

# xCELLigence RTCA Software Pro

## Software Guide



# Notices

## Manual Part Number

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## Software Revision

This guide is valid for the 2.6.0 version of the Agilent xCELLigence RTCA Software Pro and compatible programs, until superseded.

## Instrument Manufacturing

Manufactured by Agilent Biosciences (Hangzhou), Inc. Building 4, No. 208 Zhenzhong Road, Xihu District, Hangzhou, Zhejiang Province, 310030

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### WARNING

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# In This Guide

This guide covers the following module: RTCA Software Pro

## 1 Prologue

This chapter provides basic information on RTCA Software Pro guide, including overview of Trademarks, Intended Use, Software License Agreement, and Preamble.

## 2 Getting Started with RTCA Software Pro

This chapter provides information of system requirements for RTCA Software Pro, how to log into the windows operating system, install/uninstall RTCA Software Pro, activate software, and set up user account.

## 3 Software Interface, Menus & Icons

This chapter describes the software main interface, **Cradle Selection** window and functions of windows, menus, and tool bars.

## 4 Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

This chapter describes RTCA Software Pro functions that support GxP compliance and FDA 21 CFR Part 11 compliance.

## 5 Setting Up an Experiment

This chapter describes how to set up an experiment including: launch RTCA Software Pro, enter real-time or offline modes automatically, set plate configuration, record experimental notes, define plate layout (RTCA Basic and Immunotherapy Module), define experiment schedule and save/re-use experimental setups.

## 6 Running an Experiment

This chapter describes running an experiment including checking connections between the contact pins in the cradle and E-Plates and measuring impedance.

## 7 Monitoring an Actively Running Experiment

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### 8 Plotting Data

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### 9 Data Analysis

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### 10 Report, Technical Support Request and Data Export

This chapter introduces **Report, Technical Support Request**, and data export methods.

### 11 Multiple Users Working with an RTCA Instrument

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# 1

## Prologue

This chapter provides basic information on RTCA Software Pro guide, including overview of Trademarks, Intended Use, Software License Agreement, and Preamble.

### Trademarks

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### Intended Use

The xCELLIGENCE RTCA Software Pro is specifically designed for use with the xCELLIGENCE RTCA (Real-Time Cell Analyzer) DP (Dual Purpose), SP (Single Plate), MP (Multiple Plate), TP (Triple Plates), and HT (High Throughput). These xCELLIGENCE RTCA instruments are intended for label-free, real-time, automated monitoring of cell number, cell size/shape, and cell-substrate attachment quality in a variety of cell-based assays, using the proprietary electronic microtiter plates (E-Plates) developed by Agilent Technologies. The xCELLIGENCE RTCA instruments must be used exclusively by laboratory professionals who are trained in laboratory techniques and have studied the instructions for use of the respective instrument.

### NOTE

RTCA Software Pro 2.6.0 only support HT offline analysis with HT Immunotherapy and/or HT Basic license, but not the online mode for data acquisition.

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Read the following terms and conditions of this Software License Agreement (“Agreement”) carefully before installing or using the RTCA Software Pro (hereinafter referred to as “Software”). Proceeding with the installation of the Software or using the Software will constitute acceptance of the terms and conditions of this Agreement. By accepting the terms and conditions of this Agreement, the end user (“Licensee”) assumes all responsibility and liability for the selection of this Software to achieve the intended results, and for its installation and subsequent use. If Licensee is not willing to be bound by the terms and conditions of this Agreement, the RTCA Instrument (including the RTCA Software Pro package) must be promptly returned to Agilent (“Supplier”) with a copy of the receipt against refunding of the purchase price for this Software.

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### **8 Miscellaneous**

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The parties agree that the courts of San Diego, CA in the United States of America shall have exclusive jurisdiction over any dispute arising out of or in connection with this Agreement.

# Preamble

Before setting up the xCELLigence RTCA instruments that of the RTCA Software Pro is designed to interface with, users should read both this RTCA Software Pro Guide and the appropriate instrument Operator's Guide thoroughly. Non-observance of the instructions contained in this guide may lead to safety hazards.

This Software Guide was prepared using the Windows 10 operating system. When using other supported operating systems, the screenshots and/or process step(s) may differ slightly. Consult your operating system manual and/or IT support for help if needed.

## 2 Getting Started with RTCA Software Pro

This chapter provides information of system requirements for RTCA Software Pro, how to log into the windows operating system, install/uninstall RTCA Software Pro, activate software, and set up user account.

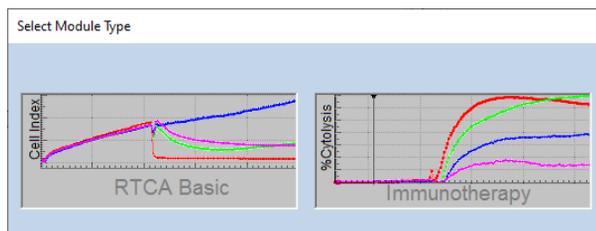
RTCA Software Pro is an integrated software package for complete operation (experiment set up, data acquisition, and data analysis) of the xCELLigence RTCA SP, MP, and DP instruments.

### NOTE

RTCA Software Pro 2.6.0 only support HT offline analysis with HT Immunotherapy and/or HT Basic license, but not the online mode for data acquisition.

The software contains different modules used for running and analyzing different experiments. The RTCA Software Pro has two modules: RTCA **Basic Module** and **Immunotherapy Module**. Immunotherapy module provides data analysis methods and functionalities specific for immunotherapy and immuno-oncology, in addition to all the functions that the Basic module provides. If there is license other than the Basic License, there will be a Module Selection window shown to ask user to select which module to be used.

For example, if the Immunotherapy license is activated for the software, the **Module Selection** window will appear when you access a **Layout** or **Data Analysis** page.



## Getting Started with RTCA Software Pro

### NOTE

If you choose **RTCA Basic**, and your experiment was performed with **RTCA Basic**, you can convert your basic experiment to an immunotherapy experiment at a later date.

Since RTCA Software Pro is compatible with previous versions of RTCA Software, the data files generated by previous versions of RTCA Software can be opened and analyzed by RTCA Software Pro.

### NOTE

If your software is licensed with **RTCA Basic**, you have an option to upgrade your old data file to RTCA Pro Basic format when you open it. The original experiment file will be saved as a .bak file.

### NOTE

If your software is licensed with **RTCA Immunotherapy**, you have an option to upgrade your old data file to either RTCA Pro Basic or Immunotherapy format when you open it. The original experiment file will be saved as a .bak file.

### NOTE

A Basic data file (including old RTCA data file converted to Basic) can also be upgraded to Immunotherapy later by selecting:

- 1 Launch the Software
- 2 Enter **Immunotherapy Module**
- 3 Open the Basic file
- 4 Choose upgrading option

## System Requirements for RTCA Software Pro

When delivered, all xCELLigence RTCA instruments have xCELLigence-specific software pre-installed on their workstation. The software contains all the functionality of previous xCELLigence software and data analysis tools and experimental layout templates facilitating immunotherapy assays if the Immunotherapy license is activated. The RTCA Software Pro 2.6.0 also provides functionalities and features to support GxP/FDA 21 CFR Part 11 Compliance if the **Compliance** license is activated. If RTCA Software Pro is going to be installed to replace the existing RTCA software, or if it is simply being installed as new software on a different computer, the following requirements for the computer to run the software efficiently must be met.

### Hardware (Computer)

- CPU speed: minimum 2 GHz

## Getting Started with RTCA Software Pro

- RAM: minimum 8 GB
- Hard Drive free space: minimum 20GB
- Monitor Resolution: 1366 × 768 or higher

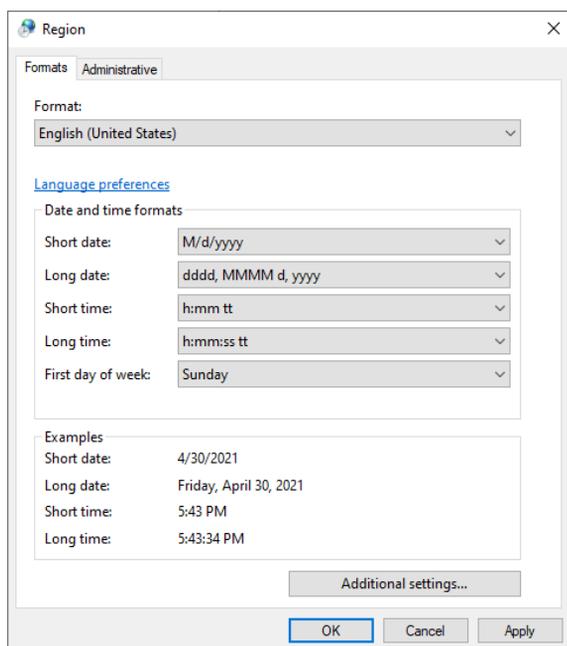
## Software

- Windows 10, Windows 7
- Microsoft Office 2007 or newer (for data export to Excel)

## Windows System Settings

### Control Panel > Clock and Region > Region

Language should be set to **English (United States)**. Date/Time should also be set to U.S. styles as shown below. Alternative settings might lead to problems in data analysis and software instability.



## Settings of the xCELLigence RTCA SP/MP/DP Workstation

Since the xCELLigence RTCA instruments acquires data in real-time, the computer power scheme must be set as follows: **Never** turn off hard disks; **Never** standby;

## Getting Started with RTCA Software Pro

**Never** hibernates. Setting “**Turn off monitor**” to **Never** is optional. For the Windows 10 operating system these settings are accessed through:

**Control Panel > System and Security > Power Options.**

It is highly recommended that an xCELLigence RTCA SP/MP/DP workstation (pre-configured computer from Agilent Technologies, Inc.) is used to run your experiments.

## Logging into Windows Operating System

The xCELLigence RTCA SP/MP/DP workstation is delivered with the following Windows Operating System users preinstalled:

Username	Default Password	Group	Description
Operator	no password (leave field empty)	Administrators	Default user account for instrument operation. The software is able to run properly without the Windows administrator privilege.

## Installing RTCA Software Pro

### Software Download

Make sure the computer with which RTCA Software Pro will be registered is connected to the Internet. Go to <https://www.agilent.com/chem/rtca> in a web browser.

- Fill out the form with the serial number of xCELLigence station then click **Next**.
- Click the download link to download the RTCA Software Pro.

### Software Installation

The xCELLigence workstation provided with each xCELLigence instrument already has the RTCA Software pre-installed for running and analyzing experiments. However, if the workstation is bought without an instrument, the RTCA Software Pro may or may not be installed. If you wish to upgrade the existing RTCA Software on the workstation, or if you wish to install RTCA Software Pro on a different computer, follow the installation protocol listed below.

## Getting Started with RTCA Software Pro

### NOTE

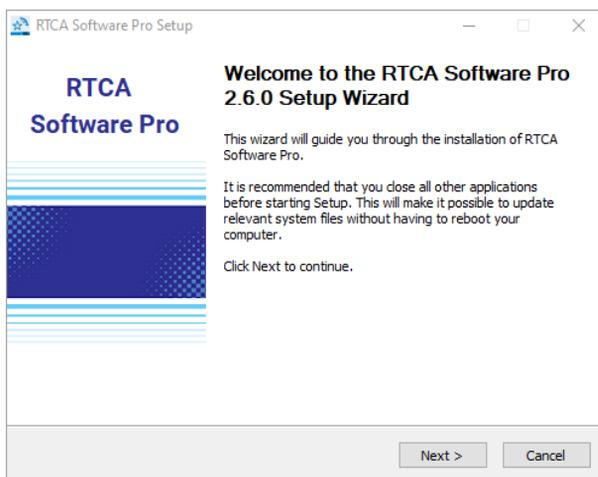
To install the RTCA Software Pro on a computer, the Windows login user must be the “Administrator” of that computer. You may need to ask your IT to grant you “**Local Administrator**” privileges or ask your IT to install the software.

### WARNING

**When RTCA Software Pro is installed, previously installed RTCA Software, including xIMT Software, may still remain on the computer. It is recommended that previous versions of RTCA Software are uninstalled manually.**

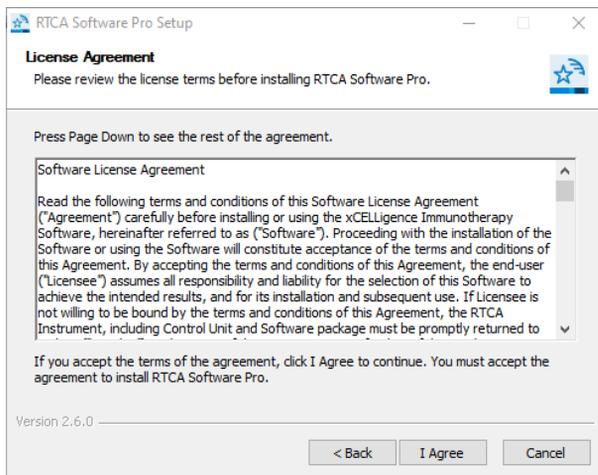
The RTCA Software Pro setup package is a zipped file. After the zip file is downloaded and saved properly, unzip the file, then double-click “**Setup.exe**” file from the unzipped folder.

- 1 The **Setup Wizard for RTCA Software Pro** will now appear. Click the **Next** button.



- 2 After reading the license agreement thoroughly, click the **I Agree** button.

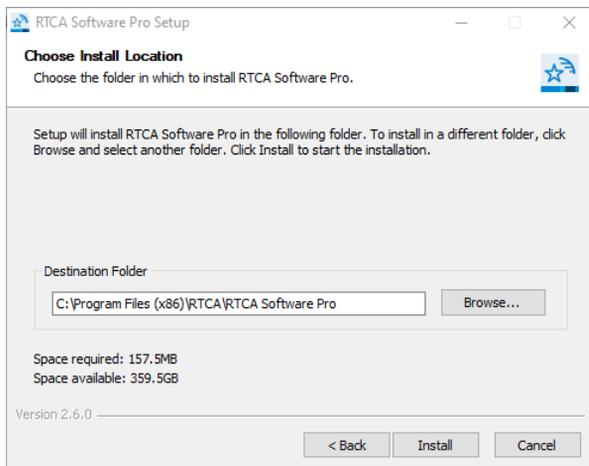
## Getting Started with RTCA Software Pro



- 3 By default, the RTCA Software Pro is saved to the following location:

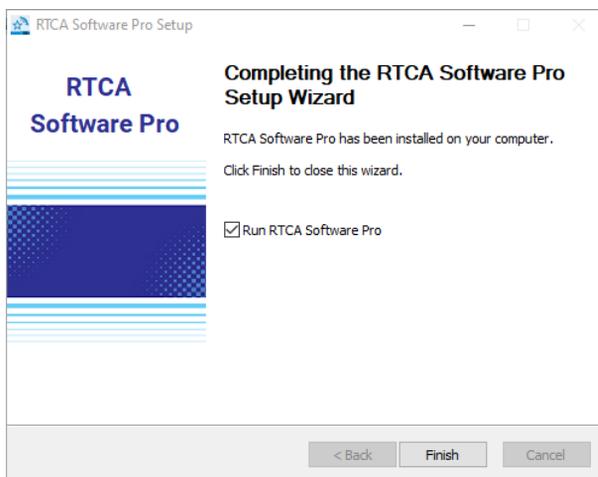
**C:\Program Files (x86)\RTCA\RTCA Software Pro**

If this location is acceptable, click the **Install** button.



- 4 Complete the installation by clicking the **Finish** button in the **Software Setup Wizard**. To skip running the RTCA Software Pro after the installation, uncheck **Run RTCA Software Pro**.

## Getting Started with RTCA Software Pro



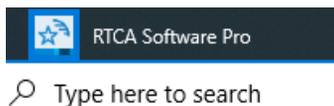
## Launching RTCA Software Pro

After installation, two shortcuts are available for launching the RTCA Software Pro:

- Computer Desktop



- The Programs menu accessed via the Windows Start button



## Software Activation

The software is available for a 30-day free trial. Registration will be needed to use the software after the trial expiration. The RTCA Software Pro License Key comes

## Getting Started with RTCA Software Pro

with the xCELLigence RTCA SP, MP or DP instrument, and can also be purchased separately from Agilent Technologies.

Immunotherapy licenses are specific to the instrument type. The DP Immunotherapy license allows for the use of the immunotherapy module on xCELLigence RTCA DP instruments; the SP immunotherapy license allows for the use of the immunotherapy module on xCELLigence RTCA SP and DP instruments; and the MP immunotherapy license allows for the use of the immunotherapy module on xCELLigence RTCA MP, SP and DP instruments. The activation of the immunotherapy license for any instrument type allows for the use of the basic module on xCELLigence RTCA MP, SP, and DP instruments.

### RTCA Software Pro Registration

The **Software Registration** window will automatically pop up when starting the software if the software is not registered yet. You can also click **Help**, then **Software License, Registration...** to open a dialog:

There are two ways to register the software.

- If the computer is connected to the Internet:  
Enter a valid Agilent Technologies issued license key and your company/institution email address, then click the **Register** button to register the software.

RTCA Registration

Software trial evaluation period will be valid for 2 day(s).

Please input your License Key from Agilent to register software license on this computer, and provide a valid email address from your institution/company according to the license agreement. Software updates will be sent to the email provided.

License Key: 12345-67890-12345-67890

Email Address: user@company.com

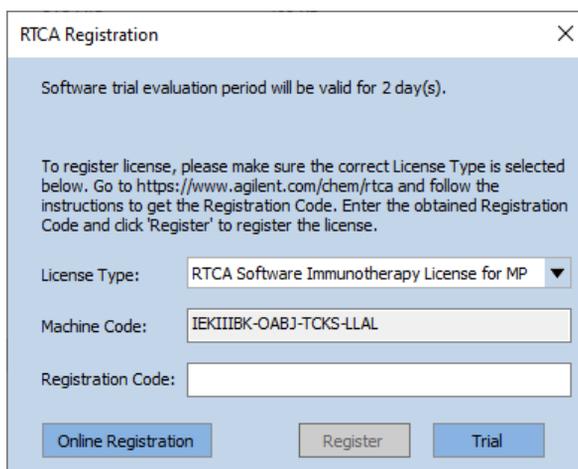
Offline Registration Register Trial

- If the computer is not connected to the Internet:  
Click the **Offline Registration** button to switch to offline registration mode. Select the **License Type** that you have purchased from the drop-down menu and copy the **Machine Code** displayed in the window.

## Getting Started with RTCA Software Pro

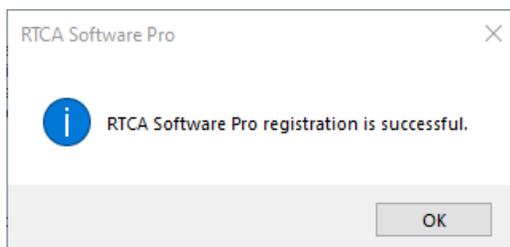
On another computer that is connected to the Internet, go to <https://www.agilent.com/chem/rtca> in a web browser:

- Fill out the form with the serial number of xCELLigence station then click **Next**.
- In “**Download and Registration**” page, click “**2. Get Registration Code**”.
- Enter valid **Machine Code** and **License Key**.
- Click **GET REGISTRATION CODE**.
- Copy the **Registration Code**, enter it to the **Software Registration** window, and click **Register** button to register the software.



The screenshot shows a dialog box titled "RTCA Registration" with a close button (X) in the top right corner. The text inside the dialog reads: "Software trial evaluation period will be valid for 2 day(s). To register license, please make sure the correct License Type is selected below. Go to <https://www.agilent.com/chem/rtca> and follow the instructions to get the Registration Code. Enter the obtained Registration Code and click 'Register' to register the license." Below the text are three input fields: "License Type:" with a dropdown menu showing "RTCA Software Immunotherapy License for MP", "Machine Code:" with the text "IEKIIIBK-OABJ-TCKS-LLAL", and "Registration Code:" which is empty. At the bottom of the dialog are three buttons: "Online Registration" (blue), "Register" (gray), and "Trial" (blue).

- A message box will pop up, indicating the result of the registration.



### NOTE

A valid email address of your company/institution must be provided according to the license agreement. Software update information will be sent to the email provided here.

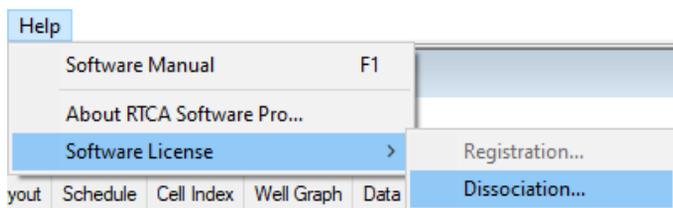
## Getting Started with RTCA Software Pro

### NOTE

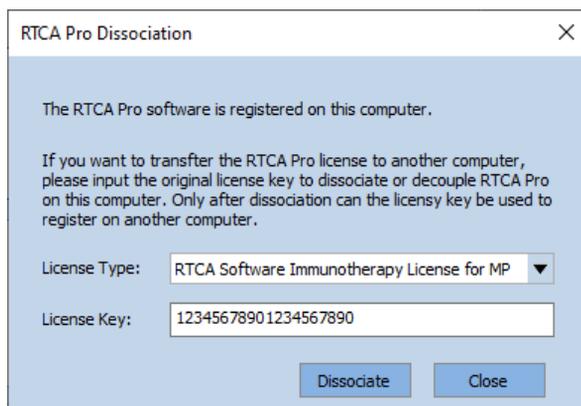
A license key is required for software registration or license transfer. Keep a safe record of the license key assigned to each computer or user. You can continue to register other licenses after registering one license successfully.

## RTCA Software Pro Dissociation

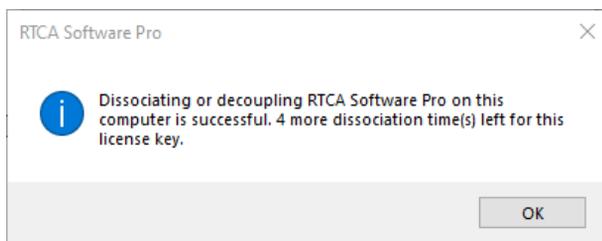
The software supports license transfer to another computer. Each license may be transferred up to 5 times. If you want to transfer the Software license to another computer, click **Help > Software License > Dissociation...** to open a dialog:



Select the **License Type** that you want to dissociate from the drop-down menu, and the **License Key** will be automatically filled in the text box. You can also enter the license key manually. Click the **Dissociate** button to dissociate or decouple the software from this computer. After dissociation, the license key can be used to register the software on another computer.



## Getting Started with RTCA Software Pro

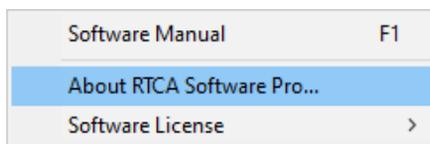


### NOTE

Please connect the workstation to the internet when dissociating the license. Contact Agilent technical support for how to dissociate the license if connection to internet is not available.

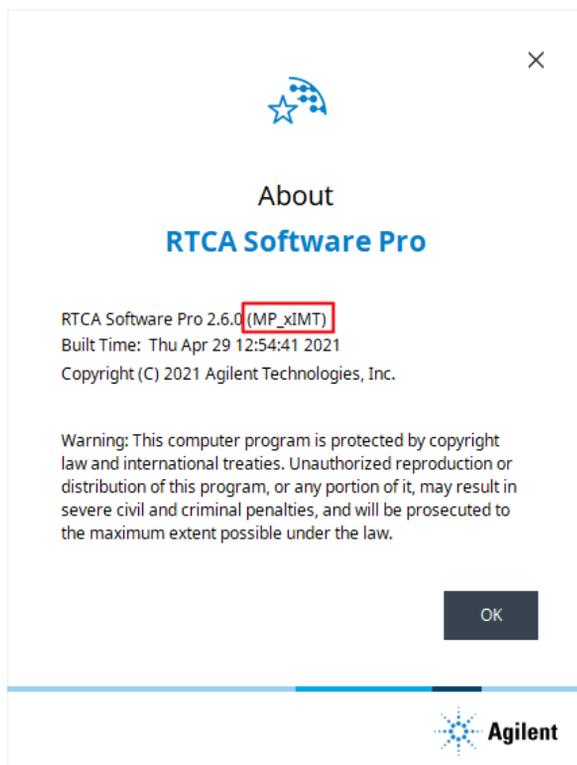
## Checking the Software Version

Click **Help** in the menu bar of the RTCA Software Pro, and select **About RTCA Software Pro**.



A pop-up window will display the software version and license that is installed and registered in the computer.

## Getting Started with RTCA Software Pro



If there are multiple software licenses registered, only the highest license is displayed. For example, if there are **Basic**, **DP\_xIMT**, and **MP\_xIMT** licenses are registered in the computer, only the **MP\_xIMT** license is shown (see above figure).

## Uninstalling RTCA Software Pro

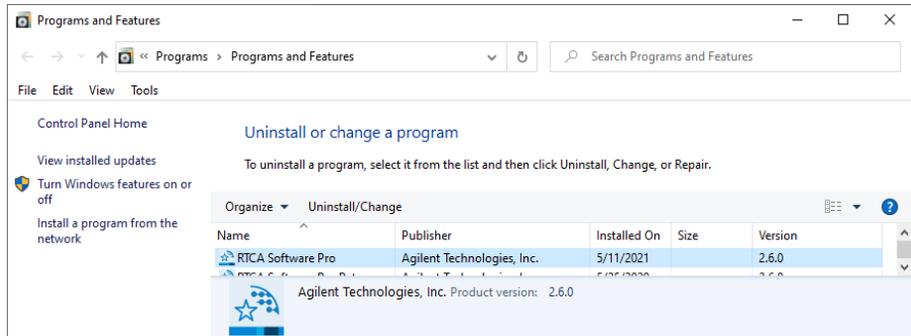
There are two ways to uninstall the RTCA Software Pro from your computer:

- Click **Start** on the Windows toolbar, and then follow the steps shown below:

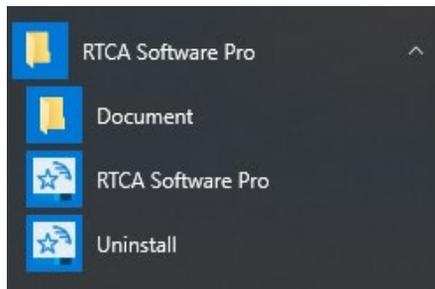
**Start > Control Panel > Programs > Programs and Features**

Within **Programs and Features**, click on **RTCA Software Pro** to highlight it, and then click **Uninstall**.

## Getting Started with RTCA Software Pro



- Alternatively, use the Uninstall feature within the RTCA Software Pro folder located within: **Start > All Programs**



## User Management

By default, there are initially two built-in user accounts within the RTCA Software Pro. The usernames, and the default passwords are:

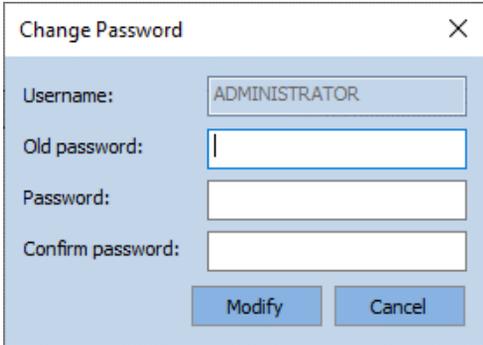
Username	Password	Description
ADMINISTRATOR	administrator	The default user account with all permissions
ServiceEngineer	[Nonpublic]	This account is used by service engineer only

## Change Password

- Login to RTCA Software Pro with a valid username and password.
- Click **Change Password** within the **System** menu.

## Getting Started with RTCA Software Pro

- Enter current password in **Old Password** box.
- Enter new password in **Password** box.
- Re-enter new password in **Confirm Password** box.
- Click **Modify** button.



The screenshot shows a 'Change Password' dialog box with the following fields and buttons:

- Username:** ADMINISTRATOR
- Old password:** (empty)
- Password:** (empty)
- Confirm password:** (empty)
- Modify** button
- Cancel** button

### NOTE

Please note that the password length and complexity can be configured in the **Security Settings** window.

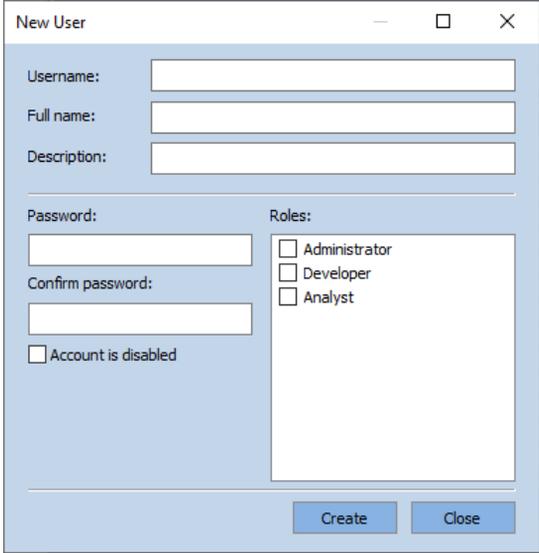
### NOTE

The user with “**Change Access Control Setting**” permission has the ability to overwrite/reset other users’ passwords. Please refer to [Users Page](#) for how to reset users’ passwords.

## Add a New User

- Login as user with “**Change Access Control Setting**” permission.
- Click **Security Settings** within the **System** menu.
- Click **Access Control**, then **Users**.
- Click **New**.
- In the pop-up window, enter a new user’s name in **Username** box and select a role. Password is not required.

## Getting Started with RTCA Software Pro



The screenshot shows a 'New User' dialog box with the following fields and options:

- Username: [Text Input]
- Full name: [Text Input]
- Description: [Text Input]
- Password: [Text Input]
- Confirm password: [Text Input]
- Account is disabled
- Roles:
  - Administrator
  - Developer
  - Analyst
- Buttons: Create, Close

- Click **Create**.

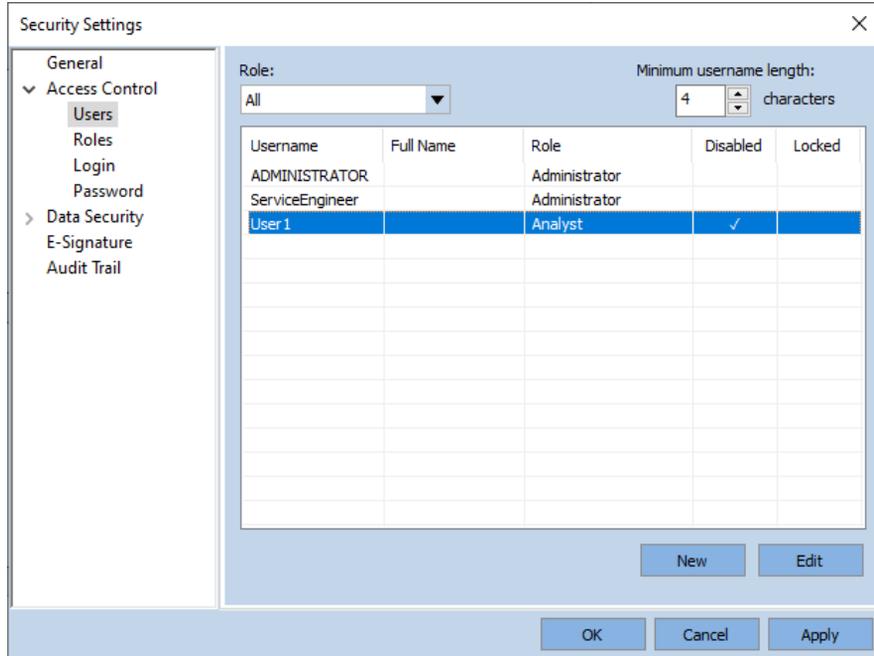
### NOTE

One user account can have different roles with the combined permissions from all the roles he/she has. Please refer to [Roles Page](#) for permissions of each role.

### Disable a User

- Login as user with “**Change Access Control Setting**” permission.
- Click **Security Settings** within the **System** menu.
- Click **Access Control**, then **Users**. Select a user and click **Edit**.
- Check the **Account is disabled** box and click **OK**.
- Administrator can check if a username is disabled in **Security Settings**.

## Getting Started with RTCA Software Pro



### NOTE

Only user with "Change Access Control Setting" permission can disable users.

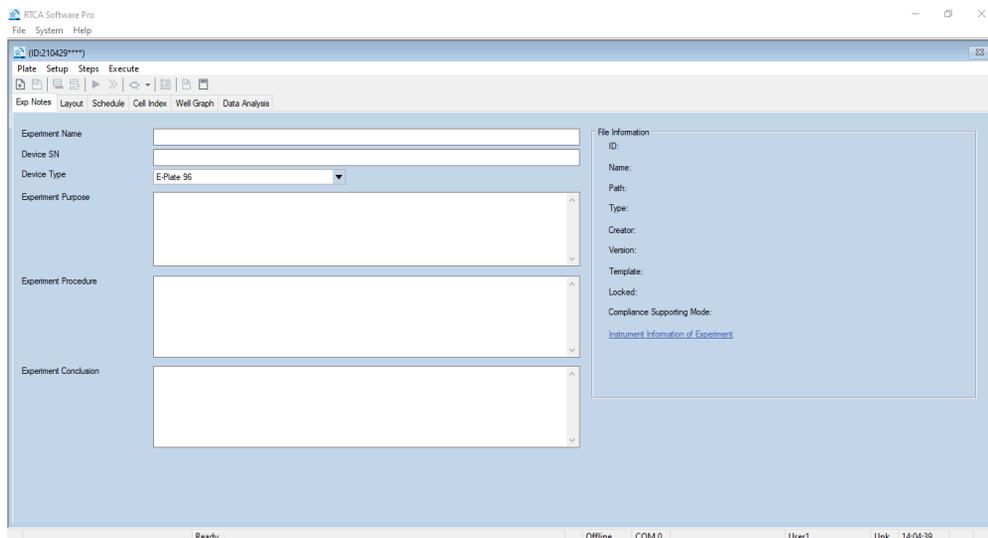
## 3 Software Interface, Menus & Icons

This chapter describes the software main interface, **Cradle Selection** window and functions of windows, menus, and tool bars.

The main interface of the RTCA Software Pro is shown below. This chapter describes key features of this page and the menus that are available, in addition to explaining the function of key icons.

### Interface Layout

The main user interface of the RTCA Software Pro contains, from top to bottom, the following: Main Window Menu, Plate Window Menu, Tool Bar, Page Tabs, and Status Bar.

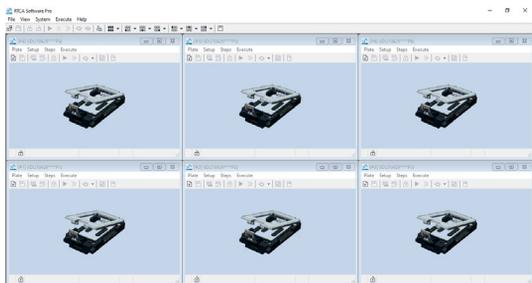
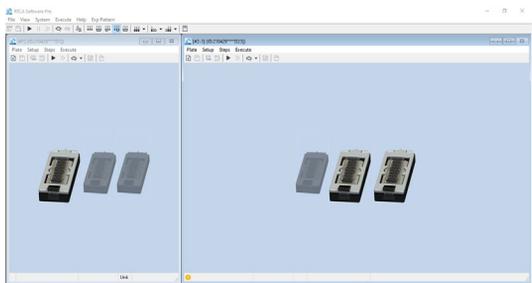


Main user interface for the xCELLigence RTCA SP instrument.

## Cradle Selection Window

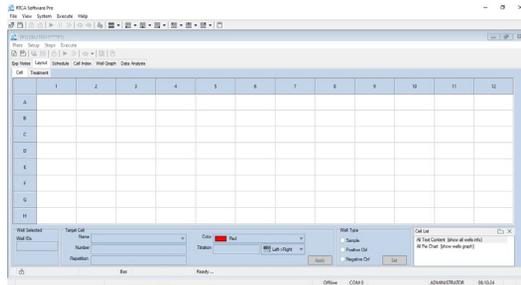
While the xCELLigence RTCA SP and HT contain a single plate cradle, the xCELLigence RTCA DP and MP contain three and six plate cradles, respectively. The **Cradle Selection** window (images of which are shown in the next section) enables the user to define which cradle of the xCELLigence RTCA DP or MP they wish to work with while setting up an experiment, analyzing data, etc. The **Cradle Selection** window is only available when i) in online mode while an xCELLigence RTCA DP or MP are connected to the computer, ii) selected by the user while working offline, or iii) when opening a .plt data file obtained from an xCELLigence RTCA DP or MP.

## Function of Windows, Menus, and Tool Bars

Item	Example	Major Function
Cradle Selection Window (for RTCA MP instrument)		Handles all the functionalities relevant to an E-Plate 96/E-Plate View 96/E-Plate WOUND 96/E-Plate 96 PET in each of the six cradles.
Cradle Selection Window (for RTCA DP instrument)		Handles all the functionalities relevant to an E-Plate 16/E-Plate 16 PET/E-Plate View 16 in each of the three cradles. The layout changes based on pattern selection.

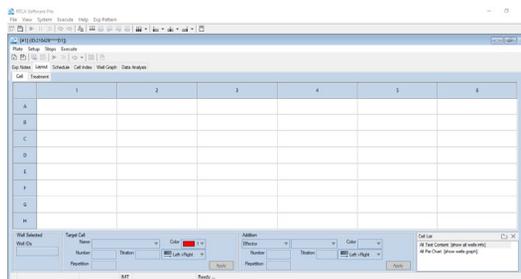
## Software Interface, Menus & Icons

Plate Window (SP/MP)



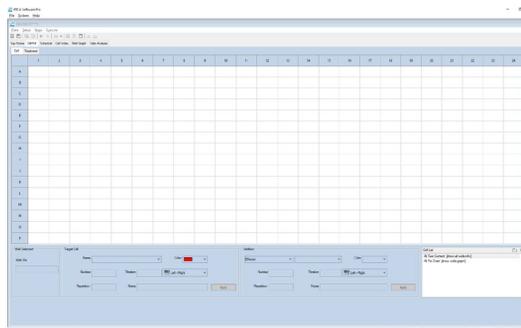
Handles all the functionalities relevant to a single E-Plate 96/E-Plate View 96/E-Plate WOUND 96/E-Plate 96 PET.

Plate Window (DP)



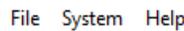
Handles all the functionalities relevant to each of the three E-Plate 16/E-Plate 16 PET/E-Plate View 16 simultaneously. The layout changes based on cradle combination pattern selected.

Plate Window (HT)



Handles all the functionalities relevant to a single E-Plate 384.

Main Window Menu



User actions to operate RTCA Software.

Main Window Tool Bar



Shortcuts for frequently used menu functions.

Plate Window Menu



User actions to handle all functionalities for a single E-Plate.

Plate Window Tool Bar



Shortcuts for frequently used menu functionalities for a single E-Plate.

Page Tabs



Work area for a single E-Plate to define the layout schedule and review and analyze acquire data.

Status Bar

Offline COM 0 User1 14:23:41

Displays the working status of the instrument.

### NOTE

Depending on the model of the instrument connected or offline mode, some menu items and main window tool bar may not be accessible.

## Functions within the Main Window Menu

The main window menu contains following items: **File**, **View**, **System**, **Execute**, **Help**, and **Exp Pattern**.

File View System Execute Help Exp Pattern

The functions available within each of these menu items are as follows:

Menu Item	Function
<b>File</b>	
Open All Last	Open last experiments on all cradles
New from Template...	Load a template file
Release All	Clear Information on all pages of all cradles
Reserve All	Reserve all cradles for later use
Save All	Save experiments on all cradles
Clone Exp Notes...	Copy <b>Exp Notes</b> settings from a PLT file on all cradles
Clone Layout	Copy <b>Layout</b> settings from a PLT file on all cradles
Clone Schedule	Copy <b>Schedule</b> settings from a PLT file on all cradles
Technical Support Request...	Generate technical support file
Lock Out...	Lock out to protect the experiment. The experiment file remains open and can run in the background.
Log Out...	Log out the software and return to log in window. The experiment files opened by the user will be closed.
Exit	Close RTCA Software Pro
<b>View</b>	
All Plates	View all plates with specified page
Plate 1	Maximize <b>Plate 1</b> window

## Software Interface, Menus & Icons

<b>Plate 2</b>	Maximize <b>Plate 2</b> window
<b>Plate 3</b>	Maximize <b>Plate 3</b> window
<b>Plate 4</b>	Maximize <b>Plate 4</b> window
<b>Plate 5</b>	Maximize <b>Plate 5</b> window
<b>Plate 6</b>	Maximize <b>Plate 6</b> window
<b>System</b>	
<b>User Settings...</b>	Open <b>User Settings</b> window
<b>Change Password...</b>	Open <b>Change Password</b> window to change password for the current user
<b>System Settings...</b>	Open <b>System Settings</b> window
<b>Security Settings...</b>	Open <b>Security Settings</b> window
<b>System Audit Trail...</b>	Open <b>System Audit Trail</b> window
<b>QC Test</b>	Run QC test
<b>QC Report</b>	Open <b>QC Test Report</b>
<b>Instrument Information</b>	Show information of current connected instrument
<b>Execute</b>	
<b>Start All</b>	Start experiments on all plates
<b>Pause All</b>	Pause experiments on all plates
<b>Abort All</b>	Abort experiments on all plates
<b>Scan All</b>	Scan all plates
<b>Stop Scan All</b>	Stop scan all plates
<b>Lock All</b>	Lock all cradles
<b>Unlock All</b>	Unlock all cradles
<b>Help</b>	
<b>Software Manual</b>	Open this Software Manual in PDF format
<b>About RTCA Software Pro...</b>	Information about RTCA Software
<b>Software License</b>	Register / Dissociate Software License(s)
<b>Exp Pattern</b>	DP only
<b>  D1   D2   D3  </b>	Use the three DP cradles separately as three experiments
<b>  D1-D2-D3  </b>	Use the three DP cradles together as a single experiment
<b>  D1   D2-D3  </b>	Use the D2 and D3 cradles together and D1 separately as two experiments

## Software Interface, Menus & Icons

D1-D2   D3	Use the D1 and D2 cradles together and D3 separately as two experiments
D2   D1-D3	Use the D1 and D3 cradles together and D2 separately as two experiments

### NOTE

Depending on the model of the instrument connected or offline mode, some menu items may not be accessible.

## Functions within the Plate Window Menu

The **Plate Window** menu contains four items: **Plate**, **Setup**, **Steps**, and **Execute**.



The functions within each of these menu items are described in the below table:

Menu Item	Function
<b>Plate</b>	
<b>Open</b>	Open an experiment
<b>New from Template</b>	Load a template file to create a new experiment
<b>Release</b>	Clear all the information on all the pages
<b>Reserve</b>	Reserve the plate for later use
<b>Save</b>	Save current experiment
<b>Save as Template...</b>	Save current experiment setup as a Template
<b>Sign...</b>	Sign current experiment electronically. Compliance License is required.
<b>Signature Log...</b>	Show the history of electronic signature for the current experiment file. Compliance License is required.
<b>File Audit Trail...</b>	Show file audit trail
<b>Technical Support Request...</b>	Generate a technical support file
<b>Export Experiment Info...</b>	Export experiment details to an Excel or text file
<b>Report</b>	Export experiment report to a PDF file
<b>Print Current Page</b>	Print the currently displayed page

## Software Interface, Menus & Icons

<b>Print All Pages</b>	Print all pages
<b>Setup</b>	
<b>Allow Everyone to Read</b>	Allow all users to read current experiment
<b>Allow Everyone to Write</b>	Allow all users to edit current experiment
<b>Lock File...</b>	Lock current experiment file. Compliance License is required.
<b>Frequency</b>	Set up measurement frequency. (Single Frequency or All 3-Frequencies; default is all 3-Frequencies: 10, 25, and 50 kHz)
<b>Clone Exp Notes...</b>	Copy <b>Exp Notes</b> settings from a PLT file
<b>Clone Layout...</b>	Copy <b>Layout</b> settings from a PLT file
<b>Clone Schedule...</b>	Copy <b>Schedule</b> settings from a PLT file
<b>Curve Adjustment...</b>	For adjusting <b>Cell Index</b> curves
<b>Analysis Methods...</b>	Manage <b>Analysis Methods</b>
<b>Steps</b>	
<b>Add a Step</b>	Add a new step to experiment
<b>Delete a Step</b>	Delete a selected step
<b>Execute</b>	
<b>Start/Continue</b>	Start a step, or resume step that is currently paused
<b>Pause</b>	Pause current step
<b>Abort Step</b>	Skip remaining sweep(s) of currently running step
<b>Scan Plate</b>	Perform an impedance measurement on all the active wells in the plate
<b>Stop Scan Plate</b>	Stop plate scanning, ignore the scanned data
<b>Lock Plate</b>	Lock E-Plate (MP only)
<b>Unlock Plate</b>	Unlock E-Plate (MP only)

## Icon Functions

Operations associated with icons are as follows:

Icon	Description
	Open an experiment .plt file

## Software Interface, Menus & Icons

	Apply changes made in the <b>Layout</b> or <b>Schedule</b> page
	Add a step
	Delete a step
	Lock Plate (MP only)
	Start a step
	Pause a step
	Abort current running step
	Scan Plate
	Stop Plate Scan
	Analysis Methods
	File Audit Trail

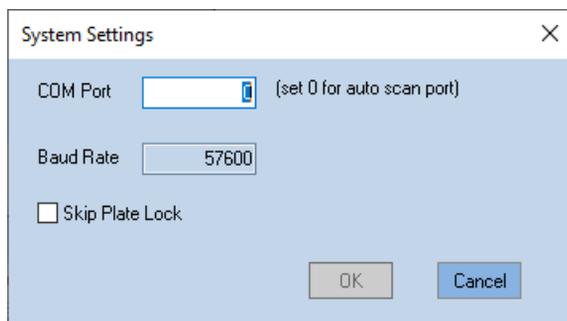
### NOTE

Often there are several ways to access functions in this Software (“right-clicking”, “menus” or “icons”). Although this guide describes one method, users should familiarize themselves with the other available methods and use the one they prefer.

