RTCA Software Manual

Software Version 2.1.0

Version November 2018



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Prologue

I. Revision History

Manual Version	Revision Date
1.0	June 2008
2.0 (RTCA Software 1.1)	September 2008
3.0 (RTCA Software 1.2)	March 2009
3.1 (RTCA Software 1.2)	November 2009
4.0 (RTCA Software 2.0)	January 2013
4.1 (RTCA Software 2.1.0)	August 2017
4.2 (RTCA Software 2.1.0)	April 2018
4.3 (RTCA Software 2.1.0)	November 2018

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Questions or comments regarding the contents of this Manual can be directed to the address below or to your local ACEA representative.

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San Diego, CA 92121
USA
softwaresupport@aceabio.com

Every effort has been made to ensure that all the information contained in the RTCA Software Manual is correct at the time of publishing.

For new features and a list of remaining issues, please refer to the RTCA Software Release Note document located in the RTCA Software installation folder (By default under "C:\Program Files (x86)\RTCA\RTCA Software x.x\Document").

However, ACEA Biosciences, Inc. reserves the right to make any changes necessary without notice as part of ongoing product development.

II. Contact Information

	Manufacturer	ACEA Biosciences, Inc. 6779 Mesa Ridge Road, Suite 100 San Diego, CA 92121 USA
	China Distributor	ACEA Bio (Hangzhou) Co., Ltd. Building 5, 2# Xiyuan Wu Road, Xihu District Hangzhou, Zhejiang Province, P.R. China 310030
	International Distributors	Please contact ACEA Biosciences, Inc., or visit www.aceabio.com for a complete list of international distributors

III. Trademarks

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Other brands or product names are trademarks of their respective holders.

IV. Intended Use

The xCELLigence RTCA (Real-Time Cell Analyzer) SP (Single Plate), MP (Multiple Plate) and DP (Dual Plate) Instrument is intended for label-free, real-time, automated monitoring of cell status in a variety of cell-based assays, using proprietary microelectronic sensor technology developed by ACEA Biosciences. They can be used for both high throughput screening and research laboratory environments.

The xCELLigence RTCA SP, MP and DP Instrument are intended for life science research and must be used exclusively by laboratory professionals who are trained in laboratory techniques and have studied the instructions for use of this instrument. **The xCELLigence RTCA SP, MP and DP Instrument are not intended for use in diagnostic procedures.**

V. Software License Agreement

Read the following terms and conditions of this Software License Agreement ("Agreement") carefully before installing or using the RTCA Software, 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 xCELLigence RTCA Instrument, including xCELLigence RTCA SP/MP/DP Control Unit and Software package must be promptly returned to ACEA Biosciences, Inc. ("Supplier") with a copy of the receipt against refunding of the purchase price for this Software.

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b) modifying the infringing Software without affecting its functions and/or with effects that are acceptable to Licensee; or

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7. Import, Export and Use of the Software

Licensee shall be exclusively responsible for ensuring compliance with the relevant legislation relating to its rights to import, export or use the Software.

8. Miscellaneous

Should any part of this Agreement be declared void or unenforceable by a court of competent jurisdiction, the remaining terms shall remain in full force and effect. Failure of Supplier to enforce any of its rights in this Agreement shall not be considered a waiver of its rights, including but not limited to its rights to respond to subsequent breaches. By opening and using this Software Licensee acknowledges that he has read this Agreement, understands it, and agrees to be bound by its terms and conditions. Licensee further agrees that this Agreement is the complete and exclusive statement of the Agreement between Licensee and Supplier and supersedes any proposal or prior agreement, oral or written, any other communications between Licensee and Supplier relating to the subject matter of this Agreement. The headings of the several Clauses of this Agreement are intended for convenience of reference only and are not intended to be a part of or to affect the meaning or interpretation of this Agreement.

9. Governing Law and Place of Jurisdiction

This Agreement shall be governed by and construed in accordance with the laws of the United States of America, without giving effect to any choice of law principles thereof. The parties agree that the United Nations Convention on Contracts for the International Sale of Goods (1980) is specifically excluded from application to this Agreement.

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.

VI. Preamble

This RTCA Software Manual must be used in conjunction with the xCELLigence RTCA SP (or MP, or DP Instrument) Operator's Manual. Before setting up the xCELLigence RTCA SP (or MP, or DP Instrument), users should read the RTCA Software Manual and the xCELLigence RTCA SP (or MP, or DP Instrument) Operator's Manual thoroughly and completely. Non-observance of the instructions contained in this manual may lead to safety hazards.

VII. Usage of the Instrument Software Manual

This Software Manual will help users operate the xCELLigence RTCA SP (or MP, or DP Instrument). It contains the following chapters:

Prologue contains RTCA Software version history and license agreement information.

Chapter A: About RTCA Software contains basic information about the RTCA Software.

Chapter B: Software Function describes the detailed RTCA Software functions and procedures for programming xCELLigence RTCA SP (or MP, or DP Instrument) runs and performing data analysis.

Chapter C: Appendix contains xCELLigence RTCA SP (or MP, or DP) Products Ordering Information.

VIII. Conventions Used in this Manual

Text Conventions

To impart information that is consistent and easy to understand, the following text conventions are used in this Software Manual:

Numbered Listing	Steps in a procedure that must be performed in the order listed.
Italic type, gold	Refers to a different chapter in this Software Manual that should be consulted.
Italic type	Describes buttons, icons or functions when operating the RTCA Software. In addition, important notes and information notes are shown in italic style.

Symbols

In this Software Manual symbols are used as an optical signal to point out important things.

Symbol	Heading	Description
	IMPORTANT NOTE	Information critical to the success of the procedure or use of the product.
	INFORMATION NOTE	Additional information about the current topic or procedure.
▶▶▶		Table continued on next page.
■		End of table.

A About RTCA Software 2.1.0

RTCA Software 2.1 is an integrated software for operating the following 3 xCELLigence RTCA instruments:

- xCELLigence RTCA SP (Single Plate) (here and after refer as “SP”)
- xCELLigence RTCA MP (Multiple Plate) (here and after refer as “MP”)
- xCELLigence RTCA DP (Dual Plate) Instrument (here and after refers as “DP”)

The basic GUI (Graphic User Interface) and operation of the SP, MP and DP are similar. To reduce redundancy, this manual describes only once the common functionalities of all 3 instruments (SP, MP, and DP). The descriptions for common features are based on the SP instrument. MP and DP users should read the chapters specifically prepared for your instrument, then refer to the related chapters for common functionalities and operations.

The RTCA Software has two work modes: Real-Time and Offline mode.

- Real-Time mode: RTCA Software is synchronizing with the xCELLigence RTCA Instrument (SP, MP, or DP), even when there is no experiment running.
- Offline mode: RTCA Software is not connected to xCELLigence RTCA Instrument, or the connection between the xCELLigence RTCA SP/MP/DP Control Unit and instrument is absent.

1. System Requirements and RTCA Software Installation

1.1 System Requirements

The xCELLigence RTCA SP/MP/DP Control Unit is delivered with the xCELLigence RTCA Instrument. The following Hardware and Software requirements are only necessary if you plan to install the RTCA Software on an additional computer.

Hardware (Computer):

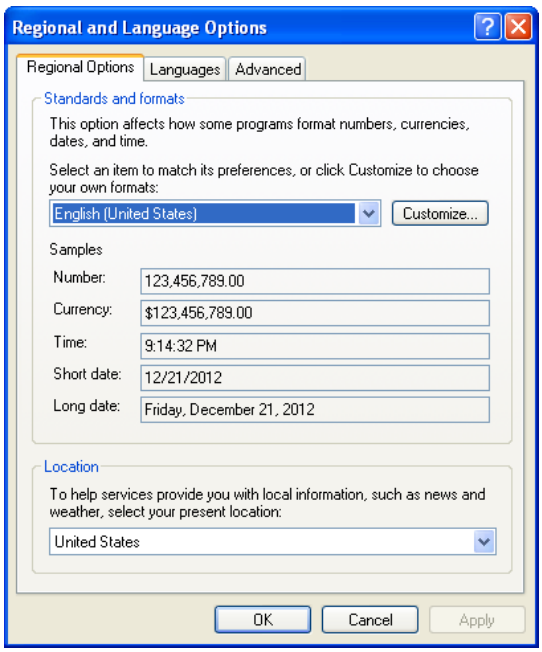
- CPU speed: minimum 1 GHz
- RAM: minimum 256 MB
- Hard Drive free space: minimum 100 MB
- Monitor Resolution: 1024 × 768 or higher

RTCA Software 2.1 is designed to handle one E-Plate with the xCELLigence RTCA MP Instrument, multiple E-Plates with MP Instrument, and multiple E-Plates 16 or CIM-Plate 16 with xCELLigence RTCA DP Instrument.

Software:

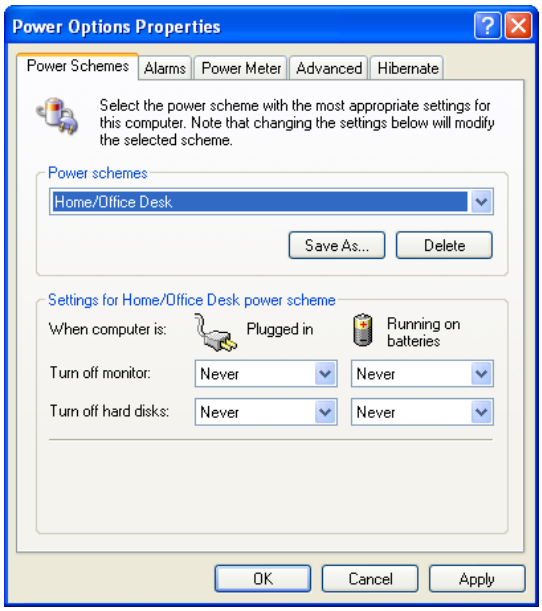
- Windows XP/Windows Vista/Windows 7/Windows 10
- Microsoft Office 2003 or newer (optional, for better data analysis performance)

Regional and Language Options should be set to English (United States). Date/Time/Number should also be set to U.S. styles. Any other settings might lead to problems in data analysis and instability of the Software.



Settings of the xCELLigence RTCA SP/MP/DP Control Unit:

Since the xCELLigence RTCA MP Instrument is a real-time data measuring system, the computer power scheme must be set as follows: ***Never*** *turn off hard disks;* ***Never*** *standby;* ***Never*** *hibernates. Setting "Turn off monitor" to* ***Never*** *is optional.*



Logon to Windows Operating System

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

User Name	Default Password	Group	Description
RTCAOperator	No password	Power Users	Default user for instrument operation. Click *RTCAOperator* (No password) to login Windows when Windows starts.
RTCAdmin	sandiego	Administrators	Administrator is able to perform administrative operations such as: ► Change date and time settings ► Perform software updates This password should only be distributed to IT or lab administrators.

How to Change Windows Users

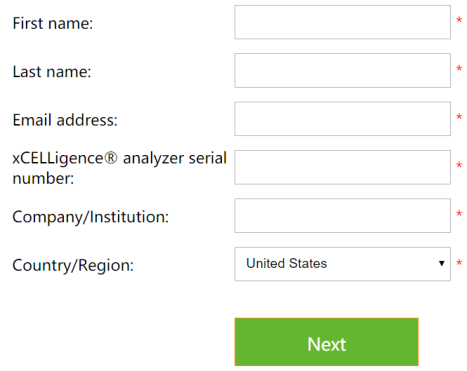
1. Start the xCELLigence RTCA SP/MP/DP Control Unit
2. Click *RTCAOperator* (No password), or *RTCAdmin*. If *RTCAdmin* is clicked, password is required in order to login.

A

1.2 Install RTCA Software

1.2.1 Software download

1. Make sure the computer with which you want to register RTCA is connected to the Internet. Go to https://www.aceabio.com/software/rtca in a web browser.
2. Copy the serial # of the RTCA station, then use it to log in and download the software.



3. Click the *Download RTCA Software* tab to download the RTCA software.



1.2.2 Software Installation

1. Unzip the installation package.
   Run Setup.exe and follow the instructions to install the RTCA Software.
   Select the installer language on the menu that appears:



   Click *OK* to continue.

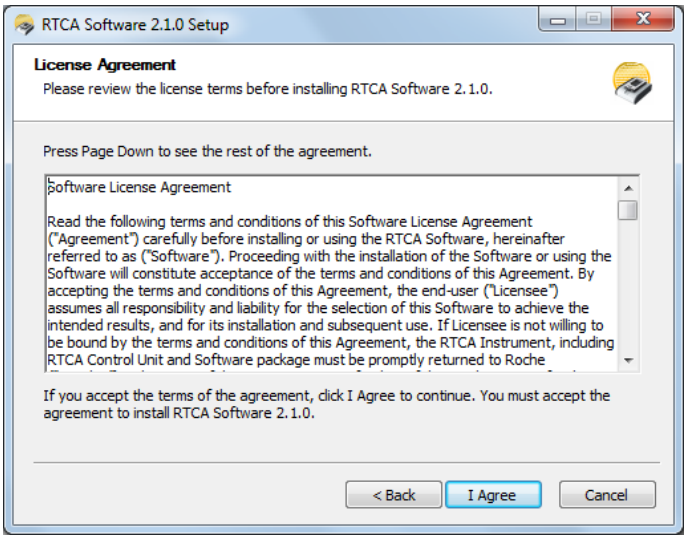
A

2 Start the RTCA Software Setup wizard. This page will appear automatically after step 1:



Click *Next* to go to next step.

3 Read the terms of the license agreement carefully. If you agree, click on *I Agree* and go to the next page to select the installation folder.



4 Select Installation Folder.

On this page, the user can browse-select or type in the name of a folder (location) where the RTCA Software will be installed.

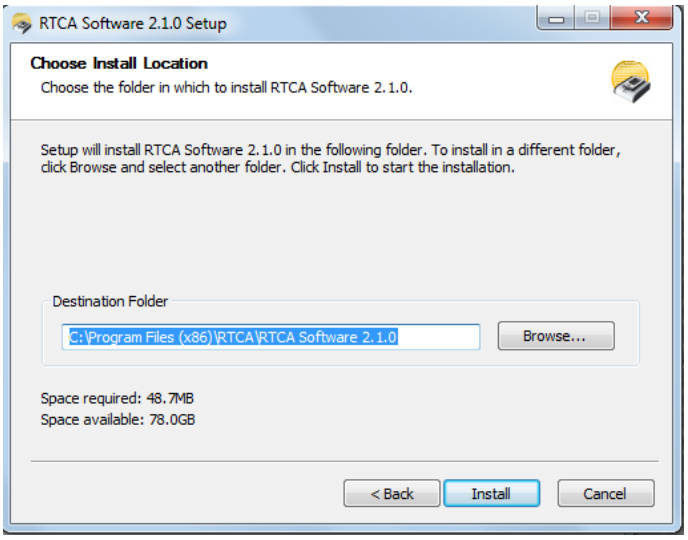
- To browse-select a folder: Click Browse… and choose an existing folder
- To type in folder name: click the text box under Destination Folder

If the folder does not exist, the installation program will create the new folder.

The folder name should not include "?", "-" or "*"

The default installation folder is C:\Program Files (x86)\RTCA\RTCA Software 2.1.0.

▶▶▶



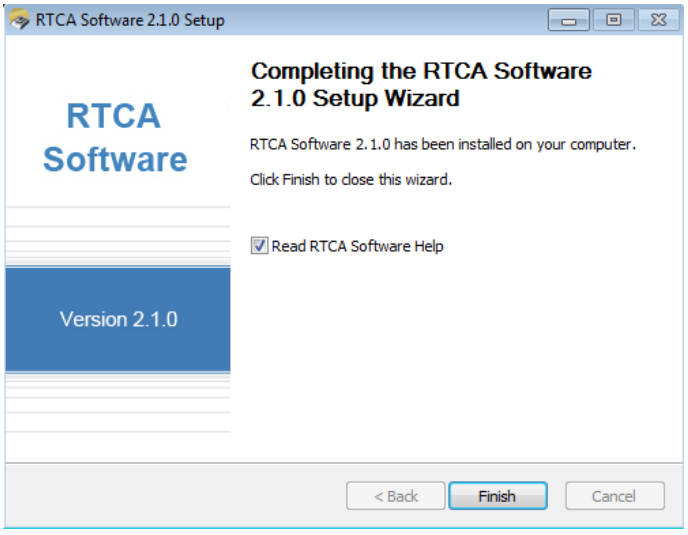
After choosing the installation folder, click *Install* to confirm installation.

5 If the computer has not previously installed Microsoft Office 2003 Web Components, a page with a license agreement will appear automatically. Otherwise go to the next step.

Check *I accept the terms in the License Agreement* and click *Install* to install Microsoft Office 2003 Web Components automatically.

6 **Finish the installation**

Click *Finish* to complete the installation, and RTCA Software Help will be shown. To skip showing the RTCA Software Help, uncheck *Read RTCA Software Help*.



1.2.3 Launch RTCA Software

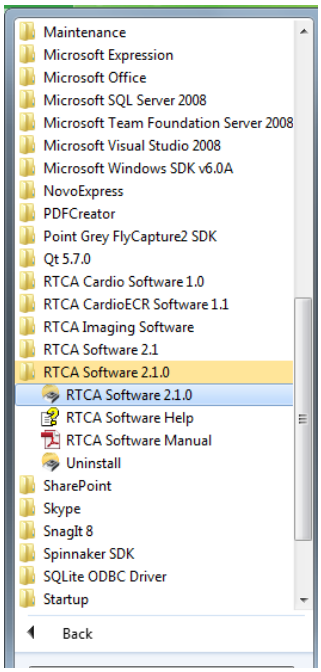
Two short-cuts for launching RTCA Software are available after installation:

- Computer Desktop



A

- Computer Program Menu



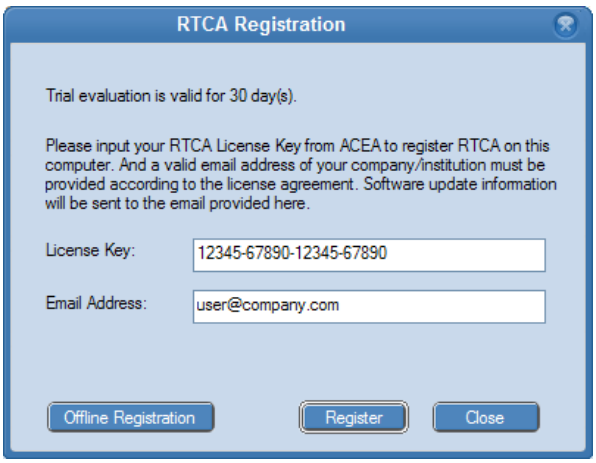
1.3 RTCA License

The RTCA software is available for a 30-day free trial. Registration will be needed to use the software after the trial expiration. RTCA Software License Key comes with the RTCA SP, MP or DP instrument, and can also be purchased separately from ACEA.

1.3.1 RTCA Registration

The RTCA Registration window will automatically pop up when starting the RTCA software if the software is not registered yet. You can also click *Help* → *About* → *Licensing* to open the *RTCA Registration* window. There are two ways to register the software.

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



A

- If the computer is not connected to the Internet, click the *Offline Registration* button to switch to offline registration mode. Write down the *Machine Code* displayed in the window, go to another computer that is connected to the Internet and open the *Get Registration Code* web page (https://www.aceabio.com/software/rtca). On the web page, enter the *Machine Code* into the specified textbox, type a valid ACEA Biosciences issued license key and your company/institution email address, then, click *Get Registration Code*. Write down the *Registration Code*, enter it to the *RTCA Registration* window, and click *Register* button to register the RTCA software.

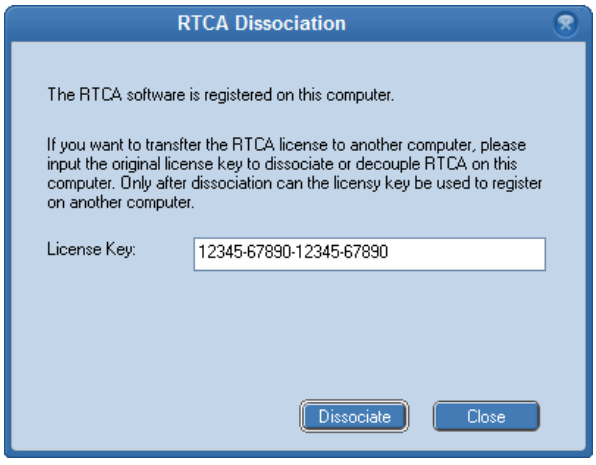


A valid email address of your company/institution must be provided according to the license agreement. Software update information will be sent to the email provided here.

License key is required for RTCA software registration or license transfer. Keep a safe record of license key assigned to each computer or user.

1.3.2 RTCA Dissociation

The RTCA software supports license transfer to another computer. Each license may be transferred up to 5 times. If you want to transfer the RTCA license to another computer, click *Help* → *About* → *Dissociation* to open the *RTCA Dissociation* window.



Enter the original license key in the text box and click the *Dissociate* button to dissociate or decouple the RTCA from this computer. After dissociation, the license key can be used to register the RTCA software on another computer.

Please connect the control unit to the Internet when dissociating the license. Contact ACEA technical support for how to dissociate the license if no connection to internet is available.

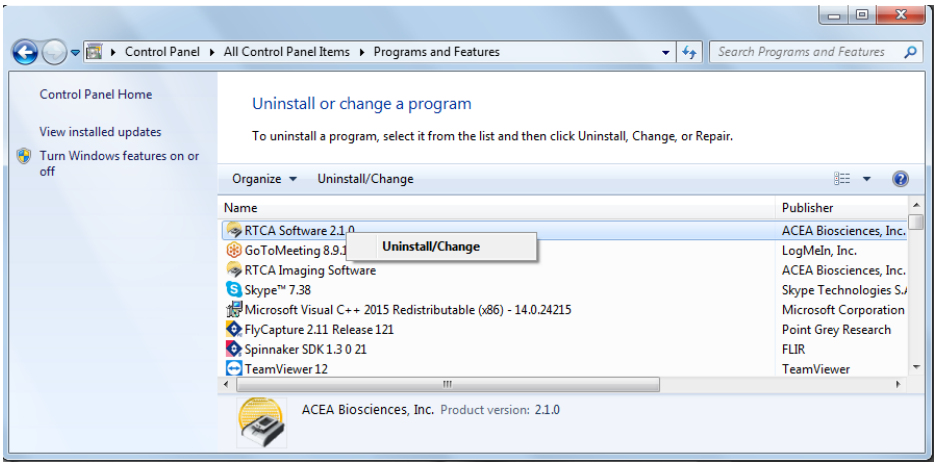
A

1.4 Uninstall RTCA Software

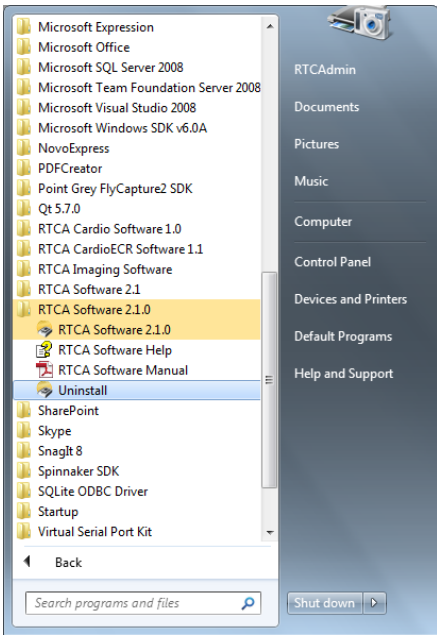
There are two ways to uninstall RTCA Software from the computer:

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

  Start → *Settings* → *Control Panel* → *Add or Remove Programs*, then highlight *RTCA Software 2.1.0*, click *Uninstall/Change*.



- Alternatively, use the Computer Program Menu:



1.5 User Management

There are three built-in usernames in the RTCA Software. They are: ***Administrator, User1, and User2.***

To change a user's password:

- Login to RTCA Software with a valid username and password.
- Click User Management from Setup menu.

A

- Enter current password in Old Password box.
- Enter new password in New Password box.

Please note that the password of Administrator should contain 4-20 characters.

- Re-enter new password in Confirm Password box.
- Click Change Password button.



The default password of the built-in user ADMINISTRATOR is administrator (all lower case). The default passwords of built-in users User1 and User2 are not set (empty).

The Administrator can change/reset all other users' passwords without knowing those passwords.

To add a new user:

- Login as Administrator.
- Enter a new user's username in User Name box.
- Click Add User button.

The default new user's password is the same as the username (all lower case).

To delete a user:

- Login as Administrator.
- Select a username from the User Name drop down list.
- Click Delete User button.
- In the pop-up window "Are you sure to delete?", click Yes to delete, click No to cancel.

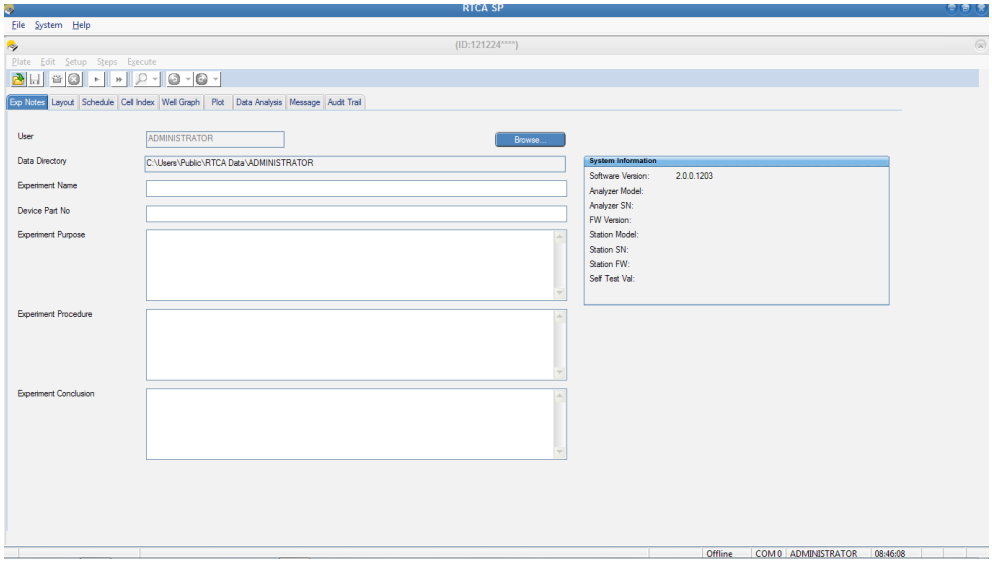
Only the Administrator can delete users.

A

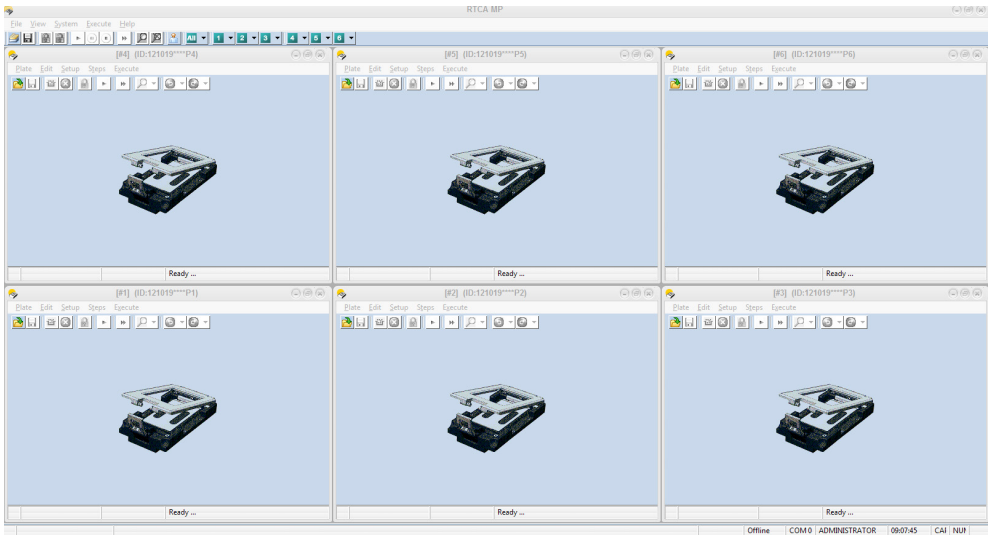
2. Entering SP, MP or DP Real-Time Mode Automatically

When the xCELLigence RTCA Instrument is powered on and connected to xCELLigence RTCA SP/MP/DP Control Unit, the RTCA Software will enter real-time mode directly.

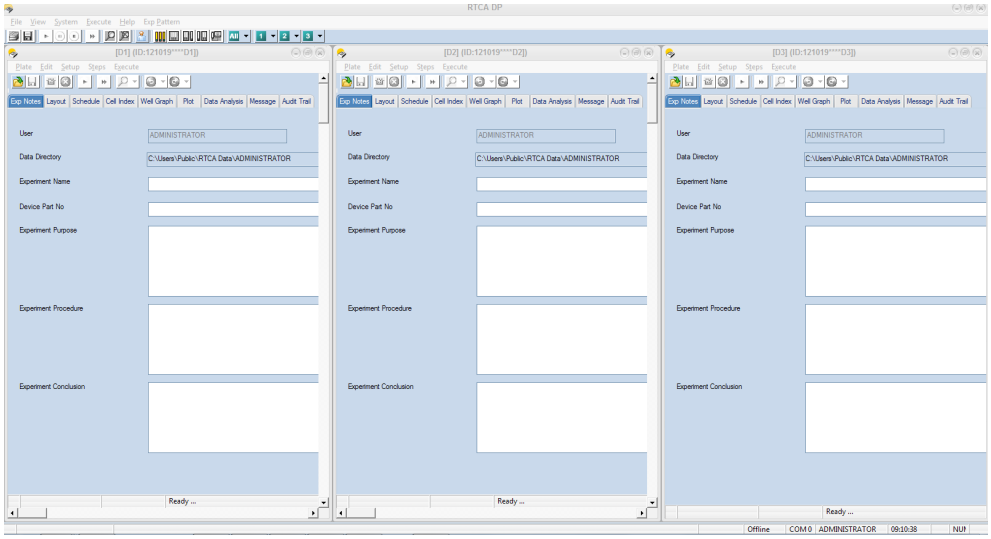
When the RTCA Software starts, it will automatically detect the xCELLigence RTCA Instrument connected to the xCELLigence RTCA SP/MP/DP Control Unit (computer). Based on the instrument configuration, the RTCA Software will launch RTCA SP, or RTCA MP, or RTCA DP user interface directly.



RTCA SP User Interface



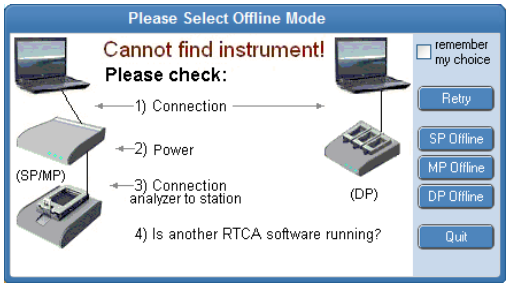
RTCA MP User Interface



RTCA DP User Interface

3. Entering SP, MP or DP Offline Mode

When the RTCA Software starts, if no xCELLigence RTCA Instrument is connected to the xCELLigence RTCA SP/MP/DP Control Unit, or the connected xCELLigence RTCA Instrument is not turned on, the RTCA Software will show a dialog window that allows users to choose the RTCA Software running mode:



Dialogue Window for the RTCA offline mode selection

With this window, the user can click one of five different buttons (from top to bottom):

- Retry Button: The RTCA Software will try to reconnect to xCELLigence RTCA Instrument.

