden plots, or rearrange plots within the main area.

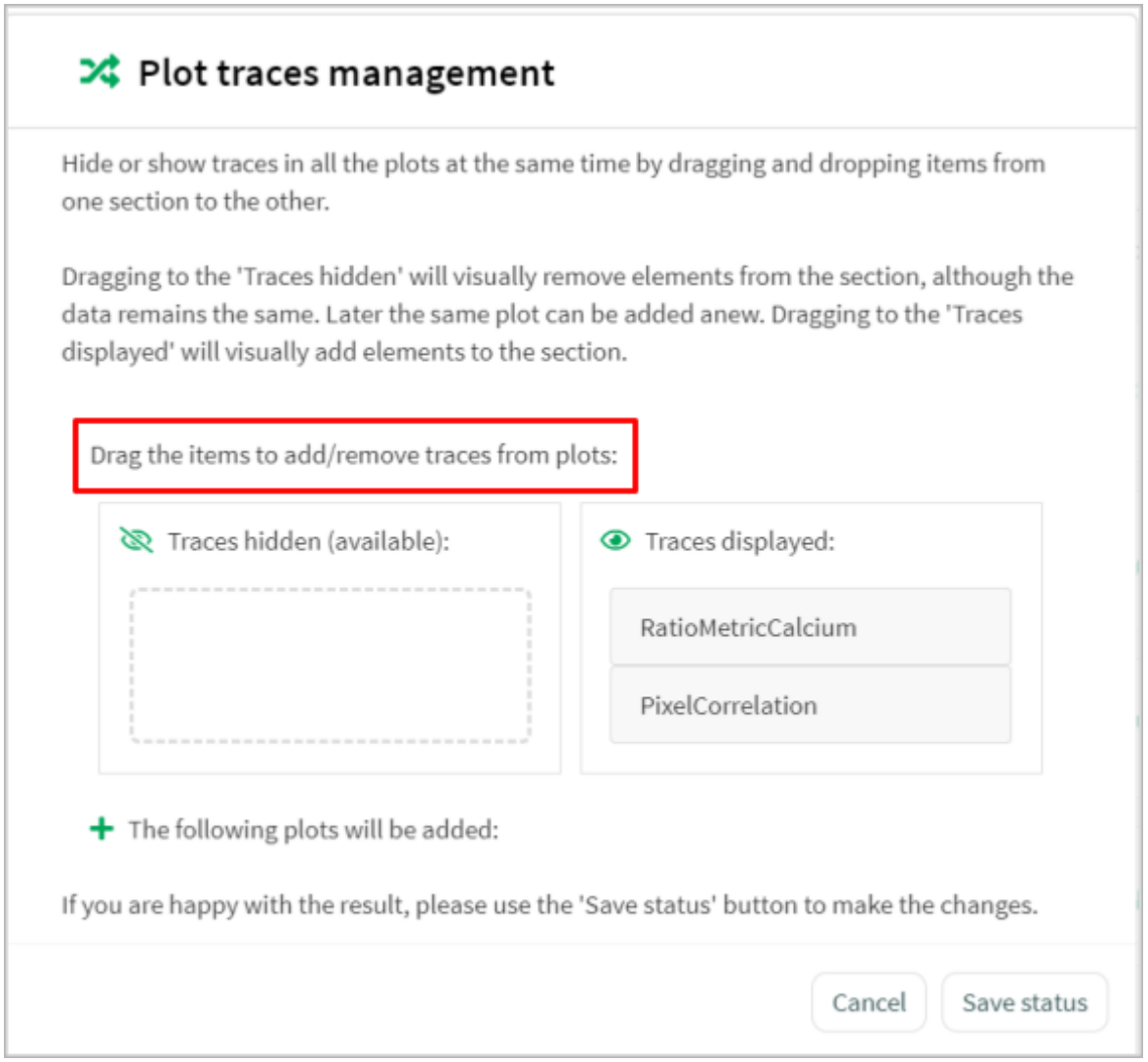


Figure 156. DataVisualization (DataVis) plot traces management.

If you choose "Display Side by Side" from the settings window, all plots will appear next to each other, causing them to become considerably smaller than in the default view where they are displayed below one another.
Subplots have synchronized axes, which implies that they share the same scale and labels. When the "Free axis" option is activated, each subplot will have its own independent axis and labels.
By selecting "What do the plots mean?" in the settings, a pop-up window titled "Plots" will appear, providing details on several plots and their purpose.
Finally, choosing "Graph tips" will open another pop-up window labeled "Graph plots," offering helpful hints for navigating the graphs.

In DataVisualization (DataVis) tool, the primary section where plots are shown is referred to as the “main area”. By default, all data points within a project are displayed in a single graph.

Figure 157. DataVisualization (DataVis) main area location.

You can modify your data display by using toolboxes on the right-hand side of the page. These toolboxes are standard for every plot and include Data, Plotting, Filters, and Actions. Certain plots may also have additional toolboxes located above the standard ones. We will delve further into these toolboxes in subsequent sections.

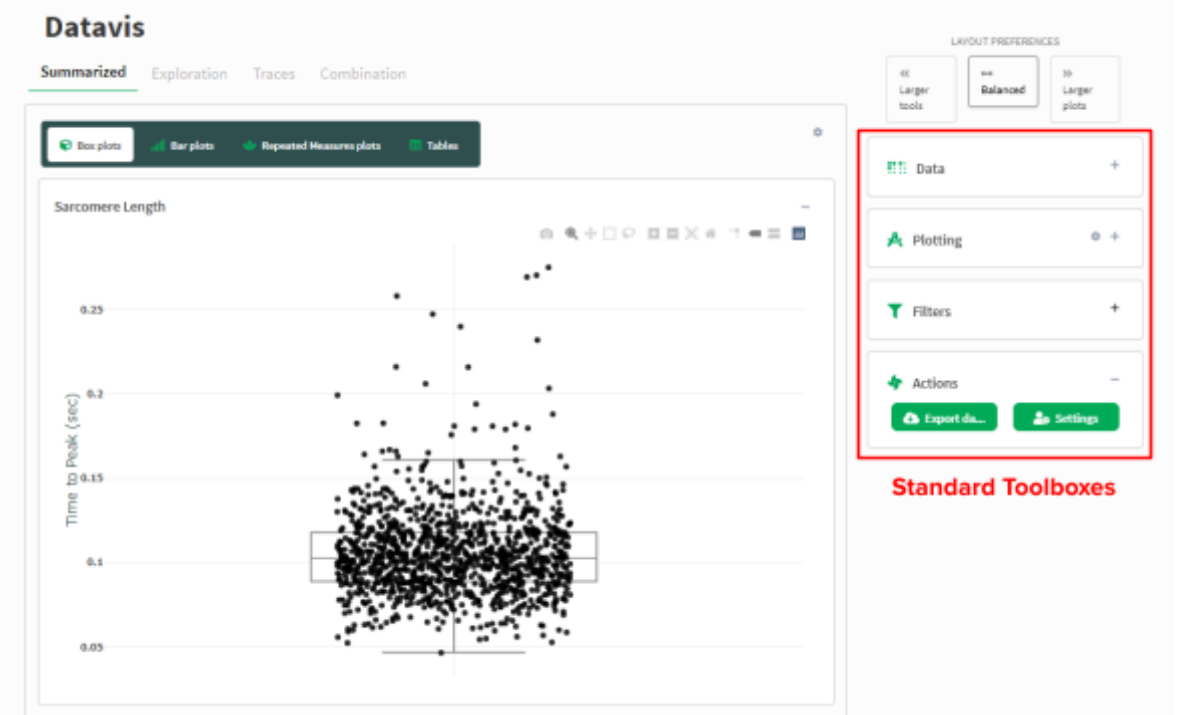


Figure 158. DataVisualization (DataVis) standard toolboxes location.

6.6.2 Understanding concepts used in the Data visualization tool

6.6.2.1 What is a factor?

A factor is a column in the data obtained from CS that typically serves as a grouping level and is not considered a variable or an "essential core value," such as an ID or sample ID. Factors often include levels of any annotations and additional items such as "filename" and "collection date."

6.6.2.2 What is annotation?

Annotation in DataVisualization (DataVis) does not entail actively adding descriptive information such as labels or tags to data. Rather, it involves using the pre-assigned tags to color code, organize data, and create subplots on the graphs, thereby facilitating data analysis and visualization.

IMPORTANT

It is essential to annotate your data appropriately using the "annotation" feature **prior to** opening DataVisualization (DataVis) to extract valuable insights from the graphs

6.6.2.3 What is raw data?

“Raw data” refers to the initial, unrefined measurements or observations obtained during a scientific investigation or study. Within our systems, this would entail unprocessed recordings of various parameters such as fluorescence (which usually denotes calcium measurements), sarcomere length, and pixel intensity, among others recorded on IonWizard software.

6.6.2.4 What are repeated measurements?

“Repeated measures” are data points that have been collected from a single sample under different conditions. Using DataVisualization (DataVis) you can Link the same sample over all repeated measures which helps track effects of a variable being studied on the response. This approach reduces data variability, increases statistical power, and helps determine statistical significance.

6.6.3 Tabs and plots

6.6.3.1 What is a tab?

In DataVisualization (DataVis) “Tabs” are graphical elements used to organize and display multiple sections or pages of content within a single user interface, enabling users to swiftly switch between different sets of information or tasks. Clicking on a tab displays the corresponding information, with each tab typically representing a different section or page of content.

6.6.3.2 What is a plot?

In DataVisualization (DataVis), a "Plot" is a graphical representation of data that is used to visually analyze and explore patterns and trends. There are various types of plots, each of which is better suited to different types of data and analytical tasks. We combined several types of plots on our platform, to help users identify outliers, detect trends and anomalies, and draw meaningful conclusions from their data,

6.6.3.2.1 Plots area visualization settings

Plot area visualization settings are a crucial aspect of data visualization tools, as they enable users to explore and analyze data with ease. These settings include features such as downloading plots, zooming in on a particular section of a plot, box selection, lasso selection, autoscaling the plots, and resetting the axes.

Figure 159. Plots area visualization settings banner.

The visualization settings banner for the plot area (shown above) will be present for each plot within the main area, situated in the top right corner of the plot. It becomes visible after the

cursor enters the graph area. Each plot has its own banner, enabling you to explore data in one plot without impacting the default view of other plots.

Below we will explain the functionality of each icon within the visualization settings banner.

Icon	Definition	Functionality
	Download Plot	Exports data in the format of Scalable Vector Graphics (SVG).
	Zoom	Enables you to select a specific section on the graph for more detailed analysis of clusters. If there are subplots, zooming in on one will also zoom in on the others.
	Pan	Allows you to move the graph around within its plot area, either up and down or left and right. If there are subplots, they will all move together in parallel.
	Box Select	Enables you to choose an area and blur out the rest of the graph, affecting only the plot you drew the box on.
	Lasso Select	Lasso select works similarly to the Box select feature, but with a free-form area instead.
	Zoom in	Magnifies the entire plot.
	Zoom out	Reduces the size of the entire plot.
	Autoscale	Restores the plot to its default scale, but if you haven't modified the scaling, clicking this icon will not alter anything.
	Reset Axes	Similar to Autoscale
	Toggle Spike Lines	Creates a horizontal and vertical line at the selected data point, and provides the X and Y axis values in a black pop-up text box.
	Show closest data on hover	Shows a pop-up text box with the X and Y axis values of the closest data points to the cursor.
	Compare data on hover	Shows a pop-up text box with the X and Y axis values of the closest two data points to the cursor.
	Produced with Plotly	Opens plotly website

NOTE

Box and Lasso select are not available for Distribution plots under the **Exploration** tab, or any of the plots under **Traces** tab; Average Traces, Normalized Traces, and Velocity Traces.

