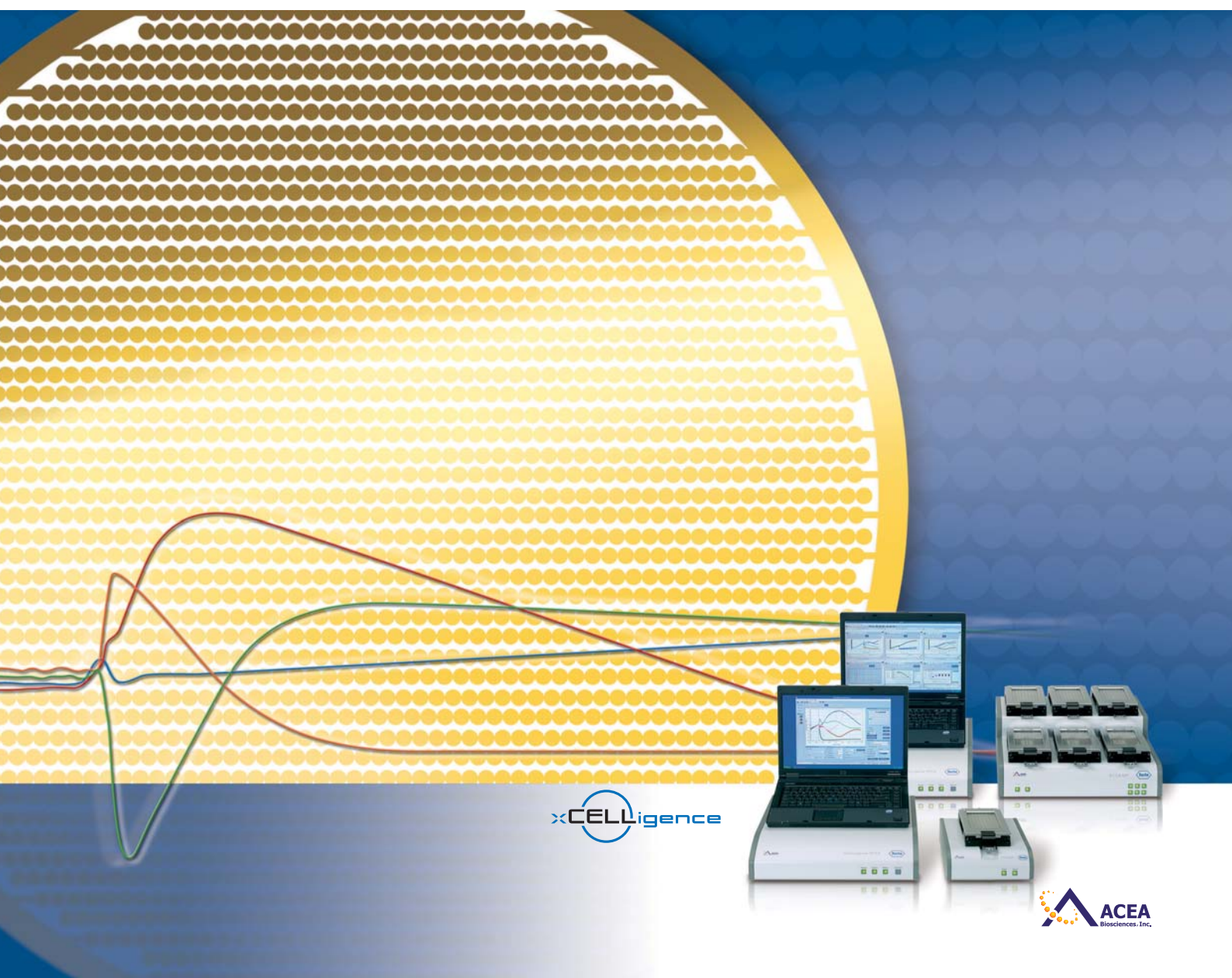


# RTCA Software Manual

Software Version 1.1

Version September 2008





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# Prologue

## I. Revision History

Manual Version	Revision Date
1.0	June 2008
2.0 (RTCA Software 1.1)	September 2008

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Customer Support  
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82372 Penzberg, Germany

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

However, Roche Diagnostics GmbH and ACEA Biosciences, Inc. reserve the right to make any changes necessary without notice as part of ongoing product development.

## II. Contact Addresses



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The RTCA (Real-Time Cell Analyzer) SP (Single Plate) and MP (Multi Plate) Instruments are 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 RTCA SP and MP Instruments 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 RTCA SP and MP Instruments are not intended for use in diagnostic procedures.**

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The parties agree that courts of Basel, Switzerland, 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 RTCA SP (or MP) Instrument Operator's Manual. Before setting up the RTCA SP (or MP) Instrument, users should read the RTCA Software Manual and the RTCA SP (or MP) 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 RTCA SP (or MP) Instrument. It contains the following chapters:

**Preface** 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 RTCA SP (or MP) Instrument runs and performing data analysis.

**Chapter C Appendix** contains Ordering Information.

## VIII. Conventions Used in this Manual



*In this manual referral to the term "RTCA Instrument" indicates applicability of the respective description to both RTCA SP Instrument and RTCA MP Instrument.*





### Text Conventions

To impart information that is consistent and memorable, 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	Points to a different chapter in this Software Manual which 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.

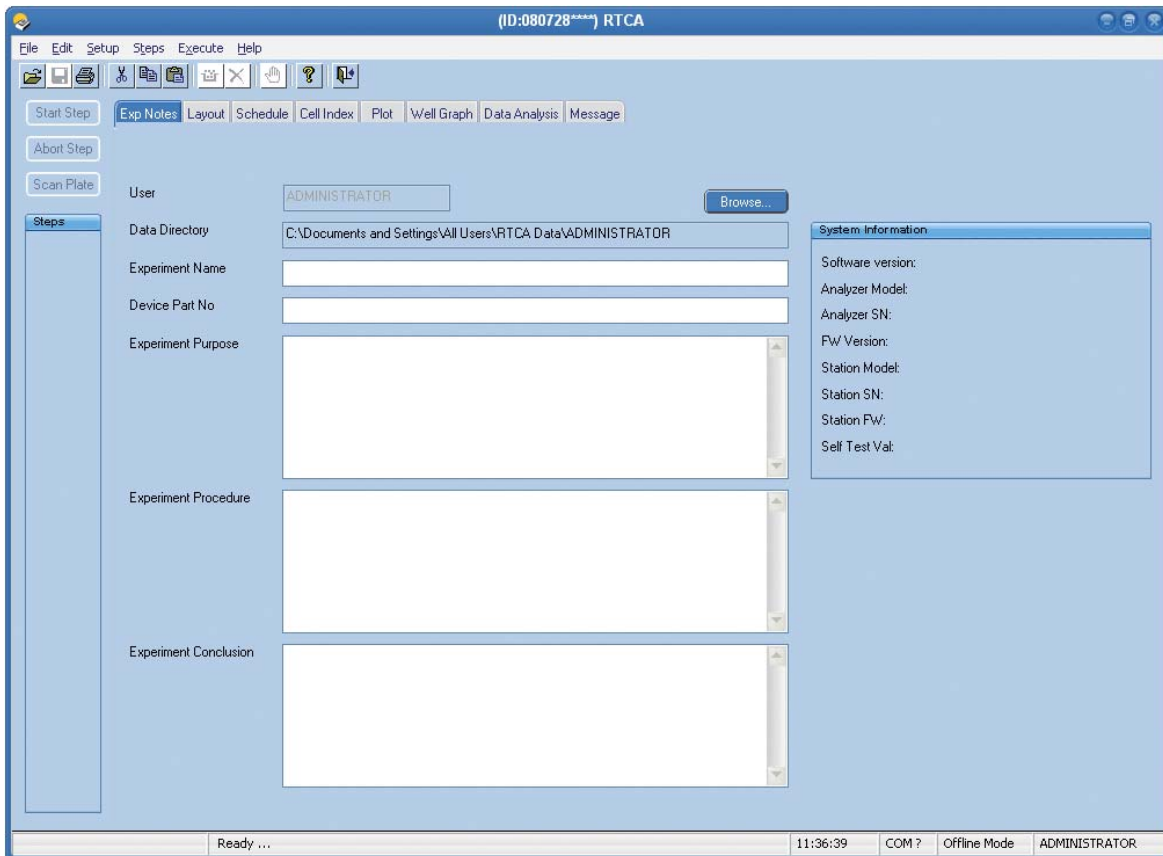
Symbol	Heading	Description
	IMPORTANT NOTE	Information critical to the success of the procedure or use of the product.
	INFORMATION NOTE	Additional information about the current topic or procedure.
		Table continued on next page.
		End of table.

## A About RTCA Software 1.1

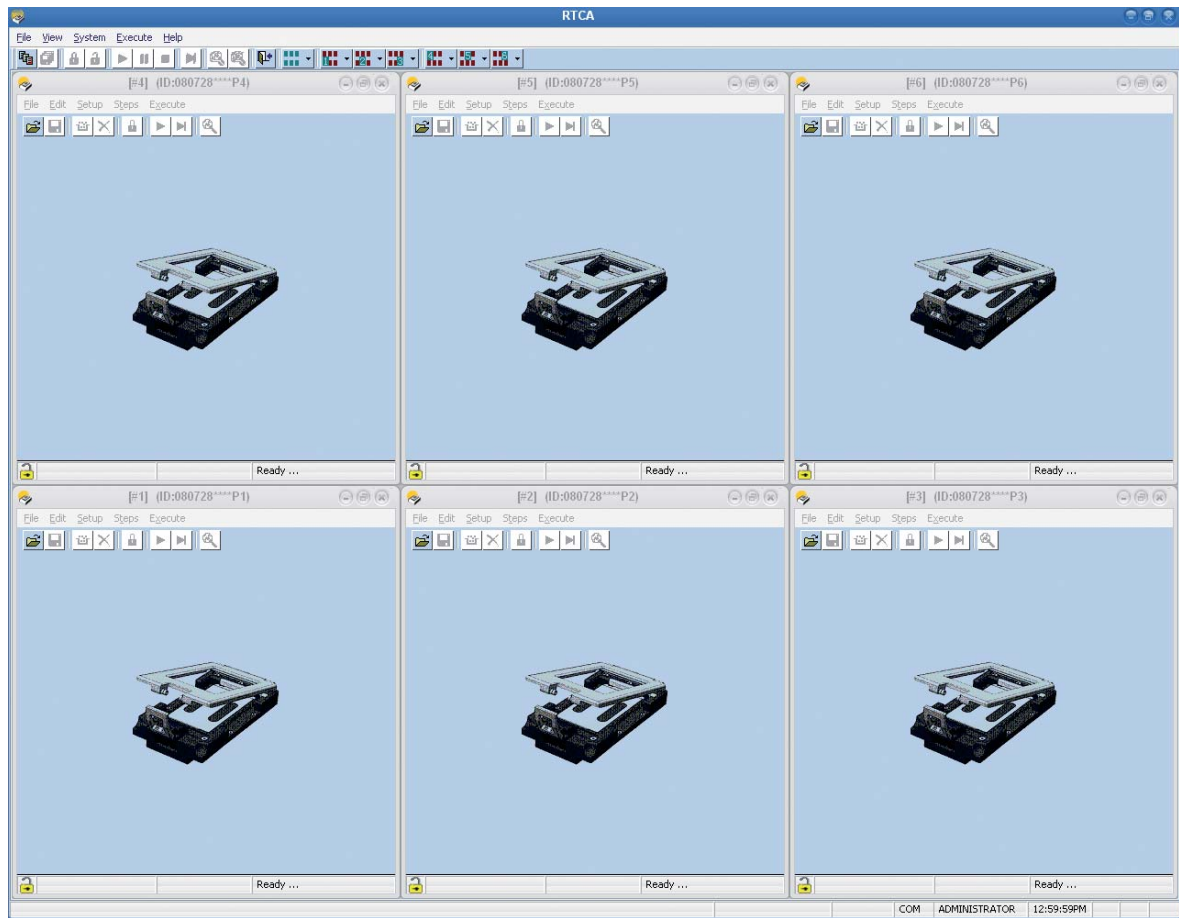
RTCA Software 1.1 is an integrated software for both the RTCA SP (Single Plate) Instrument (here and after refer as “SP”) and the RTCA MP (Multi Plate) Instrument (here and after refer as “MP”).

### 1. Entering SP or MP Real-Time Mode Automatically

When the RTCA Software starts, it will automatically detect the RTCA Instrument connected to the RTCA Control Unit. Based on the instrument configuration, the RTCA Software will launch either the RTCA SP or RTCA MP user interface.



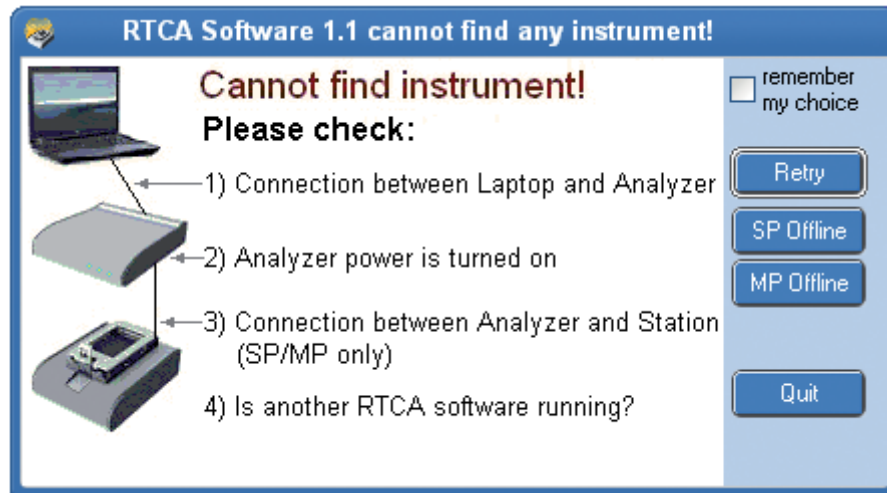
**RTCA SP User Interface**



RTCA MP User Interface

## 2. Entering SP or MP Offline Mode

When the RTCA Software starts, if no RTCA Instrument is connected to the RTCA Control Unit, or the connected RTCA Analyzer is not turned on, the RTCA Software will show a dialog window that allows user to choose the RTCA Software running mode:



At this window, user can click one of the four buttons:

- ▶ **Retry**      The RTCA Software will try to reconnect to RTCA Instrument.

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

- ▶ The serial communication cable between RTCA Control Unit and RTCA Analyzer is connected firmly;
  - ▶ The RTCA Analyzer is turned on;
  - ▶ A RTCA SP Station or RTCA MP Station is connected to the RTCA Analyzer;
  - ▶ No other RTCA Software is running.
- 
- ▶ **SP Offline**      The RTCA Software will enter SP offline mode.
  - ▶ **MP Offline**      The RTCA Software will enter MP offline mode.
  - ▶ **Quit**              The RTCA Software will close.



*If the “remember my choice” box is clicked and SP Offline or MP Offline is selected, the RTCA Software will enter SP or MP offline mode directly next time if no RTCA Instrument is detected.*



*The Offline preference does not affect real-time mode, i.e. even if “remember my choice” was selected, if the RTCA Software detects connected a RTCA SP or MP Instrument next time, it will enter RTCA SP or MP real-time mode, depending on which instrument has been detected.*



*The Offline preference settings can be cleared by using the command line parameter “x” (see below)*

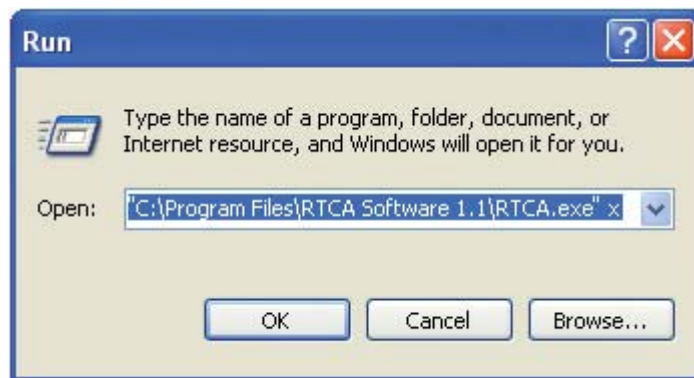
### 3. RTCA Software Command Line Parameters

RTCA Software supports the following command parameters:

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

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

(From Windows status bar) Start → Run, type “C:\Program Files\RTCA Software 1.1\RTCA.exe” x in the “Open” box, and click OK.



### 4. How to Use this Software Manual

The basic functions and operations of RTCA SP Instrument and RTCA MP Instrument are similar. In *Chapter B Software Functions*, the common functions and operations of RTCA Software are described in Sections 1 through 12, using the SP real time mode as an example. Then, the functions and operations exclusive to RTCA MP Instrument are covered in Section 13.

The RTCA SP Instrument user should focus on Sections 1 through 12, ignoring Section 13. The RTCA MP Instrument user can consult Section 13 first to become familiar with the RTCA Software MP user interface, then read Sections 1 through 12 for details on RTCA Software functions.



## B Software Function

In this chapter the main steps and functions of the RTCA Software are described.

Since RTCA MP Instrument uses 6 individual E-Plates 96 and the function and operation of each E-Plate is similar to those of RTCA SP Instrument, Sections 1 through 12 of this Chapter describe just the RTCA Software functions available in SP real-time mode. The functions and operations unique to RTCA MP Instrument are described in Section 13.

Sections 1 through 12 start with an overview of the RTCA Software, followed by the experiment set-up, data monitoring and data analysis functions.