## System Settings

Go the **System > System Settings** to open the **System Settings** window.

## Software Interface, Menus & Icons



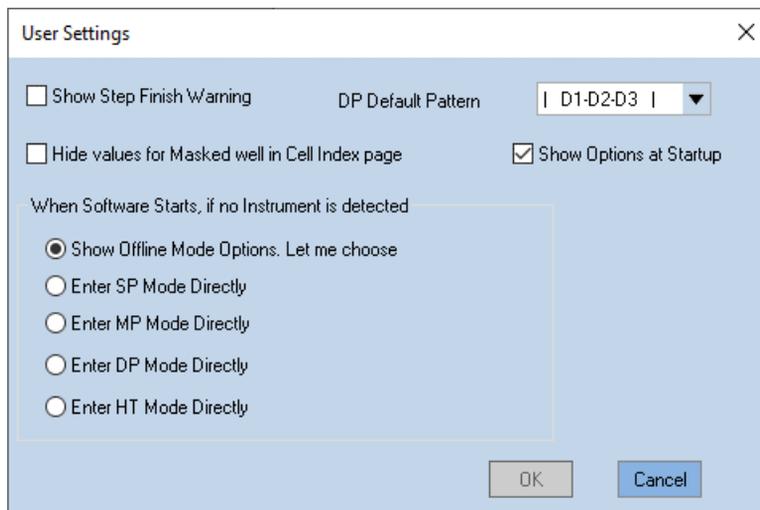
**Com Port:** Set the COM port that the software use to communicate with the instrument. If 0 is set, the software will scan all the available COM ports to search the connected instruments.

**Baud Rate:** The Baud Rate of the COM port. This field is read-only.

**Skip Plate Lock (MP only):** If this is checked, the software will not lock the MP cradle automatically when running the experiment.

## User Settings

Go the **System > User Settings** to open the **User Settings** window.



## Software Interface, Menus & Icons

**Show Step Finish Warning:** If checked, the software will show a flash text on **Schedule** page one minute before a step is going to be finished.

**Hide value for Masked well in Cell Index page:** If checked, the Masked wells will not show Cell Index values on the **Cell Index** page.

**DP Default Pattern** (DP only): Set the default pattern for DP experiments.

**Show Options at Startup** (DP only): If checked, the software will show the pattern setup window every time the software starts with DP instrument connected.

**When Software Starts, if no Instrument is detected:** Choose the default instrument type if no instrument is detected when the software starts.

## 4

# Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

This chapter describes RTCA Software Pro functions that support GxP compliance and FDA 21 CFR Part 11 compliance.

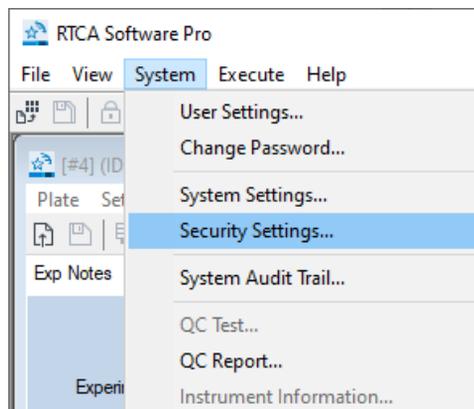
RTCA Software Pro 2.6.0 provides a series of functions that support GxP compliance and FDA 21 CFR Part 11 compliance. To access these functions, RTCA Software Pro needs to be registered with RTCA Software Compliance license. For registration steps, please refer to [RTCA Software Pro Registration](#) section.

## Security Settings

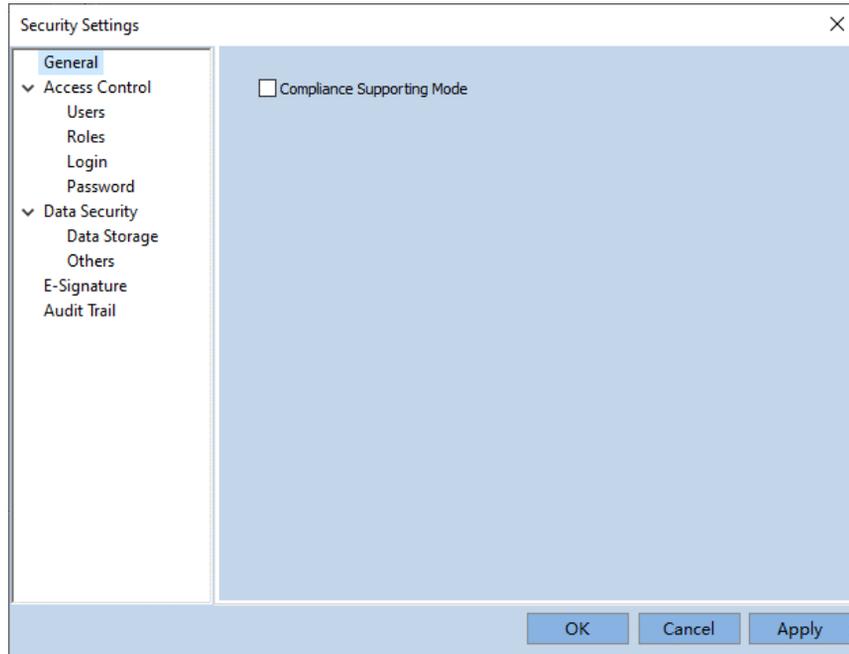
### NOTE

Most of the settings in **Security Settings** window require RTCA Software Compliance License.

Click **System > Security Settings...** to open **Security Settings** window.



### General

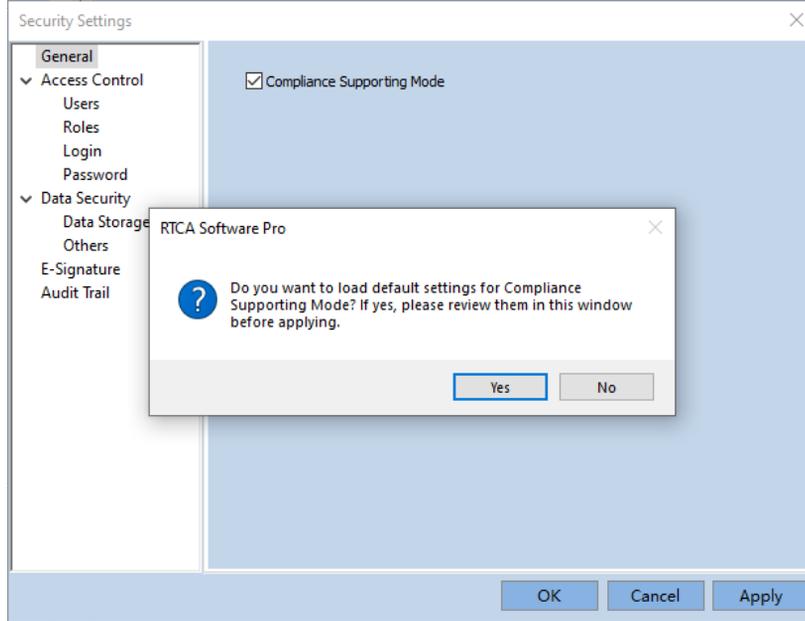


**Compliance Supporting Mode:** Check this box and click **Apply** button to quickly load the default settings for **Compliance Supporting Mode**. You can review the settings on this window before applying. RTCA Software Compliance License is required to turn this feature on.

#### NOTE

Please close any plt file opened before changing this option, otherwise, the checkbox may be disabled.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance



When **Compliance Supporting Mode** is turned on for the first time, it is required to create a Windows Account to allow the software to write and read data in the secure data folder. Input password that comply to the Windows system password policy (if applicable ) and then click **Create** to continue.



### Access Control

#### Users Page

This page is used for administrator to manage the user accounts.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

The Security Settings dialog box features a left-hand navigation pane with the following items: General, Access Control (expanded), Users (selected), Roles, Login, Password, Data Security (expanded), E-Signature, and Audit Trail. The main area is titled 'Security Settings' and includes a 'Role:' dropdown menu set to 'All' and a 'Minimum username length:' spinner box set to '4' characters. Below these is a table with columns for Username, Full Name, Role, Disabled, and Locked. The table contains three rows: ADMINISTRATOR (Role: Administrator), ServiceEngineer (Role: Administrator), and User 1 (Role: Analyst, Disabled: checked). At the bottom right of the main area are 'New' and 'Edit' buttons. At the very bottom of the dialog are 'OK', 'Cancel', and 'Apply' buttons.

Username	Full Name	Role	Disabled	Locked
ADMINISTRATOR		Administrator		
ServiceEngineer		Administrator		
User 1		Analyst	✓	

- **Role:** select a role to filter users list below. Select **All** to list all users.
- **Minimum username length:** set the minimum username length requirement when creating new user account.
- **New:** click to open **New User** window.

The New User dialog box contains the following fields and options: Username: jsmith; Full name: John Smith; Description: (empty); Password: (masked with 7 dots); Confirm password: (masked with 7 dots); Account is disabled: ; Roles:  Administrator,  Developer,  Analyst. At the bottom are 'Create' and 'Close' buttons.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

- **Username:** unique identification of a user and serves as the User ID.
- **Full name:** a non-unique name of a user. The **Full Name** together with **Username** will show in the report.
- **Description:** serves as a memo for a user account.
- **Roles:** assign a functional role to the new user account.
- **Edit:** click to open **Edit User** window.

The screenshot shows the 'Edit User' dialog box with the following details:

- Username:** jsmith
- Full name:** John Smith
- Description:** (empty)
- Account is disabled:**
- Account is locked:**
- Roles:**
  - Administrator
  - Developer
  - Analyst
- Buttons:** Reset Password, OK, Cancel

- **Username:** Username cannot be changed once created.
- **Full Name:** can be edited.
- **Description:** can be edited.
- **Roles:** edit the role for the user.
- **Account is disabled:** select the checkbox in front of **Account is disabled** to disable the selected user. Once a user account is disabled, the login or e-signature with that account will become unavailable. User account can be re-enabled by administrator after it is disabled. Therefore, at least one user account which has the **“Change Access Control Settings”** permission will be forced to be in active status.
- **Account is locked out:** unselect this checkbox to unlock a user account. A user account may be locked out due to reach specified number of failed login attempts.



## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

The screenshot shows a 'New Role' dialog box with the following elements:

- Name:** A text input field.
- Description:** A text input field.
- Role is disabled
- Permissions:** A section with two tabs: 'Permissions' (selected) and 'Members'. Below the tabs is a tree view of permissions:
  - Permissions
    - >  Instrument Control
    - >  Edit Exp Notes
    - >  Edit Layout
    - >  Edit Schedule
    - >  Cell Index
    - >  Well Graph
    - >  Data Analysis
    - >  Data Read
    - >  Data Write
    - >  Data Import
    - >  Data Export
    - Change System Setting
    - >  Change Security Setting
    - Register/Dissociate Software License

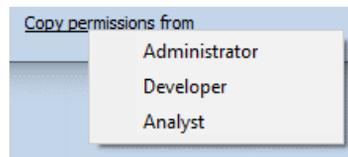
At the bottom of the dialog, there is a link 'Copy permissions from', and two buttons: 'Create' and 'Close'.

- **Name:** the name of the role.
- **Description:** a detailed description of the role. This field is optional.
- **Permissions:** check the box in front of each item to enable a specific permission for this role. Refer to [Permission List](#) for the complete list of all available permissions under each category.
- **Members:** check the box to assign users as members of this role.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

Username	Full Name	Role	Disabled	Locked
<input type="checkbox"/> ADMINISTRATOR		Administrator		
<input type="checkbox"/> jsmith	John Smith	Developer		
<input type="checkbox"/> ServiceEngineer		Administrator		
<input type="checkbox"/> User1		Analyst	✓	

- **Copy permissions from:** click to quickly clone permissions from another role.



- **Edit:** click to open **Edit Role** window.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

Edit Role

Name: Developer

Description:

Role is disabled

Permissions Members

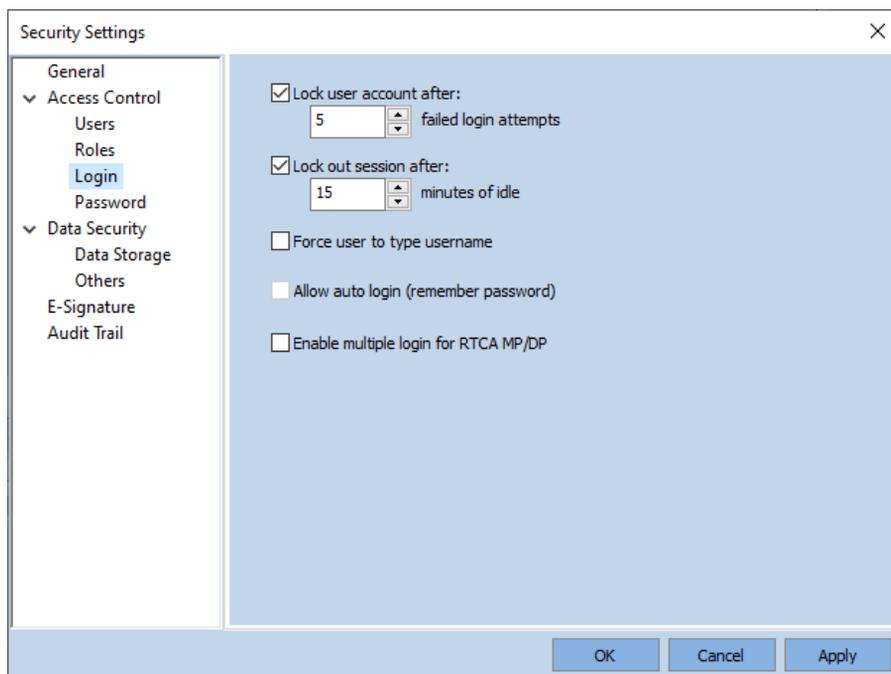
Permissions

- >  Instrument Control
- >  Edit Exp Notes
- >  Edit Layout
- >  Edit Schedule
- >  Cell Index
- >  Well Graph
- >  Data Analysis
- >  Data Read
  - >  Data Read
  - >  Data Write
- >  Data Import
- >  Data Export
  - >  Data Export
- >  Change System Setting
- >  Change Security Setting
- >  Register/Dissociate Software License

[Copy permissions from](#) OK Cancel

- **Role is disabled:** check to disable a role. One role can only be disabled when there are no members associated with it.

### Login Page



- **Lock user account after:** when checked, user account will be locked with specified number of failed login attempts. The software will inform user how many failed attempts left when a login fails.
- **Lock out session after:** when checked, after specified minutes of idle (without any interaction within the software), the software will be locked, and login window will show.
- **Force user to type username:** when not checked, user is able to select username in the login and sign window from the drop-down list. When checked, user needs to type in username manually using the keyboard.
- **Allow auto login:** this option is not available if **Compliance Supporting Mode** is on.
- **Enable multiple login for RTCA MP/DP:** this option allows multiple users to use their own credentials to log into their own experiments (cradles).

### Password Page

The screenshot shows a 'Security Settings' dialog box with a tree view on the left and configuration options on the right. The 'Password' option is selected in the tree view. The configuration options on the right are:

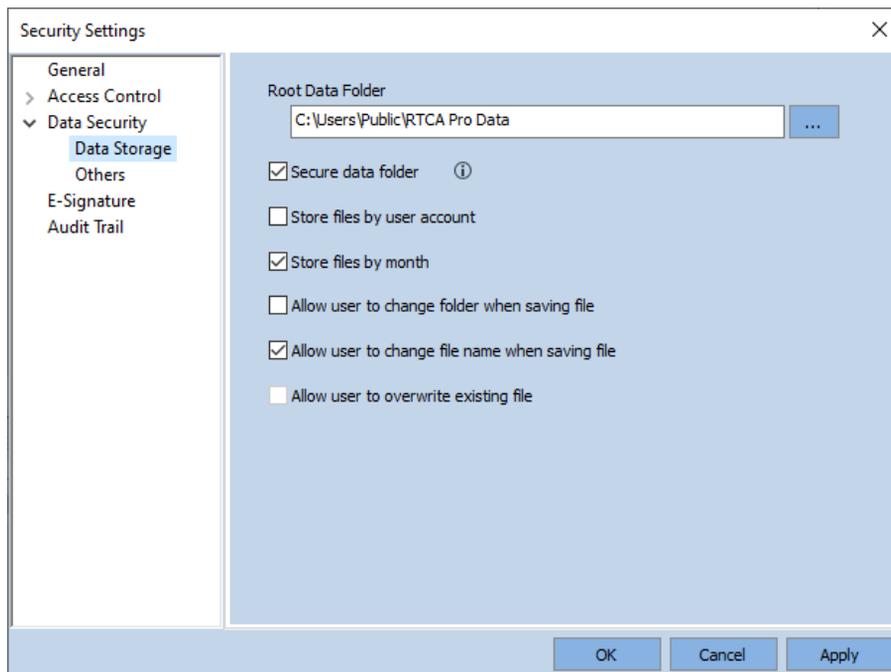
- Minimum password length: 8 characters
- Enforce complicated password ⓘ
- Password expires after 90 days
- Advise user to change password 15 days before password expires
- Forbid to reuse password: remember last 6 passwords
- User must change password at first logon and after password reset

Buttons at the bottom: OK, Cancel, Apply.

- **Minimum password length:** define the minimum length of password that a user needs to set.
- **Enforce complicated password:** when checked, a password must contain characters from three of the following categories: uppercase letters, lowercase letters, digits, and special characters.
- **Password expires after:** when checked, it requires user to change the password when login after specified days.
- **Forbid to reuse password:** when checked, system will remember the last specified number of user passwords and the user is not allowed to reuse them.
- **User must change password at first logon and after password reset:** requires user to change the password under these situations.

### Data Security

#### Data Storage Page



- **Root Data Folder:** set the default root data folder when saving experiment files. This can be changed only when **Secure data folder** is not checked.
- **Secure data folder:** If checked, Windows normal user is not able to delete, move or rename files in the root data folder or its subfolders through Windows Explorer. When this setting is turned on and saved, the software ask user to create a Windows account for the software to write and read data to and from that folder. If system administrator changes the password of this Windows account, RTCA Software Pro will prompt to input the updated password.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance



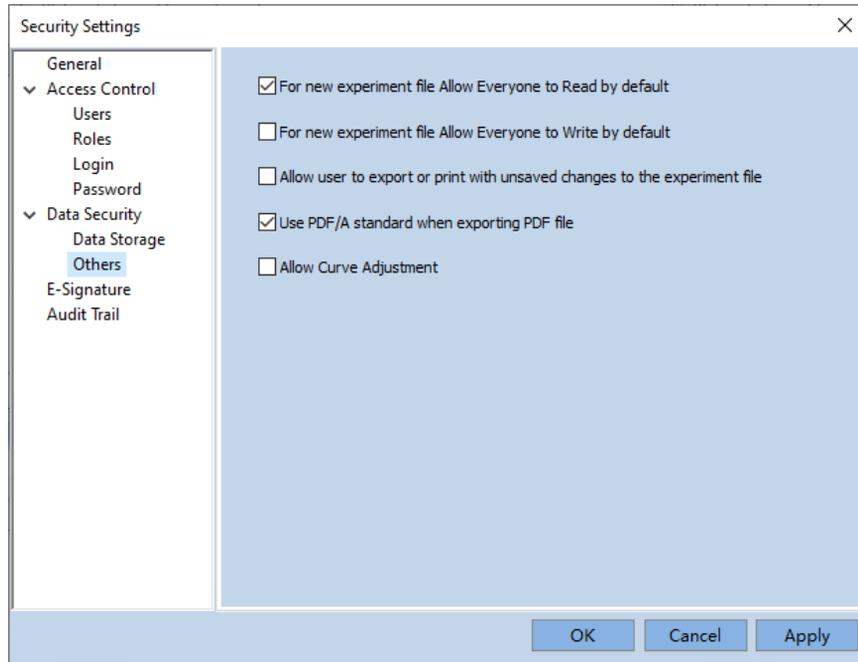
- **Store files by user account:** If checked, the software create folder under root data folder by Username (i.e. unique User ID) and save the experiment files created by the user to his/her corresponding folder.
- **Store files by month:** If checked, the software creates folders under root data folder by month according to when the experiment files are created and save experiment files under that folder by default.

### NOTE

If both **Store files by user account** and **Store files by month** are checked, the User Account will be the parent folder with sub-folders organized in month according to who created the experiment files and when the experiment files are created.

- **Allow user to change folder when saving file:** If not checked, new experiment files are saved to the default folder defined either by **Store files by user account** or **Store files by month**. If checked, user can choose the folder when saving new experiment files. But if secure data folder is used, only root data folder or its subfolders can be used.
- **Allow user to change file name when saving file:** if not checked, software created default file name is used when saving new experiment files. The default experiment file name is consisted of current date and time with experiment name with the format of YYMMDDhhmmPx<sub>B</sub>\_ExpName.plt where x is plate number and B stands for basic mode.
- **Allow user to overwrite existing file:** this option is not available if **Compliance Supporting Mode** is on.

### Others Page

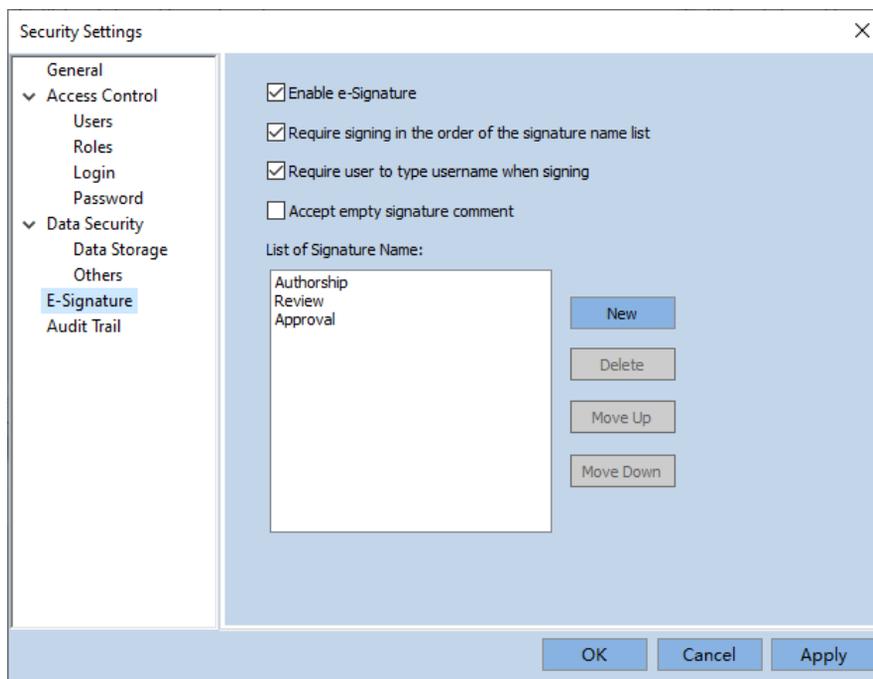


- **For new experiment file Allow Everyone to Read by default:** if checked, the new created file will have **Allow Everyone to Read** be checked by default.
- **For new experiment file Allow Everyone to Write by default:** if checked, the new created file will have **Allow Everyone to Write** be checked by default.
- **Allow user to export or print with unsaved changes to the experiment file:** if not checked, when user try to export or print when there are unsaved changes, an error message will prompt. User has to save the changes to be able to export and print in this case.
- **Use PDF/A standard when exporting PDF file:** if checked, PDF/A standard will be used when exporting PDF files.
- **Allow Curve Adjustment:** if not checked, the **Curve Adjustment** function will be disabled.

### e-Signature

Used for administrator to manage e-Signature settings.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance



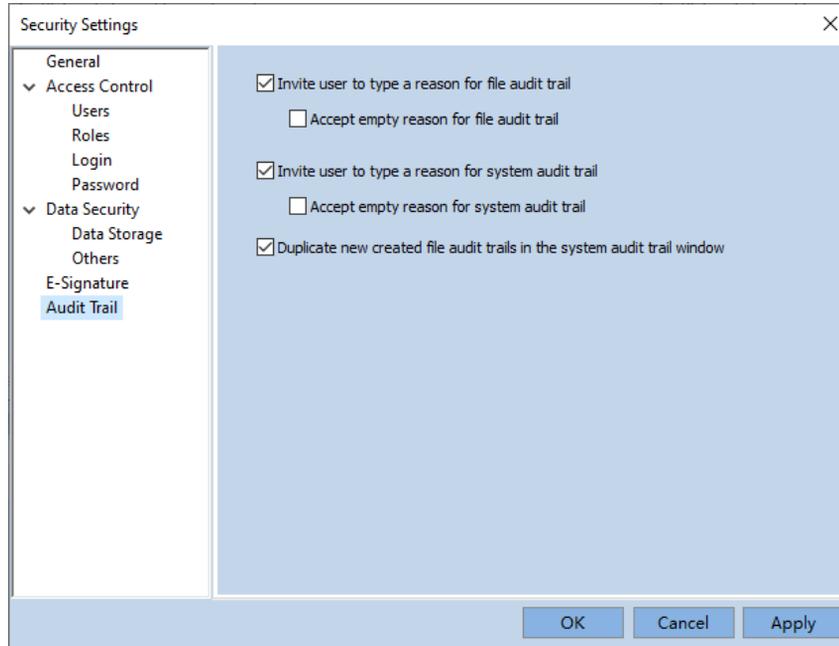
- **Enable e-Signature:** if not checked, the sign and signature log menu item will not be available. RTCA Software Compliance License is required to turn this feature on.
- **Require signing in the order of signature name list:** if checked, the software require users to sign a file in the order of Signature Name list defined at the lower part of this page.
- **Require user to type username when signing:** If checked, when signing a file, user need to type the username, along with the password.
- **Accept empty signature comment:** if checked, comment can be empty when signing.
- **List of Signature Name:** an editable list for different signature name (i.e. **Authorship, Review, Approval**, etc.) applicable when signing a file. The order of different **Signature Name** also can be edited in order to create a signing workflow.
  - **New:** click to create a new signature name.
  - **Delete:** click to delete the selected signature name from the list. The signature that has already been applied to an experiment file is not affected by any change of deletion here. It will only affect when user performs new signing.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

- **Move up:** move the selected signature name up in the list.
- **Move down:** move the selected signature name down in the list.

### Audit Trail

Used for administrator to manage audit trail settings.



- **Invite user to type a reason for file audit trail:** when checked, any time when a file audit trail is recorded, the following window will pop up to ask user to input the reason for the changes. User can review the changes or actions in the table.



## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

- **Accept empty reason for system audit trail:** when checked, reason for system audit trail is optional, user can leave it empty and click **OK** to continue in the above **Input System Audit Trail Reason** window.
- **Duplicate new created file audit trails in the system audit trail window:** if checked, all the file audit trails will be also shown in the system audit trial. Changing this setting will only affect the new created audit trails.

### NOTE

Audit Trail cannot be turned off. The software always records file and system audit trails. File audit trails are embedded into the data file (\*.plt) and system audit trails are saved in the **SystemLog.sys** file under software installation folder.

## Signing File Electronically

### Sign File Window

Click **Plate > Sign** menu item in a **Plate Window** to open the **Sign the File** window.

Sign the File

The current file will be signed. The signature is equivalent to a handwritten signature. Please enter your password and signature-related comments.

Username: Input Current Username

Password: [Empty]

Signature Name: Authorship

Comments: [Empty]

Lock file at the same time

Sign Cancel

Enter the **Username** and **Password** for the signature. Only the current login user account should be input.



## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

records software configuration changes, instrument operation activities, software run logs and so on.

### Audit Trail Category list

The following table lists all the available audit trail categories.

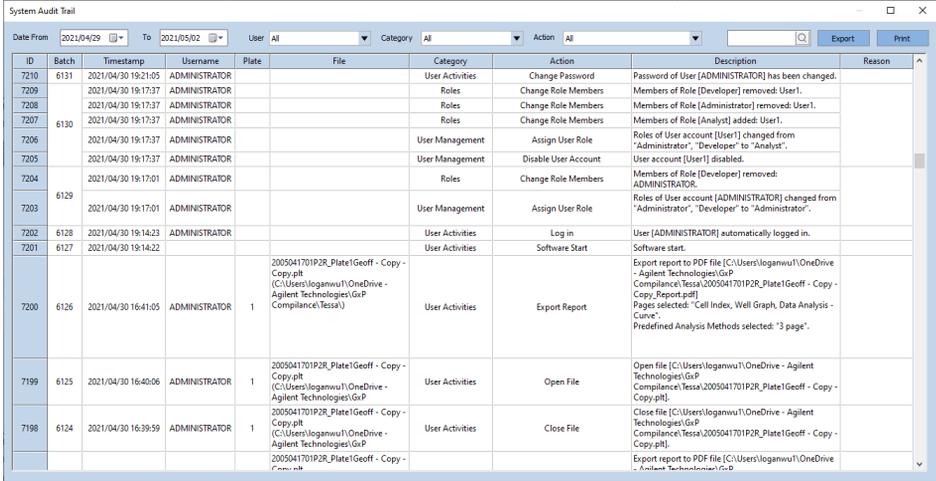
#### NOTE

If “**Duplicate new created file audit trails in the system audit trail window**” is turned on in **Security Settings**, all file audit trails will also be logged into **System Audit Trail**.

ID	Category Name	System Audit Trail	File Audit Trail
1	System Settings	Yes	
2	Security Settings	Yes	
3	User Management	Yes	
4	Roles	Yes	
5	User Activities	Yes	
6	User Settings	Yes	
7	Experiment Operation	Yes	Yes
8	Information Message	Yes	Yes
9	Warning Message	Yes	Yes
10	Error Message	Yes	Yes
11	File Settings		Yes
12	Exp Notes		Yes
13	Layout		Yes
14	Schedule		Yes
15	Cell Index		Yes
16	Well Graph		Yes
17	Data Analysis		Yes
18	Analysis Method		Yes

### System Audit Trail

Click **System > System Audit Trail** or **System Audit Trail** toolbar button  to open the **System Audit Trail** window. User needs to have the “**View System Audit Trail**” permission to open this window.



ID	Batch	Timestamp	Username	Plate	File	Category	Action	Description	Reason
7210	6131	2021/04/30 19:21:05	ADMINISTRATOR			User Activities	Change Password	Password of User [ADMINISTRATOR] has been changed.	
7209		2021/04/30 19:17:37	ADMINISTRATOR			Roles	Change Role Members	Members of Role [Developer] removed: User1.	
7208		2021/04/30 19:17:37	ADMINISTRATOR			Roles	Change Role Members	Members of Role [Administrator] removed: User1.	
7207	6130	2021/04/30 19:17:37	ADMINISTRATOR			Roles	Change Role Members	Members of Role [Analyst] added: User1.	
7206		2021/04/30 19:17:37	ADMINISTRATOR			User Management	Assign User Role	Roles of User account [User1] changed from "Administrator", "Developer" to "Analyst".	
7205		2021/04/30 19:17:37	ADMINISTRATOR			User Management	Disable User Account	User account [User1] disabled.	
7204		2021/04/30 19:17:01	ADMINISTRATOR			Roles	Change Role Members	Members of Role [Developer] removed: ADMINISTRATOR.	
7203	6129	2021/04/30 19:17:01	ADMINISTRATOR			User Management	Assign User Role	Roles of User account [ADMINISTRATOR] changed from "Administrator", "Developer" to "Administrator".	
7202	6128	2021/04/30 19:14:23	ADMINISTRATOR			User Activities	Log in	User [ADMINISTRATOR] automatically logged in.	
7201	6127	2021/04/30 19:14:22				User Activities	Software Start	Software start.	
7200	6126	2021/04/30 16:41:05	ADMINISTRATOR	1	2005041701P2R_Plate1Geoff - Copy - Copy.pdf (C:\Users\logannwu\OneDrive - Agilent Technologies\GxP Compliance\Tessa\2005041701P2R_Plate1Geoff - Copy - Copy_Report.pdf)	User Activities	Export Report	Export report to PDF file [C:\Users\logannwu\OneDrive - Agilent Technologies\GxP Compliance\Tessa\2005041701P2R_Plate1Geoff - Copy - Copy_Report.pdf] Page selected: "Call Index, Well Graph, Data Analysis - Curve". Predefined Analysis Methods selected: "3 page".	
7199	6125	2021/04/30 16:40:06	ADMINISTRATOR	1	2005041701P2R_Plate1Geoff - Copy - Copy.pdf (C:\Users\logannwu\OneDrive - Agilent Technologies\GxP Compliance\Tessa\2005041701P2R_Plate1Geoff - Copy - Copy.pdf)	User Activities	Open File	Open file [C:\Users\logannwu\OneDrive - Agilent Technologies\GxP Compliance\Tessa\2005041701P2R_Plate1Geoff - Copy - Copy.pdf].	
7198	6124	2021/04/30 16:39:59	ADMINISTRATOR	1	2005041701P2R_Plate1Geoff - Copy - Copy.pdf (C:\Users\logannwu\OneDrive - Agilent Technologies\GxP Compliance\Tessa\2005041701P2R_Plate1Geoff - Copy - Copy.pdf)	User Activities	Close File	Close file [C:\Users\logannwu\OneDrive - Agilent Technologies\GxP Compliance\Tessa\2005041701P2R_Plate1Geoff - Copy - Copy.pdf].	
					2005041701P2R_Plate1Geoff - Copy - Copy.pdf (C:\Users\logannwu\OneDrive - Agilent Technologies\GxP Compliance\Tessa\2005041701P2R_Plate1Geoff - Copy - Copy.pdf)			Export report to PDF file [C:\Users\logannwu\OneDrive - Agilent Technologies\GxP Compliance\Tessa\2005041701P2R_Plate1Geoff - Copy - Copy_Report.pdf] Page selected: "Call Index, Well Graph, Data Analysis - Curve". Predefined Analysis Methods selected: "3 page".	

- **Date From:** select the start date to filter the records. The default **Date From** depends on the number of records in the time period.
- **To:** select the end date to filter the records. By default, it is set to be the current date.
- **User:** select a user to filter the records only associated with one specific user.
- **Category:** select the category to filter the records.
- **Action:** select the type of action to filter the records.
- : input a keyword and click to search the records. Only contents in the **File**, **Description**, and **Reason** are searchable.
- **Export:** click to export the records shown in the table to an Excel file.
- **Print:** click to print the records shown in the table.
- **Context menu:** right click the table header row to select columns to show.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

ID	Batch	Timestamp	Username
8673	✓ Batch		
	Session		
8672	✓ Timestamp		
8671	Computer		
8670	Software		
8669	SW Version		
8668	SW Compliance Supporting Mode		
8667	✓ Username		
8666	✓ Plate		
8665	✓ File		
	File Version		
8664	✓ Category		
8663	✓ Action		
8662	✓ Description		
8661	✓ Reason		

### NOTE

Some records may have multiple line of contents. You can move the cursor to the row header to resize the row to view the full contents.

9	5
8	
7	

## File Audit Trail

Click **Plate > File Audit Trail** or **File Audit Trail** toolbar button  to open the **File Audit Trail** window.

### NOTE

**File Audit Trail** window is only available when a PLT file is open and current user has the “**View File Audit Trail**” permission.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

ID	Batch	Timestamp	Experiment Time	Username	Category	Action	Description	Reason
20	11	2021/05/01 19:26:17	225:30:41	ADMINISTRATOR	File Settings	Sign	Signed by User [ADMINISTRATOR], Signature Name "Review", Comments "reviewed".	
19	10	2021/05/01 19:24:42	225:29:06	ADMINISTRATOR	File Settings	Sign	Signed by User [ADMINISTRATOR], Comments "re-sign after edit the experiment conclusion".	
18	9	2021/05/01 19:23:10	225:27:34	ADMINISTRATOR	Cell Index	Change Cell Index Settings	Raw Data Type set to "Raw Scan Data", Raw Scan Data Index "1".	Test
17	8	2021/05/01 19:23:10	225:27:34	ADMINISTRATOR	Cell Index	Change Cell Index Settings	Time point set to "00:00:00", data type set to "Cell Index".	
16	8	2021/05/01 19:18:02	225:22:26	ADMINISTRATOR	Exp Notes	Change Exp Notes	Experiment Conclusion changed from "" to "the new conclusion".	aaa
15	7	2021/05/01 19:12:46	225:17:10	ADMINISTRATOR	File Settings	Sign	Signed by User [ADMINISTRATOR], Signature Name "Authorship", Comments "complete the run".	sign the file
14	6	2021/04/24 17:18:09	55:22:33	ADMINISTRATOR	Analysis Method	Create Analysis Method	Analysis Method [22] created, Includes "Data Analysis", Time Resolve Method "Sweep Index in Step", Notes "".	
13	5	2021/04/24 17:17:34	55:21:58	ADMINISTRATOR	Data Analysis	Change X Axis Settings	Normalization Time changed from "" to "00:00:05".	
12	4	2021/04/24 17:17:34	55:21:58	ADMINISTRATOR	Data Analysis	Change X Axis Settings	Time To changed from "" to "00:00:05".	
11	3	2021/04/24 17:17:34	55:21:58	ADMINISTRATOR	Data Analysis	Change X Axis Settings	Time From changed from "" to "00:00:00".	
10	2	2021/04/24 17:17:34	55:21:58	ADMINISTRATOR	Data Analysis	Change Y Axis Settings	Y Axis parameter changed from "Cell Index" to "SControl", Normalization enabled.	
9	1	2021/04/24 17:17:34	55:21:58	ADMINISTRATOR	Data Analysis	Plot Wells	Well(s) [D3:F3] added to Curve area, Curve Marker Size "0".	
8	1	2021/04/24 17:17:34	55:21:58	ADMINISTRATOR	Analysis Method	Delete Analysis Method	Delete Analysis Method [m2].	
7	1	2021/04/24 17:17:34	55:21:58	ADMINISTRATOR	Data Analysis	Load Analysis Method	Load Analysis Method [m2].	
6	1	2021/04/24 17:17:34	55:21:58	ADMINISTRATOR	Analysis Method	Edit Analysis Method	Time Resolve Method of Analysis Method [m1] changed from "Sweep Index in Step" to "Nearest Time".	
5	4	2021/04/22 09:55:45	00:00:09	ADMINISTRATOR	Information Message	Finish Step	Step [Step 1] finished.	
4	3	2021/04/22 09:55:41	00:00:05	ADMINISTRATOR	Warning Message	Run Experiment	Measure Background. Following wells may have background problems:	

- **User:** select a user to filter the records only associated with one specific user.
- **Category:** select the category to filter the records.
- **Action:** select the type of action to filter the records.
- **Insert Note:** click to create a new record with self-input notes.

Input Note ✕

User defined note:

some memo for the experiment with this timestamp

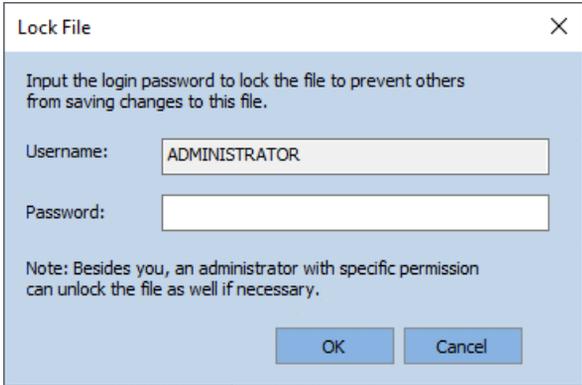
OK
Cancel

ID	Batch	Timestamp	Experiment Time	Username	Category	Action	Description
29	15	2021/05/02 09:13:23	239:17:47	ADMINISTRATOR	File Settings	Insert Note	User defined note: "some memo for the experiment with this timestamp".

- **Export:** click to export the records shown in the table to an Excel file.
- **Print:** click to print the records shown in the table.
- **Context menu:** right click the table header row to select columns to show.



## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance



Lock File

Input the login password to lock the file to prevent others from saving changes to this file.

Username: ADMINISTRATOR

Password:

Note: Besides you, an administrator with specific permission can unlock the file as well if necessary.

OK Cancel

Once the file is locked, the **File Information panel** on **Exp Notes** page will show the file lock information as shown below. Refer to [File Information](#) for more information regarding the file information.

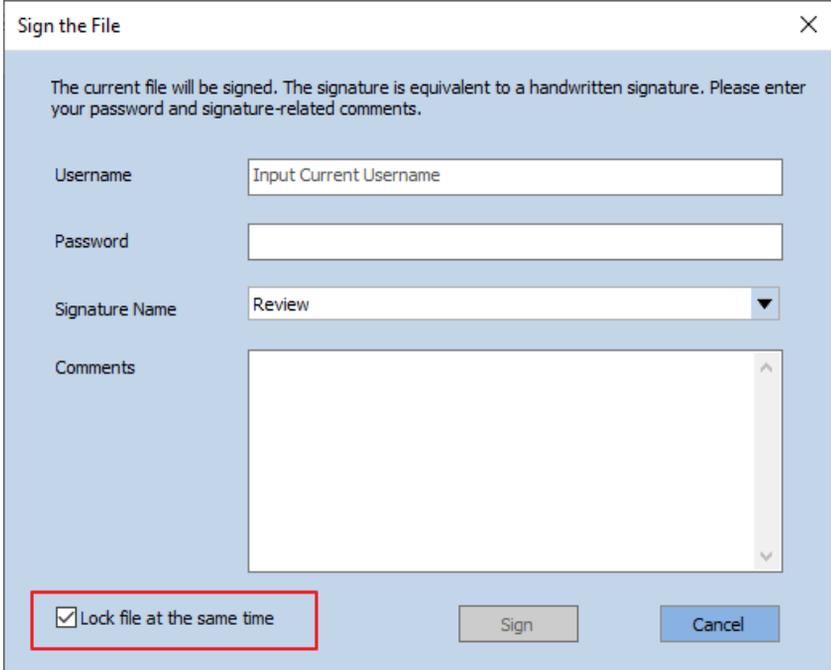
Locked: Yes, by ADMINISTRATOR

The status (annotated by **(locked)**) will also show at the end of the experiment file on the top of the plate window.

 2101131427B\_Test.plt (ID:2101131427B) (locked)

With RTCA Software compliance license, users can also lock the file at the same time when signing the file. Check the **“Lock file at the same time”** check box on the **Sign the File** window.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance



The current file will be signed. The signature is equivalent to a handwritten signature. Please enter your password and signature-related comments.

Username:

Password:

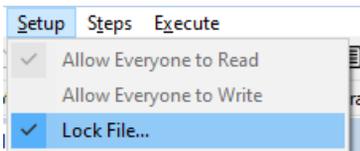
Signature Name:

Comments:

Lock file at the same time

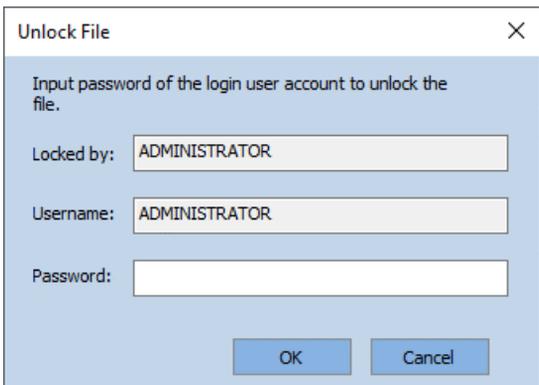
Sign Cancel

To unlock a locked file, click **Setup > Lock File** again. Enter the password of login account to unlock it. Only user who lock the file or user who has the permission of **“Unlock File Locked by Others”** can unlock the file.



Setup Steps Execute

- Allow Everyone to Read
- Allow Everyone to Write
- Lock File...



Unlock File

Input password of the login user account to unlock the file.

Locked by:

Username:

Password:

OK Cancel

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

### NOTE

The **Lock File** menu item is visible only when RTCA Compliance License is registered.

When a file is locked, it cannot be saved (for changes) and signed. To save and sign a locked experiment file, unlock it first.

## QC Test

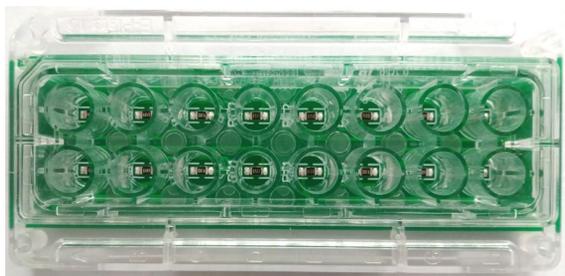
RTCA QC Plate Kit is a set of eight resistor plates which are used for xCELLigence RTCA instruments quality control to check if the instrument meets the design specifications.

### RTCA QC Plate Kit Product Information and Classification

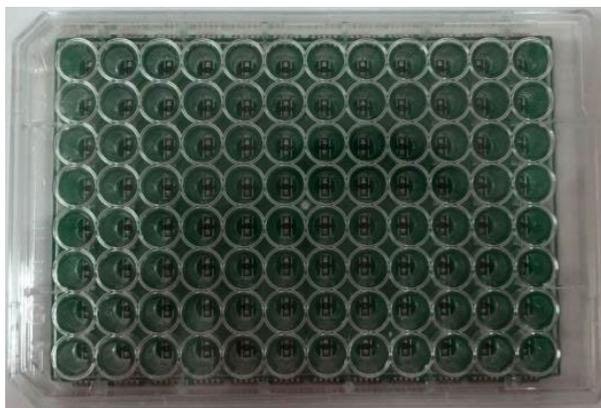
There are two RTCA QC Plate Kits available: RTCA QC Plate Kit 16X and RTCA QC Plate Kit 96X. The resistor value for each plate is specified in the table below:

Type	Resistor Value ( $\Omega$ )			
Well	A <sub>i</sub> H <sub>i</sub>	B <sub>i</sub> G <sub>i</sub>	C <sub>i</sub> F <sub>i</sub>	D <sub>i</sub> E <sub>i</sub>
X-1			0	
X-2	37.4	64.9	91.0	115.0
X-3 CI1	52.4	79.9	106.0	130.0
X-3 CI2	67.4	94.9	121.0	145.0
X-3 CI5	112.4	139.9	166.0	190.0
X-3 CI7	142.4	169.9	196.0	220.0
X-3 CI12	217.4	244.9	217.0	295.0
X-3 CI15	262.4	289.9	316.0	340.0

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance



RTCA QC Plate 16X-1



## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance



RTCA QC Plate 96X-1

### Operating Precautions

- Make sure that RTCA QC Plates are placed firmly against the cradle.
- There may be some wells are out of specified range and it could be caused by bad contact between the connector pads at the back side of the QC Plate and the contact pins on the RTCA instrument. Check the connector pads on the plate and the contact pins. Make sure that all the 120 connector pads of the QC Plate are clean and free of any dust or dirt particles. If not, blow the dust off the contact pads with compressed air and then directly clean the connector pad with a clean, lint-free cotton swab. Also, make sure that the contact pins which constantly have connection issues are clean and straight without any bending. Change the pogo-pins which constantly have connection issues.