6.6.3.2.2 Plots under Summarized tab

CytoSolver 3.0's DataVisualization (DataVis) offers a "**Summarized**" tab that provides multiple visualization options for previously analyzed parameters from the transient analysis tool, such as "**Box Plots**," "**Bar Plots**," "**Repeated Measures Plots**," and "**Tables**".

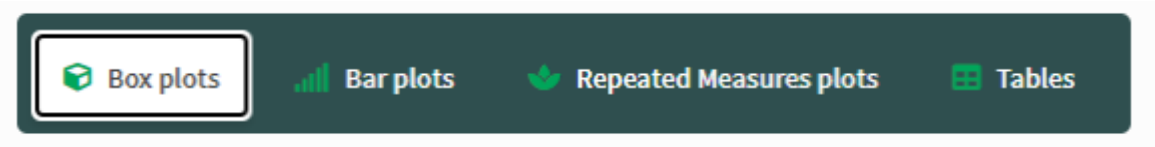


Figure 160. Summarized tab plots.

"**Box Plots**" present data using the central tendency measures such as median, quartiles, minimum and maximum values, along with individual data points. Additionally, they display standard error of the mean (SEM) to represent variability of data. However, users cannot modify error bars to display standard deviation (SD).

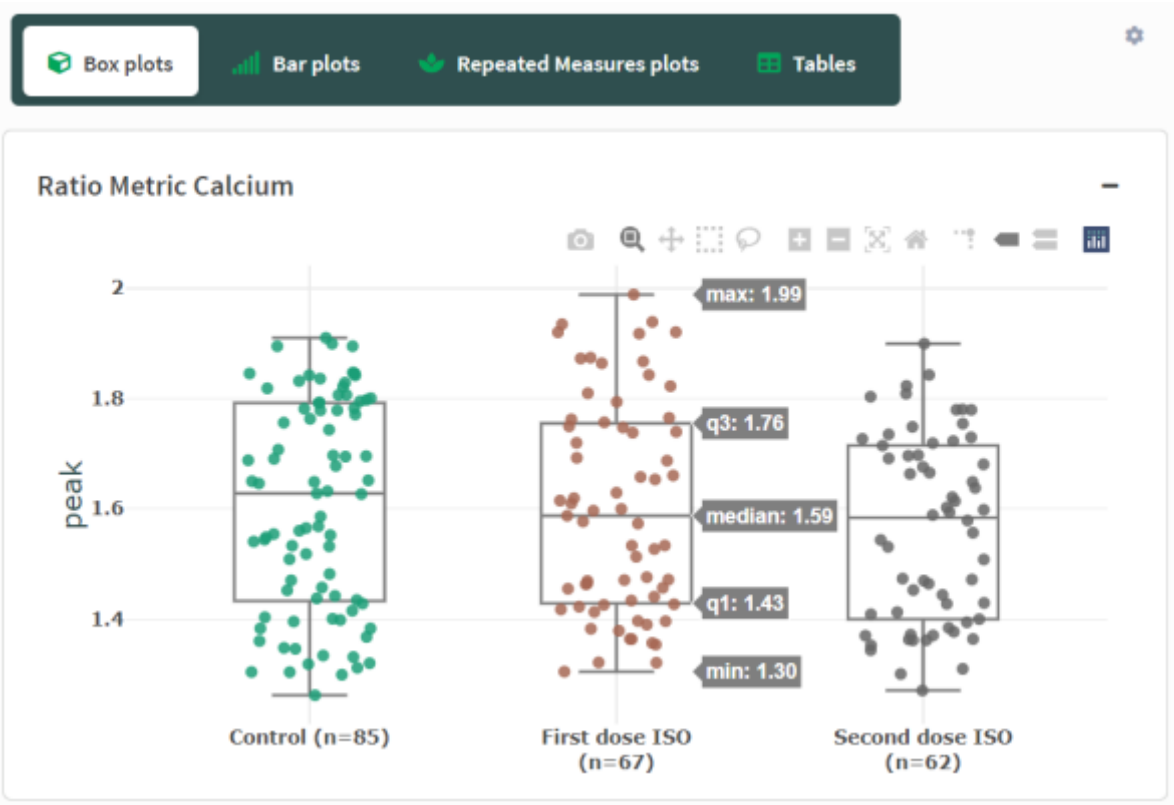


Figure 161. Box plots - color coded based on treatment conditions.

NOTE

Outliers are omitted in this graph.

"**Bar Plots**" shows data as average values with SEM, but does not display individual data points. To include SD error bars or individual data points, users can make use of the "**Bar Plots Extras**" toolbox, situated at the top of standard toolboxes on the right side of graphs.

Figure 162. Bar Plots - displaying individual data points and averages.

In the "**Repeated Measures Plots**" display, data is grouped into subcategories based on repeated measures. Users can access an additional toolbox called "**Repeated Measures Extras**" located at the top of standard toolboxes on the right-hand side. This toolbox provides various options, such as linking data points and filtering cells that do not include all conditions, among other features.

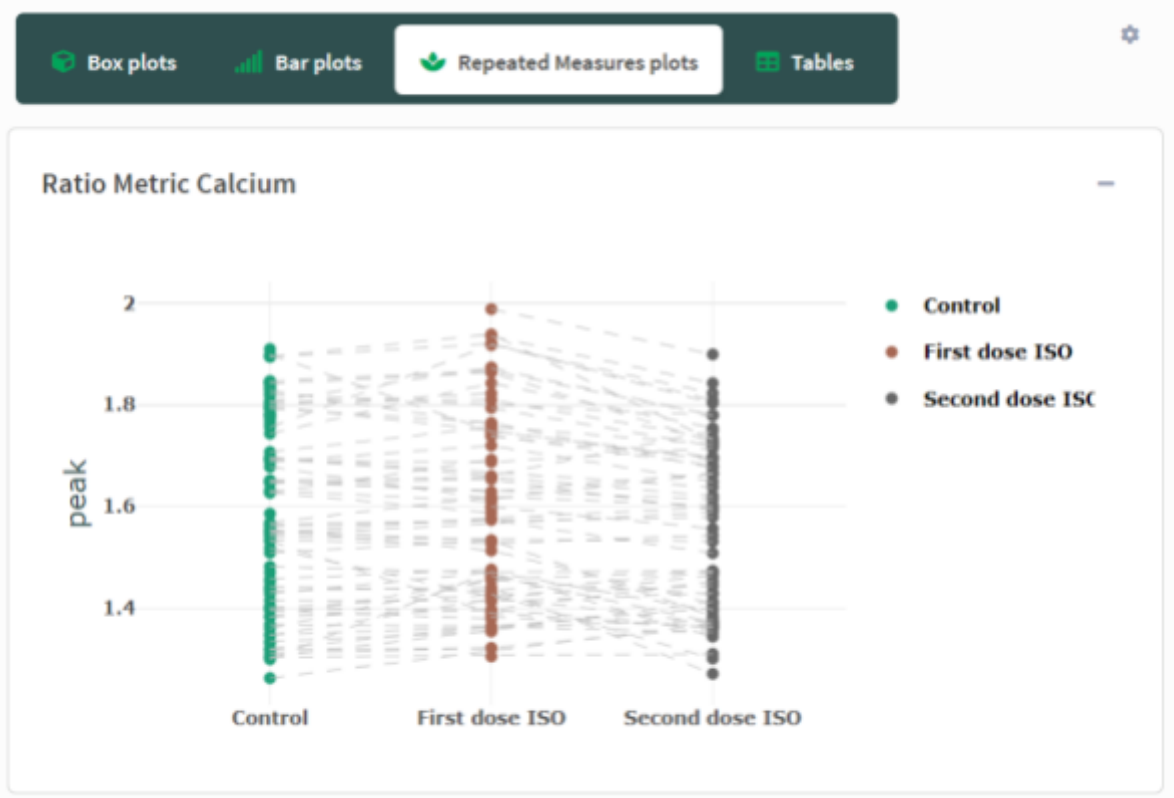


Figure 163. Repeated measures plot - displaying connections between repeated measures.

"**Tables**" tab displays numerical data in a central table format. This table presents a summary of selected variables for each trace based on user preferences and filters. Table columns show the mean, standard deviation or standard error of mean, and number of data points (N) for each variable. The left-hand side of the table contains additional tools such as "**Variables**" and "**Options**" toolboxes.

6.6.3.2.3 Plots under Exploration tab

"**Exploration**" tab on CytoSolver 3.0's DataVisualization (DataVis) allows users to examine relationships between variables in previously analyzed parameters through scatter plots, distribution plots, and correlation plots, helping them to identify patterns, trends, and outliers. The display layout of the exploration plots is consistent across all types and features a central area for graphs and standard toolboxes located on the right side of the plots.

Figure 164. Exploration tab plots.

"**Scatter Plots**" display individual data points on the graph, with X and Y axes exhibiting all of the chosen variable's data points. The X axis can display either the number of data points found in a project's data or collection time.

NOTE

Scatter plots are distinctive for using time on the X axis

By examining scatter plots, users can identify any potential outliers or clusters, providing valuable insights into the data and highlighting areas that require further investigation.

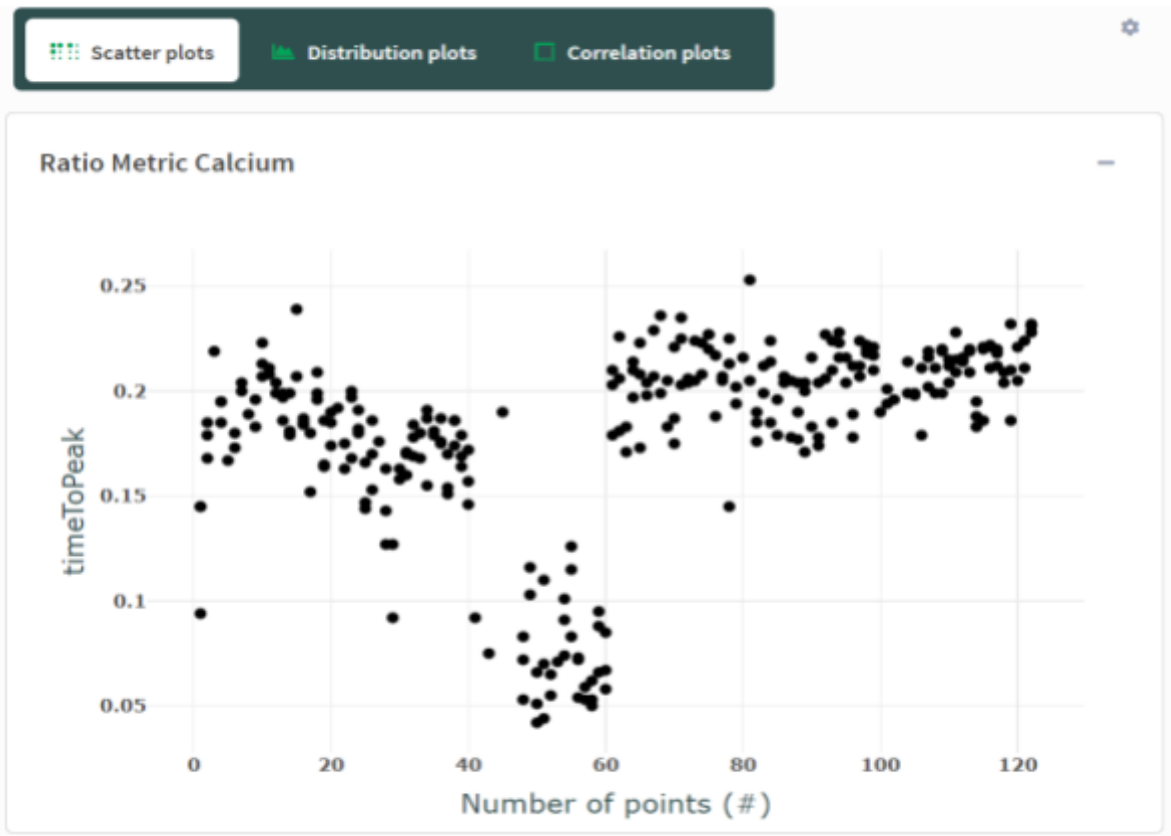


Figure 165. Scatter plot - displaying all data points without filtering.