  Make sure the 4 conditions for entering SP or MP real-time mode have been met:

  1. The serial communication cable between xCELLigence RTCA SP/MP/DP Control Unit and xCELLigence RTCA SP/MP Analyzer (in short "SP/MP Analyzer") must be connected securely;
  2. The SP/MP Analyzer is turned on;
  3. A SP Station or MP Station is connected to the SP/MP Analyzer;
  4. No other RTCA Software is running.

  Make sure the 2 conditions for entering DP real-time mode have been met:

  1. Make sure the USB cable between the xCELLigence RTCA SP/MP/DP Control Unit and the xCELLigence RTCA DP Instrument is connected securely, and that at least one of the DP LED indicators is on.
  2. No other RTCA Software is running.

- SP Offline Button: The RTCA Software will enter SP offline mode.
- MP Offline Button: The RTCA Software will enter MP offline mode.
- DP Offline Button: The RTCA Software will enter DP offline mode.
- Quit Button: The RTCA Software will close.

When either SP Offline, MP Offline, or DP Offline is selected with the "remember my choice" box being checked, the RTCA software will enter the selected offline mode (SP, MP, or DP offline) directly if no xCELLigence RTCA Instrument is connected.

The Offline preference does not affect the real-time mode, i.e,. even when the " remember my choice " option was selected, the RTCA Software will detect a connected RTCA SP, MP or DP Instrument the next time, and it will enter into the RTCA SP, MP or DP real-time mode automatically(depending on which instrument has been detected).

The Offline preference settings can be cleared using the command line parameter "x" (see below).

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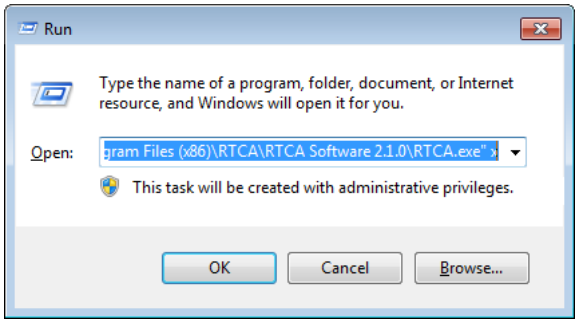
4. RTCA Software Command Line Parameters

RTCA Software supports the following command parameters:

- S Enter SP offline mode directly
- M Enter MP offline mode directly
- D Enter DP offline mode directly
- X Clear Offline Preference setting

Example: Run "RTCA.exe" with parameter "x" to clear offline preference setting.

(From the Windows status bar) Start →Run, type "C:\Program Files (x86)\RTCA\RTCA Software 2.1.0\RTCA.exe" x in the "Open" box, and click OK.



The command must include the quotation mark (") and there is a space between the command and the parameter x.

A

5. How to Use this Software Manual

The basic functions and operations of xCELLigence RTCA MP Instrument, xCELLigence RTCA MP Instrument and xCELLigence RTCA DP Instrument are similar. In *Chapter B Software Functions*, the common functions and operations of RTCA Software are described in Sections 1 through 13, using the SP offline mode as an example. Thereafter, the functions and operations for exclusively the xCELLigence RTCA MP Instrument and xCELLigence RTCA DP Instruments, are covered in Section 14 and Section 15, respectively.

The xCELLigence RTCA MP Instrument user should thus read only Sections 1 through 13, ignoring Sections 14 and 15. The xCELLigence RTCA MP Instrument user can consult Section 14 first to become familiar with the RTCA Software MP user interface; the xCELLigence RTCA DP Instrument user can consult Section 15 first to become familiar with the RTCA Software DP user interface, but both RTCA MP and DP users must then read Sections 1 through 13 for details on RTCA Software functions.

B Software Function

This chapter describes the main functions of the RTCA Software for configuring, monitoring, and analyzing the experiment.

B

1. Overview of the RTCA Software

The user-friendly RTCA Software provides outstanding instrument control for flexible experiment set-ups, data acquisition and data analysis. All instrument and experiment controls are contained in the software to simplify experiment set-up and operation. The RTCA Software consists of nine different functional pages:

Experiment Set-up pages:

- *Exp Notes* page
- *Layout* page
- *Schedule* page

Experiment Monitoring and Data Analysis pages:

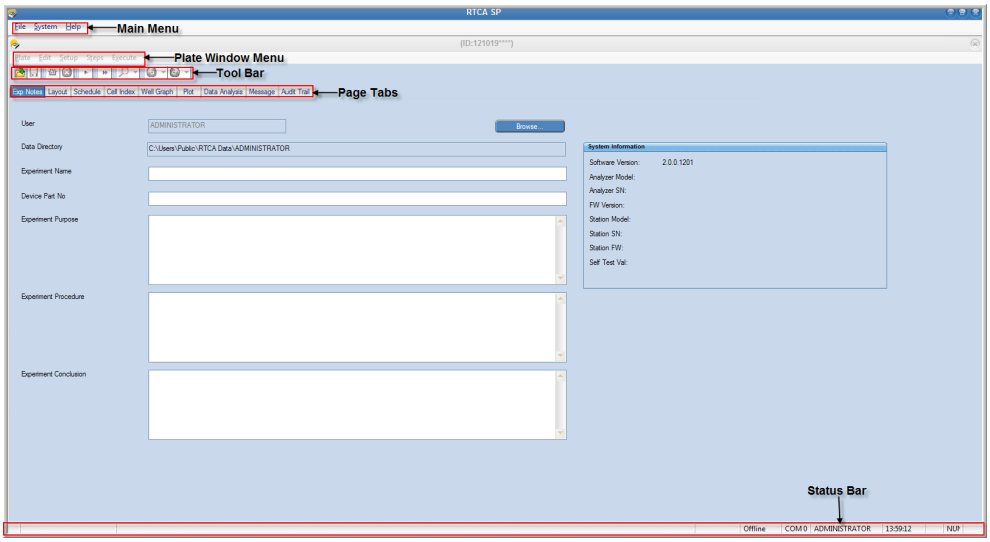
- *Cell Index* page
- *Plot* page
- *Well Graph* page
- *Data Analysis* page
- *Message* page
- *Audit Trail page*

The first three pages contain functions required for running experiments. The other six pages contain functions for monitoring and analyzing experiments. Special features programmed into the RTCA Software provide the flexibility needed for dynamic measurement of cell status in the wells. Based on experiment requirements, the Software can be programmed to take measurements over any time interval, from less than one minute to over a day, and at any frequency, from hours to days or weeks. The real-time data acquisition, data normalization, and data plotting functions allow the user to monitor experiments in real-time. The Software can analyze experimental results, such as Cell Index doubling time, IC_{50} values, or EC_{50} values, at a given time interval, or determine time-dependent IC_{50} or EC_{50} values for the entire treatment period after compound addition.

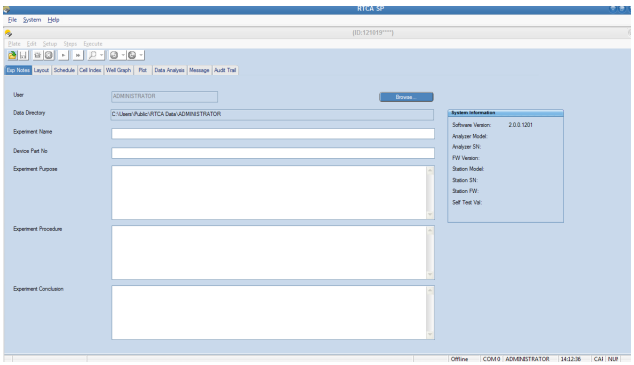
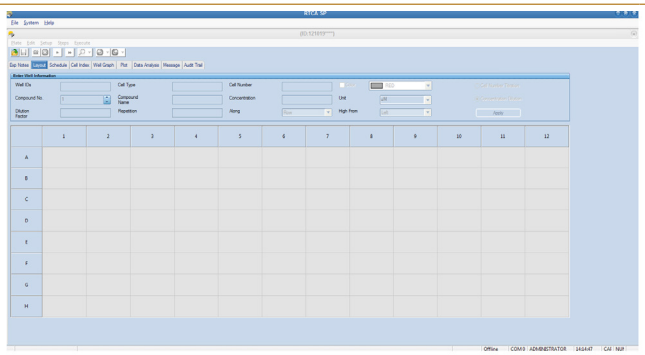
1.1 Software Functions

The following describes the functions and utilities of the RTCA Software that control the xCELLigence RTCA Instrument.

The user interface of the RTCA Software in the SP Instrument contains (from the top to bottom): Main Menu, Plate Window Menu, Tool Bar, Page Tabs, and Status Bar.



RTCA SP Software main user interface

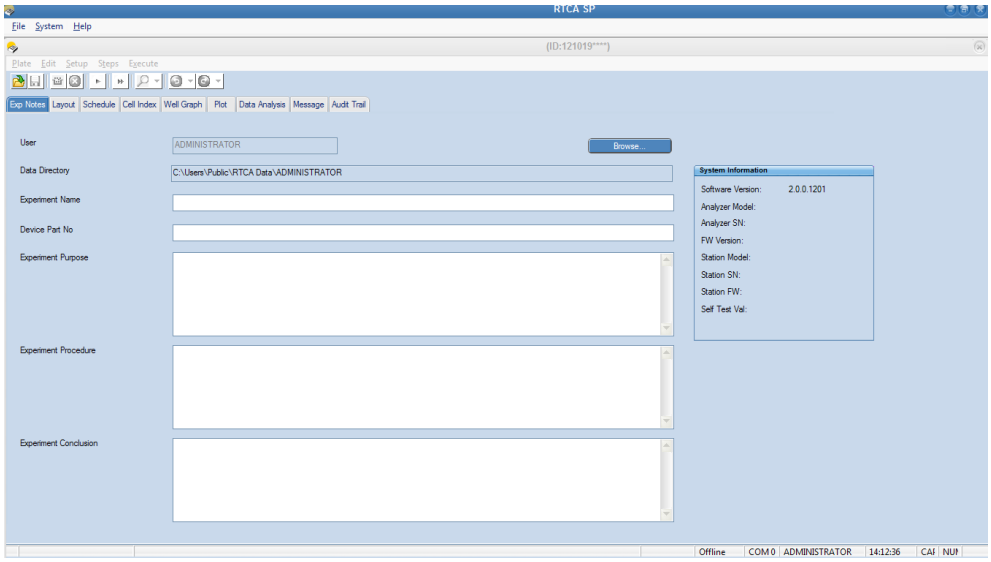
Item	Example	Major Function
Main Window		
Plate Window		Handles all functionalities of an individual experiment on one E-Plate
Main Menu		User actions to operate RTCA Software
Plate Window Menu		User actions to handle all functionalities on one or multiple E-Plates
Plate Window Tool Bar		Short-cuts of frequently used menu functions

►►►

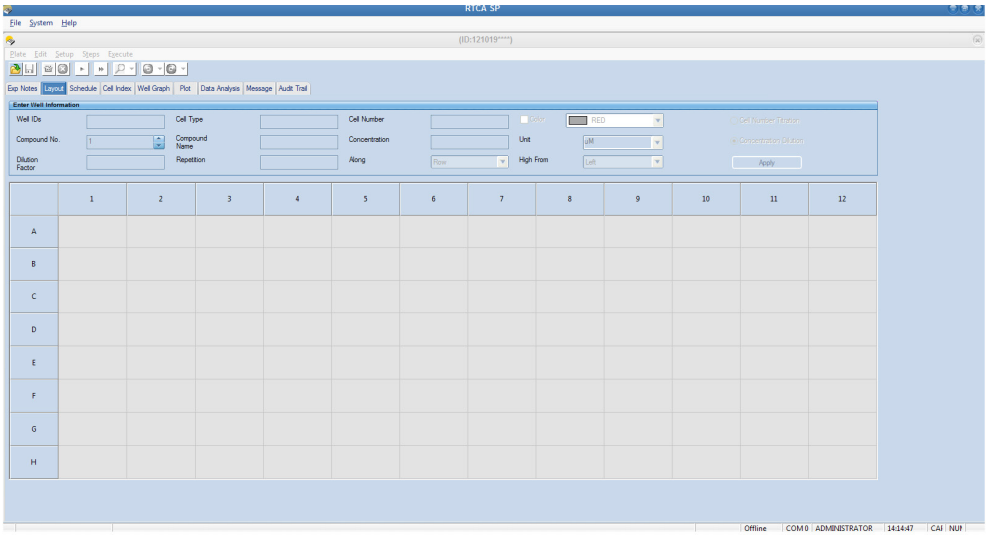
Page Tabs	Exp Notes | Layout | Schedule | Cell Index | Well Graph | Plot | Data Analysis | Message | Audit Trail	Work area; user-defined information
Status Bar	Offline | COM 0 | ADMINISTRATOR | 14:18:52 | CAI | NUI	Displays Instrument working status

Icon	Description
	Open an experiment .plt file
	Apply changes made in the *Layout* or *Schedule* page
	Add a step
	Delete a step
	Start a step
	Pause a step
	Abort current running step
	Scan Plate
	Stop Plate Scan
	Undo
	Redo

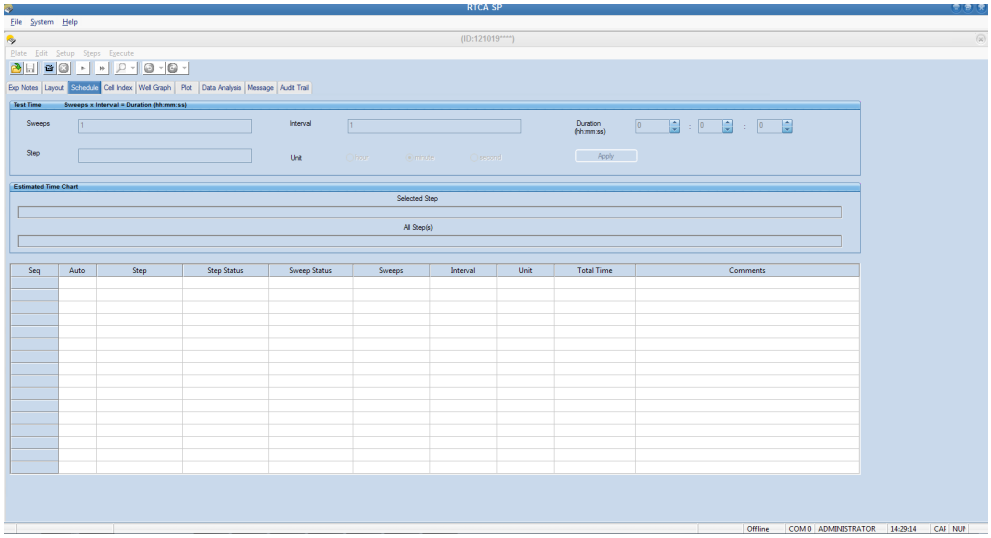
1.1.1 RTCA Software Functions Required for Running Experiments



***Exp Notes* Page: Record key information about the experiment**

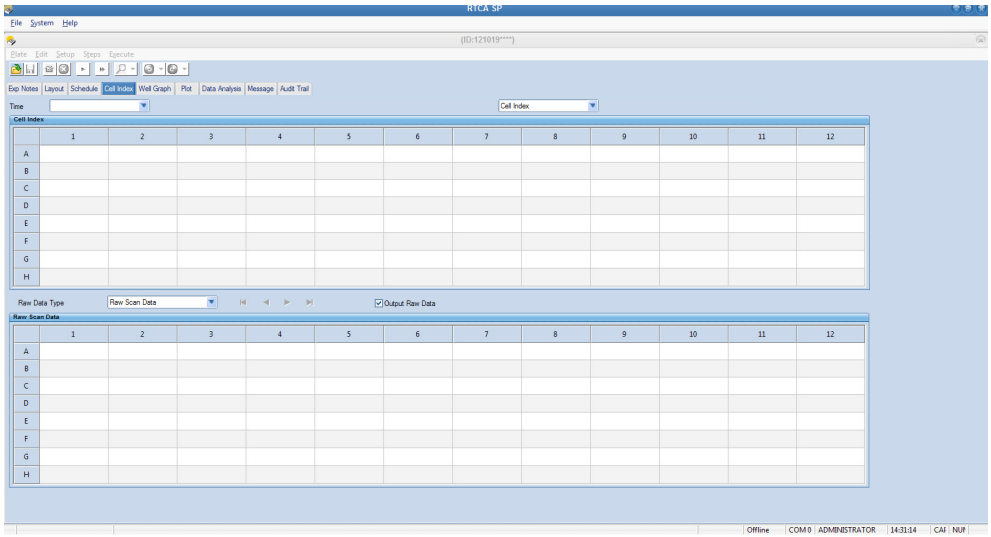


***Layout* Page: Define arrangement of samples on E-Plate 96 (e.g. , cell type, cell number, compound name, concentration)**



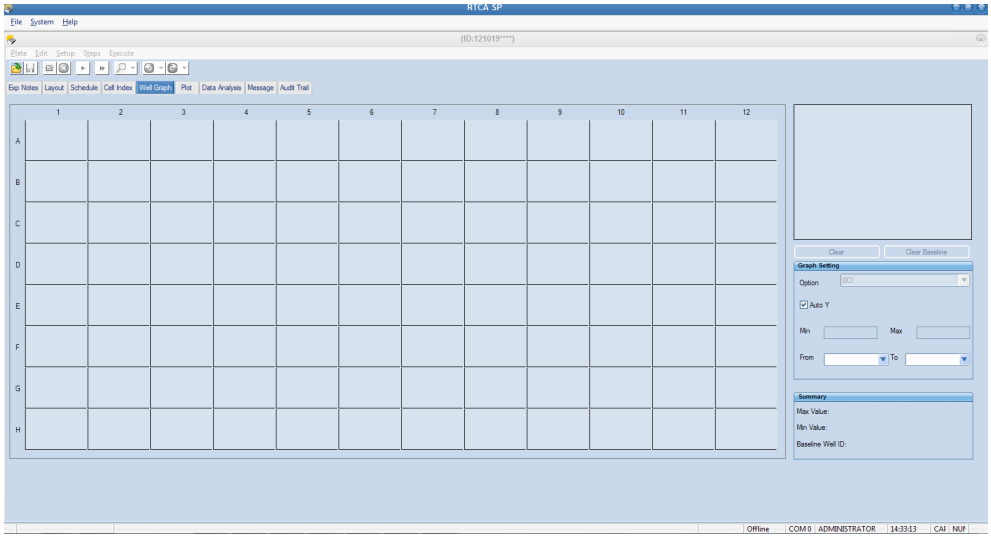
***Schedule* Page: Set up parameters for real-time data acquisition, such as the number of sweeps and time interval for each sweep, for each experiment step.**

1.1.2 RTCA Software Functions Required for Monitoring Experiments

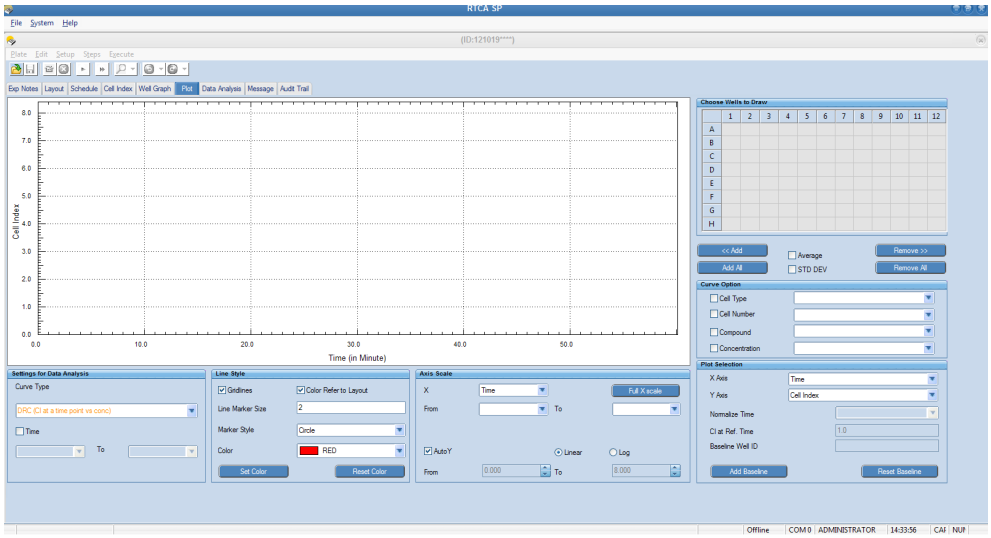


***Cell Index* Page: Real-time data acquisition and monitoring.**

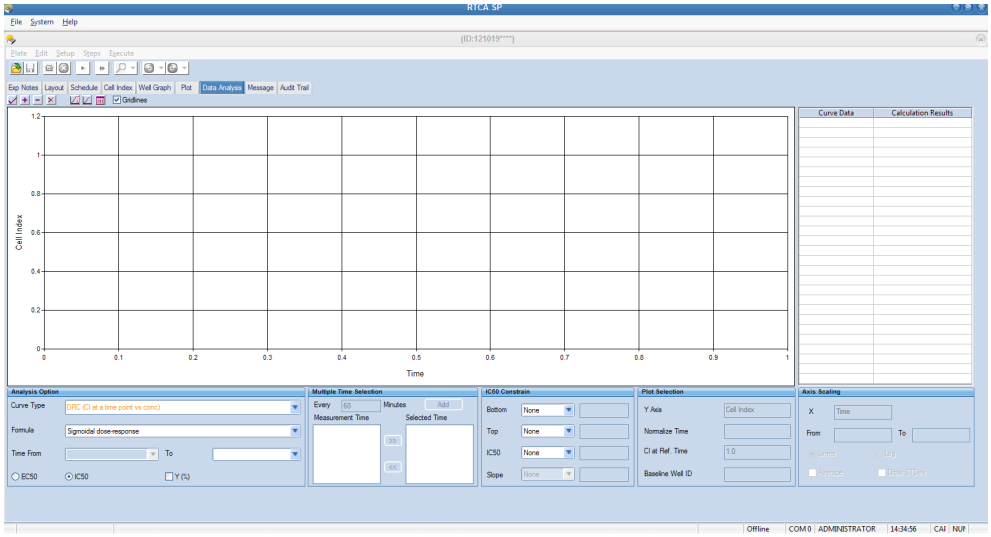
B



***Well Graph* Page: Shows all graphs of all well data in E-Plate format (8 × 12).**

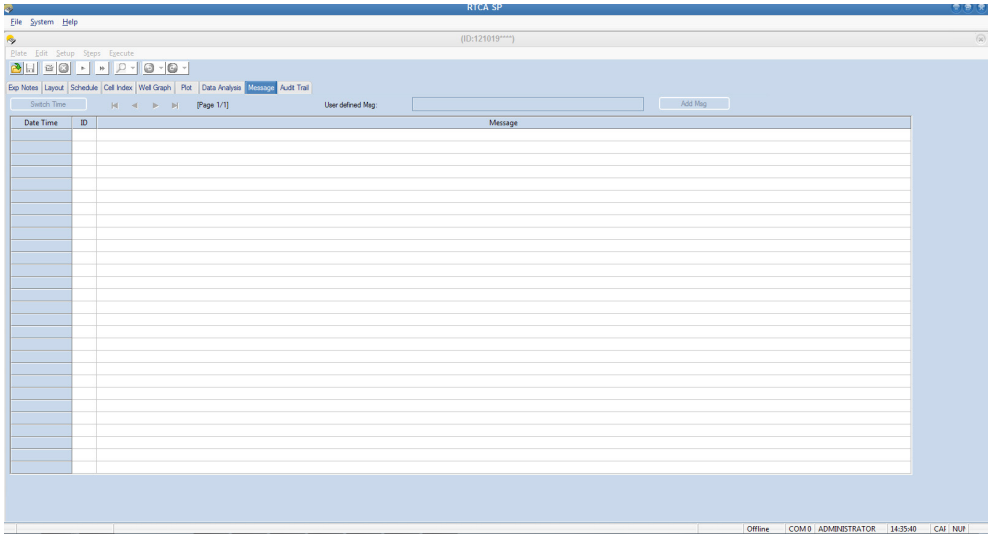


***Plot* Page: Multiple data plot and data export functions.**

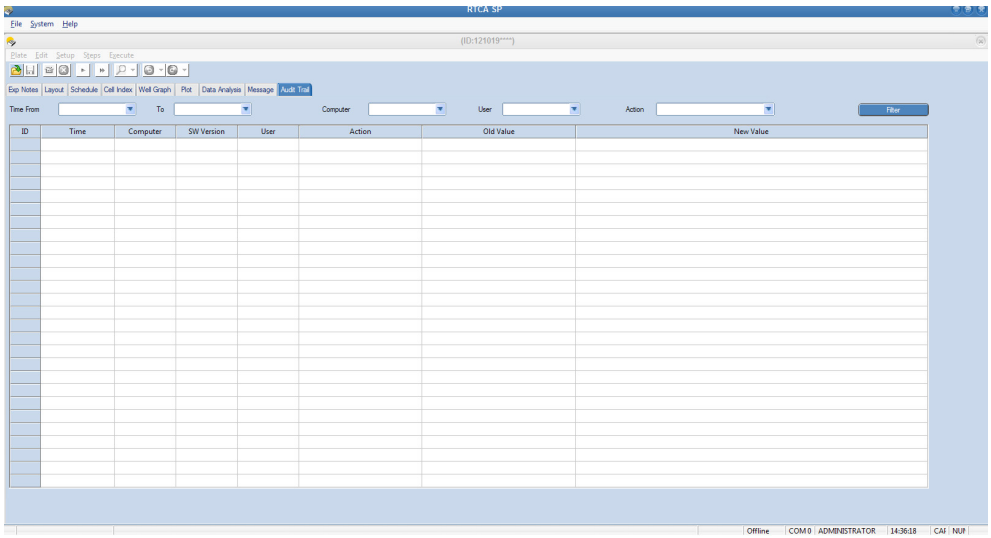


***Data Analysis* Page: Multiple data analysis functions (e.g., IC_{50}/EC_{50}, Cell Index doubling time, Slope, etc.)**

B



***Message* Page: Record and report the major steps of the experimental process.**



***Audit Trail* Page: Record and report every action user has made that would modify the experiment file.**

2. Starting an Experiment

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

2.1 Launch the RTCA Software

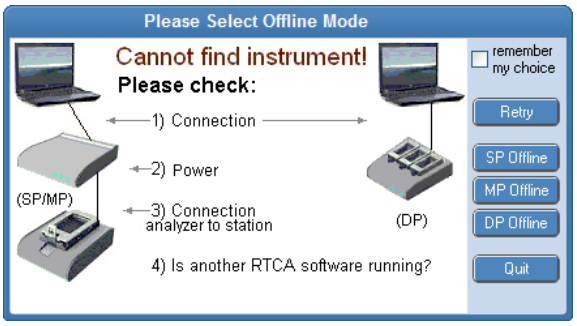
Double-click the RTCA Software 2.1.0 Icon 

on the desktop to launch the application. A login window appears. All user names are shown in a drop-down list. Choose a user name and enter the corresponding password to login to the RTCA Software.

*The default password of the built-in user ADMINISTRATOR is **administrator** (all lower case). The default passwords of built-in users User1 and User2 are not set.*

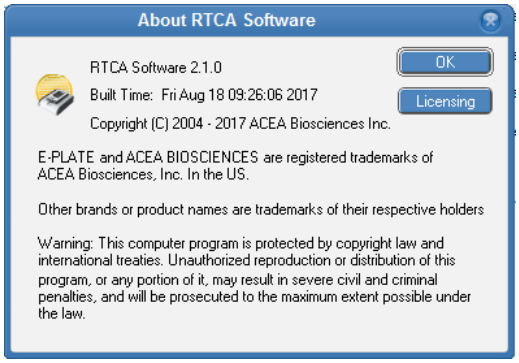
After launch, the RTCA Software will initialize the COM port, using previously saved settings. One of the following will happen:

- If xCELLigence RTCA Instrument (SP/MP Analyzer, SP/MP Station, and DP Instrument) is powered on and connected to the xCELLigence RTCA SP/MP/DP Control Unit properly, the RTCA Software will try to communicate with the xCELLigence RTCA Instrument and obtain related information. As soon as the connection is successfully established, the software will display the instrument information, such as *Analyzer Model*, *Analyzer SN* (serial number), *Station Model*, *Station SN*, etc. on the main interface. Also the Software will enter Real-Time mode directly.
- If the designated port number is not available, or the set port is available, but the Software fails to communicate with the SP/MP Analyzer, the Software will provide the user with five options: (1) select *Retry* to try to reconnect to the xCELLigence RTCA Instrument, (2) select *SP Offline* mode, (3) select *MP Offline* mode, and (4) select *DP Offline* mode; and (5) Quit the RTCA Software.



2.2 Check the Software Version

Click *Help* in the Menu bar of the RTCA Software  and use the menu to select *About RTCA*. This will activate a pop-up box displaying the Software version that is installed on the xCELLigence RTCA SP/MP/DP Control Unit or computer.



2.3 Menus of Main Window and Plate Window

2.3.1 Main Menu

The main window menu contains three sub-menus: *File*, *System,* and *Help*.



Here are menus specifically for the main menu:

File

Exit: Close RTCA Software.

System

Logout: Logout to protect the experiment

Setup: Change system settings

User Management: Change password, add / delete users

Help

Help Topics: Providing on-line help functions

Software Manual: Open Software Manual in PDF format. (Adobe Reader is required)

About RTCA: Information about RTCA Software

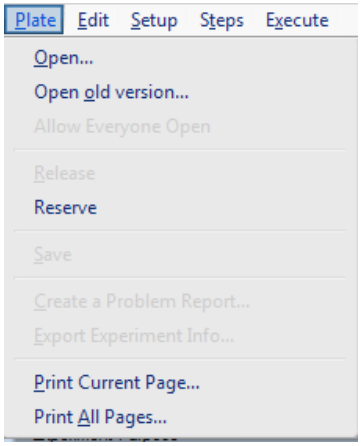
2.3.2 Plate Window Menu

The Plate Window menu has five sub-menus including File, Edit, Setup, Steps, and Execute.



Plate Menu and Tool bar

Plate



Open: Open an experiment

Open Old Version: Open an experiment from an old version of RTCA Software

Allow Everyone Open: Remove protection of currently opened experiment

Release: Clear all the information on all the pages

Reserve: Reserve one plate for later use

Save: Save current experiment

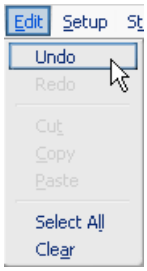
Create a Problem Report: Create a problem report for sending to ACEA representative

Export Experiment Info: Export experiment details to an Excel file

Print Current Page: Print displayed page

Print All Pages: Print all pages

Edit



Undo: Rollback changes at Layout page

Redo: Reapply changes at Layout page

Cut: Cut information

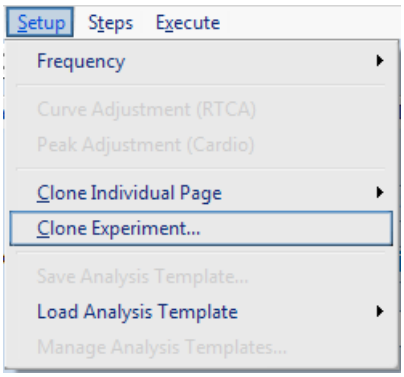
Copy: Copy information

Paste: Paste information

Select All: Select all information or all wells

Clear: Clear information

Setup



Frequency: Set up measurement frequency. (Single Frequency or All 3-Frequency; default is All 3-Frequency)

Curve Adjustment Tools: For adjusting Cell Index curves

Clone Individual Page: Copy settings for individual page (Exp_Notes, Layout, and Schedule)

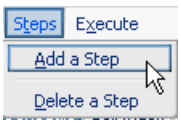
Clone Experiment: Copy all settings for ExpNotes, Layout, and Schedule pages

Save Analysis Template: Save data analysis items as a template to be used to analyze the other data file

Load Analysis Template: Load the saved data analysis template

Manage Analysis Template: Change the setting for the saved data analysis template

Steps



Add a Step: Add a new step to experiment

Delete a Step: Delete selected step

Execute

Start/Continue: Start a step, or resume step that is currently paused

Pause: Pause current step

Abort Step: Skip remaining sweep(s) of currently running step

Scan Plate: Perform a measurement on all selected wells in the plate

Stop Scan Plate: Stop plate scanning, ignore the scanned data

Lock Plate: Lock E-Plate (MP only)

Unlock Plate: Unlock E-Plate (MP only)

2.4 Experiment Record

The *Exp Notes* page contains fields in which the user can record experimental details.