### Operating Procedures

- 1 Open the RTCA Software Pro.
- 2 Click **System** > **QC Test** menu item to open the **QC Test** window.
- 3 Input the **QC Plate Kit Lot** and select the **Cradle ID** to start the test. Click **Next** to continue.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

QC Test

Operator Name:

Instrument Name:

Analyzer SN:

Station SN:

QC Plate Kit Lot:

Cradle ID:

### NOTE

Available “**Cradle ID**” from the drop-down list varies based on the model of the connected RTCA instruments.

- Place the correct QC Plate as indicated on the following window into the corresponding cradle.

QC Test

**Electronic Switch Impedance Measurement**

Plate: RTCA QC Plate 96X-1 Pass Criteria: 2.00~5.00

Impedance

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

- After the QC Plate is placed in the cradle, the software will conduct an auto-scan and the results will be shown in the table.

QC Test

**Electronic Switch Impedance Measurement**

Plate: RTCA QC Plate 96X-1

Auto Scan Data Pass Criteria: 0.0~500.0

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.1185	3.1314	3.1430	3.1515	3.1361	3.1623	3.1480	3.1475	3.1654	3.1744	3.2125	3.2043
B	3.1118	3.1394	3.1303	3.1555	3.1173	3.1524	3.1326	3.1485	3.1561	3.1727	3.1895	3.2104
C	3.1424	3.1492	3.1519	3.1638	3.1398	3.1706	3.1479	3.1655	3.1721	3.1936	3.2098	3.2284
D	3.1532	3.1654	3.1720	3.1792	3.1648	3.1654	3.1739	3.1705	3.1850	3.1821	3.2234	3.2256
E	3.1239	3.1012	3.1426	3.1222	3.1335	3.1241	3.1472	3.1336	3.1789	3.1529	3.2260	3.2114
F	3.1156	3.0972	3.1436	3.1175	3.1392	3.1170	3.1494	3.1304	3.1746	3.1533	3.2224	3.2056
G	3.0922	3.0666	3.1219	3.0840	3.1115	3.0855	3.1255	3.1009	3.1506	3.1261	3.1930	3.1844
H	3.0906	3.0772	3.1078	3.1021	3.1038	3.0980	3.1082	3.1155	3.1419	3.1208	3.1891	3.1969

Run      Next

- Click **Run** to measure data of this QC Plate. After impedance measurement completes, the table shows the results. If a well passes the **Pass Criteria**, the well background is white, otherwise a yellow background will show to indicate Failure on that well.

QC Test

**Electronic Switch Impedance Measurement**

Plate: RTCA QC Plate 96X-1

Impedance Pass Criteria: 2.00~5.00

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.0919	3.1027	3.1043	3.1083	3.1094	3.1404	3.0818	3.0872	3.1085	3.1258	3.1200	3.1205
B	3.0974	3.1073	3.1007	3.1117	3.1051	3.1253	3.0715	3.0835	3.1034	3.1268	3.1187	3.1299
C	3.1025	3.1217	3.1155	3.1102	3.1203	3.1473	3.0982	3.0938	3.1253	3.1467	3.1427	3.1418
D	3.1295	3.1259	3.1344	3.1308	3.1511	3.1458	3.1262	3.1042	3.1414	3.1372	3.1546	3.1452
E	3.0856	3.0566	3.1012	3.0802	3.1173	3.0959	3.0820	23443.7910	3.1281	3.1145	3.1451	3.1326
F	3.0936	3.0702	3.1034	3.0791	3.1184	3.0940	3.0956	3.0624	21177.7402	3.1150	3.1514	3.1241
G	3.0561	3.0316	3.0675	3.0426	3.0898	3.0603	3.0628	3.0297	3.1039	3.0799	3.1241	3.1108
H	3.0570	3.0313	3.0551	3.0528	3.0940	3.0705	3.0540	19246.6094	3.1015	3.0844	3.1256	3.1132

Run      Next

- Click **Next** to repeat this step for the rest of the QC Plates.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

- 8 When all 8 QC Plates are measured, click **Show Report** to view the **QC Report**.

QC Test

**Cell Index Measurement**

Plate Info: RTCA QC Plate 96X-3 CI 15

Cell Index:  Maximum  Minimum  Individual 1

Pass Criteria: 14.85~15.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	15.0057	15.0021	15.0065	15.0072	15.0079	15.0036	15.0051	15.0065	14.9968	15.0009	15.0109	15.0089
B	15.0076	15.0002	14.9997	15.0035	15.0083	15.0064	15.0044	15.0126	15.0015	15.0078	15.0068	14.9984
C	15.0091	15.0055	14.9964	14.9974	15.0116	15.0001	15.0005	15.0039	15.0053	15.0057	15.0051	15.0008
D	15.0100	15.0109	15.0032	14.9895	15.0064	15.0035	15.0162	15.0014	15.0024	14.9958	15.0156	15.0149
E	15.0058	15.0014	15.0003	14.9982	14.9986	15.0060	15.0049	14.9939	15.0062	15.0097	14.9995	15.0065
F	14.9934	15.0024	14.9964	14.9973	14.9990	14.9991	15.0047	15.0025	14.9997	15.0001	15.0052	14.9989
G	15.0031	15.0059	15.0051	15.0029	14.9995	15.0044	15.0055	15.0021	15.0017	15.0017	15.0068	15.0027
H	15.0012	15.0039	15.0033	15.0069	14.9981	15.0001	15.0022	15.0009	15.0106	15.0042	14.9986	15.0023

Run Show Report

- 9 Values in red color in the **QC Report** indicate failed result.

Test Report

Open Data File Export to Excel Export to PDF Print New Test

**QC Test Report**

Operator: ServiceEngineer Test date: 2021/04/27 10:02:28  
 Instrument: xCELLigence RTCA MP Cradle ID: 1  
 Analyzer SN: 691200410452 Station SN: Z91190913960  
 Software Version: 2.6.0 QC Plate Kit Lot: 20201201

Item: Electronic Switch Impedance (Ohm)  
 Test Time: 2021/04/27 11:24:36  
 Pass Criteria: 2.00~5.00

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.0919	3.1027	3.1043	3.1083	3.1094	3.1404	3.0818	3.0872	3.1085	3.1258	3.1200	3.1205
B	3.0974	3.1073	3.1007	3.1117	3.1051	3.1253	3.0715	3.0835	3.1034	3.1288	3.1187	3.1299
C	3.1025	3.1217	3.1155	3.1102	3.1203	3.1473	3.0982	3.0938	3.1253	3.1467	3.1427	3.1418
D	3.1295	3.1259	3.1344	3.1308	3.1511	3.1458	3.1262	3.1042	3.1414	3.1372	3.1546	3.1452
E	3.0856	3.0568	3.1012	3.0802	3.1173	3.0959	3.0820	-23443.7910	3.1281	3.1145	3.1451	3.1326
F	3.0936	3.0702	3.1034	3.0791	3.1184	3.0940	3.0956	3.0624	-21177.7402	3.1150	3.1514	3.1241
G	3.0561	3.0316	3.0675	3.0426	3.0898	3.0603	3.0628	3.0297	3.1039	3.0799	3.1241	3.1108
H	3.0570	3.0313	3.0551	3.0528	3.0940	3.0705	3.0540	-19246.6094	3.1015	3.0844	3.1256	3.1132

Item: Background Impedance (Ohm)  
 Test Time: 2021/04/27 11:26:16  
 Pass Criteria: AH(37.80~43.90); BG(64.90~71.80); CF(90.60~98.40); DE(114.30~122.70)

- 10 Scroll down the report to see the total results table. **P** indicates pass and **F** indicates failed.

## Functions Supporting GxP and FDA 21 CFR Part 11 Compliance

Test Report

Open Data File Export to Excel Export to PDF Print New Test

D	14.9885	14.9913	14.9900	14.9795	14.9772	14.9829	14.9970	14.9851	14.9825	14.9783	14.9868	14.9906
E	14.9863	14.9792	14.9775	14.9774	14.9792	14.9888	14.9858	14.9644	14.9866	14.9869	14.9774	14.9864
F	14.9822	14.9842	14.9778	14.9817	14.9814	14.9799	14.9841	14.9845	14.9832	14.9878	14.9865	14.9800
G	14.9878	14.9804	14.9946	14.9875	14.9837	14.9867	14.9911	14.9893	14.9832	14.9842	14.9881	14.9860
H	14.9861	14.9935	14.9901	14.9935	14.9863	14.9904	14.9918	14.9940	14.9917	14.9856	14.9864	14.9928

Total Results: **Failed**

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	P	P	P	P	P	P	P	P	P	P	P
B	P	P	P	P	P	P	P	P	P	P	P	P
C	P	P	P	P	P	P	P	P	P	P	P	P
D	P	P	P	P	P	P	P	P	P	P	P	P
E	P	P	P	P	P	P	P	P	F	P	P	P
F	P	P	P	P	P	P	P	P	F	P	P	P
G	P	P	P	P	P	P	P	P	P	P	P	P
H	P	P	P	P	P	P	P	P	F	P	P	P

- Click **New Test** on the report window to start a new QC Test for another cradle. It takes about 10~15 minutes to complete the QC Test for one cradle.

## Query QC Test Report

Click **System > QC Report** menu item to open the **QC Test Report** window to query the history QC test report and review the Levey-Jennings Plot.

## QC Test Report

QC Test Report

Operator: ADMINISTRATOR  
Instrument: xCELLigence RTCA MP  
Analyzer SN: 281120300221  
Software Version: 2.6.0

Test date: 2021/01/04 09:27:42  
Plate: 1  
Station SN: 291200714567  
QC Plate Kit Lot: 20200102

Item: Electronic Switch Impedance (Ohm)  
Test Time: 2021/01/04 09:30:55  
Pass Criteria: 2.00~5.00

	1	2	3	4	5	6	7	8	9	10	11	12
A	3.0244	3.0489	3.0664	3.0648	3.0525	3.0645	3.0636	3.0594	3.0769	3.0818	3.1093	3.1240
B	3.0258	3.0555	3.0479	3.0617	3.0383	3.0587	3.0471	3.0599	3.0698	3.0816	3.1105	3.1337
C	3.0501	3.0621	3.0733	3.0819	3.0546	3.0818	3.0710	3.0718	3.0922	3.0997	3.1384	3.1462
D	3.0691	3.0774	3.0991	3.0860	3.0714	3.0756	3.0687	3.0889	3.1063	3.0993	3.1418	3.1442
E	3.0284	3.0100	3.0578	3.0403	3.0435	3.0360	3.0617	3.0430	3.0877	3.0719	3.1325	3.1238
F	3.0327	3.0159	3.0642	3.0310	3.0484	3.0268	3.0720	3.0338	3.0877	3.0682	3.1367	3.1233
G	3.0041	2.9820	3.0377	3.0068	3.0192	2.9968	3.0387	3.0056	3.0668	3.0388	3.1061	3.0922
H	3.0002	2.9957	3.0222	3.0112	3.0259	3.0085	3.0219	3.0248	3.0522	3.0340	3.1080	3.1120

Item: Background Impedance (Ohm)  
Test Time: 2021/01/04 09:33:49  
Pass Criteria: AH(37.80~43.90); BG(64.90~71.80); CF(90.60~98.40); DE(114.30~122.70)

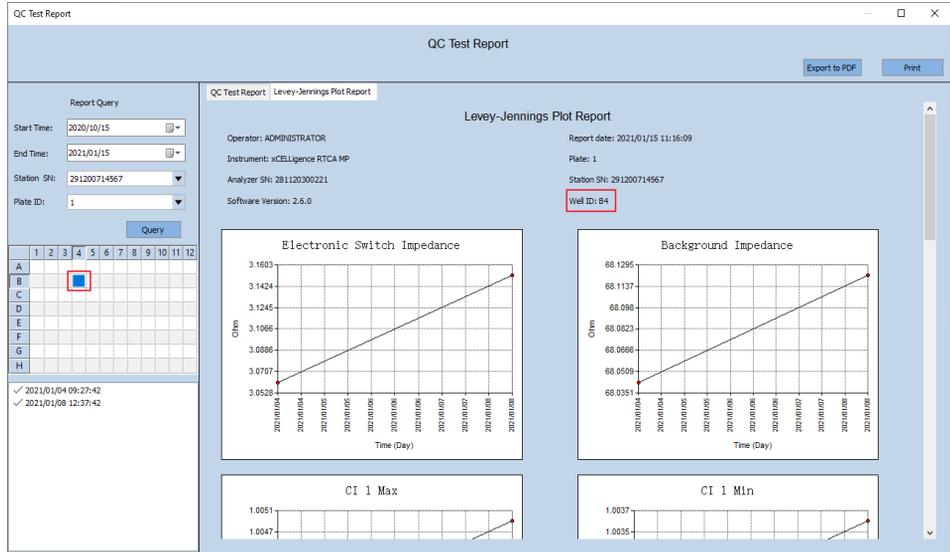
In the **Report Query** panel, input the **Start Time**, **End Time**, **Station SN** (if multiple instrument results exist) and **Plate ID**, then click **Query** button to query the history QC test results.

All the QC test which meets the query condition will be listed in the table at the bottom in the format of **YYYY/ MM/ DD hh:mm:ss** which is the date and time when the QC test is started. The icon ✓ indicates the QC Test result is Pass and the icon ✗ indicates the QC Test result is Failed. Select the QC test from the list to view its results. The QC test report will show on the right part of the window.

## Levey-Jennings Plot

Click the **Levey-Jennings Plot Report** tab to review the Levey-Jennings Plot. Click the well grid on the left part to select the well to view its data on the right plot at different time when a QC test is conducted.

# Functions Supporting GxP and FDA 21 CFR Part 11 Compliance



## 5 Setting Up an Experiment

This chapter describes how to set up an experiment including: launch RTCA Software Pro, enter real-time or offline modes automatically, set plate configuration, record experimental notes, define plate layout (RTCA Basic and Immunotherapy Module), define experiment schedule and save/re-use experimental setups.

This chapter describes how to set up an experiment in RTCA Software Pro step-by-step, using the xCELLigence RTCA SP as an example. In the instances where the steps for the xCELLigence RTCA DP or MP are different from what is reported for the xCELLigence RTCA SP, such differences will be discussed in the text.

### Launch RTCA Software Pro

Double-click the RTCA Software Pro icon on the desktop (or in the Start menu) to launch the application. A login window appears, and all usernames are shown in a drop-down list. Choose a username and enter the corresponding password to login.

#### NOTE

The default password for the built-in user ADMINISTRATOR is administrator (all lower case).

### Enter Real-Time or Offline Modes Automatically

When the xCELLigence RTCA SP/MP/DP workstation is connected to an xCELLigence RTCA instrument (that is powered on), launching the RTCA Software Pro causes the xCELLigence system to automatically enter the real-time mode. Within the real-time mode the workstation actively communicates with the connected instrument, and all software functionalities are available.

## Setting Up an Experiment

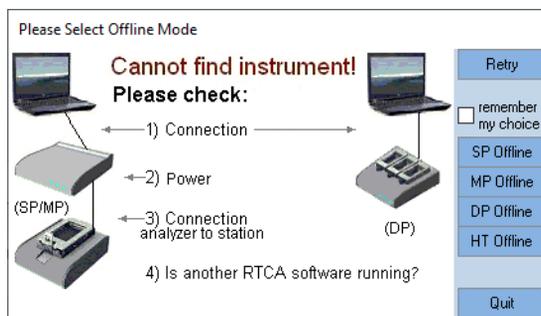
### NOTE

The software automatically identifies the model of the instrument that the workstation is connected to and displays the corresponding plate map and functionality.

If the software does not automatically enter real-time mode with RTCA instrument connected and powered on, check the following settings:

- 1 The serial communication cable that connects the xCELLigence RTCA SP/MP/DP workstation and xCELLigence RTCA SP/MP Analyzer (in short “SP/MP Analyzer”) and RTCA DP Instrument must be connected securely.
- 2 The SP/MP Analyzer is turned on.
- 3 The SP and MP Station is connected to the SP/MP Analyzer.
- 4 No other RTCA Software is running.

If the communication between the software and the RTCA instrument fails, RTCA Software Pro will automatically enter offline mode where it is only possible to create/ save experimental templates and analyze pre-existing data. Depending on the type of software license(s) registered, you can choose the SP, MP, DP, or HT interface in the offline mode.



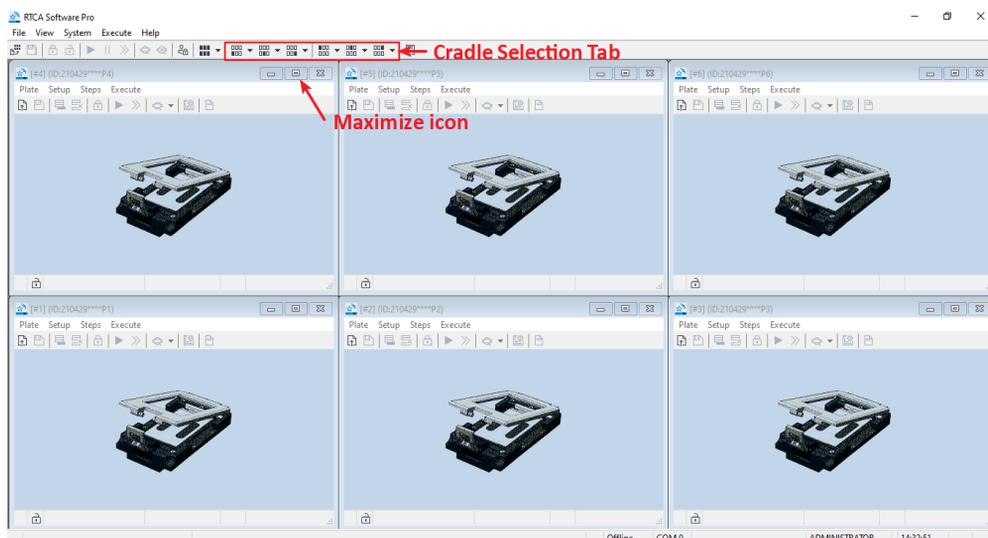
## Plate Configuration

While the xCELLigence RTCA SP accommodates a single 96-well E-Plate, the xCELLigence RTCA DP, MP and HT accommodate three 16-well E-Plates, six 96-well E-Plates and a single 384-well E-Plate, respectively. For the xCELLigence RTCA DP and MP instrument, the user must choose which of the plate positions to use for their experiment.

## Setting Up an Experiment

### Cradle Selection When Using an xCELLigence RTCA MP

After the software has been launched, the **Cradle Selection** interface will show with an image of a cradle on the available **Plate Window**. Depending on the presence or absence of an E-Plate inside the cradle, the cradle image will display an occupied or empty status. A specific cradle (experiment) can be selected either by clicking the corresponding icon on the **Cradle Selection Tab**, or by clicking the maximize icon on the top right corner of the **Plate Window**. Once a specific cradle has been enlarged, it is possible to switch to another cradle by clicking the corresponding icon in the **Cradle Selection** tab, or by clicking the  icon to show the main **Cradle Selection** interface and then selecting a different cradle.



- Plate Release

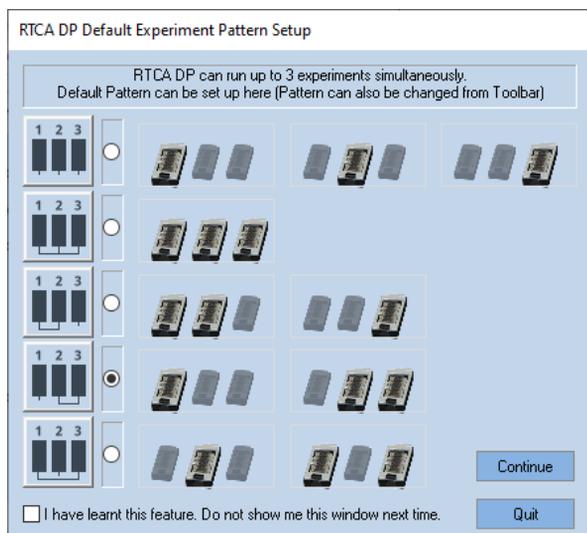
At the end of the experiment, the plate should be released to make the cradle available for another experiment.

- a In the **Plate Window**, select the **Plate** drop-down menu.
- b Select **Release**.

### Cradle Selection When Using an xCELLigence RTCA DP

When starting the software while the workstation is connected to an xCELLigence RTCA DP instrument, **Experiment Pattern Setup** (shown below) will show up. As illustrated below, the user can choose from a variety of configurations where data will be acquired from 1, 2, or 3 cradles.

## Setting Up an Experiment



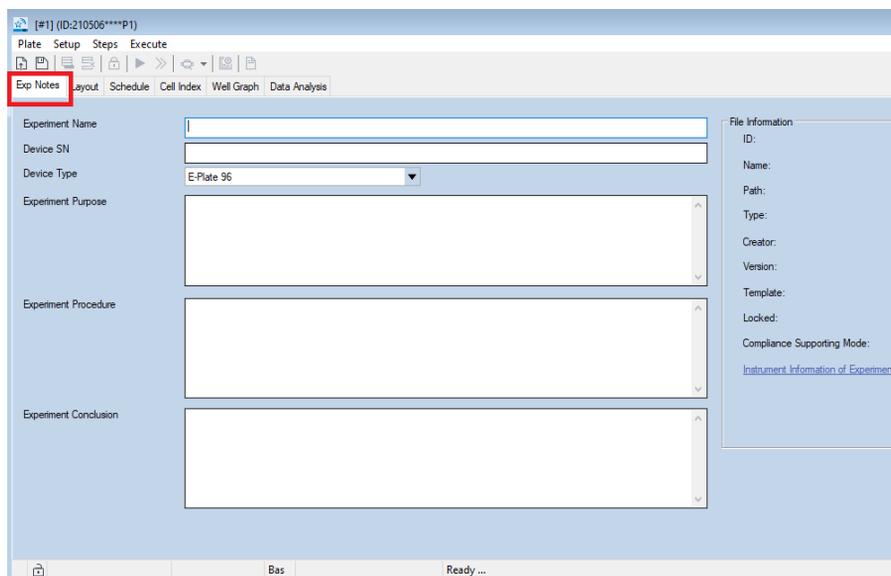
To select a specific cradle configuration, click on the radio button next to the desired configuration. Once a selection is made, click the **Continue** button to enter DP software.

For more information about multiple users sharing with an xCELLigence RTCA MP/DP instrument, please refer to [Multiple Users Working with an RTCA Instrument](#) section.

## Record Experimental Notes

In the **Plate Window**, click the **Exp Notes** tab to record information related to the planned experiment.

## Setting Up an Experiment



### NOTE

The information (except **Device Type**) recorded on the **Exp Notes** page is not required for running an experiment. However, this information is useful for later reference.

## Entering Information

Below is a list of the fields included in the **Exp Notes** page and the types of information that can be recorded in each field.

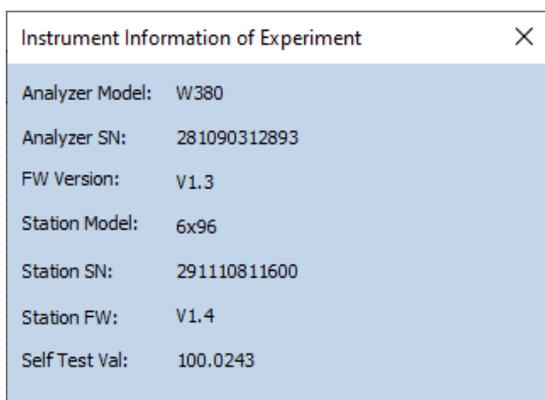
- **Experiment Name:** User-defined information. The **Experiment Name** is saved as part of the experiment file name.
- **Device SN:** The serial number of the E-Plate.
- **Device Type (SP/MP only):** The type of the E-Plate.
- **Experiment Purpose:** User-defined information.
- **Experiment Procedure:** User-defined information.
- **Experiment Conclusion:** User-defined information. At the end of an experiment, you can use this field to record the main conclusions.

## File Information

The file information is shown in the **File Information** panel, including:

## Setting Up an Experiment

- **ID:** The experiment ID with the format of **YYMMDDhhmmB** or **YYMMDDhhmmR**, where **YYMMDD** is the date and **hhmm** is the time when the experiment file created. The last character (B or R) indicates the **Module Type** chosen when the experiment file is created. B indicates Basic module and R indicates Immunotherapy module.
- **Name:** The current experiment file name.
- **Path:** The file path that the file currently stored.
- **Type:** Experiment File or Template File.
- **Creator:** The username of the account who created the file.
- **Version:** The version of the file, which starts from 1 and increases 1 each time when file is saved with any changes.
- **Template:** The path and name of the template file that the current experiment file is created from.
- **Locked:** “No” means the file is not locked. “Yes, by username” means the file is locked by the indicated user.
- **Compliance Supporting Mode:** “Yes” means the file is created and modified by the software with **Compliance Supporting Mode** turned on. “No” means it is not.
- **Instrument Information of Experiment:** Click to view the information of the instrument on which the experiment file is generated. The instrument information is stored within the experiment file when the experiment is started.



Instrument Information of Experiment	
Analyzer Model:	W380
Analyzer SN:	281090312893
FW Version:	V1.3
Station Model:	6x96
Station SN:	291110811600
Station FW:	V1.4
Self Test Val:	100.0243

## Saving the Recorded Information

The information logged into the **Exp Notes** fields will be automatically saved into the experiment file when the user clicks the **Start/Continue** ► icon to initiate an experiment. The default name for the experiment file is

## Setting Up an Experiment

YYMMDDhhmmB\_ExperimentName.plt or YYMMDDhhmmR\_ExperimentName.plt, where “**ExperimentName**” is the user-defined text from the **Experiment Name** box of the **Exp Notes** page. However, a different name can be entered if desired.

### WARNING

**After an experiment is started (i.e. data acquisition has been initiated), any changes made on the Exp Notes page must be saved manually using the Save Changes  icon.**

### NOTE

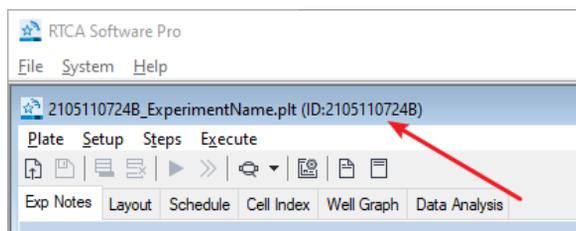
When the RTCA data file (\*.plt) is generated, a backup copy (\*.bak) is generated at the same time in the **Auto Backup** folder under the root data folder. For Windows 7 and Windows 10, the default root data folder for the RTCA data file is **C:\Users\Public\RTCA Pro Data**, and the default directory for the backup copy of the RTCA data file is **C:\Users\Public\RTCA Pro Data\Auto Backup**.

## Editing the Information Recorded

The information logged into the **Exp Notes** page can be edited while the experiment is running or after the experiment has finished. Once the experiment has started, if the user adds or changes information on the **Exp Notes** page, these changes must be saved manually by clicking the **Save Changes**  icon.

## Define the Experiment ID

The **Experiment ID** is displayed at the top of the **Plate Window** (highlighted by the red arrow in the image below). Before an experiment is configured and started, the default ID is the current date (YYMMDD) and time (\*\*\*\*). Once a user sets up and initiates an experiment, this default ID changes to the current date and time (YYMMDDhhmmB or YYMMDDhhmmR). The last character (B or R) indicates the **Module Type** chosen when the experiment file is created. B indicates Basic module and R indicates Immunotherapy module. And the experiment file name becomes YYMMDDHHmmB\_ExperimentName.plt or YYMMDDHHmmR\_ExperimentName.plt, where “**ExperimentName**” is the user-defined information from the **Experiment Name** box.



## Setting Up an Experiment

During an experiment or after completion of an experiment, the **Experiment ID** will not change even if the experiment file name is changed.

## Define Plate Layout (RTCA Basic)

If multiple modules are active within the software license, when selecting the **Layout** (or **Data Analysis**) tab, the software will automatically ask which module should be used for plate layout and data analysis. Select **RTCA Basic** module for general RTCA experiments and select **Immunotherapy** module for immunotherapy experiments.

### NOTE

If the Basic license module is the only license activated, the software will automatically open with the RTCA Basic module. No Software Module Selection will be shown when only Basic license is activated.

### NOTE

For the xCELLigence RTCA MP and DP configurations, the initial module selection (Basic or Immunotherapy) is only applied to the specified plate or cradle. To apply the same module selection to all plates or cradles, check the “**Also apply my module selection to...**”

When the RTCA Basic module has been selected, it is possible to switch to the Immunotherapy module by releasing the plate, selecting the **Layout** tab, and performing the Module Selection process again.

Select the **Layout** tab in a **Plate Window** to enter detailed information (cell type, drug treatment, etc.) for each well of an E-Plate 96. Each box in the **Plate Map** represents the corresponding well of an E-Plate 96. Entering the correct information for each well is essential for accurately monitoring the impedance signal during the course of data acquisition and for data analysis.

### NOTE

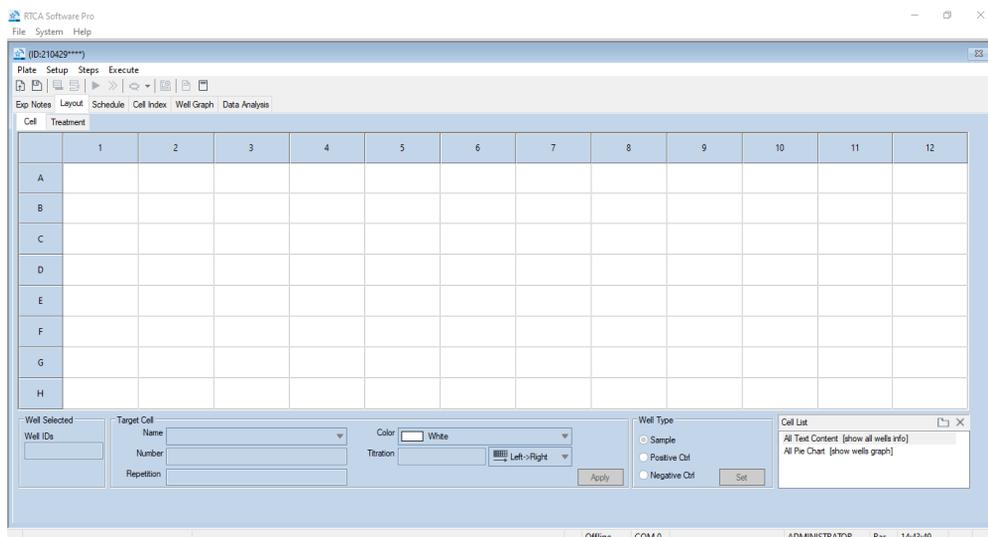
Information entered in the **Layout** page is saved automatically when the experiment is started. The user can enter or edit the layout information before the experiment starts, during the experiment or after it is finished. To save any changes made in the **Layout** page after an experiment is finished, click the **Save Changes**  button.

### WARNING

**To prevent loss of data, the RTCA Software Pro does not allow the user to turn any well on or off (see below) while an experiment is running.**

## Setting Up an Experiment

**Layout** page: Used for defining the contents and treatments for each well within the E-Plate 96 (e.g. target cell type, cell number, treatment name, concentration, etc.). The **Layout** page contains two subpages: **Cell** page and **Treatment** page.



The **Well IDs** box highlights the **Plate Map** region of the page – where information about the contents of each well is entered. The other boxes represent the fields for inputting well information which will populate in the **Plate Map**.

## Adding Experimental Information to the Wells in the Plate Map: Overview

### NOTE

The following text describes how to define the plate layout for 96-well E-Plates (used in the xCELLigence RTCA SP and MP). However, the same procedure is applicable for the xCELLigence RTCA DP instrument, with the difference that the plate map configuration may include 16, 32, or 48 wells depending on how many cradles have been selected.

In order to measure the impedance signal in a given well of the E-Plate 96, you must “enable” it. Add information to define the contents/conditions of the well, and then “activate” the well as follows:

- Enable the wells you will be using on the E-Plate by highlighting the corresponding boxes on the **Plate Map**. There are many ways to highlight different boxes on the map:
  - Holding the **Ctrl** key while clicking enables the selection of multiple boxes.

## Setting Up an Experiment

- Holding the **Shift** key while clicking enables the selection of a range of boxes.

### NOTE

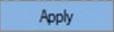
These functions work in the same way as the “select multiple files” function of the Microsoft Windows operating system.

- Clicking on one box and dragging across others will highlight multiple boxes.
- Clicking a column number will highlight the entire column of boxes.
- Clicking a column number and then dragging the mouse across other column numbers will highlight multiple columns.
- Clicking a row letter will highlight the entire row of boxes.
- Clicking a row letter and then dragging the mouse across others row letters will highlight multiple rows.

Enabled wells will turn dark blue.

- Go to the fields for inputting well information and type in the information such as **Target Cell** name, **Cell Number**, etc. for the enabled wells. (This step is optional; see details below).
- Click the **Apply** button  to simultaneously transfer the information to the highlighted wells in the **Plate Map**. After the button has been clicked, the activated wells on the **Plate Map** will be highlighted in color (no longer dark blue).

### WARNING

The information introduced into the text boxes will not be transferred to the Plate Map if the Apply button  is not clicked.

## Adding Experimental Information to the Wells in the Plate Map: Details

### WARNING

Entering experimental information in the Plate Map is optional. You can still perform an experiment even if no information appears in the Plate Map as long as the wells are activated (white color). However, while the experiment can be performed with incomplete information, certain functions in the Data Analysis page will be unavailable without complete information entered in the Layout page.

## Setting Up an Experiment

### NOTE

When typing information into these fields, spaces are not permitted. However, an underscore “\_” can be used as a spacer.

### Target Cells Information in the Cell Sub Page

The **Cell** subpage is utilized to introduce the target cell name and seeding density. One single cell type can be added to each well. Cell name and seeding densities are utilized in combination with treatment names and concentrations for data analysis and grouping replicate wells.

- Enter target cell information at **Cell** section:
  - On the **Plate Map**, highlight the interested well(s) where you want to enter the target cell information.
  - Type or select the target cell name in the **Name** box. (Only enter a~z, A~Z, 0~9, and underscore “\_”, other characters will be ignored).
  - Select a color you want to assign to the target cell you are entering.
  - Enter the target cell number in the **Number** box.
  - Enter the notes in the **Notes** box.
  - Click **Apply** to save the target cell information for the highlighted well(s).

When a new target cell is added to the **Plate Map**, it is also added to the **Cell List** box on the bottom right of the **Layout** page.

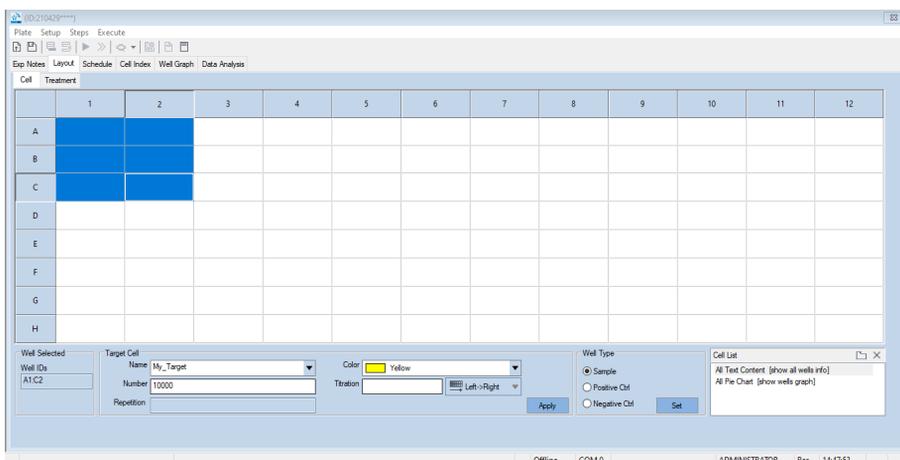
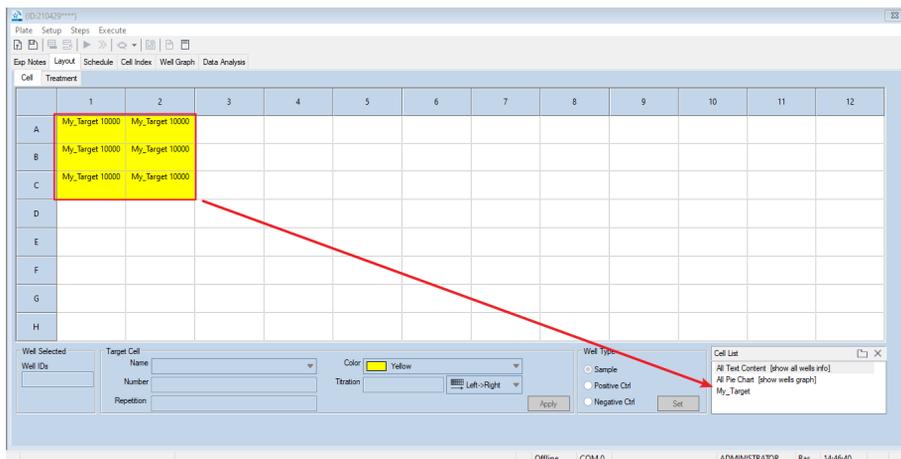


Plate Map with target cell name and seeding density entered, but not applied.

## Setting Up an Experiment

After the information has been applied, the wells are populated with the information and highlighted with the selected color (yellow). The target cell name is also added to the cell list (indicated by the red arrow).



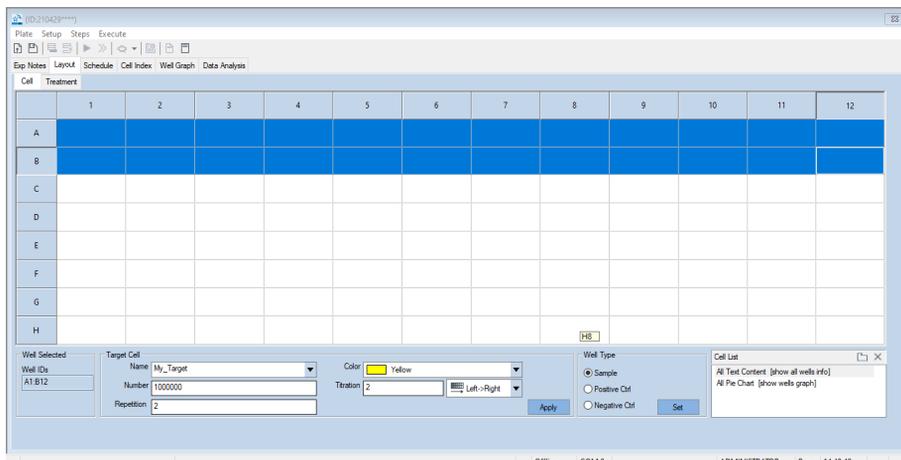
**Plate Map** with target cell name and seeding density entered and applied.

- Target Cell Seeding Density Titration

When a portion of the plate layout should be populated with a seeding titration, instead of introducing the single cell density manually, the software can automatically introduce the correct cell number.

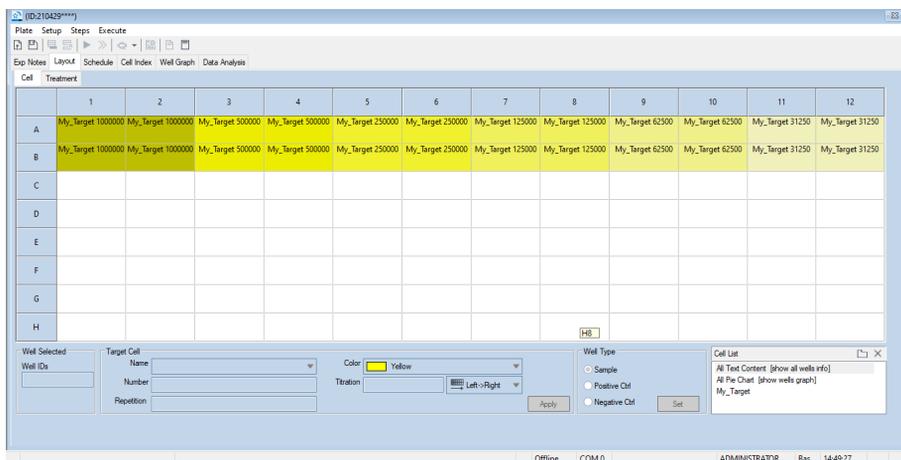
- Highlight the well(s) where you want to enter the target information on the **Plate Map**.
- Type or select the target cell name in the **Name** box.
- Select a color you want to assign to the target cells you are entering.
- Enter the highest target cell number in the **Number** box.
- Enter the cell titration factor to the **Titration** box.
- Select the titration direction from the drop-down list.
- Enter the repetition number in the **Repetition** box.
- Enter the notes in the **Notes** box.
- Click **Apply** button  to transfer the target cell information to the highlighted well(s).

## Setting Up an Experiment



**Plate Map** with target cell name and seeding density titration entered but not applied is shown below.

After the information has been applied, the wells are populated with the information and highlighted with the selected color. The color gradient reflects the seeding density as shown below.



**Plate Map** with target cell name and seeding density titration entered and applied.

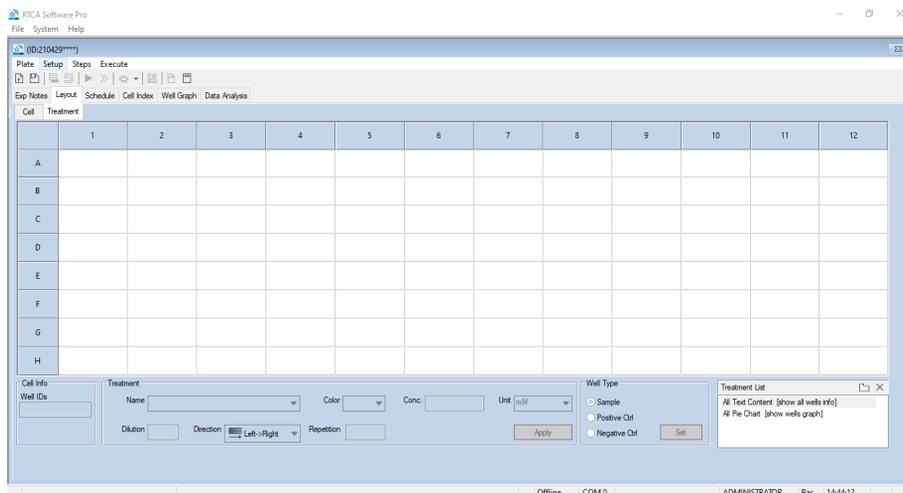
### WARNING

The seeding density titration can only be applied when a region is selected, and the number of wells matches the number of necessary wells for each dilution.

## Setting Up an Experiment

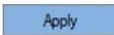
### Treatment Information on Treatment Sub Page

The **Treatment** subpage is utilized to introduce up to nine different treatments in the same wells. Cell name, seeding densities, treatment names, and concentrations are combined for data analysis and grouping replicate wells.



- Enter treatment information at **Treatment** section.

Entering the Treatment information is similar to the Target Cell editing.

- Highlight the well(s) where you want to enter the treatment information on the **Plate Map**.
- Type or select from the drop-down menu the name for the treatment information. (Only a~z, A~Z, 0~9, and underscore “\_” are acceptable, other characters will be ignored).
- Select a color you want to assign to the item you are entering.
- Enter the concentration in the **Conc. (Concentration)** box.
- Select the concentration unit in the **Unit** box.
- Enter the notes in the **Notes** box.
- Click **Apply** button  to transfer the treatment information to the highlighted well(s)

When a new treatment is added to the **Plate Map**, it is also added to the **Treatment List** box on the bottom right of the **Layout** page.

## Setting Up an Experiment

### NOTE

If a concentration unit is not listed in the drop-down menu, the **-define-** unit can be selected and a new unit can be entered.

### NOTE

A treatment concentration titration can be defined with the same operations described above for cell seeding density titration.

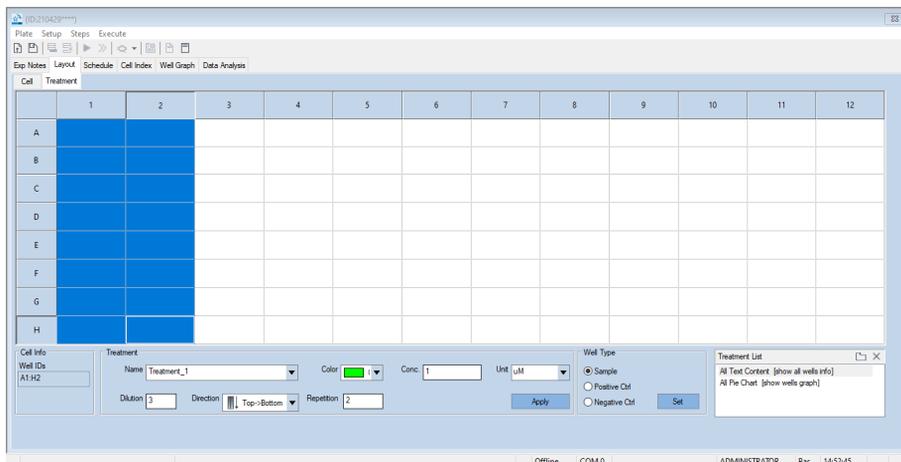


Plate Map with treatment concentration titration entered but not applied.

After the information has been applied, the wells are populated with the information and highlighted with the selected color. The color gradient reflects the treatment concentration gradient as shown below.