"**Distribution Plots**" display density distribution of a chosen Y-variable. Analyzing the distribution of a dataset can help identify important characteristics, such as skewness or kurtosis. Curve's shape can also provide insights into underlying data, such as whether it is normal, bimodal, or skewed.

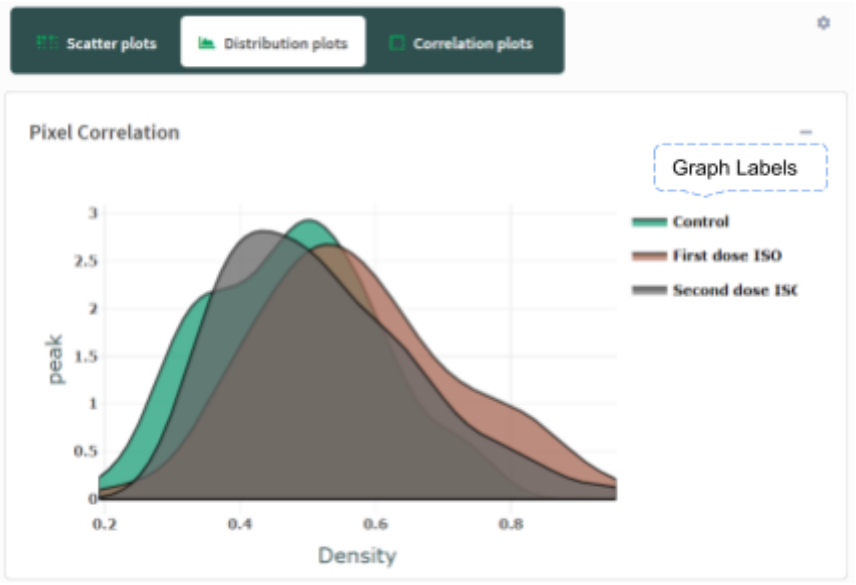


Figure 166. Distribution plots - displaying data filtered based on treatment conditions.

NOTE

To toggle visibility of a density graph for a specific label on distribution plots, simply click on the corresponding label. This feature applies universally to any graph that has been labeled.

"**Correlation plots**" allow users to explore relationships between different variables and identify potential trends through a matrix of scatter plots. This visualization shows correlation between two variables by plotting their relationship, including each individual data point and the linear regression line.

Figure 167. Correlation plots - displaying linear regression line of pixel correlation baseline vs peak (data filtered by treatment conditions).

6.6.3.2.4 Plots under Traces tab

"**Traces**" tab in CytoSolver 3.0's DataVisualization (DataVis) feature allows examining trace behavior over time, such as fluorescence traces (usually ratiometric calcium) and sarcomere length, among others. These traces can be examined in three unique formats: "Average trace," "Normalized trace," and "Velocity traces." Data points used to generate these figures are the accepted traces post-analysis, rather than individual parameters extracted from each trace.

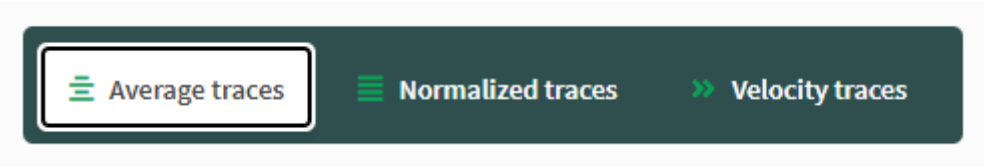


Figure 168. Traces tab plots.

"**Average traces**" view traces in their raw format, such as ratiometric calcium and pixel correlation among others, rather than displaying extracted parameters from each trace. Average traces depict the mean value of all traces within a group over time, with error bars indicating the degree of variability. These error bars are depicted in the same opaque color as the trace.

NOTE

Examining data in this way can be very informative for identifying variations in baseline or absolute values.

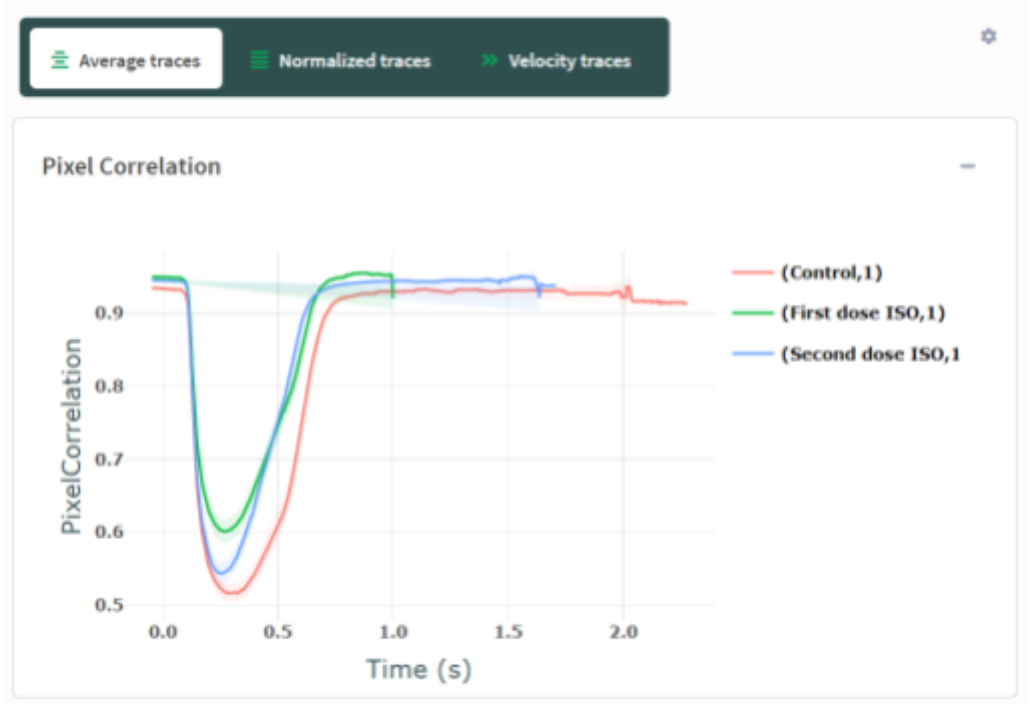


Figure 169. Average traces plot - displaying pixel correlation values of data filtered by treatment conditions.

"**Normalized traces**" allows users to improve accuracy of averaged traces by accounting for differences in baseline values or absolute magnitudes between individual traces. DataVisualization (DataVis) aligns all traces to a single point on the Y-axis.

NOTE

Data is normalized to the average of all baseline values.

Essentially, the shape of traces remains identical as traces presented on "average traces", but it is shifted on the Y-axis. Normalizing traces enables researchers to identify subtle differences in dynamics or behavior of parameters that may not be noticeable when looking at absolute values alone.

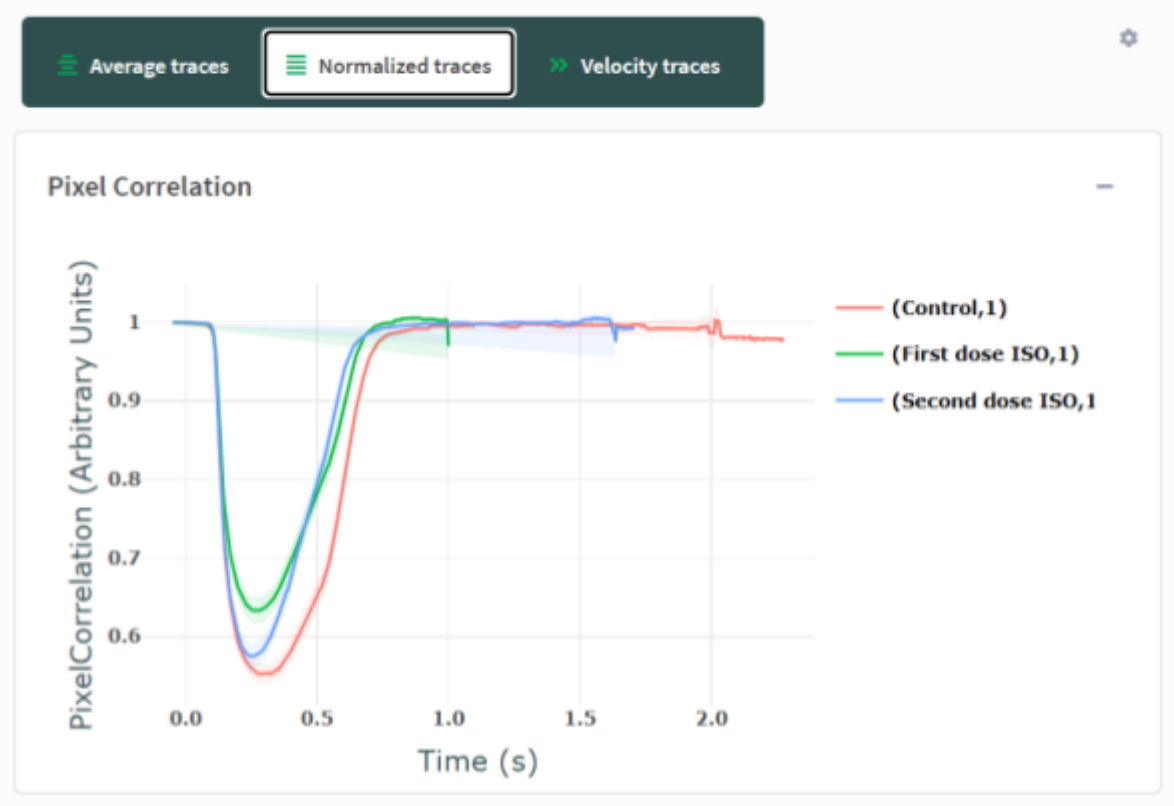


Figure 170. Normalized traces plot - displaying normalized pixel correlation values of data filtered by treatment conditions.

“**Velocity traces**” depict the first derivative of the average trace, which is a measure of rate of change of a signal over time. This representation is based on user preferences and filters, allowing for customized analysis of data. In other words, velocity traces provide information on the speed or rate of change of a signal being measured, allowing for a more in-depth analysis of the underlying data.