### 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 instrument set-up and operation. The RTCA Software consists of 8 function pages:

Experiment Set-up pages:

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

Experiment Monitoring and Data Analysis pages:

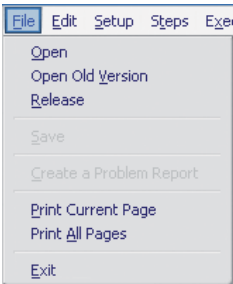

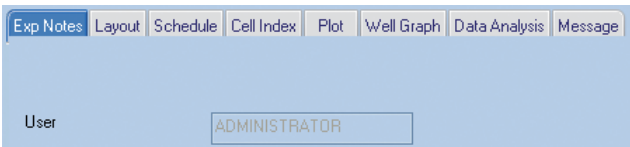

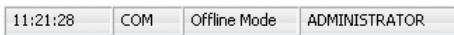
- ▶ *Cell Index* page
- ▶ *Plot* page
- ▶ *Well Graph* page
- ▶ *Data Analysis* page
- ▶ *Message* page







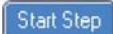
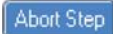

The first 3 pages contain functions required for running experiments. The latter 5 pages contain functions for monitoring and analyzing experiments. Features programmed into the RTCA Software provide the flexibility needed for dynamic measurement of cell status in the wells. Based on experiment requirements, 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 summarize 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 RTCA Instruments.

The user interface of the RTCA Software in the SP real-time mode contains (from top to bottom): Menu bar, tool bar, pages, and status bar:

Item	Example	Major Function
<b>Menu Bar</b>		User actions, system settings, software help functions
<b>Tool Bar</b>		Short-cuts of frequently used menu functions
<b>Pages</b>		Work area; user-defined information
<b>Experimental status bar</b>		Displays current experimental status
<b>Status Bar</b>		Displays Instrument status

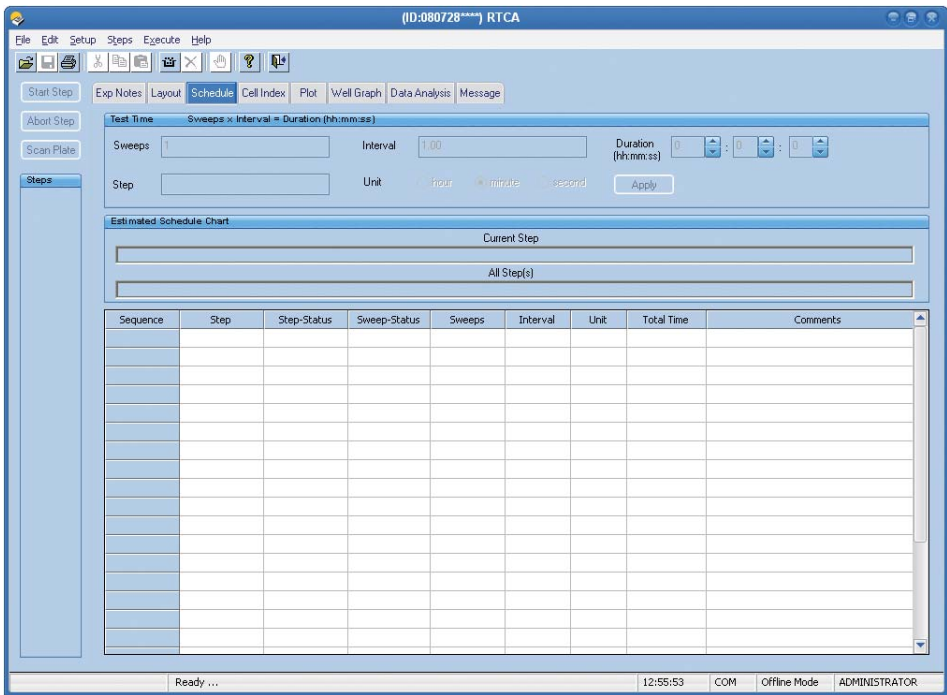
Icon	Description
	Add a step
	Delete a step
	Abort current running step
	Help function of the Software
	Logout
	Apply changes made in the <i>Layout</i> or <i>Schedule</i> page
	Start a step
	Abort current running step
	Scan Plate

### 1.1.1 RTCA Software Functions Required for Running Experiments

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

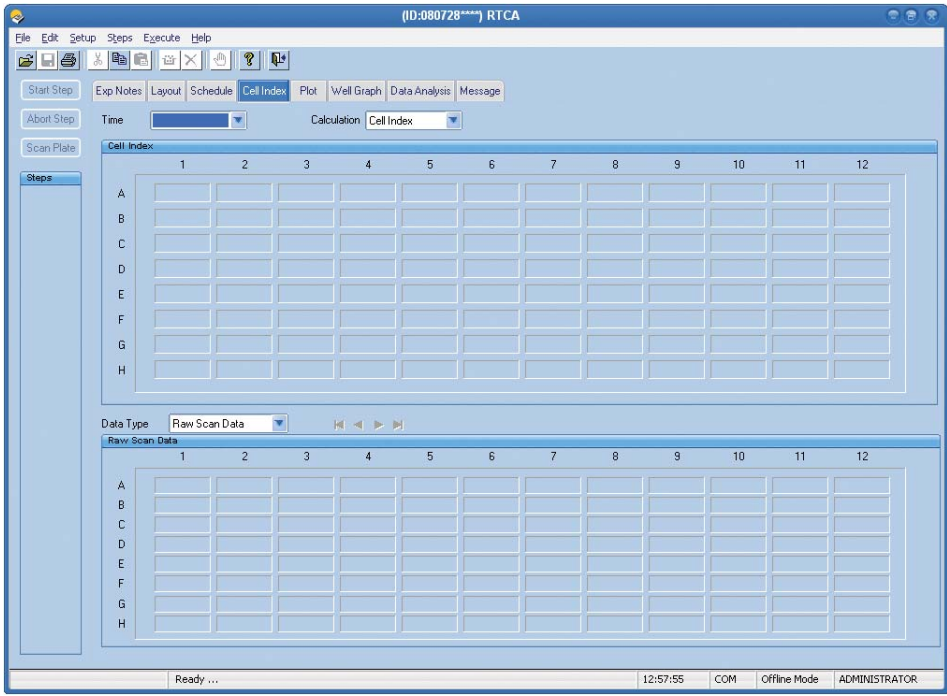
**Layout Page:**

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

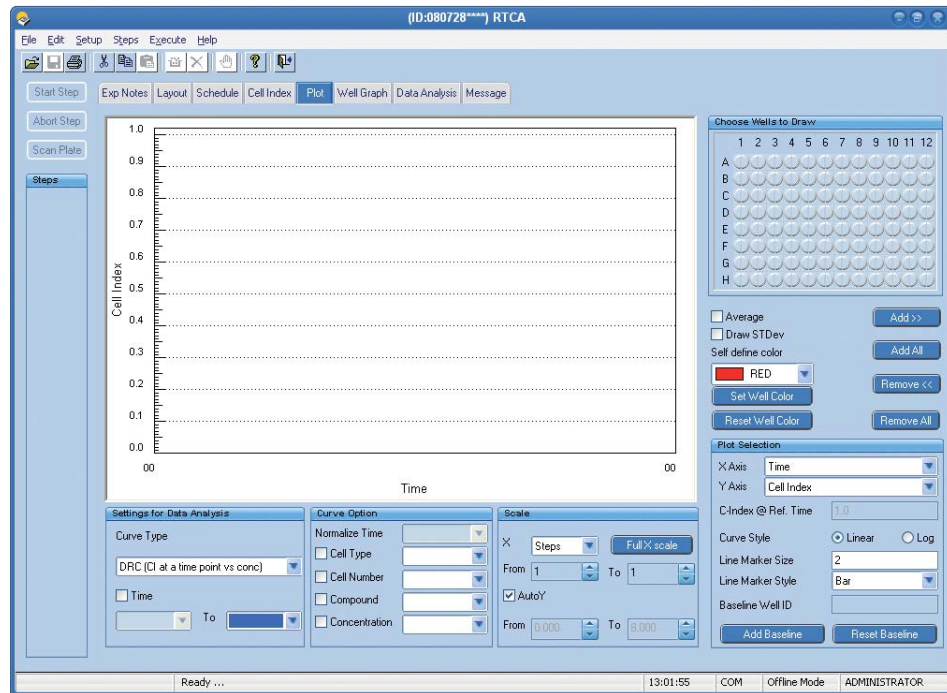


**Schedule Page:** Program experiment steps and frequency of real-time data acquisition

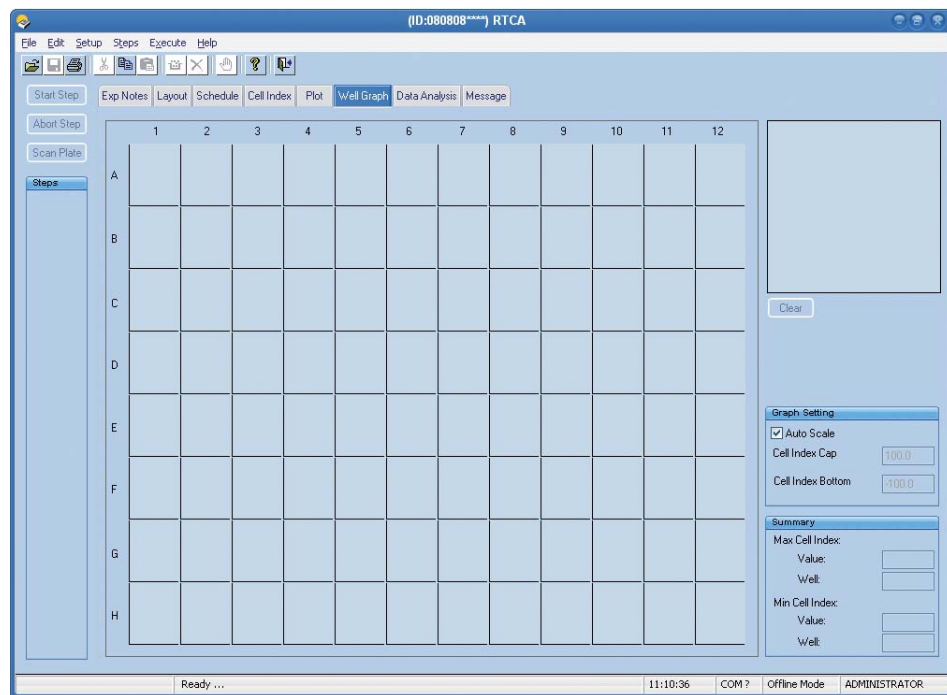
**1.1.2 RTCA Software Functions Required for Monitoring Experiments**



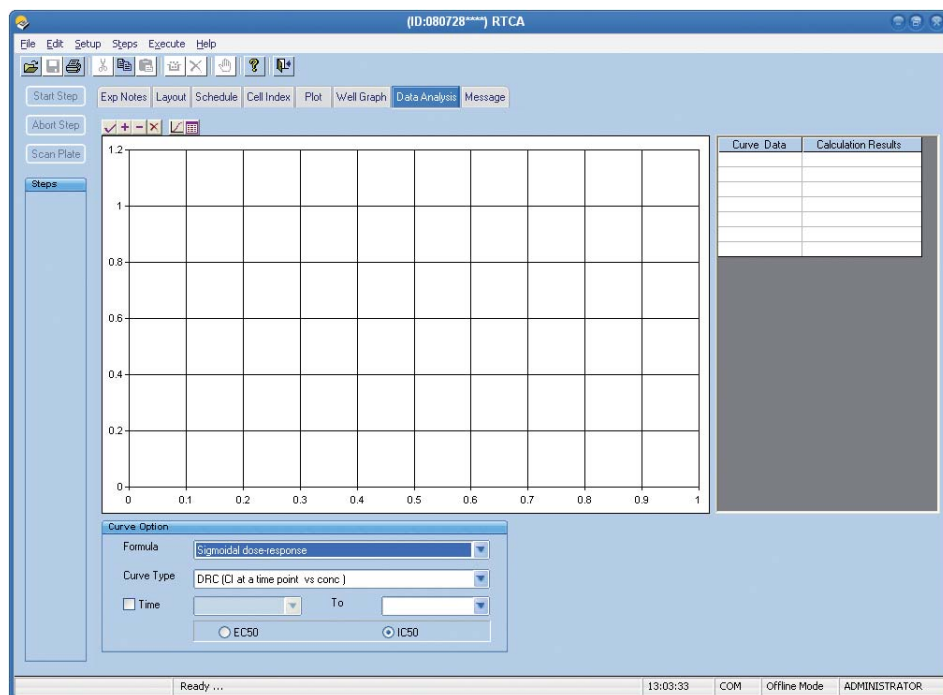
**Cell Index Page:** Real-time data acquisition and monitoring



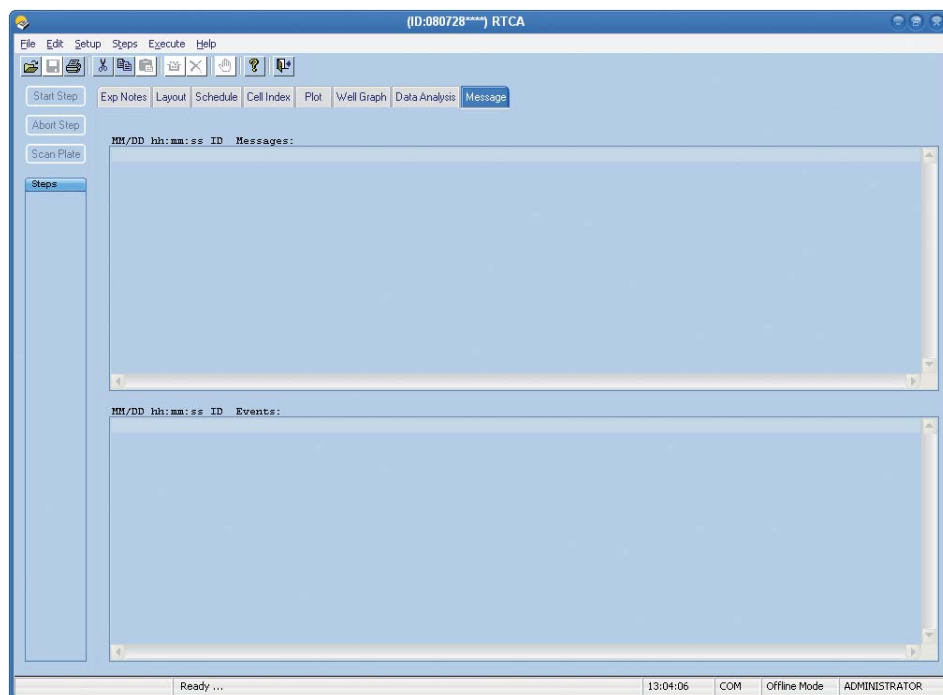
**Plot Page: Multiple data plot and data export functions**



**Well Graph Page: Showing all graphs of all well data in E-Plate format (8x12)**



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



**Message Page:**  
Record and report the quality of the experimental process, i.e., E-Plate 96/RTCA SP Instrument function



## 1.2 System Requirements and RTCA Software Installation

### 1.2.1 Requirements



Since the preinstalled RTCA Control Unit is delivered with the RTCA Instrument, the following Hardware and Software requirements are only necessary if you plan to install the RTCA Software on an additional computer.

#### Installation on additional computers:

##### Hardware (Computer):

- ▶ CPU speed  $\geq 1$  GHz
- ▶ RAM  $\geq 256$  MB
- ▶ Hard Drive free space  $\geq 100$  MB
- ▶ Monitor Resolution  $1024 \times 768$  or higher



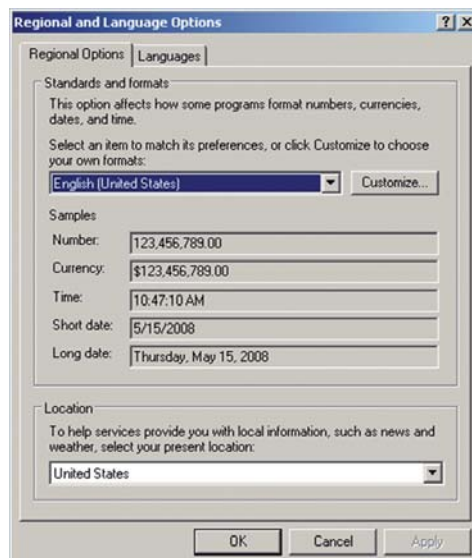
RTCA Software 1.1 in SP mode is a dialog-based program, therefore its window is not resizable. If a monitor is set to a higher resolution than  $1024 \times 768$ , the RTCA Software SP mode window will not fill the entire screen. For a better view, you should set the screen resolution to  $1024 \times 768$  when using the SP mode.

##### Software:

- ▶ Windows XP, Windows 2000
- ▶ Microsoft Office 2003 or newer (optional, for better data analysis performance)



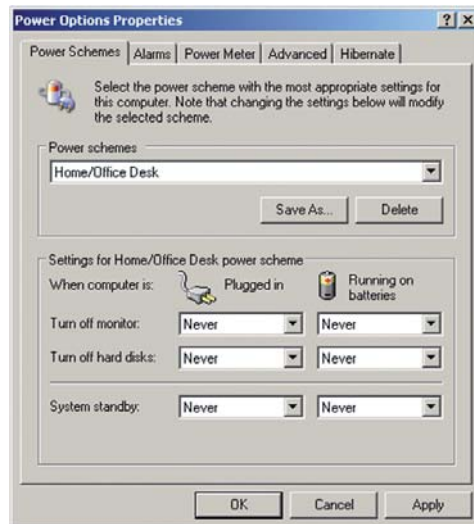
Regional and Language Options should be set to English (United States), Date/Time/Number should also be set to U.S. styles. Any other settings might lead to problems in data analysis and instability of the Software.



### Settings of the RTCA Control Unit:



Since the RTCA SP 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 XP Operating System

The RTCA Control Unit is delivered with the following Windows XP Operating System users preinstalled:

User Name	Default Password	Group	Description
RTCAOperator	No password	Power Users	Default user for instrument operation. This user is used if the RTCA Control Unit is started through auto-logon.
RTCAAdmin	penzberg	Administrators	Administrator is able to perform administrative operations such as: <ul style="list-style-type: none"> <li>► Change date and time settings</li> <li>► Perform software updates</li> </ul> This password should only be distributed to IT or lab administrators.