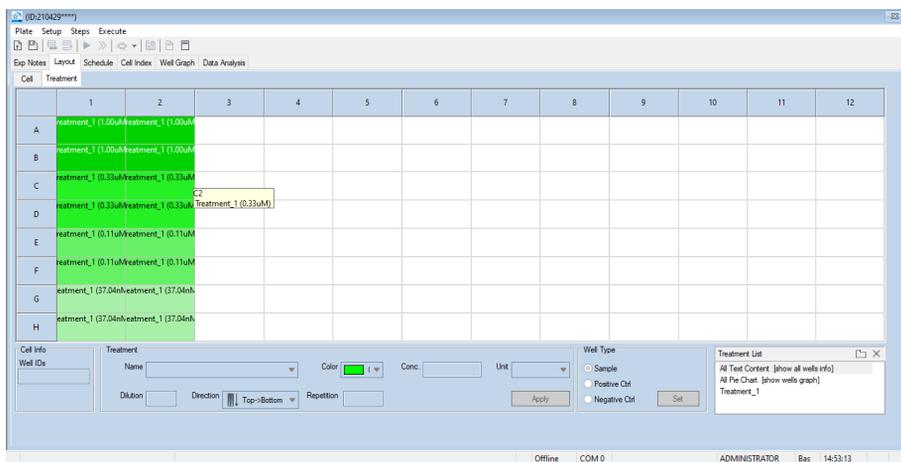


Plate Map with treatment concentration titration entered and applied.

## Setting Up an Experiment

### NOTE

In the **Cell** subpage, it is possible to distinguish wells that have a treatment from wells without treatment through the presence of a border around the well. Wells that contain a treatment have a border while wells without treatment have no border.

The **Plate Map** below shows four wells containing the same target cell line (My\_Target). Treatments are applied in wells A1, A2. No treatments are applied in wells B1, B2. The treatments can be identified by selecting the **Treatment** subpage.

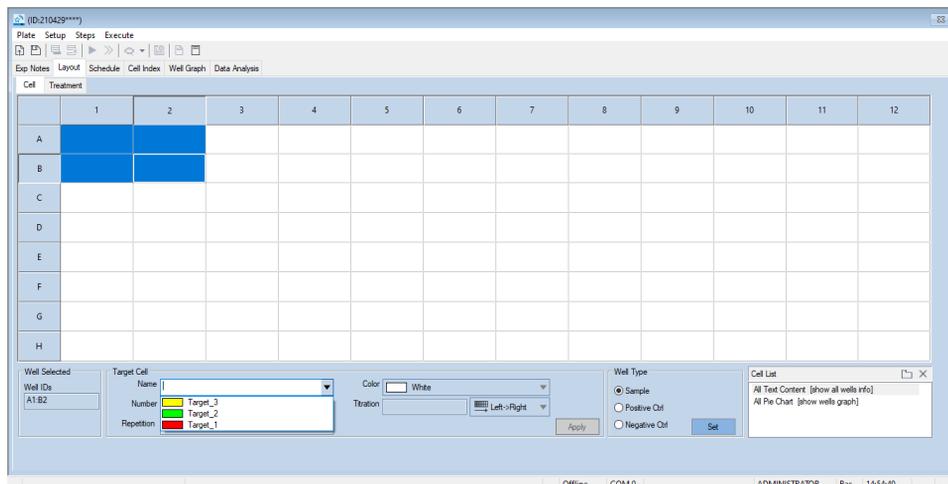
Exp Notes	Layout	Schedule	Cell Index	Well Graph	Data
Cell	Treatment				
	1	2			
A	My_Target	My_Target			
B	My_Target	My_Target			
C					

### Using the Cell and Treatment Manager to Define a Well

The RTCA Software Pro contains an internal database that stores the name of target cells and treatments that have been used previously. It is therefore possible to introduce a previously used cell line or treatment without entering the information again. Simply click the drop-down menu beside the **Target Cell Name** or **Treatment Name** box and select the desired item.

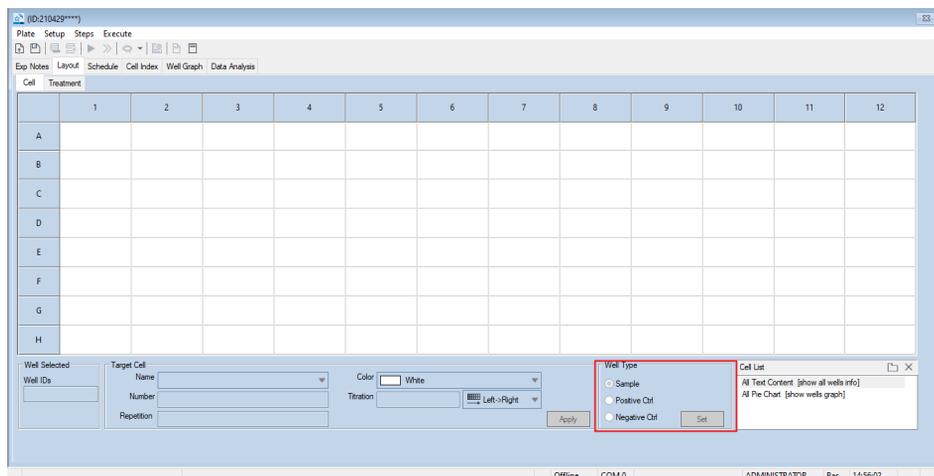
When the drop-down menu of the **Target Cell Name** is clicked, the drop-down list displays the names that have been used previously and stored in the database.

## Setting Up an Experiment



### Defining Sample Type

A well or group of wells can be defined either as a positive or negative control to plot the % of signal relative to references (see [Plotting Data](#) section), which can be defined in the **Well Type** area.



**Well Type** selection area.

### Defining positive and negative controls:

- Populate the wells with standard information, similar to any other sample well.
- Select the groups of wells that need to be defined as positive or negative controls and select the radio button corresponding to the desired control type.

## Setting Up an Experiment

Once a well is defined as a control, the boundary of the well will change according to the status:

- Wells defined as positive controls containing no treatment will be identified by a red long-dashed border.
- Wells defined as positive controls containing treatment will be identified by a red dotted border.
- Wells defined as negative controls containing no treatment will be identified by a green long-dashed border.
- Wells defined as negative controls containing treatment will be identified by a green dotted border.

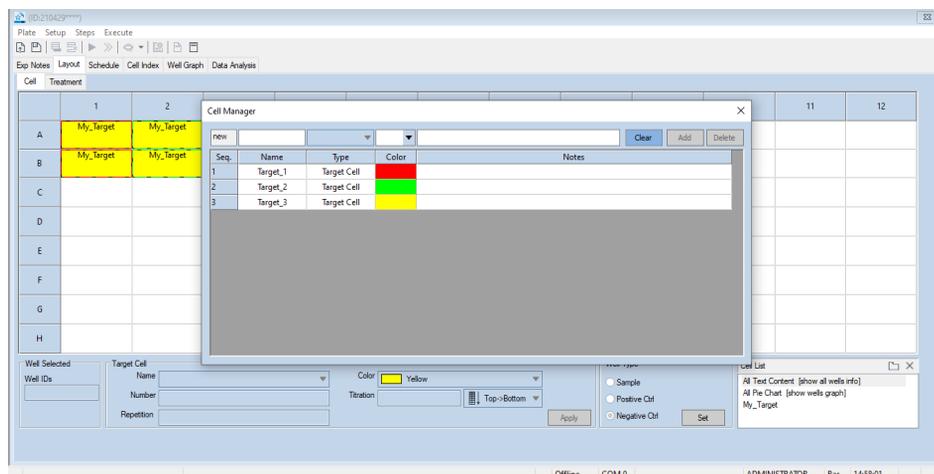
Plate map below shows the layout of different control wells with the same target cell: B1 is a positive control well with no treatment; A1 is a positive control well with a treatment added in the **Treatment** subpage; B2 is a negative control well with no treatment; A2 is a negative control well with an added treatment.

Exp Notes	Layout	Schedule	Cell Index	Well Graph	Data Analysis
Cell	Treatment				
	1	2	3		
A	My_Target	My_Target			
B	My_Target	My_Target			
C					

### Editing Cell and Treatment Manager Lists

The **Cell Manager** or **Treatment Manager** window can be viewed by clicking the icon  above the **Cell List** or **Treatment List** panel. In the **Cell Manager** and **Treatment Manger** window, it is possible to add, remove or edit an entry in the list.

## Setting Up an Experiment



### Cell List/Treatment List: Edit Target Cells and Treatments

The **Cell List** or **Treatment List** on the bottom right includes all the target cells or treatments that have been entered in the plate map when the **Cell** or **Treatment** subpage is selected. Selecting **All Text Context** shows the names, cell density and location of all target cells or treatments, while selecting **All Pie Chart** will show the target cells or treatments based on color in a pie chart format. The distribution of a specific target cell/treatment can be viewed by directly selecting the name in the **Cell List** or **Treatment List**.

#### NOTE

When multiple treatments are present in a well, the well background will be set to white. However, when a specific treatment is selected in the **Treatment List**, only that specific treatment will be listed in the well and the color will change to the defined color.

The selection of **All Text Context** in the **Treatment List** shows the name, concentration, and location of all treatments. Wells with multiple treatments have a white background, as shown below.

## Setting Up an Experiment

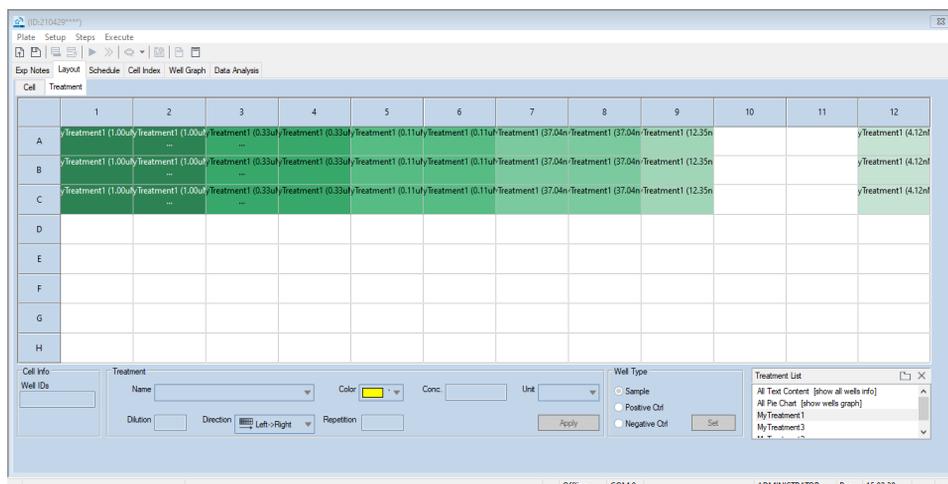
The screenshot shows the software interface with a plate layout table. The table has columns for wells 1-12 and rows for treatments A-H. Each cell contains a list of treatments and their concentrations. For example, well A1 contains 'Treatment1 (1.00u)', 'Treatment2 (1.00u)', and 'Treatment3 (0.33u)'. The 'Treatment List' dropdown is open, showing options like 'All Text Content (show all wells info)', 'All Pie Chart (show wells graph)', 'MyTreatment1', and 'MyTreatment2'. A red arrow points to the 'All Pie Chart' option.

The selection of **All Pie Chart** in the **Treatment List** shows the location of each treatment represented as a color in the pie chart. The color gradient reflects the treatment concentration. Wells with multiple treatments have multiple colors in the pie chart, as shown below.

This screenshot shows the same plate layout, but instead of text, each well contains a pie chart. The colors of the pie charts correspond to the treatments listed in the previous screenshot. For example, well A1 has a pie chart with three segments: green, red, and yellow. The 'Treatment List' dropdown is open, showing options like 'All Text Content (show all wells info)', 'All Pie Chart (show wells graph)', 'MyTreatment1', and 'MyTreatment2'. A red arrow points to the 'All Text Content' option.

The selection of a specific treatment in the **Treatment List** shows the name, concentration, and location of the wells with the selected treatment.

## Setting Up an Experiment



- Renaming Target Cell/Treatment

To rename an item of **Cell** or **Treatment**:

- Use **Update** function in **Cell Manager** or **Treatment Manager** window. (refer [Editing Cell and Treatment Manager Lists](#) for details).
- Or double click an item in the **Treatment List** panel and enter the new name.

- Removing an Item from Cell/Treatment Sub Page

To remove an item from the plate layout:

- In the **Cell/Treatment List** located on the bottom right, click the item you want to remove from the current plate.
- Click the **Delete** icon **X** in the **Cell/Treatment List** tool bar.
- In the confirmation window, click **Yes** to remove the item you selected, click **No** to cancel.

## Rapidly Viewing Well Information within the Plate Map

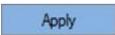
To access the full list of information entered for a specific well in the subpage, hover the mouse over the well and a tooltip will show up, listing content in the selected well.

## Editing Information within a Well

To edit a specific field in a well or a group of wells, select the desired wells and edit the corresponding box.

## Setting Up an Experiment

To edit a well from the plate layout:

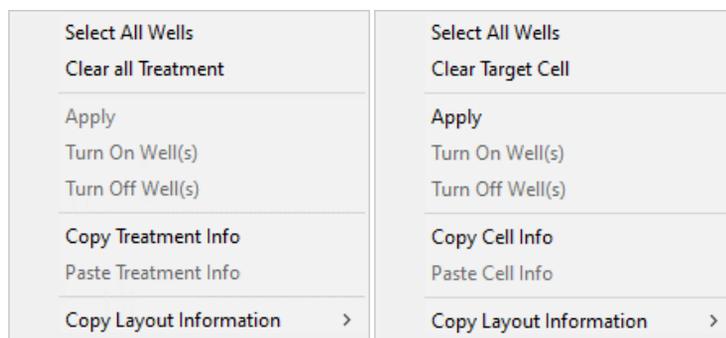
- In the **Cell/Treatment** subpage, select a well or group of wells
- Click the field that needs to be edited
- Edit the field contents or select a new option
- Click **Apply** button  to implement the changes

### WARNING

**When a different treatment is selected or entered in the Treatment subpage, clicking the Apply button will insert the treatment as a second treatment and will not substitute the original. To substitute the treatment, clear the contents of the well using the layout right click menu first and then enter the new treatment information.**

## Layout Right Click Menu

Select a well or a group of wells and right click to access the right click menu as shown below.



The items in the right click menu allow the user to copy, paste, select all, clear all or apply changes to a well or a group of wells.

- **Select All Wells:** Highlights all wells in the **Plate Map**.
- **Clear Target Cell (Target Cell subpage):** Clear all **Target Cell** information from the selected well(s).
- **Clear all Treatment (Treatment subpage):** Clear all **Treatment** information from the selected well(s).
- **Apply:** Apply changes made in selected wells (Same as clicking the **Apply** button).
- **Turn On Well(s):** Turn on selected wells for an experiment. Wells are turned on by default. Experiment can be set up without entering detailed well

## Setting Up an Experiment

information. However, detailed well information is essential for data analysis and it is highly recommended to enter the correct layout information.

- **Turn Off Well(s):** Turn off previously turned on well(s). Well(s) cannot be turned off after an experiment has started.
- **Copy Cell Info (Target Cell subpage):** Copy **Target Cell** information from the high- lighted well(s) to the clipboard.
- **Paste Cell Info (Target Cell subpage):** Paste **Target Cell** information to the destination well(s).
- **Copy Treatment Info (Treatment subpage):** Copy **Treatment** information from the highlighted well(s) to the clipboard.
- **Paste Treatment Info (Treatment subpage):** Paste **Treatment** information to the destination well(s).
- **Copy Layout Information:** Copy **Target Cell** or **Treatment** layout information to the clipboard in either **List Format** or **Block Format**.

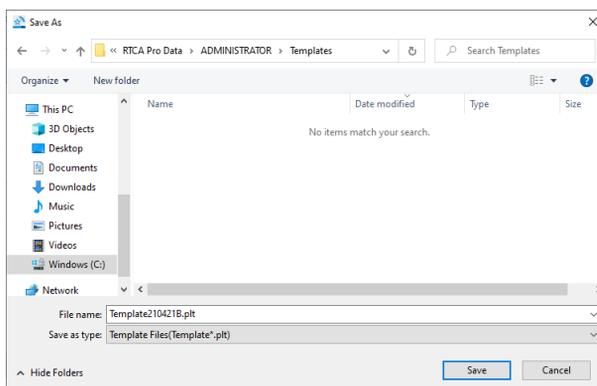
## Saving and Loading a User-defined Plate Layout Template

To save an experiment layout, including experiment notes and schedule, select the **Save** option in the **Plate** drop-down menu in a **Plate Window**. It is also possible to load a full experiment template or only the plate layout from a saved template or existing experiment.

### 1 Saving a User-defined Experiment Template

To save a defined experiment into an Experiment Template, including notes, plate map and schedule:

- From any window, select the **Plate** drop-down menu on the top left of the screen
- Select the **Save as Template** option



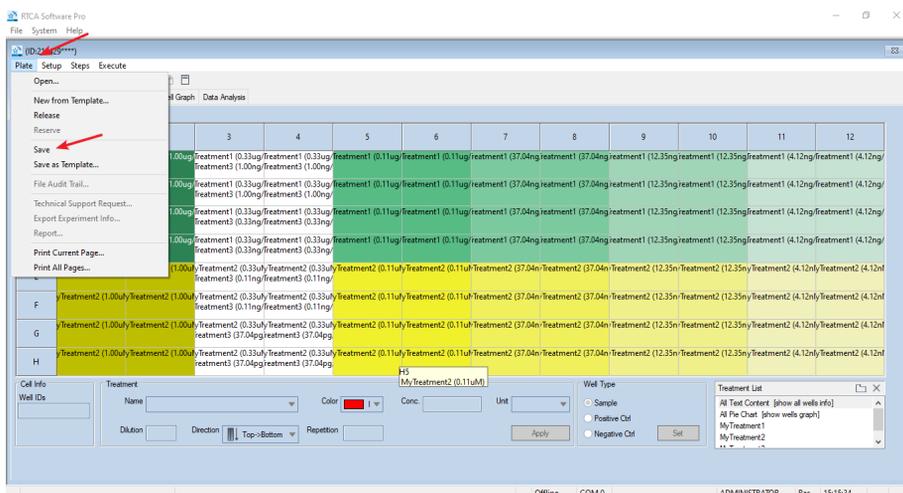
## Setting Up an Experiment

- Select the desired folder to store the experiment template
- Enter the name of the experiment template in the **File name** field
- Click the **Save** button to save the experiment as a template

### NOTE

By default, the software generates a standard Template name followed by the date in the **File name** field. The name can be edited.

Position of the **Plate** drop-down menu and **Save** option in the screen:



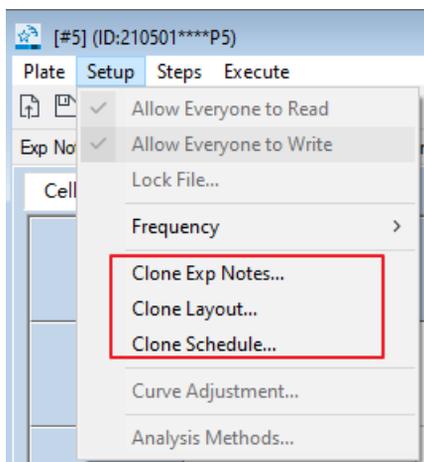
## 2 Loading a Template or Experiment Setting from a Previous Experiment

To load a full experiment or a specific setting from a predefined template or a previous experiment:

- In the **Plate Window**, select the **Setup** drop-down menu
  - Select **Clone Exp Notes...** to copy the **Exp Notes** setting
  - Select **Clone Layout...** to copy the **Layout** setting
  - Select **Clone Schedule...** to copy the **Schedule** setting
- Select the folder and open either a template file or experiment file
- Click the **Open** button and the **Exp Notes** or **Layout** or **Schedule** settings of the selected file will be copied to the new experiment.

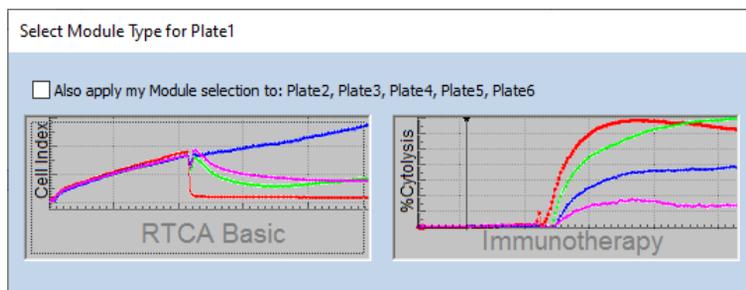
Position of the **Setup** drop-down menu and all clone options:

## Setting Up an Experiment



## Define Plate Layout (Immunotherapy Module)

If multiple modules are active with valid software license, when selecting the **Layout** (or **Data Analysis**) tab, the software will automatically ask which module should be used for plate layout and data analysis. Select **Immunotherapy** to enter **Immunotherapy Module**.



### NOTE

If only the Basic license module is activated, the software by default will not show the **Module Type** selection window and the plate map will open with the basic layout.

### NOTE

For the xCELLigence RTCA MP and DP configurations, the initial module selection (**Basic** or **Immunotherapy**) is only applied to the specified cradle. To apply the same module selection to all cradles, check the “**Also apply my module selection to...**”

## Setting Up an Experiment

When the **Immunotherapy Module** has been selected, it is possible to switch to the RTCA **Basic** module by releasing the plate, selecting the **Layout** tab again and reselect the module.

Defining the plate layout inside the **Immunotherapy Module** of RTCA Pro follows the same general rules of the RTCA **Basic Module**. However, there are additional functions in **Layout**, **Plots**, and **Data Analysis** which are specific for immunotherapy assays.

## Adding Experimental Information to the Wells in the Plate Map: Overview

In the **Cell** subpage, enter the properties of **target cells** (e.g. cell name and seeding density) and other additions such as effector cells (**Effector**), mock control cells (**Mock Effector**) or any compound that induces rapid death of the target cells (**Full Lysis**). Effector cells, in combination with treatments, constitute the samples of interest for analysis purposes. Mock effector cells and full lysis are used as reference controls to determine the % cytolysis. These are automatically included in the calculation algorithm based on the cytolysis parameters selected (see [Plotting Data](#) section).

Two different sections are dedicated in the **Cell** subpage to manage the target and effector cells/controls:

The screenshot shows the RTCA Pro software interface. At the top, there is a menu bar with 'Plate', 'Setup', 'Steps', and 'Execute'. Below the menu bar is a toolbar with various icons. The main area is a plate map grid with columns 1-12 and rows A-H. Below the grid, there are two configuration panels. The left panel is titled 'Target Cell' and contains fields for 'Name', 'Color', 'Number', 'Titration', and 'Repetition', along with 'Left-Right' and 'Apply' buttons. The right panel is titled 'Addition' and contains fields for 'Effector', 'Color', 'Number', 'Titration', and 'Repetition', along with 'Left-Right' and 'Apply' buttons. A 'Cell List' panel is visible on the far right. Two red arrows point from text annotations to the 'Target Cell' and 'Addition' panels.

**This section controls the definition and characteristics of target cells**

**This section controls the definition and characteristics of effector cells, mock effector cells, and full lysis controls**

The **Treatment** subpage has the same layout and features already described for the RTCA Basic software.

### Adding Experimental Information to the Wells in the Plate Map: Details

#### Enter Target Cell Information in the Cell Subpage

The introduction of **Target Cells** follows the same modality described for RTCA Basic:

- On the **Plate Map**, highlight interested well(s) where you want to enter the target cell information.
- Type or select the target cell name in the Name box of the Target Cell field. (Only enter a~z, A~Z, 0~9, and underscore "\_", other characters will be ignored).
- Select a color you want to assign to the target cell you are entering.
- Enter the target cell number in the **Number** box.
- Enter the notes in the **Notes** box.
- Click **Apply** to save the target cell information for the highlighted well(s).

When a new target cell is added to the plate map, it is also added to the **Cell List** box on the bottom right of the screen.

#### Enter Addition Information in the Cell Subpage

To introduce an **Addition**:

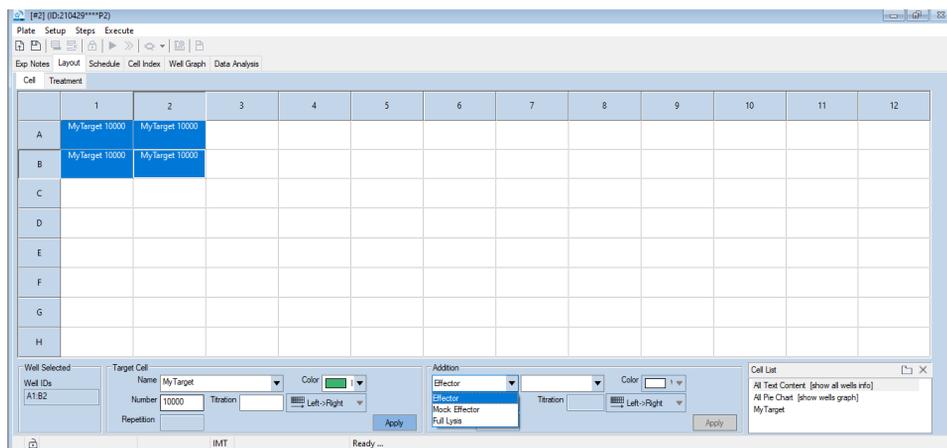
- On the **Plate Map**, highlight interested well(s) where you want to enter the addition information.
- Select between **Effector**, **Mock Effector** and **Full Lysis** in the drop-down menu of the **Addition** field.
- Type or select the name in the box next to the drop-down menu. (Only enter a~z, A~Z, 0~9, and underscore "\_", other characters will be ignored)
- Select a color you want to assign to the addition you are entering.
- Enter the cell number in the **Number** box; the field will be inactivated if **Full Lysis** is selected.
- Enter the notes in the **Notes** box.
- Click **Apply** button to save the addition information for the highlighted well(s).

## Setting Up an Experiment

### WARNING

For each well, only one addition category can be introduced when applying Effector, Mock Effector or Full Lysis options. Furthermore, when Full Lysis is selected, a “Full Lysis” label will be added to the well in the Cell and Treatment subpages. The software will block the addition of other Treatments for the same well.

The drop-down menu defines the type of **Addition** to the well in the **Cell** subpage:



### Enter Treatment Information at Treatment Subpage

Entering the **Addition** information follow the same rules of the RTCA Basic:

- On the **Plate Map**, highlight interested well(s) where you want to enter the treatment information.
- Type or select from the drop-down menu the treatment name (Only enter a~z, A~Z, 0~9, and underscore “\_”, other characters will be ignored).
- Select a color you want to assign to the addition you are entering.
- Enter the concentration in the **Conc.** (Concentration) box.
- Select the concentration unit in the **Unit** box.
- Enter the notes in the **Notes** box.
- Click **Apply** button to save the addition information for the highlighted well(s).

### NOTE

When a new treatment is added to the plate map, it is also added to the **Treatment List** box on the bottom right of the **Layout** page.

## Setting Up an Experiment

### Copying, Pasting and Exporting Data

These operations can be accessed through right-click menu and are generally executed in the same way described for the RTCA Basic. The only difference is that at the level of the **Cell** subpage, clearing, copying, and pasting will affect both the **Target Cell** and **Addition** fields.



### Example of a Completed Layout

The following example shows a typical complete experiment **Layout** page containing two different target cells tested with effector and mock effector cells at different Effector:Target (E:T) ratios. The **Cell** subpage shows the location of **Effector** and **Mock Effector** alone as control wells, as well as **Full Lysis** control wells. The **Treatment** subpage identifies the position and concentration of an antibody and small molecule treatments. The small molecule is tested in combination with the antibody across the different E:T ratios.

**Cell** subpage with two different **Target Cells** seeded in combination with **Effector** and **Mock Effector** cells at several densities, effector alone controls and full lysis controls:

## Setting Up an Experiment

Target+Effector at different E:T ratios

Target+Effectors at different E:T ratios containing also treatments (border is present)

Target+Mock Effectors at different E:T ratios containing also treatments (border is present)

Full Lysis controls

Target+Mock Effectors at different E:T ratios

Target alone controls

Effector alone controls matching different seeding densities

Mock Effector alone controls matching different seeding densities

Light gray wells are inactive wells

Cell	Treatment	1	2	3	4	5	6	7	8	9	10	11	12	
A	MyTarget1 (10000)	Target1 (10000)	Target1 (10000)	Target1 (10000)	MyEffector 20000	MyEffector 5000	MyEffector 2500	MyEffector 1250	MyMock 20000	MyMock 10000	MyMock 5000	MyMock 2500	MyMock 1250	
B	MyTarget1 (10000)	Target1 (10000)	Target1 (10000)	Target1 (10000)	MyEffector 20000	MyEffector 5000	MyEffector 2500	MyEffector 1250	MyMock 20000	MyMock 10000	MyMock 5000	MyMock 2500	MyMock 1250	
C	MyTarget1 (10000)	Target1 (10000)	Target1 (10000)	Target1 (10000)	MyEffector 20000	MyEffector 5000	MyEffector 2500	MyEffector 1250	MyMock 20000	MyMock 10000	MyMock 5000	MyMock 2500	MyMock 1250	
D	MyTarget2 (50000)	Target2 (50000)	Target2 (50000)	Target2 (50000)	MyEffector 20000	MyEffector 5000	MyEffector 2500	MyEffector 1250	MyMock 20000	MyMock 10000	MyMock 5000	MyMock 2500	MyMock 1250	
E	MyTarget2 (50000)	Target2 (50000)	Target2 (50000)	Target2 (50000)	MyEffector 20000	MyEffector 5000	MyEffector 2500	MyEffector 1250	MyMock 20000	MyMock 10000	MyMock 5000	MyMock 2500	MyMock 1250	
F	MyTarget2 (50000)	Target2 (50000)	Target2 (50000)	Target2 (50000)	MyEffector 20000	MyEffector 5000	MyEffector 2500	MyEffector 1250	MyMock 20000	MyMock 10000	MyMock 5000	MyMock 2500	MyMock 1250	
G					MyEffector 20000	MyEffector 10000	MyEffector 5000	MyEffector 2500	MyEffector 1250	MyMock 20000	MyMock 10000	MyMock 5000	MyMock 2500	MyMock 1250
H					MyEffector 20000	MyEffector 10000	MyEffector 5000	MyEffector 2500	MyEffector 1250	MyMock 20000	MyMock 10000	MyMock 5000	MyMock 2500	MyMock 1250

Pie Chart for the same plate map: **Target Cell** are represented on the top half of the pie chart, while **Effector**, **Mock Effector** and **Full Lysis** are on the bottom half of the pie chart.

Target+Effectors at different E:T ratios

Target+Effectors at different E:T ratios containing also treatments (border is present)

Target+Mock Effectors at different E:T ratios containing also treatments (border is present)

Full Lysis controls

Target+Mock Effectors at different E:T ratios

Target alone controls

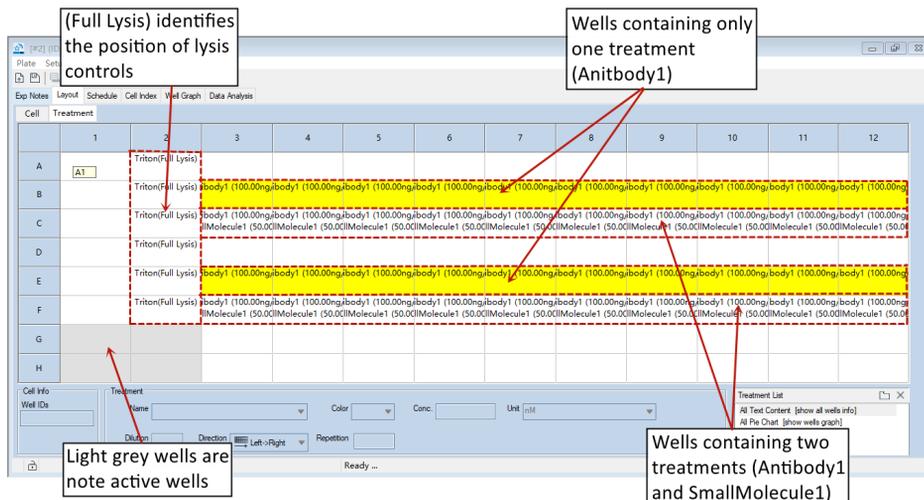
Effector alone controls matching different seeding densities

Mock Effect alone controls matching different seeding densities

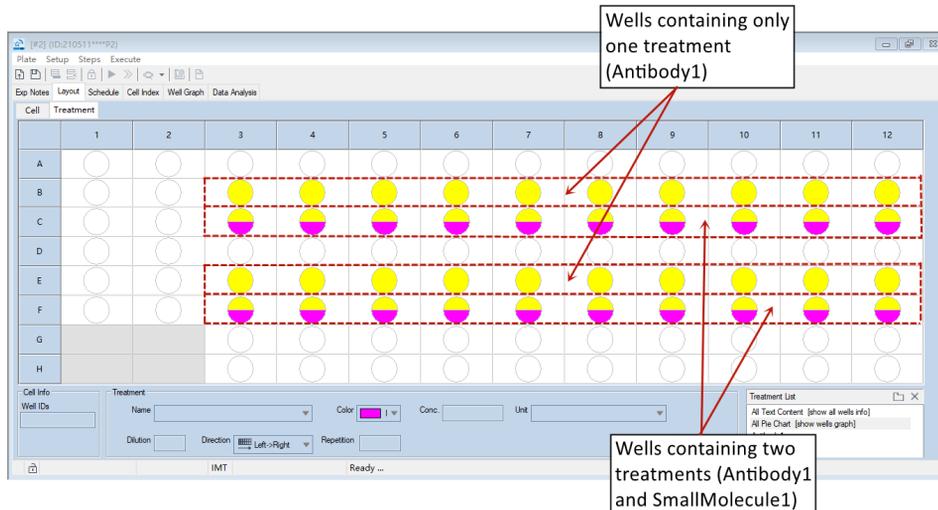
Light gray wells are inactive wells

## Setting Up an Experiment

**Treatment** subpage showing the position of a tested antibody and a small molecule. The **Full Lysis** control is identified also in the plate map.



The same **Treatment** subpage as pie chart.



# Define Experiment Schedule

The xCELLigence RTCA instruments can be programmed to acquire impedance data at different time intervals (every 15 seconds, every 5 minutes, etc.), and for different durations of time (minutes, hours, days, etc.). Before initiating an experiment, the user must define these parameters for data acquisition within the **Schedule** page (the tab for this page is highlighted by the red arrow in the image below).

In the RTCA Software Pro, a “step” is defined as a distinct phase of data acquisition during the course of an experiment. Experiments typically consist of at least three different steps:

- Background measurement.
- Target cell addition and monitoring the target cell attachment and proliferation.
- Addition of a test compound/effector cells and monitoring the cellular response to the treatment.

Within the **Schedule** page you can:

- Add (or delete) experiment steps.
- Set the measurement parameters for each step.

As shown in the image below, within the **Schedule** page there are three main areas. From top to bottom, these include:

- A set of fields for setting schedule parameters.
- A set of fields that display the estimated elapsed time for both the currently running experimental step and for the experiment as a whole.
- A table that displays the details for each of the steps in the experimental schedule.

## Setting Up an Experiment

Seq	Auto	Step	Step Status	Sweep Status	Sweeps	Interval	Unit	Total Time	Comments
1	<input type="checkbox"/>	Step_1	IDLE	0	1	1	minute	00:00:01	
2	<input type="checkbox"/>	Step_2	IDLE	0	100	15	minute	24:45:01	
3	<input checked="" type="checkbox"/>	Step_3	IDLE	0	100	15	minute	49:30:01	
4	<input type="checkbox"/>	Step_4	IDLE	0	100	15	minute	74:15:01	

Functional regions within the Schedule page. The Schedule tab and the Apply button are highlighted by red and green arrows, respectively.

## Adding, Defining, and Editing Steps in the Schedule Page

When setting up a new experiment, the **Schedule** page is initially empty (i.e. no steps are present in the **Step Information Table** at the bottom of the page). There are three different ways to add steps to the **Schedule** page:

- Click the **Add a Step** icon  on the tool bar, or
- Click **Steps** within the **Plate Window** menu; then select **Add a Step**, or
- Hover the cursor over the **Step Information Table**, right-click, and then select **Add a Step** from the pop-up menu.

After a step is added to the **Step Information Table**, it will be highlighted (with the entire row turning dark blue).

### Step #1 is Automatically Used for Background Measurement

#### WARNING

The first step added to the Schedule page is automatically commandeered by the Software for the purpose of taking a background/reference reading. This background step must be performed when each well of the E-Plate contains only tissue culture media (i.e. before cells are added).

When a user adds the first step to the **Schedule** page, the Software automatically sets the parameters for this step as follows: **Sweeps** = 1 and **Interval** = 1 minute (see section below for an explanation of these parameters). The user should not change these parameters for the background measurement step.

## Setting Up an Experiment

### Defining Step Parameters

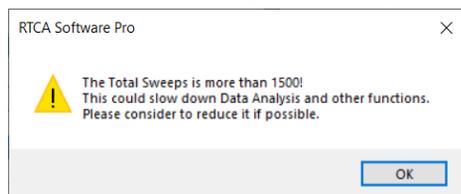
To define the parameters for a step in the data acquisition schedule, first highlight the step of interest in the table by clicking on it; the entire row will turn dark blue. The text boxes and buttons at the top of the **Schedule** page (within the red **Setting Schedule Parameters** box in the above image) are now used for defining the parameters. The fields/terms used for this purpose are as follows:

- **Step:** This field allows you to name each step of the experiment. If steps aren't given a unique name by the user, by default they will be named as "**Step\_1**", "**Step\_2**", etc.
- **Sweeps:** A "sweep" is defined as a single round of acquiring impedance data for each of the selected wells of an E-Plate (i.e. those wells that in the **Plate Map** of the **Layout** page have been chosen to acquire data from).

#### NOTE

The number of **Sweeps** should be given within a reasonable value, therefore the max sweeps for a step is limited to 9999.

When the total **Sweeps** for all steps exceed 1500, a warning message is shown:



- **Interval:** Interval defines how regularly impedance data is acquired (i.e. the frequency at which Sweeps are conducted). The **Interval** can have units of hours, minutes, or seconds. As an example, if the **Interval** is set to be 10 minutes, one sweep will be initiated every 10 minutes.
- **Duration:** **Duration** defines how long a step will last for. The duration of a step will automatically be calculated when the number of sweeps and the sweep interval have been input. For example, if a step is programmed to conduct 3 sweeps at an interval of 10 minutes the Duration field will automatically display a time of 20 minutes, corresponding to the following sequence:

sweep#1 > 10-minute lapse > sweep#2 > 10-minute lapse > sweep#3

Alternatively, when the duration of a step is defined along with the interval, the number of sweeps will automatically be determined.

In short, the details of each step in the experiment are easily defined using the fields and buttons at the top of the Schedule page. To quickly gain proficiency with defining step parameters, it is most efficient to simply enter values into a given

## Setting Up an Experiment

field and then observe how other fields automatically adjust in response to this entry.

### NOTE

Once the parameters for a step have been input, click the **Apply** button (highlighted by the green arrow in the above image) to save/transfer this information to the step information table at the bottom of the **Schedule** page.

### Starting a Step Manually vs. Automatically

It is often advantageous to record impedance at different time intervals over the course of an experiment. This is accomplished by acquiring data in distinct steps, where each one has its own unique data acquisition interval. Importantly, the experiment schedule can be programmed so that the transition from one data acquisition step to the next occurs automatically (requiring no user action) or manually (where the user must initiate the next step after a previous step has been completed).

In the image below, selecting the **Auto** box for the “**Growth**” step causes this step (in which measurements are made once per hour) to begin immediately after the “**Adhesion**” step (in which measurements are made every 5 minutes) has been completed.

Seq	Auto	Step	Step Status	Sweep Status	Sweeps	Interval	Unit
1		Background	IDLE	0	1	1	minute
2	<input type="checkbox"/>	Adhesion	IDLE	0	50	5	minute
3	<input checked="" type="checkbox"/>	Growth	IDLE	0	24	1	hour
4	<input type="checkbox"/>	Treatment	IDLE	0	100	15	minute

Once a step has been completed, if the **Auto** box for the next step is not selected the instrument will simply idle. When idling, no data is actively being collected but the experiment is still open, and the next step of data acquisition can be manually initiated by clicking the **Start/Continue** ► button.

### Building in Flexibility by Including an “Extra” Step

When the final step of data acquisition is completed the Software terminates the experiment and generates a finalized version of the data file. Once this happens, though it is still possible to acquire new data using the same plate, all the new data will be stored in a different data file. Because of this, when scheduling an experiment, it is best to program the final step to run for a longer period of time than the user deems necessary. This gives the user the chance to review the current status of the experiment and, on the basis of this, decide whether to continue or terminate data acquisition. As an alternative to employing an extra-long duration for the final step, simply add, at the end of the experiment, an extra step that will not start automatically. In this scenario, when the Software finishes the penultimate step it will simply idle, waiting for instruction from the user. If the

## Setting Up an Experiment

user then determines (the next morning, for example) to continue monitoring impedance, all subsequent data will still be stored in the same contiguous data file.

### Editing the Schedule of a Step

To change the schedule of a previously entered/saved step, click the step of interest in the table (causing it to turn dark blue). Then, simply make the necessary changes in the fields at the top of the page (sweeps, interval, duration, etc.) and click the **Apply** button. The new settings will then appear in the table.

### Deleting a Step

To delete a step, click the step of interest in the table (causing it to turn dark blue). Then, click the  button. Alternatively, click the step of interest in the table, right click within the highlighted row, and then select **Delete a Step** from the drop-down menu that appears. As a final option, after clicking on the step of interest in the table, click on **Steps** within the page window menu and then click **Delete a Step**.

### Inserting a Step into an Existing Schedule

To insert a step into an existing experiment schedule, within the step information table at the bottom of the **Schedule** page, click the step that you'd like your new step to follow (e.g., click on Step 2 if you want to insert a step between Step 2 and 3). Next, click the  button. Alternatively, go to the **Steps** menu and select **Add a Step**.

#### NOTE

Although the newly inserted step will be named Step\_X, the user can change the name.

#### NOTE

The steps below the inserted step are automatically moved down a row and the number of each step is reassigned accordingly.

### “Total Time/Finish At” Column

The **Total Time** column in the schedule table indicates the estimated total time needed for the experiment to reach a step. Clicking on the **Total Time** heading in the table will change the heading to be **Finish At**, which indicates the time at which a step will finish.

## Setting Up an Experiment

Seq	Auto	Step	Step Status	Sweep Status	Sweeps	Interval	Unit	Finish At	Comments
1	<input type="checkbox"/>	Background	IDLE	0	1	1	minute	2021/04/29 16:19	
2	<input type="checkbox"/>	Adhesion	IDLE	0	50	5	minute	2021/04/29 20:24	
3	<input checked="" type="checkbox"/>	Growth	IDLE	0	24	1	hour	2021/04/30 20:24	
4	<input type="checkbox"/>	Treatment	IDLE	0	100	15	minute	2021/05/01 21:09	

### NOTE

The specific times provided for **Total Time** and the **Finish At** are estimates only. The actual time may be different when:

- Pause/Continue/Abort a step is selected during the run.
- A step is added/deleted.
- Sweeps/Intervals are changed.
- Intervals assigned to a step are too short (for example, if a 1 second interval is assigned to steps).

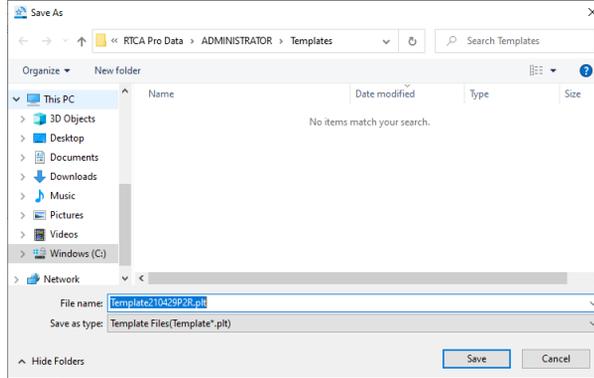
## Saving and Re-Using Experimental Set-ups

As it has been described in detail throughout this chapter, a large number of parameters are defined during the course of setting up an RTCA experiment. Because of this, re-using experimental setups can be a big timesaver. There are two ways to quickly set up an experiment which are described below.

### Creating a Template

While setting up an experiment (but before initiating data acquisition), if it seems likely that the information contained within the **Exp Notes**, **Layout** and **Schedule** pages could be helpful in setting up future experiments, save this set-up information as a Template file. Do this by clicking the **Save** button in **Plate** drop-down menu. A **Save As** dialog will appear:

## Setting Up an Experiment



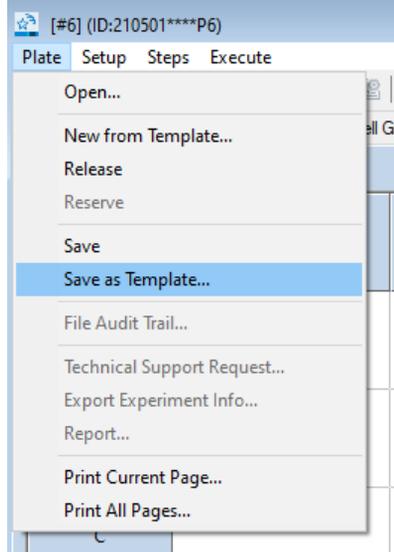
For the xCELLigence RTCA SP the default file name is “TemplateYYMMDD”, although the user can change this to be whatever they wish. The template file contains all the information contained within the **Exp Notes**, **Layout**, and/or **Schedule** pages.

Template files can be used to quickly set up future experiments, by using the template file settings directly or modifying certain settings.

### Saving as Template

Instead of creating a template before data acquisition, user can also save an existing experiment file as a template. To do this, click **Plate > Save as Template**, then select the folder and enter the name of the template file in the prompted window.