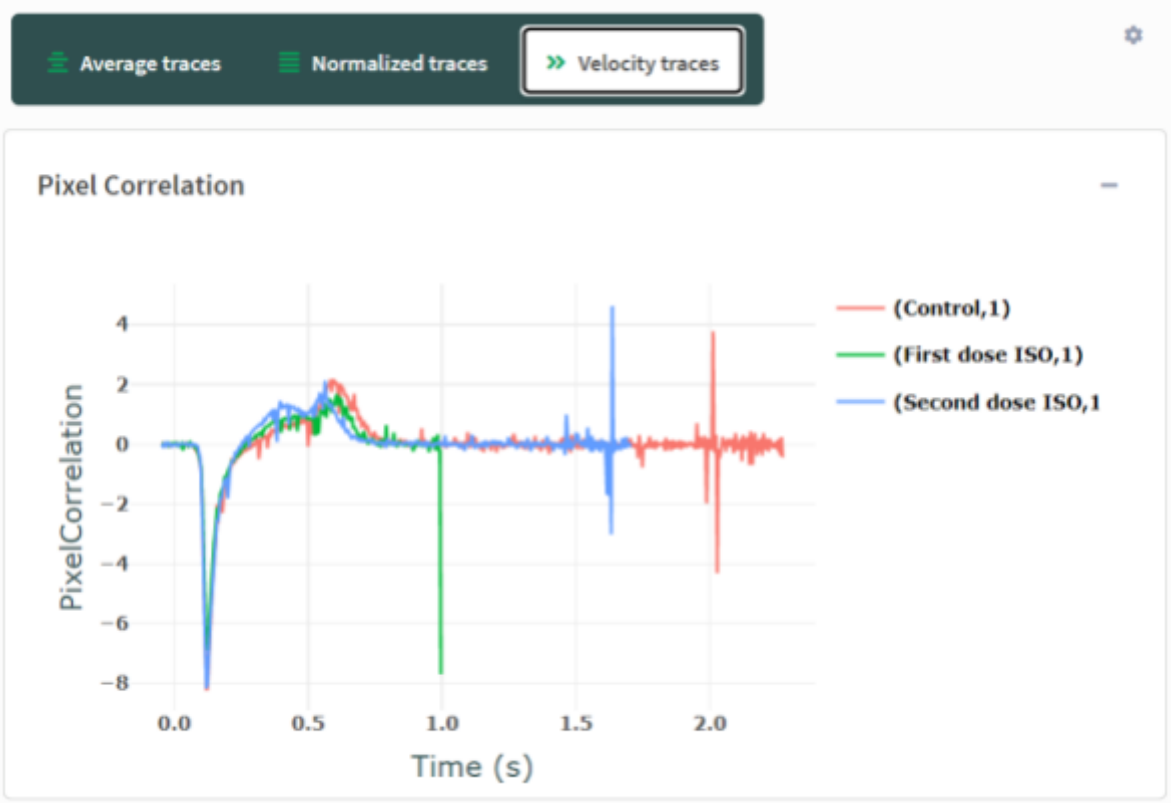


Figure 171. Velocity traces plot - displaying first derivative of the average pixel correlation trace.

6.6.3.2.5 Plots under Combination tab

The combination tab in DataVisualization (DataVis) offers two types of plots. "Correlation traces" plots create scatter plots that display the relationship between two different variables from separate traces, while "Contractility traces" are plots that investigate cardiomyocyte function during activation cycles.

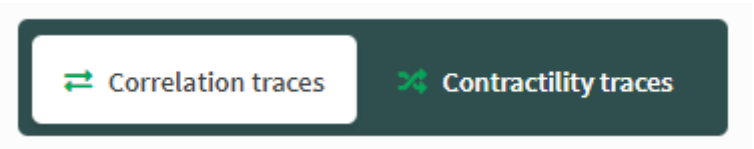


Figure 172. Combination tab plots.

"**Correlation traces**" enable creating scatter plots that display the relationship between two different variables from two separate traces. The "Combination Extras" toolbox can be used to select the variables of interest. This visualization method shows the correlation between two variables by displaying their relationship, along with each individual data point and a linear regression line.

By examining the shape of a scatter plot, researchers can thoroughly analyze the interrelationship between the two variables, enabling identification of any underlying patterns or associations.

Figure 173. Combined correlation Traces plot - displaying linear regression lines of ratiometric calcium and pixel correlation peak values (data filtered by treatment conditions).

“Contractility traces”, also known as phase loops, can be created using DataVisualization (DataVis) and are a useful tool for investigating cardiomyocyte function during activation cycles. These loops are generated by plotting the concentration of calcium ions against cardiomyocyte length during each cycle.

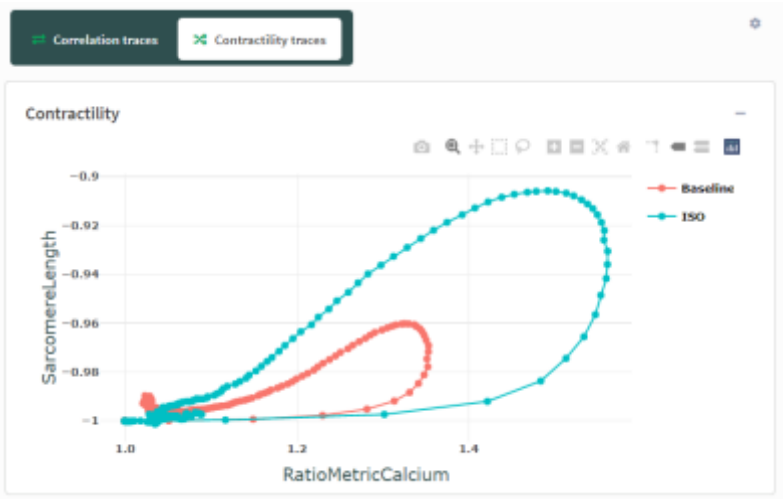


Figure 174. Contractility traces plot - phase loops displaying cardiomyocyte function during activation cycles (reverse plot setting was used to generate the graph in this format).

The descending part of the loop, which corresponds to the relaxation phase, is particularly crucial as it provides insight into myofilament calcium sensitivity.

NOTE

Unlike correlation traces, contractility traces do **NOT** allow you to pick X and Y variables for each trace.

6.6.4 Toolboxes

Cytosolver 3.0 DataVisualization (DataVis) tool provides a comprehensive toolbox for data management and analysis, including data source management, table view, variable and data creation, value calculations, plotting tools, data filtering, deletion/restoration of created data, export options, and access to additional toolboxes. Each component of the toolbox offers various possibilities for users to efficiently analyze their data. In subsequent sections, we will explore each feature in more detail.

6.6.4.1 Layout management

DataVisualization (DataVis) provides layout management options on the right side of the screen above toolboxes.

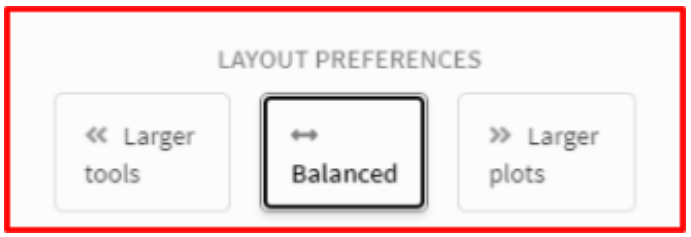


Figure 175. DataVisualization (DataVis) layout preferences navigator.

Users can customize size and position of toolboxes and plots. You can choose to make toolboxes larger on the right-hand side of the screen, this will make plots smaller. Or you may increase plot size, which pushes toolboxes further to the right. Alternatively, you can choose to keep the default balanced setting. These options provide flexibility in organizing workspace to fit individual preferences and needs.

6.6.4.2 Managing data source

6.6.4.2.1 Automatic data import

When you access DataVisualization (DataVis) in CytoSolver 3.0, all data files related to your project will be imported automatically. You can observe this process in real-time; two brief pop-up windows will appear, “Loading” followed by an "Importing project: *project name*" message at the center of your screen. The entire import process usually takes only a few seconds.

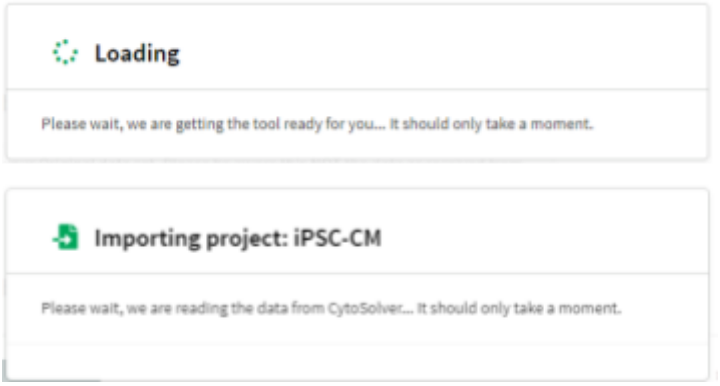


Figure 176. DataVisualization (DataVis) data import pop-up windows.

NOTE

After importing, plots won't appear immediately on your screen. Instead, you can observe the real-time progress of the plots through small windows on the right-hand side of the screen. This step is very quick as well.

After data files have been fully imported DataVisualization (DataVis) by default shows you Box Plots under the Summarized tab.

6.6.4.2.2 Viewing your data (table format)

To view or adjust imported data, use the “**Data**” toolbox.

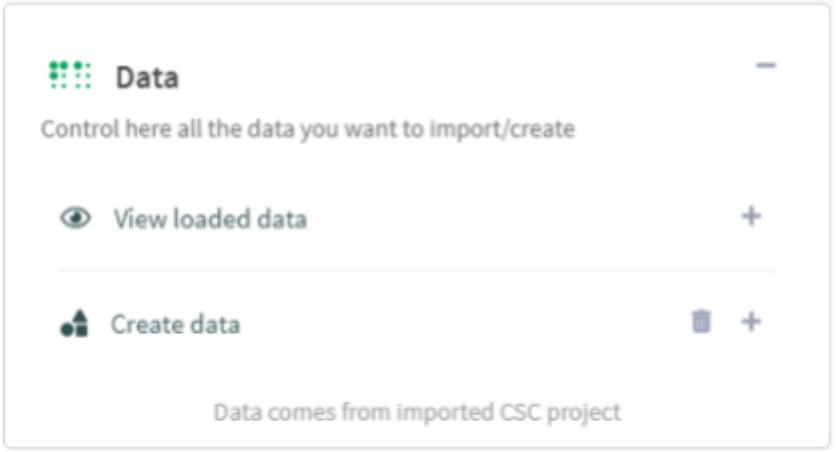


Figure 177. Data toolbox general features.

If you expand “**View loaded data**” you will see the current source of data, clicking the "**Display Data**" button will display all analyzed data from previously imported ZPT files in the project, presented in a tabular format.

6.6.4.2.3 Creating new data

If you expand “**Create data**” you can either show data from original data set (all analyzed ZPT files in the project) or you can create new:

1. Variables
2. Data Sets

6.6.4.2.3.1 Creating new variables

At present, users can only create a new Y-variable within the software. By selecting this option, a pop-up window titled "**Create new variable**" will appear. This feature permits creation of a personalized Y-variable, which involves combining existing variables and constants using a mathematical formula of user's choice.

6.6.4.2.3.2 Creating new data sets using annotation

Users have the option of creating normalized data or creating delta data

6.7.4.2.3.2.1 Calculating value normalizations (a.k.a *norms*)

With this feature, you can create a 'normalized' (μ) data set by normalizing a variable. The new data set will use your original data set, imported from the transient analysis page, as a reference point.

IMPORTANT

To carry out this procedure, data needs to be annotated, and the formula will be applied to all variables present in the original data set.

Depending on your preferences, it will perform a division operation by dividing rows labeled with a tag chosen under 'targets' by average values of rows labeled with a tag selected under 'baseline'.

Steps:

After opening the “Calculate time-based normalization” pop-up window by selecting “Normalized data” from the drop-down menu under “Data sets”.