The xCELLigence RTCA Instrument can run an experiment even if Exp Notes page is blank.

The information on the *Exp Notes, Layout,* and *Schedule* pages will automatically be saved in an experiment file when the user clicks the *Start/Continue*  icon before starting a new experiment. The default file name is:

- YYMMDDHHmm_ExperimentName.plt for SP
- YYMMDDHHmm PPlateNumber_ExperimentName.plt for MP
- YYMMDDHHmm DPlateNumber_ExperimentName.plt for DP

where Experiment Name is the user-entered text in the *Experiment Name* box on the *Exp Notes* page.

The information on the *Exp Notes* page can be edited while the experiment is running or after the experiment has finished. After the experiment has started, if the user adds or changes information on the Exp Notes page, they must save this page manually by clicking the *Save Changes*  icon.

To save the information on the Exp Notes page manually before the experiment is started, click the Save Changes button  *. A Save As window will open, displaying a default page name in the file name box. The default name is TemplateYYMMDD.plt for SP, TemplateYYMMDDPPlateNumber for MP, and TemplateYYMMDD DPlateNumber for DP. plt. You can type a different name if desired. Click the Save button on the window. The Exp Notes page information will be saved in the file. Such a file can be opened as a template for future experiments.*

To insert the cursor in a text box, left-click the mouse in that box. Once the cursor has been inserted into one text box, use the Tab key to move from text box to text box, or, alternatively, use the mouse again to insert the cursor in the next text box.

A backup copy of the RTCA data file (.bak) will be generated at the same time as the RTCA data file (*.plt). The backup copy of the data file can be saved either to the default directory of "[HKEY_LOCAL_MACHINE\SOFTWARE\Microsoft\Windows\CurrentVersion\Explorer\Shell Folders] key "Common Documents" or to a user-defined directory.*

For example, for Windows7 English version, the default directory of the RTCA data file is: C:\Users\Public\RTCA Data, and the default directory of the backup copy of the RTCA data file is: C:\Users\Public\RTCA Backup Data.

2.5 Text Boxes on Exp Notes Page

- ***User***: The login user name is displayed in this box and cannot be changed (read-only).
- ***Data Directory***: the default path is to C:\Users\Public\RTCA Data \login username. To direct the path to a different folder on the xCELLigence RTCA SP/MP/DP Control Unit, use the Browse… button to locate and open the folder.

Since an experiment can continue for a long time, the RTCA Software reads from and writes to the experiment data file from time to time. Therefore, the file directory must be a fully accessible folder stored on the local hard drive.

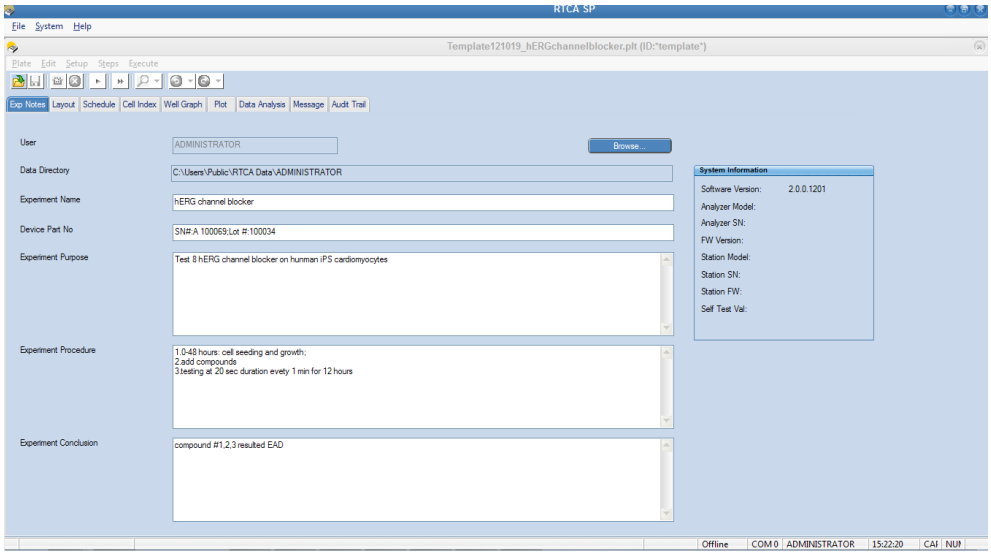
- ***Experiment Name***: User-defined information. Use the mouse or Tab key to move the cursor to this text box. This information will be saved as part of the experiment file name.
- ***Device Part No.***: Entering the serial number of the E-Plate here will allow the user to monitor the quality of the experiment and track any possible issues with the E-Plate Plate. Entering the E-Plate serial number is highly recommended. Use the mouse or Tab key to move the cursor to this text box.
- ***Experiment Purpose***: User-defined information. Use the mouse or Tab key to move the cursor to this text box.
- ***Experiment Procedure***: User-defined information. Use the mouse or Tab key to move the cursor to this text box.
- ***Experiment Conclusion***: User-defined information. Entered after experiment has finished to record the main conclusions about the experiment. Use the mouse or tab key to move the cursor to this text box. Most likely this information would be added after the experiment has finished. So, in order to save the added information, click the *Save Changes* button  while the Software is displaying the Exp Notes page.

After the experiment starts, any changes made on the Exp Notes page must be saved manually using the Save Changes button  .

The Experiment ID is displayed on the top bar of the software window. Before an experiment starts, the default ID is the current date (YYMMDD) and time (asterisks). When an experiment is started, the default ID changes to the current date and time (YYMMDDHHmm), and the experiment file name is YYMMDDHHmm_ExperimentName.plt for SP, YYMMDDHHmm PPlateNumber_ExperimentName.plt for MP, and YYMMDDHHmm DPlateNumber_ExperimentName.plt for DP, where Experiment Name is the user-defined information from the Experiment Name box. During an experiment or after completion of an experiment, the Experiment ID will not change even if the experiment file name is changed.

The information recorded or edited on the Exp Notes page is not required for running an experiment. However, such information is very useful for later reference.

2.6 Example of a Completed Exp Notes Page



An example of a completed RTCA Exp Notes page.

3. Design an Experiment

3.1 Experiment Layout

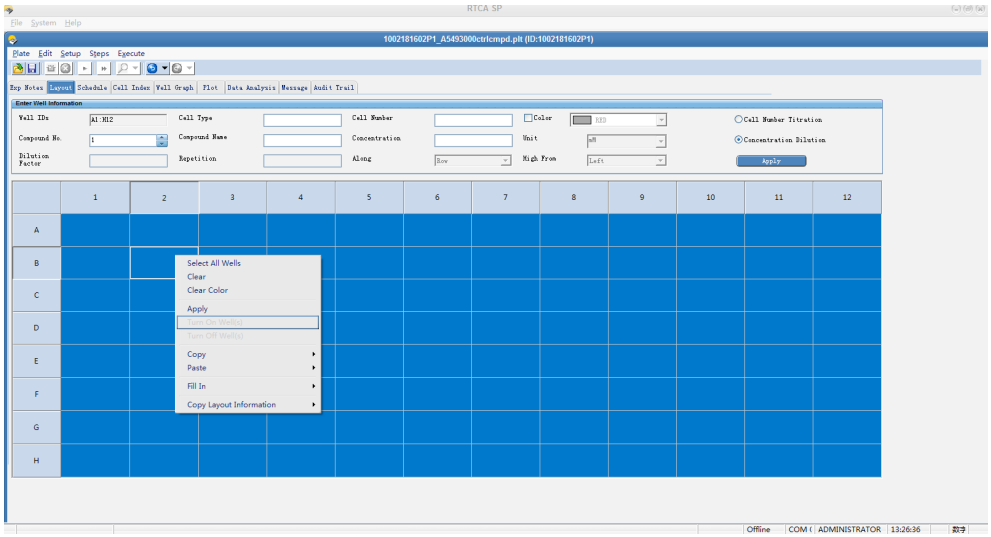
Click on the *Layout* page tab (shown in the figure below). This page allows the user to enter detailed information for each well. Correct information about each well is essential for monitoring experimental data on the *Plot* page during the experiment.

Layout data is saved automatically when the experiment is started.

The user can enter or edit the layout information either before the experiment starts or after it finishes.

To save any changes made in the *Layout* page after an experiment has finished, click *Save Changes* button .

To prevent invalid data or loss of data, the RTCA Software does not allow the user to turn on or turn off any wells (see below) during the experiment.



3.2 Add Experimental Information

For xCELLigence RTCA Instrument, each box on the well map represents one well of an E-Plate 96 on the SP or MP Station. Once all information about the wells has been entered in the text boxes, click the *Apply* button to add that information to the well map.

Information entered into the text boxes will not be transferred to the well map if the Apply step is omitted.

B

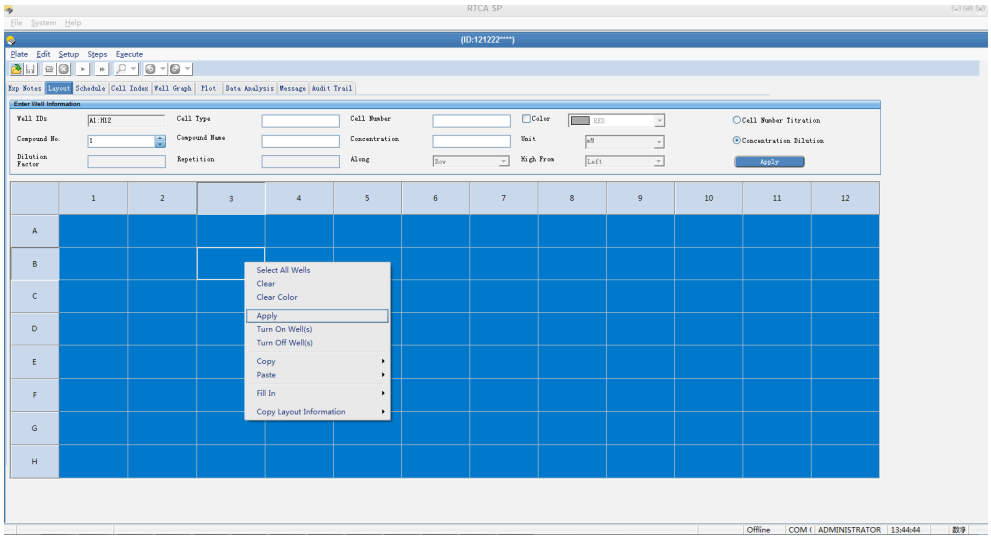
3.3 Enable Wells

In order to run an experiment on your sample wells, you must first enable, then activate those wells, as follows:

1. Enable the wells you will be using on the E-Plate by highlighting the light blue boxes on the well map that correspond to those wells. The enabled wells will turn dark blue. For convenience, there are many ways to highlight different boxes on the map:
   - Holding the *Ctrl* or *Shift* key while clicking will highlight all clicked boxes.

     These functions work the same 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, then clicking and dragging the mouse across others will highlight multiple columns.
   - Clicking a row letter will highlight the entire row of boxes.
   - Clicking a row letter, then clicking and dragging the mouse across others will highlight multiple rows.
2. Go to the information text boxes (above the well map) and type in specific information such as *Cell Type, Cell Number, Compound Name, Concentration, etc.* for the enabled wells. (This step is optional; see details below).
3. Click the *Apply* button to transfer the information from the text boxes (if any) to the highlighted wells on the well map and activate those wells. After the *Apply* button has been clicked, the activated wells on the well map will be lighter in color (no longer dark blue) and contain the information that was typed into the text boxes.

   This step must be performed before the experiment can start.



Right-clicking on the page will provide a drop-down menu that allows you to perform several commands, such as "Apply".

3.4 Information Boxes

Entering information in these text boxes is optional. You can still perform an experiment even if no information appears in the text boxes, but you will not be able to use some of the functions on the Plot and Data Analysis Pages.

► ***Cell Type:***

Enter the name of the cell types you are using.

No space / blank are allowed for the Cell Type.

► ***Cell Number:***

Enter the number of cells seeded in a well.

► ***Compound No.:***

If multiple compounds are to be added to the same well(s), you can assign each compound a number from this drop-down menu. After entering information about the first compound in the text boxes below (Compound Name, Concentration, etc.), select "2" from this menu and enter information about a second compound in the boxes. To check the names of multiple compounds assigned to the same well, the user must select the number that is assigned to each compound. Note that the well in the layout map will display each compound in a column (one beneath another).

► ***Compound Name:***

Enter compound name or names.

No space / blank are allowed for the Compound Name.

► ***Concentration:***

Enter compound concentration. This function can also be used to indicate a dilution series.

To define a dilution series, use this field in conjunction with the Dilution Factor, Repetition, Along fields and High From (which give details about the dilution series). First enter in the Concentration box the highest concentration used; then, the dilution series will start with this concentration and proceed as defined in the other fields.

► ***Unit:***

Select a unit for your compound from this drop-down menu. To add your own unit, select -**define**- from the menu and type your unit name into the text box.

If you select a user defined unit ("-define-") it is not possible to perform certain calculations on the Data Analysis page, e.g., Dose Response Curves (DRCs). An error message will appear.

► ***Dilution Factor:***

For a dilution series, you must enter the dilution factor that will be used. For example, for a 3-fold dilution from well to well, enter 3. The default value is 1 if nothing is entered in the text box.

A new function of RTCA Software for better illustration of Dilution Factor in the Layout page is by selecting the Color checkbox. To use this function, select multiple wells with Dilution Factor applied. Input the Compound Name, Concentration, and Dilution Factor information for the selected wells. Then click the Color checkbox and assign a specific color for this compound. You can also adjust the intensity of the color assigned for each concentration. Then click the Apply button. The information will be displayed on each box with specific color whose intensity corresponds to the compound concentration.

B

- ***Repetition:***

If a dilution is to have replicates, enter the number of replicates here. The default value is 1 if nothing is entered in the text box. Replicates can be down a column or across a row (as defined by the "*Along*" box below).

- ***Along:***

Indicate whether the serial dilution and/or repetition is to proceed along a row or a column.

- ***High From:***

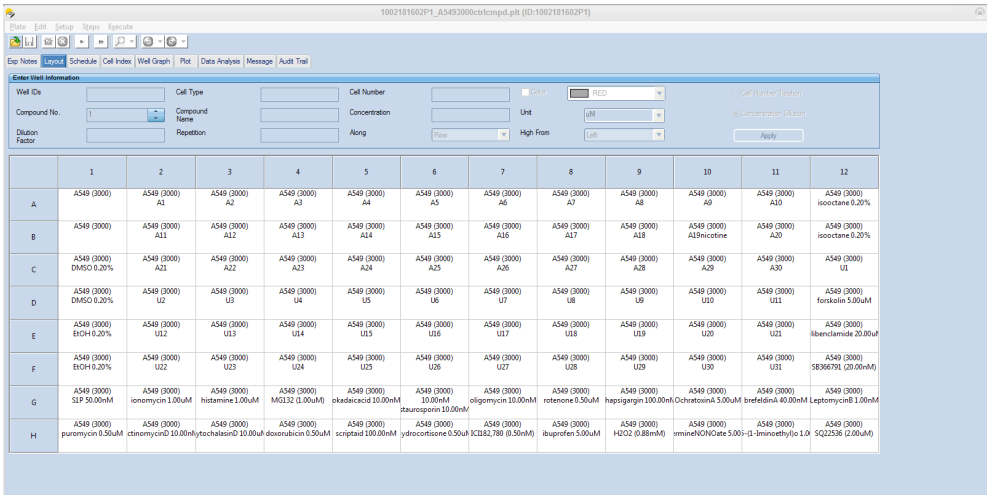
Starting point of the dilution series. If you want the dilution series to proceed along a row (as defined in the "Along" box below), select *Left* or *Right* to indicate whether the highest concentration will be in the left-most or right-most well of that row. If you want the dilution series to proceed along a column, select *Top* or *Bottom* to indicate whether the highest concentration will be in the top or bottom well of that column.

- ***Apply:***

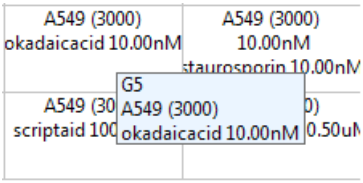
Use this button to transfer all the information from the text boxes to the well map.

Information entered into the text boxes above will not be transferred to the well map if the Apply step is omitted.

3.5 Example of a Completed Layout Page

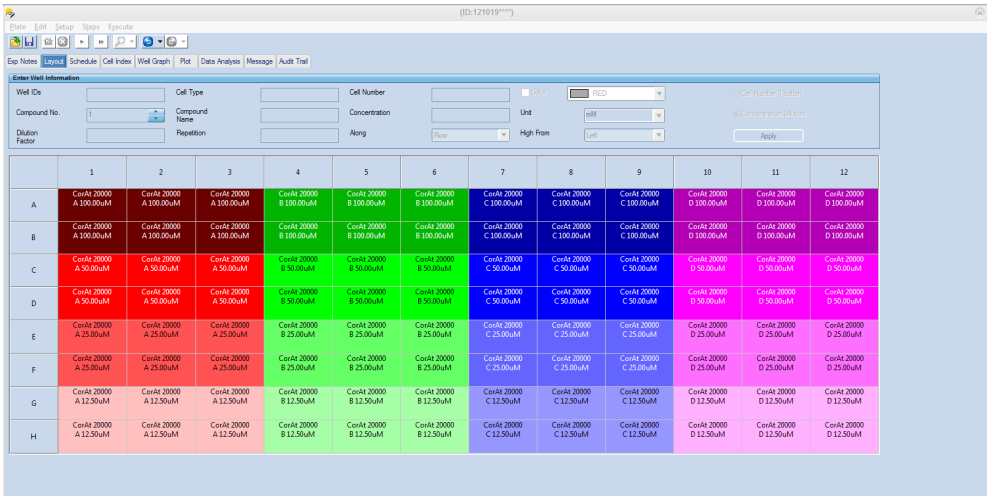


In order to view detailed information for each well, just simply place the cursor on that well, a Tool Tip Message will show to list its details.



The tooltip message showing the detailed information of each well in the layout page.

3.6 Example of Dilution Factor and Dilution Repetition



In the RTCA Software, the term "μ" as a dimension unit is always shown as "u" (e.g., uM instead of μM).

In the above figure, a dilution factor of 2 is selected. The highest concentration is 100 μM. The repetition number of 2 means the first concentration repeats 2 times, then the next concentration 2 times downward along the column etc., since Along Column was selected.

If you need to change the information inside the well map, you must re-select the wells that need to be changed, then right-click and select Clear from the pop-up menu that appears. The selected wells will still be turned on, but will contain no information. You can then enter the correct information.

If you need to change the color inside the well map, you must re-select the wells that need to be changed, then right-click and select Clear Color from the pop-up menu that appears.

If you need to add another compound to the map you can select the affected wells, then type in the compound information and click Apply. A pop-up box will appear. Select Yes if you want to add a new compound to this well. Select No if you want to replace the original compound in the well with the new compound. Select Cancel if you do not want to add this compound. An alternative way to add another compound to the map is to select "2" from the Compound No. menu, then type in the compound information and click Apply.

The Dilution function can also be used for Cell Titration.

3.7 Copy/Paste Layout Settings

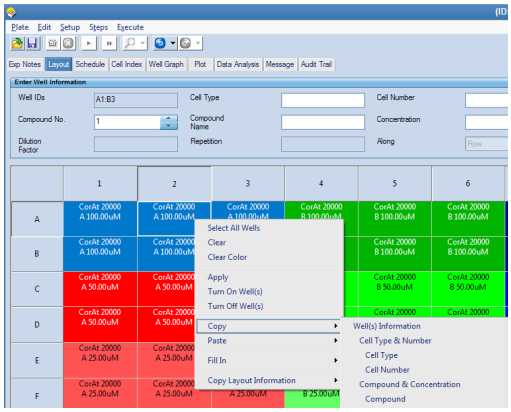
For your convenience, the RTCA Software offers *Copy* and *Paste* functions to help set up the *Layout* page.

Copy and *Paste* can be done within the *Layout* page. In addition, you can Copy to / Paste from Microsoft Excel or other Windows applications.

The following steps/figures show how these functions work.

1. Copy source well(s)
Use the left mouse key to highlight the source well(s). Then right-click, select Copy from the menu, and select the item to be copied:

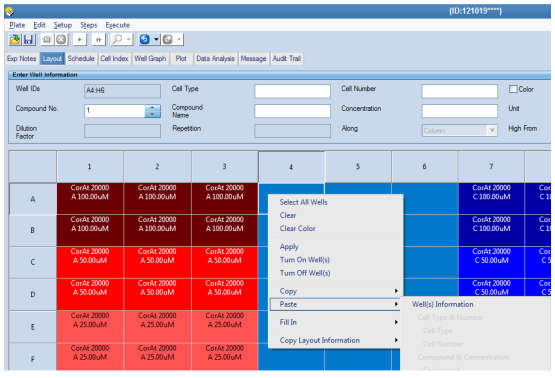
Well(s) Information	Copy all information in the well(s)
Cell Type & Number	Copy Cell Type and Cell Number only
Cell Type	Copy Cell Type only
Cell Number	Copy Cell Number only
Compound & Concentration	Copy Compound and Concentration
Compound	Copy Compound Name only
Concentration	Copy Concentration and Unit



2. Paste to target well(s)
Right-click the target cells, select *Paste* from the menu, and then select the item to be pasted:

Well(s) Information	Paste all information in the well(s)
Cell Type & Number	Paste Cell Type and Cell Number only
Cell Type	Paste Cell Type only
Cell Number	Paste Cell Number only
Compound & Concentration	Paste Compound and Concentration
Compound	Paste Compound Name only
Concentration	Paste Concentration and Unit

If an item was Copied within RTCA Software, only the Copied item will be available on the menu for Pasting.



4. Experiment Schedule

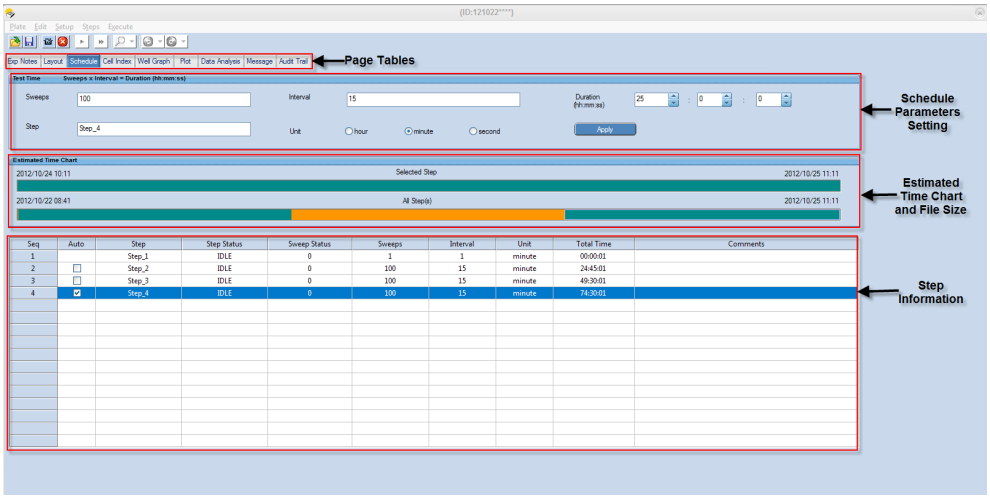
There are several ways to access functions in this Software ("right-clicking", "menus," "icon"). This Manual describes only one method, but users should familiarize themselves with the other methods so they can use the one they prefer.

Click the *Schedule* tab to display the Schedule setting page. This page allows:

- Adding (or deleting) experiment steps
- Setting the measurement parameters for each step

A Step is one part of an experimental set-up. Typically, an experiment consists of at least three different steps: 1. Background measurement; 2. Cell addition and monitoring of cell attachment and proliferation; 3. Addition of a test compound and monitoring of cellular reactions to that compound.

In the Schedule page of RTCA Software, there are three parts which designed for setting the Schedule Parameters, displaying Estimated Time Chart and File Size, and displaying the Step Information, respectively.



4.1 Add Steps and Set Measurement Parameters

There are several ways to add steps to the *Schedule* page:

- Click the *Step*  icon on the tool bar, or
- Click *Steps* from the menu; then select *Add a Step*, or
- Right-click mouse and select *Add a Step* from the pop-up menu.

After one step is added, it will be highlighted in the *Step Information* table of the *Schedule* page. Input the parameters in the Schedule Parameters Setting for this step, and click *Apply* button to finalize the setting.

To change the schedule of a step, click the step in *Step Information* table, then move the cursor to the text boxes and make the changes for each parameter. To confirm the changes, click the *Apply* button. The new settings will appear in the *Step Information* table.

B

4.1.1 Schedule Parameter Setting

The top part of the *Schedule* page is for setting the measurement parameters for each step:

- ***Step:***

The name of each step. By default, it will be Step_1, Step_2, etc. if this parameter is not changed by users.

- ***Sweeps:***

One Sweep means a continuous acquisition of resistance data from all the wells selected in the *Layout* page for a certain amount of time (*Sweep Duration*).

- ***Interval:***

Interval is a parameter to determine how often one Sweep takes place. The unit for Interval can be selected as hour, minute, or second.

For example, if the *Interval* is set to be 10 minutes, it means that one sweep will be initiated every 10 minutes.

Interval should be set larger than the Sweep Duration (see below).

- ***Duration:***

Duration determines how long that one step will last. For a fixed Interval, you can either adjust *Sweeps* or *Duration* in order to set the whole time that one step will actually last for. For *Duration*, there are three boxes that allow you to specify hour/minute/second (hh:mm:ss).

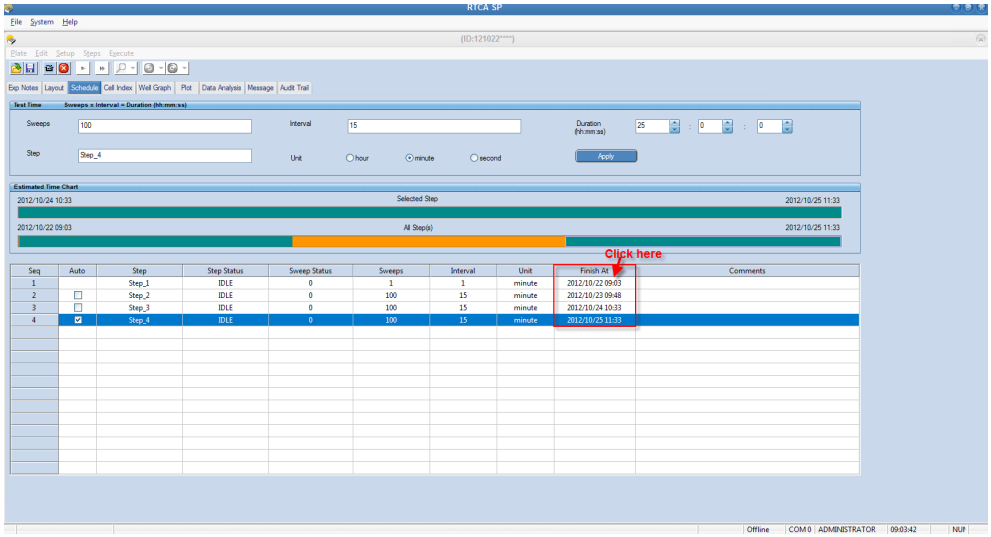
For example, if one step is designed to last for 24 hours with the *Interval* of 10 minutes, either 145 *Sweeps* or *Duration* of 24 hours can be used. RTCA Software will automatically calculate one parameter when the other one is adjusted for a fixed *Interval* value.

4.1.2 Change the Preset Schedule of a Step

To change the schedule of a step, click the step in the table or click the Step icon, then move the cursor to the text boxes and make the changes. To confirm the changes, click the *Apply* button. The new settings will appear in the table.

4.1.3 Total Time

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



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 (substep) is selected during the run
- A step or substep is added / deleted
- Sweeps / Intervals are changed
- Intervals assigned to step or substep are too short, for example: 1 second interval is assigned to Steps / Substeps

4.2 Delete Steps

To delete a step, click the step icon and then click the button. You can also use the *Steps* drop-down menu or right-click for a pop-up menu and select *Delete a Step*.

4.3 Insert Steps

To insert a step, click the Step icon below which you want to insert a new step (e.g., click Step 2 if you want to insert a step between Step 2 and 3), then go to the *Steps* menu and select *Add a Step*.

The newly inserted step will be named Step_X. The user can change the given name.

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

4.4 Set up a Background Step

You must set up a background or reference step (i.e., step 1) before setting up any other experiment steps. The background step must be performed when each well of the E-Plate contains only tissue culture media, that is, before cells are added. The RTCA Software always reserves the first step (step 1) for background or reference data collection.

When a user adds the first step in the *Schedule* page, RTCA Software automatically sets its Sweeps to be 1, Interval be 1 minute. This is the setting required for a background step. The user should not change this setting except for Resistor Plate Verification (see xCELLigence RTCA Instrument Operator's Manual).

It is recommended to add an extra step at the end of the experiment when setting experiment schedule. This will ensure that, when the Software finishes the last experimental step, it will idle, waiting for manual intervention. The user can then determine (e.g., on the next morning) whether the experiment can be terminated or needs to be continued.

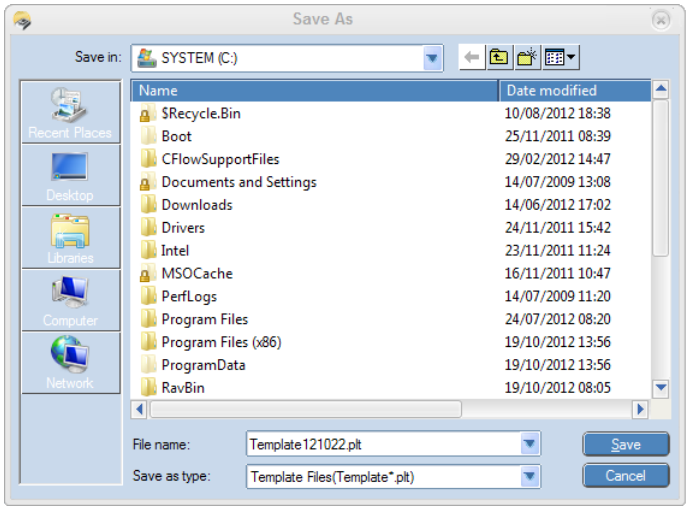
4.5 Saving Set-up(s)

As described above, the RTCA Software allows users to edit/enter various experimental set-ups. If they use a particular set-up frequently, users should save the information for that set-up, so it can rapidly be loaded into the RTCA Software each time a similar experiment is performed.

The RTCA Software automatically saves all set-up information when the experiment starts. The user does not need to manually save such information, unless they wish to save it to be "re-used" in future experiments.

There are three parts of a set-up that the RTCA Software can save: *Exp Notes, Layout* and *Schedule*.