## Setting Up an Experiment

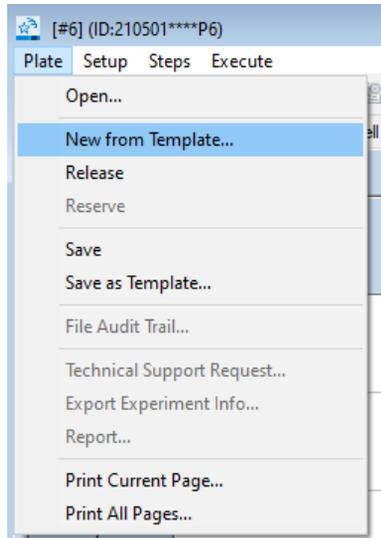


The advantage of this method is that besides **Exp Notes**, **Layout** and **Schedule**, the **Report** and **Analysis Methods** defined in the experiment data file are also saved to the template file.

### New from Template

To run a new experiment from an existing template, click **Plate > New from Template**, then choose the template file in the open file dialog. The **Exp Notes**, **Layout**, **Schedule**, **Report** and **Analysis Methods** stored in the template file will be loaded into the new experiment file.

## Setting Up an Experiment

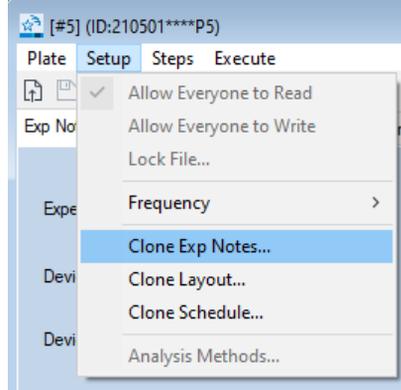


### Generating a Clone

If a user would like to run a new experiment using an experimental setup that is identical to (or similar to) a previously run experiment, but a template file containing the **Exp Notes**, **Layout**, and/or **Schedule** pages was not previously saved, a **Clone** of the previous experiment can be made by doing the following:

- 1 Open a new experiment with RTCA Software Pro.
- 2 Within the **Setup** menu of the **Plate Window**, choose from the dropdown menu either:
  - a **Clone Exp Notes**, or
  - b **Clone Layout**, or
  - c **Clone Schedule**.

## Setting Up an Experiment



A dialog box will then appear, allowing the user to select the previously saved file they wish to clone.

### NOTE

The **Clone** function will load only the experiment settings from the previous experiment. Data in the previously-run experiment file are not loaded or changed.

## 6 Running an Experiment

This chapter describes running an experiment including checking connections between the contact pins in the cradle and E-Plates and measuring impedance.

Once the layout of wells in the E-Plate has been established in the **Layout** page, and a data acquisition program has been recorded in the **Schedule** page, an experiment is ready to be run. Similar to previous chapters, this chapter describes the use of an xCELLigence RTCA SP with the RTCA Software Pro. When protocols for the xCELLigence RTCA DP and MP differ from the xCELLigence RTCA SP, instrument-specific instructions are provided.

### Checking the Connections between the Instrument and E-Plate

Small spring-loaded pins located in the station interface with the bottom of the E-Plate(s) to enable measurement of impedance in all the wells of the plate. To ensure the integrity of these interactions between the instrument and the plate, a quick scan can be made. While this scan can be initiated manually, the instrument also does this automatically each time a plate is placed inside the cradle.

#### Checking Connections: Scanning Plate Manually

When a well has been activated in the **Layout** page, the Scan Plate  icon in the tool bar will become active; the **Scan Plate** option within the **Execute** menu will also become active. Initiating Scan Plate by either of these options causes the Software to immediately measure the resistance of activated wells.

#### NOTE

The **Scan Plate** button is disabled while the SP/MP Analyzer is performing a measurement.

The **File Audit Trail** window will display the results of the scan and indicate if there is any problem with the connections between the instrument and the E-Plate. A faulty connection will give rise to an impedance measurement that is outside the normal range; if this occurs the identity of the problematic well(s) will be listed in

## Running an Experiment

the **File Audit Trail** window. Instructions for correcting connection issues are described in a later section of this chapter.

Each time the **Scan Plate** function is initiated manually (either before or during an experiment), the event is recorded in the **File Audit Trail** window. If the actual experiment has not yet been initiated, the scan data are saved to the following location: **C:\Users\Public\RTCA Pro Data\Username\ScanPlateData** and will have a file name with the following format: PlateScanningYYMMDD.txt

If more than one scan is performed on the same day, the data from all of these scans are saved in the same file, with the newest data being appended to the end of the existing scan data file.

Once the actual experiment (where impedance is being monitored according to the parameters defined by the user) is initiated, the data from the last 10 manually-initiated plate scans will be saved, together with the experimental impedance data in the experiment's \*.plt file. During the course of the experiment, the data from any new manually-initiated plate scan will be saved in the same \*.plt file.

## Checking Connections: Scanning Plate Automatically

To facilitate monitoring the integrity of an E-Plate's connection to the Station, the RTCA Software Pro has a built-in plate scanning function. Each time it detects an E-Plate being placed into the Station, the Software will automatically perform a Scan Plate measurement. This scan data is automatically saved and can be viewed in the same way as scan data generated manually (described above).

## Scanning Plate: Example Results

Once the **Scan Plate** function has been completed, the results are shown in two locations. First, the **Cell Index** page (tab is highlighted by the red arrow in the below image) shows the impedance and Cell Index values for all scanned wells.

## Running an Experiment

The screenshot shows the RTCA Software Pro interface. The 'Raw Data Type' dropdown menu is set to 'Plate 96'. Below it, the 'Raw Scan Data' window displays a table of impedance values for a 96-well plate. The table has columns for well indices 1 through 12 and rows for plate sections A through H. The values represent impedance measurements for each well.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0822	0.1749	0.1160	0.0986	0.1111	0.0950	0.1682	0.1793	0.5069	0.6592	5.5193	4.1351
B	-0.0431	-0.0413	0.0005	-0.0255	-0.0440	-0.0476	0.0002	-0.0027	0.1488	0.1648	4.4306	3.5164
C	0.0300	0.0841	0.0312	0.0133	0.0636	0.0987	0.2218	0.0523	1.4852	0.5359	4.0604	2.7861
D	-0.0116	0.0352	0.1536	0.0711	0.0392	0.0373	0.1603	0.1850	1.0644	1.7568	4.0446	3.0842
E	0.4752	1.3537	2.3864	1.9554	2.9028	2.5234	2.7662	3.4019	3.7147	2.2787	3.5623	3.5613
F	0.8883	0.3744	1.9817	1.6766	2.5141	2.5246	2.3919	2.5499	3.5044	4.0666	3.6059	4.6153
G	4.4948	1.8425	4.9221	1.0082	5.0449	0.7789	4.9587	0.0541	4.7317	-0.0933	3.9015	4.3634
H	4.3852	0.4122	5.6301	0.1971	5.8877	0.3934	5.6230	0.1442	5.7318	0.0424	5.3189	5.6981

Second, the **File Audit Trail** window indicates that the **Scan Plate** function has been used. If no problems are detected, the following message will be listed: **“Plate Scanned. Connections OK”**.

The screenshot shows the File Audit Trail window. It displays a log of system events. The 'Scan Plate' action is highlighted, with the description 'Plate scanned. Connections OK.' The log includes columns for ID, Batch, Timestamp, Experiment Time, Username, Category, Action, Description, and Reason.

ID	Batch	Timestamp	Experiment Time	Username	Category	Action	Description	Reason
8	7	2021/05/06 10:35:35	00:00:23	ADMINISTRATOR	Information Message	Scan Plate	Plate scanned. Connections OK.	
7	6	2021/05/06 10:35:34	00:00:24	ADMINISTRATOR	Experiment Operation	Scan Plate	Scan plate.	
6	5	2021/05/06 10:35:22	00:00:12	ADMINISTRATOR	Information Message	Finish Experiment	Experiment finished.	
5	4	2021/05/06 10:35:22	00:00:12	ADMINISTRATOR	Information Message	Finish Step	Step [Step_1] finished.	
4	3	2021/05/06 10:35:17	00:00:07	ADMINISTRATOR	Warning Message	Run Experiment	Measure Background: Following wells may have background problems: A1,A3,A4,A5,A6,A8,A9,A11,A12,C2,C6,C7,C9,C10,D3,D4,E	
3	2	2021/05/06 10:35:10	00:00:00	ADMINISTRATOR	Information Message	Start Experiment	Experiment started.	
2	1	2021/05/06 10:35:10	00:00:00	ADMINISTRATOR	Experiment Operation	Start experiment	Start experiment.	
1	1	2021/05/06 10:35:10	00:00:00	ADMINISTRATOR	File Settings	Select Module Type	Select module type: RTCA Basic.	

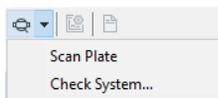
However, if the impedance value of a well is higher than 500 ohms, the **File Audit Trail** window will report that this well may have a connection problem.

## Diagnosing a Connection Problem Using a Resistor Plate

If a connection warning is shown in the **File Audit Trail** window, this can often be resolved by removing the E-Plate and gently wiping the connecting pads on the

## Running an Experiment

PCB at the bottom of the E-Plate with a soft lint free cloth. If the connection problem persists, one can determine whether the problem is with the E-Plate or the xCELLigence contact pins. To do this, remove the E-Plate from the instrument, insert a Resistor Plate into the cradle, and click the **Check System** option from the **Scan Plate** drop-down list.



The RTCA Software will perform a resistor scan, the results of which will be shown on both the **Cell Index** page and the **File Audit Trail** window.

## Fixing Connection Issues

### NOTE

For detailed instructions on resolving E-Plate Contact Pin connection problems, please refer to your [RTCA Instrument Operator's Manual](#). Specifically, the 'Maintenance and Care' section includes instructions on cleaning and exchanging the xCELLigence Contact Pins.

## Measuring Impedance

The **Layout** page and **Schedule** page must be set up before an experiment can be initiated. Once these two pages have been set up, the **Start/Continue** ► button becomes active. Click this button to begin measuring impedance.

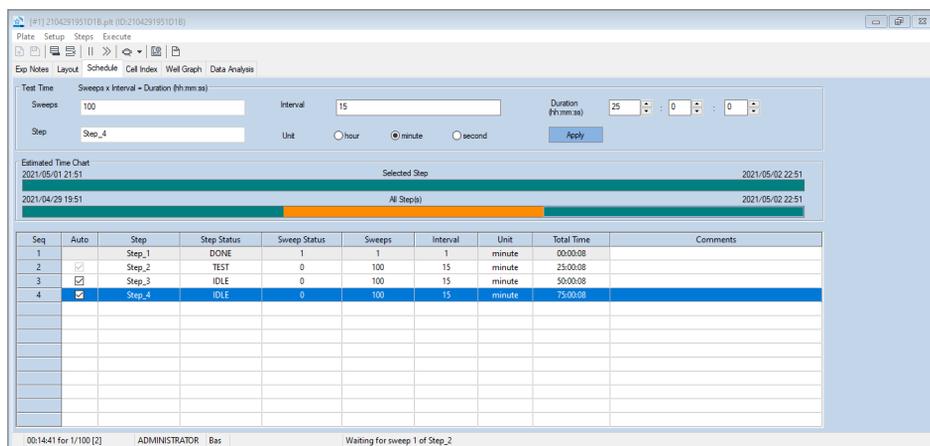
### WARNING

**When the experiment starts, the Software measures the background first. Do not remove the E-Plate while the Software is measuring the background.**

## Tracking an Experiment's Progression

As data acquisition progresses, the status of the experiment can be monitored in the **Step Status** column on the **Schedule** page (see image below). The status can be listed as **DONE** (step is finished), **TEST** (step is in progress), **IDLE** (step has not started yet) or **PAUSE** (step is paused).

## Running an Experiment



## Continuing a Terminated Experiment

If an experiment is accidentally terminated, or if it has been manually paused by the user (see below), it can be resumed by doing the following:

- 1 Launch the RTCA Software Pro.
- 2 Within the **Plate** menu, select **Open**.
- 3 Select the original experiment file of the terminated experiment and click the **Open** button.
- 4 After the Software has successfully loaded the experiment file (.plt), the **Start** ► button will become the **Continue** ► button (as long as there are unfinished steps left in the data acquisition schedule).
- 5 By clicking the **Continue** ► button the xCELLigence instrument will automatically continue measuring impedance and all calculations will be performed using the original background data (i.e. the background measurement taken before the experiment was terminated). This new data gets appended to the original experiment file. Neither the experiment file name, nor the experiment ID, nor the file location will be changed.

### WARNING

**Do not use this Continue function if the experiment was terminated before the initial background measurement was completed. When this is the case, always set up the experiment again from the beginning.**

## Running an Experiment

### Pausing an Experiment

After an experiment has been initiated, the start ► button automatically changes into the pause || button. Click this button to pause the current unfinished step (and the whole experiment). Because impedance data is automatically saved as a sweep is performed, it isn't necessary to save the experiment file before pausing an experiment.

#### NOTE

**Pause** temporarily stops a step, so the user can perform actions, such as adding compounds, checking connections, etc. without data being collected. The user can subsequently resume the experiment and complete the remaining sweeps of the paused step.

#### NOTE

After the user has clicked the pause || button, the button will automatically change into the ► Continue button. This indicates that the RTCA Instrument is ready to resume the experiment. Once the user clicks the ► button, it automatically changes back into the || button, making it possible to pause the experiment again.

### Starting an Idle Step

As explained in detail in the section describing the **Schedule** tab, an experimental step can be scheduled to automatically initiate after the preceding step is completed. In order for this automatic progression to occur, the **Auto** box for the step of interest must be selected within the scheduling table. If this box is not selected for a given step, that step will simply sit idle until the user initiates it manually. In this context, the ► button will become active, and clicking it will initiate the next step.

### Aborting a Step

To terminate the currently running step, click the Abort Step >> button. You can terminate the current step and initiate the next step at any time.

#### WARNING

**The Abort Step button stops the current step and discards all the unfinished (remaining) sweeps of the step. Once aborted, a step cannot be resumed. To continue the experiment, you must manually start the next step, if there is one.**

#### NOTE

Aborting a step does NOT lead to invalidation or loss of data.

# 7

## Monitoring an Actively Running Experiment

This chapter describes monitoring an actively running experiment using status bar, **Cell Index** page and **Well Graph** page.

In addition to analyzing impedance data upon completion of an experiment, it is often useful to analyze the data while the experiment is still in progress. This chapter shows how to do this when using an xCELLigence RTCA SP. The protocol is the same when using an xCELLigence RTCA MP, except that the steps need to be applied to each of the six plates individually. When using an xCELLigence RTCA DP instrument the only difference will be in the well layout configuration, where instead of 96 wells there will be 16, 32, or 48 wells (depending on how the user has set up the experiment).

The status of an experiment (which step it is currently running), the timing of pending data acquisition steps, impedance values at the last time point, and many other parameters can all be monitored while an xCELLigence instrument is actively running an experiment. The primary tools for monitoring an actively running experiment are detailed below.

### Status Bar

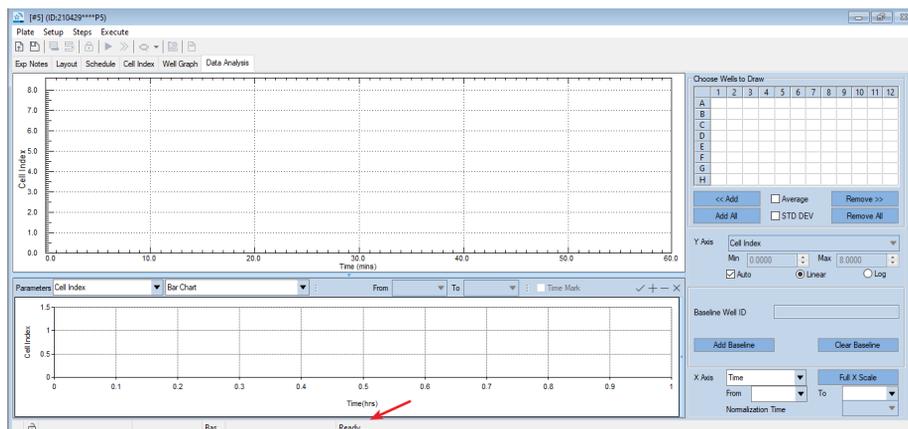
The RTCA Software Pro status bar, which reports the current state of the experiment, is located on the bottom left corner of the Software window (denoted by the red arrow in the figure below). The status bar displays one of the following messages:

- **Ready** (before the start of an experiment)
- **Ready for starting next step** (waiting to start the next step after completing a step)
- **Executing sweep # of step #** (while the instrument is performing a sweep measurement)
- **Waiting for sweep # of step #** (waiting for the start of the next sweep)

During a sweep measurement, a portion of the status bar will display **Test Col #**, which denotes the column number (in the E-Plate) that is presently being

## Monitoring an Actively Running Experiment

measured. Between sweeps, a portion of the status bar will count down the time remaining until the next sweep is initiated.



## Cell Index Page

The impedance data recorded by xCELLigence is reported using a unitless parameter called Cell Index, which is defined as follows:

$$\text{Cell Index} = (R_{tn} - R_{t0})/Fi$$

Where:

$i = 1, 2, \text{ or } 3;$

$F1 = 15\Omega, F2 = 12\Omega, F3 = 10\Omega;$

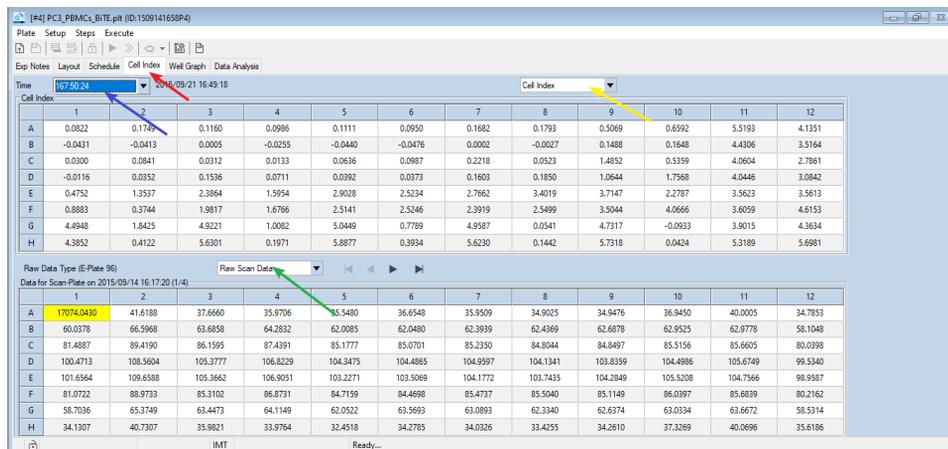
and  $n = 0, 1, 2, \dots, N$  (time points)

### NOTE

$R_{t0}$  is the background resistance measured at time point  $T_0$ ,  $R_{tn}$  is the resistance measured at a time point  $T_n$ .

The impedance data acquired at any time point can be viewed in a tabular format on the **Cell Index** page (the tab for which is highlighted by the red arrow in the below image). Use the **Time** drop-down menu (highlighted by the blue arrow) to define which time point the table displays data for.

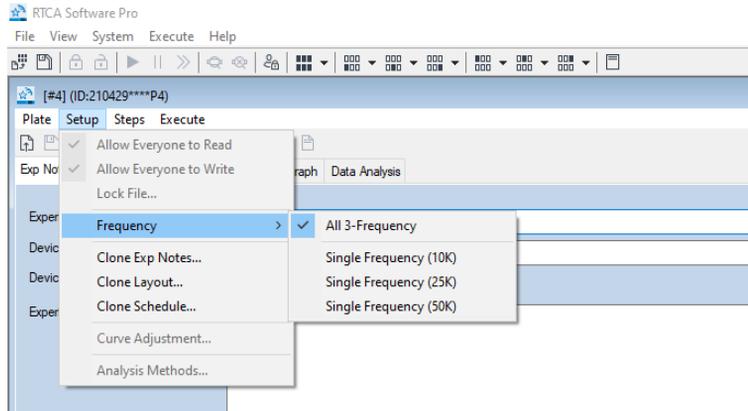
## Monitoring an Actively Running Experiment



The upper table on the **Cell Index** page reports Cell Index values, calculated as described above, for all wells in the E-Plate which are activated (i.e. only those wells selected for data acquisition on the **Layout** page). Depending on which option is selected using the drop-down menu highlighted by the green arrow, the lower table on the **Cell Index** page reports either the raw resistance data or the background data.

Depending on the nature of the cells being analyzed and the type of assay being conducted, the quality and consistency of impedance assays can vary slightly depending on the frequency of the alternating current that is used in making the measurements. Because of this, xCELLigence measures impedance at three different alternating current frequencies: **Cell Index** is measured at 10 kHz, **Cell Index-II** is measured at 25 kHz, and **Cell Index-III** is measured at 50 kHz. Define which of these three data sets is displayed in the **Cell Index** page by using the drop-down menu highlighted by the yellow arrow in the above image. Note that, because it is the most universally useful frequency, by default xCELLigence uses the data acquired at 10 kHz (for plots, etc.) unless the user specifies otherwise. To monitor at a frequency other than the default 10 kHz, prior to starting an experiment the user must specify this within the **Setup** menu, as shown below.

## Monitoring an Actively Running Experiment



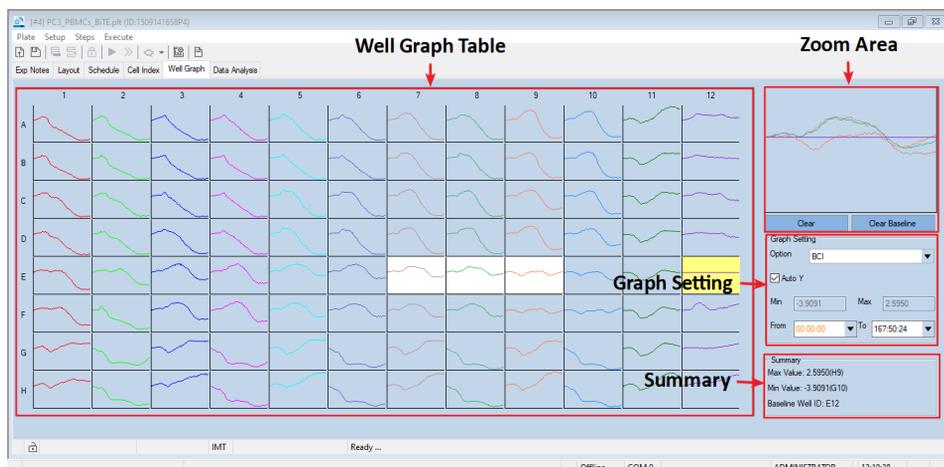
## Exporting Cell Index Data/Raw Data

In the event that the user would like to analyze the Cell Index data and Raw data using a different software, these data tables can readily be copied and pasted into text files or spreadsheets. Simply select the time point of interest (using the drop-down menu), and then use Ctrl+C to copy selected table contents in **Cell Index** page. Click the top left corner of table to quickly select entire table.

## Well Graph

The **Well Graph** page provides an overview of the Cell Index curves for all active wells by displaying them in an 8 x 12 matrix format.

## Monitoring an Actively Running Experiment



The four sections of this page, each of which is enclosed by a red box in the above figure, serve distinct purposes which are described in the sections that follow.

### Well Graph Table

For an experiment that was previously run or that is currently open, any impedance data that was acquired is displayed as Cell Index curves within the Well Graph Table (see above image). While an experiment is running (i.e. when data is actively being acquired) these curves are updated in real-time. Though the Cell Index curves for all the wells in the plate can also be displayed within the **Data Analysis** page, the unique tabular format in the **Well Graph** page can be useful for quickly making comparisons between different wells or sets of wells. To take full advantage of the functionality of this page, the **Zoom Area** should be utilized, as described in the next section.

### Zoom Area

The user can generate an expanded view of specific curves from the **Well Graph** Table, or compare multiple curves side-by-side, by using the **Zoom Area** located at the top right part of the page (see above image). To do so, simply left click on the wells to be expanded/ compared. A color change from light blue to white indicates which wells are being displayed in the **Zoom Area**. To remove a selected well from the **Zoom Area**, left click on the well within the table; at this point its color will change from white back to light blue. To rapidly clear all the curves displayed within the **Zoom Area**, click the **Clear** button located directly beneath it. To use one of the wells as a baseline/reference that all the other curves in the **Zoom Area** are plotted relative to, right click on it within the table. The color of this well will change from light blue to yellow, and the impedance values from this real-time data trace

## Monitoring an Actively Running Experiment

will now be subtracted from the other curves being displayed in the **Zoom Area**. In the above figure, the data traces from wells E7, E8, and E9 are being plotted with well E12 being used as the background/reference. When a background/reference curve is being used, the impedance traces for all the other curves are calculated, at each time point, using:

$$CI_{new} = CI_{original} - CI_{reference}$$

Using RTCA Basic mode, in addition to being highlighted in yellow within the **Well Graph Table**, the identity of the well(s) being used as the baseline/reference will be displayed in the **Summary** area of the **Well Graph** page.

### Graph Setting

Precisely what is being plotted in the **Well Graph Table** and **Zoom Area** can be controlled using the functions within the **Graph Setting** area (see image above). These different functions are explained below:

- **Option:**

The **Option** function is available only when one or more wells in the **Well Graph Table** are being used as a background/reference. In this case, three different plot types are selectable within the **Option** field. These three plot types differ from one another in the way that data traces from sample wells are plotted relative to the background/reference well(s).
- **Auto Y vs. Min and Max:**

Selecting the **Auto Y** box causes the software to automatically adjust the Y-axis of the plots. Deselecting **Auto Y** enables manual adjustment of the Y-axis; the minimum and maximum values can be defined using the **Min** and **Max** fields, respectively.

#### NOTE

Please note that the value of **Min** must be less than that **Max**.

- **From XXX to XXX:**

Use these drop-down menus to select the time range being plotted within the **Well Graph Table** and **Zoom Area**.

### Summary

The **Summary** area lists information about the plots in the **Well Graph Table** and **Zoom Area**. The minimum Cell Index value, and the ID of the well that this occurs in, is listed. The maximum Cell Index value, and the ID of the well that this occurs

## Monitoring an Actively Running Experiment

in, is also listed. In the above figure, out of all 96 wells in this plate the well that has the highest Cell Index value is H4, and its value is 2.554. The well that has the lowest Cell Index value is F11, with a value of -0.2182. Also included in the **Summary** area is a list of which (if any) wells are being used as background/reference.

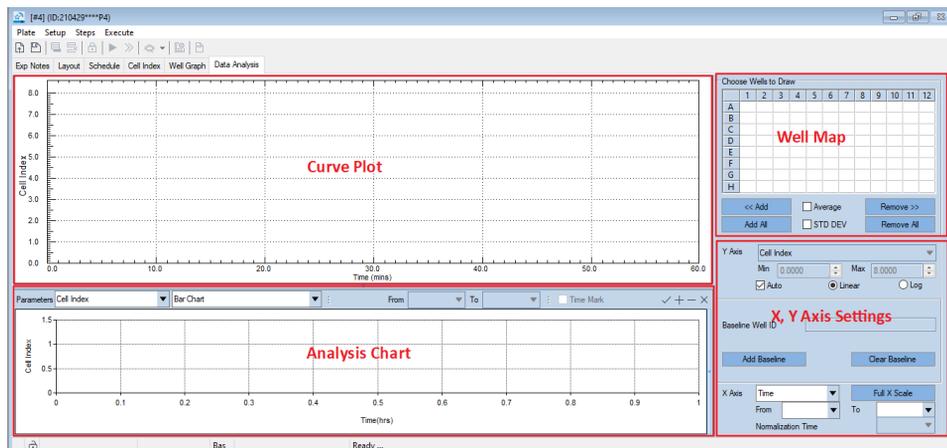
## 8 Plotting Data

This chapter describes how to plot data including: open experiment data, select displayed curve type (RTCA Basic and Immunotherapy module), use curve adjustment tools and copy plot information.

The **Data Analysis** page (shown below) allows to display collected data and to measure parameters across the temporal scale. The page layout automatically updates depending by which of the software module is active (Basic or Immunotherapy).

This page contains four main functional areas:

- **Well Map** is used to select wells, the data for which will be displayed in the Curve Plot.
- **Curve Plot** displays Cell Index or other parameters as a function of time or treatment. Data can be plotted for individual wells or for the average of well replicates.
- **Analysis Chart** reports information, such as Cell Index at a specific time point for wells that have been selected in the **Well Map**. Time ranges for analysis to be performed in this area are selected in the **Curve Plot**.
- **X, Y Axes Settings** enables to change how data are displayed within the **Curve Plot**. Functionalities include defining the type of curve that is plotted (**Cell Index**, **Normalized Cell Index**, etc.) and the scale of the curves.



# Opening Experimental Data

Depending on how often impedance is being recorded during an experiment, it may be possible to perform data analysis while an experiment is still running.

### NOTE

If data acquisition is infrequent (for example, if the time interval is every 30 minutes or more), then data analysis can be performed at the same time that an experiment is running.

### WARNING

**If data acquisition is occurring frequently (e.g., a time interval of 5 minutes or less), data analysis should not be performed online (because the analysis uses computer resources that are required for running the experiment).**

Data Plotting and Analysis can be performed after opening any existing RTCA experiment data file. Follow these steps to load existing experiment data:

- 1 Click **Plate > Open** in the plate menu.
- 2 Select the experiment file (.plt) from the **Open File** dialog.
- 3 Click **Open** to load the experiment data into the RTCA Software.

## Open Data File by Double-Click

You can open a data file (.plt) by double-click the file from Windows Explore

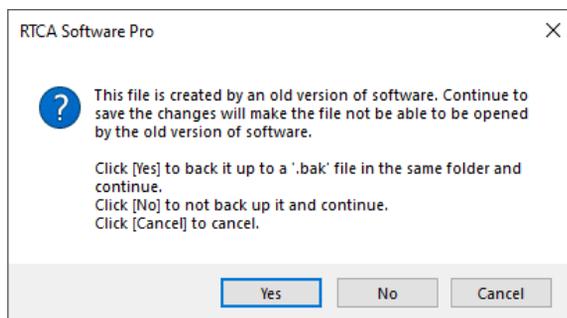
## Open Data File by Drag-Drop

You can also open a data file (.plt) by drag-drop, i.e. drag the .plt file, then drop it to the RTCA Software Pro icon on Windows desktop.

## Open Data File Created by Previous RTCA Software

The RTCA Software Pro is compatible with data files generated by previous RTCA software. When saving a data file generated by previous RTCA software, a dialog window is shown

## Plotting Data



Choose **Yes** to convert the old plt to new software plt file format. Meanwhile, a copy of the original file is saved in the same folder with “.bak” file name. Choose **No** to convert without creating the backup.

## Well Map

Within the **Data Analysis** page, the **Well Map** is used to select the well or wells whose curves will be displayed in the **Curve Plot**. To plot the impedance data from a single well, double-click that well in the **Well Map** (located in the upper right corner of the **Data Analysis** page). The impedance trace for that well of the E-Plate will immediately be displayed within the **Curve Plot** area of the page.

To display the impedance traces from multiple wells, click on one well and drag the pointer across the desired set of wells, causing all of them to turn dark blue. Next, click the **<< Add** button. Alternatively, clicking the **Add All** button will cause data from every well measured in the experiment to be displayed in the **Curve Plot**. Similarly, clicking the **Remove >>** or **Remove All** buttons will remove selected wells or all wells, respectively, from the **Curve Plot**.

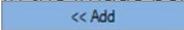
### NOTE

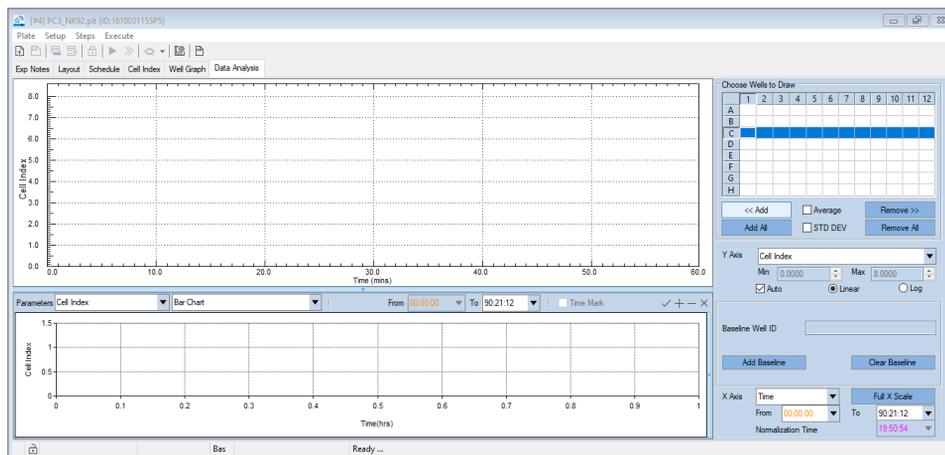
The white color of wells in the **Well Map** indicates that they have been activated on the **Layout** page for this experiment and are therefore amenable to having their data plotted.

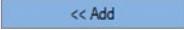
Once wells are displayed, their color in the **Well Map** will change to reflect the color that is displayed in the **Curve Plot**; if well average is selected, wells with the same content will show same color and average value will be plotted.

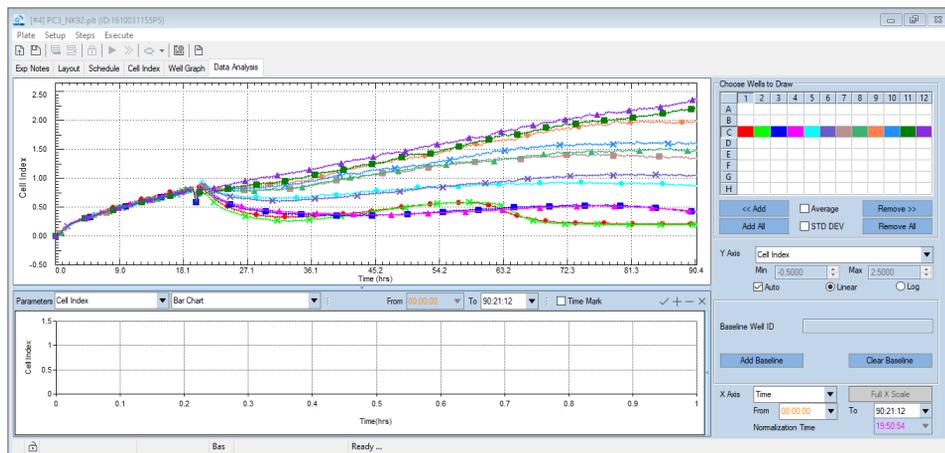
## Plotting Data

### Example of Using the Well Map to Add Data to the Curve Plot

In the image below wells have been selected for graphical display, but the  button has not been clicked yet:



The  button is clicked, and each well is represented by a colored line in the **Curve Plot**.



### Plotting Average and Standard Deviation for Well Replicates

- **Average:**

If some of the plotted impedance traces are from replicate wells (i.e., wells with the same cell type, cell number, compound name, and concentration on the Layout page), checking the **Average** box results in an average of these

## Plotting Data

replicates to be plotted in a single color. The same color is used to highlight the replicate wells within the **Well Map**.

- **STD DEV:**

Checking the **STD DEV** box adds standard deviation error bars to any averaged data trace.

**Average** and **STD DEV** box are checked in the **Well Map** to show average and standard deviation between well replicates:

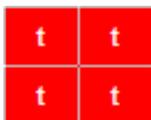
### Specific Functions of the Well Map in the Immunotherapy Module

In the Immunotherapy Module, when **% Cytolysis** is displayed in the **Curve Plot**, the **Well Map** area shows the localization of reference and subtraction wells that are used in the calculation through letters with matching color to the sample wells.

These colored letters give the indication of the property of the wells (reference or background to be subtracted). This map allows for intuitive and rapid identification of controls/references position and mislabeled or misplaced wells.

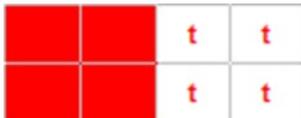
The following rules are utilized when plotting the position of the controls:

- **Text:** The text is used to identify which control is located in the well:
  - Lowercase letters indicate the presence of reference wells
    - t:** target alone
    - te:** target + effectors
    - tr:** target + treatment
    - tm:** target + mock
  - Capital letters are used for background subtraction
    - F:** target + Full Lysis
    - E:** effector alone
    - M:** Mock control alone
- **Color:** The color of the text indicates which wells are utilizing that specific control:
  - If a control well/average group are plotted and utilized only for those wells, the text appears inside the colored wells with a white color.



## Plotting Data

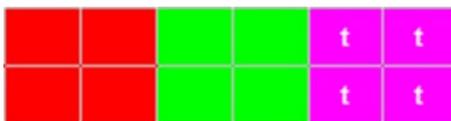
- If a control well/average group or subtraction wells are not plotted but utilized for one single group of wells, the text appears in the well map with the color of the plotted samples they are utilized for calculation.



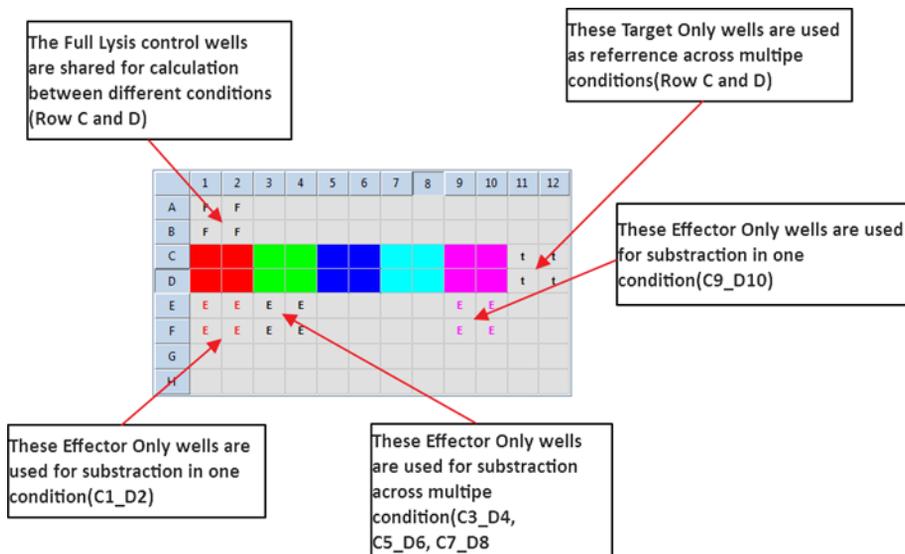
- If a control well/average group or subtraction wells are not plotted but utilized for more than one group of wells, the text appears in the well map with the black color.



- If a control well/average group or subtraction wells are plotted, the text appears inside the well map with the white color.



The following example shows how reference and subtraction wells are identified in the **Well Map** relative to the number and position of the sample wells for which they are utilized in the **% Cytolysis** calculation.

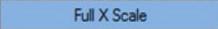


## Plotting Data

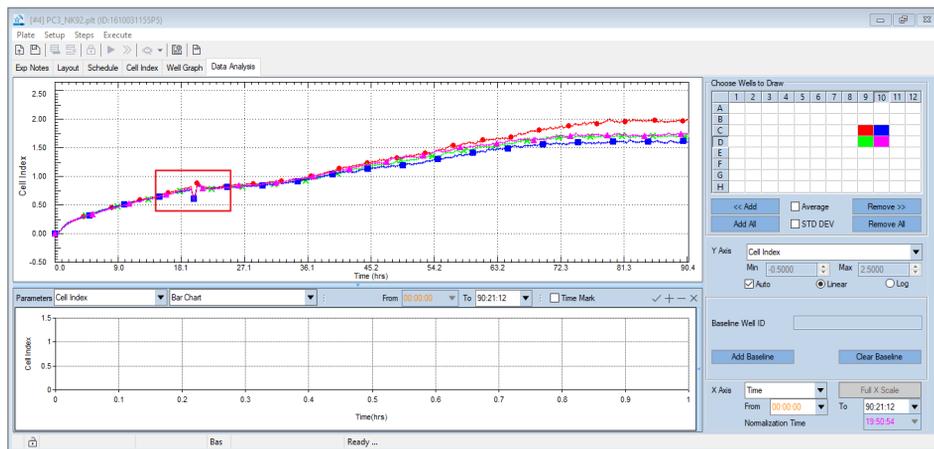
### Curve Plot Area

Once impedance traces are displayed within the **Curve Plot** area of the **Data Analysis** page, use the zoom in/zoom out functions to focus on specific regions of the curves.

### Zoom In/Zoom Out

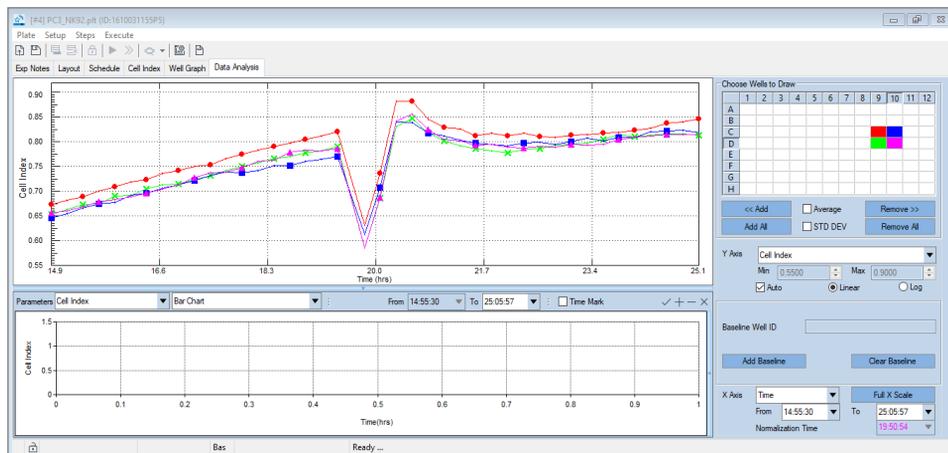
Within the **Curve Plot** area, hold down the left mouse button while dragging the cursor to draw a rectangle around the specific area of interest within the plot. When you release the button, the curve within this area will be zoomed in (magnified). Press the Esc key or click **Full X Scale**  to zoom out (return the plot to its original scale).

Zoom In:



After Zoom In:

## Plotting Data



### NOTE

The **Zoom In** function may take a few seconds to complete when there are a large number of data points within the area that has been selected in the zoomed area.

### NOTE

The **Zoom In** function is unavailable if any part(s) of the **Plot** area is blocked by any pop-up window(s).

## X Axis Grouping and Scale

To change how the wells are grouped on the X axis, click on the X axis drop-down menu on the X axis manager on the bottom right of the **Data Analysis** page. Wells can be grouped by **Target name**, **Target Number**, **Addition/Treatment**, **Number/Concentration**, **Row** and **Column**. Wells with the same selected conditions will be displayed in the same group.

Cell Index value is displayed for single wells according to the row position:

## Plotting Data

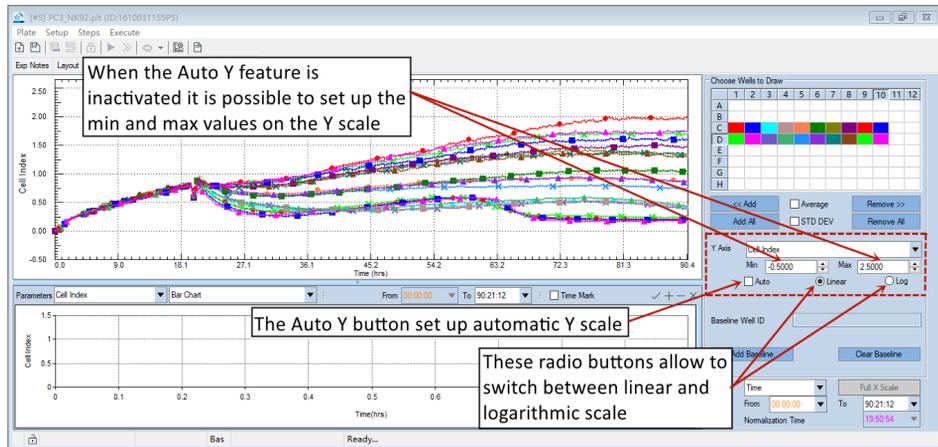


When a time curve is displayed, the scale of the X axis and normalization point can also be manually changed through the **From**, **To** and **Normalization Time** drop-down lists.

## Y Axis Scale

The Y axis scale can be switched between linear and logarithmic by selecting the corresponding radio buttons in the Y axis manager area. Unchecking the **Auto** button allows also to set up the **Min** and **Max** values on the Y scale.

Features of the Y axis manager area:



# Selecting Displayed Curve Type (RTCA Basic)

The RTCA Software Pro can display different types of curves in the **Curve Plot** area depending on which software module is active and the samples selected to be plotted. The selection of the curve type is done through the drop-down list in the Y axis manager area. The following curves are available in the RTCA Basic version of the software:

## Cell Index

The Cell Index curve plots the single well or average Cell Index traces for selected samples. Cell Index can be plotted using the following steps:

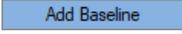
- Select **Cell Index** from the **Y-Axis** drop-down list.
- Select well(s) on the well map.
- Click the **Apply** button to draw the curve(s).

### 1 Adding a Baseline to Cell Index and other Plots

For **Cell Index** and some derived parameters (**Normalized Cell Index**, **Delta Cell Index**, **Scaled Cell Index** and **Baseline Normalized Cell Index**) it is possible to design a specific group of wells as “Baseline reference”, causing all the other samples to be plotted as variation relative to the baseline reference. Once a baseline is chosen, all points on the data curves will be calculated by subtracting the **Baseline Cell Index** from the original Cell Index:

$$CI_{new} = CI_{original} - CI_{baseline}$$

The baseline reference will always have 0 value across the temporal scale. Follow these steps to draw a **Baseline Cell Index** curve:

- On the **Well Map** area, select a well or wells to be used as baseline wells. If more than one curve is selected, the average Cell Index of the selected curves will be used as the baseline.
- Click the **Add Baseline button**  to set the wells as baseline. The **Y-axis** will be labeled as **Baseline Cell Index**.
- Select the well(s) to be plotted on the well map.
- Click the **Add** button to add curves to the plot.