1. Select a name that will serve as an identifier for your new dataset. This name will be accompanied by an internal ID that will appear just before it, creating a format like this: [ID: Your_data_set_name].
2. Select a baseline and targets for division. In this example, we chose to normalize data based on treatment conditions and use “control” as our average for normalization.
3. Choose an algorithm to calculate the average of 'baseline' rows. Two algorithms are available:
    a. Average of all baselines: calculates average of cells annotated as baseline, and subtracts the value to all cells annotated with target (including baseline)
    b. Average of closest baseline in time: groups each target-annotated cell with its closest baseline in time. Then, the average of each group's baseline is subtracted from the rest of the group (including itself)
4. Calculate results and preview your new dataset before saving it.
5. Close or save your data

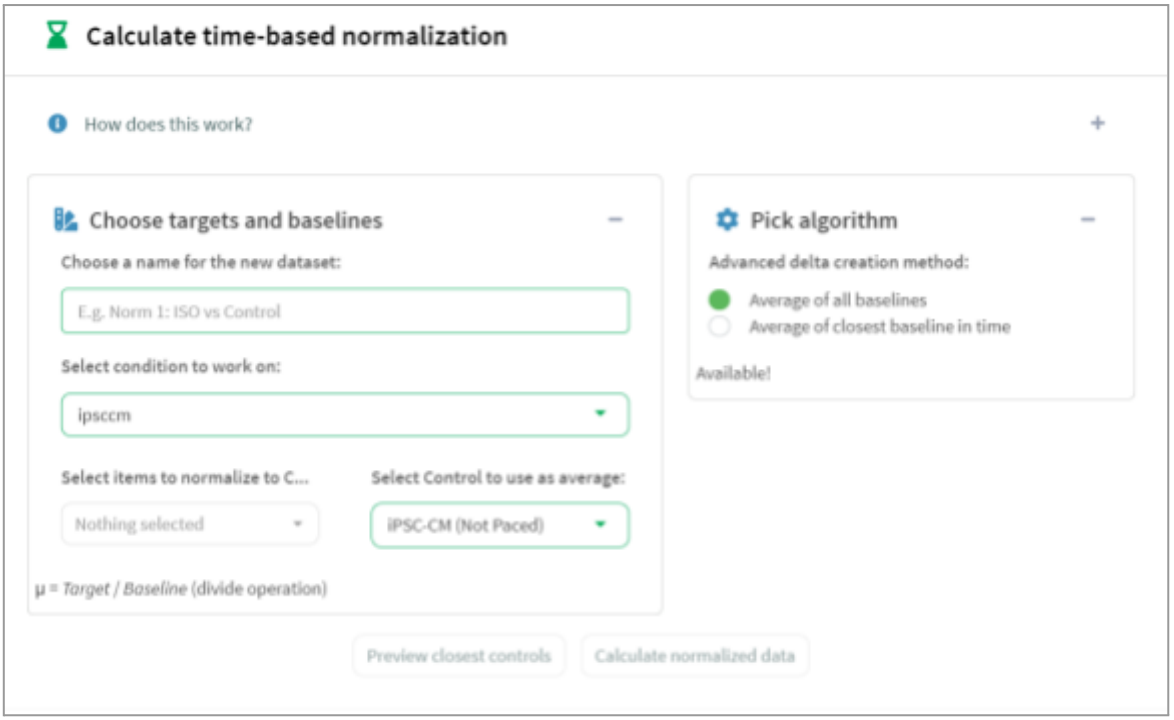


Figure 178. Calculate time-based normalization pop-up window - opens when creating normalized data.

6.7.4.2.3.2.2 Calculating value changes (a.k.a *deltas*)

With this feature, you can create a 'delta' (Δ) data set that represents a variable's change over time. The new data set will utilize your original data set, imported from the transient analysis page, as a reference point.

IMPORTANT

To carry out this procedure, data needs to be annotated with **at least 2 factors**, and the formula will be applied to all variables present in the original data set.

Depending on your preferences, it will perform a subtraction operation by subtracting average values of rows labeled with a tag chosen under 'baseline' from rows labeled with a tag chosen under 'targets'.

Steps:

After opening the “Calculate deltas” pop-up window by selecting “Delta Data” from the drop-down menu under “Data sets”.

1. Choose a name to identify your new data. The dataset will receive an internal ID, which will be displayed as [ID: Your_data_set_name].
2. Select a baseline to be subtracted from targets.
3. Choose an algorithm to calculate the average of 'baseline' rows. Three algorithms are available:
    a. True repeated measurements: subtracts baseline to target only for cells that have true repeated measurements
    b. Average of all baselines: calculates the average of cells annotated as baseline, and subtracts the value to all cells annotated with target (including baseline).
    c. Average of closest baseline in time: groups each target-annotated cell with its closest baseline in time. Then, the average of each group's baseline is subtracted to the rest of the group (including itself)
4. Calculate results and preview your new dataset before saving it.
5. Close or save your data.

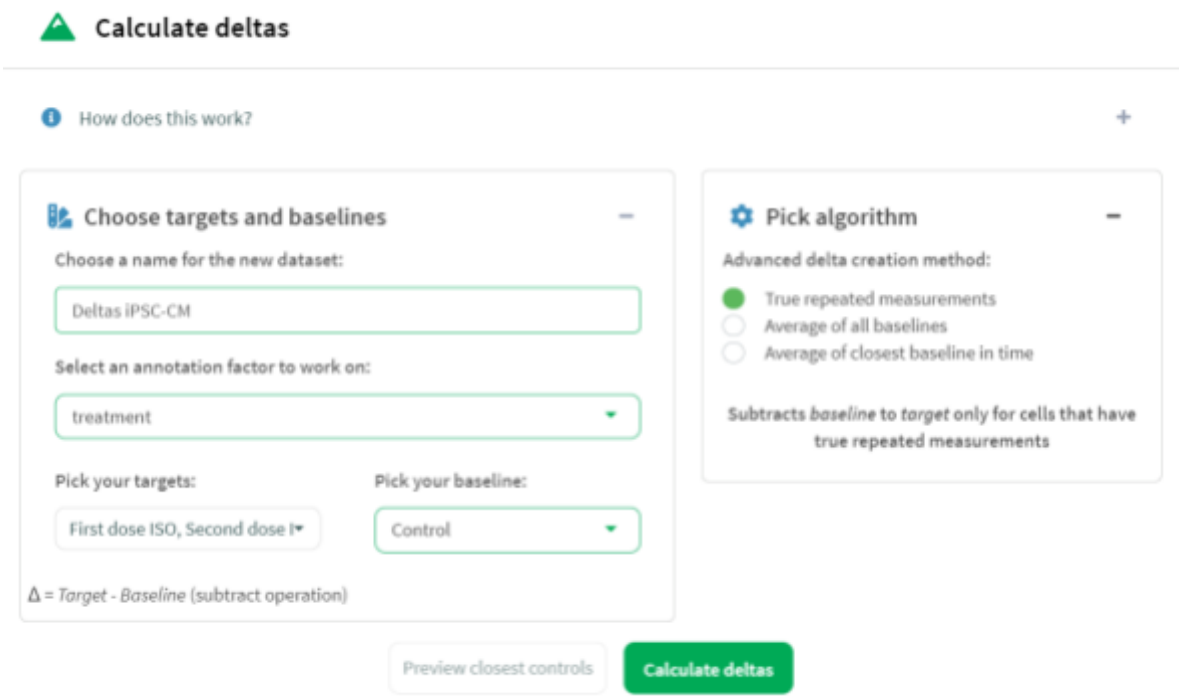


Figure 179. Calculate deltas pop-up window - opens when creating delta data.

6.6.4.2.4 Deleting created data

If you want to delete any previously created data, you can do so by clicking on the waste container icon. This will open a drop-down menu from which you can choose to clear individual variables, normalized data, delta data, or all of the created sets.

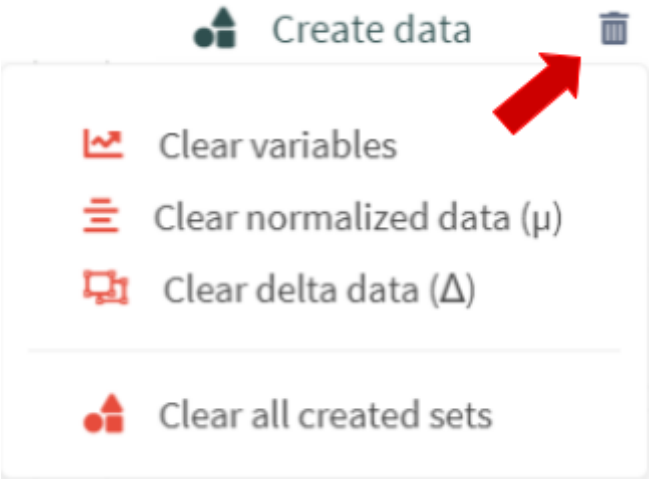


Figure 180. Deleting created data on DataVisualization (DataVis)- loaded from data toolbox (create data).

6.6.4.2.5 Restoring created data

The Data visualization page allows you to configure all kinds of settings, options, and configurations which can differ per project, and session. For this, we've included the optional toolbox for saving, loading, and clearing your preferences.

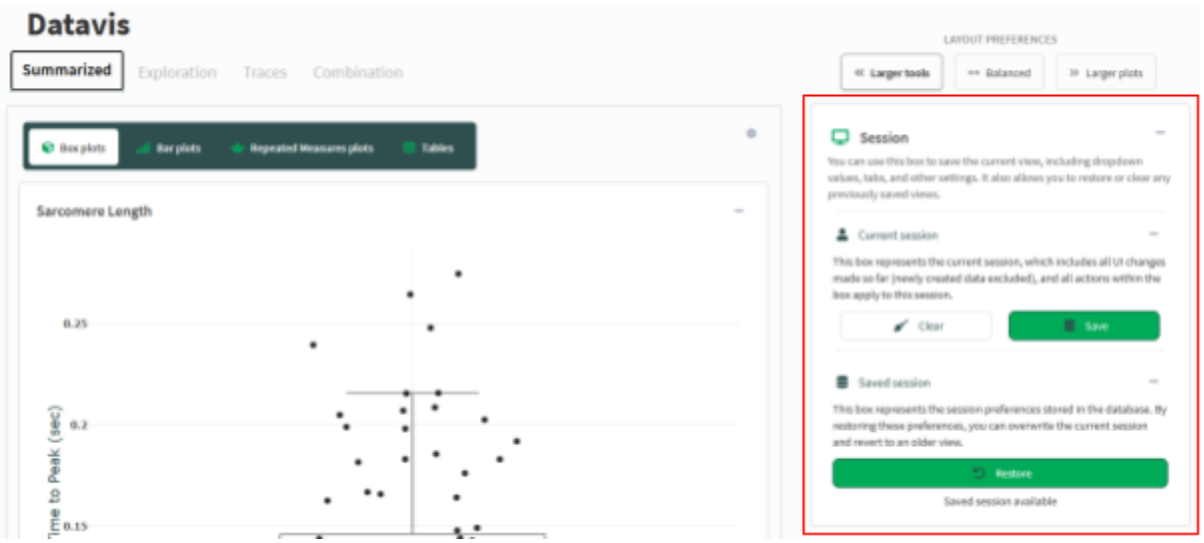


Figure 181. User preferences saving, restoring, clearing toolbox.