Once they enter or change the settings, users can save all the set-up information as a template file for later use, referred to as a Clone. To create a template, launch the RTCA Software, edit one or more pages (*Exp Notes, Layout,* and/or *Schedule*), then click the *Save Changes* button to save the settings on the edited page(s). A *Save As* dialog will appear:



The default file name is "TemplateYYMMDD" for SP, TemplateYYMMDDPx" for MP (x is the plate number: 1 to 6), and TemplateYYMMDDDx for DP (x is DP pattern number: 0 to 3. refer to Section 15 for details). The user can change the template file name, as appropriate. The template file contains all the information added to the *Exp Notes, Layout*, and/or *Schedule* pages.

4.6 Opening a Template (Clone Settings)

The user can open a previously saved template or clone the experiment setup from a previously-run experiment by selecting *Setup* from the main menu and choosing *Clone Individual Page* or *Clone Experiment*. A dialog box will appear. The user can navigate to the previously saved directory to open or clone from the saved template(s).

If *Clone Individual Page* is used, only the designated page is cloned from the selected template or experiment. Thus, the user can create a new experiment by cloning the different pages (*Exp Notes, Layout,* or *Schedule*) from up to 3 different templates and/or previously-run experiments.

If *Clone Experiment* is used, all three experiment setting pages (*Exp Notes, Layout,* and *Schedule*) will be cloned from a given template or an experiment file from a previously-run experiment.



Clone Individual Page or Clone Experiment will load only the experiment settings, so a new experiment can be run. Data in the previously-run experiment file are not loaded or changed.

5. Start an Experiment

5.1 Scan Plate

5.1.1 Manual Scan Plate

When a well has been activated in the *Layout* page, the *Scan Plate*  icon in the tool and the "*Scan Plate*" from "*Execute*" menu will become active. When the Scan Plate function is initialized, the Software measures the resistance of each of the defined wells. This will verify that the proper connections are made between the E-Plate 96 and the SP Station.

The Scan Plate button is disabled when the SP/MP Analyzer is performing the measurement.

The *Message* page will display the results of the scan and indicate any connection problems by listing the specific wells whose contact resistance is out of normal range. Each time when *Scan Plate* function is activated (either before or during the experiment), it will be recorded in the *Message* Page.

If the experiment has not started, the scan data are saved in:

C:\Users\Public\RTCA Data \Username\ScanPlateData

The Software creates the file name in the following format:

PlateScanningYYMMDD.txt (SP)

PlateScanningYYMMDDPx.txt (MP, x = 1 to 6)

PlateScanningYYMMDDDx.txt (DP, x = 0 to 3)

If more than one scan is performed on the same day, all scan data made during that day will be saved in one file. The new scan data will be appended to the end of the existing scan data file.

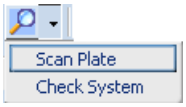
At the beginning of an experiment, the data from the last 10 scans will be saved, together with the experiment data in the experiment *.plt file. After that, all new scan data will be saved in the experiment *.plt file.

5.1.2 Automatic Scan Plate

To facilitate monitoring of the E-Plate connection to the SP/MP Station or DP Instrument, the RTCA Software has a built-in scan function. Each time it detects an E-Plate being placed on the SP/MP Station or DP Instrument, the Software will automatically perform a *Scan Plate* measurement. The scan data will be saved and can be viewed in the same way as scan plate data generated manually.

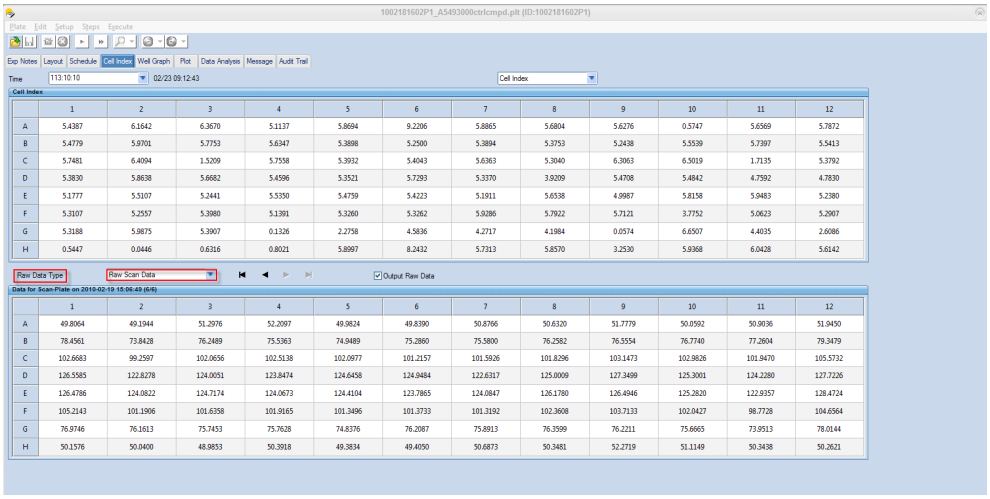
5.1.3 Check System

If there is a connection warning shown in the *Message* page, and you want to determine whether it is a device problem or an RTCA Contact Pin (system) issue, you can use the *Check System* function. To do this, insert a Resistor Plate into the cradle, and click *Check System* from *Scan* drop-down list. The RTCA Software will perform a resistor scan. The check result will be shown at both the *Cell Index* page and the *Message* page.



5.2 Example of Results from Scan Plate

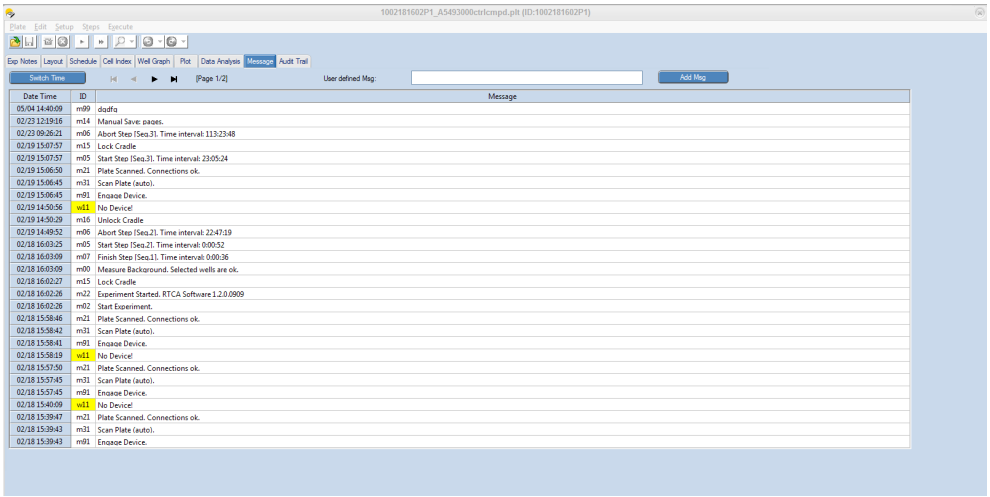
The *Cell Index* page shows the resistance values for all scanned wells.



	1	2	3	4	5	6	7	8	9	10	11	12
A	5.4387	6.1642	6.3670	5.1137	5.8694	9.2206	5.8865	5.6804	5.6276	0.5747	5.6569	5.7872
B	5.4779	5.9701	5.7753	5.6347	5.3898	5.2500	5.3894	5.3753	5.2438	5.5539	5.7397	5.5413
C	5.7481	6.4094	1.5209	5.7558	5.3932	5.4043	5.6363	5.3040	6.3063	6.5019	1.7135	5.3792
D	5.3830	5.8638	5.6682	5.4596	5.3521	5.7293	5.3370	3.9209	5.4708	5.4842	4.7592	4.7830
E	5.1777	5.5107	5.2441	5.5350	5.4759	5.4223	5.1911	5.6338	4.9987	5.8158	5.9483	5.2380
F	5.3107	5.2557	5.3980	5.1391	5.3260	5.3262	5.9286	5.7922	5.7121	3.7752	5.0623	5.2907
G	5.3188	5.9875	5.3907	0.1326	2.2758	4.5836	4.2717	4.1984	0.0574	6.6507	4.4035	2.6086
H	0.5447	0.0446	0.6316	0.8021	5.8997	8.2432	5.7313	5.8570	3.2530	5.9368	6.0428	5.6142

	1	2	3	4	5	6	7	8	9	10	11	12
A	49.8064	49.1944	51.2976	52.2097	49.9824	49.8390	50.8766	50.6320	51.7779	50.0592	50.9036	51.9450
B	78.4561	73.8428	76.2489	75.5363	74.9489	75.2860	75.5800	76.2582	76.5554	76.7740	77.2604	79.3479
C	102.6683	99.2597	102.0656	102.5138	102.0977	101.2157	101.5926	101.8296	103.1473	102.9826	101.9470	105.5732
D	126.5585	122.8278	124.0051	123.8474	124.6458	124.9484	122.6317	125.0009	127.3499	125.3001	124.2280	127.7226
E	126.4786	124.0822	124.7174	124.0673	124.4104	123.7865	124.0847	126.1780	126.4946	125.2820	122.9357	128.4724
F	105.2143	101.1906	101.6358	101.9165	101.3496	101.3733	101.3192	102.3608	103.7133	102.0427	98.7728	104.6564
G	76.9746	76.1613	75.7453	75.7628	74.8376	76.2087	75.8913	76.3599	76.2211	75.6665	73.9513	78.0144
H	50.1576	50.0400	48.9853	50.3918	49.3834	49.4050	50.6873	50.3481	52.2719	51.1149	50.3438	50.2621

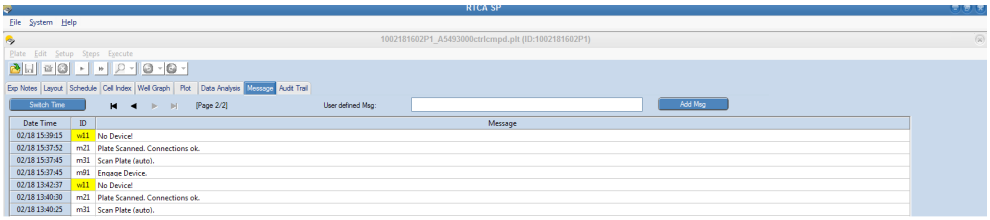
The *Message* page will report that the *Scan Plate* function was used, and that the result was recorded as “Plate Scanned. Connections ok”.



Date Time	ID	Message
05/04 14:40:09	m99	dqdfq
02/23 12:19:16	m14	Manual Save: paaes.
02/23 09:26:21	m06	Abort Step [Seq.3]. Time interval: 113:23:48
02/19 15:07:57	m15	Lock Cradle
02/19 15:07:57	m05	Start Step [Seq.3]. Time interval: 23:05:24
02/19 15:06:50	m21	Plate Scanned. Connections ok.
02/19 15:06:45	m31	Scan Plate (auto).
02/19 15:06:45	m91	Engage Device.
02/19 14:50:56	w11	No Device!
02/19 14:50:29	m16	Unlock Cradle
02/18 14:49:52	m06	Abort Step [Seq.2]. Time interval: 22:47:19
02/18 16:03:25	m05	Start Step [Seq.2]. Time interval: 0:00:52
02/18 16:03:09	m07	Finish Step [Seq.1]. Time interval: 0:00:36
02/18 16:03:09	m00	Measure Background. Selected wells are ok.
02/18 16:02:27	m15	Lock Cradle
02/18 16:02:26	m22	Experiment Started. RTCA Software 1.2.0.0909
02/18 16:02:26	m02	Start Experiment.
02/18 15:58:46	m21	Plate Scanned. Connections ok.
02/18 15:58:42	m31	Scan Plate (auto).
02/18 15:58:41	m91	Engage Device.
02/18 15:58:19	w11	No Device!
02/18 15:57:50	m21	Plate Scanned. Connections ok.
02/18 15:57:45	m31	Scan Plate (auto).
02/18 15:57:45	m91	Engage Device.
02/18 15:40:09	w11	No Device!
02/18 15:39:47	m21	Plate Scanned. Connections ok.
02/18 15:39:43	m31	Scan Plate (auto).
02/18 15:39:43	m91	Engage Device.

However, if the resistance value of a well is higher than 500 ohms, the *Message* page will report that this well may have a connection problem.

The following figure displays an example of a plate scan where potential connection problems are found using the *Scan Plate* function.



Date Time	ID	Message
02/18 15:39:15	w11	No Device!
02/18 15:37:52	m21	Plate Scanned. Connections ok.
02/18 15:37:45	m31	Scan Plate (auto).
02/18 15:37:45	m91	Engage Device.
02/18 13:42:37	w11	No Device!
02/18 13:40:30	m21	Plate Scanned. Connections ok.
02/18 13:40:25	m31	Scan Plate (auto).

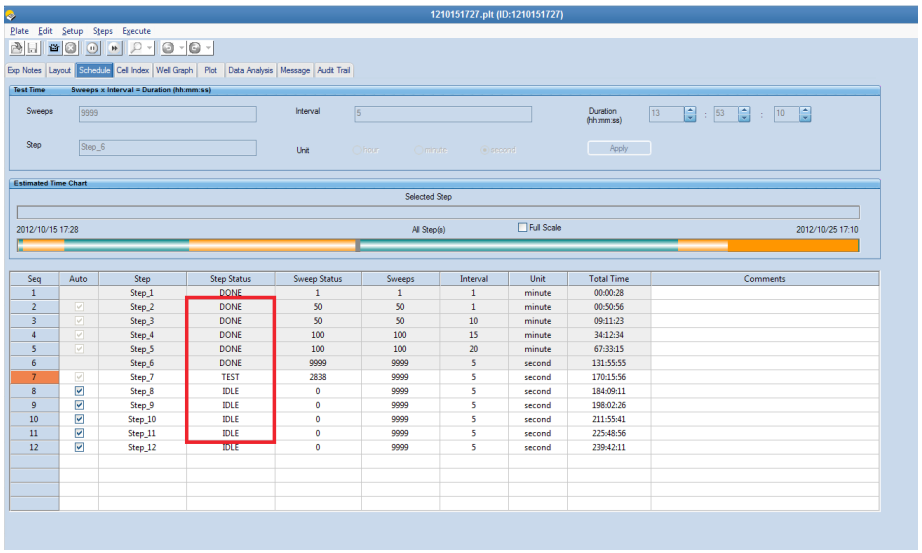
5.3 How to Handle Connection Issues

For detailed instructions on resolving E-Plate 96 (used with SP and MP Instruments) or E-Plate 16 or CIM-Plate 16 (used with DP Instruments) Contact Pin connection problems, please refer to your specific RTCA SP, MP or DP Instrument Operator's Manual, under the section in 'Maintenance and Care', on cleaning and exchanging the RTCA Contact Pins.

5.4 Start Measurement

The Layout page and *Schedule* page must be set up before the experiment can be started. Once these two pages have been set, the button in the upper left corner becomes active. Click on the button to start the experiment.

The Step Status on the Schedule page shows the individual status of the steps included in the experiment. The status can be IDLE (indicates that the step has not started yet), TEST (step is in progress) or DONE (step is finished).



Seq	Auto	Step	Step Status	Sweep Status	Sweeps	Interval	Unit	Total Time	Comments
1		Step_1	DONE	1	1	1	minute	00:00:28	
2		Step_2	DONE	50	50	1	minute	00:50:56	
3		Step_3	DONE	50	50	10	minute	09:11:23	
4		Step_4	DONE	100	100	15	minute	34:12:34	
5		Step_5	DONE	100	100	20	minute	67:33:15	
6		Step_6	DONE	9999	9999	5	second	131:55:55	
7		Step_7	TEST	2838	9999	5	second	170:15:56	
8		Step_8	IDLE	0	9999	5	second	184:09:11	
9		Step_9	IDLE	0	9999	5	second	198:02:26	
10		Step_10	IDLE	0	9999	5	second	211:55:41	
11		Step_11	IDLE	0	9999	5	second	225:48:56	
12		Step_12	IDLE	0	9999	5	second	239:42:11	

Check cable connections before starting the experiment to ensure that all plugs and cables of the xCELLigence RTCA Instrument are securely connected.

The xCELLigence RTCA Instrument will initiate measurements automatically, starting from the first step.

When the experiment starts, the Software measures the background first. Please do not remove the E-Plate while the Software is measuring the background. Otherwise, the experiment result will be incorrect.

5.5 Continue the Experiment

If the experiment is accidentally terminated (or after the experiment has been manually paused by the user, see below), the experiment can be resumed. To resume the experiment, launch the Software, go to *File* menu and select *Open*. Select the original experiment file of the accidentally terminated experiment and click the *Open* button. After the Software has successfully loaded the experiment, the  button will become the *Continue* button, when there are any unfinished steps left to run. The xCELLigence RTCA Instrument automatically continues the measurement and performs calculations using the original background data. The new data is appended to the original experiment file. Neither experiment file name, nor experiment ID, nor the file location will be changed.

Do not use this 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.

5.6 Pause the Experiment

After an experiment is started and as long as a step has not finished, the  button automatically changes into the  button. Click the  button to pause the current unfinished step (and the whole experiment). All experimental data is automatically saved in the experiment file as a sweep measurement is performed. Thus, the user does not need to save the experiment file before pausing the experiment.

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

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

5.7 Start a Step

After a step is finished, it can directly start the next step if the Auto checkbox is selected. If the Auto checkbox is not selected for this step, the xCELLigence RTCA Instrument will stay IDLE to wait for the user's interaction. In this case, the  button will become active. In order to start the next step, click the  button, and the system will initiate the next step.

5.8 Abort a Step

To terminate the current step, click the *Abort Step*  button. You can terminate the current step and initiate the next step at any time depending on your experimental requirements.

Abort Step stops the current step and discards the unfinished (remaining) sweeps of this step. Therefore, the user cannot resume this step. To continue the experiment, you must manually start the next step, if there is one.

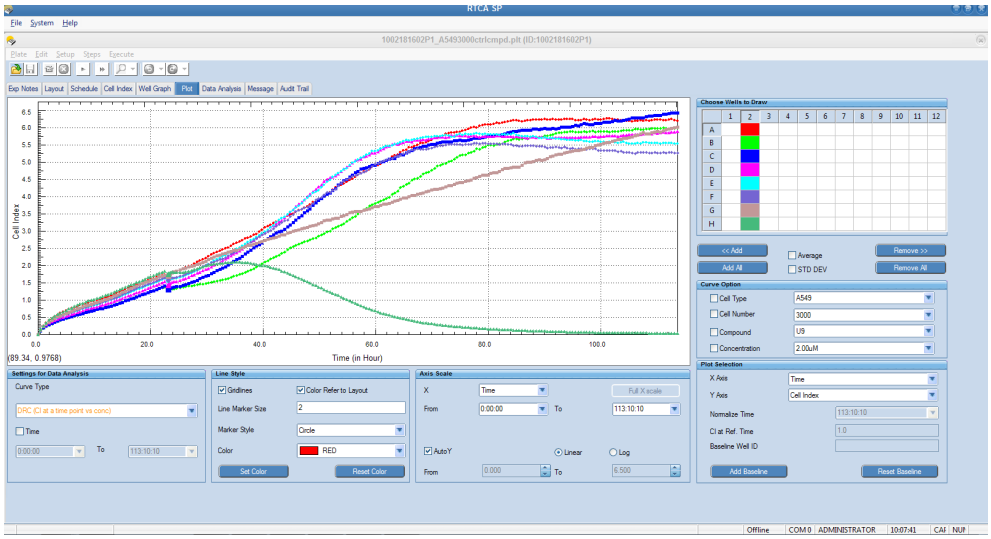
Aborting a step does NOT lead to invalidation or loss of data.

5.9 Monitoring the Current Experiment Status

The status bar on the bottom of the Software states the current status of the run. It will report 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)
- ***Testsweep#ofstep#***(whilethexCELLigenceRTCAInstrumentisperformingasweepmeasurement)
- ***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 #* (the column number that is being measured). Between sweeps, a portion of the status bar will count down the time before start of the next sweep.



6. Monitor an Experiment in Cell Index Page

Real-time resistance measurement data will be recorded and presented with a unitless parameter called *Cell Index*. There are three cell indices taken at different frequencies: Cell Index-I at 10 kHz, Cell Index-II at 25 kHz, and Cell Index-III at 50 kHz. The Cell Index is defined as follows:

Cell Index = $(R_{tn}–R_{t0})/ F_i$

Where:

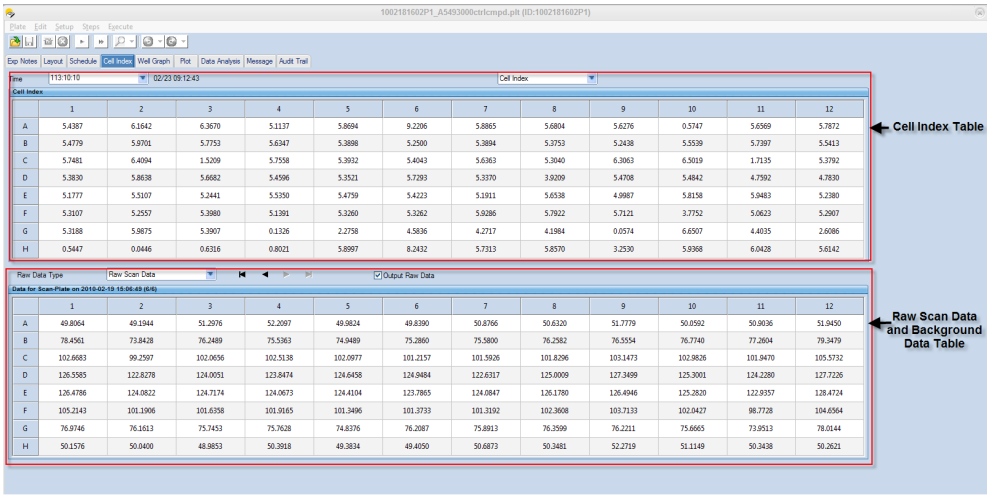
i = 1, 2, or 3;

F_1 = 15Ω, F_2 = 12Ω, F_3 = 10Ω;

and n = 0, 1, 2, …, N (time points)

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

Cell Index page archives the *Cell Index* for all the finished *Sweeps* of the experiment in Cell Index Table, and the *Raw Scan Data* and *Background Data* in Raw Scan Data and Background Data Table.



6.1 Cell Index Table

There is one drop-down lists in the Cell Index table. The *Time* drop-down list allows you to select the time point at which one Sweep was started during an experiment. Calculated *Cell Index* will be displayed in the Cell Index table for the selected wells in the *Layout* page.

When an experiment is running, the *Cell Index* table shows the most recent Cell Index obtained. To view previously collected data, select a particular measurement time point from the *Time* drop-down menu. If more than nine time points have been recorded, then use the scroll bar to view the hidden time points.

B

6.2 Scan Plate Data and Background Data Table

Raw Data Type drop-down list allows you to toggle between *Raw Scan* Data and *Background* Data to be displayed in the table.

Raw Scan Data:
Measured resistance data of all 96 wells (or 16/32/48 wells for xCELLigence RTCA DP Instrument) each time the Scan Plate function is performed.

Compare these data to the scan plate results on the Message Page to confirm which wells, if any, may have connection issues.

Raw Scan Data is also used for Resistor Plate Verification. For more information please refer to the xCELLigence RTCA Instrument Operator's Manual.

If the Scan Plate function was performed more than once, click the arrow buttons in  *icon to switch through each Raw Scan Data.*

Background Data:
Background resistance data for all 96 wells (or 16/32/48 wells for xCELLigence RTCA DP Instrument) at time point 0.

Check the background measurement message on the Message page, to discover which wells, if any, may have background problems.

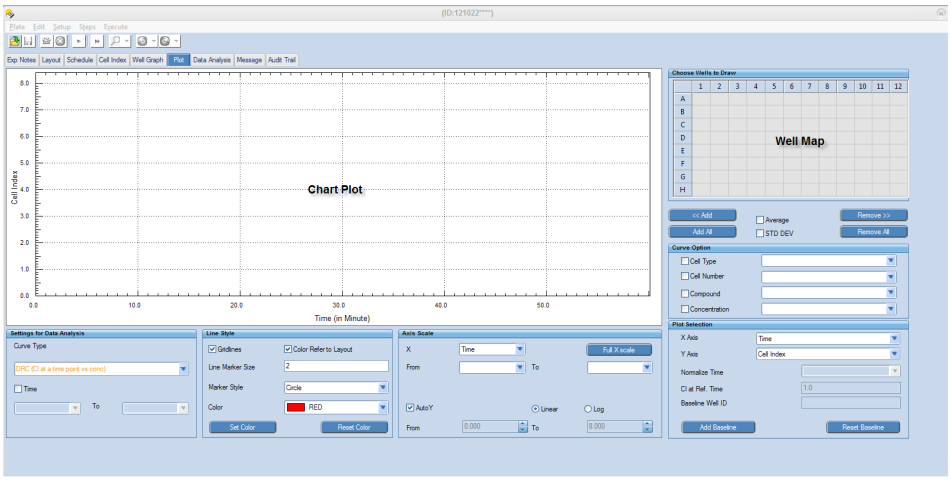
6.3 Copy Cell Index Data and Raw Data

The Cell Index data and raw data (including Background Data and Raw Scan Data) can be copied and paste to a text file and spreadsheet. To do so, just select the Time you want to copy and paste in the Cell Index Table. If you want to copy the raw data as well, select either *Raw Scan Data* or *Background Data* from the *Raw Data Type* drop-down list and enable the *Output Raw Data* checkbox in the *Raw Scan Data* and *Background* Table. In the Plate window Menu, click *Edit* ✳ *Copy*. The desired data at desired time are now ready to be pasted to a text file and spreadsheet.

7. Plot Experiment Data

The *Plot* page displays and analyzes the collected data. To plot experiment data from a single well, double-click the well button that represents the well position on the well map; the **Chart Plot** will display the curve for that well.

To display multiple data points on the chart, click on one well and drag the pointer across the desired selection of wells. (The selected wells turn dark blue.) Then, click the << Add button. The experiment data collected from the selected wells will be displayed on the Chart Plot. Clicking the Add All button will cause data from every well measured in the experiment to be plotted on the Chart Plot. (See section 7.5 for information on removing well data from the Chart Plot.)



7.1 Plot Selection

Use the options in this section to change the way plots of well data are displayed:

- ***X-axis:*** From this drop-down menu, select the parameter to be plotted on the X-axis of the Chart Plot.
- ***Y-axis:*** From this drop-down menu, select *Cell Index*, *Normalized Cell Index* or *Delta Cell Index* as the parameter to be plotted on the Y-axis.

  If you select *Normalized Cell Index*, you must also choose a specific time point for normalizing the data. You can select this time point from the *Normalization Time* drop-down menu under *Curve Options* (section 7.2). (A suitable time point might be, *e.g.*, the last time point before compound addition.) The *Normalized Cell Index* for all wells will be set to one (1) at the normalization time point.

  Similarly, if you select *Delta Cell Index*, you must choose a **Delta time** from the *Normalization Time* drop-down menu (section 7.2). 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 by using the box just below the *Y-axis* box. Note that you use the same input box (*Normalize Time*) to select both the Delta Time and the Normalization Time.

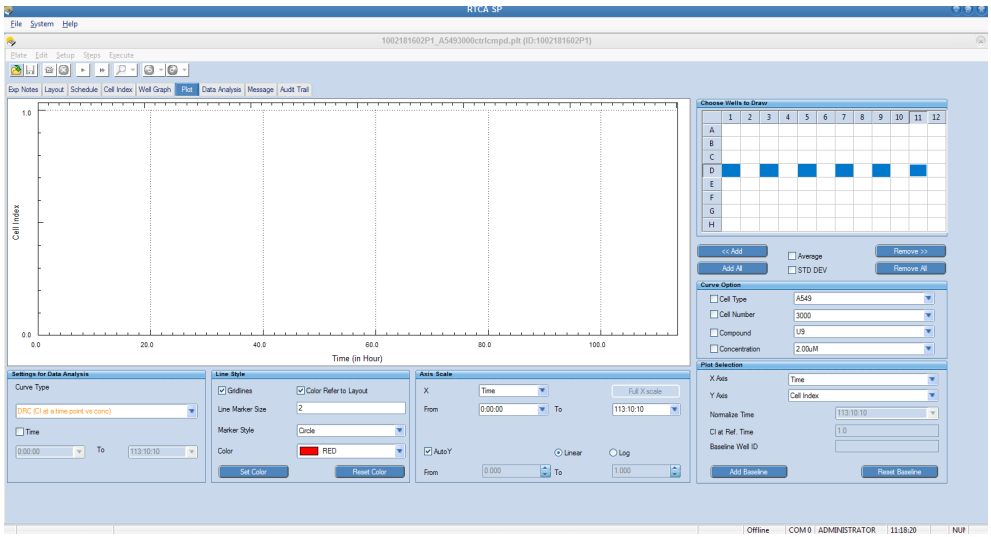
- ***Curve Style:*** A user can choose to have data plotted on a *Linear* (default) or *Log* scale by clicking one of the radio buttons. Then, the curves will automatically use this scale on the Chart Plot.

- ***Line Marker Size:*** Users can change the line size used on the plots. To do this, first click the button(s) on the well map corresponding to the well(s) you want to plot (selected well or wells will turn dark blue). Once the wells are selected, go to the *Line Marker Size* box and enter a smaller or larger value than is currently displayed. Smaller numbers will make the lines appear thinner and larger numbers will make the lines appear thicker.
- ***Line Marker Style:*** Users can change the type of symbol used to represent data on the plot. To do this, first click the button(s) on the well map corresponding to the well(s) you want to plot (selected well or wells will turn dark blue). Once the wells are selected, select the symbol from the *Line Marker Style* drop-down menu.

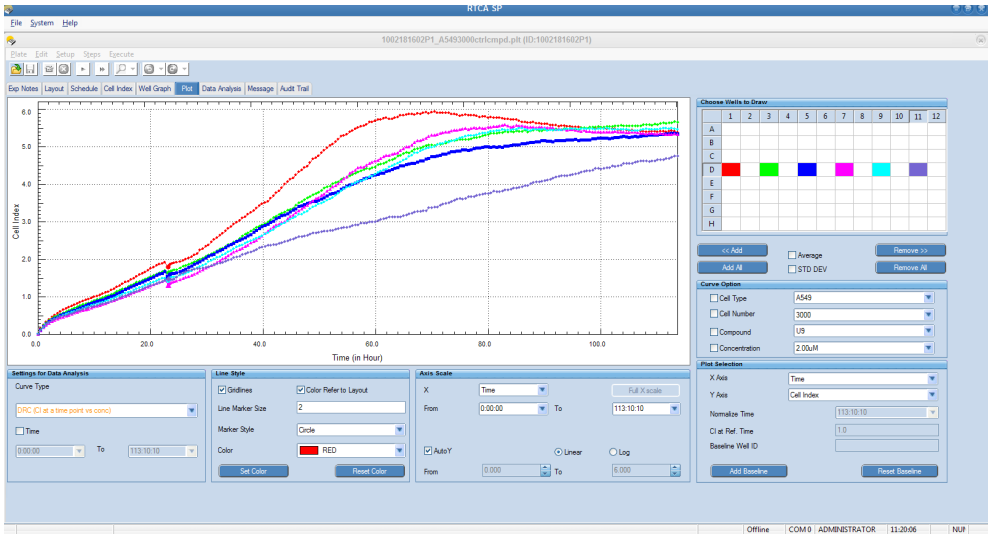
B

Example of Plotting Curve Data

Notice that the dark blue color of well buttons D1, D3, D5, D7, D9 and D11 in the well map indicates that these wells have been selected.



Clicking  button displays the Cell Index curves for the six selected wells. (Note that the colors of the selected wells have changed to match the colors of the corresponding curves on the Chart Plot.)