In normal operation no user name and password is required since the Windows XP Operating System is started with an **auto-logon**.

### How to Change Windows XP Users

- 1 Start RTCA Control Unit (Auto logon to RTCAOperator).
- 2 Click *Start* → *Shutdown*.
- 3 In the *Shut Down Windows* dialog select *Log off RTCAOperator* and click *OK*.
- 4 Press and hold the *SHIFT* key during the log off procedure. After a few seconds the *Log On to Windows* dialog window will open. If you do not hold the *SHIFT* key, the RTCAOperator will automatically be logged on again.
- 5 In the *Log On to Windows* dialog enter user name and password of the user you want to log on as.

### 1.2.2 Software Installation



*The RTCA Software is already preinstalled on the RTCA Control Unit.*



*If you upgrade from RTCA Software 1.0 to RTCA Software 1.1, installation of RTCA Software 1.1 will automatically lead to uninstallation of RTCA Software 1.0.*

#### 1. Quick Installation (for general users)

The RTCA Software CD is an auto-run installation CD. For “Quick Installation” (recommended), do the following:

- 1 Insert the RTCA Software CD into the computer CD drive. The auto-run set-up program will start automatically. If not, double-click on the *Setup.exe* file in the RTCA Software CD window to launch it manually.
- 2 Click *I accept the terms of the license agreement* when asked. Please read the license agreement thoroughly.
- 3 Click *Next* when the button becomes available (*i.e.*, is not grayed out).
- 4 Repeat 3) until the installation is complete.

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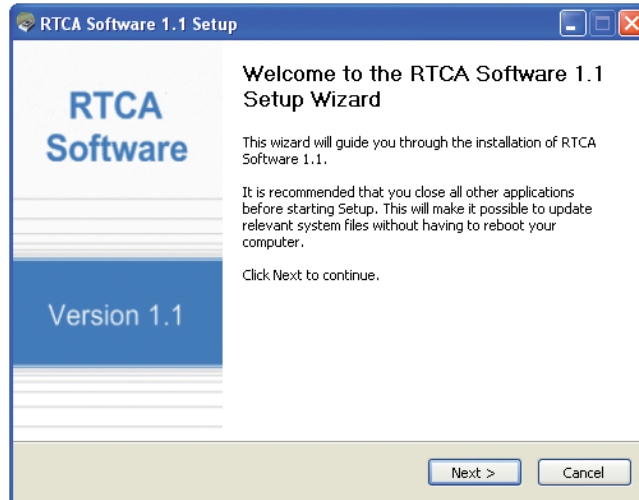
#### 2. Customized Installation (for advanced users)

If a user wants to install the RTCA Software to an assigned directory, installation can be customized as follows:

- 1 Insert the RTCA Software CD into the computer's CD drive.  
The auto-run setup program will start automatically. If not, double-click on the *Setup.exe* file in the RTCA Software CD window to launch it manually.  
Select the installer language on the menu that appears:

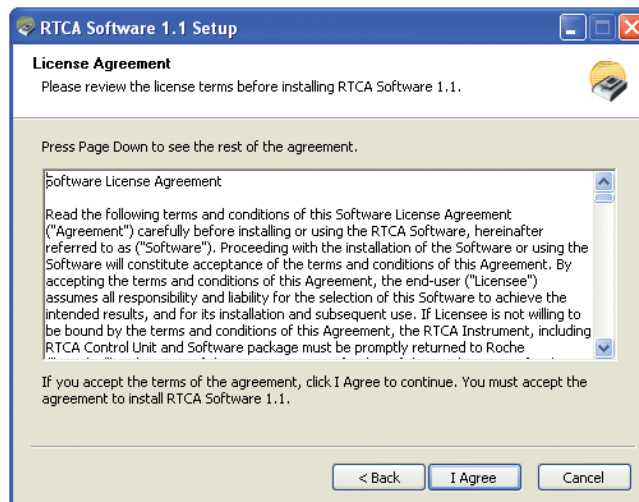


- 2 Start the RTCA Software Setup wizard.  
This page will appear automatically after step 1:



Click on *Next*; go to next page.


- 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.



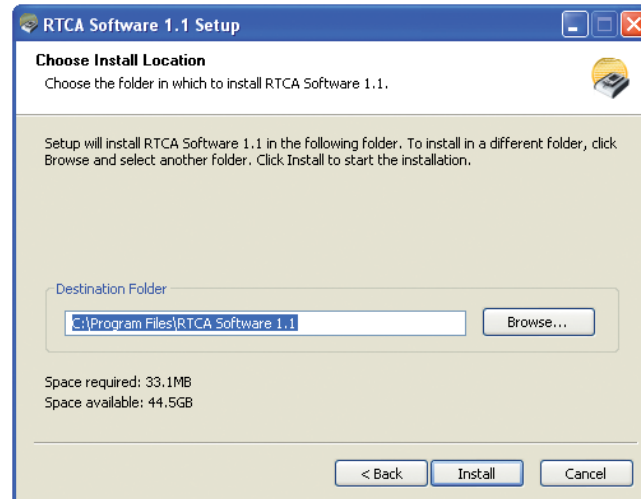
**4** Select Installation Folder.

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\RTCA Software 1.1*

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 step 6.
- 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*.

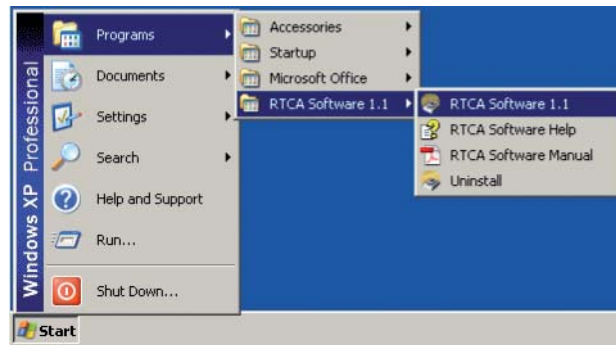


Two short-cuts for launching RTCA Software1.1 are now available:

► Computer Desktop



► Computer Program Menu



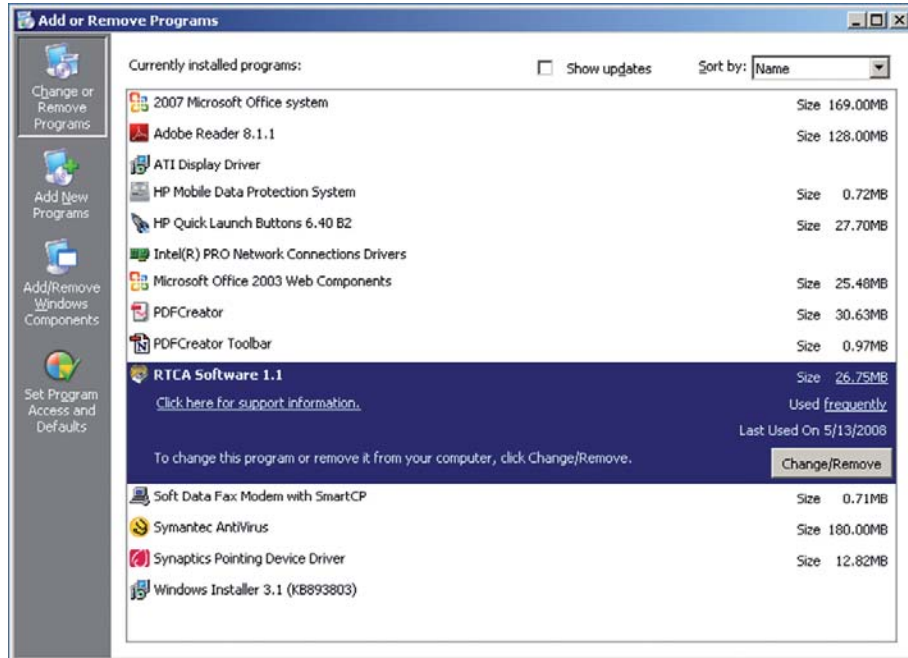


### 1.2.3 Software Uninstallation

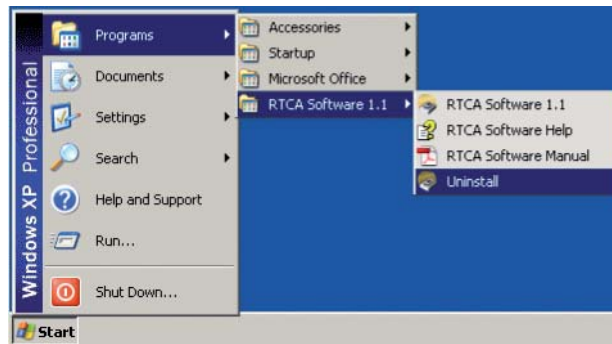
There are two ways to uninstall RTCA Software from the computer:

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

*Start* → *Settings* → *Control Panel* → *Add or Remove Programs*, then highlight *RTCA Software 1.1*, click *Remove*.



- Alternatively, use the Computer Program Menu:



### 1.3 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.
- ▶ Enter current password in *Old Password* box.
- ▶ Enter new password in *New Password* box.
- ▶ 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.
- ▶ When asked *Are you sure to delete?* click *Yes* to delete, click *No* to cancel.




Only the Administrator can delete users.

## 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 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 the designated port number is available, the RTCA Software will try to communicate with the RTCA Analyzer and obtain information about the RTCA Instrument. 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. Then the Software will enter *Normal* mode and start the Auto-Scan monitor.
- ▶ If the designated port number is not available, or the set port is available, but the Software fails to communicate with the RTCA Analyzer, the Software will provide the user four options: (1) select *Retry* to try to reconnect to the RTCA Instrument, (2) select *SP Offline* mode, (3) select *MP Offline* mode, and (4) *Quit* the RTCA Software.

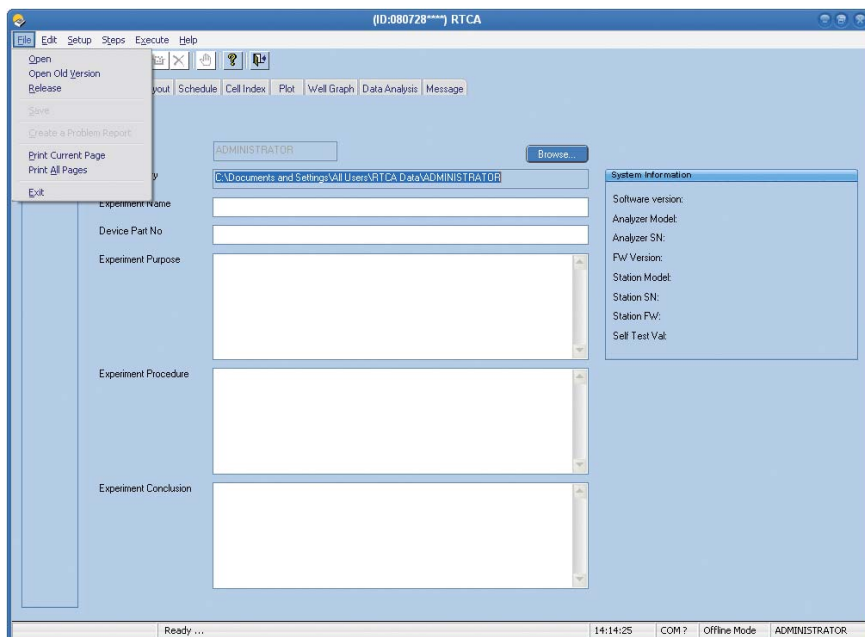
### 2.2 Check the Software Version

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 RTCA Control Unit or computer.

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## 2.3 Main Menu

At the top of the RTCA Software window appears a main menu bar, with options including *File*, *Edit*, *Setup*, *Steps*, *Execute* and *Help*.



### File Menu

**Open:** Open an experiment

**Open Old Version:** Open an experiment from an old version of the Software

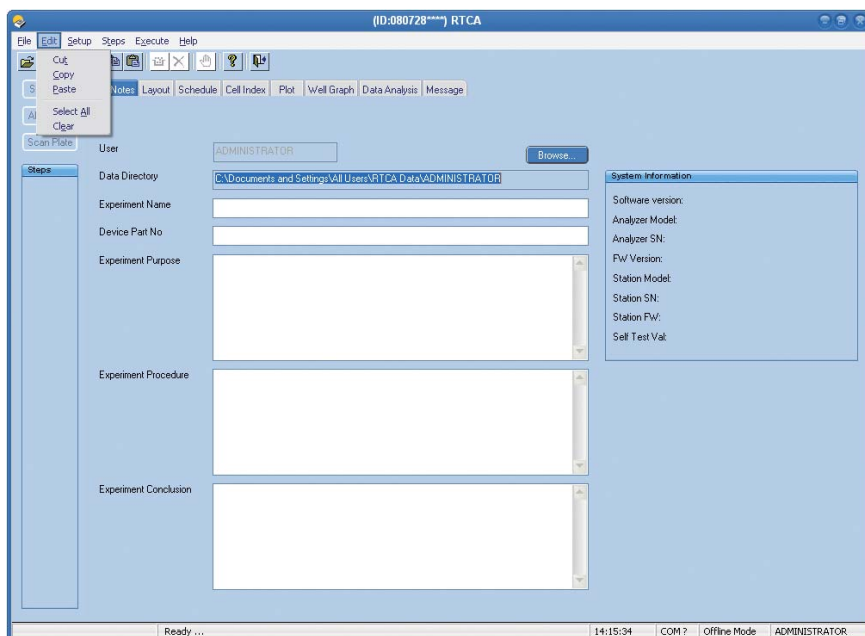
**Release:** Clear all the information on all the pages

**Save:** Save current experiment

**Create a Problem Report:** Create a problem report for sending to Roche representative

**Print Current Page:** Print displayed page

**Print All Pages:** Print all pages



### Edit Menu

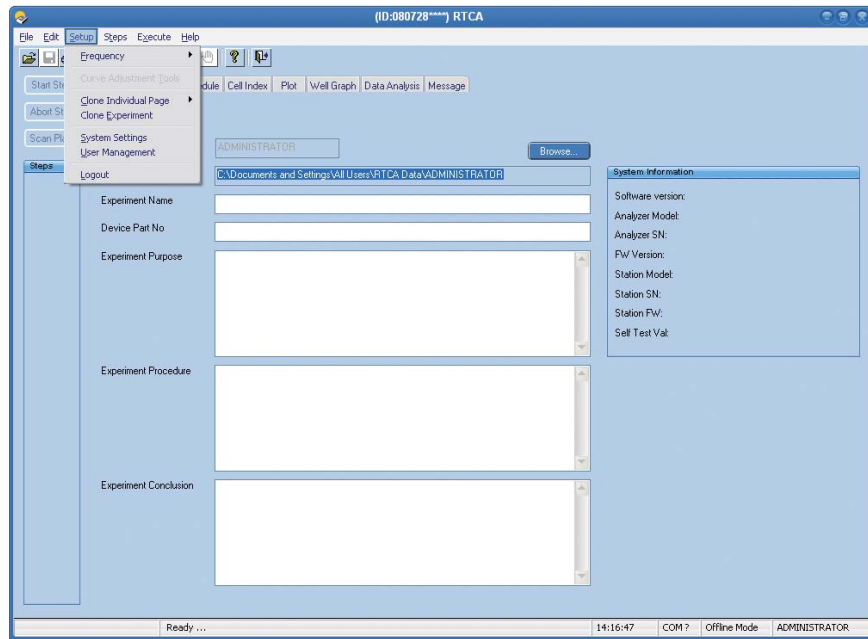
**Cut:** Cut information

**Copy:** Copy information

**Paste:** Paste information

**Select All:** Select all information or all wells

**Clear:** Clear information



### Setup Menu

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

**Curve Adjustment Tools:** For adjusting Cell Index curves

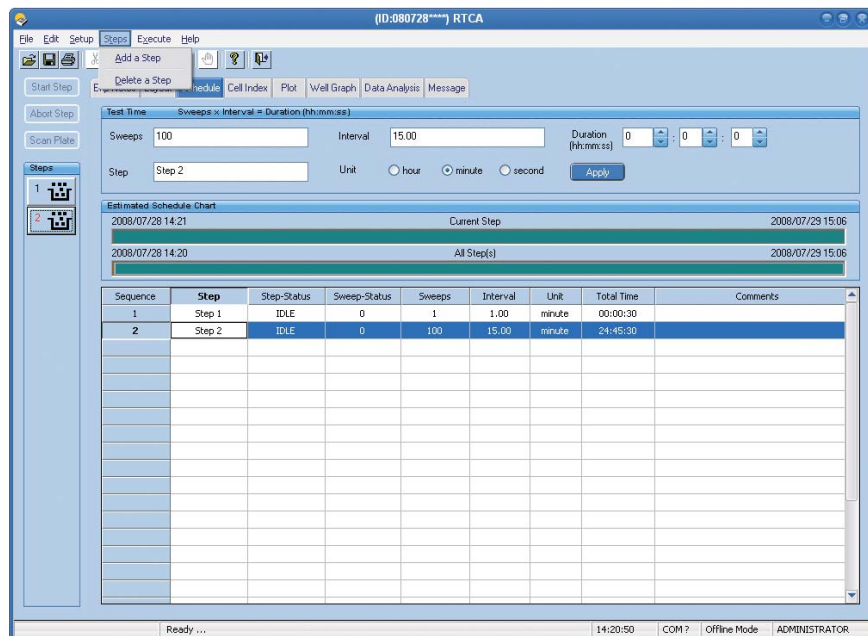
**Clone Individual Pages:** Copy settings for individual page