As mentioned, the Data visualization preferences can be applied to your current session and also project-wide. What this means is that when you open the Data visualization page, and

start modifying any filtering, sorting, coloring, etc., you'll be presented with the visualizations based on those preferences.
Now, if you would decide to leave the Data visualization page you could potentially lose those preferences as they are only applied to your current session. A session is a time that you're spending on the page. To make sure not to lose your preferences you can save them with the data saving toolbox. What's important to note is that the preferences are saved project-wide which means that when visiting the Data visualization page and choosing to load the preferences from the data saving toolbox you could load your preferences or those of someone else that saved them after you've saved yours. We currently only support a single project-wide preferences profile to be saved.

6.6.4.3 Plotting data

If you want to customize the appearance of your plots, you can utilize the "Plotting" toolbox available in all graphs. This toolbox allows you to select axes, group and color your data to achieve the desired look.

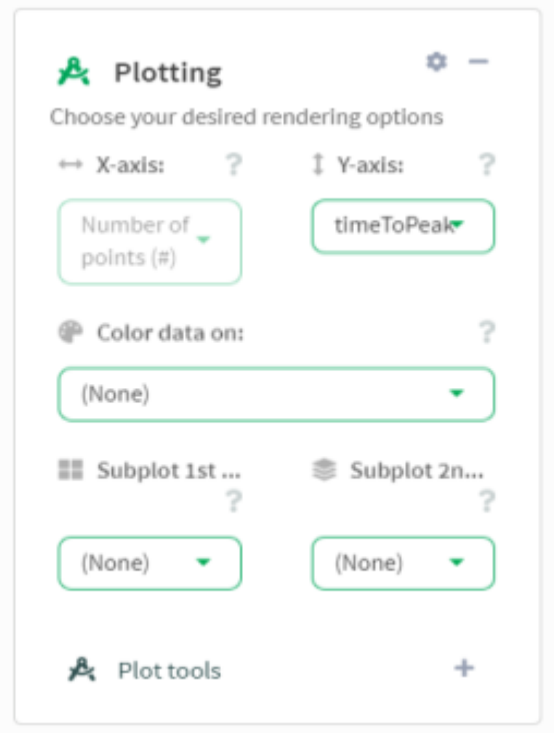


Figure 182. Plotting toolbox.

6.6.4.3.1 Selecting Axes

Selecting the X and Y axis is straightforward. Click on the box below an axis name, and if applicable, a drop-down menu will appear.
The X-axis can include number of points, density, time, or extracted parameters from analyzed data. In some plots, the X-axis is locked, so you won't be able to adjust it. The Y-axis always represents extracted parameters from analyzed data.

6.6.4.3.2 Grouping and Coloring Data

If you want to group data without creating subplots, you can utilize the "color data on" button. This button opens a drop-down menu that enables you to color code and group data based on factors or annotations. Creating subplots is a similar process to coloring data, as it also depends on factors or annotations.

NOTES

1. Coloring data creates labels on the graph.
2. Creating subplots does not color code data, rather splits the plot into subsections

IMPORTANT

Please note that in bar and box plots, **subplot options** will merely **reorganize** data and **NOT** generate subdivisions within the same plot

6.6.4.3.3 More plotting tools

Expanding the “Plot tools” section will give you access to control plot height, the angle of X and Y labels on the graph, and wrapping figures preferences.

Figure 183. Plot tools section from plotting toolbox expanded.

6.6.4.4 Filtering data by contained values

Using DataVisualization (DataVis), you can filter your data based on values it holds. Available filtering options comprise content-based filtering, time-based filtering, and hiding specific data points.

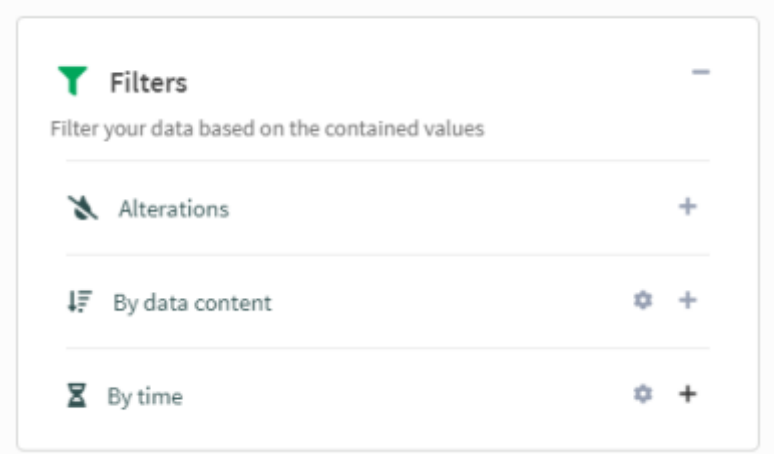


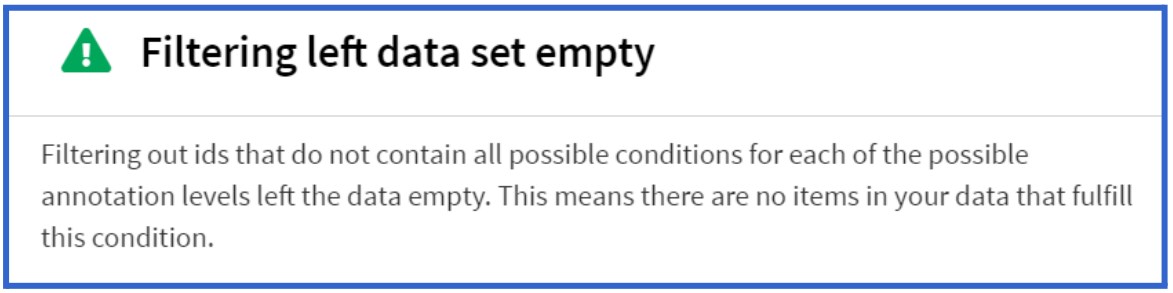
Figure 184. Filters toolbox general view.

6.6.4.4.1 Filter by Alterations

You have the option to remove samples that don't have prior annotations by choosing “Remove NAs from groups”. This action will exclude such samples from groups and colored results. By choosing "remove IDs with no full conditions," you can also remove samples that do not include all available annotation values/options for each annotation item.

NOTE

If your dataset doesn't contain any data with complete conditions, you will receive a notification with this message.



6.6.4.4.2 Filter by variables or factors values

DataVisualization (DataVis) offers the capability to add multiple factors for filtering your data. To add a new factor, you can select "Add new factor," which will present you with two drop-down menus. The "Factor" drop-down menu provides a range of options to choose from, such as annotation levels, date, well description, and more. Once you have selected your desired factor, you can choose one of the options under that factor by selecting "include in plots."

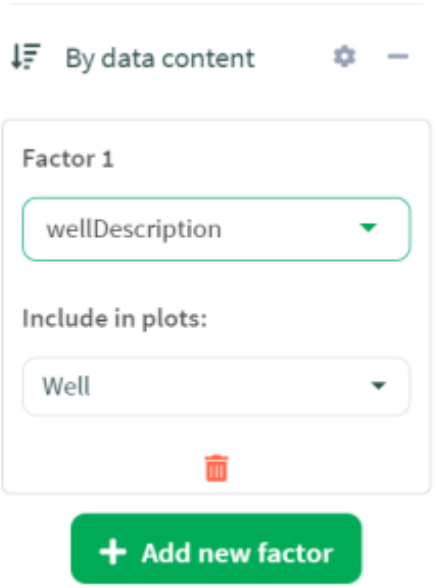


Figure 185. By data" content section from "Filters" toolbox expanded.

6.6.4.4.3 Filter by date and time

If you want to filter your data based on acquisition date, you can use "By time" filter option. This feature enables you to choose a specific time frame of acquisition within a defined date range.

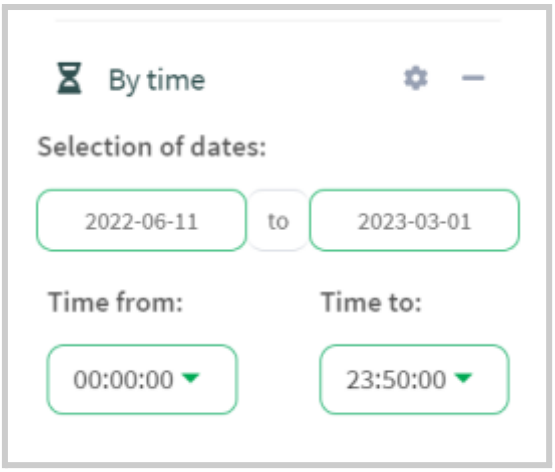


Figure 186. "By time" section from "Filters" toolbox expanded.

6.6.4.5 Actions

Actions toolbox in DataVisualization (DataVis) enables you to export data or modify your preferred settings when launching the application.

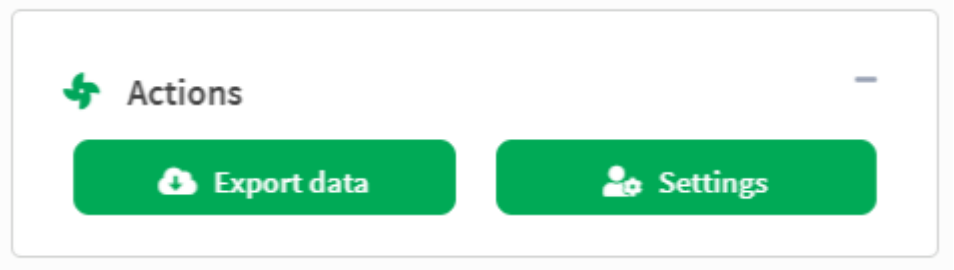


Figure 187. Actions toolbox general view.

6.6.4.5.1 Export calculated averaged data

Exporting data from DataVisualization (DataVis) allows you to choose variables and traces you want to export. You can save your files in Excel, CSV, or TSV format. When you export data, a zip folder will be downloaded with two files.

First file is the Individual Data sheet, exported as full_individual, which contains the original dataset you uploaded. You can apply a filter to your data and select whether to export raw data (columns tagged with "y"). If your data doesn't have raw traces, the option to include them will be hidden. You can filter using already applied factors or choose to ignore them.

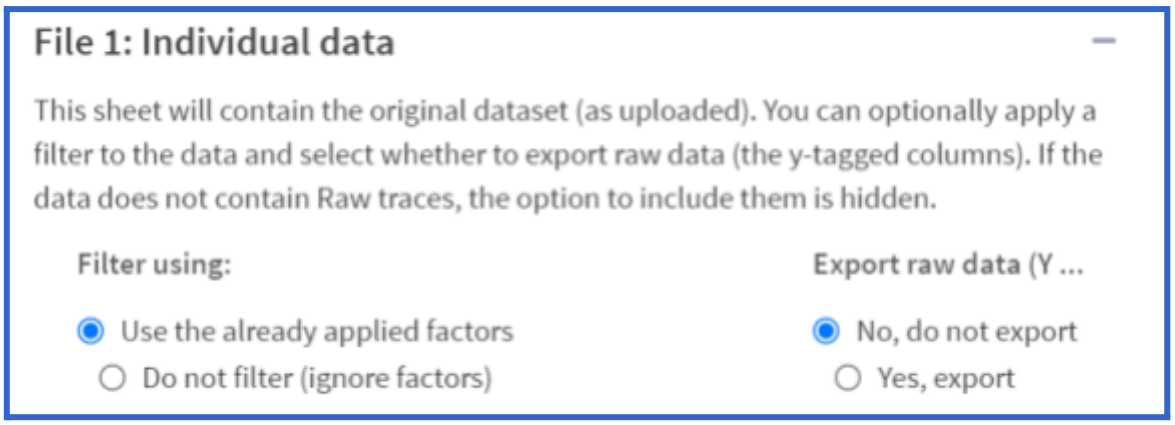


Figure 188. Exporting data from DataVisualization (DataVis) pop-up window (shows after selecting export data from Actions toolbox) - displaying the first type of files to be exported.