7.2 Curve Options

Use the drop-down menus in this section to change the type of data displayed:

- ***Normalize Time:*** This drop-down menu is not always available. It is only available when users select *Normalized Cell Index* or *Delta Cell Index* for the Y-axis (see *Y-axis* in section 7.1 above). In those cases, this drop-down menu displays all time points at which experiment data have been collected. Any time point selected in the drop-down menu will then be used as the normalization or delta time.
- ***Cell Type:*** If the experiment involves more than one type of cell, this menu lets users view data from a particular cell type (The different types must first be listed on the *Layout* page).
- ***Cell Number:*** If the experiment involves different numbers of cells, this menu lets users view data from a specific number of cells (as listed on *Layout* page).
- ***Compound:*** If multiple compounds were tested, this menu lets users view data generated from a particular compound (as listed on *Layout* page).
- ***Concentration:*** If multiple concentrations of a compound were used, this menu lets users view data generated from a particular concentration (as listed on *Layout* page).

7.3 Scale

Use the options in this section to change the range of data displayed:

- ***Steps:*** If the experiment consists of multiple steps, this function will facilitate viewing of only a few of those steps. For example, to view data from step 2 through step 5, first select *Steps* from the ***X*** menu, and then select 2 on the *From* menu and 5 on the *To* menu.
- ***Time:*** This function will facilitate viewing data recorded over a certain period of time. For example, to view data recorded from 2 hours, 10 minutes, to 5 hours, 30 minutes of the experiment, first select *Time* from the ***X*** menu, and then select 02:10:00 on the *From* menu and 05:30:00 on the *To* menu.

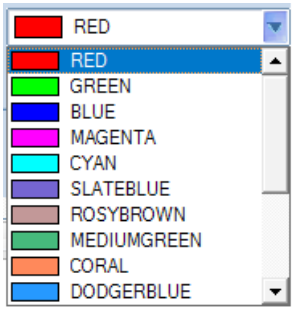
  Choices are limited to the actual time points (hh:mm:ss) of measurement sweeps.
- ***Full X scale:*** Use this button to undo the *Steps* or *Time* selections (above) and restore the full range of data (*i.e.*, display all the data points on the Chart Plot).
- ***Auto Y:*** Placing a checkmark in the *AutoY* box turns the auto-scaling function on and lets the software choose the best Y-axis for data display. To turn this function off, click on the box, and the checkmark will be removed.

Turning the auto-scaling function off will activate the *From* and *To* boxes below the *AutoY* box, allowing users to manually set the Y-axis scale. (Either use the up or down arrows next to these boxes, or type in numbers to set the desired Y-axis scale.)

7.4 Curve Color Selections

By default, if you click << Add or Add All, the curves will be drawn in colors that the system selects. To select different colors for each curve, use the ***Set Well Color*** *button. 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 drop-down list above the *Set Well Color* button is used to select plot colors. To change the color of a specific curve, first select the well button on the **Well Map** by clicking on it (the selected well will turn dark blue). Then, choose the desired color from the color drop-down list and click *Set Well Color* to apply that color to the well data plot.



- ***Set Well Color:*** Click this button to change the color used to plot well data.
- ***Reset Well Color:*** Click this button to return plot colors to their default settings.

7.5 Other Function Buttons

- ***Average:*** If some data plotted on the chart are replicates (as defined in the *Layout* page), checking the *Average* box causes an average of these replicates to be plotted.
- ***STD DEV:*** Checking the *STD DEV* box adds error bars to any averaged data plotted on the Chart Plot (see section 7.8).
- ***<<Add:*** Once a well button or buttons are selected, clicking the *<<Add* button will plot the data from the selected wells on the Chart Plot (See also section 7).
- ***Add All:*** Clicking the *Add All* button will cause data from every well measured in the experiment to be plotted on the Chart Plot.
- ***Remove>>:*** Once a well button or buttons are selected, the *Remove>>* button removes the corresponding curves from the Chart Plot.
- ***Remove all:*** Clicking the Remove all button will remove all the curves from the Chart Plot.

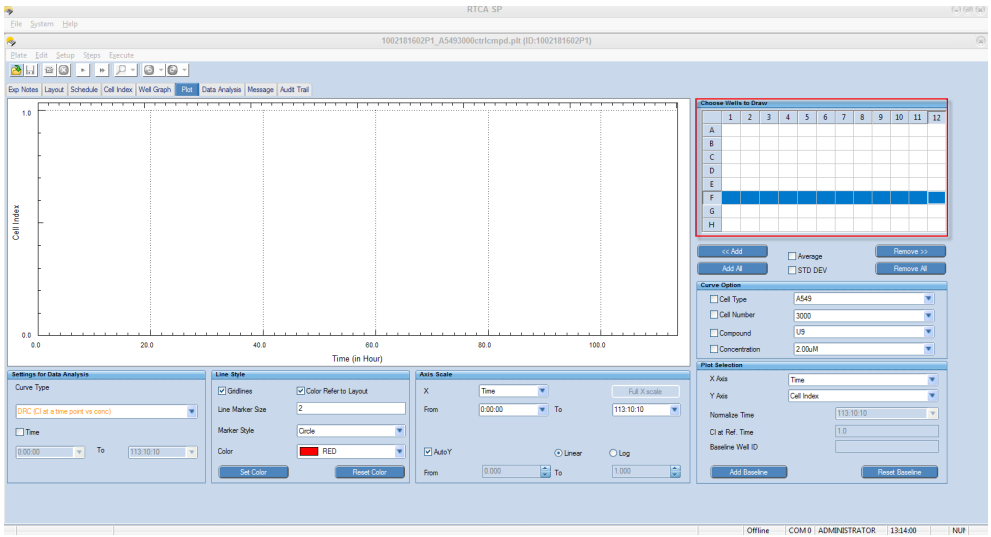
7.6 Well Map

On the ***Plot*** page, the well map is used to select the well or wells whose curves will be displayed on the **Chart Plot**. Select an individual well by simply clicking on the well button. Select multiple wells by clicking and dragging across the desired well buttons. Individual wells can also be double-clicked to add data for that well to the Chart Plot.

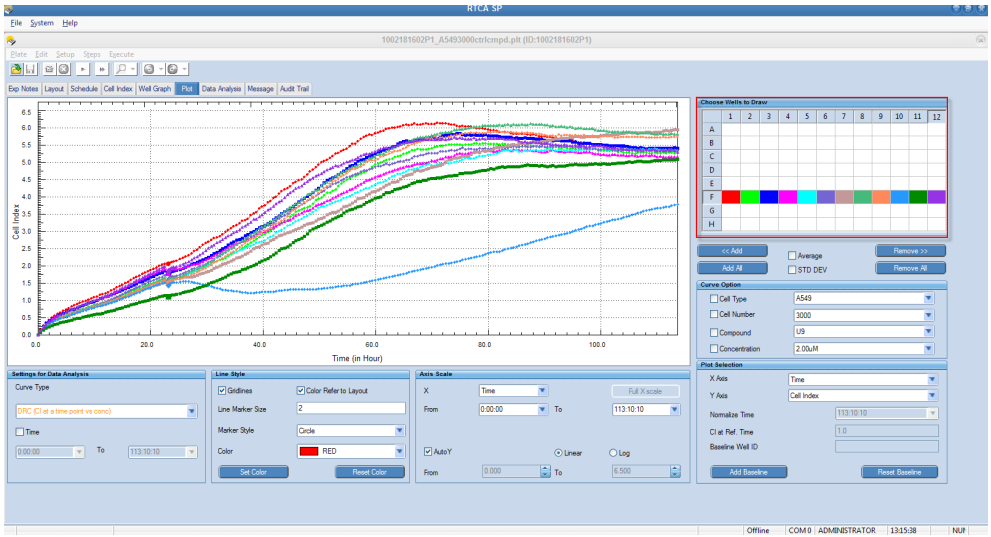
The light grey color of wells on the well map indicates that these wells have been activated on the ***Layout*** *page for this experiment.*

7.7 Example of Adding Wells to the Chart

In the example below, wells have been selected for graphical display, but the << *Add* button has not been clicked yet.

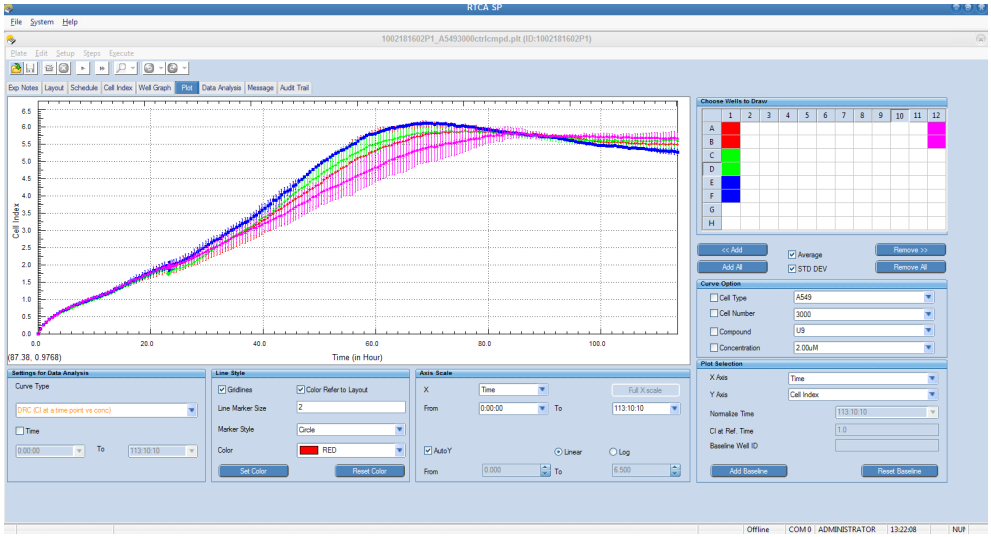


After the << *Add* button is clicked, each well is represented by a colored line on the Chart Plot:



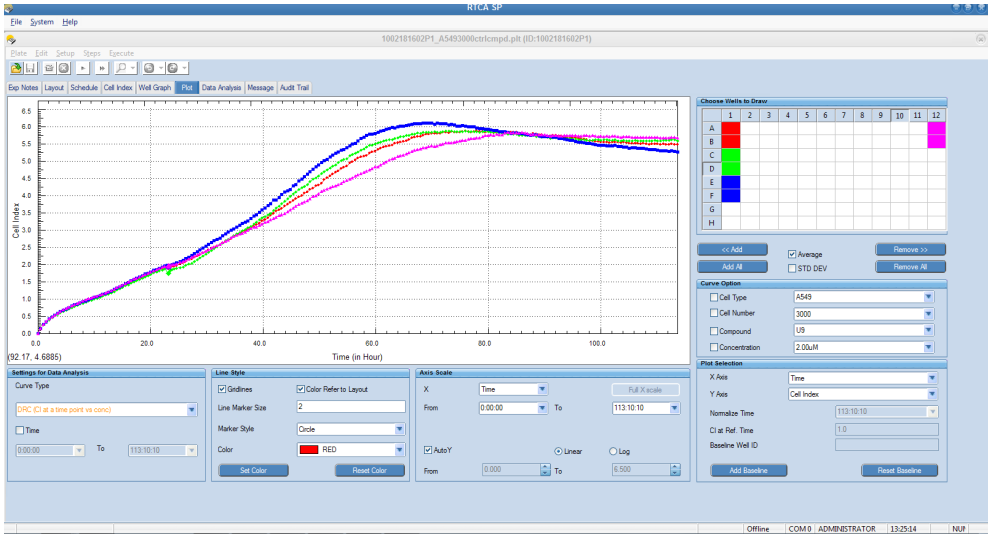
7.8 Example of the Average Function

The *Average* function calculates the average of replicate wells (*i.e.*, wells with the same cell type, cell number, compound name and concentration on the *Layout* page) at each time point and displays the averaged data in a single color. The same color is used to highlight the replicate wells on the well map. The *STD DEV* function adds error bars (representing standard deviation the Software calculates for the average) to the graph.



7.9 Example of the Log Scale

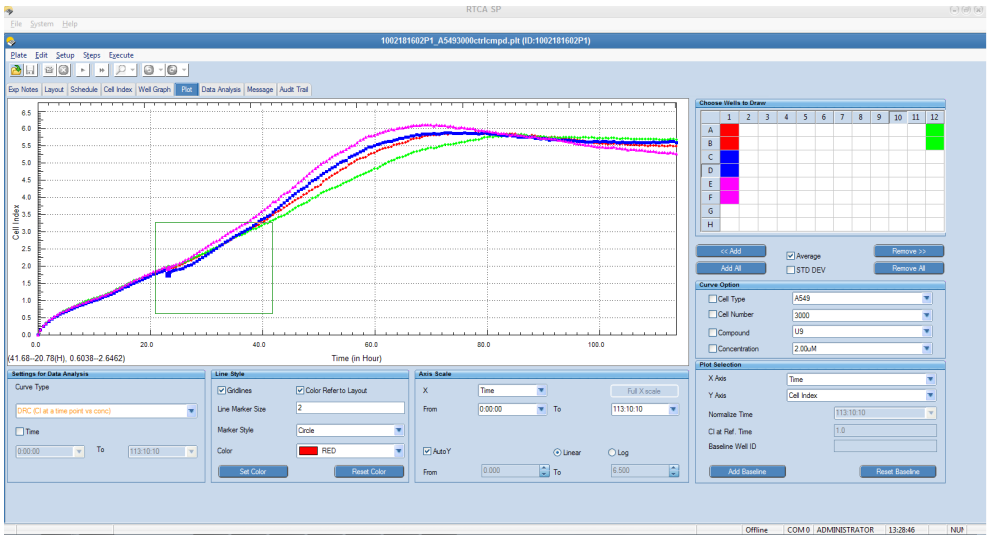
The data below is displayed on a chart with a logarithmic Y-axis. To redraw a plot this way, select the ***Log*** button (in *Axis Scale*).



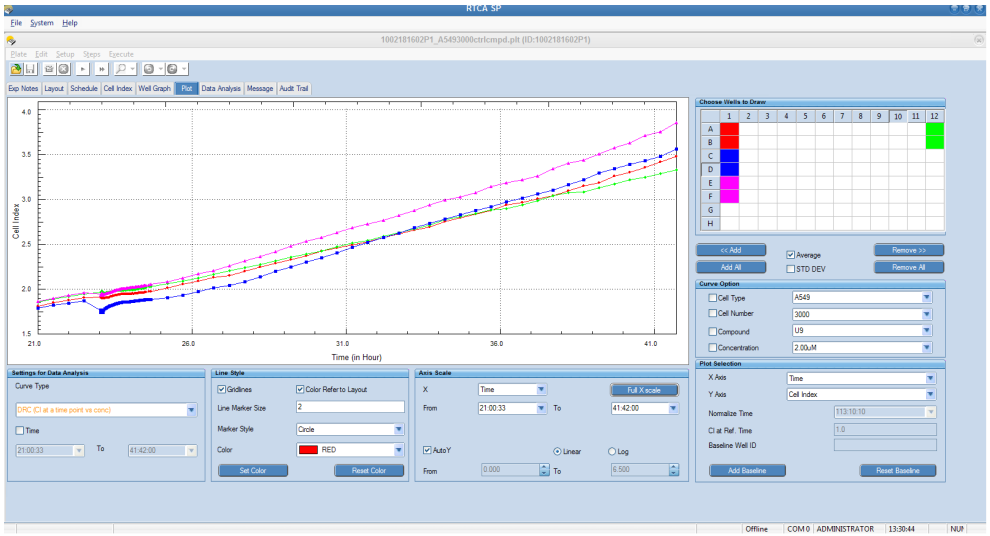
7.10 Zoom In/Zoom Out

Click the left mouse button, then drag the cursor to draw a rectangle around an area on the plot. When you release the button, the curve within this area will be zoomed in (magnified). Press the *Esc* key to zoom out (return the plot to its original scale).

Before **Zoom In**:



After **Zoom In**:



Zoom In may take a longer time when there are a lot of data points in the area selected for zooming.

7.11 Curve Repair Function

If for any reason the experimental curve is not smooth (most likely due to poor RTCA Contact Pin connections that occurred when a plate was taken out and re-engaged during an experiment), the RTCA Software offers *Curve Adjustment Tools* for repairing those problem curves.

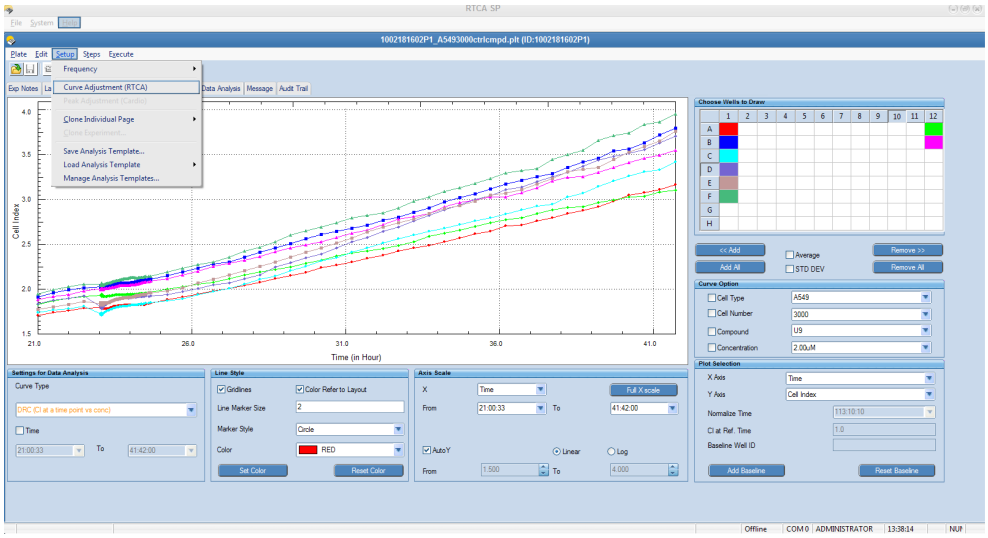
***Curve Adjustment Tools** are not available when an experiment is running. Curve repair must be done only after the experiment has finished or paused.*

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

***Curve Adjustment Tools** are available only when Cell Index data is plotted on the Y-axis. After curve adjustment, the user can plot **Normalized Cell Index** or **Delta Cell Index** based on the adjusted Cell Index data.*

To repair problematic curves, select *Curve Adjustment Tools* from the *Setup* menu.

Curves before adjustment:



After *Curve Adjustment Tools* is selected, the *Adjust peak* panel will appear in the bottom-left corner. Two types of curve adjustment tools are offered, *Shift* and *Line fit*.

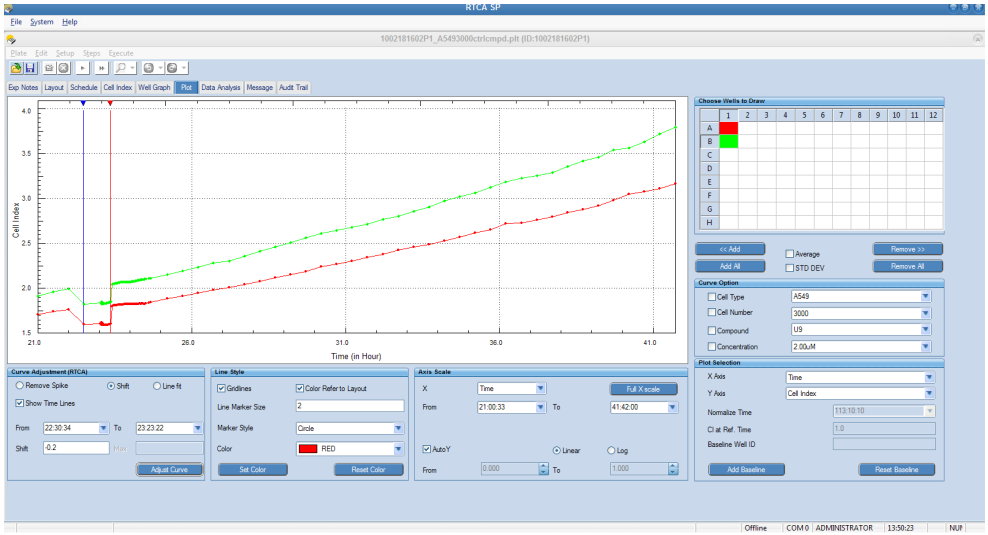
7.11.1 Shift of Curves

To shift curves, follow these steps:

- Select curve(s) to be shifted by selecting wells from the well map and adding their curves to the plot.
- Select the time range over which to shift curve(s), using the time drop-down menu to set the *From* and *To* time points.
- Type in the value (positive or negative) by which the curves will be shifted.
- Choose the *Shift* button.
- Click the *Adjust Curve* button.

After the *Shift* adjustment, all the curves in the selected time range will be shifted by the *Shift* value.

Example: The curves have been shifted by -0.2 for all time points between 22:30:34 and 23:23:22.



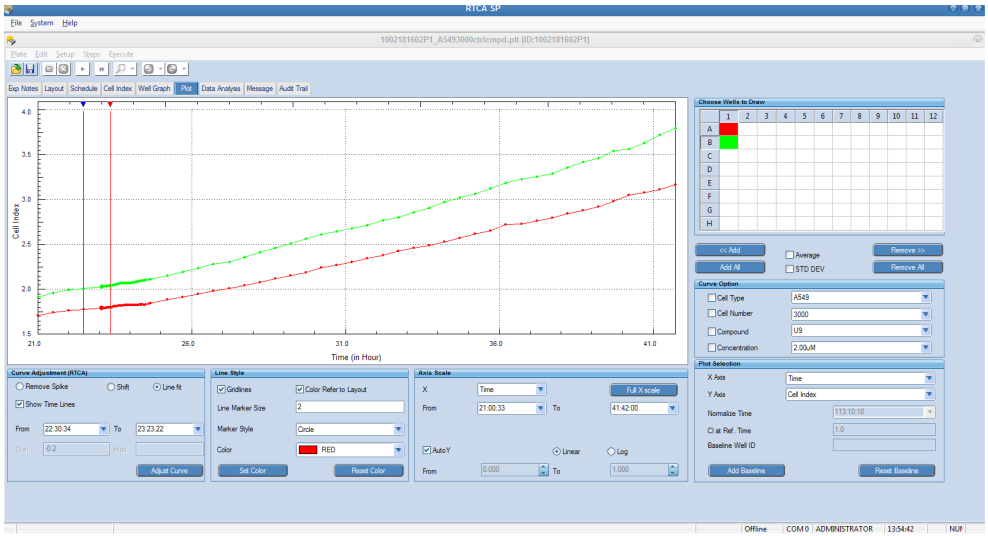
7.11.2 Line Fit

To do a *Line fit*, follow these steps:

- Select curve(s) to be adjusted by selecting wells from the well map, and adding their curves to the plot.
- Select the time range over which to fit curve(s), using the time drop-down menu to set the *From* and *To* time points.
- Choose the *Line fit* button.
- Click the *Adjust Curve* button.

After the *Line fit*, the portion of each curve within the selected time range becomes a smooth line.

Example: The portion of the curves between 22:30:34 and 23:23:22 are adjusted with a line fit.



7.12 Baseline Cell Index

Users can choose one or more curve(s) as a **baseline** (if more than one curve is given, the average Cell Index of the given curves will be used as the baseline). 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, *i.e.*:

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

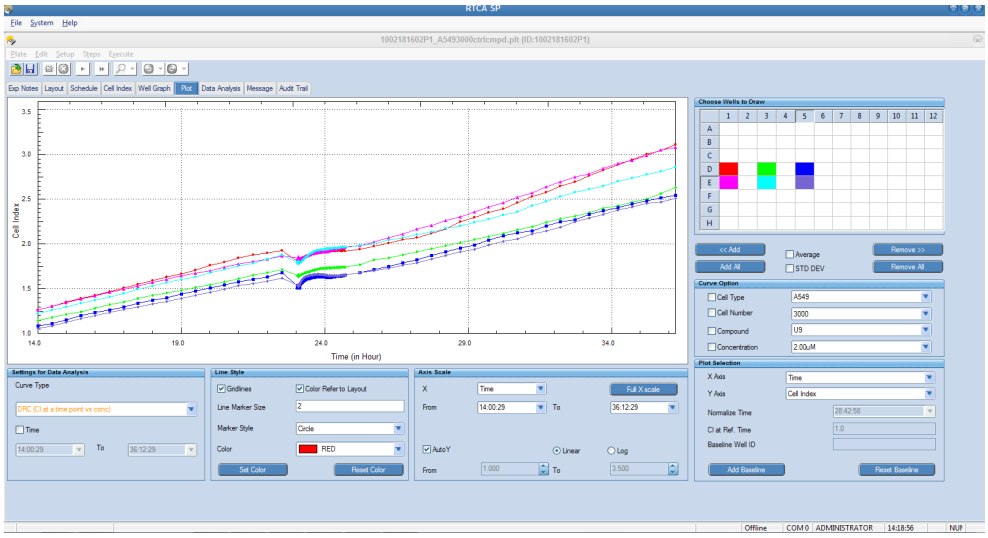
The Y-axis of the plot is now labeled *BaseLine Cell Index* (*BaseLine Normalized Cell Index*, or *BaseLine Delta Cell Index*).

Follow these steps to draw a Baseline Cell Index curve:

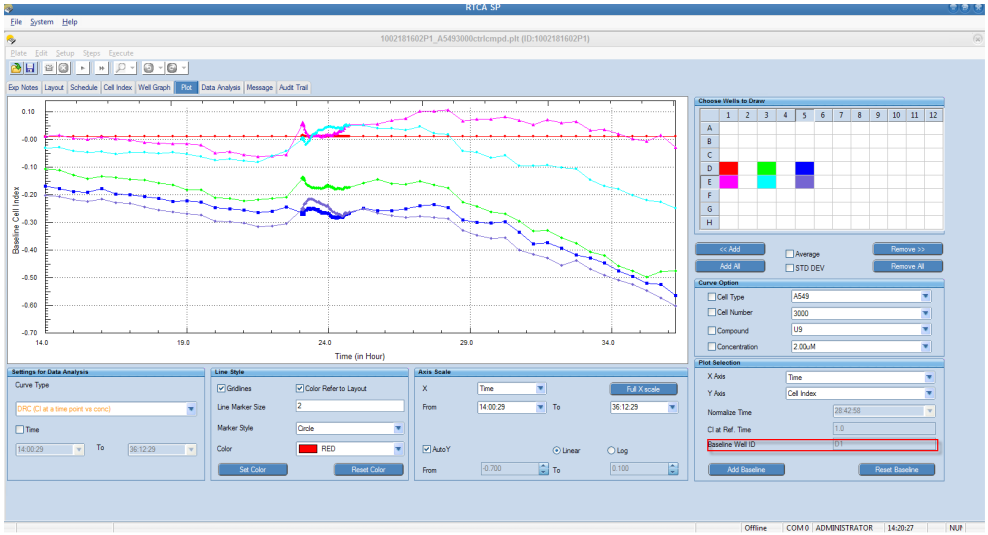
- On the well map, select a well or wells to be used as baseline wells.
- Click the *Add BaseLine* button (the selected well(s) will be shown in the *BaseLine Well ID* box).
- Select the well(s) to be plotted on the well map.
- Click the << *Add* button to add curves to the plot. The Y-axis will be labeled *BaseLine Cell Index*.

Click *Reset BaseLine* to remove the baseline and to restore plots of actual Cell Index values.

Example: Six wells are selected (D1, E1, D3, E3, D5 and E5) and displayed without and with D1 as a baseline well.



Cell Index curves are plotted for D1, E1, D3, E3, D5 and E5 wells.



BaseLine Cell Index curves are plotted for D1, E1, D3, E3, D5 and E5, with D1 well as baseline well.

7.13 Normalized Cell Index

For each well, the **Normalized Cell Index** (NCI_{ti}) is calculated as the Cell Index CI_{ti} at a given time point divided by the Cell Index CI_{nml_time} at the normalization time point (nml_time), as below:

$$NCI_{ti} = CI_{ti}/CI_{nml_time}$$

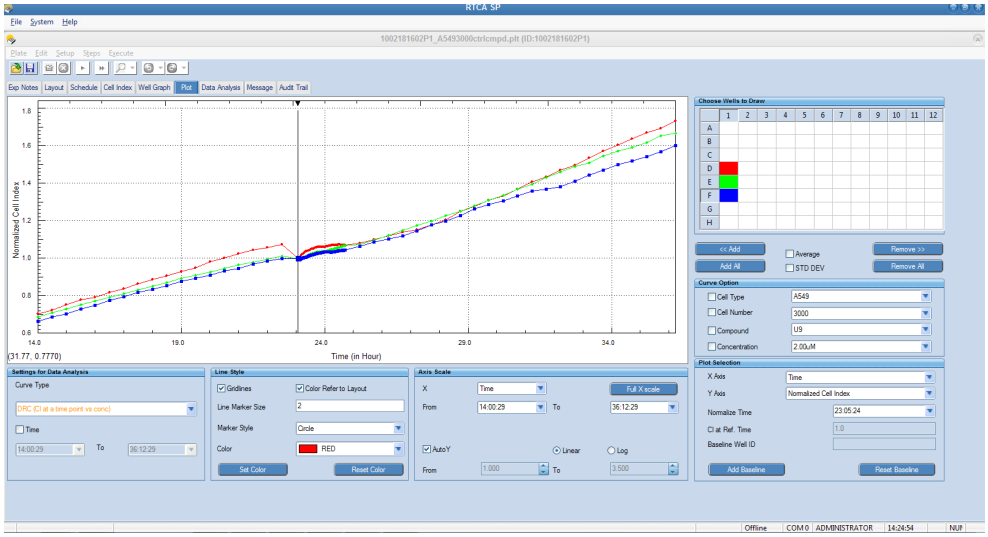
Thus, the Normalized Cell Index for all wells must equal one (1) at the normalization time point.

Normalized Cell Index is only effective for RTCA mode.

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

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

Example: Normalized Cell Index plot for wells D1, E1, F1. *Normalize Time* is 23:05:24.



7.14 Delta Cell Index

For each well, the **Delta Cell Index** (DCI_{ti}) is calculated as the Cell Index CI_{ti} at a given time point 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 below:

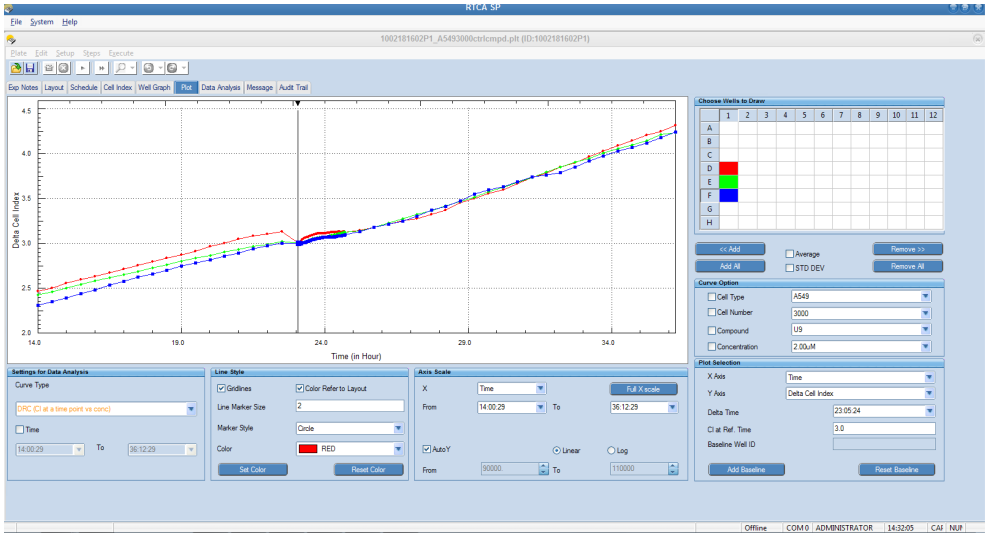
$$DCI_{ti} = CI_{ti} + (DCI_{reference} - CI_{Delta_time})$$

Thus, the Delta Cell Index for all the wells is the reference DCI value at the Delta time point. The default value of the *CI at Ref. Time* is one (1), but the user can change that value.