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

**System Settings:** Select or set up COM port

**User Management:** Add/Delete users, change passwords

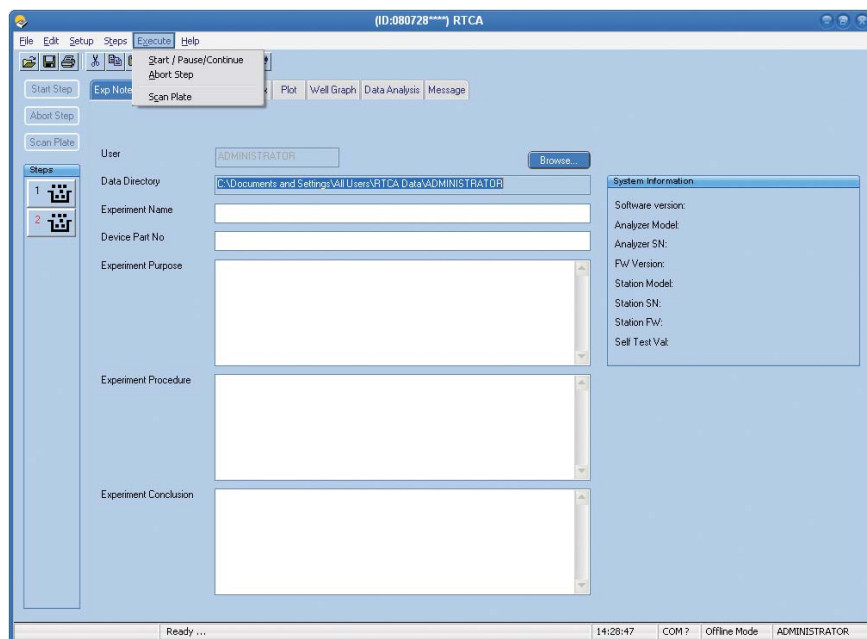
**Logout:** Logout RTCA Software



### Steps Menu (for editing steps in experiment schedule)

**Add a Step:** Add a new step to experiment

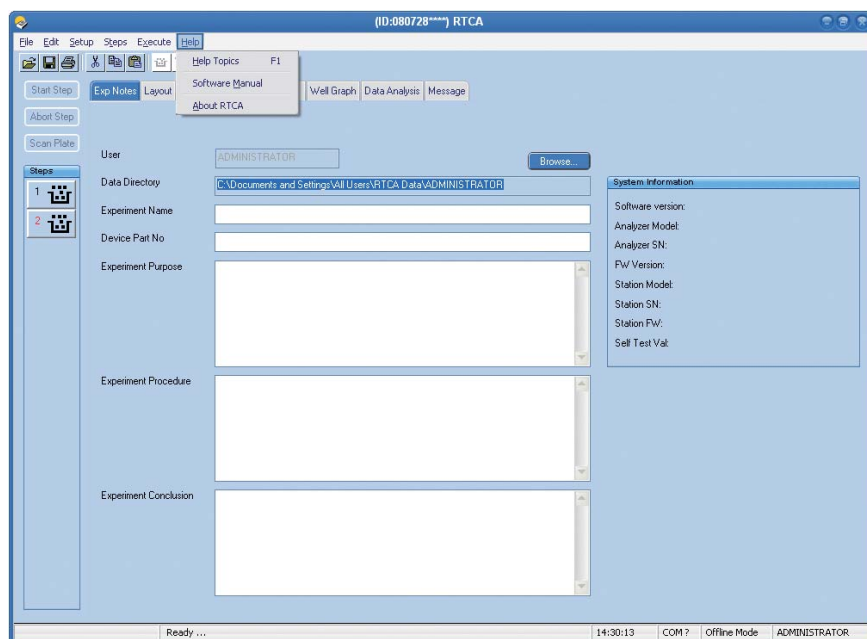
**Delete a Step:** Delete selected step

**Execute Menu (for running an experiment)**

**Start/Pause/Continue:** Start a step, or pause current step, or resume step that is currently paused

**Abort Step:** Abort current step

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

**Help Menu**

**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

## 2.4 Experiment Record

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



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

The information on the *Exp Notes* page (and *Layout* page and *Schedule* page) will automatically be saved in an experiment file when the user clicks the *Start Step* button. The default file name is YYMMDDHHmm\_ExperimentName.plt for SP, and YYMMDDHHmmPPPlateNumber\_ExperimentName.plt for MP, where ExperimentName is the user-entered text in the *Experiment Name* field, YYMMDDHHmm is current date and time and PlateNumber is the E-Plate 96 position on the RTCA MP Station (1-6). 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 should save this page manually by clicking the *Save Changes* button



*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, and TemplateYYMMDDPPPlateNumber for MP. Type in 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.*

Exp Notes Tab



*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.*




## 2.5 Text Boxes on Exp Notes Page

- ▶ **User:** The login user name is displayed in this box and cannot be changed (read-only).
- ▶ **File Directory:** The default path is to C:\Documents and Settings\All Users\RTCA Data\login username. To direct the path to a different folder on the RTCA 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 96 here will allow the user to monitor the quality of the experiment and track any possible issues with the E-Plate 96. Entering the E-Plate 96 serial number is highly recommended.
- ▶ **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; to be entered after experiment has finished. 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 changes to YYMMDDHHmm\_ExperimentName.plt for SP, and YYMMDDHHmmPPlateNumber\_ExperimentName.plt for MP, where ExperimentName is the user-defined information from the *Experiment Name* field. 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 may be highly useful for later reference.*

## 2.6 Example of a Completed Exp Notes Page

The screenshot displays the RTCA software interface, specifically the 'Exp Notes' page. The window title is '(ID:080728\*\*\*\*) RTCA'. The menu bar includes File, Edit, Setup, Steps, Execute, and Help. The toolbar contains icons for Start Step, Abort Step, Scan Plate, and various data analysis functions. The 'Exp Notes' tab is active, showing fields for User (ADMINISTRATOR), Data Directory (C:\Documents and Settings\All Users\RTCA Data\ADMINISTRATOR), Experiment Name (Cell Titration with HeLa Cells), Device Part No (C9827 NT L/N: 080202), Experiment Purpose (Assessment of suitable cell concentration for further experiments), Experiment Procedure (1. Addition of culture media, 2. Addition of cells and monitoring of cell proliferation, 3. Compound addition), and Experiment Conclusion. A 'System Information' panel on the right lists Software version, Analyzer Model, Analyzer SN, FW Version, Station Model, Station SN, Station FW, and Self Test Val. The status bar at the bottom shows 'Ready ...', '14:53:51', 'COM?', 'Offline Mode', and 'ADMINISTRATOR'.

### 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 ends.*

After an experiment starts, changes made in the *Layout* page must be saved manually via the *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) after the experiment has started.*

#### 3.2 Add Experimental Information



Each box on the well map (see picture of *Layout* page below) represents one well of an E-Plate 96 on the RTCA SP Station. You can use the text boxes on the page to define the conditions of each well (see the *Enable Wells* procedure below). 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.*

### 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 96 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 light and contain the information that was typed in the text boxes.
    -  *This step must be performed before experiment can start.*
- 

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

B

### 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.
- ▶ **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.
- ▶ **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*, *High From* and *Along* fields (which give details about the dilution series). First enter in this *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. 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.
- ▶ **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).
- ▶ **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.
- ▶ **Along:** Indicate whether the serial dilution and/or repetition is to proceed along a row or a column.
- ▶ **Apply Button:** 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

Example of a complete experimental layout:

(ID:080728\*\*\*\*) RTCA

File Edit Setup Steps Execute Help

Start Step Exp Notes Layout Schedule Cell Index Plot Well Graph Data Analysis Message

Abort Step

Scan Plate

Steps

Enter Well Information

Well IDs:  Cell Type:  Cell Number:  ☐ Cell Number Titration

Compound No.:  Compound Name:  Concentration:  Unit:  ☐ Concentration Dilution

Dilution Factor:  Repetition:  Along:  High From:

	1	2	3	4	5	6	7	8	9	10	11	12
A	HeLa 4000 A 10.00nM	HeLa 4000 A 10.00nM	HeLa 4000 A 10.00nM	HeLa 4000 A 10.00nM	HeLa 4000 A 10.00nM	HeLa 4000 A 10.00nM	CHO 5000 A 10.00nM	CHO 5000 A 10.00nM	CHO 5000 A 10.00nM	CHO 5000 A 10.00nM	CHO 5000 A 10.00nM	CHO 5000 A 10.00nM
B	HeLa 4000 B 50.00uM	HeLa 4000 B 50.00uM	HeLa 4000 B 50.00uM	HeLa 4000 B 50.00uM	HeLa 4000 B 50.00uM	HeLa 4000 B 50.00uM	CHO 5000 B 50.00uM	CHO 5000 B 50.00uM	CHO 5000 B 50.00uM	CHO 5000 B 50.00uM	CHO 5000 B 50.00uM	CHO 5000 B 50.00uM
C	HeLa 4000 C 20.00ng/ml	HeLa 4000 C 20.00ng/ml	HeLa 4000 C 20.00ng/ml	HeLa 4000 C 20.00ng/ml	HeLa 4000 C 20.00ng/ml	HeLa 4000 C 20.00ng/ml	CHO 5000 C 20.00ng/ml	CHO 5000 C 20.00ng/ml	CHO 5000 C 20.00ng/ml	CHO 5000 C 20.00ng/ml	CHO 5000 C 20.00ng/ml	CHO 5000 C 20.00ng/ml
D	HeLa 4000 D 30.00nM	HeLa 4000 D 30.00nM	HeLa 4000 D 30.00nM	HeLa 4000 D 30.00nM	HeLa 4000 D 30.00nM	HeLa 4000 D 30.00nM	CHO 5000 D 30.00nM	CHO 5000 D 30.00nM	CHO 5000 D 30.00nM	CHO 5000 D 30.00nM	CHO 5000 D 30.00nM	CHO 5000 D 30.00nM
E	HeLa 4000 E 80.00ppM	HeLa 4000 E 80.00ppM	HeLa 4000 E 80.00ppM	HeLa 4000 E 80.00ppM	HeLa 4000 E 80.00ppM	HeLa 4000 E 80.00ppM	CHO 5000 E 80.00ppM	CHO 5000 E 80.00ppM	CHO 5000 E 80.00ppM	CHO 5000 E 80.00ppM	CHO 5000 E 80.00ppM	CHO 5000 E 80.00ppM
F	HeLa 4000 F 70.00ug/ml	HeLa 4000 F 70.00ug/ml	HeLa 4000 F 70.00ug/ml	HeLa 4000 F 70.00ug/ml	HeLa 4000 F 70.00ug/ml	HeLa 4000 F 70.00ug/ml	CHO 5000 F 70.00ug/ml	CHO 5000 F 70.00ug/ml	CHO 5000 F 70.00ug/ml	CHO 5000 F 70.00ug/ml	CHO 5000 F 70.00ug/ml	CHO 5000 F 70.00ug/ml
G	HeLa 4000 G 15.00mM	HeLa 4000 G 15.00mM	HeLa 4000 G 15.00mM	HeLa 4000 G 15.00mM	HeLa 4000 G 15.00mM	HeLa 4000 G 15.00mM	CHO 5000 G 15.00mM	CHO 5000 G 15.00mM	CHO 5000 G 15.00mM	CHO 5000 G 15.00mM	CHO 5000 G 15.00mM	CHO 5000 G 15.00mM
H	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control

Ready ... 15:03:46 COM ? Offline Mode ADMINISTRATOR

B



### 3.6 Example of Dilution Factor and Dilution Repetition



In the RTCA Software, the term “ $\mu$ ” as a dimension unit is always shown as “u” (e.g.,  $\mu\text{M}$  instead of  $\mu\text{M}$ ).

The concentration for compound A in the first 2 wells in column 1 is 100  $\mu\text{M}$  and in the next 2 wells it is 50  $\mu\text{M}$  as indicated by the dilution factor of 2. The repetition factor of 2 means the first concentration repeats 2 times, then the next concentration 2 times down 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 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

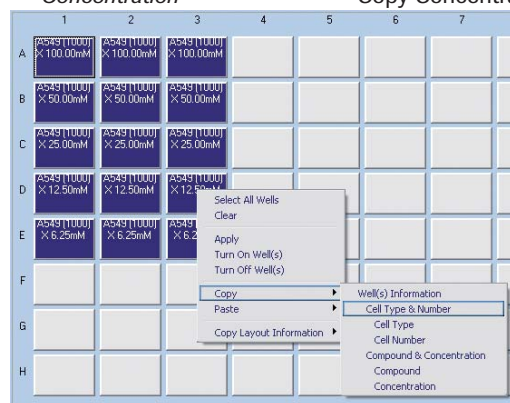
For convenience, the RTCA Software offers *Copy* and *Paste* functions to help set up the *Layout* page.

*Copy* and *Paste* can be done within the Layout page, or between Plate Windows (for MP only). 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 left mouse key to highlight source well(s). Then right-click, select *Copy* from the menu, and select the item to be copied:

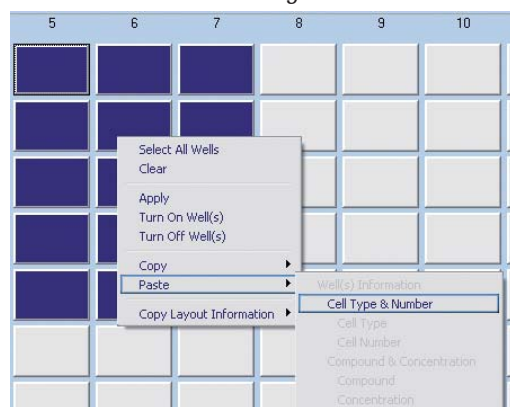
<i>Well(s) Information</i>	Copy all information in the well(s)
<i>Cell Type &amp; Number</i>	Copy Cell Type and Cell Number only
<i>Cell Type</i>	Copy Cell Type only
<i>Cell Number</i>	Copy Cell Number only
<i>Compound &amp; Concentration</i>	Copy Compound and Concentration
<i>Compound</i>	Copy Compound Name only
<i>Concentration</i>	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:

<i>Well(s) Information</i>	Paste all information in the well(s)
<i>Cell Type &amp; Number</i>	Paste Cell Type and Cell Number only
<i>Cell Type</i>	Paste Cell Type only
<i>Cell Number</i>	Paste Cell Number only
<i>Compound &amp; Concentration</i>	Paste Compound and Concentration
<i>Compound</i>	Paste Compound Name only
<i>Concentration</i>	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. Schedule Setting



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 schedule for each step

Schedule Tab

Process bar (Selected step)

Process bar (All steps)

Sequence	Step	Step-Status	Sweep-Status	Sweeps	Interval	Unit	Total Time	Comments
1	Step 1	IDLE	0	10	1.00	minute	00:09:17	
2	Step 2	IDLE	0	10	1.00	minute	00:18:17	
3	Step 3-1	IDLE	0	10	1.00	minute	00:27:17	
3	Step 3-2	IDLE	0	10	1.00	minute	00:37:17	
4	Step 4-1	IDLE	0	10	1.00	minute	00:46:17	
4	Step 4-2	IDLE	0	10	1.00	minute	00:56:17	

### 4.1 Add Steps

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

- ▶ Click the *Step* icon on the toolbar, 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.

Once the step is added, the step icon will appear on the left part of the window; it will be labeled for the first step and for second step and so on. To rename the step, move the cursor into the Steps column, highlight the text (e.g., Step 1), type the desired step name and click *Apply*.



For simplicity, always edit the *Schedule* of the current step, including intervals and sweeps, before adding the next step.

#### 4.1.1 Set the Schedule for Each Step



There are three related items involved in setting the schedule for a step, called sweeps, interval and duration. Enter any two of them in the text boxes at the top of the page, and the third can be calculated automatically.

- ▶ Enter interval time (time between two measurements) by moving the cursor to the *Interval* text box, and entering the desired time. Then select the unit (hour or minute or second) by clicking the appropriate button.
- ▶ Enter the number of sweeps (a sweep is one measurement of an E-Plate 96) in the *Sweeps* text box.
- ▶ Enter the total time needed for the step/sub-step in the *Duration* text boxes. There are three boxes that allow you to specify hour/minute/second (from left to right).
- ▶ Click the *Apply* button to confirm the setting. Once the setting is confirmed, the detailed setting information will appear in the list table below the boxes, including *Step*, *Step-Status*, *Sweep-Status*, *Sweeps*, *Interval*, *Unit* and *Total Time* for the experiment.
- ▶ For any step or substep, you can click the *Comments* column (in the list table) and enter any comments you wish.

#### 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 screenshot shows the RTCA software interface. At the top, there's a menu bar (File, Edit, Setup, Steps, Execute, Help) and a toolbar. Below the toolbar, there's a 'Test Time' section with input fields for 'Sweeps' (10), 'Interval' (1.00), and 'Duration' (0:09:00). There are also radio buttons for 'Unit' (hour, minute, second) and an 'Apply' button. Below this is an 'Estimated Schedule Chart' showing a timeline from 2008/07/22 12:31 to 2008/07/22 13:09. The main part of the interface is a table with the following columns: Sequence, Step, Step-Status, Sweep-Status, Sweeps, Interval, Unit, Total Time, and Comments. The table contains several rows of data, including 'Step 3-1', 'Step 3-2', 'Step 4-1', and 'Step 4-2'. The 'Total Time' column shows values like 00:27:17, 00:37:17, 00:46:17, and 00:56:17. At the bottom, there's a status bar showing 'Scan Plate finished. Connections ok', the time '12:13:35', 'COM 1', and the user 'ADMINISTRATOR'.