Second file is the Mean Data sheet, exported as grouped_means, which includes new calculated columns based on color and wrap options you selected in DataVisualization (DataVis). This table will be similar to the one under "Summarized > Tables." You can select additional calculations to generate, and a new column will be added for each checkbox per variable.

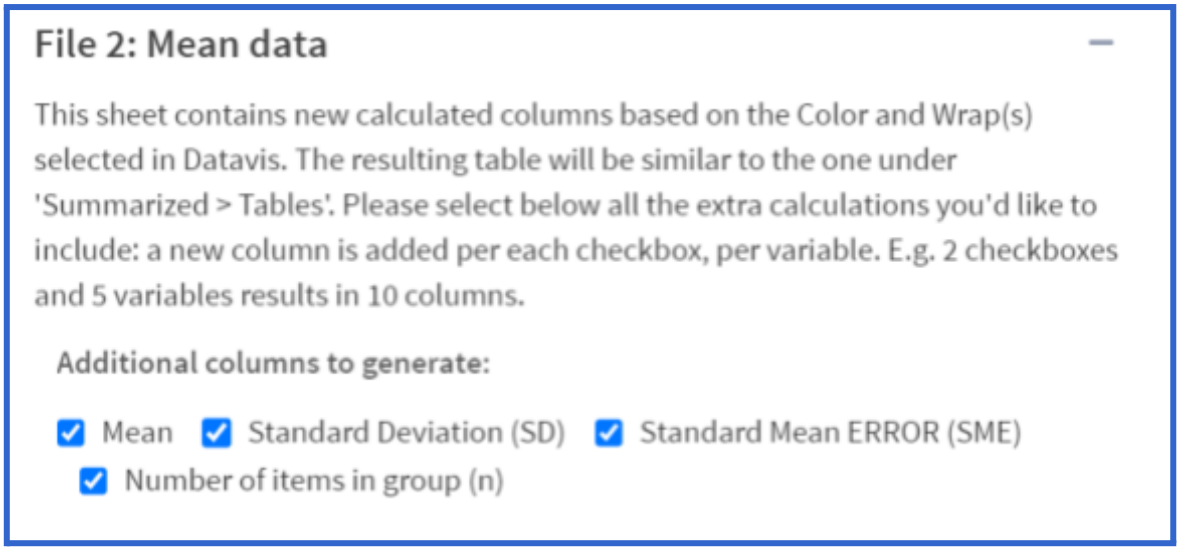


Figure 189. Exporting data from DataVisualization (DataVis) pop-up window (shows after selecting export data from Actions toolbox) - displaying the second type of files to be exported.

For instance, selecting 2 checkboxes and 5 variables will result in 10 columns. The additional columns you can generate include Mean, Standard Deviation (SD), Standard Mean Error (SEM), and Number of Items in Group (n).

6.6.4.5.2 Tool's settings

Settings pop-up window in Data Visualization allows users to make customized adjustments to the tool's interface and behavior. These settings are only applied to Data Visualization features and do not affect the CytoSolver platform.

Visual settings include options to hide: CytoSolver projects dropdown, "Upload from local" tool, and left-side tools. Enabling these options restricts users from loading projects or data outside of CytoSolver and displays plots in full screen.

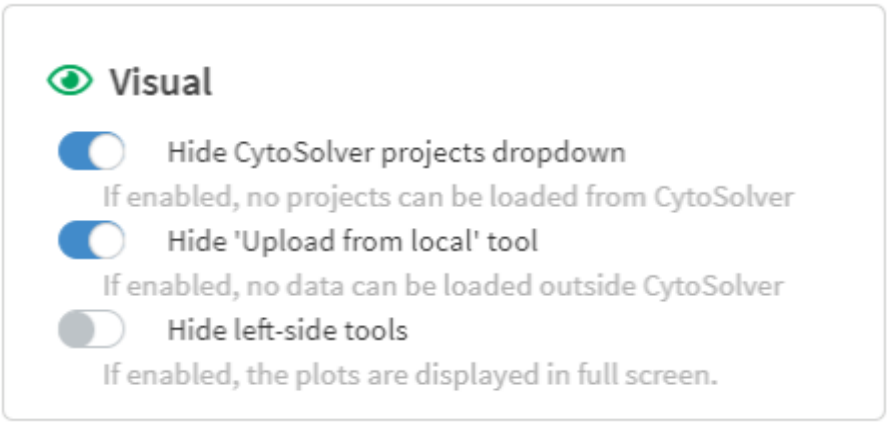


Figure 190. Visual section of "settings" pop-up window selected from actions toolbox.

Behavior settings include the ability to set 'Traces' as default tab when raw data is detected, overriding the 'Preferred start tab' option. The 'Preferred start tab' option allows users to select their preferred start tab.

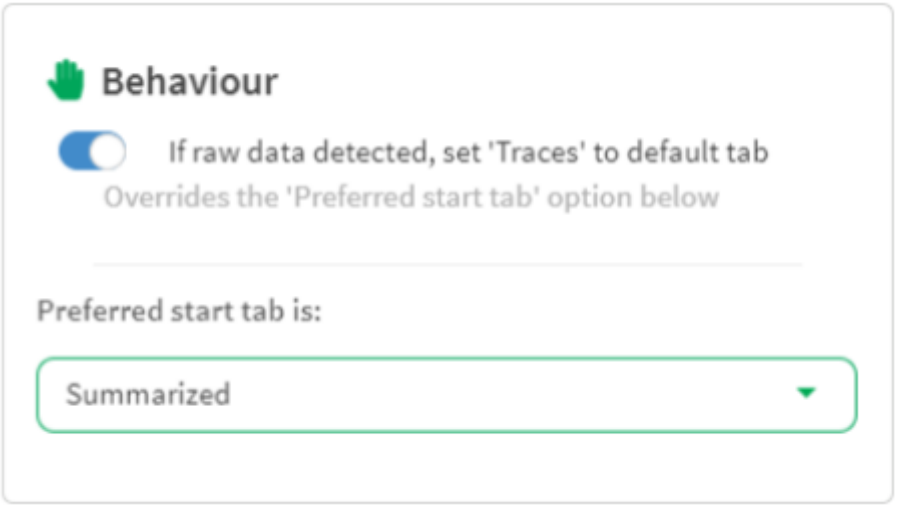


Figure 191. Behavior section of "settings" pop-up window selected from actions toolbox.

Plots settings allow you to change plot height, providing greater customization of the visualization.

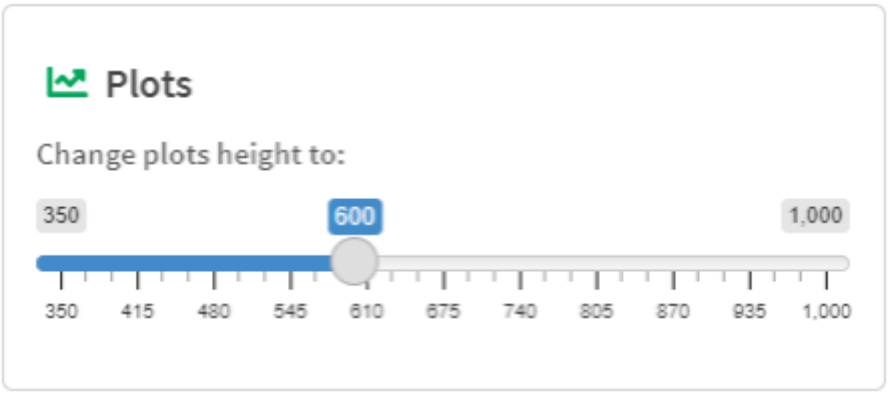


Figure 192. Plots section of "settings" pop-up window selected from actions toolbox.

6.6.4.6 Additional toolboxes

6.6.4.6.1 Bar plots extras toolbox

The additional features of bar plots allows you to visualize data using either standard mean error or standard deviation. Additionally, you can choose to display individual data points and/or show the averages. If you opt to display the averages, you must specify the criterion for averaging the individual dots, which includes the calculated parameters.

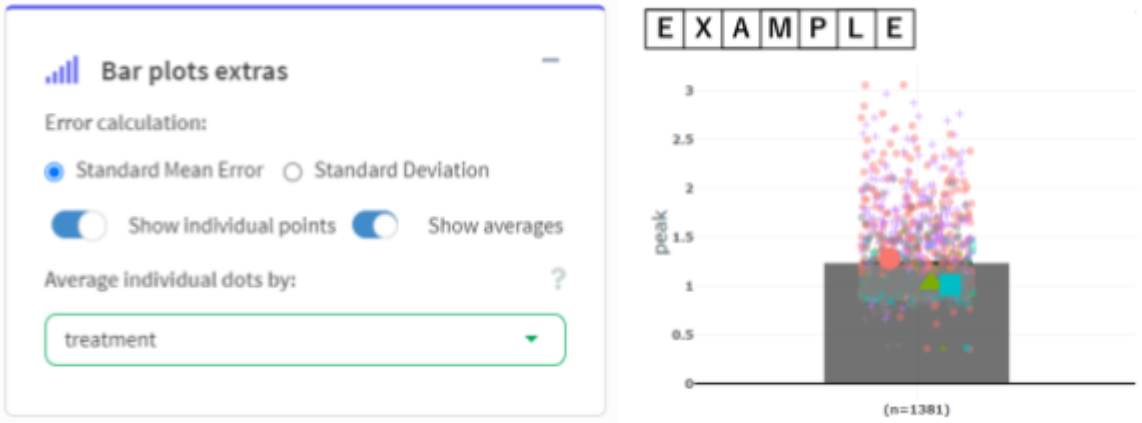


Figure 193. Bar plots extras toolbox - only available with bar plots, the example shows how graphs would look like if both individual data points and averages are selected to be displayed.

It's worth noting that the averages are depicted as a large dot with the same shape and color as the individual data points of that shape.

6.6.4.6.2 Repeated measurements extras toolbox

The additional features provided by the repeated measures extras toolbox enable you to analyze the impact of variable changes on measurements of the same cell over a period of time.

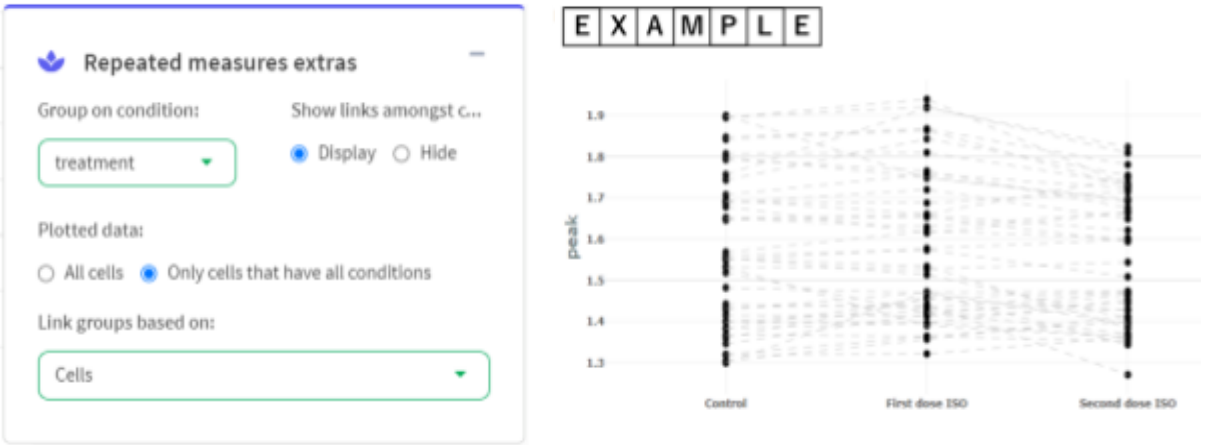


Figure 194. Repeated measures extras toolbox - only available with repeated measures plots, the example shows how graphs would look like if you chose to display links between cells.