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

- Select *Delta Cell Index* from Y-Axis drop-down list.
- Select well(s) on the well map.
- Click the <<*Add* button to draw the *Delta Cell Index* curve(s).
- Select Delta time point from the *Delta Time* drop-down list (the same list as the *Normalize Time* list).
- Put in the required Reference DCI by typing in the value in the *CI at Ref. Time* box.

Example: Delta Cell Index plot for wells H1, I1, H13, and I13; *Delta Time* point is 23:41:57 and $DCI_{reference}$ = 3.0.



7.15 Settings for Data Analysis

In the *Settings* of the *Data Analysis* section of the *Plot* page, the user can set up some parameters for the *Data Analysis* page, including **curve type** and **time period** to analyze. The options in *Settings for Data Analysis* section are the same as those under IC_{50} *Option* on the *Data Analysis* page. For more details, refer to section 10.2.4, *Curve Types and Curve-Fit Formula.*

B

8. Copying Experiment Data

8.1 Copy Experiment Data to Microsoft Excel

There are two ways to export data to Microsoft Excel.

First, launch the RTCA Software and open the experiment data file (*.plt), then:

- 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. Select *Copy* from the *Edit* menu. The Cell Index values will be copied and ready for pasting into an Excel worksheet. Open Microsoft Excel and select *Paste* from the *Edit* drop-down menu to paste the copied data to Microsoft Excel.
- Go to the *Plot* page, select the wells to be analyzed on the well map and click << *Add* to display curves for the selected wells. Once the curves are displayed, right-click the mouse, and select *Copy Data in List Format from the pop-up menu*. The data on the chart will be copied (as a list of data points) and ready for pasting to an Excel worksheet. Open Microsoft Excel and select *Paste* from the *Edit* drop-down menu, to paste the copied data to Microsoft Excel.

8.2 Copy Graphic Charts or Data on Charts

To copy graphic charts or data on charts to Microsoft PowerPoint, Microsoft Word or Microsoft Excel, follow these steps:

- Launch the RTCA Software and open the experiment data file (*.plt).
- Add the desired curves to the Chart Plot.
- Point the cursor at the Chart Plot and right-click the mouse.
- Select *Copy Charts* (or other items) from the pop-up menu.
- Open the program (typically Microsoft Word/Excel/PowerPoint) to which you want to paste the chart or data from the chart.
- Select *Paste* on the *Edit* drop-down menu.

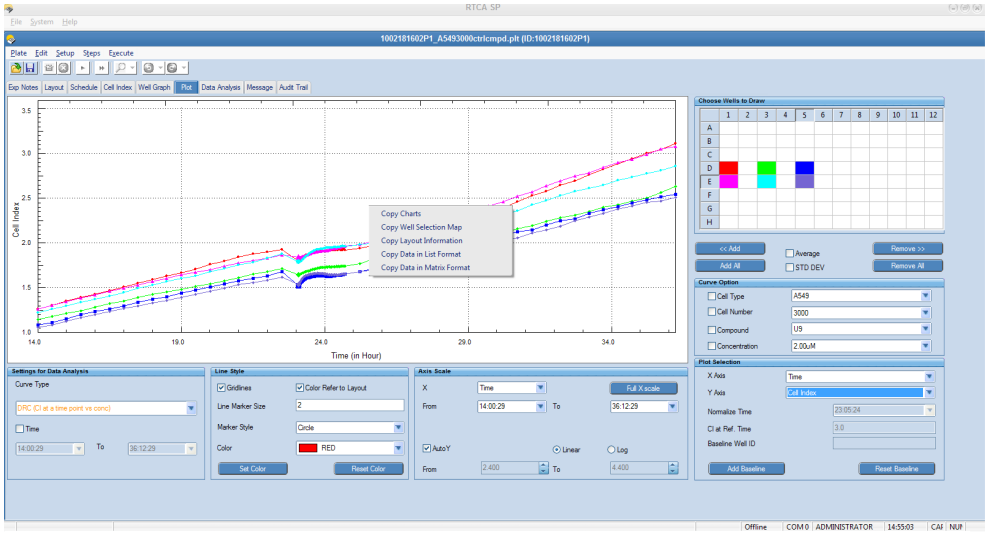
The charts are imported as pictures. Therefore, their curves and labels cannot be modified. If the chart is to be modified, use the ***Copy Data from Chart*** *function to paste the data into Microsoft Excel (or other data analysis software), then use Microsoft Excel's graphing function to re-plot the data.*

8.3 Example of a Right-Click Menu

On the *Plot* page, right-clicking the mouse will open a menu with the following options:

- ***Copy Charts:*** *Copy the chart as a picture.*
- ***Copy Well Selection Map:*** *Copy the well map.*
- ***Copy Layout Information:*** *Copy all layout information for the selected wells.*
- ***Copy Data in List Format:*** *Copy (as a simple list) the Cell Indexes for the selected wells and for time points currently displayed on chart.*
- ***Copy Data in Matrix Format:*** *Copy (as a matrix) the Cell Indexes for the selected wells and for time points currently displayed on chart.*

After clicking one of the menu items described above, the user can go to Excel and paste the copied chart or all the copied data.



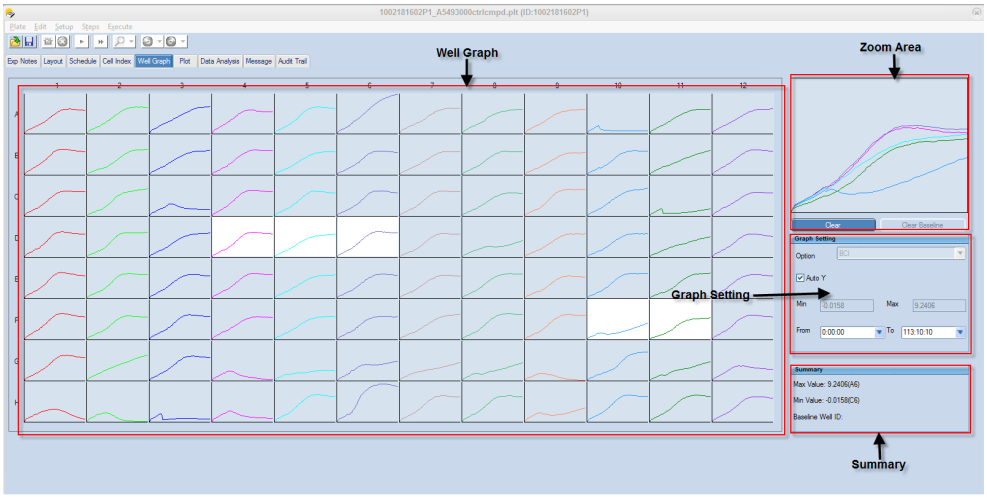
9. Well Graph

The *Well Graph* page provides an overview of Cell Index curves for all the wells by displaying them in 8×12 matrix format. The user can display and compare some of the curves in the **Zoom Area** (top-right part of page) by just clicking on the wells to be compared. The user can also select one of the wells as a reference.

The Well Graph display is only effective for RTCA mode.

9.1 User Interface

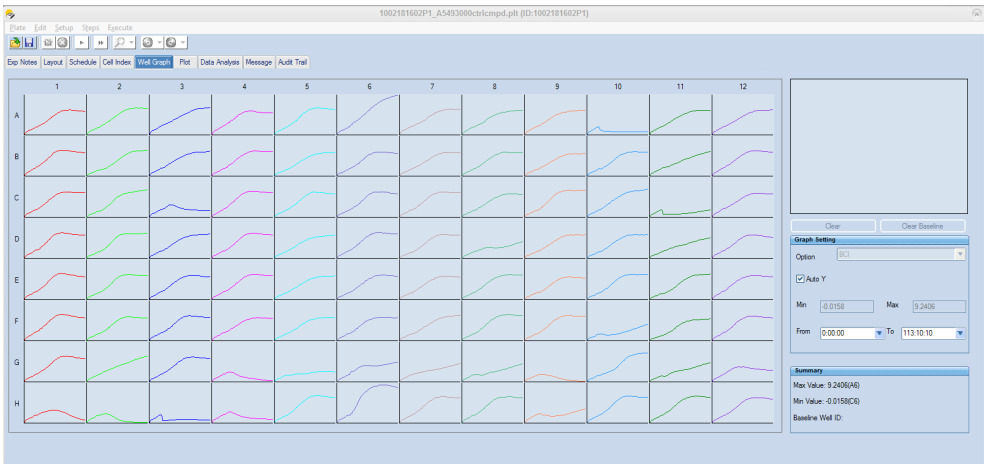
The *Well Graph* Page is divided into four main areas: **Well Graph**, **Zoom Area**, **Graph Setting** and **Summary**.



9.2 Well Graph Display

If there is Cell Index data in the experiment that is currently open, all the Cell Index curves will be displayed on the Well Graph. If any data is added or modified during or after the experiment, the curves on this display will automatically be updated in real time.

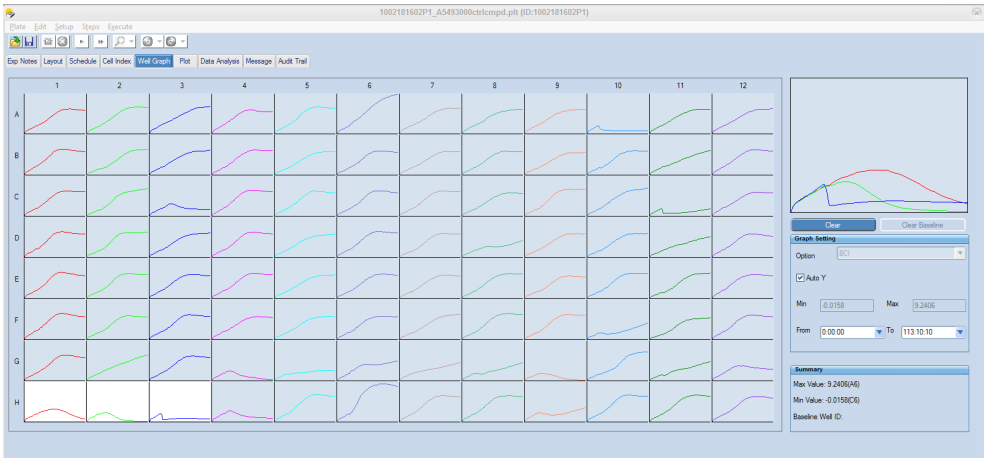
The following figure is an example of a Well Graph display during an experiment.



9.3 Zoom Area

The Zoom Area can display expanded versions of curves that the user selects from the Well Graph. To select one or more well curves to display in the Zoom Area, simply click the corresponding wells on the Well Graph. The color of the chosen well(s) will change to white. To remove a curve from the Zoom Area, just click again and the color of this well will change to the original. To clear all the added curves from the Zoom Area, click the *Clear* button.

The following figure is an example of a Zoom Area display.



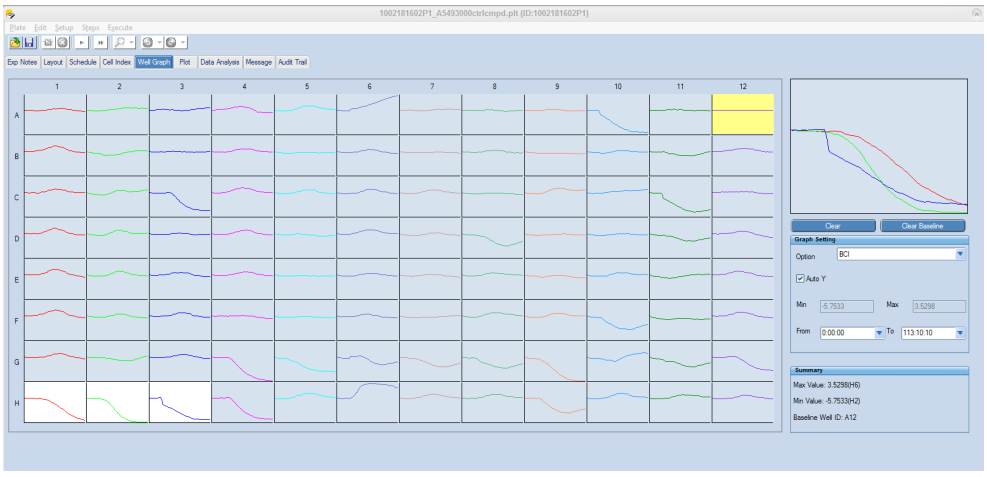
Users can also choose one of the curves on the Well Graph as a reference. Once a reference curve is chosen, the curves in the Zoom Area will be calculated by subtracting the *reference Cell Index* from the *original Cell Index*, *i.e.*:

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

Follow these steps to draw a Reference Cell Index curve:

- Move the mouse cursor to the well that you want to set as a reference
- Right-click the mouse. The select reference well will turn to yellow. This reference well ID will also show in *Baseline Well ID* in the *Summary* area of the well graph.
- Add other wells to the Zoom Area by clicking them.

The following figure is an example of *reference curves*.



In the above figure, well A12 has been selected as a *reference*, so the wells (*i.e.*, H1, H2, H3) added to the Zoom Area are plotted as *Reference Cell Index* lines.

B

9.4 Graph Setting

Users can adjust the setting for Well Graph and Zoom Area by using the functions in the *Graph Setting* area.

- ***Auto Scale:***

Automatically adjust the Y-axis of the Well Graph by the software. De-selecting *Auto Scale* allows users to adjust the Y-axis scale manually. Typing numbers in the boxes of *Cell Index Cap* and *Cell Index Bottom* set the upper and lower limits of the Y-axis for the Well Graph plot.

Please note that the value of Cell Index Bottom must be less than that of Cell Index Cap.

- ***Draw Baseline:***

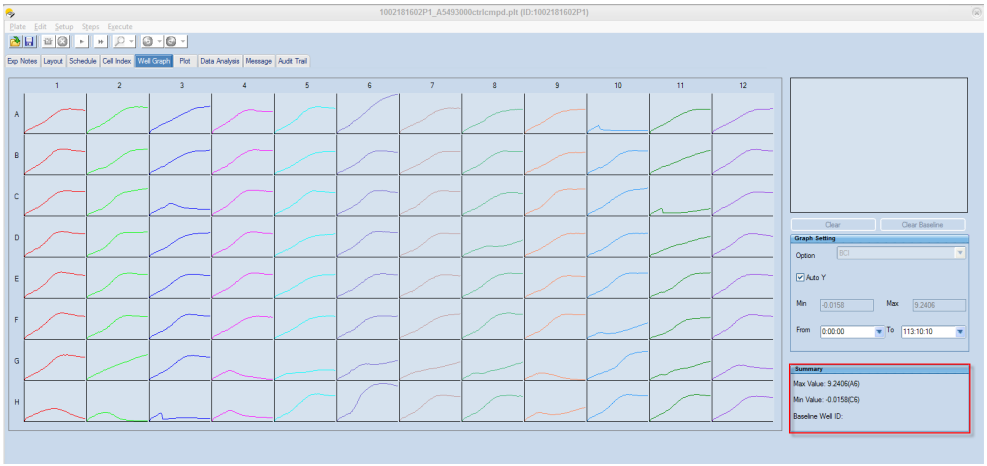
If a reference well is selected, select this Draw Baseline box will add the curve of the reference well to each of the Well Graph in order to make direct comparison to the reference well possible.

- ***From XXX to XXX:***

Selection of the time period to plot the well graph.

9.5 Summary

There is a *Summary* area displaying information about the current experiment, including *maximal Cell Index* value and the corresponding well ID, *minimal Cell Index* value and the corresponding well ID. The following figure is an example of a summary.

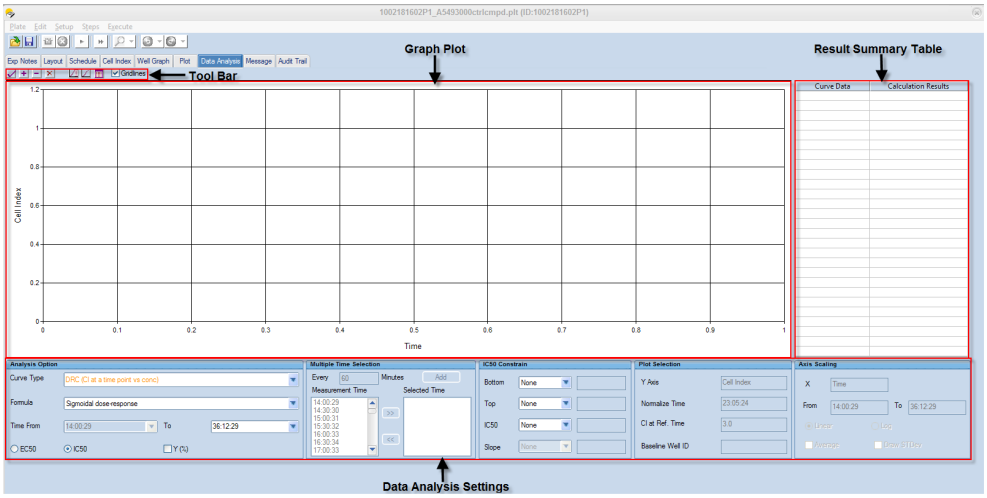


In the above figure, the well that has the *Maximal Cell Index* is A6, and its value is 9.2406, and the well that has the *Minimal Cell Index* is C6, and its value is -0.0158.

10. Data Analysis

The *Data Analysis* Page is used to analyze data obtained by the xCELLigence RTCA Instrument and to calculate relevant parameters for cell-based assays.

The *Data Analysis* Page is divided into four main areas: Tool Bar, Graph Plot, Data Analysis Settings and Result Summary Table.



10.1 Tool Bar Button Definitions

10.1.1 Draw Curve, Add Curve and Clear Curve

Four buttons in the tool bar are used to draw, add, remove and clear data analysis curves.

Draw a New Curve:	After the drawing parameters have been selected, clicking will first clear all curves on the graph area, then draw the new curve.
Add a Curve:	After the drawing parameters have been selected, clicking will add the curve(s) to the existing graph. This button is usually used for curve comparison.
Remove Last Curve:	Clicking will remove the last curve added to the existing graph.
Clear Chart:	Clicking will clear all the curves from the graph plot, as well as the corresponding data from the summary table.

10.1.2 Copy Page, Copy Chart and Copy Data on Chart

Three buttons are used to copy page, copy charts and data on the *Data Analysis* page to the Windows clipboard.

▶ *Copy Page:*	Click will copy the page to the Windows clipboard, where they can be pasted to other application software, such as Microsoft Excel, Word, PowerPoint, etc.
▶ *Copy Chart:*	Click will copy the chart(s) to the Windows clipboard, where they can be pasted to other application software, such as Microsoft Excel, Word, PowerPoint, etc.
▶ *Copy Data on Chart:*	Click will copy chart data [parameters, curve-fit equation, source data, curve-fit data points (max. 240 data points)] to the Windows clipboard, where they can be pasted to other application software, such as Microsoft Excel, Word, PowerPoint, etc.

10.2 Data Analysis Functions

The Data Analysis Page is used to analyze data obtained by the xCELLigence RTCA Instrument and to calculate relevant parameters for cell-based assays. Major functions include:

- Calculate and display **IC_{50}/EC_{50}** values for experimental data, when a series of compound concentrations is used. Various methods are used to derive IC_{50}/EC_{50} values.
- Calculate and display **Cell Index Slope** and **Doubling Time** within a given time period.
- Calculate and display **time-dependent IC_{50}** values.
- Export **Dose-Response Curves** and other graphs to Microsoft Excel or Microsoft PowerPoint and other application software.
- Export **curve-fit data** to Microsoft Excel and other application software.

10.2.1 Experimental Data for Analysis

Data Analysis can be performed after opening any existing RTCA experiment data file.

Follow these steps to load existing experiment data:

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

Depending on how often data is measured during an experiment, it is possible to perform data analysis when the experiment is still running.

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

It is not advisable to perform data analysis during an experiment that is acquiring data very frequently (e.g., time interval is every 5 minutes or less). In this case, the on-line data analysis would use computer resources that are required for running the experiment.

10.2.2 Well Selection for Analysis

After loading a set of experiment data, go to the ***Plot*** page and select wells which will be used for data analysis.

*For **IC_{50}/EC_{50}** calculation, selected wells must all contain the same compound (which must be listed on the **Layout** page). If the **Layout** page shows that the wells contain more than one type of compound, go to the **Plot** page and put a checkmark in the **Compound** box, then select the compound you want. Then, add the desired wells to the plot.*

10.2.3 An Example of Data Analysis: Dose-Response Curve (DRC) at a Time Point

After selecting wells on the *Plot* page, go to the *Data Analysis* page. To obtain a *dose-response curve* at a single time point, select the following items:

1. Select RTCA mode.
2. Select *DRC (CI at a time point vs conc)* from the *Curve Type* drop-down list.
3. Choose *Sigmoidal dose-response* from the *Formula* drop-down list.
4. Select a single Time Point from the *Time* drop-down list.

Click or on the tool bar. The graph will display a dose-response curve (X-axis: concentration; Y-axis: identical to the Y-axis shown on the *Plot* Page, *e.g.*, **Cell Index** or **Normalized Cell Index**) with discrete points for selected wells. Furthermore, the RTCA Software uses the "*sigmoidal dose-response equation*" to apply curve-fitting to the experimental data points, and then calculates IC_{50}/EC_{50} values, as well as values for other parameters in the equation. The calculated IC_{50}/EC_{50} values are shown in the *Result Summary Table* on the right side of the Page.

The number in the yellow box next to each curve provides the order of the curves that have been drawn /added to the graph area. This number is used to distinguish between different curves on the graph area. The details for each curve are shown next to the corresponding number in the Table.

*Not all information can be displayed in the Result Summary Table due to space limitations. There are **tooltips** available for each cell. If you want to see more information about each cell, just click on the respective cell. Then, a pop-up window containing information on the corresponding cell will open.*

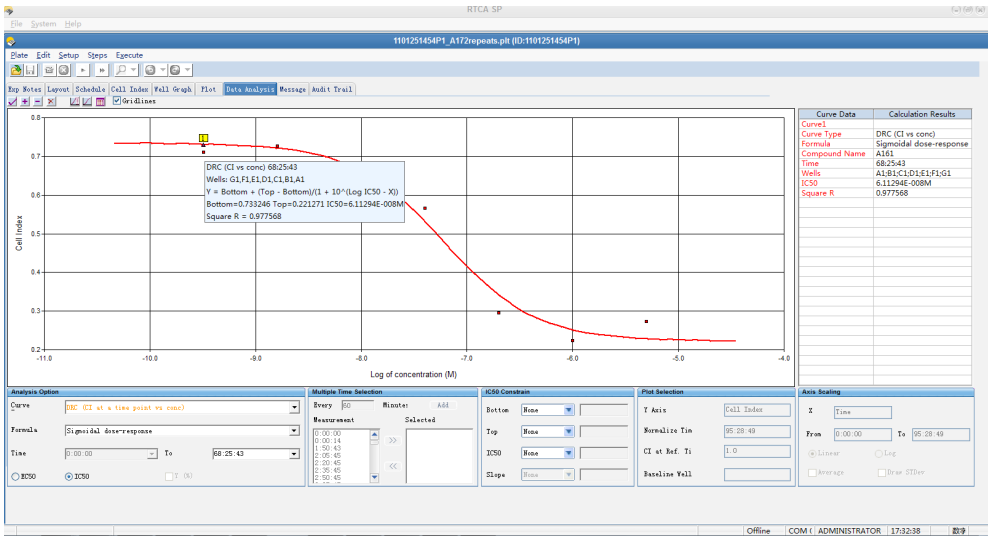
*If you have selected a **user defined unit** ("Ur Unit"), it is not possible to perform certain calculations on the **Data Analysis** page, such as DRCs. An error message will appear.*

Curve Types and Time period used for can be preselected in Settings for Data Analysis on the Plot page. There is a checkbox which allows user to choose a period of time for data analysis. By default, this box is unchecked. If this box is checked, a blue line (indicating the Starting Time) and a red line (indicating the ending time), will be shown on the Plot page. These lines represent the time period covered by the calculation. If the selected curve type is DRC at a time point, only the red line is shown, to indicate the time point selected on the Plot page.

You can either select the Stating Time and Ending Time by clicking the drop-down list or by simply dragging the blue line and red line by along the X-axis on the Chart Plot.

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To show curve-fitting details, move the cursor close to the yellow box on the Graph Plot. The detailed information about **formula**, **curve type**, **time point**, and **fitted parameters** will be shown in a *yellow box*.



10.2.4 Curve Types and Curve-Fit Formula

10.2.4.1. Curve Types

There are eight built-in **curve types** available. Not all the eight curves will be suitable for a particular user with a specific assay, but one or more of them should be appropriate for a given application. Based on your experiment, you may choose one or more of the curves for your experimental data analysis.

Group ID	Curve ID	Curve
1	1	Slope
2	2	Doubling time
3	3	Time dependent IC_{50}
4 DRCs	4	DRC (CI at a time point vs conc)
	5	DRC (max CI in a time period vs conc)
	6	DRC (min CI in a time period vs conc)
	7	DRC ((max-min) CI in a time period vs conc)
	8	DRC (area-under-curve in a time period vs conc)

*All calculations and curve-fitting on the **Data Analysis** page are performed using Cell Index or Normalized Cell Index or Delta Cell Index values identical to those displayed on the Y-axis on the **Plot** page. For example, if Normalized Cell Index is selected as the Y-axis on the Plot page, all calculations will use Normalized Cell Index values.*

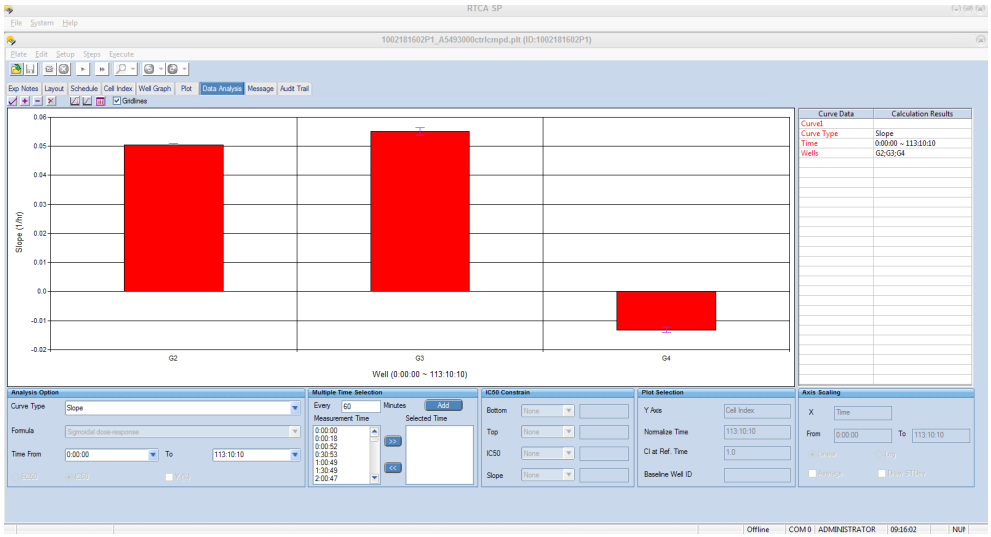
Curve Types and Time Period can be preselected in the Settings for Data Analysis on the Plot page.

► ***Slope:***

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

interested in the rate of change of the Cell Index for cells after cytotoxic compound treatment, then Slope could be chosen for such analysis.

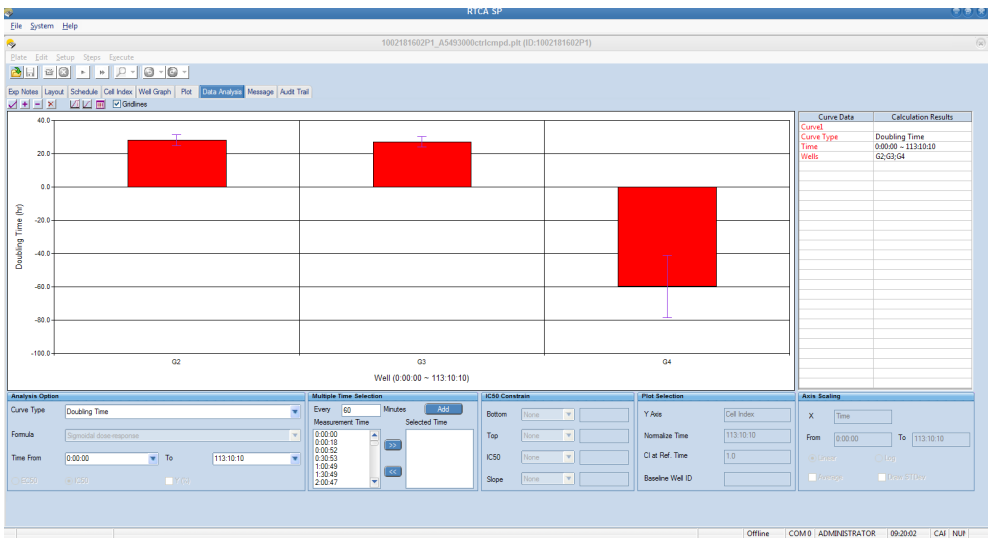
For each selected well, the Software calculates the Slope of the Cell Index (or Normalized Cell Index, or Delta Cell Index) curve in the chosen *Time* period, after fitting the points to a straight line. The slope values are shown in the bar chart. Please see the example illustrated in the screenshot below:



► *Doubling Time:*

The ***Doubling Time*** is used to describe the period of time required for a curve Cell Index value to double or to be half ("*half*" only if the slope of the curve is negative within the selected time period). The *Doubling Time* parameter may be used for cell proliferation and other cell-based assays. For example, if one is interested in how the Cell Index changes during the log-growth phase of cells in E-Plate, the Doubling-Time can be used for such analysis.

For each selected well, the Software calculates the doubling-time for the Cell Index (or Normalized Cell Index) in the chosen time period, by fitting the curve to an exponential equation. The *doubling time* is the time required for the Cell Index (or Normalized Cell Index) to double, based on the curve-fit. If the doubling time is negative, it means that Cell Index (or Normalized Cell Index) decreases with time. In this case, the doubling time is the time required for Cell Index (or Normalized Cell Index) to halve, based on the curve-fit. The calculated Cell Index Doubling Time values are shown in the bar chart. Please see the example illustrated in the screenshot below:

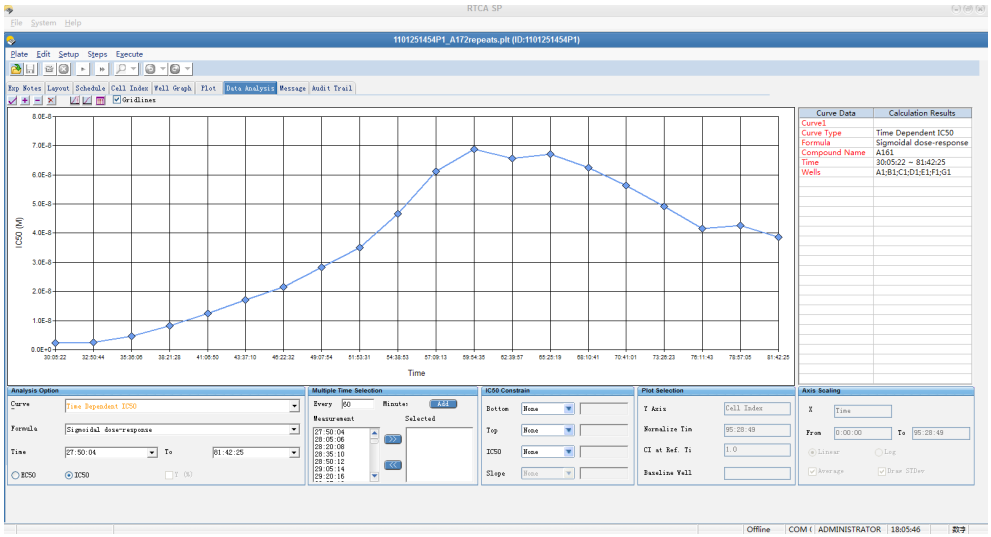


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► ***Time Dependent IC_{50}:***

The **Time Dependent IC_{50}** describes how the IC_{50} changes within a given time period following a compound treatment. While end-point assays can only provide a single IC_{50} value, the calculation of the **Time dependent IC_{50}** is one of the important features of xCELLigence RTCA Instrument, allowing the user to determine and derive the kinetic dependency parameter for compound effectiveness and potency. The **Time-dependent IC_{50}** is used in cell-based assays, when compound treatment has been performed on the cells in multiple wells and at different concentrations. Applicable cell-based assays include compound-induced cytotoxicity, compound-effected cell adhesion, receptor-activation and many other compound-added assays.