Sequence	Step	Step-Status	Sweep-Status	Sweeps	Interval	Unit	Total Time	Comments
1	Step 1	IDLE	0	10	1.00	minute	00:09:17	
2	Step 2	IDLE	0	10	1.00	minute	00:18:17	
3	Step 3-1	IDLE	0	10	1.00	minute	00:27:17	
3	Step 3-2	IDLE	0	10	1.00	minute	00:37:17	
4	Step 4-1	IDLE	0	10	1.00	minute	00:46:17	
4	Step 4-2	IDLE	0	10	1.00	minute	00:56:17	



The *Total Time* and the *Finish At* are estimates only. They may change 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\_a. The user can change the given name.*

You can also insert a substep within a step. Move the mouse cursor to the chosen step and select that step by left-clicking it. Then right-click the mouse and choose *Add a Substep* from the pop-up menu.



*Normally, the program requires the user to manually start each step. However, substeps can be used to group parts of the experiment that you want to run continuously without manual intervention. Substeps are useful when you want to have different durations for sweeps, for example after compound addition (e.g., 60× 2 minutes, followed by 100× 15 minutes).*

## 4.4 Set up Multiple Substeps in the Same Step

The Software allows the user to set up multiple substeps within the same step, so the RTCA Instrument will change the time interval of the measurement without manual intervention.

To use this function, simply click the step (left-click either the icon in the left margin or in the table) you want to divide into substeps. Then right-click on the *Schedule* page, and select *Add a Substep* from the menu.

If the selected step has the time interval and sweep number entered, then the added substep will have the same sweeps and intervals as those for the highlighted step. If the user wants to change the settings for any of the substeps, they can click that substep, enter the appropriate information in the corresponding text boxes, then click the *Apply* button. The information will be transferred into the table.



*Although all steps are executed in sequence, only substeps in a step will continue automatically. After each normal step, the program will pause and wait for the user to manually start the next step.*

## 4.5 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 96 contains only cell culture media, that is, before cells are added. The Software always reserves the first step (step 1) for background or reference data collection.*

When a user adds the first step to the scheduling table, the Software automatically sets its sweep to one (1) and interval to one (1) minute. This is the setting required for a background step. The user should not change this setting except for *Resistor Plate Verification* (see RTCA SP Instrument Operator's Manual).



*When scheduling, you should add an extra step at the end of the experiment. This will ensure that, when the software finishes the last experimental step, it will idle, waiting for manual intervention. The user can then decide (e.g., on the next morning) whether the experiment can be terminated or needs to be continued.*



*After a step has been started, its settings (except for Comments) cannot be edited.*

## 4.6 Text Box and Button Functions

► **Interval:** Time duration between two measurements, user-defined value.




*The minimum interval time for measurement of a complete E-Plate 96 is 15 seconds. If a smaller interval is programmed, the number of wells included in the measurement must be reduced, since the time needed for measurement is a function of the number of wells (e.g., 7.5 seconds for 48 wells).*

► **Sweeps:** Number of times that the interval will be repeated during a step, user-defined value.

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

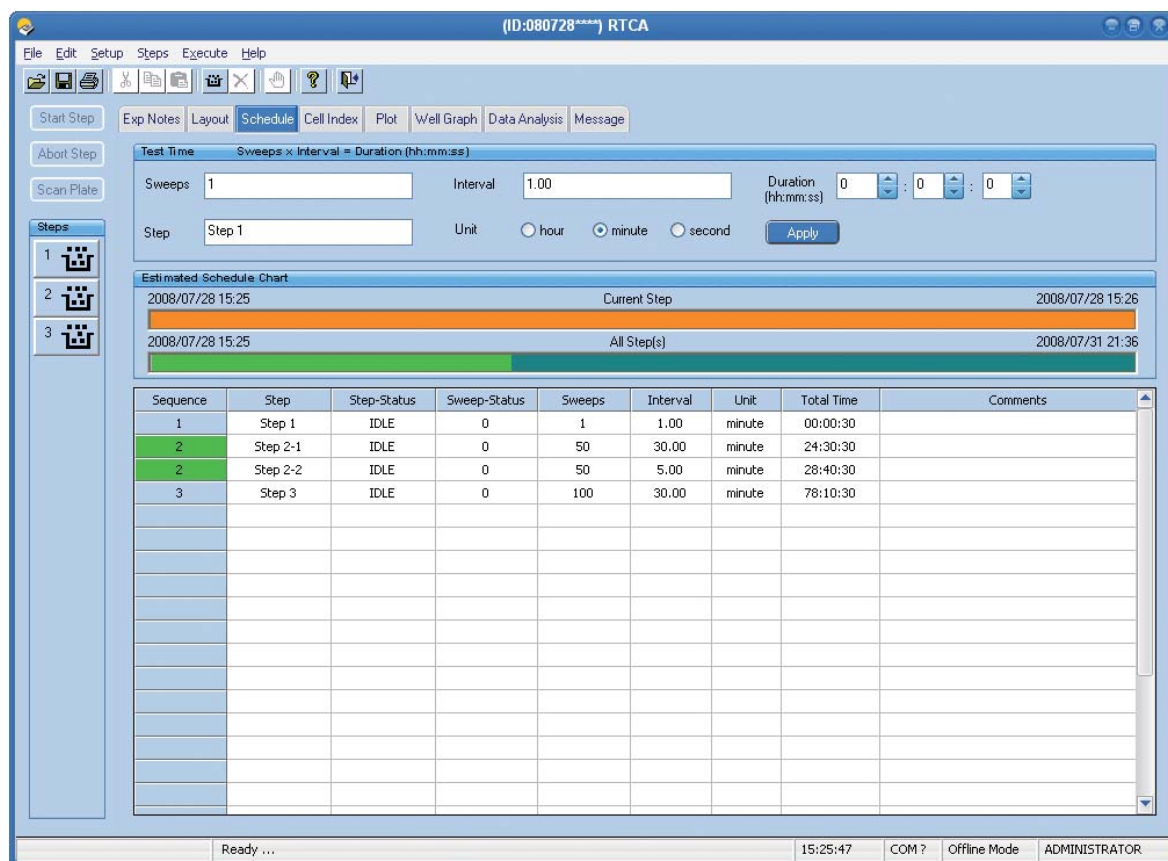
► **Duration:** the total time needed for a step/substep.

► **Apply:** Confirms settings and transfers them from the text boxes to the table beneath the boxes. The *Apply* function also generates the  icon in the left-hand margin.

B

## 4.7 Example of Multiple Substeps in one Experiment

In the example below, the Software will run step 1 when the *Start Step* button is clicked. After the completion of step 1, the Software will wait (in idle mode) for the user to click the *Start Step* button to begin step 2. Once the *Start Step* button is clicked, the system will run step 2, which has 2 substeps. When step 2 is completed the system will wait (in idle mode) for the user to click *Start Step* before running step 3.




## 4.8 Saving Set-up(s)

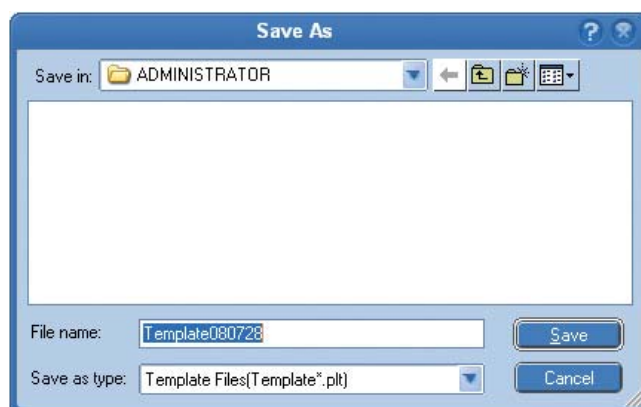
As described above, the RTCA Software allows users to edit/enter various experimental set-ups. If they use a particular set-up frequently, users should save the information for that set-up, so it can rapidly be loaded into the RTCA Software each time a similar experiment is performed.



*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 all the settings, the user can save all the set-up information as a template file for later use (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, and “TemplateYYMMDDP-PlateNumber” for MP. 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.

## 4.9 Opening a Template (Clone Settings)

The user can open a previously saved template or clone from a previously-run experiment by selecting *Setup* from the menu bar 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 (*Exp Notes*, *Layout*, and *Schedule*) is not loaded or changed.*

## 5. Start an Experiment

### 5.1 Scan Plate

#### 5.1.1 Manual Scan Plate

Once any well is activated in the *Layout* page the *Scan Plate* button will become active (lettering turns black). When *Scan Plate* is run, the software measures the resistance of all the activated wells. This will verify that the connections between the E-Plate 96 and the RTCA SP Station are operating properly.



*The Scan Plate button is disabled when the RTCA Analyzer is collecting data.*

The *Message* page will display the results of the scan and indicate any connection issues by listing the specific positions that did not have normal resistance. A scan will be recorded in the Events log each time the *Scan Plate* function is activated (before or during the experiment).

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

C:\Documents and Settings\All Users\RTCA Data\Username\ScanPlateData

The Software creates the file name in the following format:

PlateScanningYYMMDD.txt

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 96 connection to the RTCA SP Station, the RTCA Software has a built-in scan function. Each time it detects an E-Plate 96 being placed on the RTCA SP Station, 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.

## 5.2 Example of Results from Scan Plate

The *Cell Index* page shows resistance values for all scanned wells (Background Data).

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
B	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
C	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
D	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
E	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
F	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
G	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
H	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

	1	2	3	4	5	6	7	8	9	10	11	12
A	14.3218	14.2503	14.3276	14.6589	14.4088	14.2140	14.3386	14.2226	14.3588	14.4070	14.4354	14.3564
B	14.1197	14.1160	14.1584	14.4127	14.2868	14.0669	14.2240	14.2046	14.3583	14.1497	14.4086	14.2625
C	13.2354	12.9832	13.2587	13.6023	13.4652	13.2735	13.3985	13.2042	13.5578	13.2951	13.2605	13.4325
D	14.3806	14.3557	14.4313	14.7399	14.5041	14.3021	14.4013	14.3236	14.5029	14.5301	14.5799	14.4437
E	14.5828	14.4378	14.5861	14.6722	14.5617	14.3400	14.4922	14.5019	14.4361	14.4985	14.5756	14.6077
F	13.3945	13.0430	13.3298	13.5982	13.5256	13.2867	13.3642	13.4457	13.1998	13.5774	13.2784	13.5376
G	14.3938	13.9840	14.2622	14.3524	14.3907	14.0108	14.2888	14.2744	14.1740	14.2848	14.4054	14.3455
H	14.3682	14.2678	14.4445	14.5616	14.4104	14.2339	14.2960	14.3257	14.3203	14.4234	14.3638	14.4639

The *Message* page will report that the *Scan Plate* function was used and that the result was recorded as “Connections ok”. However, if the resistance value of a well is higher than 500 ohm, the *Message* page will report that this well may have a connection problem.

“Connections OK”  
displayed if all  
connections are fine.

Scan Plate Record

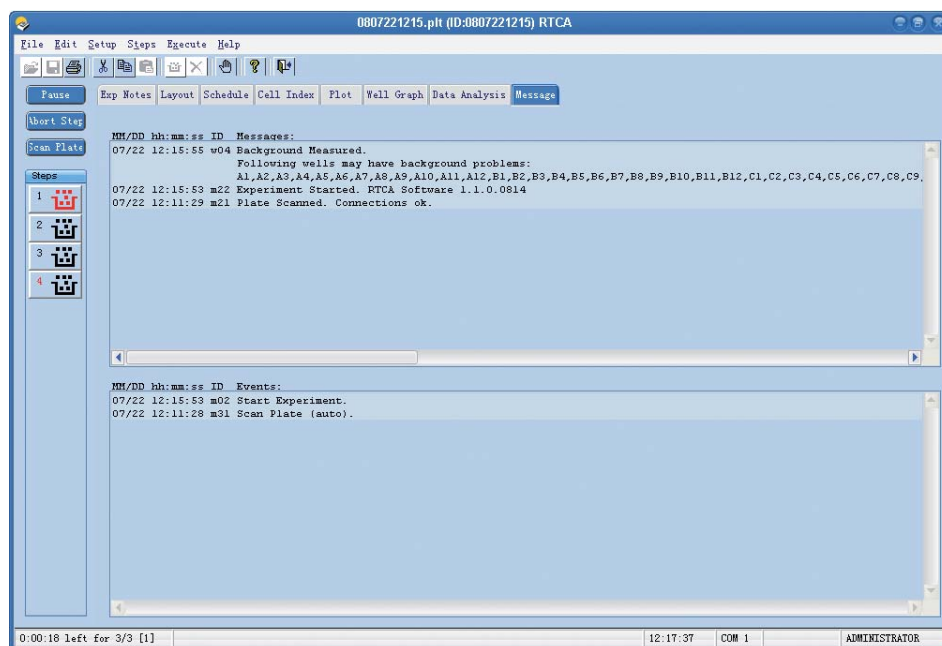
MM/DD hh:mm:ss	ID	Messages:
06/05 14:54:46	m21	Plate Scanned. Connections ok.
06/05 14:50:11	m00	Background Measured. Selected wells are ok.
06/05 14:49:41	m22	Experiment Scanned. RTCA Software 1.1.0.0814
06/05 14:48:44	m21	Plate Scanned. Connections ok.
06/05 14:48:08	m21	Plate Scanned. Connections ok.

MM/DD hh:mm:ss	ID	Events:
06/05 14:55:29	m08	Finish Experiment.
06/05 14:54:42	m31	Scan Plate (auto).
06/05 14:54:42	m31	Engage Device.
06/05 14:54:00	w11	No Device!
06/05 14:49:41	m02	Start Experiment.
06/05 14:48:40	m31	Scan Plate (auto).
06/05 14:48:40	m31	Engage Device.
06/05 14:48:10	w11	No Device!
06/05 14:48:04	m31	Scan Plate (auto).
06/05 14:48:04	m31	Engage Device.
06/05 14:47:10	w11	No Device!



The following Figure displays a typical result where potential connection issues are found by the *Scan Plate* function.



### 5.3 How to Handle Connection Issues



For instructions on handling E-Plate 96 connection issues, please refer to the RTCA Instrument Operator's Manual.

## 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 *Start Step* button in the upper left corner becomes active. Click on the *Start Step* button to start the experiment.

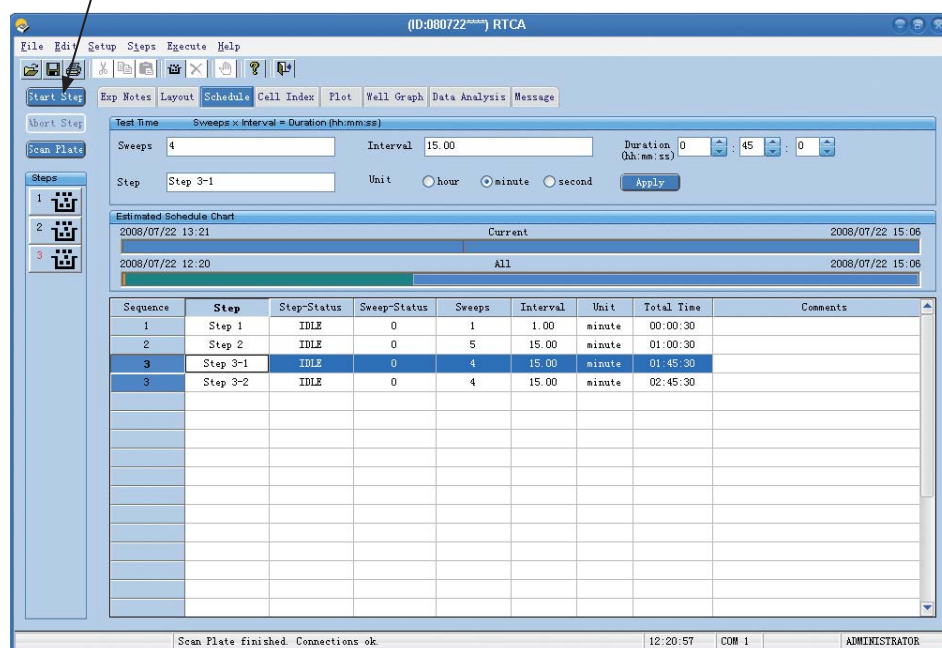


The *Step Status* on the *Schedule* page shows the individual status of the steps included in the experiment. The status can be **IDLE** (indicates that the step has not started yet), **TEST** (step is in progress) or **DONE** (step is finished).



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

Start Step Button



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



When the experiment starts, the Software measures background first. Please do not remove the E-Plate 96 while the Software is measuring the background. Otherwise, the experiment result will be incorrect.

### Continue the Experiment

If the experiment is accidentally terminated (or after the experiment has been manually paused by the user, see below), the whole 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 *Start Step* button will become the *Continue* button if there are any unfinished steps left to run. The RTCA SP 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. Set up the experiment again.

## 5.5 Pause the Experiment

After an experiment is started and as long as a step has not finished, the *Start Step* button automatically changes into the *Pause button*. Click the *Pause* 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 *Pause* button, the button will automatically change into a *Continue* button. This indicates that the Instrument is ready to resume the experiment. Once the user clicks *Continue*, the button automatically changes back into a *Pause* button, making it possible to pause the experiment again.*

## 5.6 Start Step

The RTCA SP Instrument will stay *IDLE* after a step is completed. At this time, the *Start Step* button will become active. To move to the next step, click the *Start Step* button, and the system will automatically initiate the next step. The RTCA SP Instrument will remain idle as long as necessary between steps; it will never restart automatically.