By grouping cells, you can investigate changes based on conditions, which can be displayed using connecting lines. Furthermore, this toolbox allows for exclusion of cells that do not have all conditions (all repeated measures).

IMPORTANT

If data is not annotated, repeated measures plots will endlessly loop without showing any graphs.

6.6.4.6.3 Tables extras toolbox

With tables extra toolbox, you have flexibility to personalize your data view on a table. You can accomplish this by using the "variables" feature, which allows you to select parameters you wish to include or exclude from the table. Additionally, the "options" feature provides data viewing in a single page, transposed, or normal view.

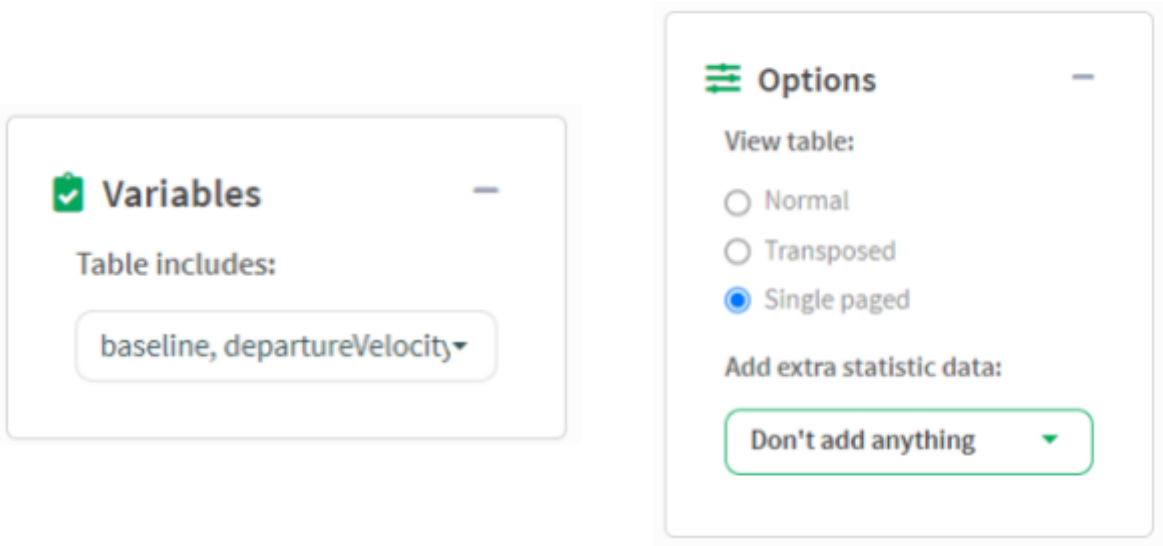


Figure 195. Tables extras toolbox general view - located on left hand side of the table under "summarized" tab.

Moreover, "options" toolbox permits inclusion of extra statistics, such as adding standard deviation (SD), standard mean error (SEM), or both.

6.6.4.6.4 Combination extras toolbox

The combination extras toolbox permits customizing variables for examining linear regression and correlation. You can adjust not only which trace is placed on the X and Y axes but also which variable within selected traces is displayed on each axis.

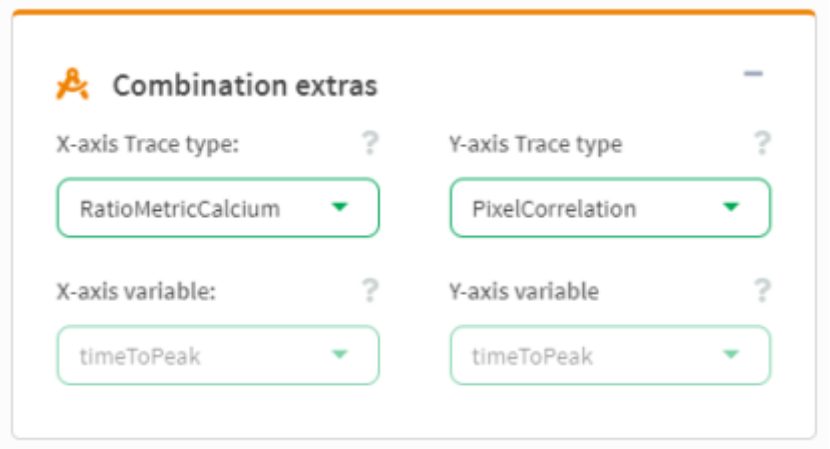


Figure 196. Combination extras toolbox - available for correlation traces plots and contractility traces plots.

7 FAQs

1. **I do not see my file(s) added to a project.**
   If you encountered an error while adding a file, it's possible that there was an issue with the internet connection. Uploading files to CytoSolver Cloud requires a stable connection, and if it's interrupted, some files may not make it to the project. Additionally, if your PC shuts down or experiences any other kind of disruption during the upload process, you may also miss some files.

   The same situation can also occur with CytoSolver Desktop. If you've experienced an interruption during the file upload process and the files haven't been processed, they may not appear in your project.
   If a file cannot be read for any reason, it will not appear in the import window when attempting to add it to the project.

   Assuming your files were successfully added and analyzed, they should appear in your project. However, if you don't see them, please reach out to our support team (info@ionoptix.com) for assistance.

2. **I do not see a segment after analysis, but it does show in IonWizard.**
   Once the analysis is complete, all accepted or rejected segments should be visible in CytoSolver. However, there are some exceptions. If a segment has been marked as "Background," it won't initially appear in CytoSolver Desktop. To display these segments, you'll need to access the "View > Background" option from the top menu and enable the checkbox.

   In CytoSolver Cloud, "Background" segments do not appear at all.

3. **Why is my transient rejected?**
   Transients may be excluded if the trace fails to meet the specified criteria settings. This can occur due to one or more of the following reasons:
   - T0 shift: This refers to a situation where the initiation time of the calcium transient does not align with that of the contractility transient. T0 shift is particularly relevant in unpaced iPSC-CM, especially when the cells have a slow beating rate.
   - Stringent criteria settings that are overly restrictive.
   - Low-quality data, which can be attributed to acquisition or sample preparation issues.

   To obtain suggestions on how to address this issue, please review our application note titled "Analyzing Calcium and Contractility Traces of unpaced iPSC-CM using CytoSolver 3.0."

4. **Where can I find created data/variables after I save it on DataVisualization (DataVis)?**

The location of the data depends on its type.

- If you generated a Y-variable, it will be accessible in the plotting toolbox by selecting the Y-axis drop-down menu.
- If you created a new dataset, such as normalized or delta data, it can be found in the data toolbox under "Create Data" and the "Show data from set" drop-down menu.

5. **Upon generation, does the newly created data (normalized or delta data) automatically populate on the graphs in DataVisualization (DataVis)?**
   The newly generated data will not appear on the graphs automatically. You need to manually choose the data from the "Show data from set" drop-down menu, located in the "Create Data" section of the data toolbox.

6. **Is it possible to reverse the direction of contractility traces?**

Yes, contractility traces direction can be reversed by selecting "**reverse Y-axis**" from the settings button on the top right corner of the graph.

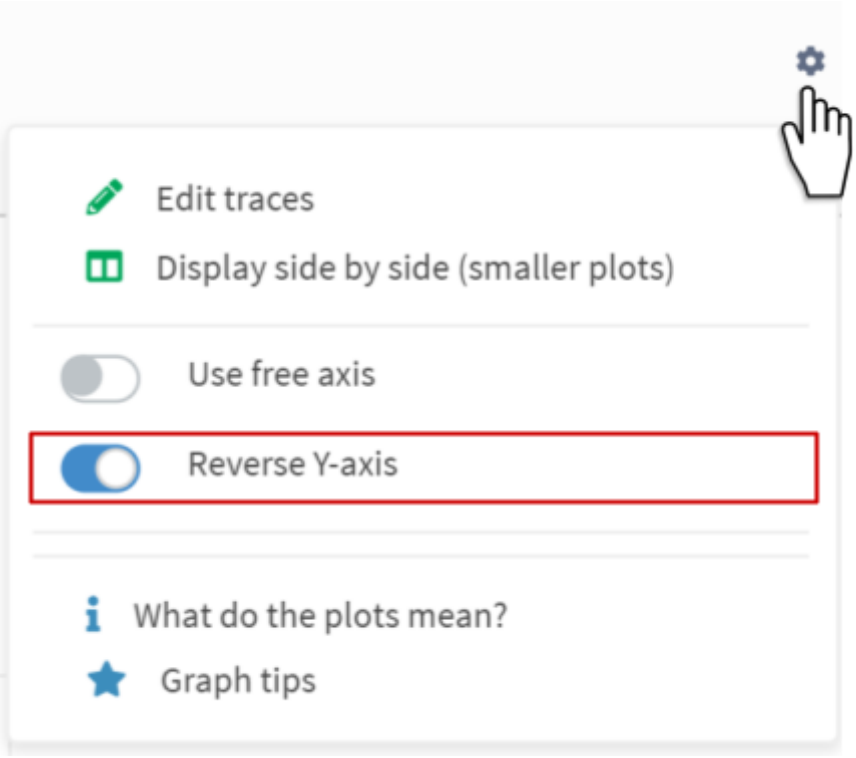
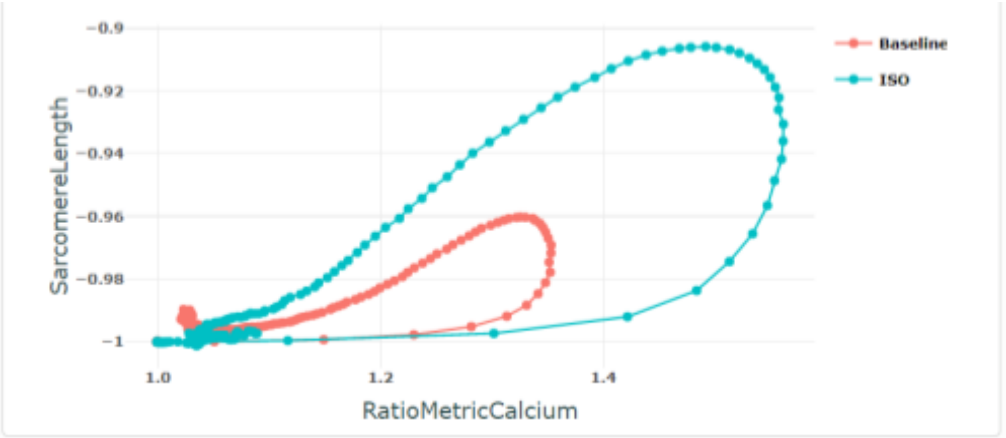


Figure 197. Reversing direction of contractility traces.

NOTES

Please be aware that only the Y axis can be reversed. To create a contractility graph (phase loops) in the most commonly used format, we *recommend* selecting contractility measurements such as sarcomere length or pixel correlation for the Y axis, and Ratiometric calcium for the X axis. Once you have done this, you can reverse the Y axis to obtain the desired graph format, as illustrated in the phase loops shown below.



8 List of figures

9 Index of tables