To calculate the Time-dependent IC_{50}, the Software automatically extracts 20 time points within the chosen time period, calculates the IC_{50}/EC_{50} values at each of these points, and then draws a curve showing the time-dependence of IC_{50} or EC_{50} values (X-axis: time point; Y-axis: IC_{50}/EC_{50} values). If there are fewer than 20 time points in the chosen period, then IC_{50} values for all the points will be calculated. Please see the example in the screenshot illustrated below:



► ***Dose-Response Curve (DRC):***

The **Dose Response Curve** is derived from Cell Index values of a group of wells (at least 3), which have the same compound treatment, but differ in concentrations. It reflects the dependency of the **Cell Index Responses** on the compound concentrations.

There are various methods for deriving the DRC, although the X-axis of the DRC is always the compound concentration. The xCELLigence RTCA Instrument carries out kinetic measurements, and the derived Cell Index or Normalized Cell Index parameters are always time-dependent. Thus, the most straightforward method in deriving DRC is the choice of *Cell Index at a time point after compound treatment* as the Y-axis parameter. This is the most widely used option for calculating the DRC when analyzing RTCA data.

There are however additional ways to analyze RTCA data to produce DRC curves based on other methods, such as **Max Cell Index** or **Minimum Cell Index** or **Difference Cell Index** or **Area Under the Curve**, within a given time period. Which analysis curve method to use is a function associated with the goals of the assays being performed and the preference of the user. For example, if the compound treatment results in cell morphological changes in a concentration-dependent and time-dependent manner, the user may be interested in the maximum alteration of cell morphology within a time period of compound treatment. In such a situation, one can choose *Max Cell Index* or *Minimum Cell Index* as the Y-axis parameter for DRC curves, depending on whether compound-induced cell morphology change corresponds to an increase or decrease in Cell Index. In other assays, the user may be interested in the overall effect of the compound treatment for a given time period. Under such

cases, *Area-under-the-Curve* can be chosen for analyzing the RTCA data.

While different types of DRCs can be used for analyzing data from any cell-based assays where compound treatment has been performed, the exact choice for the appropriate type of DRCs will depend on the assay type, assay condition and most importantly, what the user is interested in achieving in such assays. The following table summarizes the commonly used DRC curve types for different cell-based assays.

The curve types listed below are not intended to provide all possibilities but rather to provide examples for how one could choose a specific DRC curve type for the data analysis of a given assay type.

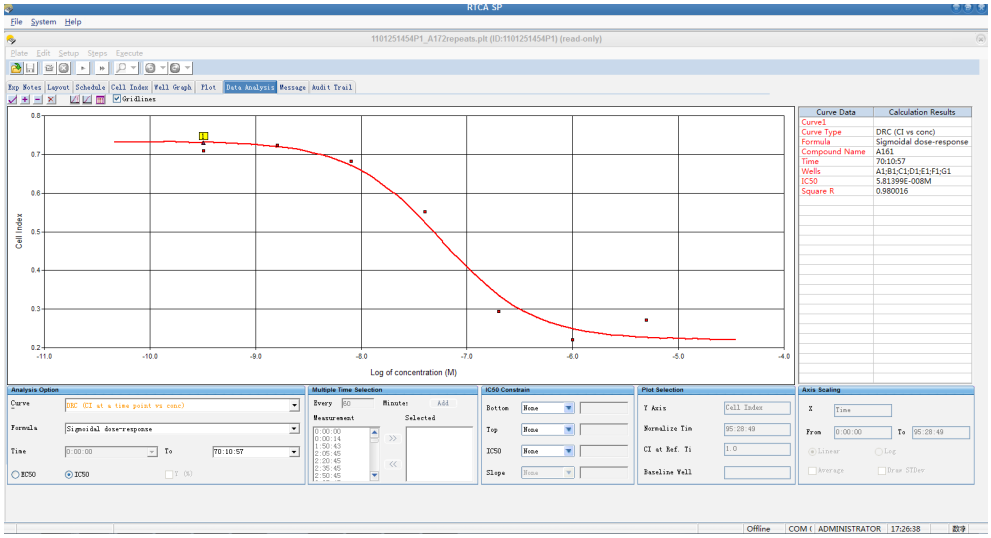
Assay Type	Commonly used DRC type
Cytotoxicity	DRC (CI at a time point vs conc), DRC (area-under-curve in a time period vs. conc)
Receptor Activation	Max (peak) Cell Index (Normalized CI, or Delta CI) within a selected time period Min (bottom) Cell Index (Normalized CI, or Delta CI) within a selected time period Difference (Max – Min) Cell Index (Normalized CI, or Delta CI) within a selected time period
Cell Adhesion	DRC (CI at a time point vs. conc)

A DRC X-axis is always the compound concentration (from low to high); its Y-axis could be any value at a selected time point or period of:

- Cell Index (Normalized CI, or Delta CI) at a selected time point
- Max (peak) Cell Index (Normalized CI, or Delta CI) within a selected time period
- Min (bottom) Cell Index (Normalized CI, or Delta CI) within a selected time period
- Difference (Max – Min) Cell Index (Normalized CI, or Delta CI) within a selected time period
- AUC (area-under-curve) of Cell Index (Normalized CI, or Delta CI) within a selected time period

- ***DRC (CI at a time point vs conc):*** Shows dose response curve [X-axis: compound concentration; Y-axis: Cell Index or Normalized Cell Index (same Y-axis as on Plot Page)] for all selected wells, and then uses the chosen formula to perform curve-fitting and calculate IC_{50}/EC_{50} values at the chosen time point.

B



- ***DRC (max CI in a time period vs conc):*** Shows dose-response curve (X-axis: compound concentration; Y-axis: maximum of Cell Index or Normalized Cell Index in the chosen time period) for all selected wells, and then uses the chosen formula to perform curve-fitting and calculate IC_{50}/EC_{50} values for that DRC.
- ***DRC (min CI in a time period vs conc):*** Shows dose-response curve (X-axis: compound concentration; Y-axis: minimum of Cell Index or Normalized Cell Index in the chosen time period) for all selected wells, and then uses the chosen formula to perform curve-fitting and calculate IC_{50}/EC_{50} values for that DRC.
- ***DRC ((max - min) CI in a time period vs conc):*** Shows dose-response curve [X-axis: compound concentration; Y-axis: (maximum minus minimum) of Cell Index or Normalized Cell Index in the chosen time period) for all selected wells, and then uses the chosen formula to perform curve-fitting and calculate IC_{50}/EC_{50} values for that DRC.
- ***DRC (area-under-curve in a time period vs conc):*** Shows dose-response curve (X-axis: compound concentration; Y-axis: area-under-curve for Cell Index or Normalized Cell Index in the chosen time period) for all selected wells, and then uses the chosen formula to perform curve-fitting and calculate IC_{50}/EC_{50} values for that DRC.

10.2.4.2. Curve Fit Formula

There are two formulas available for calculating IC_{50}/EC_{50} values:

1. **Sigmoidal dose-response**
   $Y = Bottom + (Top - Bottom)/(1 + 10\text{^}(Log\ EC_{50} - X))$

2. **Sigmoidal dose-response** (Variable slope)
   $Y = Bottom + (Top - Bottom)/(1 + 10\text{^}((Log\ EC_{50} - X)*HillSlope))$

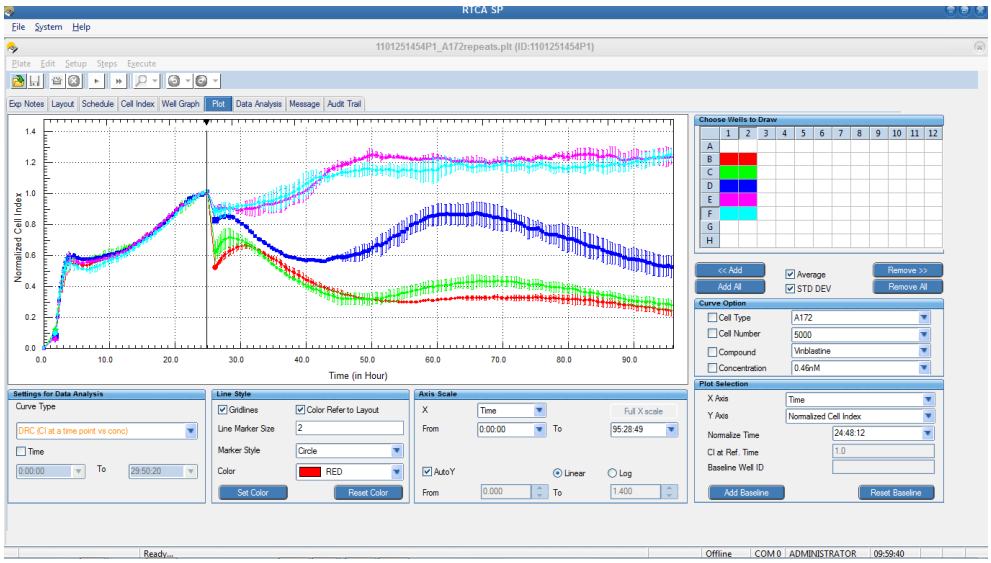
The above formulas use the EC_{50} value for the calculation. For an IC_{50} calculation, the same formulas can be used, except that IC_{50} is used in place of EC_{50}. Depending on specific applications, a user can choose to use either EC_{50} or IC_{50} in formulas by clicking one of the two radio buttons at the lower left of the *Data Analysis* page. If EC_{50} is selected, then EC_{50} will be used in the formulas, and the calculated results will be labeled EC_{50} (for example, the sixth curve type would be called **Time Dependent EC_{50}**).

The choice of EC_{50} or IC_{50} simply affects how the results are labeled and shown, but does not affect how the results are calculated.

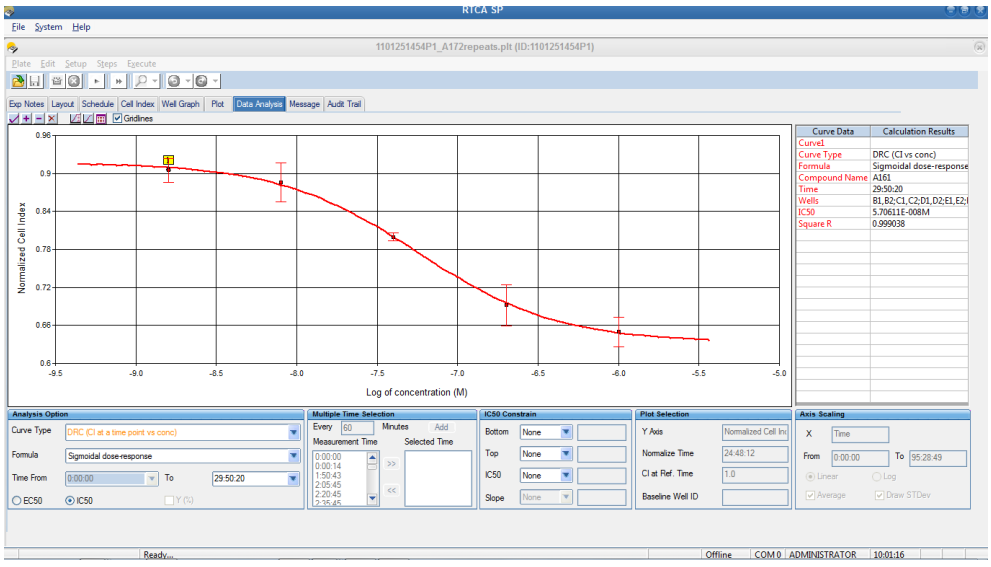
10.2.5 Data Analysis on Averaged Data for Replicate Wells

The *Data Analysis* page can process *averaged Cell Index* or *averaged Normalized Cell Index* for replicate wells. On the *Plot* page, select the wells that have the same *cell type* and the same *Compound Name* (as listed on the *Layout* page). In addition, some of the selected wells must have exactly the same compound *Concentration*. Put a checkmark in the *STD DEV* box and click the *Average* button. A graph showing *averaged Cell Index* curves (or *averaged Normalized Cell Index* curves) with standard deviations will be displayed on the *Plot* page.

In the example below, the Software has calculated and plotted the data, including average and standard deviation for each time point, for the corresponding wells in column 2 and 3 (wells B1 to F1, B2 to F2). The Cell Index in normalized at the time point before compound is added (24:28:12).



Switch to the *Data Analysis* page and then select *DRC (CI at a time point vs conc)* from the *Curve Type* box. The software will plot a dose-response curve with standard deviation for each concentration point.



Please don't use Standard Deviation function for the calculation of "Dose response curve (area-under-curve in a time period vs conc)".

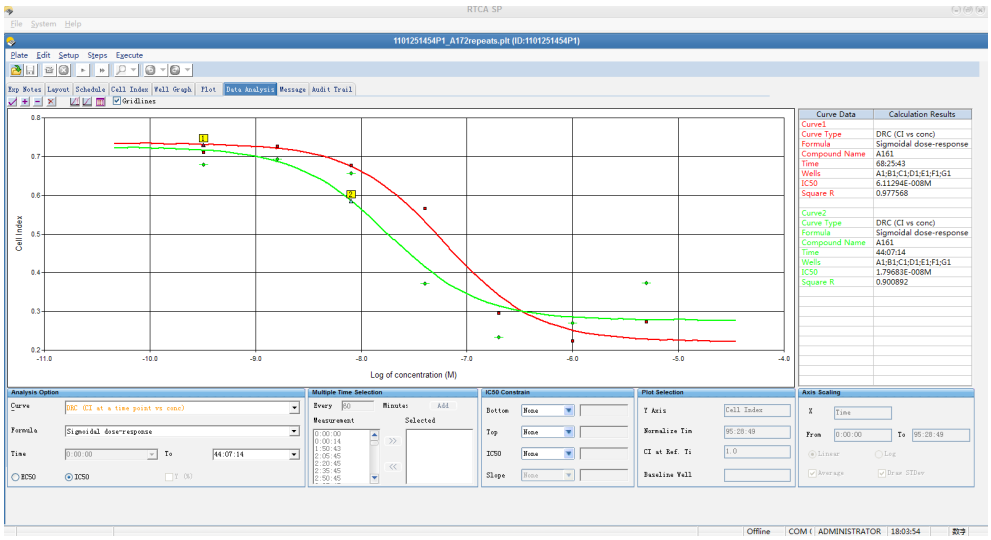
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10.2.6 Compare Dose-Response Curves and Corresponding EC_{50}/IC_{50} Values for Selected Wells at Different Time Points

To compare DRC and corresponding EC_{50}/IC_{50} values at different time points:

1. Select wells on the *Plot* page.
2. Go to the *Data Analysis* page. Choose *DRC (CI at a time point vs conc)* from the *Curve Type* box. Then select a *Time* point. Click to draw the first curve.
3. Without altering the *well selection* or *Curve Type*, select another *Time* point. Click to add another curve.
4. If needed, Repeat step 3) to add more curves for comparison.

The IC_{50}/EC_{50} data is shown in the table on the right side of the *Data Analysis* Page. Please see the example in the screenshot illustrated below:

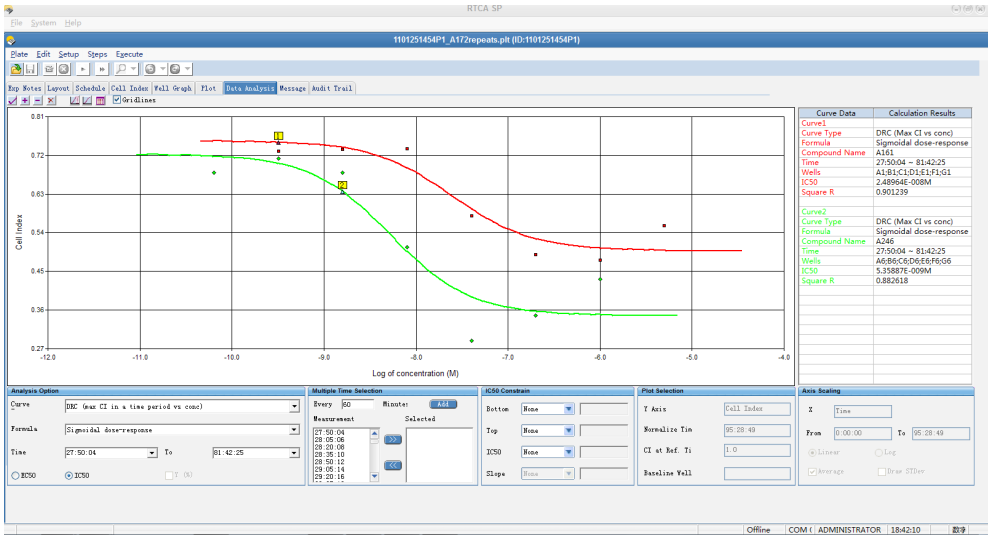


10.2.7 Compare Dose-Response Curves and Corresponding EC_{50}/IC_{50} Values for Different Wells at the Same Time Point

To compare DRC and corresponding EC_{50}/IC_{50} values for different wells at the same time point:

1. Select the first group of wells on the *Plot* page.
2. On the *Data Analysis* page, choose *DRC at a time point* as the *Curve Type* and then select a *Time* point. Click to draw the first curve.
3. Select a second group of wells on the *Plot* page.
4. On the *Data Analysis* page, without altering the *well selection* or *Time* point, click to add another curve.
5. If needed, repeat steps 3) and 4) to add more curves for comparison.

The IC_{50}/EC_{50} data are shown in the table on the right side of the Page. Please see the example illustrated in the screenshot below:

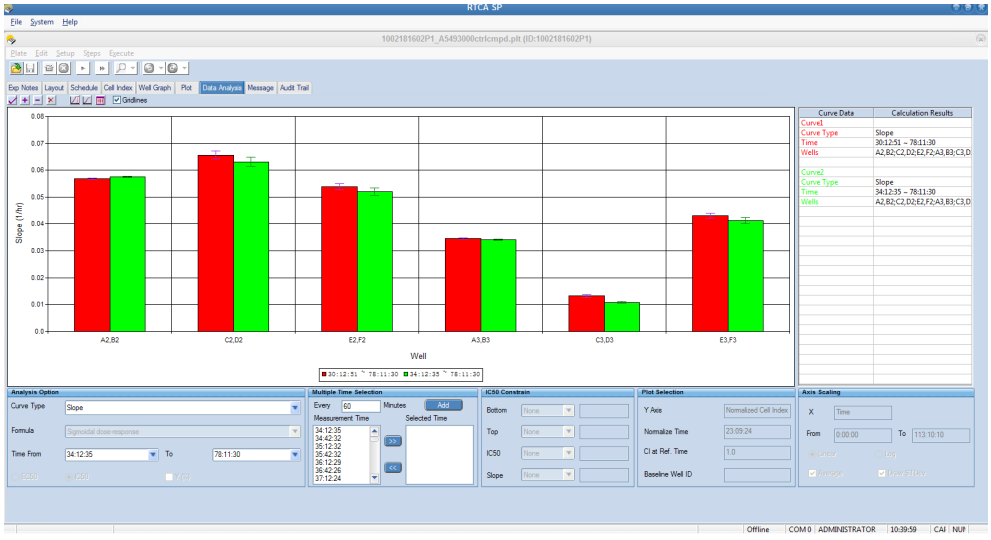


10.2.8 Compare Cell Index Slopes (or Cell Index Doubling Times)

To compare *Cell Index Slopes* or *Cell Index Doubling Times* for the same group of wells in different time periods:

1. Select a group of wells on the *Plot* page.
2. On the *Data Analysis* page, choose *Slope* (or *Doubling Time*) as the *Curve Type* and then select the first set of *Time* points (beginning time and end time). Click to draw a bar chart that shows the slope.
3. On the *Data Analysis* page, without changing the Curve Type, select a second set of *Time* points (beginning time and end time). Click to add a new slope to the existing graph.
4. If needed, repeat step 3) to add more time-periods for comparison of slopes or doubling times.

See the following example in the screenshot below that shows a comparison of slopes:



11. Message Page

The *Message* page provides information that occurs before, during, and after the experiment. The recorded information includes the start and stop time of each step, the time the scan plate was performed at, the time the experiment was paused/resumed at, etc.

Additionally, the *Message* page lists errors that occurred during an experiment and were detected by the xCELLigence RTCA Instrument, along with error codes. Some error codes are related to the use of the instrument; for example, removal of the E-Plate from the SP/MP Station or DP Instrument in the middle of a sweep-measurement would result in an error. Users can fix these instrument-use errors. Other errors may relate to hardware issues. If such error codes persist, please contact your local ACEA representative.

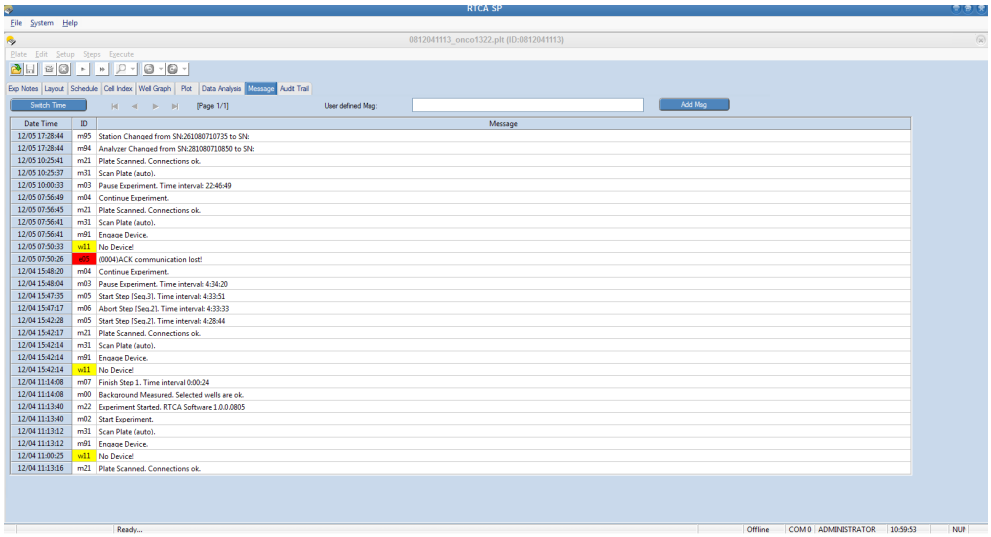
All messages are listed in the message table. Use ⏮ ◀ ▶ ⏭ *to navigate to a different message page, when there is more than one page of messages.*

Each message contains three parts:

- ***Message Time:*** When the message was recorded
- ***Message ID:*** The unique ID to identify the message

  *All messages are divided into three categories, and start with a character "**m**" (ordinary message), "**w**" (warning), or "**e**" (error).*
- ***Message Details:*** Delivers detailed information on event

Warning message IDs are highlighted in *yellow*. **Error message IDs** are highlighted *red*. The following screenshot shows a potential *Message* page appearing at the end of an experiment:



11.1 Warning Message Sign

During an experiment, if a warning was recorded, a warning sign ⚠ is shown on the left corner of the Status Bar. Clicking on this ⚠ sign, or clicking the *Message* tab will show the *Message* page with the warning, and the warning ⚠ sign will disappear.

If you have wells with connection problems, please consult the xCELLigence RTCA Instrument Operator's Manual to troubleshoot these problems.

Another warning message appears when the experiment is started, and there are wells with background problems. This message lists the wells which have background problems and helps the user in planning the layout for the experiment. If you have problem wells, please check the connection of the E-Plate and SP/MP Station or DP Instrument and follow recommendations for cleaning in the xCELLigence RTCA Instrument Operator's Manual. If problems persist, contact your local ACEA representative. Please also send appropriate data files (please refer to the xCELLigence RTCA Instrument Operator's Manual).

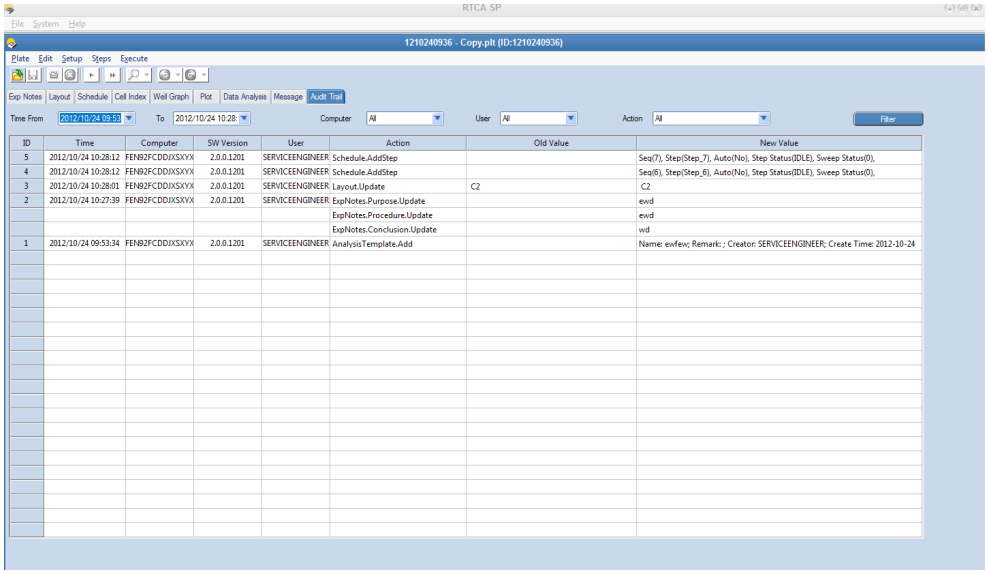
11.2 Error Message Sign

During an experiment, if there was an error recorded, an error sign  is shown on the left corner of the Status Bar. Clicking on this  sign, or clicking on the Message tab will show the Message page, and the error  sign will disappear.

Please refer Troubleshooting section in xCELLigence RTCA Instrument Operator's Manual for details to solve the problems if an error is observed.

12. Audit Trail Page

The *Audit Trail* page provides information about change history of experiment file after the experiment started, like update layout information, modify experiment notes, add a step, add or delete an analysis template, etc. Each record is one action that user did. Every action user did that would modify the experiment file will be recorded. All records are listed in time order from new to old.



Each record contains eight parts:

- ID: The unique ID of each record.
- Time: When did the user do the action.
- Computer: The name of the computer used for the experiment.
- SW Version: The software version that user used to do the action.
- User: The user that did the action.
- Action: The description of the action performed by the user.

  For example, ExpNotes. Purpose. Update means update the purpose of Exp Notes page.
- Old Value: The old value of the field before the action.

  Please note that not all actions have "Old Value", like Schedule.AddStep.
- New Value: The new value of the field after the action.

  Please note that not all actions have "New Value", like Schedule.DeleteStep

13. Experiment Reports

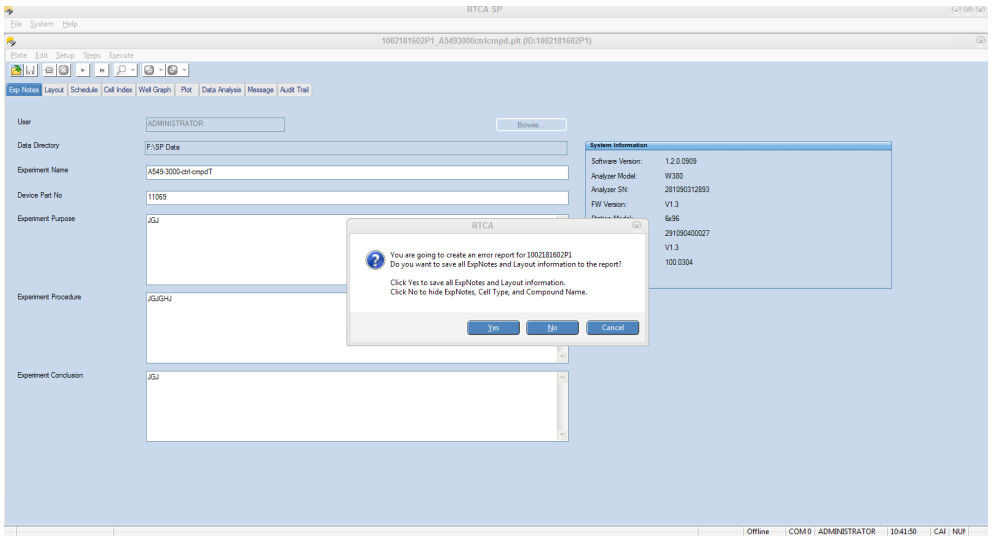
13.1 Create a Problem Report

Whenever users want to contact ACEA representative for support (*e.g.*, to solve recurring issues during an experiment), they must send a *Problem Report* file to allow precise assessment of the issue. To generate a *Problem Report* file, do the following steps:

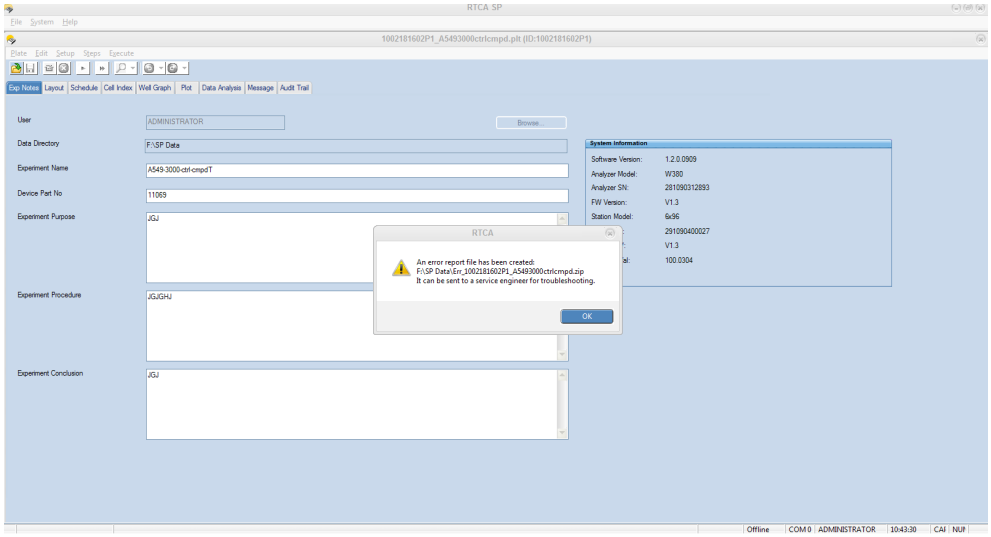
- Launch the RTCA Software.
- Open the experiment file on which you want to report any issues.
- Open the *Plate* Menu and select *Create Problem Report*.
- A dialogue window pops up asking whether it is OK to save all the *Exp Notes* and *Layout* information to the problem report file. Click the *Yes* if there is no confidential information in the experiment. Click *No* to hide all the *Cell Type* and *Compound* information. Click *Cancel* if you do not want to create the report.
- After you select either *Yes* or *No*, the RTCA Software will create a Problem Report file with the file name Err_******.zip.