## 5.7 Abort 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 can manually start the next step, if there is one.*



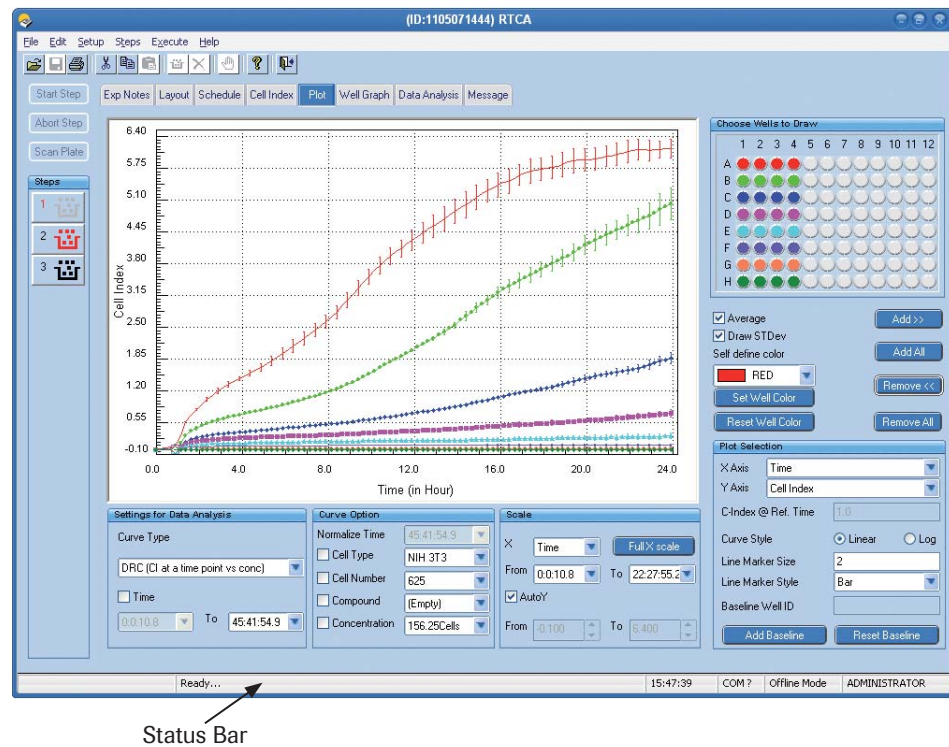
*Aborting a step does NOT lead to invalidation or loss of data.*

## 5.8 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:

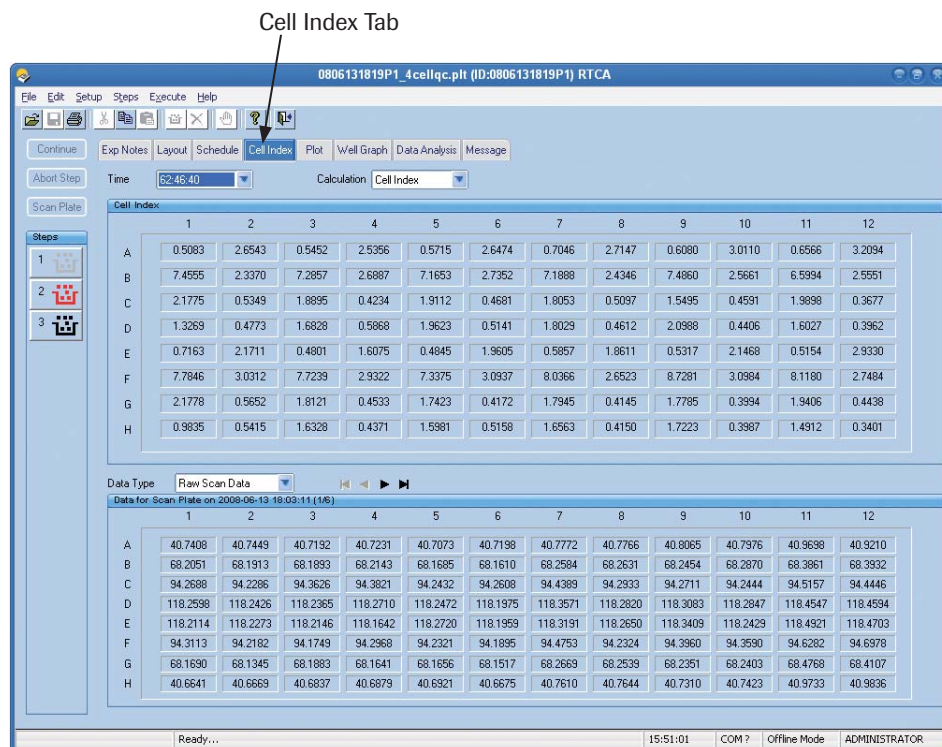
- ▶ *Ready* (before the start of an experiment)
- ▶ *Ready for starting next step* (waiting to start the next step after completing a step)
- ▶ *Test sweep # of step #* (while the RTCA SP Instrument is performing a sweep measurement)
- ▶ *Waiting for sweep # of step #* (waiting for the start of the next sweep)

During a sweep measurement, a portion of the status bar will display *Test Col #* (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

Real-time resistance measurement data will be recorded and presented as arbitrary units called Cell Indices on the *Cell Index* page. The *Cell Index* table shows the most recent measurement. To view previously collected data, select a particular measurement time point from the *Time* drop-down menu.



### 6.1 Menu Functions

- ▶ **Time:** Click the corner arrow of this drop-down menu to display measurement time points. If more than nine time points have been recorded, then use the scroll bar to view the hidden time points.
- ▶ **Calculations:** 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 indices are defined as follows:

$$\text{Cell Index}_i = (R_{t_n} - R_{t_0})/F_i$$

Where:

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

$F_1 = 15, F_2 = 12, F_3 = 10;$

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

$R_{t_0}$  is the background resistance measured at time point  $T_0$ ,  $R_{t_n}$  is the resistance measured at a time point  $T_n$ .

## 6.2 Scan Plate Data and Background Data

On the bottom half of the *Cell Index* page, you can use the *Data Type* drop-down menu to view *Raw Scan Data* and *Background Data*.

### Raw Scan Data:

Measured resistance data of all 96 wells 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 *RTCA SP Instrument Operator's Manual*.



If the *Scan Plate* function was performed more than once, click the arrow buttons to the right of the *Data Type* menu to view *Scan Plate* data at different time points.

The screenshot shows the RTCA software interface. The 'Cell Index' table is at the top, and the 'Raw Scan Data' table is at the bottom. The 'Data Type' dropdown is set to 'Raw Scan Data'. The 'Raw Scan Data' table shows resistance values for 96 wells (A-H, 1-12).


	1	2	3	4	5	6	7	8	9	10	11	12
A	39.8888	39.9144	39.8842	40.0845	39.9780	39.8799	39.9290	39.8532	39.8801	39.9475	39.9803	39.8877
B	67.3369	67.5204	67.3595	67.3289	67.4286	67.3665	67.2269	67.4055	67.4278	67.2436	67.4816	67.2922
C	93.5107	93.5291	93.5517	93.4230	93.5267	93.5891	93.4473	93.5603	93.5739	93.4016	93.2465	93.3445
D	117.7835	118.1892	117.7717	117.5305	117.7194	117.8099	117.5422	117.7260	117.5251	117.7371	117.6814	117.4596
E	117.5965	118.1642	117.7168	117.5010	117.5348	117.6799	117.4920	117.2764	117.8007	117.4664	117.4852	117.4310
F	93.4948	93.6567	93.4134	93.4416	93.6339	93.6185	93.3620	93.2235	93.4637	93.5496	93.2928	93.4839
G	67.4746	67.3583	67.3106	67.2854	67.3769	67.1323	67.3462	67.2004	67.3455	67.2785	67.3337	67.2611
H	39.9058	39.9502	39.9957	40.0546	39.8870	39.7970	39.7483	39.7238	39.9027	39.9028	39.9377	39.8972

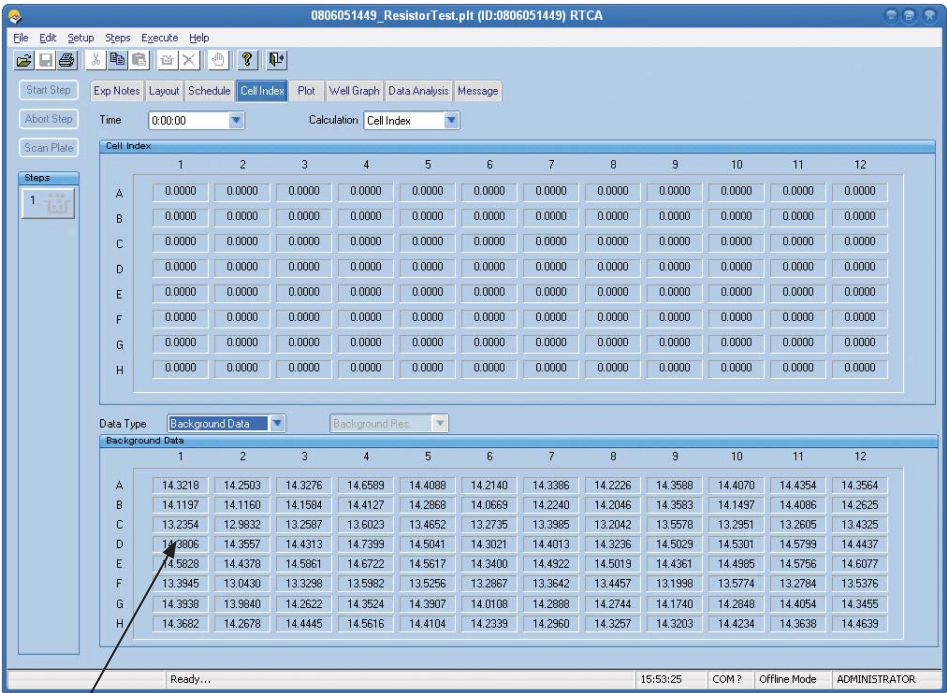
Raw Scan Data



**Background Data:**

Calculated background resistance data for all 96 wells at time point 0. In the calculation, each column of eight wells is divided into two groups, A through D and E through H.

 Check the background resistance for these 4-well groups and check the background measurement message on the Message page to discover which wells, if any, may have background issues.

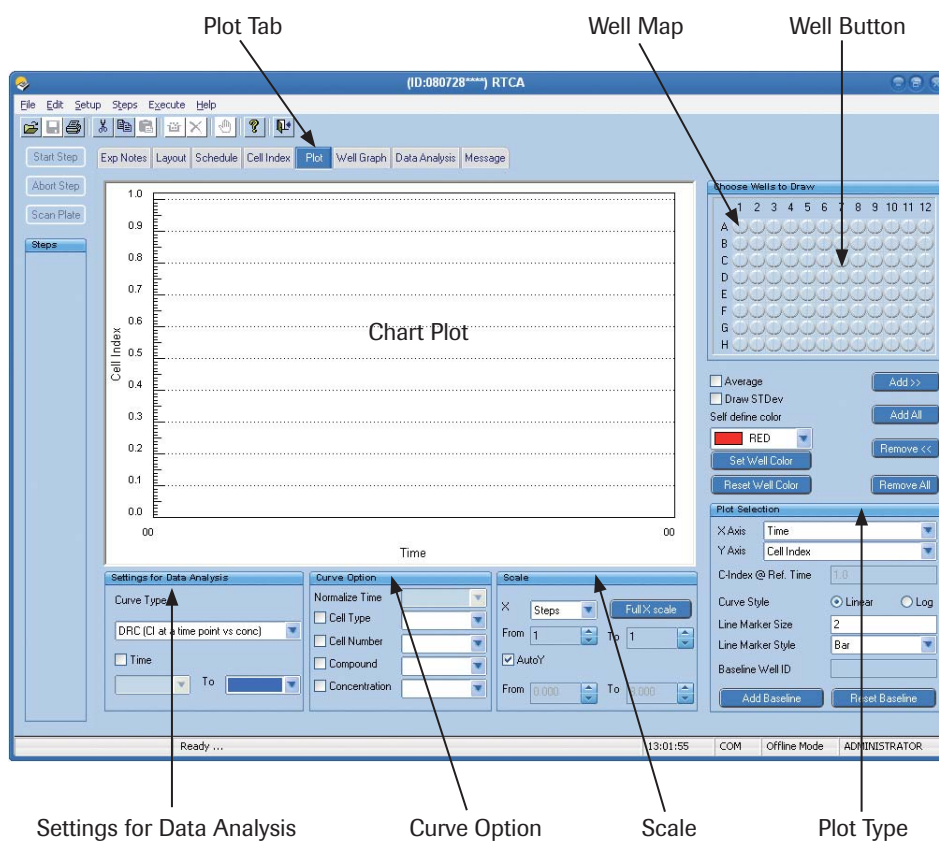


Background Data

## 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.

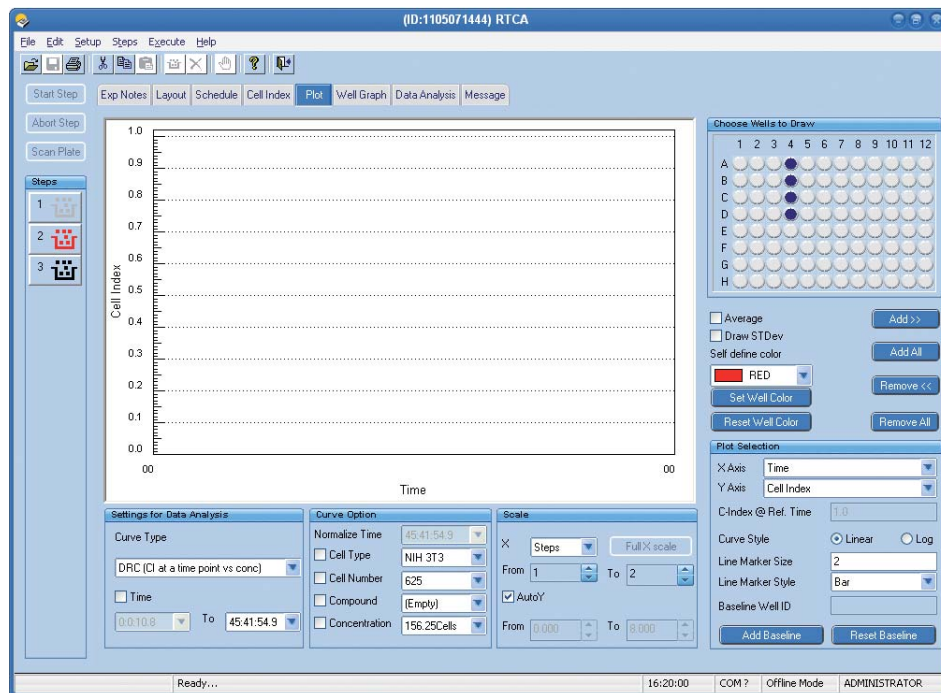
If you select *Normalized Cell Index*, you must also choose a specific time point to normalize the data to. You can select this time point from the *Normalization Time* drop-down menu under *Curve Options* (section 7.2). (A suitable time point might be, e.g., the last time point before compound addition.)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

### Example of Plotting Curve Data

Notice the dark blue color of well buttons A4, B4, C4 and D4 in the well map indicating these wells have been selected.



Clicking the *Add >>* button displays the Cell Index curves for the four 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. (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 let 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; 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.
- ▶ **Draw STDev:** Checking the **Draw STDev** 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 7.0 above.)
- ▶ **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:** The **Remove All** button removes all curves from the Chart Plot.

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## 7.6 Well Map

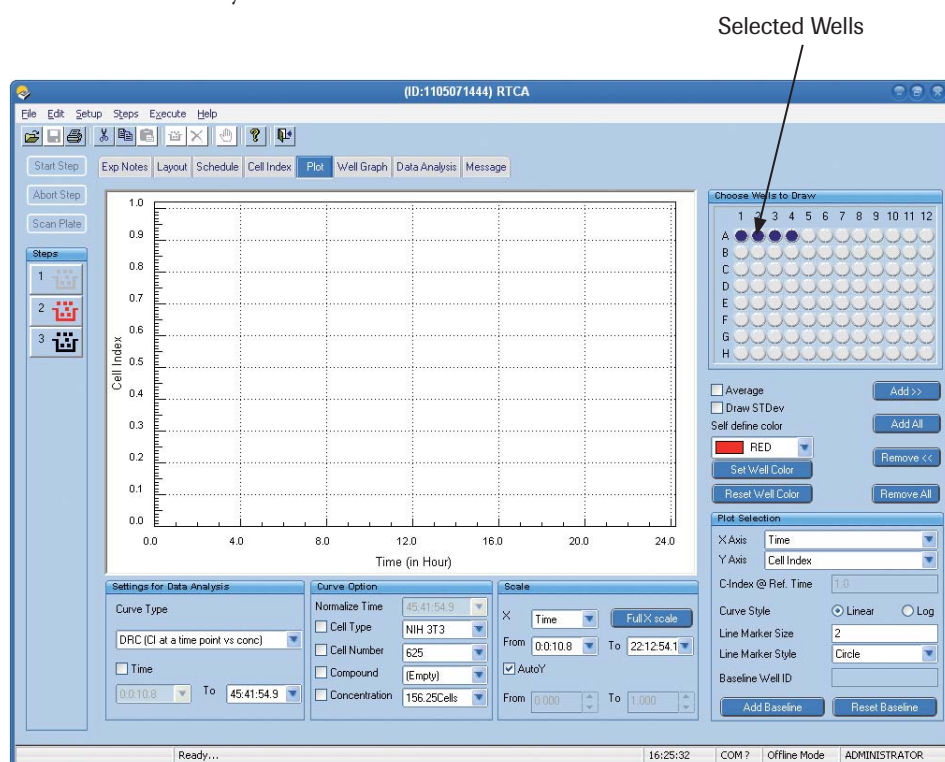
On the *Plot* page, the well map is used to select the well or wells whose curves will be displayed on the Chart Plot. Select an individual well by simply clicking on the well button. Select multiple wells by clicking and dragging across the desired well buttons. Individual wells can also be double-clicked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