#### NOTE

To remove the baseline calculation, click the **Clear Baseline** button



## Plotting Data

**WARNING**

This function is not available in the Immunotherapy Module.

In the below example the impedance traces for three different samples are plotted (each of the curves being an average of quadruplicate wells). The baseline function is not being used here.

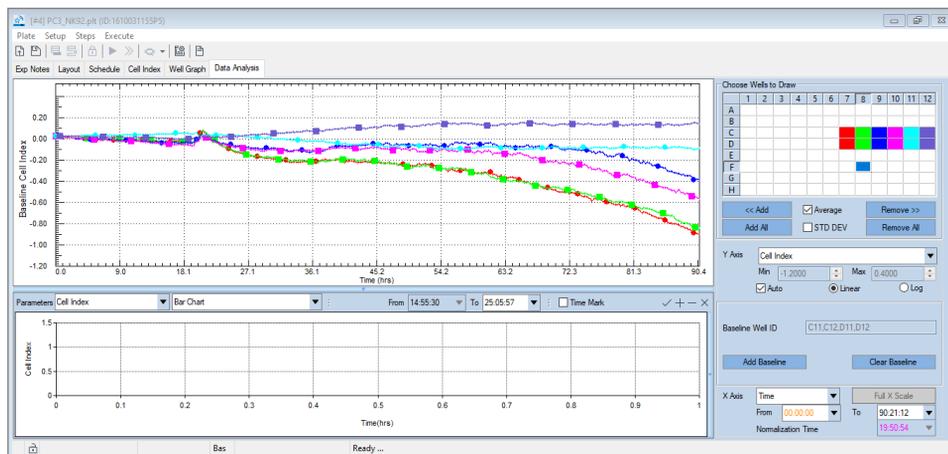


**NOTE**

To change the vertical size of the **Curve Plot** area, hover over the bottom region of the plot with the mouse until a double headed arrow appears. Click with the left mouse button and drag the arrow up or down to change the size of the plot.

In the plot below, the C11, C12, D11 and D12 are being designed as the baseline, the same data as shown in the plot above are replotted as the Y axis is defined as **Baseline Cell Index**.

## Plotting Data



## Normalized Cell Index (NCI)

The **Normalized Cell Index** plot internally divides the Cell Index for every selected well by the Cell Index at the Normalization time point:

$$NCI_{ti} = CI_{ti} / CI_{nml\_time}$$

Where  $CI_{ti}$  = CI at a specific time point "i", and  $CI_{nml\_time}$  = CI at a user-defined normalization time point.

The Normalization process removes most of the variability due to the differences in well seeding/attachment, as long as the chosen normalization point is before adding treatment.

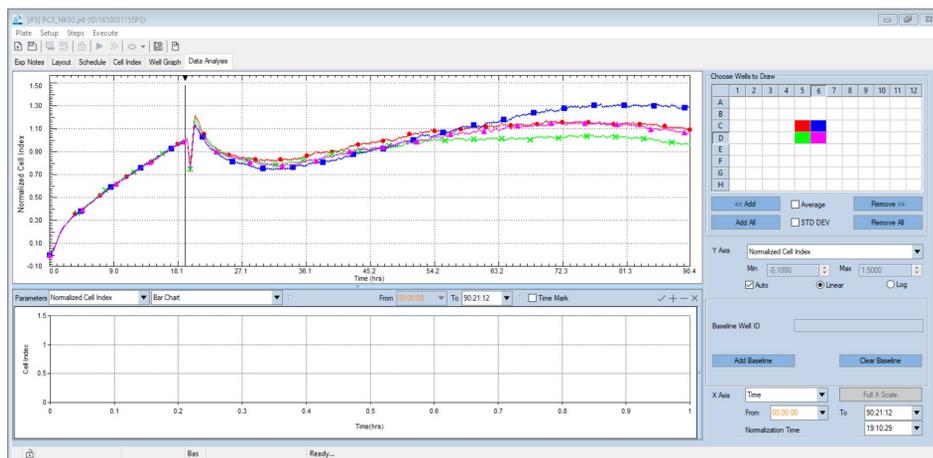
If **Normalized Cell Index** is selected from the **Y-axis** drop-down menu, a specific time point should be chosen for normalizing the data. You can select this time point from the **Normalize Time** drop-down menu. By definition, the **Normalized Cell Index** for all wells will be set to one (1) at the normalization time point. The optimal normalization time point should be the last time point before treatment addition.

Follow this procedure to plot the **Normalized Cell Index**:

- Select **Normalized Cell Index** from the **Y-Axis** drop-down menu.
- Select well(s) on the well map.
- Click the **<< Add** button to draw the curve(s).
- Select a Normalization time point from the **Normalize Time** drop-down menu or sliding the black triangle on the top of the **Curve Plot** area.

## Plotting Data

In the below example, the **Normalized Cell Index** is plotted for wells C5, C6, D5 and D6, with the **Normalize Time** set as 19:10:29. Note that a bold black vertical line and an associated black triangle show up in the plot, marking the time of normalization.



### NOTE

The normalization time marker can be changed by hovering the cursor over the black triangle at the top of the line, left clicking, and dragging it to the desired location. The normalization time can also be manually changed by selecting the drop-down list in the X axis manager area.

## Delta Cell Index (DCI)

For each well, the **Delta Cell Index** ( $DCI_t$ ) is calculated as the Cell Index  $CI_t$  at a given time point "t" plus a Delta value. The Delta value is a constant value for each well and is the difference between a reference DCI value and the Cell Index at the Delta Time point, as shown below:

$$DCI_{ti} = CI_{ti} + (DCI_{reference} - CI_{Delta\_time})$$

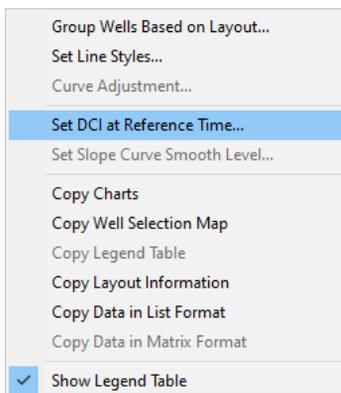
If you choose to plot **Delta Cell Index** on the **Y-axis**, the field labeled as **Normalize Time** will be relabeled as **Delta Time** and you must select a delta time point from this drop-down menu. All Cell Index data at the Delta time will be shifted to a fixed value (called the T-refer Cell Index). By default, the T-refer Cell Index will be one (1) for all wells at the Delta time point. However, you can specify a different T-refer Cell Index value using the field labeled CI at Ref. Time.

## Plotting Data

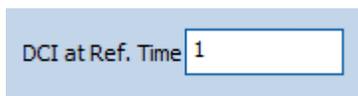
The **Delta Cell Index** basically shift all the curves, so they have the same values at the Delta time point.

Follow this procedure to plot the **Delta Cell Index**:

- Select **Delta Cell Index** from the **Y-Axis** drop-down menu.
- Select well(s) on the well map.
- Click the  button to draw the curve(s).
- Select the Delta time point from the **Delta Time** drop-down list or moving the black triangle mark above the curve plot.
- Open the curve plot **context menu** by right clicking with the mouse over the curve plot.
- Select **Set DCI at Reference Time**.



- Input the required Reference DCI by typing in the value in the **DCI at Ref. Time** box.



- Click the outside area to go back to the Plot.
- **Delta Cell Index** for the same wells described above. The DCI value is set to 1.

## Plotting Data



### Scaled Cell Index

The **Scaled Cell Index** curve plots the sample CI between the range of the positive (0) and negative (1) controls using the following equation:

$$CL_{scaled} = (CL_t - CL_{Positive Ctrl t}) / (CL_{Negative Ctrl t} - CI_{Positive Ctrl t})$$

Follow this procedure to plot the **Scaled Cell Index**:

- Select **Scaled Cell Index** from **Y-Axis** drop-down menu.
- Select well(s) on the well map.
- Click the **<< Add** button to draw the curve(s).

**WARNING**

To plot the Scaled Cell Index, Positive and Negative controls should be defined in the Plate Map layout (See [Defining Sample Type](#)).

### Baseline Normalized Cell Index (BNCI)

For each well, the **Baseline Normalized Cell Index** (BNCI(w, t)) is calculated as the Normalized Cell Index NCI(w, t) at a given time point based on the averaged Normalized Cell Index of corresponding control wells NCI(c, t), as below:

$$BNCI(w, t) = NCI(w, t) - NCI(c, t)$$

w: sample well; c: control well; t: time

## Plotting Data

Plot **BNCI** using the following procedure:

- Select **BNCI** from the **Y-Axis** drop-down menu.
- Select well(s) on the well map.
- Click the  button to draw the curve(s).
- Select a Baseline Normalization time point from the **Normalize Time** drop-down menu.

**WARNING**

To plot the BNCI a Negative control should be defined in the Plate Map layout (See [Defining Sample Type](#)).

The following example shows **Baseline Normalized Cell Index** plot for average wells C1, C2, D1 and D2 using average wells A1, A2, B1 and B2 as Negative Control and 4:14:10 as Normalization Time.



## Percentage of Control (%Control)

**Cell Index** or **Normalized Cell Index** from a sample can be plotted as % relative to negative Control following the formula:

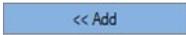
$$\%Control(s, t) = \frac{NCI(s, t)}{NCI(c, t)} * 100$$

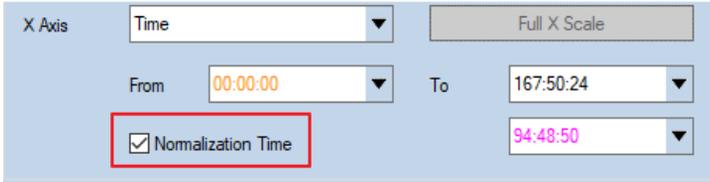
$$\%Control(s, t) = \frac{CI(s, t)}{CI(c, t)} * 100$$

Where s = sample well, c = control well, and t = a specific time point.

## Plotting Data

Follow this procedure to plot the Percentage of Control for CI or NCI:

- Select **%Control** from the **Y Axis** drop-down menu.
- Select well(s) on the well map.
- Click the  button to draw the curves.
- Select a normalization time point from the **Normalize Time** drop-down list.
- To calculate use Cell Index, uncheck the **Normalization Time**.

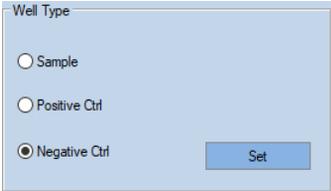


X Axis: Time (dropdown) Full X Scale (button)  
From: 00:00:00 (dropdown) To: 167:50:24 (dropdown)  
 Normalization Time (checkbox) 94:48:50 (dropdown)

### WARNING

The %Control curve cannot be plotted if a negative control has not been defined in the plate layout.

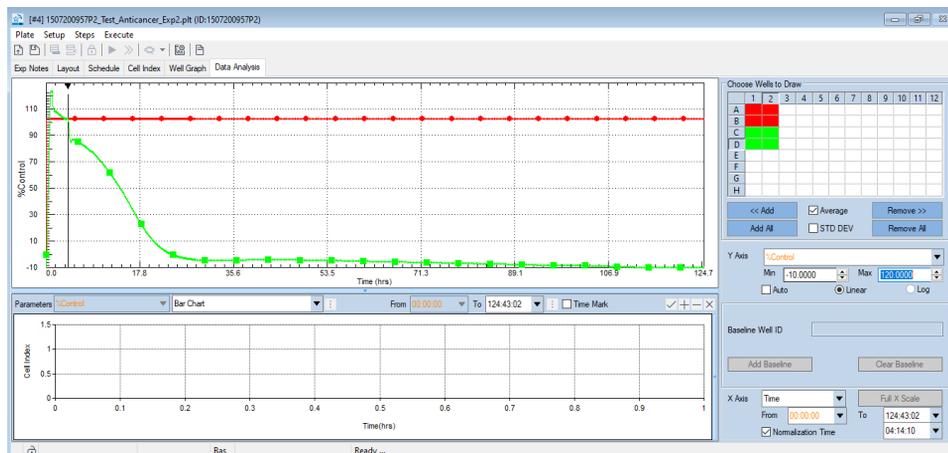
To set a negative control, go to **Layout > Cell** page. Select the wells and set as **Negative Control**.



Well Type  
 Sample  
 Positive Ctrl  
 Negative Ctrl  
Set (button)

The example shows **%Control** plot for the same samples described above. Average for sample wells C1, C2, D1 and D2 using average wells A1, A2, B1 and B2 as Negative Control and 4:14:10 as Normalization Time. The scale has been manually changed to emphasize the differences after the normalization time.

## Plotting Data



**%Control** can also be shown in grid format in **Cell Index** page. On **Cell Index** page, select **%Control** in the data type selection combo box and select target time point to show the percentage value in the following table. Note that **%Control** values in the **Cell Index** page is calculated without normalization.

Time: 90:21:12 2016/10/07 06:16:34 %Control

Cell Index	1	2	3	4	5	6	7	8	9
A	3.3681	5.7049							
B	0.5861	1.9885							
C	9.7038	9.1354	18.5810	18.9906	38.8979	46.8722	59.9945	65.8834	88.8453
D	10.9252	8.4441	17.4447	19.7947	33.1122	38.6156	59.7504	58.6182	76.0327
E									
F									
G									
H									

## Slope (Time Dependent)

For each well, the **Slope** ( $S_{ti}$ ) is calculated as the difference between the Cell Index  $Cl_{ti}$  and the Cell Index at the previous time point  $Cl_{t(i-1)}$  divided by the time interval between the two time points following the equation:

$$S_{ti} = (Cl_{ti} - Cl_{t(i-1)}) / (T_i - T(i-1))$$

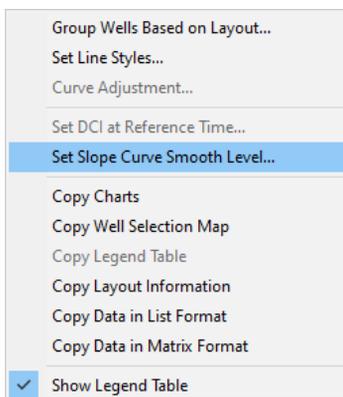
Where ( $i > 0$ )

Follow this procedure to plot the Slope:

- Select **Slope** from the **Y Axis** drop-down menu.
- Select well(s) on the well map.

## Plotting Data

- Click the **<< Add** button to draw the curves.
- Open the curve plot context menu by right clicking with the mouse over the curve plot.
- Select **Set Slope Curve Smooth Level**. Note that the default setting is **No Smooth**.

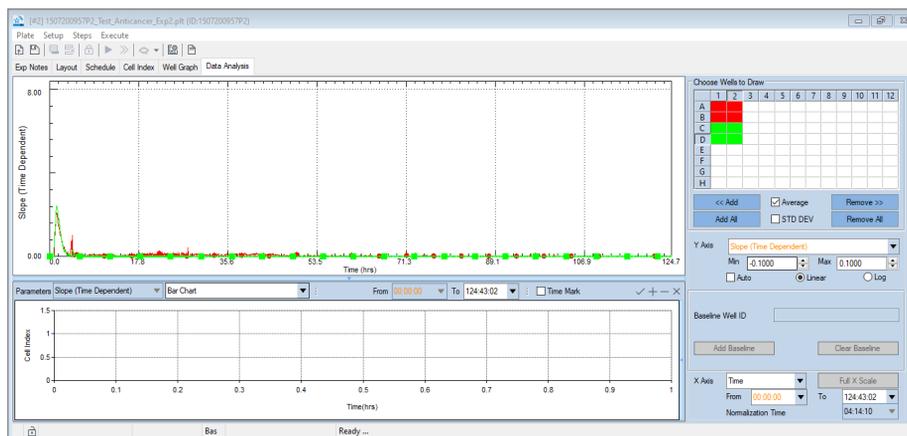


- Select the required **Smooth Level** in the box.



- Click the outside area to go back to the Plot

The example shows the Time Dependent Slope for the same samples described above. Average for sample wells C1, C2, D1, D2 and wells A1, A2, B1 and B2. The scale has been manually changed to emphasize the differences after the normalization time.



### Additional Displayed Curve Type (Immunotherapy Module)

The curves that have been described in the previous section are also available when the Immunotherapy Module is active. However, some of them have a different definition when applied to immunotherapy data analysis (see details in the following sections).

The following curves are specific to the Immunotherapy module: **Scaled Cell Index**, **%Control**, **Cell Index (Effector Subtraction)**, **Normalized Cell Index (Effector Subtraction)** and **% Cytolysis**.

#### Scaled Cell Index

The **Scaled Cell Index** curve is still used to plot the Cell Index relative to controls. However, the positive and negative controls are not selected by the user but are automatically identified based on the plate map. The software utilizes matching target alone samples as negative control (1) and full lysis samples as positive control (0).

#### WARNING

To plot the **Scaled Cell Index**, both **matching target alone** and **Full Lysis** samples should be defined in the plate map layout (See [Adding Experimental Information to the Wells in the Plate Map: Details](#)).

#### Baseline Normalized Cell Index (BNCI)

The **Baseline Normalized Cell Index** is used to plot the Normalized Cell Index  $NCI(w, t)$  at a given time point based on the averaged Normalized Cell Index of corresponding target alone control wells  $NCI(tac, t)$ , as below:

$$BNCI(w, t) = NCI(w, t) - NCI(tac, t)$$

w: sample well; tac: target alone control well; t: time

#### Percentage of Control (%Control)

This Curve follows the same calculations of the RTCA Basic, plotting the variation of the sample as % of the control. The software automatically designs the matching target alone as the reference control.

## Plotting Data

**WARNING**

The % Control curve cannot be plotted if matching target alone wells have not been defined in the plate layout.

### Cell Index (Effector Subtraction)

This new curve is used to monitor how the Cell Index changes after compensating for added effector cells. The curve plots the Cell Index from a sample containing effector or mock effector cells after the average Cell Index signal from the matching effector/mock effector alone wells has been subtracted.

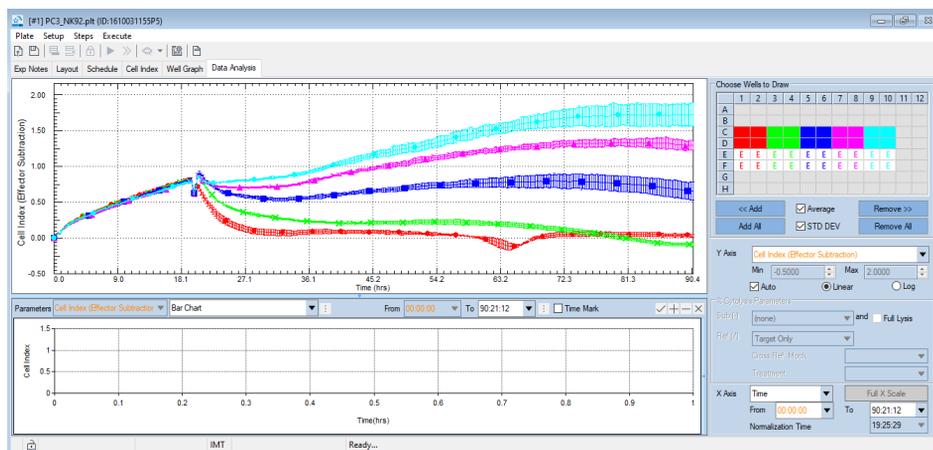
$$\text{Cell Index(Effector Subtraction)}_t = CI_t - \text{Avg}CI_{\text{effector\_alone}_t}$$

Where t is a specific time point and AvgCI<sub>effector\_alone</sub> is the average Cell Index of matching effector/mock effector alone sample wells.

**WARNING**

The curve can be plotted only for wells having matching effector/mock effector alone control wells.

**Cell Index (Effector Subtraction)** plot for a series of sample groups. The well map shows the location of matching Effector alone control wells. Every set of control wells matches one specific condition, as indicated by the colored letters **E**.



## Plotting Data

### Normalized Cell Index (Effector Subtraction)

The new curve is used to monitor how the Normalized Cell Index changes after compensating for added effector cells. The curve plots the Normalized Cell Index from a sample containing effector or mock effector cells after the average Cell Index signal from the matching effector/mock effector alone wells has been subtracted to the Cell Index.

$$\text{Normalized Cell Index (Effector Subtraction)}_t = (CI_t - \text{Avg}CI_{\text{effector\_alone}_t}) / (CI_n - \text{Avg}CI_{\text{effector\_alone}_n})$$

Where t is a specific time point, n is the normalization time point and  $\text{Avg}CI_{\text{effector\_alone}}$  is the average Cell Index of matching effector/mock effector alone sample wells.

Follow this procedure to plot the Normalized Cell Index (Eff,Sub):

- Select **Normalized Cell Index (Effector Subtraction)** from the **Y-Axis** drop-down menu.
- Select well(s) on the well map.
- Click the  button to draw the curve(s).
- Select a Normalization time point from the **Normalize Time** drop-down menu.

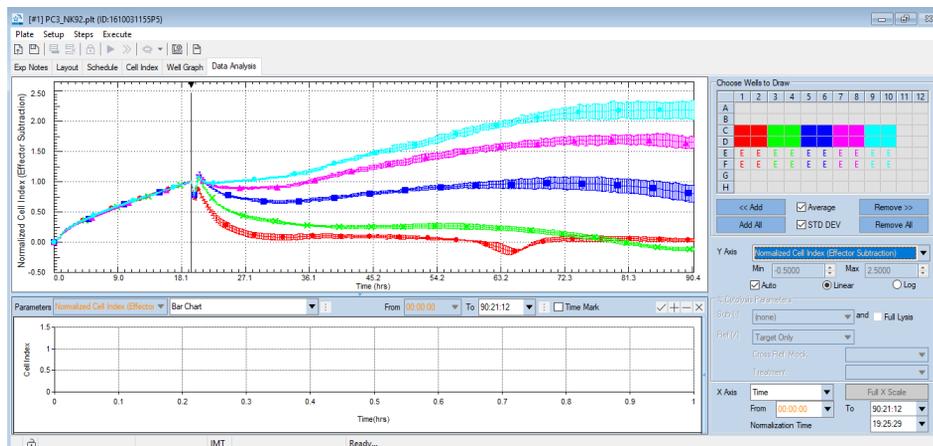
#### WARNING

**The curve can be plotted only for wells having matching effector/mock effector alone control wells, the other wells will be greyed out in the Well Map.**

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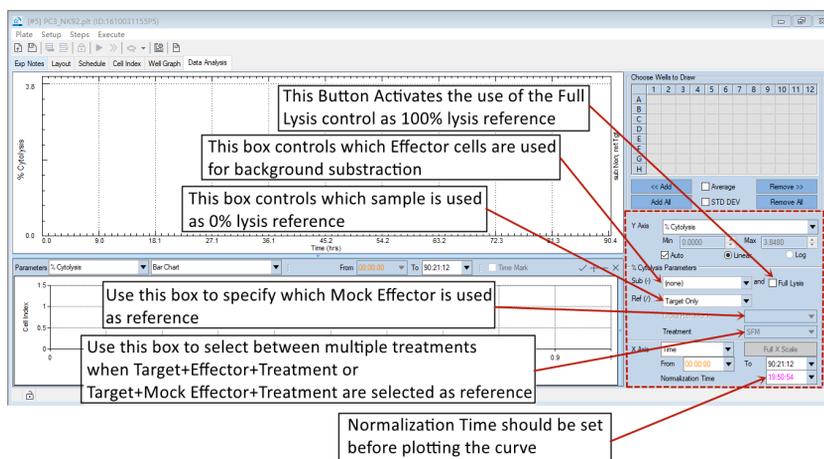
**Normalized Cell Index (Effector Subtraction)** plot for the same series of samples described above. Normalization time is 19:25:29.

## Plotting Data



## % Cytolysis

The **% Cytolysis** parameter is developed specifically for the Immunotherapy Module to quantify the percentage of cytolysis compared to the user defined controls or references. The curve reports over time the % Cytolysis using one of several controls as a reference. The curve can also subtract the signal from wells containing effector cells or from wells containing target cells and treated with a rapid killing agent (Full Lysis control) to compensate for residual background signal. Reference group and subtractions can be selected in the X, Y Axes Setting area, on the bottom right of the screen, and can be combined to generate the desired cytolysis plot.



The general equation for calculating the % Cytolysis for a sample at a time point  $t$  is:

## Plotting Data

$$\%Cytolysis_{st} = \left[ 1 - \frac{(NCI_{st})}{AvgNCI_{rt}} \right] * 100$$

Where,  $NCI_{st}$  is the Normalized Cell Index for the sample and  $NCI_{rt}$  is the average of Normalize Cell Index for the matching reference wells. This equation utilizes only the reference samples and no subtractions for calculating the % cytolysis. However, when compensations are introduced (e.g. effector alone or full lysis control), the equation assumes a different format (see below).

The % Cytolysis is 0 when the sample has the same NCI value of the average NCI of reference wells and 0 when the NCI is 0.

### WARNING

**All the % Cytolysis curve calculations are based on the Normalized Cell Index to reduce standard deviation and to improve data accuracy. For this reason, it is mandatory that the Cell Index Normalization Time Point is set correctly before generating the % Cytolysis plot.**

Follow this procedure to plot the % Cytolysis:

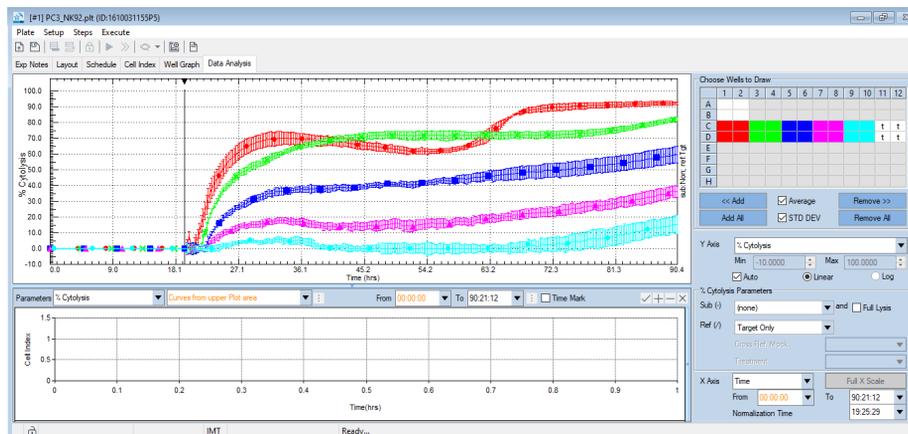
- Select **Normalized Cell Index** from the **Y-Axis** drop-down menu.
- Select well(s) on the well map.
- Click the  button to draw the curve(s).
- Select a Normalization time point from the **Normalize Time** drop-down menu or sliding the black triangle on the **Curve Plot** area (usually the last data point before adding effectors/treatments).
- Select **% Cytolysis** from the **Y-Axis** drop-down menu.
- Select Reference from the **Ref(/)** drop-down menu (**Target Only** is the default selection)
- Select Subtraction from the **Sub(-)** drop-down menu (**none** is the default selection)
- Select a specific Mock reference from the **Cross Ref. Mock** drop-down menu if **Target+Mock** is selected as reference and there are multiple Mock Effectors in the selected wells. If there is only one Mock Effector identified, the software will use it by default.
- Select a specific treatment if any condition containing treatment is selected in the **Ref(/)** and **Sub(-)** drop-down menus and there are multiple treatments in the selected wells. If there is only one treatment identified, the software will use it by default.

## Plotting Data

**WARNING**

To plot the % Cytolysis curve, at least one matching reference well is needed, otherwise wells will be greyed out in the Well Map.

% Cytolysis plot for a set of samples. The location of the shared Target Only reference wells is indicated by the black letter t in the well map



## Selecting a Reference Group

Several Reference groups are listed in the **Ref(/)** drop-down menu to be used in the formulas presented in this section. Depending by the selected reference, the curve represents either the Total Cytolysis (e.g. target alone is used as reference) or the Specific Cytolysis (e.g. target+effector is used as reference in an Antibody-Dependent Cell-Mediated Cytotoxicity experiment).

The available Reference Groups are:

- Target Only
- Target+Effector
- Target+Mock
- Target+Treatment
- Target+Effector+Treatment
- Target+Mock+Treatment

If multiple Mock Effectors or treatments are available as reference, they can be selected through the **Cross Ref. Mock** and **Treatment** drop-down menus.

## Plotting Data

### NOTE

Depending on the samples that are defined in the Plate Map layout, not all the reference group will be available in a specific experiment.

### Subtracting for Effectors/Mock Background Signal

Most of the time the effector cells that are used in immune oncology experiments are suspension cells that do not adhere to the gold electrodes embedded in the E-Plates, thus do not provide any contribute to the Cell Index parameter. However, there are cases where their contribution is minimal but measurable and may affect % Cytolysis calculation when approaching complete target cell lysis. Examples are certain cytolytic cell lines (e.g. NK92) and it is visible mostly at high Effector:Target ratios (e.g. 10:1). The signal from effector cells to a sample can be subtracted if matching effector/Mock effector alone control wells are present. The subtraction is selected in the **Sub(-)** drop-down menu.

The equation for calculating the % Cytolysis is modified as follows:

$$\%Cytolysis_{st} = [1 - Norm(CI_{st} - AvgEff_{sub\_t}) / (AvgNorm(CI_{rt} - AvgEff_{r\_sub\_t}))] * 100$$

Where  $Norm(CI_{st} - AvgEff_{sub\_t})$  is the Normalized value of the sample Cell Index minus the Average Cell Index for the matching selected Effector alone control at time t and  $AvgNorm(CI_{rt} - AvgEff_{r\_sub\_t})$  is the Average Normalized Cell Index value of the reference wells minus the Average Cell Index of the matching effectors used in the reference at time t. If target alone is used as reference, there is no subtraction of  $AvgEff_{R\_Sub\_t}$ .

### WARNING

**When subtracting effector cell signal, both matching reference well and effectors alone wells are needed. Otherwise wells will be greyed out in the Well Map.**

The available Subtraction Groups are:

- Effector
- Mock
- Effector+Treatment
- Mock+Treatment

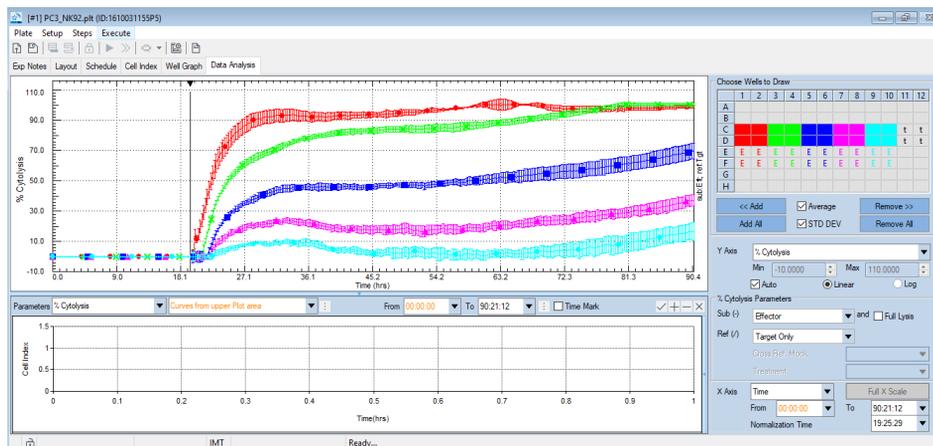
If multiple Mocks or treatments are available for subtraction, they can be selected through the **Cross Ref. Mock** and **Treatment** drop-down menus.

## Plotting Data

### NOTE

Depending on the samples that are defined in the Plate Map layout, not all the subtraction groups will be available in a specific experiment.

**% Cytolysis** plot for the same set of samples described above with Effector alone control subtraction. The location of the shared Target Only reference wells is indicated by the black letter t in the well map. Every set of effector alone control wells matches one specific condition, as indicated by the colored letter **E**.



### Compensating for Residual Cell Signal (Full Lysis Control subtraction)

When measuring the % Cytolysis, the software assumes by default that 0 Cell Index corresponds to 100% cytolysis. However, it is possible that even after complete killing, some residual impedance signal is present in the well due to cell membrane debris. The amount of residual signal is usually negligible, but it could be subtracted to provide the most accurate estimation by selecting the **Full Lysis** button in the X, Y Axes Setting area.

When the **Full Lysis** button is selected and no Effector/Mock background Signal subtraction is selected, the equation for calculating the % Cytolysis is modified as follow:

$$\%Cytolysis_{st} = \left[ 1 - \frac{NCI_{st} - AvgNCI_{FLt}}{AvgNCI_{rt} - AvgNCI_{FLt}} \right] * 100$$

Where  $NCI_{st} - AvgNCI_{FLt}$  is the Normalized Cell Index value of the sample minus the Average Normalized Cell Index of the matching Target Full Lysis control at time t and  $AvgNCI_{rt} - AvgNCI_{FLt}$  is the Average Normalized Cell Index value of the reference wells minus the Average Normalized Cell Index of the matching Target Full Lysis control at time t.

## Plotting Data

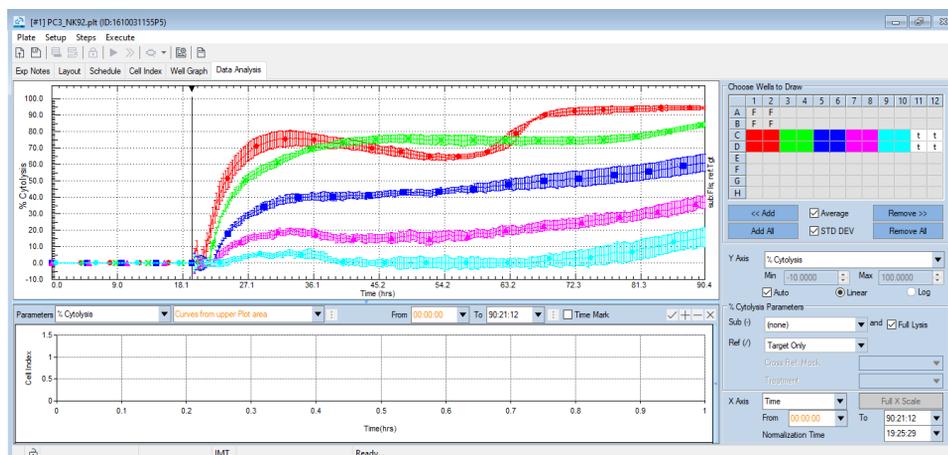
### NOTE

When samples containing Target+Effectors or Target+Mock are used as reference, the Full Lysis control that is used is still the target cells alone plus full lysis reagent.

### WARNING

**When subtracting Full Lysis control signal, both matching reference wells and Target+Full Lysis control wells are needed. Otherwise wells will be greyed out in the Well Map.**

% **Cytolysis** plot for the same set of samples described above with Full Lysis control subtraction. The location of the shared Target Only reference wells is indicated by the black letter t in the well map, while the shared Full Lysis control wells are indicated by the black letter F.



### Combined Full Lysis and Effectors Background Subtraction

To generate the % **Cytolysis** plot, subtract both the Effectors/Mock Background Signal and the Full Lysis Control by selecting the proper Effectors/Mock in the **Sub (-)** drop-down menu and checking the **Full Lysis** button.

In such case the equation used for calculating the % Cytolysis is the following:

$$\%Cytolysis_{st} = \left[ \frac{1 - \text{Norm}(CI_{st} - \text{AvgEff}_{sub,t})}{\text{AvgNorm}(CI_{rt} - \text{AvgEff}_{R,Sub,t}) - \text{AvgNCIFL}_t} \right] * 100$$

Where  $\text{Norm}(CI_{st} - \text{AvgEff}_{Sub,t})$  is the Normalized value of the sample Cell Index minus the Average Cell Index for the matching selected Effector alone control at time t;  $\text{AvgNorm}(CI_{rt} - \text{AvgEff}_{R,Sub,t})$  is the Average Normalized Cell Index value of the reference wells minus the Average Cell Index of the matching effectors used in the reference at time t and  $\text{AvgNCIFL}_t$  is the Average Normalized Cell Index of the matching Target + Full Lysis control at

## Plotting Data

time t. If Target Only is used as reference, there is no subtraction of AvgEffR\_Sub\_t.

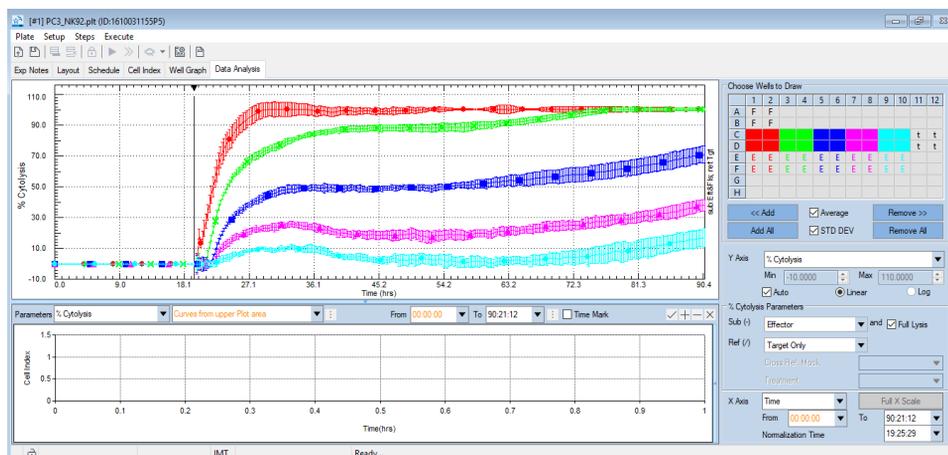
### NOTE

When samples containing Target+Effectors or Target+Mock are used as reference, the Full Lysis control that is used is still the target cells alone plus full lysis reagent.

### WARNING

**When subtracting effector cell signal and Full Lysis control signal, matching reference wells, effectors alone and target+full lysis control wells are needed. Otherwise wells will be greyed out in the Well Map.**

% **Cytolysis** plot for the same set of samples described above with Full Lysis and Effectors alone controls subtraction. The location of the shared Target Only reference wells is indicated by the black letter t in the well map. The shared Full Lysis control and single-condition matching effector alone control wells are indicated by the black letter F and colored letters E respectively.

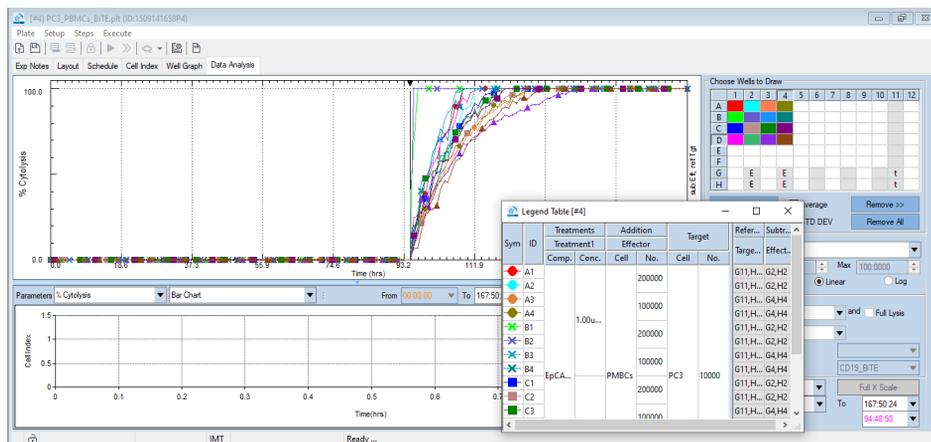


## Floating Plot Legend Table

The floating Plot Legend Window pops up every time a new plot is generated. It contains information of the content in each well or each group of wells. From left to right, the legend contains information including plot symbol and color, well ID, Treatment addition name and concentration, cell addition (effector, mock effector,

## Plotting Data

or full lysis) name and cell number, Target cell name and cell number. The software automatically groups samples to reduce table complexity. Conditions such as treatment and cell addition that are shared between multiple samples are shown as a single group that spans all samples with the same properties.



### NOTE

The position of the floating table on the screen can be changed by clicking and dragging the top of the window. The size of the table can also be changed by clicking and dragging on the side of the window.

### NOTE

The **Legend Table**, as well as other popup windows in the **Data Analysis** page will be hidden when you move to other page(s). They will be shown again when switching back to the **Data Analysis** page.

### NOTE

When the Software is running in MP or DP mode, since there are too many windows already, the **Legend Table** is displayed only when the **Data Analysis** window is maximized, otherwise it will be hidden.

In the following example, all the plotted samples have the same target, effector cells and treatment, but have differences in the seeding number of effector cells and treatment concentration.

## Plotting Data

Sym	ID	Treatments				Addition		Target	
		Treatment1		Treatment2		Effector		Cell	No.
		Comp.	Conc.	Comp.	Conc.	Cell	No.		
● A1,...									
● A3,...									
● A5,...			1.00ug/ml			PMBCs	200000		
● A7,...							100000		
● A9,...							50000		
● A1,...							25000		
● A1,...							12500		
■ C1,...									
■ C3,...							200000		
■ C5,...	EpCAM_Bi...	0.50ug/ml				PMBCs	100000		
■ C7,...							50000		
■ C9,...							25000		
■ C1,...							12500	PC3	10000
● E1,...							200000		
● E3,...							100000		
● E5,...			100.00ng/			PMBCs	50000		

Click on the **Sym** or **ID** column to highlight the fields corresponding to the selected well(s).

Sym	ID	Treatments				Addition		Target	
		Treatment1		Treatment2		Effector		Cell	No.
		Comp.	Conc.	Comp.	Conc.	Cell	No.		
● A1,...									
● A3,...									
● A5,...			1.00ug/ml			PMBCs	200000		
● A7,...							100000		
● A9,...							50000		
● A1,...							25000		
● A1,...							12500		
■ C1,...									
■ C3,...							200000		
■ C5,...	EpCAM_Bi...	0.50ug/ml				PMBCs	100000		
■ C7,...							50000		
■ C9,...							25000		
■ C1,...							12500	PC3	10000
● E1,...							200000		
● E3,...							100000		
● E5,...			100.00ng/			PMBCs	50000		

### NOTE

The **Legend Table** can be copied and pasted as image in PowerPoint or other software by right clicking with the mouse over the **Curve Plot** area and selecting **Copy Legend Table**.

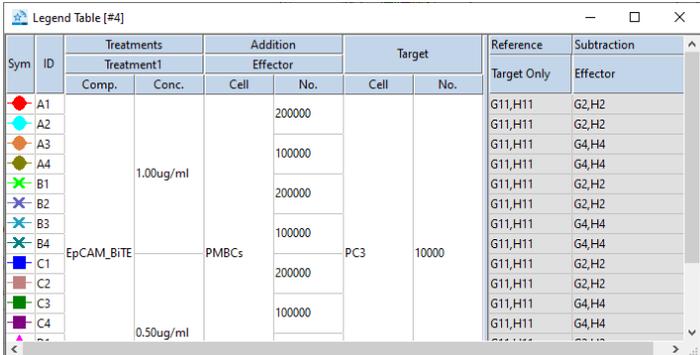
## Adding Information on Reference/Control Wells Identity

To add information on the ID of the wells that are used as reference and for background subtraction in the % Cytolysis calculation, press the **F3** key in the keyboard. After the function has been enabled, any time a % Cytolysis plot is generated, the **Plot Legend Table** will display such information in a gray background on the right

## Plotting Data

end of the window, including the wells that are utilized in that specific % Cytolysis calculation, their logical identity (e.g. sample, reference, effector alone subtraction) and content (e.g. cells/compound name and seeding density/concentration).

In the following example, for every single plotted group, the reference wells and the effector alone control wells used for background subtraction are indicated under the **Reference** and **Subtraction** columns.



Sym	ID	Treatments		Addition		Target		Reference	Subtraction
		Treatment1		Effector				Target Only	Effector
		Comp.	Conc.	Cell	No.	Cell	No.		
A1					200000		G11,H11	G2,H2	
A2							G11,H11	G2,H2	
A3					100000		G11,H11	G4,H4	
A4		1.00ug/ml					G11,H11	G4,H4	
B1					200000		G11,H11	G2,H2	
B2							G11,H11	G2,H2	
B3					100000		G11,H11	G4,H4	
B4		EpCAM_BITE		PMBCs		PC3	G11,H11	G4,H4	
C1					200000		G11,H11	G2,H2	
C2							G11,H11	G2,H2	
C3					100000		G11,H11	G4,H4	
C4		0.50ug/ml					G11,H11	G4,H4	

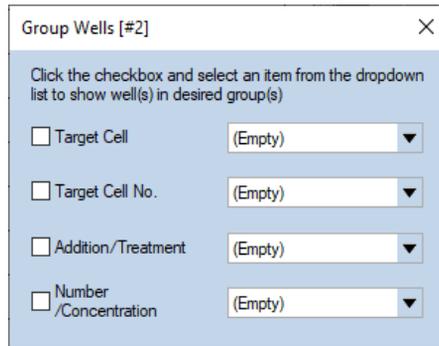
### NOTE

The function can be turned off by clicking the **F3** key again.

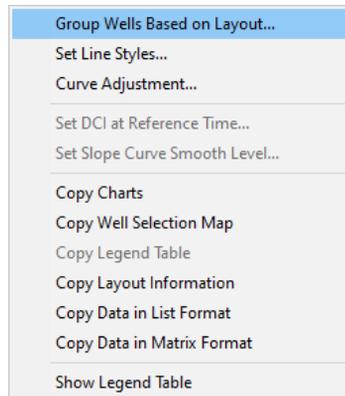
### WARNING

When clicking the **F3** button on the keyboard, be sure to have the plot window active by clicking on any point of the plot area (window border is blue) or the Plot Legend Table active (window border is blue). The function cannot be activated if the Analysis Chart is active.