*The created problem report file (Err_******.zip) is located in the same folder of its original *.plt data file.*

Example: The dialogue window asks the user whether all *Exp Notes* and *Layout* information can be sent to Customer Support.



Example: The *Message* window shows that the *Problem Report* file has been successfully generated.



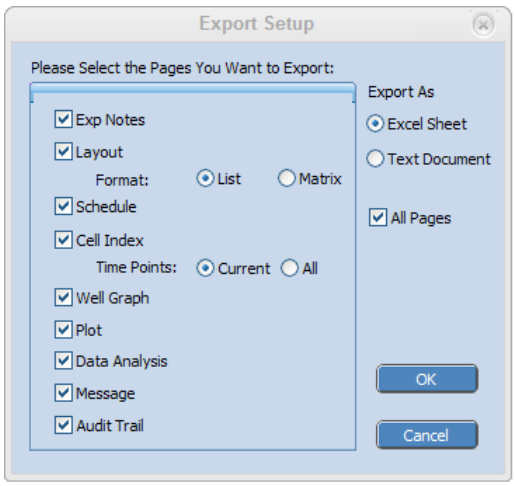
13.2 Create an Excel Format Report

RTCA Software 2.1.0 offers a convenient way to export individual pages or all the pages to an Excel file. To do so, follow the steps below:

- Select *Export Experiment Info* from *Plate* menu
- Select the Page(s) you want to export (the default is export all pages)
- Choose Format for *Layout* page
- Choose *Time* points for *Cell Index* page

> *If you choose All time points and the total time points are more than 1000, only the first 1000 time points data will be exported.*

- Choose to *Export As* either *Excel Sheet* or *Text Document*.
- Click *Ok* to export the selected experimental data to an Excel file.



14. RTCA Software Functions Unique To The xCELLigence RTCA MP Instrument

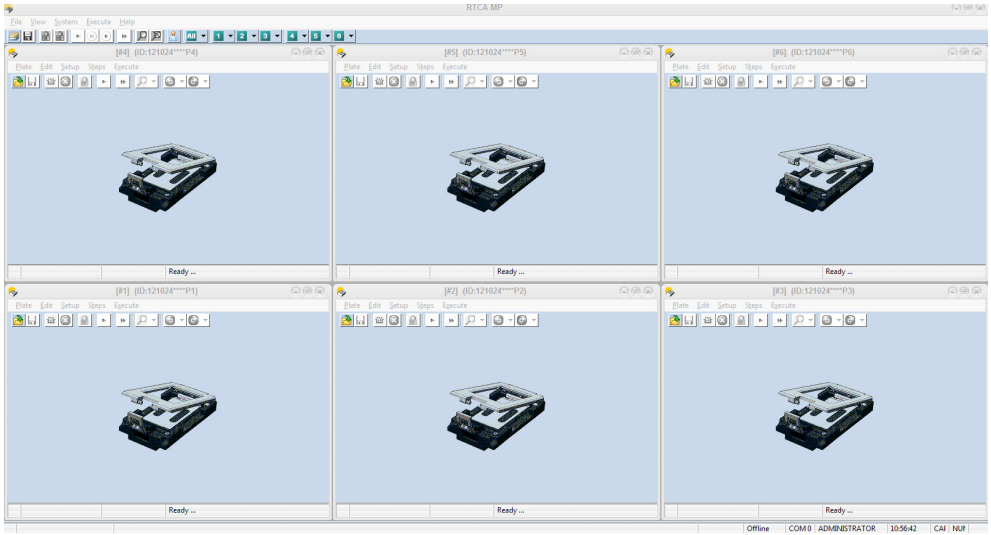
This section is for users of the xCELLigence RTCA MP Instrument only.

It describes the user graphic interfaces for the xCELLigence RTCA MP Instrument and the major differences between RTCA SP and xCELLigence RTCA MP Instruments, followed by a description of functions and operations unique to xCELLigence RTCA MP Instrument. Finally, it gives a detailed example of multiple users running experiments on the xCELLigence RTCA MP Instrument.

B

14.1 MP User Graphic Interface

The MP user graphic interface consists of a Main (parent) Window and six Plate (children) Windows (P1-P6).



Both the Main Window and the six Plate Windows are resizable.

When the Main Window is resized, the six Plate Windows are resized accordingly.

Individual Plate Windows can be resized without affecting.

B

14.2 The Major Differences of RTCA SP and xCELLigence RTCA MP Instruments

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.*

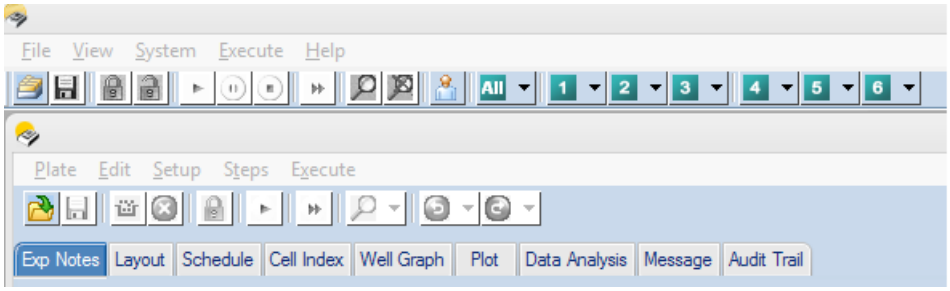
The MP Main Window contains the common functions that apply to all six Plate Windows, such as *Start All, Pause All, Continue All, Abort All and Scan All*, etc. Since the xCELLigence RTCA MP Instrument has only one E-Plate 96, those functions for ***All*** plates do not apply to the SP Instrument, and are therefore not shown in the SP main window; in addition, there is no toolbar on the SP main window.

14.3 Overview of Functions and Controls Unique to the xCELLigence RTCA MP Instrument

The xCELLigence RTCA MP Instrument can simultaneously run up to six E-Plate 96 experiments for up to six different users.

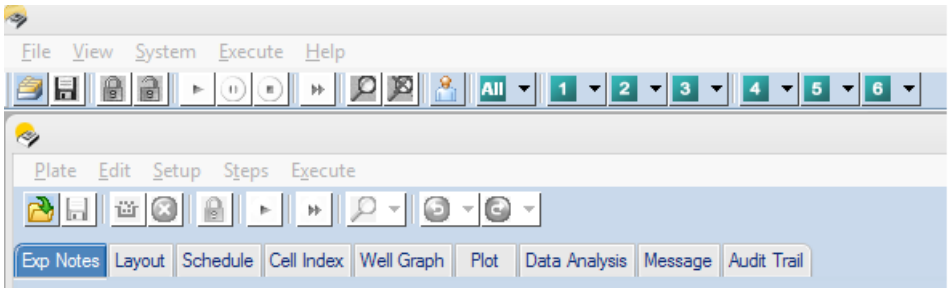
Actions taken on each Plate Window are applied to that individual Plate Window only.

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.



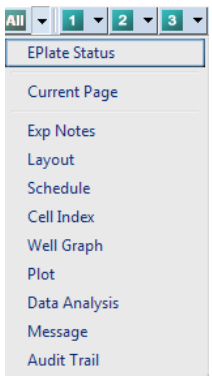
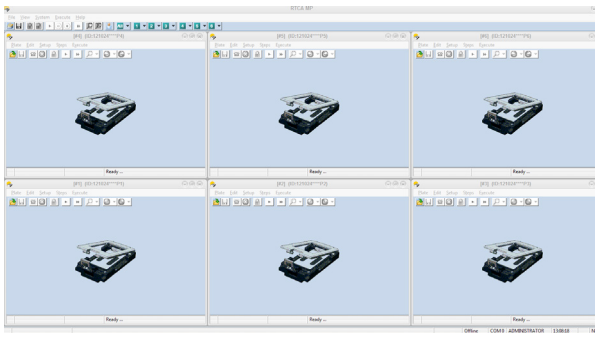
"Valid Plates" means:

1) The E-Plate 96 is owned by the login user.

2) The E-Plate 96 experiment is ready to be started.

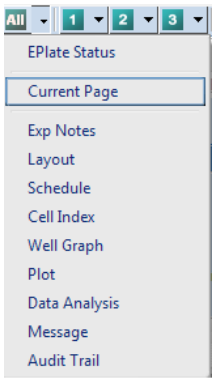
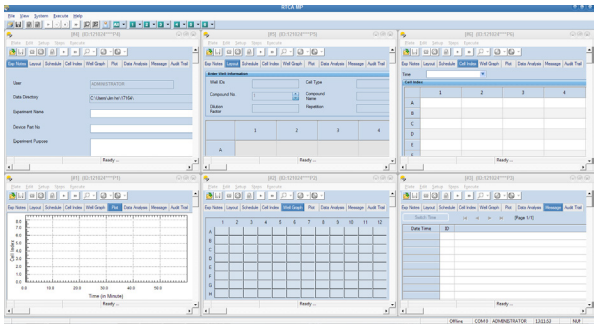
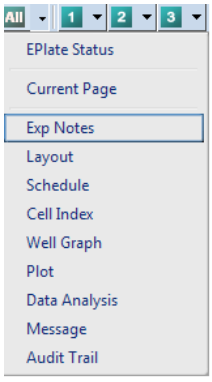
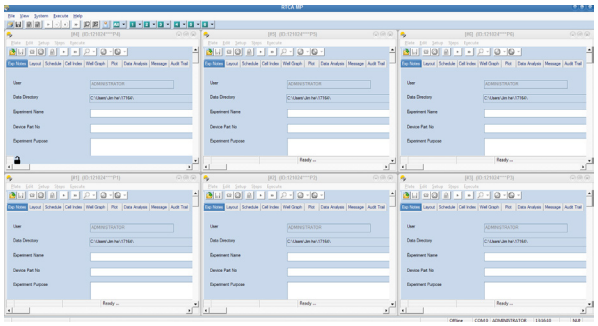
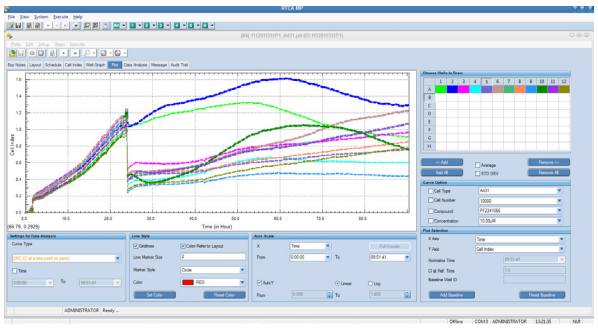
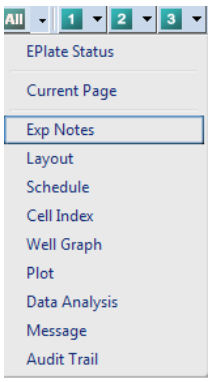
The Table below describes all the functions and controls unique to the xCELLigence RTCA

MP Instrument.

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.
	**Unlock** cradle(s)	A plate window ignores this, if it is already unlocked.
	**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 fi t 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)

►►►

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Controls	Function / Example	Comment
	**Resize** plate window(s) owned by the login user within the main window, and keep current page displayed in plate window(s). 	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.
	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 Plot 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.
	**Maximize** a plate window and show the assigned page in that window. For example, the following action (click Exp Notes under ) will maximize Plate 1 window, and show its Exp Notes page.	There are 6 numbered drop-down menus on the main window toolbar. Each controls its corresponding plate window

14.4 Launch RTCA MP Software

Double-click the RTCA Software 2.1.0 icon 

on the xCELLigence RTCA SP/MP/DP Control Unit. The RTCA Software will detect hardware connections and enter different operation modes, depending on the connections detected.

It may take a few minutes for RTCA Software to initialize the xCELLigence RTCA MP Instrument and display.

If no xCELLigence RTCA MP Instrument is connected to the xCELLigence RTCA SP/MP/DP Control Unit, or the connected xCELLigence RTCA MP Instrument is turned off, the RTCA Software may show a dialog window that allows the user to enter the Offline mode. Alternatively, if the xCELLigence RTCA MP Instrument connected to the xCELLigence RTCA SP/MP/DP Control Unit is turned on, the RTCA Software will enter the Real-Time mode automatically.

14.4.1 RTCA Software in MP Offline Mode

When the RTCA Software enters the MP offline mode, all hardware-related controls, such as *Lock / Unlock*, Scan Plate, and the 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.

Please check Section 4 of this Software Manual for details on designing an experiment template.

14.4.2 RTCA Software in MP Real-Time Mode

When the RTCA Software enters the MP Real-Time mode, it automatically performs the Scan Plate function for all E-Plates 96 engaged in the cradles of the RTCA MP Sta-tion. This automatic Scan Plate may take up to 40 seconds, depending on the number of engaged E-Plates 96.

Please check Sections 5.1 and 5.2 of this Software Manual for details regarding the Scan Plate function.

Please do not work using the RTCA MP Software while it is initializing or performing the automatic Scan Plate.

After Scanning Plates, the RTCA Software is ready for users to start experiments in either of the following ways:

1) Start a new experiment in a Plate Window by:

- Setting up new *Exp Notes* (optional), *Layout*, and *Schedule* pages

or

2) Open an unfinished experiment in a Plate window, and continue it.

Please check Sections 2.4 to 2.6 of this Software Manual to learn how to set up the Exp Notes page.

Please check Section 3 of this Software Manual to learn how to set up Layout page.

Please check Section 4 of this Software Manual to learn how to set up the Schedule page, and for Cloning Individual pages or experiments from either (1) saved templates, or(2) existing experiments.

Please check Sections 4.6 of this Software Manual for details on the Cloning of Experimental Settings.

14.5 Example of Multiple Users Running Experiments on the same xCELLigence RTCA MP Instrument

Take for example the case in which three different users are simultaneously running experiments, with two E-Plates 96 each, on the same xCELLigence RTCA MP Instrument. 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:

User1, **User2**, and **Administrator** each prepare their two respective E-Plates 96 plates.

1. The wells of each E-Plate 96 are filled with cell culture media.
2. 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.

User1 starts the experiment with their E-Plates 96 on the P1 and P2 cradles.

3. User1 launches the RTCA Software to enter **MP Real-Time** mode
4. User1 Login to the RTCA Software with username **User1**
5. User1 maximizes the **P1** *Plate Window*.
6. User1 does setup for the *Exp Notes* Page, *Layout* Page, and *Schedule* Page.
   - *Please see Sections 2.4 to 2.6 of this Software Manual for setting up the Exp Notes page.*
   - *Please see Section 3 of this Software Manual for setting up Layout page.*
   - *Please see Section 4 of this Software Manual for setting up Schedule page.*
7. Click on the **P1** *Plate Window* to start an experiment in the E-Plate 96 on **cradle 1**.
   - *Cradle 1 will be locked automatically when the experiment starts.*
8. Repeat Step 5 to Step 7 for the **P2** *Plate Window* and E-Plate 96 in **cradle 2**.
9. Remove both the E-Plates 96 from **cradle 1** and **cradle 2**, and then add cells to the appropriate E-Plate Wells.
   - *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.*
10. 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.
    - *Cradles 1 and 2 will be locked automatically when Step 2 starts.*
11. User1 should now logout.

User2 now starts their experiment with E-Plates 96 using the P3 and P4 cradles.

12. User2 performs Login to the RTCA Software with username **User2**.
    - *User2 has no rights to the plate windows of User 1, and cannot access either the P1 or P2 Plate Windows.*

▶▶▶

13 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.

14 User2 logout.

P3 and P4 cradles are locked when User2 starts the experiments.

User1 comes back to perform **compound addition** to E-Plates at **P1** and **P2**

15 User1 performs login again.

16 User1 clicks *Abort All* from the Main Window to stop the current step for experiments on the P1 and P2 Plate Windows.

User1's action Abort All does not apply to User2's E-Plates 96 on cradles 3 and 4.

17 User1 clicks *Unlock All* from the Main Window. The **P1** and **P2** cradles are unlocked.

User1's action Unlock All does not apply to User2's E-Plates 96 on cradles 3 and 4.

18 User1 now removes the E-Plates 96 from cradle 1 and cradle 2, and *adds compounds* to the appropriate wells of E-Plates 96.

19 User1 replaces these E-Plates 96 to their correct cradle 1 and 2, respectively. The RTCA Software 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).

The RTCA Software auto-scans the E-Plates 96 in the order that they were re-inserted into the cradle pockets.

20 User1 starts **Step 3** by clicking on **Main Window** for the two E-Plates.

21 User1 performs logout.

Administrator performs login to the RTCA Software to start their experiment with **all** the E-Plates 96 on MP Station, including the two new plates for the **P5** and **P6** Plate Windows.

22 **Administrator** inserts two new E-Plates 96 into **P5** and **P6** cradles respectively.

23 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.

24 Administrator checks the data on the P1, P2 P3 and P4 Plate Windows by plotting the Cell Index curves for the selected wells on the *Plot* page of each Plate Window.

Administrator has the login rights to access all other Users' experiments, concurrently running on the xCELLigence RTCA MP Instrument.

25 Administrator performs logout.

26 Experiments for all **six** E-Plates on the MP Station continue and the RTCA Software is ready for either User1, User 2 or Administrator to login.

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.

B

15. RTCA Software Functions Unique To The xCELLigence RTCA DP Instrument

This section is for users of the xCELLigence RTCA DP Instrument only.

All functions below mentioned are both applicable for E-Plate 16 and CIM-Plate 16.

It describes the connection of xCELLigence RTCA DP Instrument, followed by a description of user graphic interfaces and all experiment patterns available when using the xCELLigence RTCA DP Instrument. Finally, it discusses the functions and operations that are unique to the xCELLigence RTCA DP Instrument.

15.1 xCELLigence RTCA DP Instrument Connection

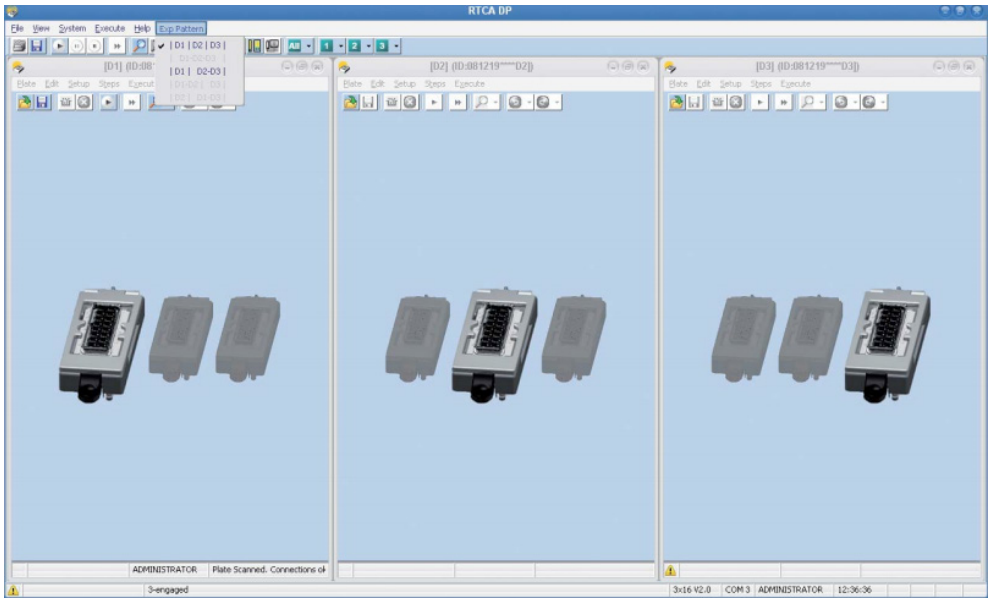
The xCELLigence RTCA DP Instrument uses a USB cable to connect to the computer. The USB cable carries out two functions:

- Communicates between the xCELLigence RTCA DP Instrument and xCELLigence RTCA SP/MP/DP Control Unit (with computer and software).
- Provides the power supply for the xCELLigence RTCA DP Instrument.

There is no extra power supply or power switch on the xCELLigence RTCA DP Instrument.

15.2 DP User Graphic Interface

The DP user graphic interface consists of a **Main Window** and three **Plate Windows**. It is similar to RTCA MP software, except that the number of windows is three, and each plate has 16 (not 96) wells.



Both the Main Window and the three Plate Windows are resizable.

When the Main Window is resized, the three Plate Windows are resized accordingly.

Individual Plate Windows can be resized without affecting other Plate Windows.

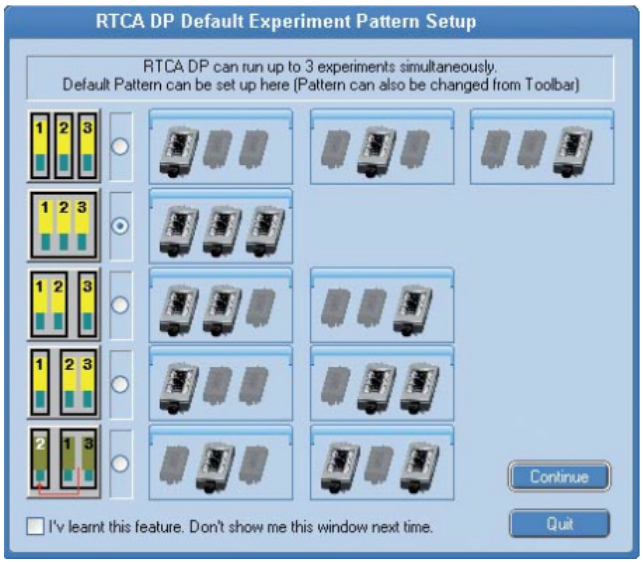
15.3 The Combination of Using Multiple E-Plates 16 or CIM-Plate 16 Within an Experiment

In terms of user interfaces and functions, each Plate Window within the **DP Main 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.

Although option 5) is available, it is highly recommended not use this experiment pattern. It can create confusion and may lead to a loss of experimental data, if the wrong cradle is inadvertently opened.



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.

*Please note that the **E-Plates 16** and **CIM-Plate 16** can* **only** *be used with the xCELLigence RTCA DP Instrument, and that the **E-Plates 96** can* **only** *be used with the xCELLigence RTCA SP and MP.*

B

15.4 Overview of Functions and Controls Unique to the xCELLigence RTCA DP Instrument

The xCELLigence RTCA DP Instrument 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).

*"**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 Instrument.

Controls	Function / Example
	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 CIM-Plate 16 #3
	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**

15.5 Launch RTCA DP Software

Double-click the RTCA Software 2.1.0 icon 

on the xCELLigence RTCA SP/MP/DP Control Unit. The RTCA Software will detect hardware connections and enter different operation modes, depending on the connections detected.

It may take a few minutes for the RTCA Software to initialize the xCELLigence RTCA DP Instrument and display.

If no xCELLigence RTCA DP Instrument is connected to the xCELLigence RTCA SP/MP/DP Control Unit, or the connected xCELLigence RTCA DP Instrument is turned off, the RTCA Software may show a dialog window that allows the user to enter the **Offline** mode. Alternatively, if the xCELLigence RTCA DP Instrument connected to the xCELLigence RTCA SP/MP/DP Control Unit is turned on, the RTCA Software will enter **Real-Time** mode automatically.

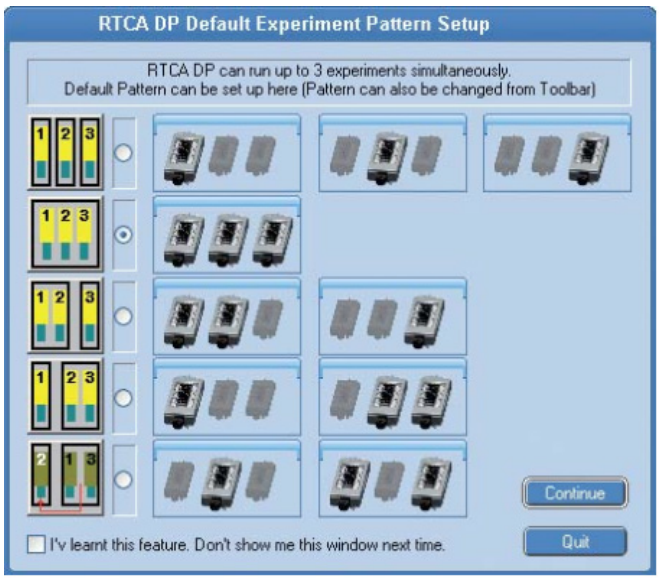
15.5.1 RTCA Software in DP Offline Mode

When RTCA Software 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.

Please check Section 4 of this Software Manual for details on designing an experiment template.

15.5.2 RTCA Software in DP Real-Time Mode

When RTCA Software starts, if an xCELLigence RTCA DP Instrument is detected, the software enters its **DP 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.



Select a desired pattern from the five available options, and then click *Continue* to enter the DP Software user interface.

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15.5.3 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 learned this feature. Don't show me this window next time."*
- Click *Continue*

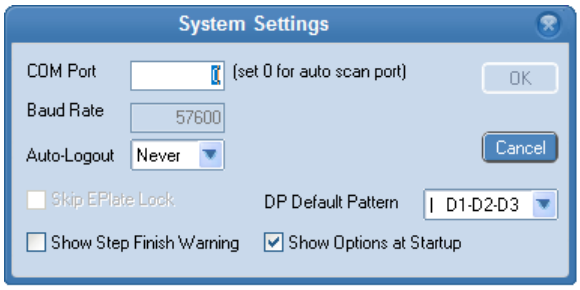
The default pattern is set and the above window will not appear when you launch the DP Software next time, and the software enters your chosen pattern directly.

The experiment pattern can also be changed by using the **toolbar** *buttons:*



If you want to set up another pattern as a default pattern when the DP Software starts, you can use the software system settings from the main window's menu:

- Choose: *System* → *Setup*



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.

15.5.4 RTCA DP Software Auto-Scan at Startup

When the RTCA Software enters the **DP 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 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.

Please check Sections 5.1 and 5.2 of this Software Manual for details of the ***Scan Plate*** *function.*

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 is ready for users to start experiments in either of the following ways:

1) Start a new experiment in a Plate Window by:

- Setting up new *Exp Notes* (optional), *Layout*, and *Schedule* pages

2) Open an unfinished experiment in a Plate window and continue it.

Please check Sections 2.4 to 2.6 of this Software Manual to learn how to set up Exp Notes page.

Please check Section 3 of this Software Manual to learn how to set up Layout page.

Please check Section 4 of this Software Manual to learn how to set up Schedule page.

Please check Section 4.6 of this Software Manual to learn how to Clone Experimental Settings, Individual pages or experiments from either (1) saved templates, or(2) existing experiments.

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15.6 Example of Multiple Users Running Experiments on the xCELLigence RTCA DP Instrument

Take for example the case in which three different users are simultaneously running experiments using the 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:

User1, **User2**, and **Administrator** prepare three separate E-Plates 16.

1. The wells of each E-Plate 16 are filled with cell culture media.
2. User1 inserts the prepared E-Plate 16 into Cradle #1.

User1 starts the experiment with their E-Plate 16 on Cradle #1.

3. User1 launches the RTCA Software to enter **DP Real-Time mode**.
4. User1 performs login to the RTCA Software with username **User1.**
5. User1 chooses from toolbar to select the **separated experiment pattern**, *i.e.,* it is possible for the DP Instrument to run **three** experiments simultaneously.
   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.
   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.
6. User1 performs setup for the *Exp Notes* Page, *Layout* Page, and *Schedule* Page
   Please see Sections 2.4 to 2.6 of this Software manual for setting up Exp Notes page.
   Please see Section 3 of this Software manual for setting up Layout page.
   Please see Section 4 of this Software manual for setting up Schedule page.
7. User1 clicks on the **D1 Window** to start an experiment in E-Plate 16 on **cradle 1**.
8. User1 removes the E-Plate 16 from **cradle 1**, then adds cells to the E-Plate 16 Wells.
   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.
9. User1 reinserts the E-Plate 16 with cells on **cradle 1**, and starts **Step 2** of the experiment by clicking on **Main Window**.
10. User1 should now logout.

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User2 now starts their experiment with E-Plates 16 on **cradle 2**.

11 User2 performs Login to the RTCA Software with username **User2**.

User2 cannot access the D1 Window that was used by User1, because only User1 has the rights to D1 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.

12 User2 works with **D2**, following the above described Steps 5 to Step 9 to start experiments with E-Plate 16 on only **cradle 2**.

13 User2 logout.

User1 comes back to perform **compound addition** to E-Plates 16 on **cradle 1**.

14 User1 performs login again.

15 User1 clicks *Abort All* from the **Main Window**. This aborts the current step for experiments on **D1 Window**.

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.*

16 User1 removes E-Plate 16 from **cradle 1**, and **adds compounds** to wells of E-Plate 16.

Since, in contrast to the xCELLigence RTCA MP Instrument, there is no automatic lock on the xCELLigence RTCA DP Instrument preventing other users from accidentally removing E-Plates 16, users sharing the xCELLigence RTCA DP Instrument should always make sure they are opening the correct cradle.

17 User1 reinsert the E-Plate 16 on **cradle 1**. The RTCA Software detects the presence of the E-Plate 16 on cradle 1, and starts a *Scan Plate* of E-Plate 16 on **cradle 1**.

When more than one E-Plate 16 is put into the instrument, the RTCA Software automaticaly scans the E-Plates 16 in the order they were re-inserted into the cradle pockets.

18 User1 starts **Step 3** by clicking on the **Main Window** for the E-Plates 16.

19 User1 performs logout.

Administrator performs login to the RTCA Software to work with **all** the E-Plates 16 on xCELLigence RTCA DP Instrument, because the Administrator has rights to all three cradles at all times.

20 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**.

21 Administrator works with the **D3 Window** and repeats the above described Step 5 to Step 9 to start the experiment for Plate#3.

22 Administrator evaluates the data produced by cells in **D1 Window** and **D2 Window** by plotting the *Cell Index* curves for selected wells on the *Plot* page of each Plate Window.

Administrator has at all times, the rights to access all the other users' experiments running on the xCELLigence RTCA DP Instrument.

23 Administrator performs logout.

▶▶▶

24 Experiments for all three E-Plates 16 on cradles 1, 2 and 3, of the xCELLigence RTCA DP Instrument continue running, and the RTCA Software is ready for User1, User2 or Administrator to login.

C Appendix

1. Ordering Information

ACEA Biosciences Service and Support

At ACEA, 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.aceabio.com

xCELLigence RTCA SP/MP Analyzer		Cat. No. 05228972001
SP Station		Cat. No. 05229057001
MP Station		Cat. No. 05331625001
xCELLigence RTCA SP/MP/DP Control Unit		Cat. No. 05454417001
RTCA Software Package		Cat. No. 05454433001
RTCA Protector Shield 96		Cat. No. 05288967001
RTCA Resistor Plate 96		Cat. No. 05232350001
RTCA Contact Pins 96 (20 units)		Cat. No. 05232384001
RTCA Frame 96		Cat. No. 05232392001
E-Plate 96	6 Units	Cat. No. 05232368001
	6 × 6 Units	Cat. No. 05232376001
xCELLigence RTCA DP Instrument		Cat. No. 05469759001
RTCA Contact Pins 16 (20 units)		Cat. No. 05471575001
RTCA Resistor Plate 16		Cat. No. 05469783001
E-Plate 16	6 Units	Cat. No. 05469830001
	6 × 6 Units	Cat. No. 05469813001
CIM-Plate 16	6 Units	Cat. No. 05665817001
	6 × 6 Units	Cat. No. 05665825001
CIM-Plate 16 Assembly Tool		Cat. No. 05665833001
E-Plate Insert 16	1 × 6 Units (6 16well inserts)	Cat. No. 06465382001
E-Plate Insert 96	1 × 6 Units (36 16well inserts)	Cat. No. 06465412001
E-Plate Insert 96 Accessories	1 × 6 Units (6 Receiver Plates & Lids)	Cat. No. 06465455001

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