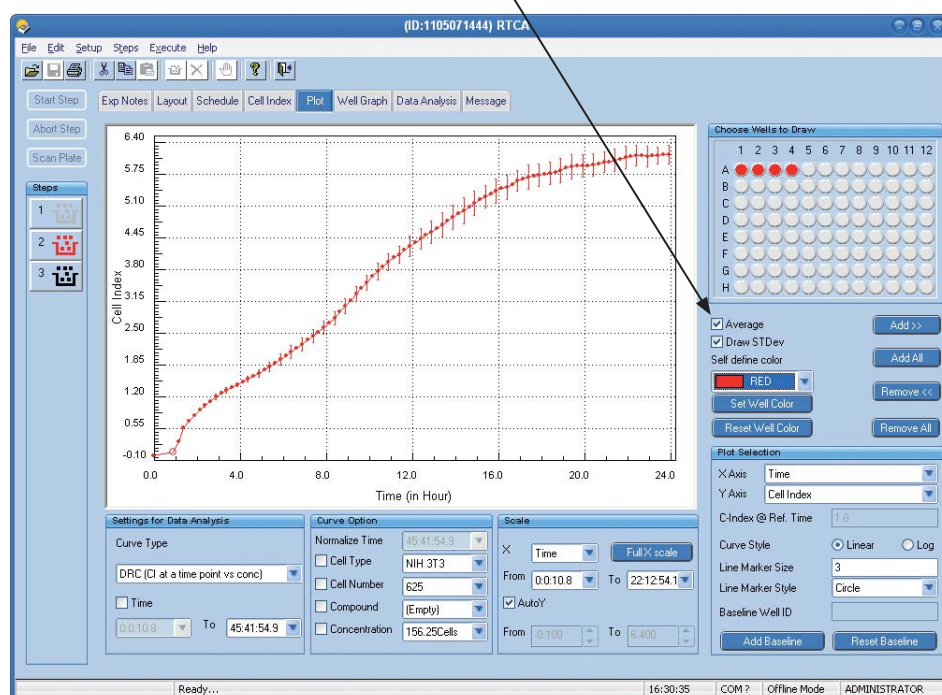
After the **Add >>** button is clicked, each well is represented by a colored line on the Chart Plot:



## 7.8 Example of the Average Function

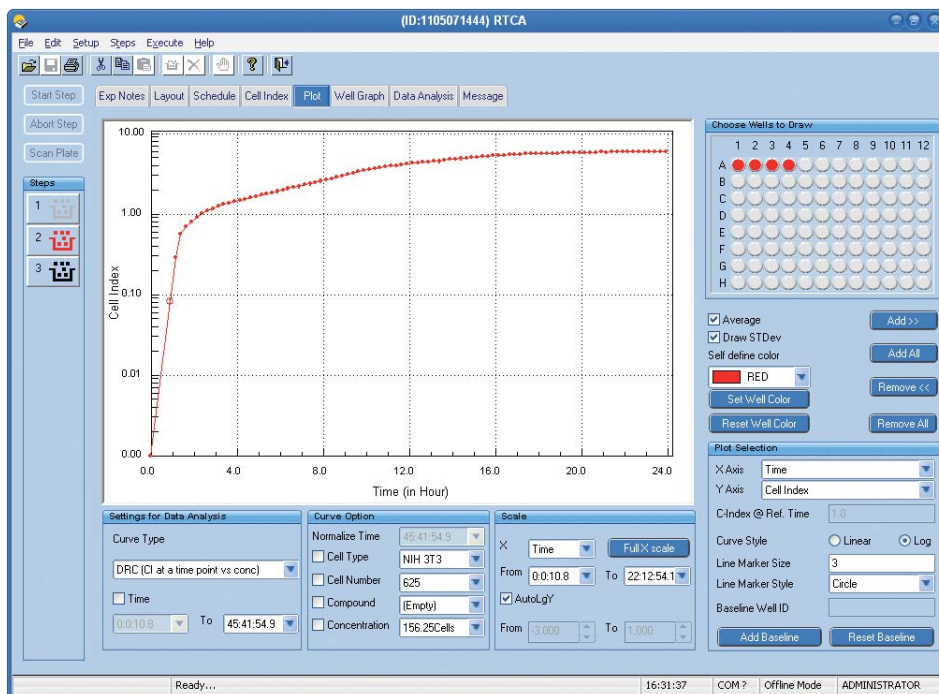
The *Average* function calculates the average of replicate wells (*i.e.*, wells with the same cell type, cell number, compound name and concentration on the *Layout* page) at each time point and displays the averaged data in a single color. The same color is used to highlight the replicate wells on the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

Average and Draw STDev Check box selected



## 7.9 Example of the Log Scale

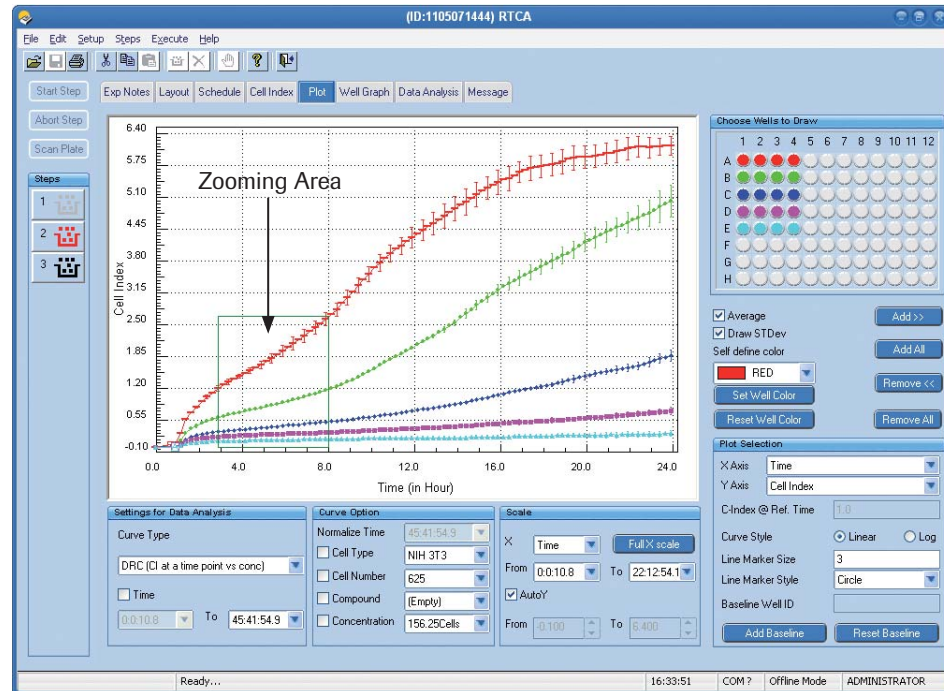
The data below is displayed on a chart with a logarithmic Y-axis. To redraw a plot this way, select the *Log* button (in “Curve Style” under “Plot Selection”).



## 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 long time if there are too many data points in the area selected for zooming.*



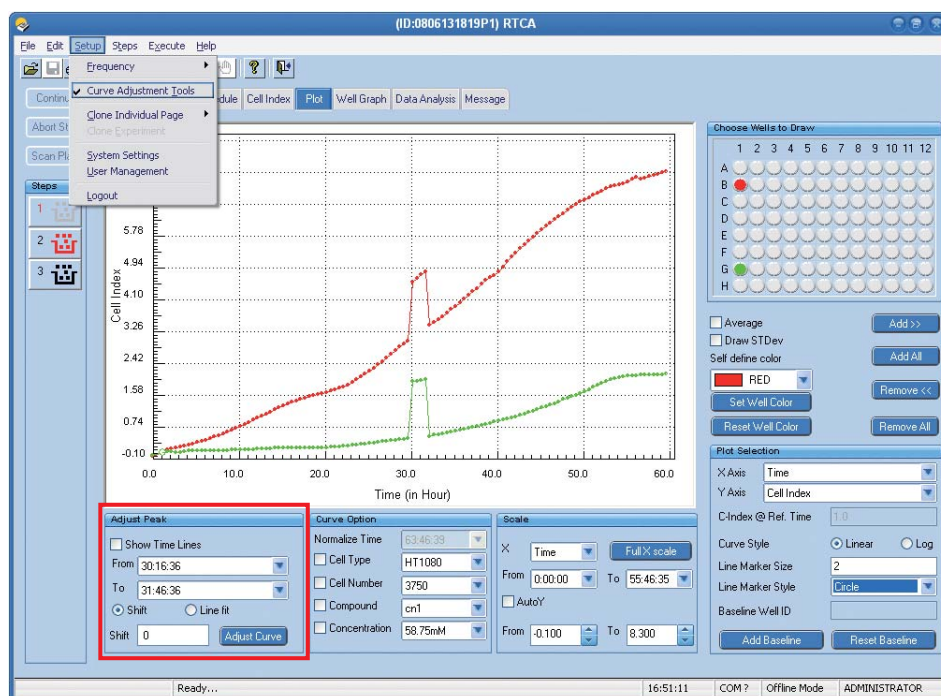
## 7.11 Curve Repair Function

If for any reason (most likely bad contacts that occurred when a device was taken out and re-engaged during an experiment) the experimental curve is not smooth, 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 should 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*.

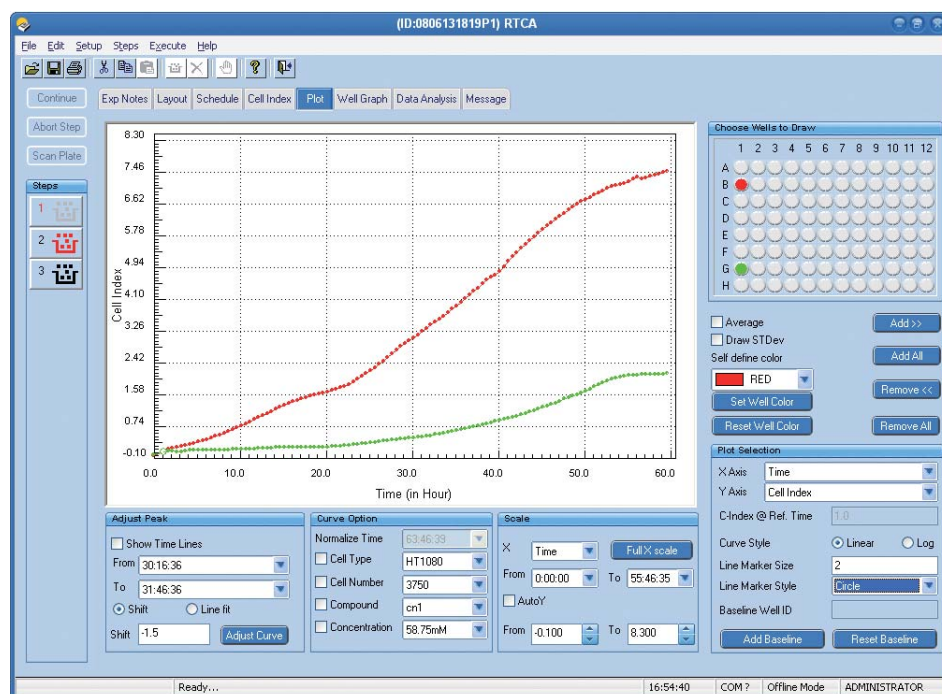
### 7.11.1 Shift of Curves

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.
- ▶ Click the *Shift* radio 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 -1.5 for all time points between 30:16:36 and 31:46:36.



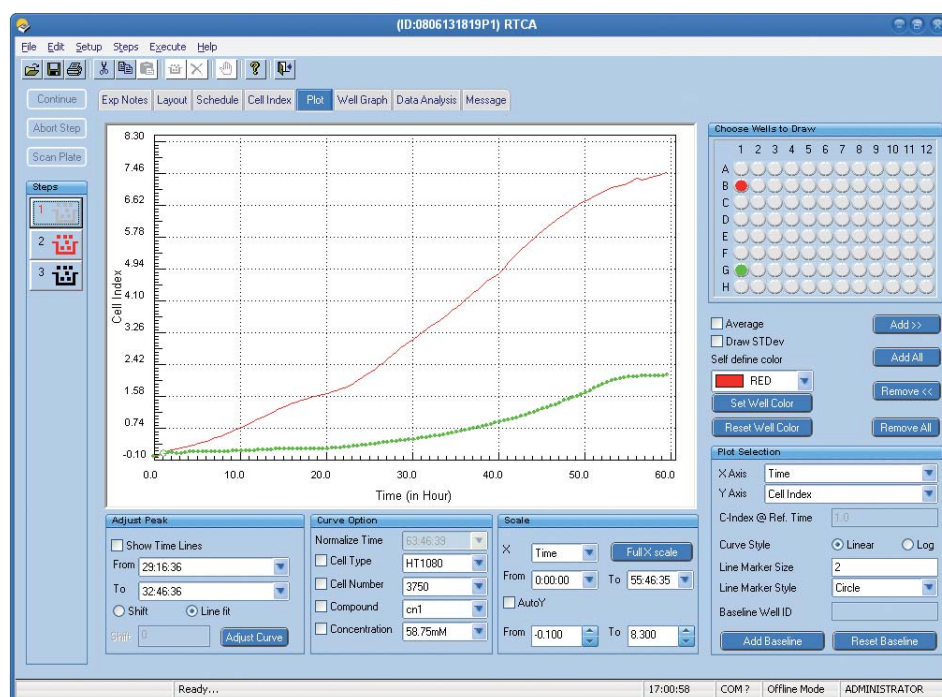
### 7.11.2 Line Fit

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.
- ▶ Click the *Line fit* radio 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 29:16:36 and 32:46:36 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.*:

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

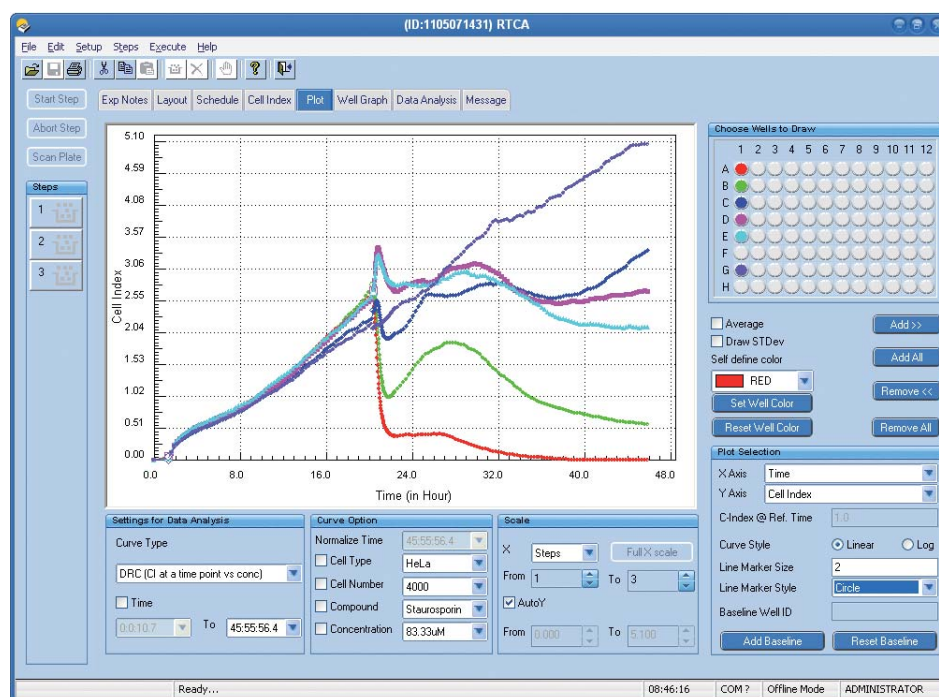
The Y-axis of the plot is now labeled *BaseLine Cell Index* (*BaseLine Normalized Cell Index*, or *BaseLine Delta Cell Index*).

Follow these steps to draw a Baseline Cell Index curve:

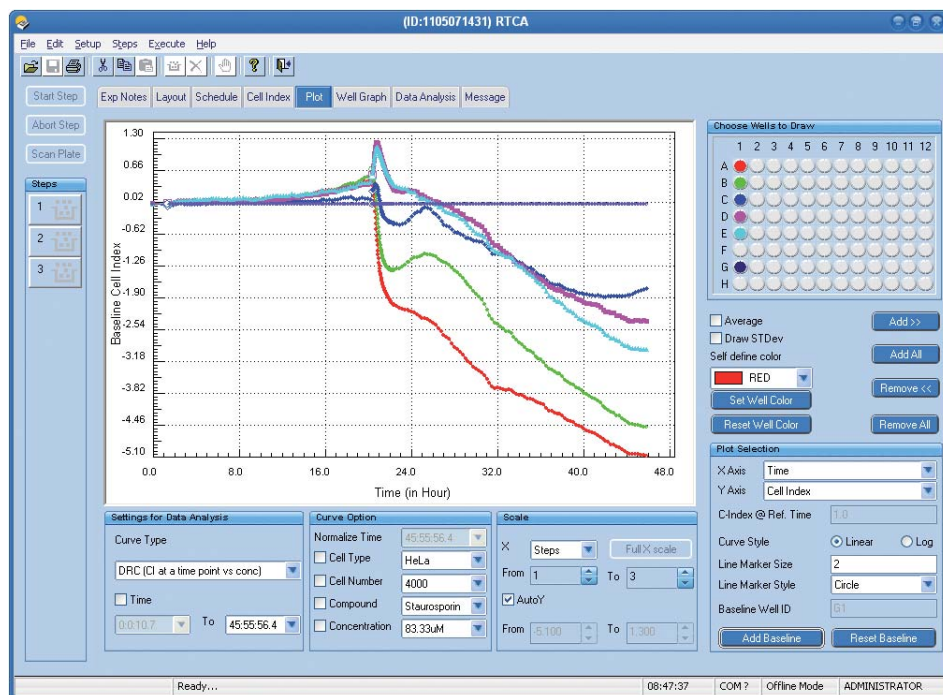
- ▶ On the well map, select a well or wells to be used as baseline wells.
- ▶ Click the *Add BaseLine* button (the selected well(s) will be shown in the *BaseLine Well ID* box).
- ▶ Select the well(s) to be plotted on the well map.
- ▶ Click the *Add >>* button to add curves to the plot. The Y-axis will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

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

Example: Six wells are selected (A1, B1, C1, D1, E1 and G1) and displayed without and with G1 as a baseline well.