## Group Wells Pop Up Window



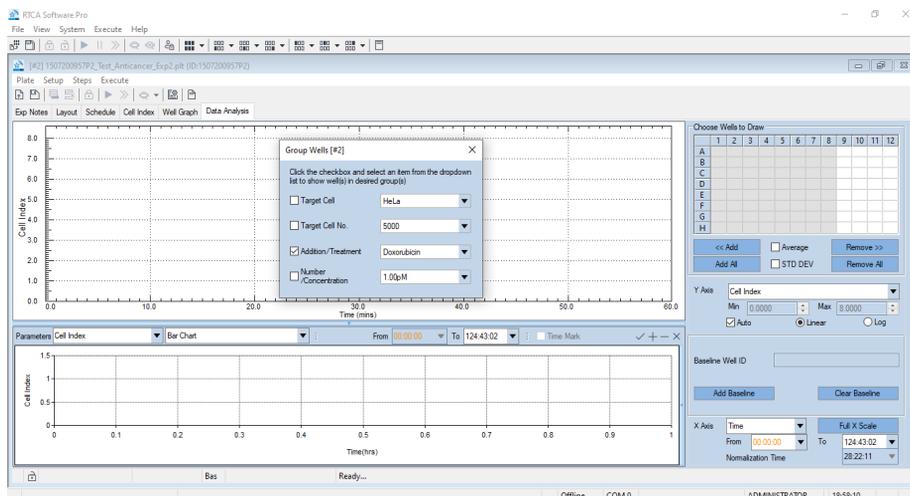
As an alternative to selecting specific wells within the **Well Map** of the **Data Analysis** page, the **Group Wells** window can be displayed by clicking the right mouse button when hovering over the **Curve Plot** area and selecting **Group Wells Based on Layout** on the pop up window.



Wells can be selected based on specific criteria such as: target cell identity, the number of target cells that were seeded, the type of addition/treatment the cells were exposed to, etc. Once the type of criteria has been selected by placing a checkmark in the appropriate box, use the corresponding drop-down menu to select the specific sample types to plot (such as 10,000 cells/well, etc.).

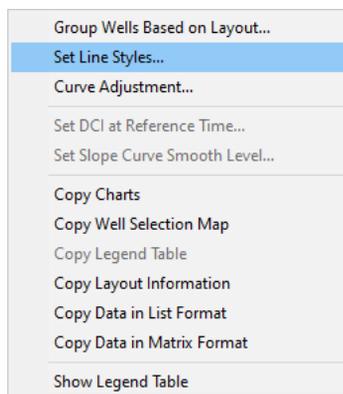
In the example below, the **Doxorubicin** treatment has been selected for plotting. The wells that fit these criteria are highlighted in white within the **Well Map**.

## Plotting Data



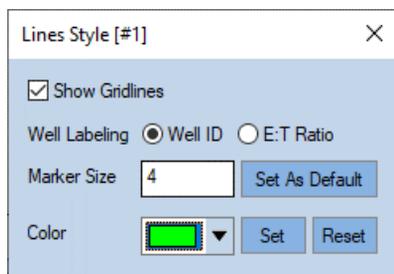
## Lines Style Pop Up Window

The **Lines Style** window is used for adjusting plot characteristics like color, thickness, and marker shape of the plotted lines. The **Lines Style** window can be opened by clicking the right mouse button when hovering over the **Curve Plot** area and selecting **Set Line Styles** on the pop-up window.



The **Lines Style** window has the following selections available.

## Plotting Data



- **Show Gridlines:** Selection of this box causes gridlines to be visible within the Curve Plot.
- **Well Labeling:** This field controls whether Well IDs or Effector:Target ratios are displayed in the **Analysis** Tab when other parameters beside steps and Time are selected in the **X Axis** (This function is available only in the Immunotherapy Module).
- **Marker Size:** Use this field to define the size of the markers used to denote data points. To adjust this parameter first highlight the well(s) within the **Well Map**, then enter a value that is larger or smaller than the value than is currently displayed. Smaller numbers will make the lines appear thinner and larger numbers will make the lines appear thicker. Click the **Set As Default** button to apply the new marker size to all samples that are plotted. The software should be closed and reopened to make the new default selection active.
- **Color:** By default, when adding impedance traces to the **Curve Plot**, using the **Add All** or **<< Add** buttons, the curves will be drawn in colors that the Software automatically selects. To plot the impedance traces using a different color, highlight the wells of interest in the **Well Map**, then select the desired color from the drop-down list of the **Color** field and click the **Set** button to change the line color.



## Plotting Data

All selected colors will be recorded in the experiment file so that, when the user logs in the next time, the same colors will be used. The color will also be changed in the **Well Map** and an "S" will be placed on top of the well to indicate that its color has been manually adjusted (see well E3 in the below image).

### NOTE

If a well(s) color is manually adjusted, %Cytolysis Subtraction or/and Reference well(s) may not be displayed.

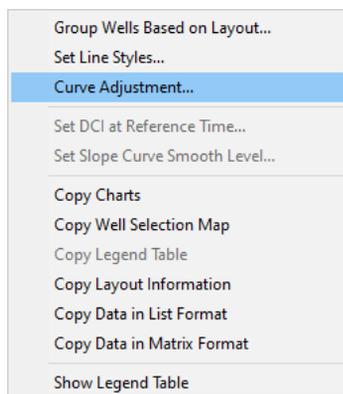
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E			S									
F												
G												
H												

For a specific well or group of wells the predetermined color can be restored by selecting the well in the **Well Map** and clicking the **Reset** button in the **Line Style** window.

## Curve Adjustment Tools

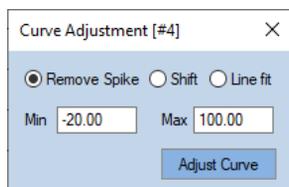
The RTCA Software Pro has a function that allows editing the **Cell Index** curve to remove spikes due to incorrect plate/pogo pins contact, to reduce noise in low impedance signals and to change curves values. Such function can be implemented opening the **Curve Adjustment** window by selecting **Curve Adjustment** in the **Setup** drop-down menu or by clicking the right mouse button while hovering the **Curve Plot** area and selecting the **Curve Adjustment** option.

## Plotting Data



As seen in the below image, three different tools are offered for curve adjustment:

- The **Remove Spike** option allows removal and fitting of rapid spikes in impedance, such as what can occur immediately after treatment addition.
- The **Shift** option enable shifting the CI of a curve in a specific time range.
- The **Line Fit** option allows removing noise by fitting a curve in a selected time range.

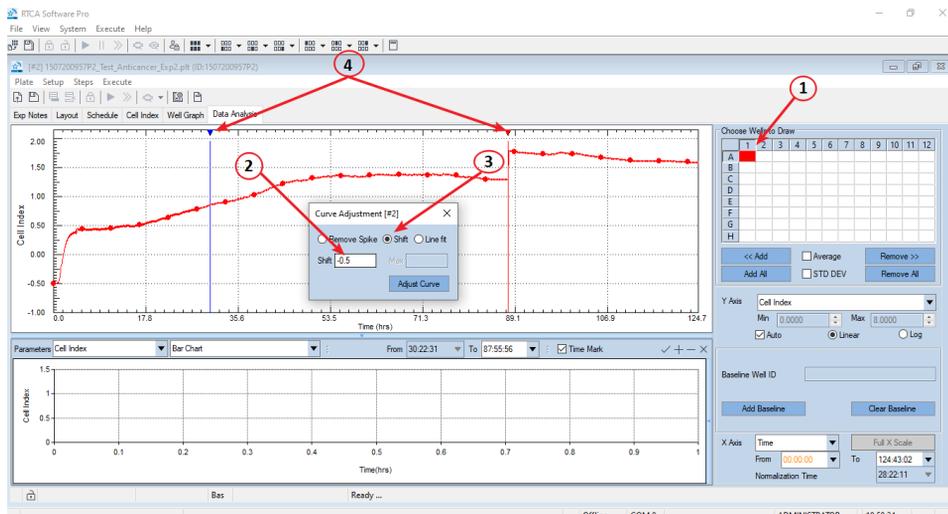


To repair problematic curves:

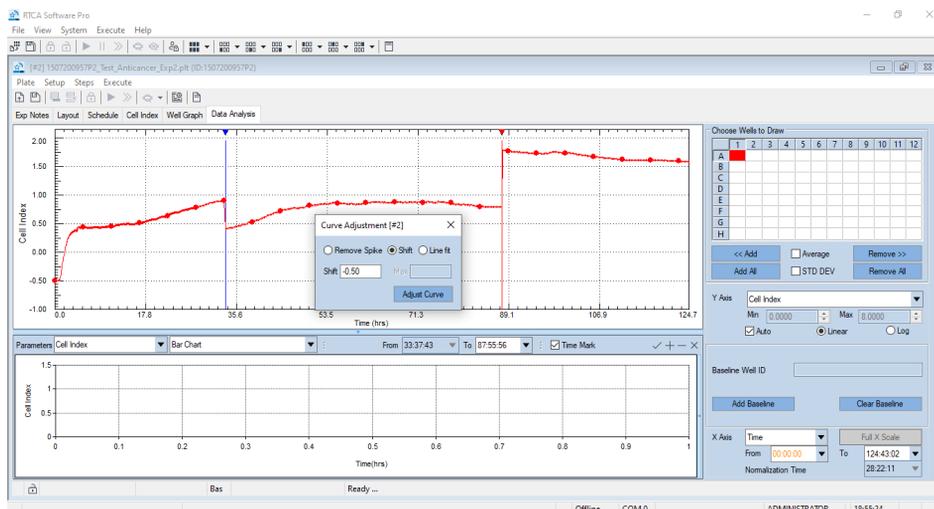
- Select the desired wells on the **Well Map**.
- Select the time range where the adjustment should be applied by checking the **Time Mark** button above the **Chart Area**.
- Two lines will appear in the **Chart Plot** to identify the selected time range where the fitting is being applied. The position of these lines can be changed by hovering the cursor over the triangle at the top of the line, left clicking, and dragging them to their desired location.
- Select the adjustment type through the dedicated radio buttons. Depending on the adjustment type that has been selected, fields in the **Min/Max** boxes or **Shift** may become active and the desired value ranges should be added.
- Click the **Adjust Curve** button to execute the adjustment.

## Plotting Data

Example of the Curve Adjustment process on well A1 (1); -0.5 value (2) has been selected for the shift curve process (3) that should be applied to the time range identified by the blue and red lines (4).



The same curve after the adjustment process has been applied.



### NOTE

Curve Adjustment Tools are not available when an experiment is running. Curve repair can be done only after the experiment has finished or paused.

## Plotting Data

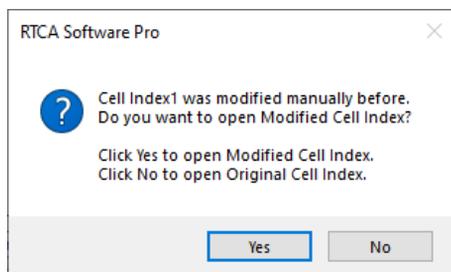
### WARNING

Always back up the original experiment data file (\*.plt) before repairing the curves.

### NOTE

The Curve Adjustment Tools can only be applied when **Cell Index** data are plotted on the **Y-axis** (i.e. not when **% Cytolysis** or other parameters are plotted on the **Y-axis**). After the **Cell Index** curve has been adjusted, the other available curve types can be plotted based on the adjusted Cell Index data.

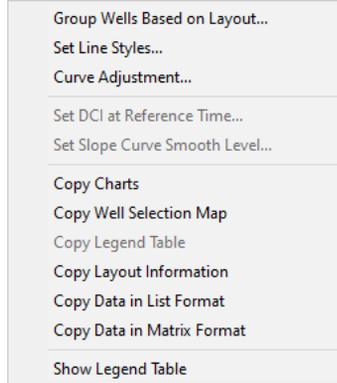
Once a curve has been modified and the data saved, the software will automatically detect the existence of a modified curve every time the experiment is opened again and will ask if the original or the modified data need to be opened.



## Copy Plots Information: Plots Charts, Wells Selection Map, Layout Information and Data

To copy the information related to the **Curve Plots**, hover over the **Plot** area. Using the right mouse button select one of the Copy options from the menu.

## Plotting Data



- **Copy Charts** allows copying a screen shot of the plotted curves.
- **Copy Well Selection Map** is used for copying a screenshot of the **Well Map** area.
- **Copy Layout Information** allows copying the content of the plotted wells. The information can be pasted in databases or Microsoft Excel Spreadsheets.
- **Copy Data in List Format** copies the content of the plot as numerical values that can be transferred to databases or Microsoft Excel Spreadsheets.

To paste the screenshot/numerical values into the software of interest, the right mouse button and paste option should be selected in the destination software.

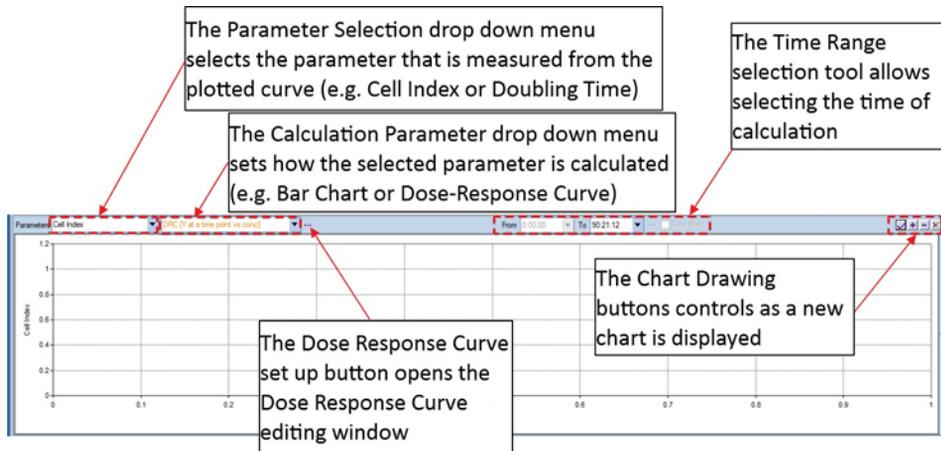
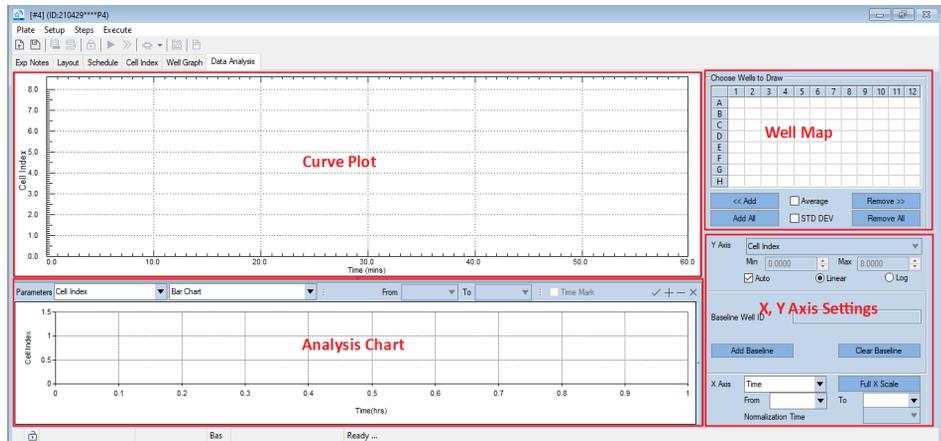
### NOTE

When using the Copy functionality, the data copied are the same data that are visualized in the **Curve Plot** area (e.g. well average, curve type and time range).

# 9 Data Analysis

This chapter describes data analysis in RTCA Software Pro including analysis chart area tools, data analysis functions (RTCA Basic and Immunotherapy Module) and copy charts and data.

Curves that are plotted in the **Curve Plot** area can be further analyzed and compared through several tools that extrapolate complex information. They are reported in the **Analysis Chart** area of the **Data Analysis** window.



## Analysis Chart Area Tools

On the top of the **Analysis Chart** area there are several drop-down menus and buttons that allow to define how the **Curve Plots** are calculated and displayed in the **Analysis Chart**.

### NOTE

The combination of parameters that are selectable changes and is a function of the selected curve plot and the combination of parameter, chart and software module that is activated.

- The **Parameter Selection menu** determines if the original curve plot parameter or derived parameters like **Slope**, **Doubling Time** or **Killing Time** are used for analysis.
- The **Calculation Parameter menu** defines if a selected parameter is displayed as a **Bar Chart** or **Dose-Response Curve (DRC)** with calculated **IC50/EC50**. Several characteristics of the selected parameter can be utilized to generate the **DRC** curve (see below).
- The **DRC Set up** button controls what formula is utilized to generate the **DRC** curve and what constrains are applied.
- The **Time Range** selection tool allows selection of the time range or time point utilized for the calculation.
- The **Chart Drawing** buttons are used to generate /remove new charts.

### Draw Chart, Add Chart and Clear Charts

To draw a chart, once the parameter and calculated parameter have been selected from the drop-down menus, select the **Draw a New Curve** button ✓.

To add a chart to an existing drawn chart, select the **Add a Curve** button +.

To remove last drawn chart, select the **Remove Last Curve** button -.

To remove all drawn chart, select the **Clear Chart** button ✕.

## Data Analysis Functions (RTCA Basic)

The following functions are available in the RTCA Basic module to derive parameters and calculations using the curves generated in the **Chart Plot** as

## Data Analysis

primary data source. The same basic functions are also available in the Immunotherapy Module.

### WARNING

**Depending on the parameter that is selected in the Y Axis box, only specific combinations of Parameter Selection and Calculation are allowed.**

---

## Parameter Selection

The **Parameter** Selection drop-down window toggles between different Cell Index derived parameter that can be utilized for generating single time bar charts, Time-dependent IC50 and Dose Response Curves (DRC).

### Cell Index/Normalized Cell Index

This parameter utilizes the **Cell Index** or **Normalized Cell Index**, in the generated charts/DRC. The impedance parameter that has been selected in the **Chart Plot** dictates which parameter is listed in the **Parameter Selection** window.

### Doubling Time

This parameter describes the period of time required for the Cell Index value of a curve to double. Alternatively, if the slope of the curve is negative within the time frame of interest, the **Doubling Time** describes the time required for the Cell Index value to drop to 50% of its initial value. Although **Doubling Time** is often used for proliferation analyses, it can be used for a wide variety of other applications.

For each selected well, the software calculates the doubling-time for the Cell Index (or Normalized Cell Index depending by the selected Chart Plot) in the chosen time period by fitting the curve to an exponential equation. The calculated Doubling Time values are displayed in a bar plot.

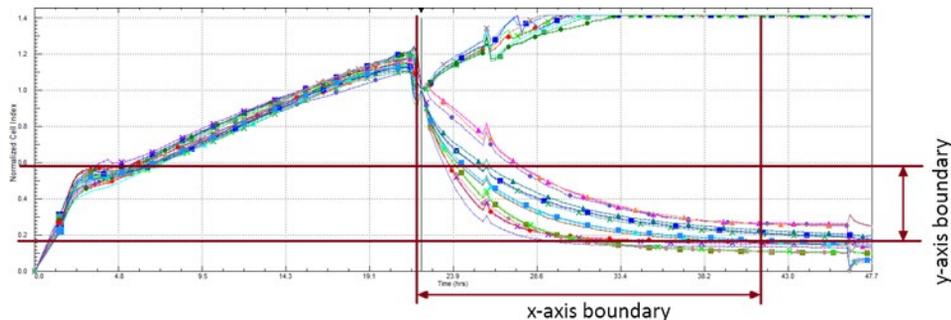
### Slope

The **Slope** is used to describe the steepness, incline, gradient, or changing rate of a curve within a given time window. The **Slope** parameter may be used for cell proliferation, cell adhesion, cytotoxicity, receptor activation, or other cell-based assays.

For each selected well, the Software calculates the Slope of the Cell Index (or Normalized Cell Index) curve over a chosen Time frame. Data points within this time frame are fit to a straight line. Slope values are displayed in a bar plot.

## Data Analysis

If Y-Axis boundary is also given, (uncheck “**Auto**” in **Y-Axis** section, i.e. do not use Auto Y function), the Slope (known as “Scaled Slope”) will be calculated within the red rectangular, shown as below:



### AUC

The **AUC** (Area Under Curve) is calculated the area between a curve and the x-axis within a given time period, i.e.:

$$AUC = \text{Sum} \left( \frac{1}{2} * (Y(t) + Y(t + 1)) * (X(t + 1) - X(t)) \right)$$

where  $t = 0, 1, 2, \dots, T$

### Calculation Parameter Selection

The **Calculation Parameter** drop-down window is utilized to select the chart that is displayed in the bottom area.

### Bar Chart

This selection is utilized to compare values (e.g. Cell Index or Slope) between single wells or well replicates at a single time point. The time point should be set by clicking the **Time Mark** button and selecting the **To** time point dragging the blue triangle/line.

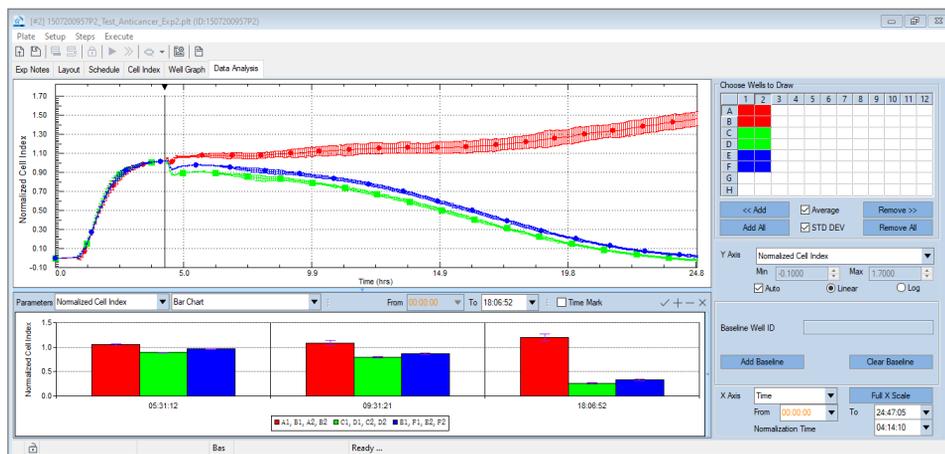
To compare Cell Indexes (Cell Index, Normalized Cell index etc.) between group of wells at different time periods:

- Select a group of wells on the **Well Map**.
- For the **Y-Axis** choose the appropriate parameter (**Cell Index, Normalized Cell Index**, etc.).
- Click the **Time Mark** button and select the desired analysis time point (in the “**To**” box) dragging the blue triangle/line.

## Data Analysis

- On the **Parameter Selection** drop-down menu select the parameter to be charted.
- On the **Calculation Parameter Selection** drop-down menu select **Bar Chart**.
- Click the **Draw a New Curve** button ✓ to generate a bar chart of the value of the curve at that specific time point. To add values from different time points to the same histogram, select a second time point and click the + button to add a new set of values to the existing chart.

In the following example the **Normalized Cell Index**, at three different time points, is compared for three different sets of wells.



### NOTE

To change the vertical size of the **Analysis Chart** area, hover over the plot region with the mouse until a double headed arrow appears. Click with the left mouse button and drag the arrow up or down to change the size of the chart.

## Chart Legend Definition

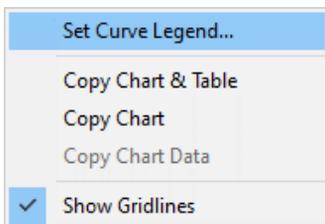
When displaying the **Bar Chart**, it is possible to edit the legend to display information in addition to well ID, including target cells name and seeding density, and treatment name and concentration.

To change the **Chart Legend** follows this procedure:

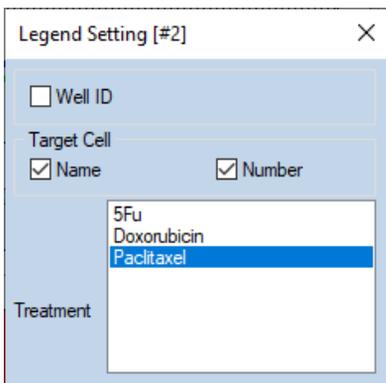
- Select a group of wells on the **Well Map**.
- For the **Y-Axis** choose the appropriate parameter (**Cell Index, Normalized Cell Index**, etc.).
- Click the **Time Mark** button and select the desired analysis time point (in the **"To"** box) dragging the blue triangle/line.

## Data Analysis

- On the **Parameter Selection** drop-down menu select the parameter to be charted.
- On the **Calculation Parameter Selection** drop-down menu select **Bar Chart**.
- Open the dial window by hovering over the **Analysis Chart** area and clicking the right mouse button.
- Select the “**Set Curve Legend**” option



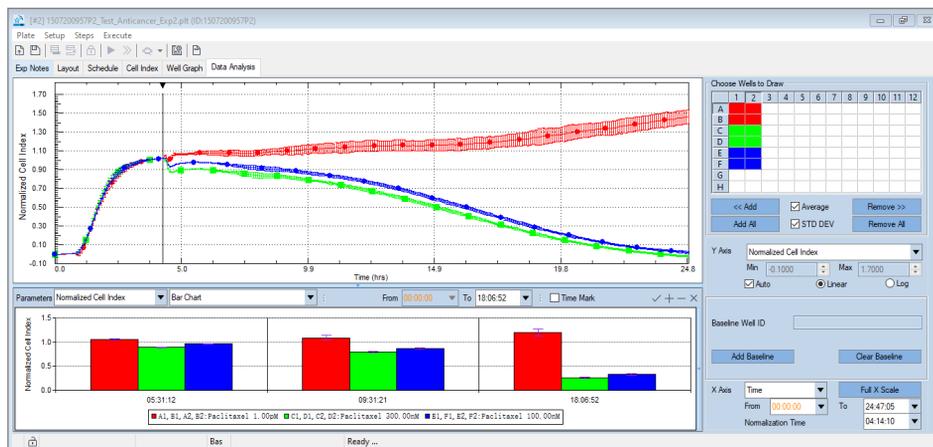
- On the **Legend Setting** window select the parameters that need to be displayed in the legend. In the following example Target cell name, number and Treatment name/concentration.



- Click the **Draw a New Curve** button ✓ to generate a bar chart of the value of the curve at that specific time point. To add values from different time points to the same histogram select a second Time point. Then, click the + button to add a new set of values to the existing chart.

The following example show the same set of samples from the previous image but with the legend changed to contain information on Target cell name (**HeLa**), seeding density (**5,000**) and treatment name/concentration (**Paclitaxel 1 pM, 300 nM and 100 nM**).

## Data Analysis



### Time Dependent IC50

The **Time Dependent IC50** describes how the IC50 changes within a given time period following a compound treatment. In contrast to end-point assays which can only provide a single IC50 value, the continuous data traces recorded by RTCA make it possible to evaluate how IC50 varies as a function of time. This enables the user to define the kinetics of a treatment's effectiveness and potency. The **Time-dependent IC50** is used in cell-based assays when compound treatment has been performed on the cells in multiple wells and at different concentrations. Applications include compound-induced cytotoxicity, compound-mediated cell adhesion, receptor-activation, etc.

To calculate the **Time-dependent IC50**, the software automatically extracts 20 data points from within the chosen time period, calculates the IC50/EC50 values at each of these points, and then draws a curve showing the time-dependence of IC50 or EC50 values (X-axis: time point; Y-axis: IC50/EC50 values). If there are fewer than 20 time points in the chosen period, then IC50 values for all the points will be calculated.

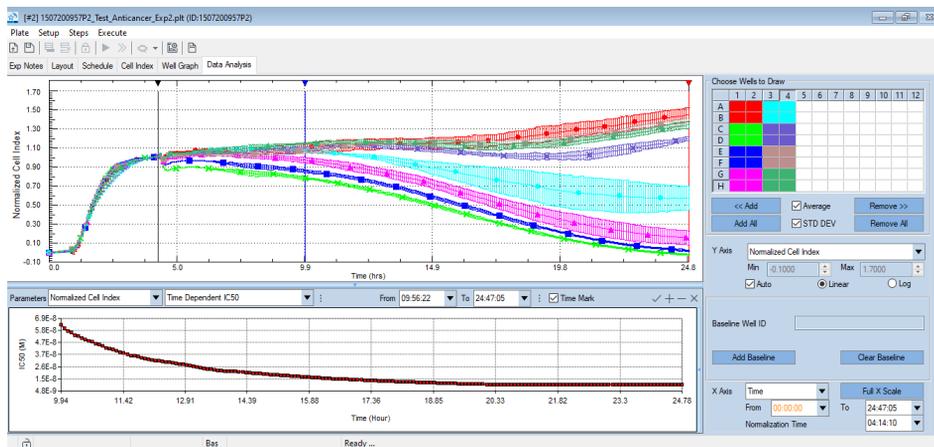
To derive a Time Dependent IC50 curve between group of wells in a time period:

- Select a group of wells on the **Well Map**.
- For the **Y-Axis** choose the appropriate parameter (**Cell Index**, **Normalized Cell Index**, etc.).
- Click the **Time Mark** button and select the desired analysis time range points (in the "**From**" and "**To**" boxes) dragging the red and blue triangles/lines.
- On the **Parameter Selection** drop-down menu select the parameter to be charted (e.g. **Normalized Cell Index**).

## Data Analysis

- On the **Calculation Parameter Selection** drop-down menu select **Time Dependent IC50**.
- Click the **Draw a New Curve** button ✓ to generate the **Time Dependent IC50**.

The following example shows a curve generated for an eight doses treatment.



## Dose Response Curve (DRC)

A Dose Response Curve displays how the Cell Index (or other parameter) varies as a function of drug/treatment concentration. Fitting a dose-response curve to either of two equations (shown below) enables calculation of IC50/EC50 values.

Although the parameter plotted on the X-axis of a DRC is always the compound concentration, different parameters can be plotted on the Y-axis. One of the most straightforward DRC formats plots the Cell Index, at a specific time point post compound addition, on the Y-axis. Other parameters that can be plotted on the Y-axis of a DRC include:

- The maximum Cell Index within a given time frame
- The minimum Cell Index within a given time frame
- The maximum Cell Index minus the minimum Cell Index within a given time frame
- The area under the Cell Index curve (AUC) within a given time frame

Which parameter is plotted on the Y-axis of a DRC is decided based on the objectives of the researcher. Consider, for example, a drug treatment that induces changes in cellular morphology. If the user wishes to analyze how maximal morphological change is dependent on drug concentration, they would use Max Cell Index or Minimum Cell Index (depending on whether the drug-induced cell morphology change corresponds to an increase or decrease in Cell Index) as the

## Data Analysis

Y-axis parameter in the DRC curve. In other assays, the user may be interested in the overall effect of the compound over a given time period. In this context, Area-under-the-Curve can be used as the parameter for the Y-axis in the DRC.

### WARNING

**If a user defined concentration unit has been selected in the plate layout, it is not possible to perform certain calculations or generate certain plots, such as dose-response curves, on the Data Analysis page. If this is attempted, an error message will appear.**

---

### WARNING

**For IC50/EC50 calculations, selected wells must all contain the same treatment (which must be listed on the Layout page). If the Layout page shows that the wells contain more than one type of treatment, go to the Group Wells Pop Up Window, and put a checkmark in the box labeled as Treatment: Cell No. or Concentration; then select the treatment of interest. This will cause the corresponding wells to be highlighted in white in the Well Map. Select these wells (causing them to turn dark blue) and then press the button ✓ to plot their data.**

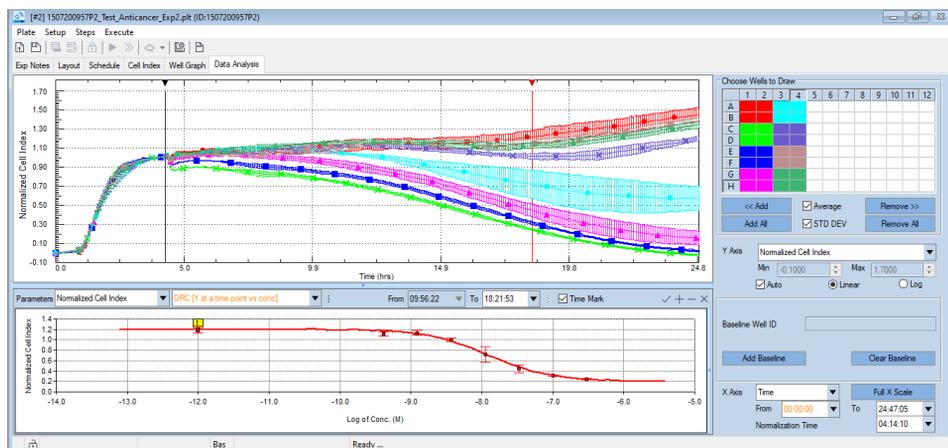
---

To generate a DRC for a treatment group at a specific time point:

- Select a group of wells with the same treatment on the **Well Map**.
- For the **Y-Axis** choose the appropriate parameter (**Cell Index, Normalized Cell Index**, etc.).
- Click the **Time Mark** button and select the desired analysis time point (in the "To" box) dragging the blue triangle/line.
- On the **Parameter Selection** drop-down menu select the parameter to be charted (e.g. **Normalized Cell Index**).
- On the **Calculation Parameter Selection** drop-down menu select one of the DRC curves (e.g. **Y at time point vs conc**).
- Click the **Draw a New Curve** button ✓ to generate the **Dose Response Curve**.
- To add another DRC curve from another compound or from a different time point select a new set of wells/second Time point. Then, click the + button to add a new curve to the existing one.

**Normalized Cell Index DRC** generated for the same eight doses treatment described above.

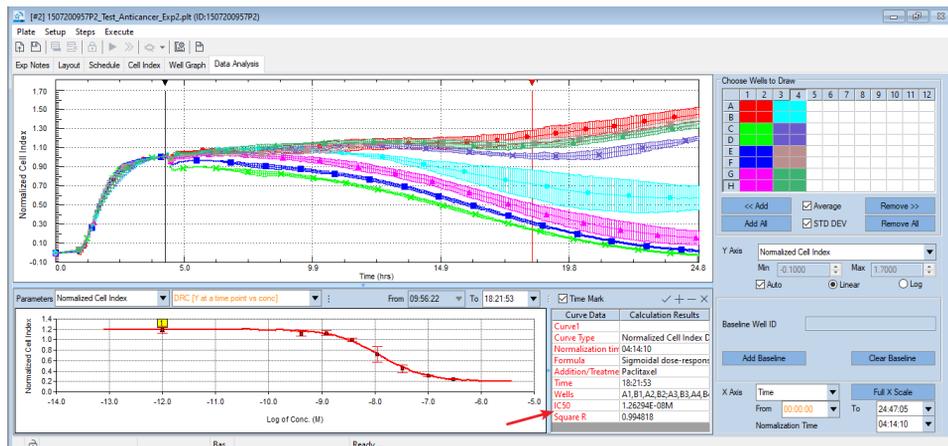
## Data Analysis



### Opening the Analysis Chart-Linked Table

To open a table with data linked to the generated chart, click on the light blue triangle at the right side of the **Analysis Chart** area. The table contains information like curve type, analyzed wells, IC50 and coefficient of determination (Square R).

Example of the **Analysis Chart Table** for the DRC curve described above. The table is opened by clicking the light blur triangle at the right side of the **Analysis Chart** Area (red arrow).



### Dose-Response Curve Fitting Formulas

There are two formulas available for calculating IC50/EC50 values:

- Sigmoidal dose-response

## Data Analysis

$$Y = Bottom + (Top - Bottom)/(1 + 10^{((Log EC50 - X) * HillSlope)})$$

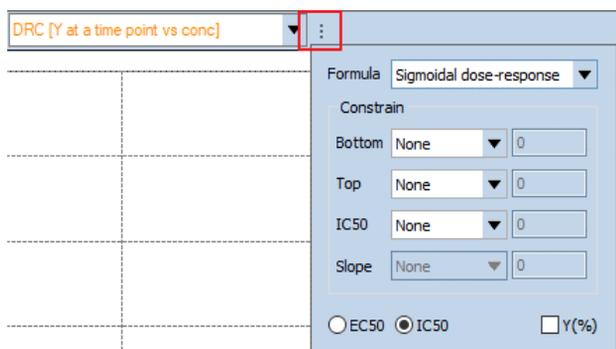
While HillSlope is fixed to -1 or 1.

- **Sigmoidal dose-response (Variable slope)**

$$Y = Bottom + (Top - Bottom)/(1 + 10^{((Log EC50 - X) * HillSlope)})$$

While HillSlope could be any number except 0.

The fitting formula, as well as constrains, are selectable through the **Dose Response Curve editing** window that can be opened by clicking the **Dose Response Curve set up** button.



Though EC50 is used in both of the above equations, this is interchangeable with IC50 by clicking one of the two radio buttons on the lower left of the **Data Analysis** page. If **EC50** is selected, then EC50 will be used in the formulas, and the calculated results will be labeled as **EC50**.

### NOTE

The choice of **EC50** or **IC50** simply affects how the results are labeled and shown but does not affect how the results are calculated.

# Additional Data Analysis Functions (Immunotherapy Module)

The Data Analysis functions that are implemented in the Basic version of the RTCA Pro are also available in the Immunotherapy module. However, there are additional analysis parameters specifically developed for Immunotherapy data analysis that are only available when the Immunotherapy module is active.

## Killing Time (KT) Calculation

One of the main characteristics of the xCELLigence platform when applied to Immuno-Oncology experiments is the access to detailed killing kinetics through high sampling rate (5 to 15 min) over a long assay time (over a week). The detailed kinetic allows “realistic” potency comparison between samples not only at a specific time point, but also across the temporal scale. The KT parameter takes full advantage of such feature and calculates, for every sample group, the time necessary to kill a specific % of the target cells based on the % Cytolysis plot and the improved accuracy reached with background subtraction. The software offers a full set of KT measurements ranging from 20% to 80%. The desired KT parameter can be selected in the Parameter Selection drop-down menu once a % Cytolysis plot has been generated. KT values are displayed through bar charts.

To generate a **Bar Chart** for the **KT50**:

- Select a group of wells on the **Well Map**.
- Display the **Normalized Cell Index** and set up the normalization time point to the last reading before adding effector cells and treatments.
- Display the **% Cytolysis** plot by selecting the parameter in the **Y-Axis** drop-down menu.
- Adjust **Reference** and **Background Subtractions** if necessary.
- Select **KT50** parameter in the **Parameter Selection** drop-down menu.
- Click the **Time Mark** button and select the desired analysis time range points (in the “**From**” and “**To**” boxes) dragging the red and blue triangles/lines. Usually such time ranges should span the whole killing kinetic, from the initial addition of effectors/treatments to the end of the experiment.
- Click the **Draw a New Curve** button ✓ to generate the **KT50 Bar Chart**.

## Data Analysis

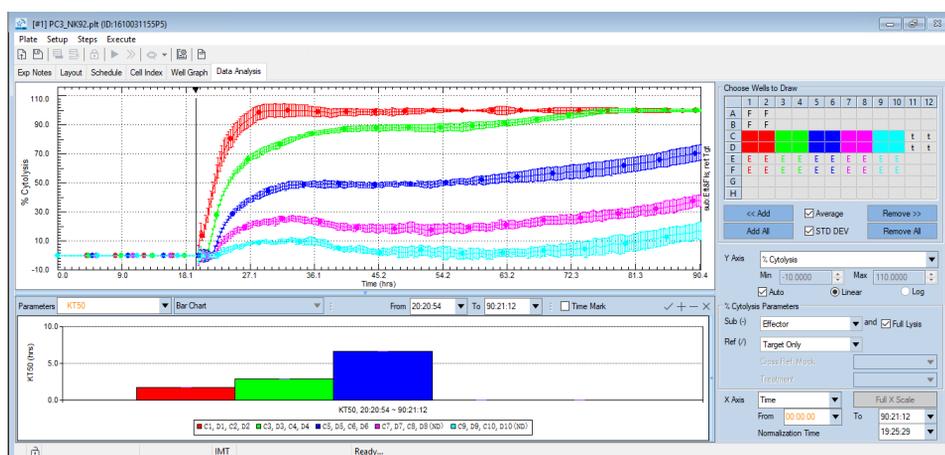
### NOTE

The KT parameter is measured as time of killing and it is calculated starting from the **Normalization Time Point** with the assumption that it corresponds to the time when effectors and other relevant conditions are added to samples.

### NOTE

When generating the KT Bar chart, if a sample never reaches the measured % killing across the set time range, the software will show 0 value for the parameter and will add the designation Not Determined (**ND**) below the sample chart.

**KT50 Bar Chart** generated for a set of samples. The Bar Chart legend shows E:T ratios and two conditions that are not reaching the 50% killing mark (**ND; E:T 1.3:1** and **0.6:1**).



## Comparing % Cytolysis Curves

The Immunotherapy module allows % Cytolysis calculation from samples with different content as reference (e.g. Target, Target+Effectors, Target+Mock Effectors) and also impedance signal subtraction from background samples (e.g. Full Lysis control or Effector alone control). Sometimes it is useful to compare the different % Cytolysis curves by having them plotted together. The Curves from the upper Plot area feature is selected in the **Calculation Parameter** drop-down menu and allows for transferring a curve that is plotted in the **Curve Plot** area to the **Analysis Chart** area. % Cytolysis curves that are generated from the same group of wells through different compensations can be added to the plots and compared in the **Analysis Chart** area.

To move a % Cytolysis plot from the **Curve Plot** area to the **Analysis Chart** area:

- Select a group of wells on the Well Map.

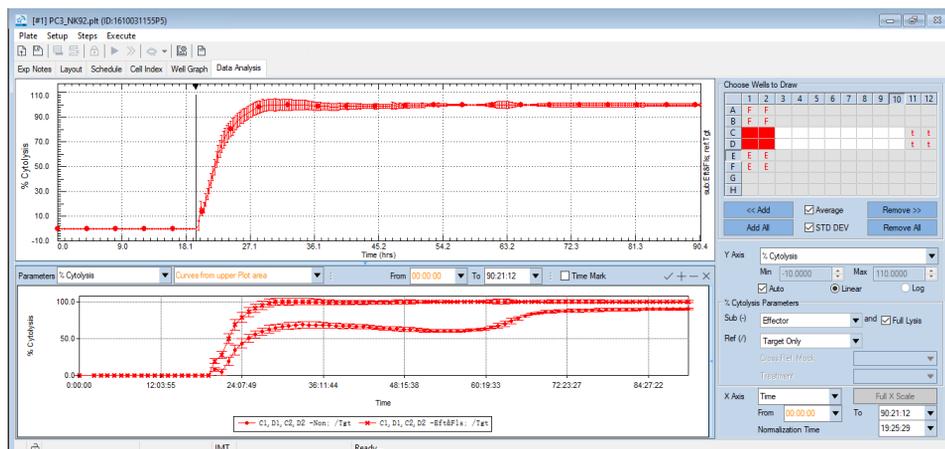
## Data Analysis

- Display the **Normalized Cell Index** and set up the normalization time point to the last reading before adding effector cells and treatments.
- Display the % Cytolysis plot by selecting the parameter in the **Y-Axis** drop-down menu.
- Adjust **Reference** and **Background Subtractions** if necessary.
- Select **% Cytolysis** in the **Parameter Selection** drop-down menu.
- Select **Curves from the upper Plot area** in the **Calculation Parameter** drop-down menu.
- Click the **Draw a New Curve** button ✓ to transfer the plot to the **Analysis Chart** area.
- Adjust **Reference** and **Background Subtractions** to generate a new % Cytolysis plot in the **Curve Plot** area
- Click the **Add a Curve** + button to add the new curve to the **Analysis Chart** area.

### WARNING

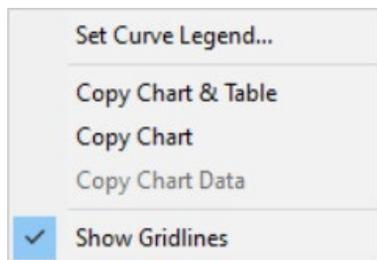
% Cytolysis curves can only be plotted together in the Analysis Chart area if they are derived from the same well groups. If a different set of wells is selected to generate a new curve, the Analysis Chart area will be cleared and will show only the last generated curve when the Add a Curve button is selected.

Two % Cytolysis curves have been generated for the same sample and superimposed for in the **Analysis Chart**. The top curve has been generated subtracting Effector alone and Full Lysis controls, while the bottom curve was not compensated.



# Copy Charts and Data

To copy the content of the **Analysis Charts**, hover over the area and click the right mouse button. Select one of the Copy options from the menu.



- **Copy Chart & Table** allows copying a screen shot of the plotted charts/curves and also of the Chart-linked table.
- **Copy Chart** is used for copying a screenshot of the plotted charts/curves.
- **Copy Chart Data** copies the content of the charts/curves as numerical values that can be transferred to databases or Microsoft Excel Spreadsheets.

To paste the screenshot/numerical values into the software of interest, the right mouse button and paste option should be selected in the destination software.

### NOTE

When using the **Copy** function, the data that are copied are the same data that are visualized in the **Analysis Chart** area (e.g. well average, curve type and time range).

## Analysis Method

**Analysis Method** provides a convenience way to manage the data analysis process and results. By using **Analysis Method**, user can quickly load a group of saved analysis parameters to the **Cell Index**, **Well Graph** and **Data Analysis** pages to restore the analysis data and charts. The **Report** can also use the pre-defined **Analysis Method** to generate a customized report.

**Analysis Methods** are stored within the experiment PLT file and will be saved to the template file when using **Save as Template** function.

Click the **Analysis Method** menu item from the **Setup** menu or the button  on the plate toolbar to open the **Analysis Method** window.



## Data Analysis

Edit Analysis Method

Note that settings of Data Analysis, Cell Index, and Well Graph pages can not be changed.

Name: Report Method 1

Includes:  Data Analysis  Cell Index  Well Graph

Time Resolve Method:  Nearest Time  Sweep Index in Step  Sweep Index in Experiment

Notes:

OK Cancel

- **New:** click to create a new **Analysis Method** from the current settings of an experiment file. Note that some curves in **Data Analysis** page rely on the **Cell** and **Treatment** settings in the **Layout** page, therefore it is high recommended to create the **Analysis Method** after the **Layout** information are entered.

## Data Analysis

New Analysis Method

Create a new Analysis Method by using the current settings of Data Analysis, Cell Index, and Well Graph pages.

Name:

Includes:  Data Analysis  Cell Index  Well Graph

Time Resolve Method:  Nearest Time  Sweep Index in Step  Sweep Index in Experiment

Notes:

OK Cancel

- **Name:** the name of the **Analysis Method**.
- **Includes:** check the checkbox to include **Data Analysis**, **Cell Index** or **Well Graph** page settings to the **Analysis Method**.
- **Time Resolve Method:** this setting is used when the method is imported to other data file. Since even two experiments are created from the same template, the **Cell Index** of every time interval may not be exactly same. This option is used to determine how the software matches the time point in the **Analysis Method** to the current experiment file.
- **Notes:** notes for this **Analysis Method**.
- **Import:** click to import **Analysis Method** from another PLT file.



# 10

## Report, Technical Support Request and Data Export

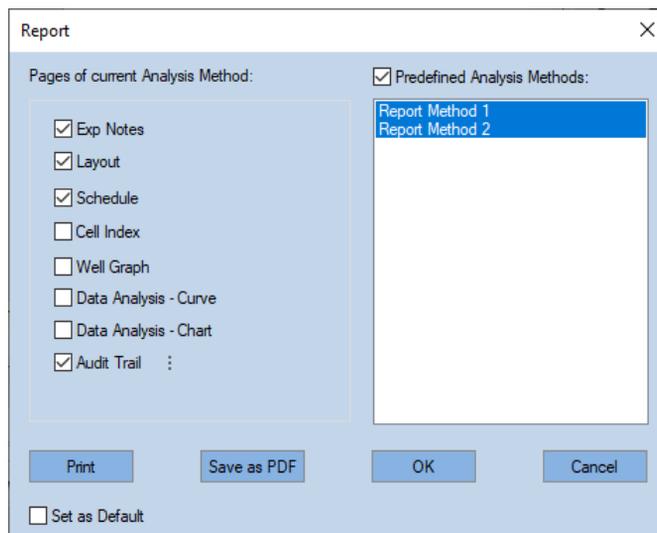
This chapter introduces **Report**, **Technical Support Request**, and data export methods.

### Report

RTCA Software Pro provides convenient ways to generate an experiment report. The report can be printed or saved as PDF file.

The report settings will be stored in the template file when using the **Save as Template** function to generate template file.

Click **Plate > Report ...** to open the **Report** window.



- **Report of current Analysis Method:** check the checkbox of each page to include the correspond information to be included in the report. For information of **Cell Index**, **Well Graph**, **Data Analysis - Curve** and **Data**

## Report, Technical Support Request and Data Export

**Analysis - Chart**, they can be reported by the predefined **Analysis Method**. The boxes checked here will report the results shown in the correspond current pages.

- : click to pop up a window to select **Audit Trail** columns to be included in the report.
- **Predefined Analysis Methods**: check this checkbox to load predefined Analysis Methods. Select Analysis Methods items below for the report to use. Press Ctrl or Shift key to select multiple items.

### NOTE

To report results from **Predefined Analysis Method**, no need to check the boxes in the left area **Pages of current Analysis Method**. For example, if an **Analysis Method** named "Report Method 1" includes **Data Analysis** page and is selected, no need to check the **Data Analysis - Curve** or **Data Analysis - Chart** check boxes to let the report to export **Data Analysis** page. Please refer to [Analysis Method](#) for more details about **Analysis Method**.

- **Print**: click to print the report to a printer.
- **Save as PDF**: click to save the report to a PDF file. PDF/A standard is available for exporting PDF files, refer to [Data Security](#) for how to enable it.
- **OK**: click to save the **Report** settings.
- **Cancel**: click to close the **Report** window without saving changes.
- **Set as Default**: check this box to set the current Report settings as the default setting for new experiment file when **OK** is clicked.

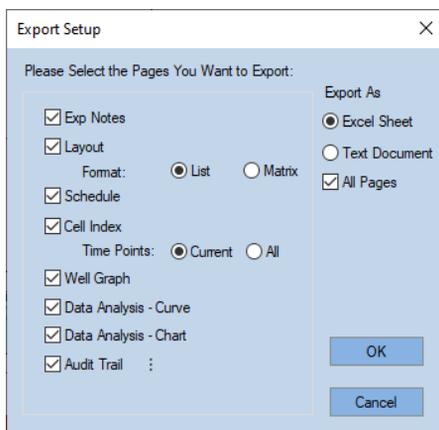
## Exporting RTCA Data

RTCA Software Pro provides convenient ways to export an entire experiment file, individual pages of an experiment file, cell index values from a specific time point, or impedance traces for selected samples.

### Exporting Complete Files or Specific Pages

- Within the **Plate** menu select **Export Experiment Info**.
- Select the Page(s) you want to export (by default, all pages are selected for export).

## Report, Technical Support Request and Data Export



- For the **Layout** category, select the desired format for the exported data: either **List** or **Matrix**.
- For the **Cell Index** category, define which time points will be exported: either **Current** or **All**.

### NOTE

If you choose All time points and the total number of time points exceeds 1000, only the first 1000 time points will be exported.

- Under the **Export As**, select either **Excel Sheet** or **Text Document**.
- Click **OK** to export the selected experimental data.

## Exporting Cell Index Values for a Specific Time Point to Microsoft Excel

- Go to the **Cell Index** page and select a time point from the time drop-down menu.
- The Cell Index values at the selected time point will be shown.
- Right click in the Cell Index table and select **Copy Cell Index**. The Cell Index values will be copied and ready for pasting into an Excel worksheet.
- Open Microsoft Excel and paste the copied data to Microsoft Excel.

## Exporting Cell Index Traces to Microsoft Excel

- Go to the **Data Analysis** page and select the wells to be analyzed on the Well Map.
- Click  to display curves for the selected wells.

## Report, Technical Support Request and Data Export

- Once the curves are displayed, right-click the mouse, and select **Copy Data in List Format** from the pop-up menu. The data for the curves displayed on the chart will be copied (as a list of data points) and ready for pasting to an Excel worksheet.
- Open Microsoft Excel and paste the copied data to Microsoft Excel.

### Exporting Graphic Charts or Data on Charts

- Add the desired curves to the **Curve Plot**.
- Hover the cursor over the **Curve Plot** and right-click the mouse.
- Select **Copy Charts** from the pop-up menu.
- Open the program (typically Microsoft Word/Excel/PowerPoint) you'd like to export to.
- Within the destination program, paste the copied chart.

#### NOTE

Using the above steps, the charts are imported as pictures. Therefore, their curves and labels cannot be modified. If the pasted chart needs to be modified in the future, use the **Copy Data from Chart** function to paste the data into Microsoft Excel (or other data analysis software), then use Microsoft Excel's graphing function to re-plot the data.

## Creating a Technical Support Request

If a user would like to contact an Agilent representative for support in dealing with a problem, they are strongly encouraged to include in their correspondence a **Technical Support Request** file. This facilitates rapid assessment of the issue. To generate a **Technical Support Request** file, follow the steps listed below:

- Launch RTCA Software Pro.
- Open the file for the experiment that has been problematic.
- Select **Plate > Technical Support Request**.
- A dialogue window will pop up asking whether it is OK to save all the **Exp Notes** and **Layout** information to the **Technical Support Request File**. Click **Yes** if there is no confidential information in the experiment. If confidential information needs to be protected, clicking **No** will hide all the **Cell Type** and **Treatment** information in the **Technical Support Request** File. Click **Cancel** if you do not want to create the report.

## Report, Technical Support Request and Data Export

- After you select either **Yes** or **No**, the RTCA Software Pro will create a **Technical Support Request File** with the file name Technical Support Request \*\*\*\*\*.zip.

### NOTE

The created **Technical Support Request File** (Technical Support Request \*\*\*\*\*.zip) is located in the same folder as the original \*.plt data file.

# 11

## Multiple Users Working with an RTCA Instrument

This chapter describes how multiple users can work with an RTCA instrument and unique functions associated with xCELLigence RTCA MP and DP.

RTCA Software Pro is designed to allow for multiple users to work with a single RTCA instrument. Different users are able to run and analyze their own experiments without interfering with each other's data.

When a user logs into the software, only E-Plates (MP) or cradles (DP) reserved by that user can be accessed.

### NOTE

**Enable multiple login for RTCA MP/DP** need to be checked in the **Security Settings** window to allow multiple users to work with the RTCA MP/DP to run their own experiments at the same time. Please refer to [Access Control](#) for this setting.

## RTCA Software Pro Functions Unique To the xCELLigence RTCA MP

### WARNING

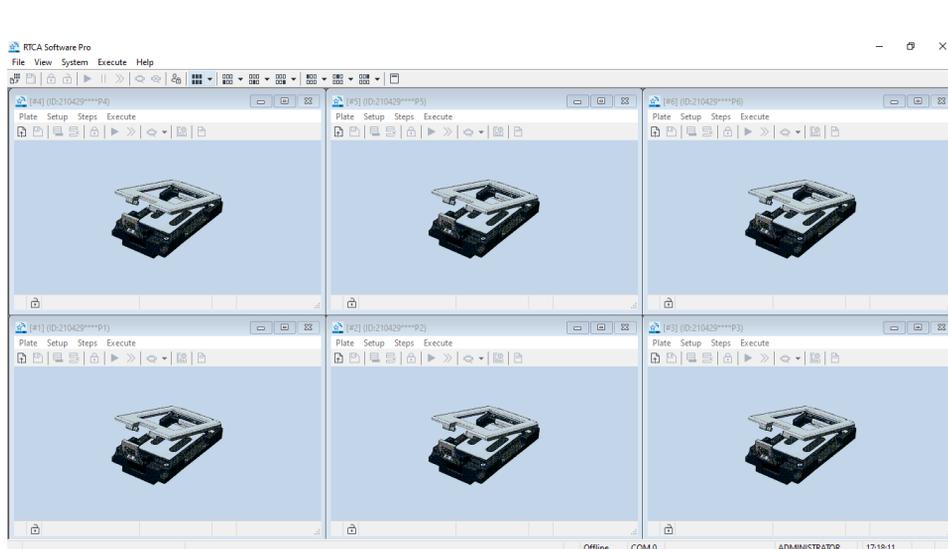
This section is for users of the xCELLigence RTCA MP only.

This section describes the user graphic interfaces for the xCELLigence RTCA MP and the major differences between xCELLigence RTCA SP and xCELLigence RTCA MP. It also gives a detailed example of multiple users running experiments on the xCELLigence RTCA MP.

### MP User Graphic Interface

The MP user graphic interface consists of a **Main Window** and six **Plate Windows** (P1-P6).

## Multiple Users Working with an RTCA Instrument



### NOTE

Both the **Main Window** and the **Plate Window** are resizable.

### NOTE

When the **Main Window** is resized, the six **Plate Windows** are resized accordingly.

### NOTE

Individual **Plate Window** can be resized without affecting other **Plate Windows**.

## The Major Differences of xCELLigence RTCA SP and xCELLigence RTCA MP

In terms of user interfaces and functions, each **Plate Window** within the MP Main Window is similar to the SP Window, and contains all individual-plate-dependent functions, such as **Start Step / Pause / Continue, Abort, Scan Plate**.

The MP Main Window contains the common functions that apply to all six Plate Windows, such as **Start All, Pause All, Continue All, Abort All and Scan All, etc.** Since the xCELLigence RTCA SP supports only one E-Plate 96, those functions for all plates do not apply to the xCELLigence RTCA SP and are therefore not shown in the SP main window. In addition, there is no toolbar on the SP Main Window.

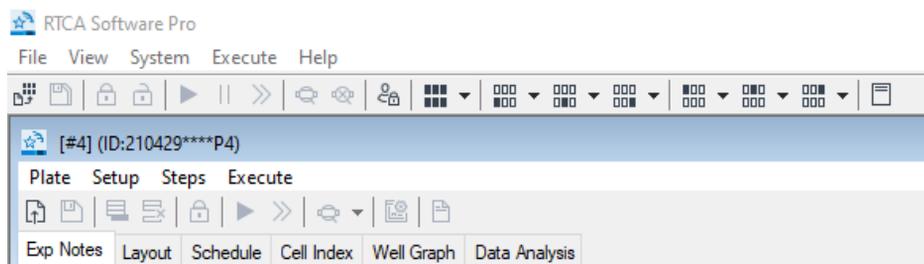
## Overview of Functions and Controls Unique to the xCELLigence RTCA MP

The xCELLigence RTCA MP can simultaneously run up to six E-Plate 96 experiments for up to six different users.

## Multiple Users Working with an RTCA Instrument

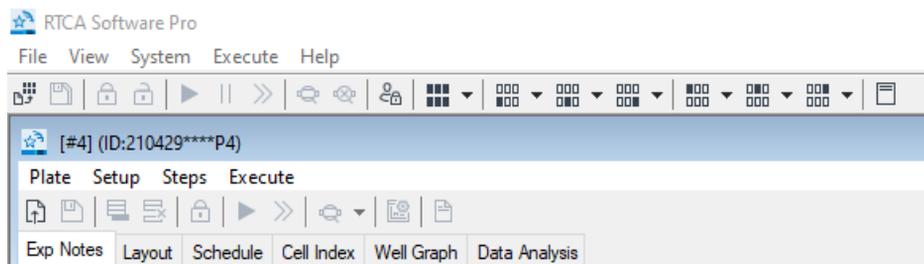
Actions taken on each **Plate Window** are applied to that individual **Plate Window** only.

For example, clicking the **Start** button  on the P4 Plate Window [#4] will start the experiment on Plate [#4] only.



Actions taken on the **Main Window** are applied to all **Plate Windows** that are owned by the current login user.

Clicking the **Start All** button  on a **Main Window** will start experiments on all valid plates.



### NOTE

“Valid Plates” means:

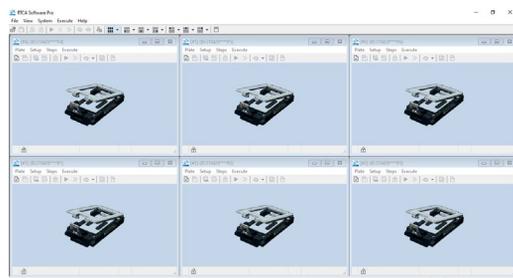
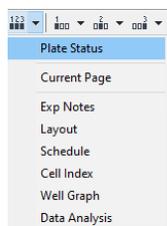
- The E-Plate 96 is owned by the login user.
- The E-Plate 96 experiment is ready to be started.

The table below describes all the functions and controls unique to the xCELLigence RTCA MP.

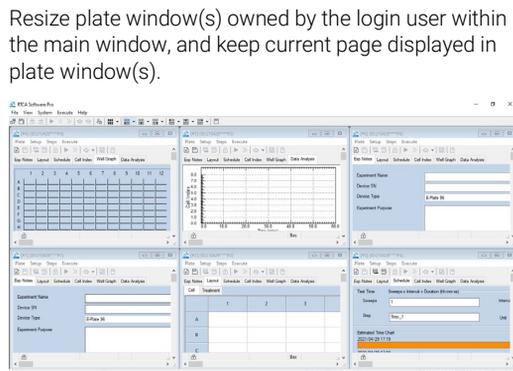
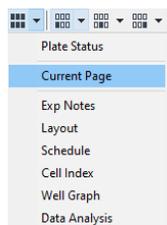
Controls	Function / Example	Comment
	Reopen the most recently opened experiment(s)	Any reserved plate window ignores this command.
	Save changes for plate window(s)	
	Lock cradle(s)	A plate window ignores this if it is already locked.

## Multiple Users Working with an RTCA Instrument

	Unlock cradle(s)	A plate window ignores this if it is already un-locked.
	Start / Continue experiment(s)	
	Pause experiment(s)	
	Cancel Pending action(s)	Pending action is an action issued, but that has not yet been executed, because the analyzer is busy with another plate window.
	Abort current running Step for plate window(s)	
	Scan plate window(s)	
	Stop Scan for plate window(s)	
	Restore all 6 plate windows to fit within the main window, and show the cradle status for each plate window (see next Control Function for an example)	This function does not require the logged-in user to be the owner of the plate windows, or the Administrator. Every login user can do this.

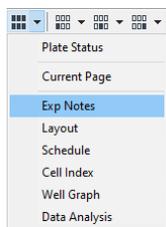


(same function as above)

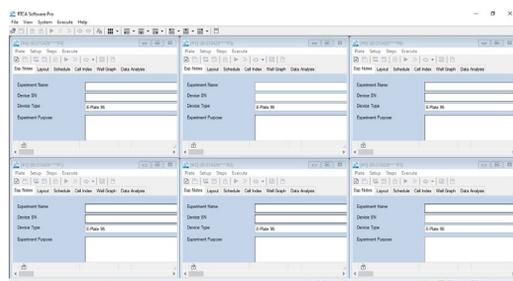


The example shows that the logged-in user is using 6 plate windows, but the 6 plate windows are on different pages. Click **Current Page** under  to resize the 6 plate windows so that they fit within the main window, and keep all current pages displayed. If you do not want to show all of the plate windows you are using, you can minimize those plate windows, and then click **Current Page**, to show only the unminimized windows.

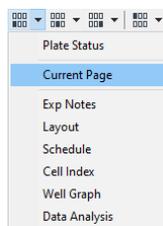
## Multiple Users Working with an RTCA Instrument



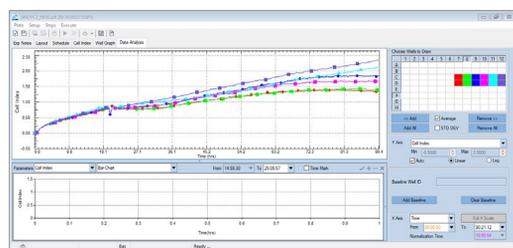
Show **Exp Notes** page(s) for the plate window(s) owned by logged-in user.



Similarly, if you choose one of the other pages (e.g., the Schedule page), the respective page is shown in the plate window(s) owned by the logged-in user.



Maximize the individual plate (P1 to P6) window.



After the selected plate window is maximized, the plate window's current page is not changed.

## Launch RTCA MP Software

Double-click the RTCA Software Pro icon on the xCELLigence RTCA SP/MP/DP workstation. The Software will detect hardware connections and enter different operation modes, depending on the connections detected.

**WARNING**

**It may take a few minutes for the Software to initialize the xCELLigence RTCA MP and becomes ready to use.**

If no xCELLigence RTCA MP is connected to the xCELLigence RTCA SP/MP/DP workstation, or the connected xCELLigence RTCA MP is turned off, the Software may show a dialog window that allows the user to enter the Offline mode. Alternatively, if the xCELLigence RTCA MP connected to the xCELLigence RTCA SP/MP/DP workstation is turned on, the Software will enter the Real-Time mode automatically.

### Example of Multiple Users Running Experiments on the xCELLigence RTCA MP

Take for example the case in which three different users are simultaneously running experiments, with two each, on an xCELLigence RTCA MP.

The three users, in this case, are: User1, User2, and Administrator.

The sequence for running such experiments can be set up using the following steps:

- 1 User1, User2, and Administrator each prepare their two respective E-Plates 96 plates.
- 2 The wells of each E-Plate 96 are filled with cell culture media.
- 3 User1 inserts two prepared E-Plates 96 into P1 and P2 cradles, User2 inserts two prepared E-Plates 96 into P3 and P4 cradles. The last two plates for Administrator will in this case be added later.
- 4 User1 starts the experiment with their E-Plates 96 on the P1 and P2 cradles.
- 5 User1 launches the RTCA Software Pro to enter MP Real-Time mode.
- 6 User1 Login to the RTCA Software Pro with username User1.
- 7 User1 maximizes the P1 **Plate** Window.
- 8 User1 does setup for the **Exp Notes** page, **Layout** page, and **Schedule** page.

#### NOTE

Please see [Record Experimental Notes](#) for setting up the **Exp Notes** page.

#### NOTE

Please see [Define Plate Layout \(RTCA Basic\)](#) and [Define Plate Layout \(Immunotherapy Module\)](#) for setting up **Layout** page.

#### NOTE

Please see [Define Experiment Schedule](#) for setting up **Schedule** page.

- 9 Click  on the P1 **Plate Window** to start an experiment in the E-Plate 96 on cradle 1.

#### NOTE

Cradle 1 will be locked automatically when the experiment starts.

- 10 Repeat Step 5 to Step 7 for the P2 **Plate Window** and E-Plate 96 in cradle 2.
- 11 Remove both the E-Plates 96 from cradle 1 and cradle 2, and then add cells to the appropriate E-Plate Wells.

## Multiple Users Working with an RTCA Instrument

### WARNING

Leave both E-Plate 96 in the tissue culture hood for 30 minutes at room temperature, so the cells settle to the bottom of the well, before returning the E-Plates 96 with cells to their cradles.

- 
- 12 Reinsert the E-Plates 96 to the appropriate positions in **cradle 1** and **cradle 2**, and then have User2 start Step 2 for the next two E-Plates by clicking on Main Window.

### NOTE

Cradles 1 and 2 will be locked automatically when Step 2 starts.

- 13 User1 should now lock out.
- 14 User2 now starts their experiment with E-Plates 96 using the P3 and P4 cradles.
- 15 User2 performs Login to the RTCA Software Pro with username User2.

### NOTE

User2 has no rights to the plate windows of User 1 and cannot access either the P1 or P2 Plate Windows.

- 16 User2 works with P3 Plate Window and P4 Plate Window, following the above steps 5 to step 10 to start experiments with E-Plates 96 on cradle 3 and cradle 4.
- 17 User2 lock out.

### NOTE

P3 and P4 cradles are locked when User2 starts the experiments.

- 18 User1 comes back to perform compound addition to E-Plates at P1 and P2.
- 19 User1 performs login again.
- 20 User1 clicks  Abort All from the Main Window to stop the current step for experiments on the P1 and P2 Plate Windows.

### NOTE

User1's action  Abort All does not apply to User2's E-Plates 96 on cradles 3 and 4.

- 21 User1 clicks  Unlock All from the Main Window. The P1 and P2 cradles are unlocked.

### NOTE

User1's action  Unlock All does not apply to User2's E-Plates 96 on cradles 3 and 4.

## Multiple Users Working with an RTCA Instrument

- 22 User1 now removes the E-Plates 96 from cradle 1 and cradle 2 and adds compounds to the appropriate wells of E-Plates 96.
- 23 User1 replaces these E-Plates 96 to their correct cradle 1 and 2, respectively. The RTCA Software Pro detects the presence of E-Plates 96 on cradle 1 and 2 and starts an auto-scan of both these E-Plates 96 (on cradle 1 and 2).

### NOTE

---

The RTCA Software Pro auto-scans the E-Plates 96 in the order that they were reinserted into the cradle pockets.

- 24 User1 starts Step 3 by clicking ► on Main Window for the two E-Plates.
- 25 User1 performs lock out.
- 26 Administrator performs login to the RTCA Software Pro to start their experiment with the P5 and P6 Plate Windows.
- 27 Administrator inserts two new E-Plates 96 into P5 and P6 cradles respectively.
- 28 Administrator works with P5 and P6 Plate Windows by carrying out the above Step 5 to Step 9 to start the new experiments for E-Plates on P5 and P6 cradles.

### NOTE

---

No one has the privilege to view others' Plate windows to access the running experiments.

- 29 Administrator performs lock out.
- 30 Experiments for all six E-Plates on the MP Station continue and the RTCA Software Pro is ready for either User1, User 2 or Administrator to login.

### NOTE

---

All six E-Plates 96 are locked, so that no one (other than User1, User2 or Administrator) can accidentally remove an E-Plate 96 without logging onto the RTCA Software Pro.

## RTCA Software Pro Functions Unique To the xCELLigence RTCA DP

### WARNING

---

This section is for users of the xCELLigence RTCA DP only.

## Multiple Users Working with an RTCA Instrument

### WARNING

All functions below mentioned are applicable for both E-Plate 16 and CIM-Plate 16.

It describes the connection of xCELLigence RTCA DP, followed by a description of user graphic interfaces and all experiment patterns available when using the xCELLigence RTCA DP. Finally, it discusses the functions and operations that are unique to the xCELLigence RTCA DP.

### xCELLigence RTCA DP Connection

The xCELLigence RTCA DP uses a USB cable to connect to the computer. The USB cable carries out two functions:

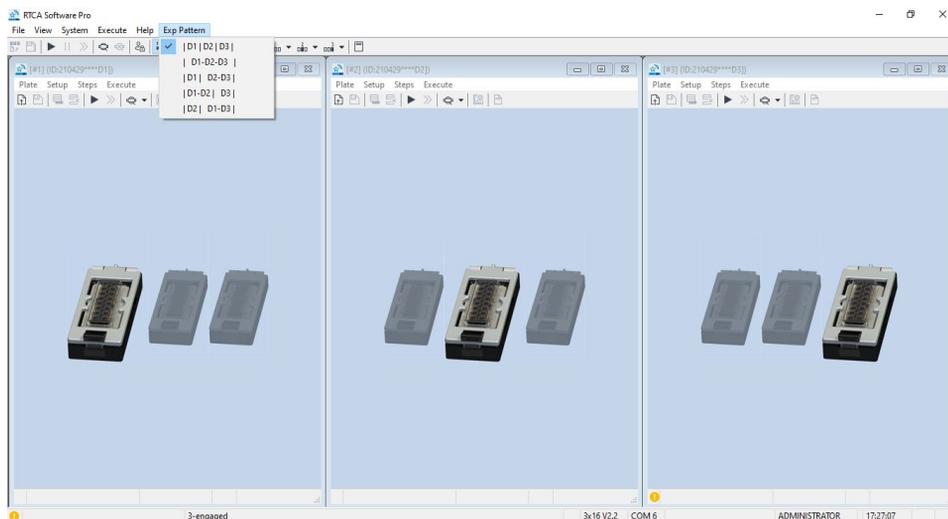
- Communicates between the xCELLigence RTCA DP Instrument and xCELLigence RTCA SP/MP/DP workstation (with computer and software).
- Provides the power supply for the xCELLigence RTCA DP.

### NOTE

There is no extra power supply or power switch on the xCELLigence RTCA DP.

### DP User Graphic Interface

The DP user graphic interface consists of a Main Window and three Plate Windows, similar to RTCA MP software, except that the number of windows is three, and each plate has 16 (not 96) wells.



## Multiple Users Working with an RTCA Instrument

### NOTE

Both the Main Window and the three Plate Windows are resizable.

### NOTE

When the Main Window is resized, the three Plate Windows are resized accordingly.

### NOTE

Individual Plate Windows can be resized without affecting other Plate Windows.

## The Combination of Using Multiple E-Plates 16 or CIM-Plate 16 within One Experiment

In terms of user interface and functions, each Plate Window within the DP Window is similar to the MP Plate Window and contains all the individual-plate-dependent functions such as **Start Step / Pause / Continue, Abort, Scan Plate**.

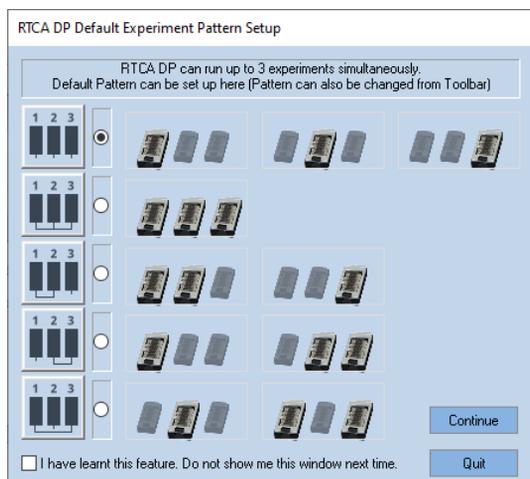
The RTCA DP Software allows users to combine up to three different E-Plates 16 or CIM-Plate 16 in a window when running an experiment. The possible combinations are:

- Run three experiments in three windows with each window containing an E-Plate 16.
- Run an experiment in a single window with all three E-Plates 16.
- Run two experiments in two windows, such that window 1 contains E-Plate #1 and #2, and window 2 contains E-Plate #3.
- Run two experiments in two windows, such that window 1 contains E-Plate 16 #1, and window 2 contains E-Plate 16 #2 and E-Plate 16 #3.
- Run two experiments in two windows, such that window 1 contains E-Plate 16 #2, and window 2 contains E-Plate 16 #1 and E-Plate 16 #3. (not recommended)

### NOTE

Although the last option is available, it is highly recommended not to use this experiment pattern. It can create confusion and may lead to a loss of experimental data, if the wrong cradle is accidentally opened.

## Multiple Users Working with an RTCA Instrument



The major difference between the DP and SP is that the DP has three Plate Windows, while the SP has only one Plate Window; the major difference between DP and MP is that the E-Plates 16 or CIM-Plate 16 can be combined within a window in DP, but not in MP.

### WARNING

**Please note that the E-Plate 16 and CIM-Plate 16 can only be used with the xCELLigence RTCA DP Instrument, and that the E-Plate 96 can only be used with xCELLigence RTCA SP and MP.**

## Overview of Functions and Controls Unique to the xCELLigence RTCA DP

The xCELLigence RTCA DP can simultaneously run up to three E-Plate 16 or CIM-Plate 16 experiments for up to three users.

Actions carried out for each Plate Window are applied to only that individual Plate Window.

For example, clicking the Start button  on the D3 Plate Window [#3] will only start the experiment on the E-Plate [#3].

Actions carried out on the main window are applied to all Plate Windows but are subject to the rights that are owned by the login user.

Clicking on the Start All button  on a Main Window will start experiments on all valid plates (meaning plates for which the logged-in user has rights, see below).

## Multiple Users Working with an RTCA Instrument

### NOTE

“Valid Plates” means:

- 1 The E-Plate 16 or CIM-Plate 16 is owned by the login user.
- 2 The E-Plate 16 or CIM-Plate 16 experiment is ready to be started.

The Table below describes all the functions and controls unique to the xCELLigence RTCA DP.

Controls	Comments
	Run three experiments in three windows with each window containing an E-Plate 16 or CIM-Plate 16
	Run one experiment in a single window with all three E-Plates 16 or CIM-Plate 16
	Run two experiments in two windows, such that window 1 contains E-Plate 16 or CIM-Plate 16 #1 and #2, and window 2 contains E-Plate 16 or
	Run two experiments in two windows, such that window 1 contains E-Plate 16 or CIM-Plate 16 #1, and window 2 contains E-Plate 16 or CIM-Plate 16 #2 and E-Plate 16 or CIM-Plate 16 #3
	Run two experiments in two windows, such that window 1 contains E-Plate 16 or CIM-Plate 16 #2, and window2 contains E-Plate 16 or CIM-Plate 16 #1 and E-Plate 16 or CIM-Plate 16 #3
	Show all windows
	Maximize the window containing E-Plate 16 or CIM-Plate 16 #1
	Maximize the window containing E-Plate 16 or CIM-Plate 16 #2
	Maximize the window containing E-Plate 16 or CIM-Plate 16 #1 and #2
	Maximize the window containing E-Plate 16 or CIM-Plate 16 #1 and #3
	Maximize the window containing E-Plate 16 or CIM-Plate 16 #2 and #3
	Maximize the window containing E-Plate 16 or CIM-Plate 16 #3

## Launch RTCA DP Software

Double-click the RTCA Software Pro icon on the xCELLigence RTCA SP/MP/DP workstation. The RTCA Software Pro will detect hardware connections and enter different operation modes, depending on the connections detected.

## Multiple Users Working with an RTCA Instrument

### WARNING

**It may take a few minutes for the RTCA Software Pro to initialize the xCELLigence RTCA DP and display.**

If no xCELLigence RTCA DP is connected to the xCELLigence RTCA SP/MP/DP workstation, or the connected xCELLigence RTCA DP is turned off, the RTCA Software Pro may show a dialog window that allows the user to enter the offline mode. Alternatively, if the xCELLigence RTCA DP is connected to the xCELLigence RTCA SP/ MP/DP workstation, the RTCA Software Pro will enter the DP mode automatically.

### RTCA Software Pro in DP Offline Mode

When RTCA Software Pro enters the DP offline mode, all hardware-related controls, such as Scan Plate, and Start / Abort Step are disabled. You can however still use the RTCA Software to analyze a previously run experiment, or design experiments for later use.

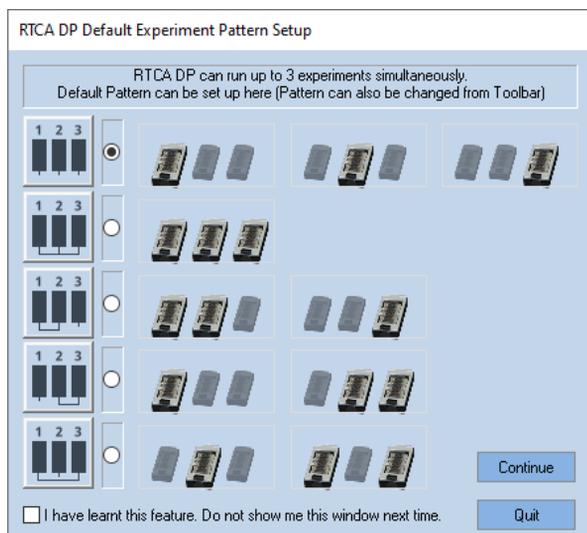
### NOTE

Please check [Saving and Re-Using Experimental Set-ups](#) for details on designing an experiment template.

### RTCA Software Pro in DP Real-Time Mode

When RTCA Software Pro starts, if an xCELLigence RTCA DP is detected, the software enters its real-time mode. Since there are five different possible experiment patterns, the software shows a dialog window where the user can set up a default pattern to launch the DP software.

## Multiple Users Working with an RTCA Instrument



Select a desired pattern from the five available options, and then click **Continue** to enter the DP Software user interface.

### Set up RTCA DP Software Default Pattern

If you do not want to choose the experiment each time the RTCA DP Software starts, you can:

- Select a default pattern.
- Check **"I have learnt this feature. Don't show me this window next time."**
- Click **Continue**.

The default pattern is set, and the above window will not appear when you launch the DP Software next time, and the software enters your chosen pattern directly.

#### NOTE

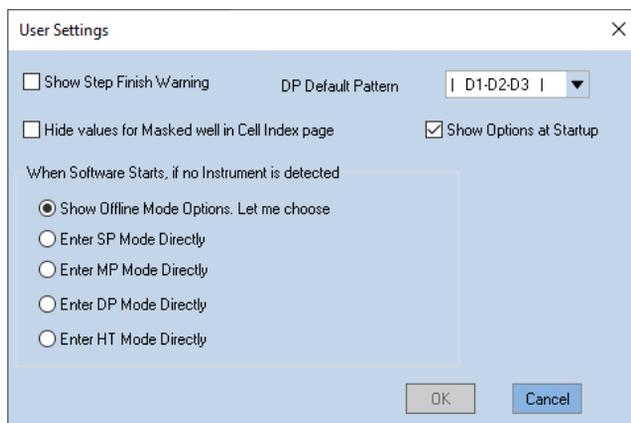
The experiment pattern can also be changed by using the buttons:



If you want to set up another pattern as a default pattern when the DP Software starts, you can use the software user settings from the main window's menu:

- Choose: **System > User Settings**

## Multiple Users Working with an RTCA Instrument



Then either check **Show Options at Startup** to recall the pattern options window or select the default pattern from the DP Default Pattern dropdown list.

### RTCA DP Software Auto-Scan at Startup

When the RTCA Software Pro enters the real-time mode, it automatically performs the Scan Plate function for all the E-Plate 16 or CIM-Plate 16 engaged in the cradles of the xCELLigence xCELLigence RTCA DP Instrument. This automatic Scan Plate may take up to 15 seconds, depending on the number of engaged E-Plates 16 or CIM-Plate 16.

The Scan Plate results will be shown in the Cell Index page after the scanning finishes.

#### NOTE

Please check [Checking the Connections between the Instrument and E-Plate](#) for details of the Scan Plate function.

#### WARNING

**Please do not work with the RTCA DP Software while it is initializing or performing the automatic Scan Plate.**

After Scanning the Plates, RTCA Software Pro is ready for users to start experiments in either of the following ways:

- Start a new experiment in a Plate Window by:
  - Setting up new **Exp Notes** (optional), **Layout**, and **Schedule** pages
- Open an unfinished experiment in a Plate window and continue it.

#### NOTE

Please check [Record Experimental Notes](#) to learn how to set up **Exp Notes** page.

## Multiple Users Working with an RTCA Instrument

### NOTE

Please check [Define Plate Layout \(RTCA Basic\)](#) and [Define Plate Layout \(Immunotherapy Module\)](#) to learn how to set up **Layout** page.

### NOTE

Please check [Define Experiment Schedule](#) to learn how to set up **Schedule** page.

### NOTE

Please check [Saving and Re-Using Experimental Set-ups](#) to learn how to clone experimental settings, individual pages or experiments from either (1) saved templates, or (2) existing experiments.

## Example of Multiple Users Running Experiments on the xCELLigence RTCA DP

Take for example the case in which three different users are simultaneously running experiments using the same xCELLigence RTCA DP instrument, with each user running one E-Plate 16. The three users are: User1, User2, and Administrator

The sequence for running such experiments can be done according to the following steps:

- 1 User1, User2, and Administrator prepare three separate E-Plates 16.
- 2 The wells of each E-Plate 16 are filled with cell culture media.
- 3 User1 inserts the prepared E-Plate 16 into Cradle #1.
- 4 User1 starts the experiment with their E-Plate 16 on Cradle #1.
- 5 User1 launches the RTCA Software Pro to enter DP Real-Time mode.
- 6 User1 performs login to the RTCA Software Pro with username User1.
- 7 User1 chooses  from toolbar to select the separated experiment pattern, i.e., it is possible for the DP Instrument to run three experiments separately. At this time, User1 can use cradle 1, or cradle 2, or cradle 3 to run their experiment. User1 maximizes the Plate 1 Window, and thereby chooses cradle 1.

### WARNING

**Always select only the number of cradles you need to run an experiment. If you are only running a single E-Plate 16, do not choose the option  to occupy all three cradles; selecting more cradles than you are actually going to use, prevents you and others from starting another experiment with unused cradles while this experiment is running.**

- 8 User1 performs setup for the **Exp Notes** page, **Layout** page, and **Schedule** page.

## Multiple Users Working with an RTCA Instrument

### NOTE

Please see Record Experimental Notes for setting up the **Exp Notes** page.

### NOTE

Please see Define Plate Layout (RTCA Basic) and Define Plate Layout (Immunotherapy Module) for setting up **Layout** page.

### NOTE

Please see Define Experiment Schedule for setting up **Schedule** page.

- 9 User1 clicks  on the D1 Window to start an experiment in E-Plate 16 on cradle 1.
- 10 User1 removes the E-Plate 16 from cradle 1, then adds cells to the E-Plate 16 Wells.

### WARNING

**Please always leave the E-Plate 16 in the tissue culture hood for 30 minutes at room temperature, so the cells can settle to the bottom of the well before returning the E-Plate 16 with cells to its cradle.**

- 11 User1 reinserts the E-Plate 16 with cells on cradle 1 and starts Step 2 of the experiment by clicking  on Main Window.
- 12 User1 should now lock out.
- 13 User2 now starts their experiment with E-Plates 16 on cradle 2.
- 14 User2 performs Login to the RTCA Software Pro with username **User2**.

### NOTE

User2 has no rights to the plate windows of User 1 and cannot access P1 Plate Window. At this time, User2 can run a single Device experiment on either cradle 2 or cradle 3. User2 can also run a two E-Plate 16 experiment since both cradle 2 and cradle 3 are available.

- 15 User2 works with D2, following the above described Steps 5 to Step 9 to start experiments with E-Plate 16 on only cradle 2.
- 16 User2 lock out.
- 17 User1 comes back to perform compound addition to E-Plates 16 on cradle 1.
- 18 User1 performs login again.
- 19 User1 clicks  Abort All from the Main Window. This aborts the current step for experiments on D1 Window.

## Multiple Users Working with an RTCA Instrument

### NOTE

User1's action **» Abort All** does not apply to User2's E-Plates 16 on cradles 2. Because User1 only has rights to run an experiment on cradle 1, the command Abort All from the Main Window is identical to clicking Abort from the D1 Window. If User1 had more experiments running, then clicking **Abort All** from Main Window would have aborted all the running experiments for which User1 had rights.

**20** User1 removes E-Plate 16 from cradle 1 and adds compounds to wells of E-Plate 16.

### NOTE

Since, in contrast to the xCELLigence RTCA MP, there is no automatic lock on the xCELLigence RTCA DP preventing other users from accidentally removing E-Plates 16, users sharing the xCELLigence RTCA DP should always make sure they are opening the correct cradle.

**21** User1 reinsert the E-Plate 16 on cradle 1. The RTCA Software Pro detects the presence of the E-Plate 16 on cradle 1 and starts a Scan Plate of E-Plate 16 on cradle 1.

### NOTE

When more than one E-Plate 16 is put into the instrument, the RTCA Software Pro automatically scans the E-Plates 16 in the order they were re-inserted into the cradle pockets.

**22** User1 starts Step 3 by clicking **▶** on Main Window for the two E-Plates 16.

**23** User1 performs lock out.

**24** Administrator performs login to the RTCA Software Pro to work with their experiment with E-Plates 16 on cradle 3.

**25** Administrator can only use cradle 3 to run a single E-Plate 16 experiment, because the other two cradles are already occupied by User1 and User2. Administrator inserts a new E-Plate 16 into cradle 3.

**26** Administrator works with the D3 Window and repeats the above described Step 5 to Step 9 to start the experiment for Plate#3.

### NOTE

No one has the privilege to view others' Plate windows to access the running experiments.

**27** Administrator performs lock out.

**28** Experiments for all three E-Plates 16 on cradles 1, 2 and 3, of the xCELLigence RTCA DP continue running, and the RTCA Software Pro is ready for User1, User2 or Administrator to login.

# 12

## Version History

**Table 1** History of updates

Date/Version	Changed by	Version
2021/05		Revision for 2.6.0 version of the software. Functions supporting GxP compliance and FDA 21 CFR Part 11 are added.

## Appendix A: Permission List

Category	Permission
Instrument Control	Start/Continue/Pause/Abort Experiment
	Scan/Stop Scan Plate
	Lock/Unlock Plate
	Reserve Plate
	Change Measurement Frequency
	QC Test
Edit Exp Notes	Edit Experiment Name
	Edit Device SN
	Edit Device Type
	Edit Experiment Purpose
	Edit Experiment Procedure
	Edit Experiment Conclusion
Edit Layout	Turn On Wells
	Turn Off Wells
	Change Well Type
Edit Layout -> Edit Cell	Change Cell Name
	Change Cell Color
	Change Cell Number
Edit Layout -> Edit Treatment	Add Treatment to Wells
	Remove Treatment from Wells
	Change Treatment Name
	Change Treatment Color
Edit Schedule	Change Treatment Concentration
	Insert Step
	Delete Step
Edit Schedule -> Modify Step	Change Step Name
	Change Step Auto

## Permission List

	Change Step Sweeps/Duration
	Change Step Interval
	Change Step Comments
Cell Index	Change Settings on Cell Index Page
Well Graph	Change Settings on Well Graph Page
	Mask/Unmask Wells
Data Analysis	Change Settings on Data Analysis Page
	Curve Adjustment
	Load Analysis Method
	New Analysis Method
Data Analysis -> Analysis Method	Import Analysis Method
	Delete Analysis Template
	Edit Analysis Method
	Open/Read Experiment File
	Open/Read Template File
Data Read	Open/Read File Owned by Others even File is not Set to Allow Everyone to Read
	View QC Report
	View System Audit Trail
	View File Audit Trail...
	Write to Experiment File
	Write to Template File
	Save Changes to File Owned by Others even File is not Set to Allow Everyone to Write
	Change Allow Everyone to Write Setting of File
	Change Allow Everyone to Read Setting of File
Data Write	Lock/Unlock Experiment File
	Lock/Unlock Template File
	Unlock File Locked by Others
	Save Changes of Cell Index Page
	Save Changes of Well Graph Page
	Save Changes of Data Analysis Page

## Permission List

	New from Template
Data Import	Clone Exp Notes
	Clone Layout
	Clone Schedule
	Change Report Settings
	Set Report Settings as System Default
Data Export	Export Report
	Export File Audit Trail
	Export System Audit Trail
	Print Pages
Data Export -> Export Experiment Information	Export Experiment Information in TXT Format
	Export Experiment Information in Excel Format
Data Export -> Export QC Report	Export QC Report in Excel Format
	Print QC Report or Export in PDF Format
Change System Settings	Change System Settings
	Change Access Control Settings
Change Security Settings	Change Data Security Settings
	Change E-Signature Settings
	Change Audit Trail Settings
Register/Dissociate Software License	Register/Dissociate Software License

## Appendix B: Ordering Information

At Agilent, we are committed to providing innovative, high-quality products and excellent customer service. For a complete overview of our products or to find a local representative, please visit [www.agilent.com](http://www.agilent.com).

Description	Catalogue Number
RTCA Software Basic License for SP/MP/DP (Single)	Cat. No. 5454433001
RTCA Software Basic License for SP/MP/DP (Site)	Cat. No. 310100200
RTCA Software Basic License for HT (Single)	Cat. No. 310100390
RTCA Software Basic License for HT (Site)	Cat. No. 310100400
RTCA Software Immunotherapy License for MP (Single)	Cat. No. 310100230
RTCA Software Immunotherapy License for MP (Site)	Cat. No. 310100240
RTCA Software Immunotherapy License for DP (Single)	Cat. No. 310100270
RTCA Software Immunotherapy License for DP (Site)	Cat. No. 310100280
RTCA Software Immunotherapy License for SP (Single)	Cat. No. 310100290
RTCA Software Immunotherapy License for SP (Site)	Cat. No. 310100300
RTCA Software Immunotherapy License for HT (Single)	Cat. No. 310100410
RTCA Software Immunotherapy License for HT (Site)	Cat. No. 310100420
RTCA Software Compliance License (Single)	Cat. No. S2807-90004
RTCA Software Compliance License (Site)	Cat. No. S2807-90005

# In This Book

This guide describes the following:

- Prologue
- Getting Started with RTCA Software Pro
- Software Interface, Menus & Icons
- Functions Supporting GxP Compliance and FDA 21 CFR Part 11 Compliance
- Setting Up an Experiment
- Running an Experiment
- Monitoring an Actively Running Experiment
- Plotting Data
- Data Analysis
- Report, Technical Support Request and Data Export
- Multiple Users Working with an RTCA Instrument

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