Cell Index curves are plotted for A1, B1, C1, D1, E1 and G1 wells.



BaseLine Cell Index curves are plotted for A1, B1, C1, D1 and E1, with G1 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:

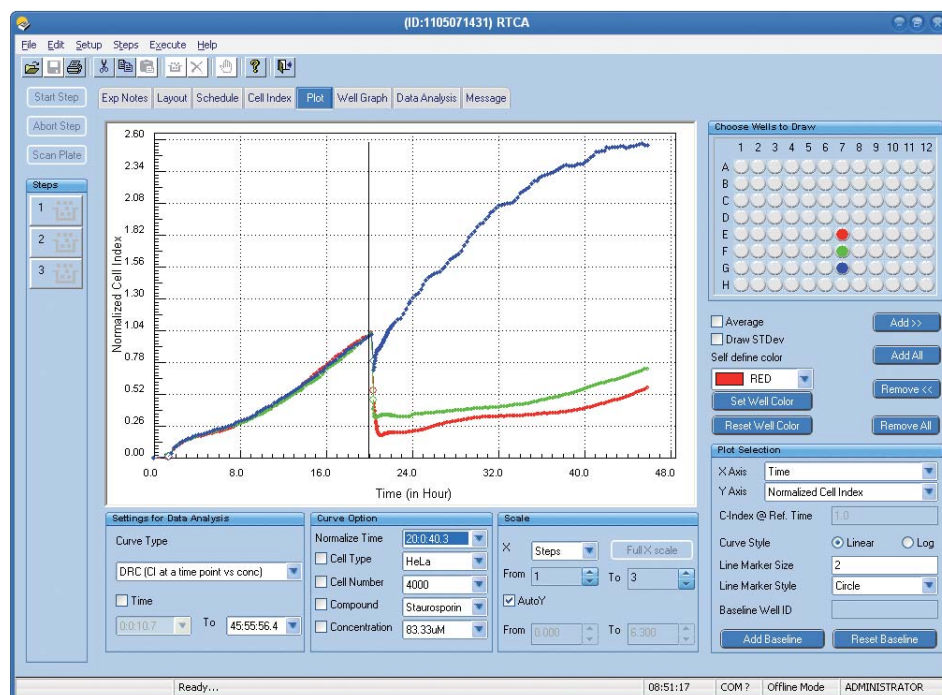
$$NCI_{ti} = CI_{ti} / CI_{nml\_time}$$

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

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 E7, F7 and G7; normalization time is 20:00:40.



## 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 reference Delta Cell Index is 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 *C-Index @ Ref. Time* box.

Example: Delta Cell Index plot for wells E7, F7 and G7; Delta time point is 20:00:40 and  $DCI_{reference} = 3.0$ .



## 7.15 Settings for Data Analysis

In *Settings for 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 *Curve Option* on the *Data Analysis* page. For more details, refer to section 10.5, *Curve Types and Curve-Fit Formula*.



## 8. Export Experiment Data

### 8.1 Export 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.*

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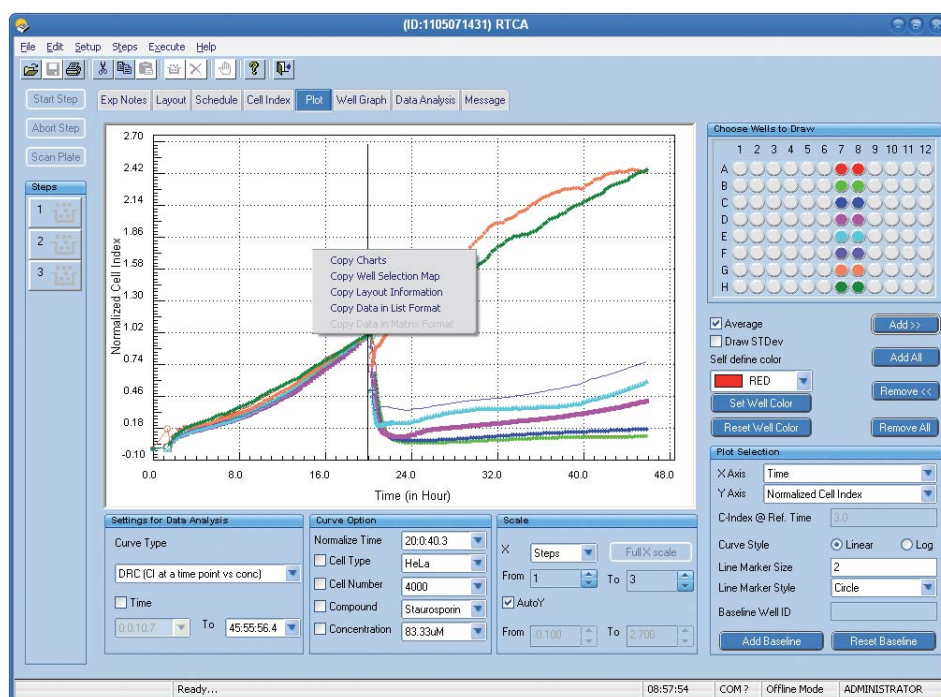
### 8.3 Example of a Right-Click Menu

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.

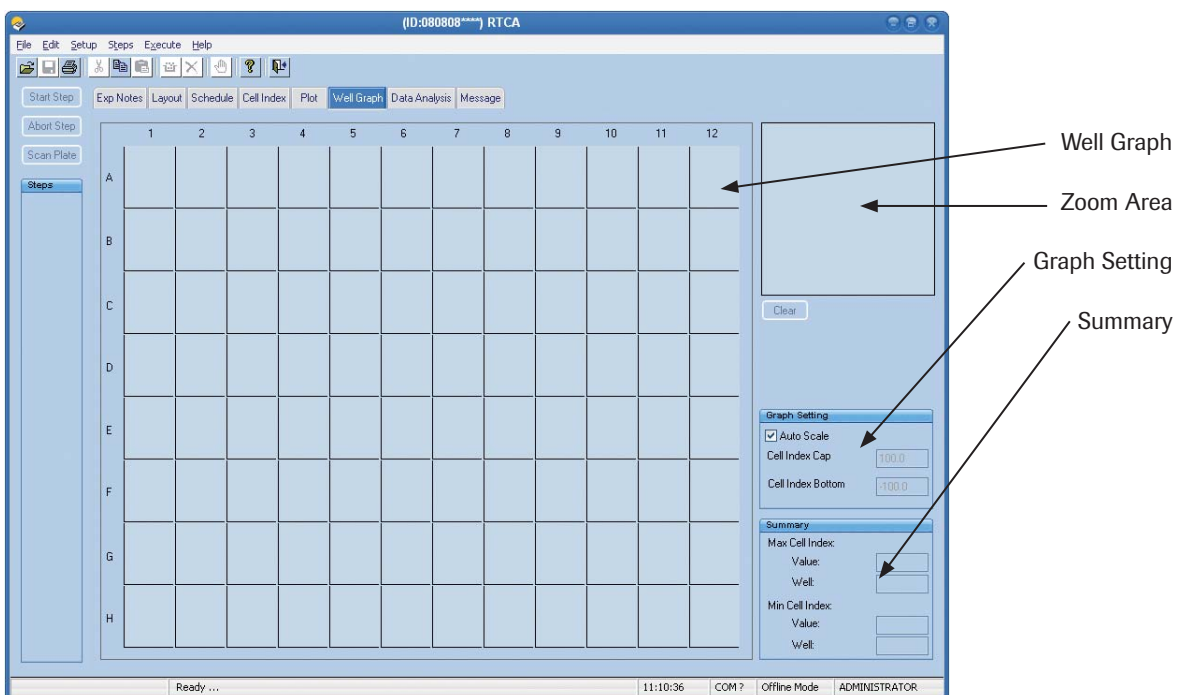


## 9. Well Graph

The *Well Graph* page provides an overview of Cell Index curves for all the wells by displaying them in an 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.

### 9.1 User Interface

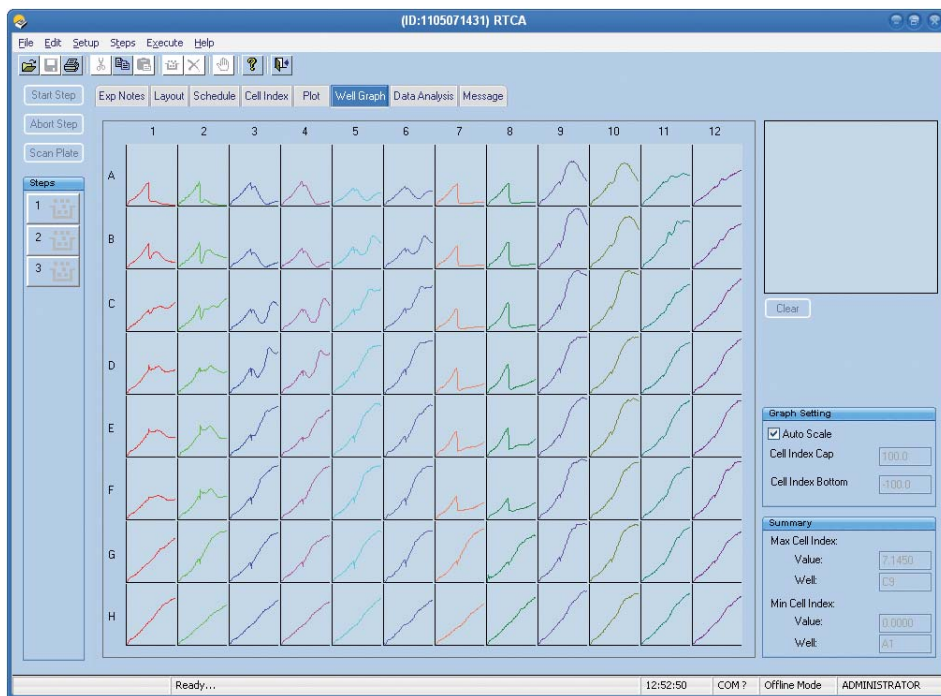
The *Well Graph* Page is divided into four main areas: Well Graph, Zoom Area, Graph Setting and Summary.



## 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.

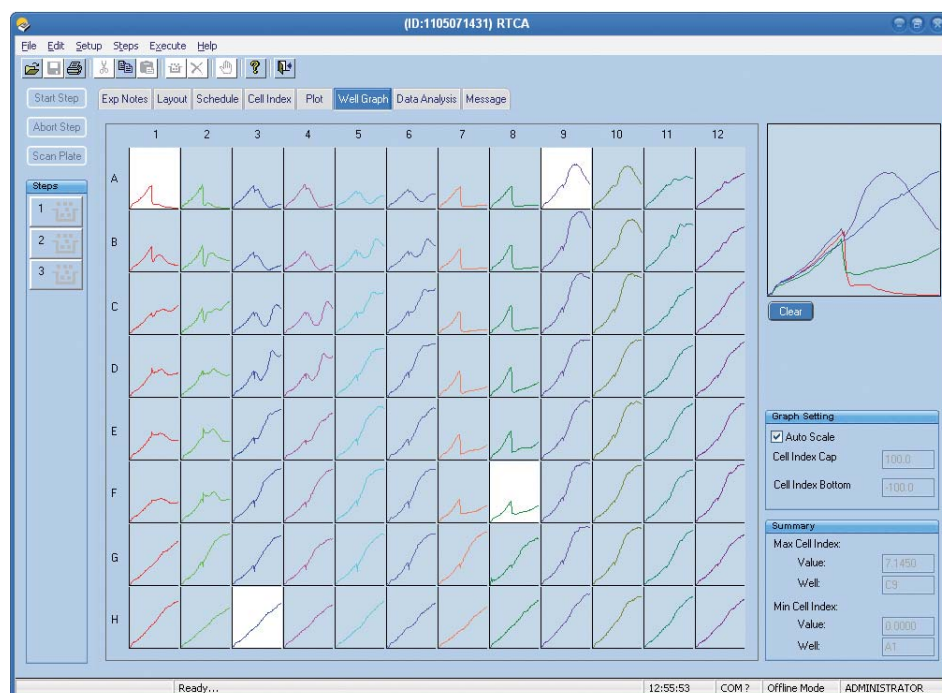


### 9.3 Zoom Area

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.

Users can also remove all the added curves (except the reference well curve) from the Zoom Area by clicking the *Clear* button.

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



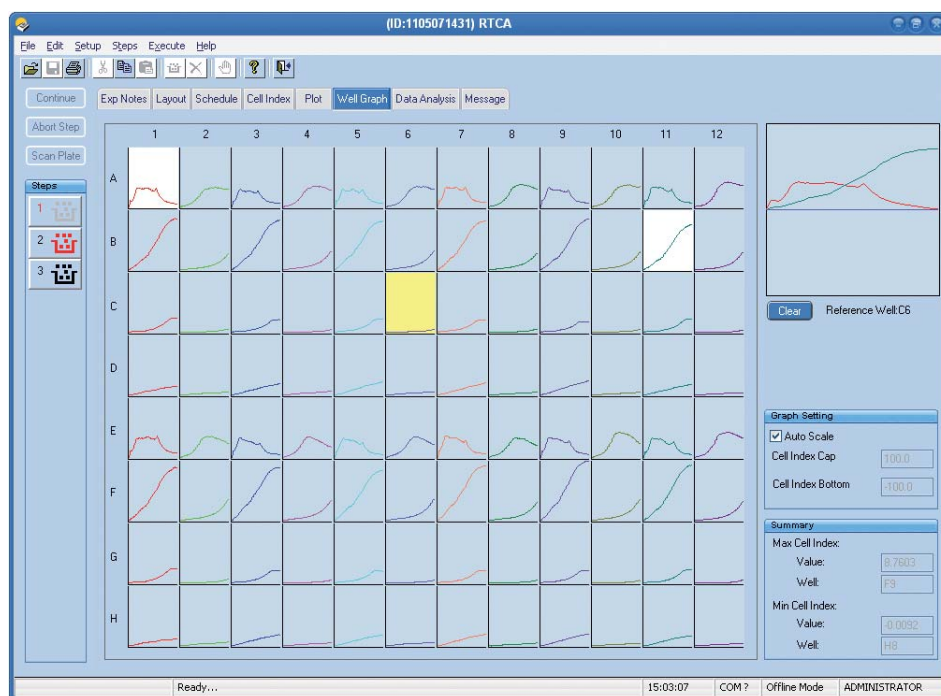
Users can also choose one of the curves on the Well Graph as a reference. Once a reference curve is chosen, points on the curves in the Zoom Area will be calculated by subtracting the reference Cell Index from the original Cell Index, *i.e.*:

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

### Follow these steps to draw a Reference Cell Index curve:

- ▶ Move mouse cursor to the well that you want to set as a reference
- ▶ Right-click the mouse to display a pop-up menu.
- ▶ Choose “select \*\* as a reference well”, where “\*\*” is the well ID (reference well will turn yellow).
- ▶ Add other wells to the Zoom Area by clicking them.

The following figure is an example of reference curves.



In the above figure, well C6 has been selected as a reference, so the wells (i.e.: A1 and B11) added to the Zoom Area are plotted as Reference Cell Index lines.

## 9.4 Graph Setting

Users can set the scale of the curves (both for Well Graph and Zoom Area) by using the functions in the *Graph Setting* area.

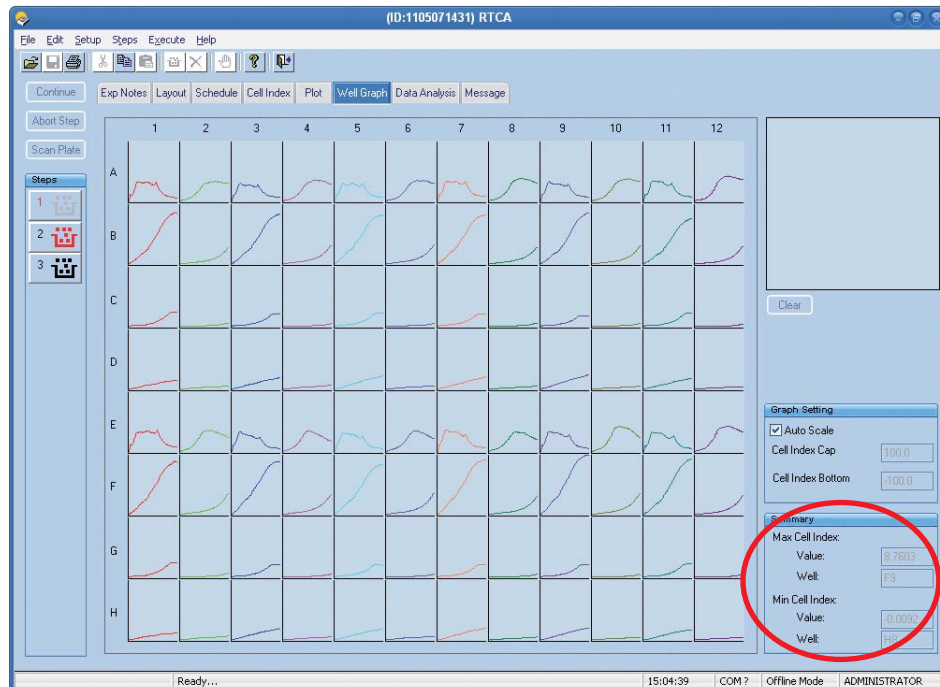
When the box for *AutoY* contains a checkmark, the Y-axis auto-scaling function is on. To turn it off, click on the box and the checkmark will be removed. When auto-scaling is off, users can change the Y-axis scale by typing numbers in the boxes for *Cell Index Cap* and *Cell Index Bottom*.



*The value of Cell Index Bottom must be less than that of Cell Index Cap.*

## 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 F9 and its value is 8.76003, and the well that has the minimal Cell Index is H8 and its value is -0.0092.



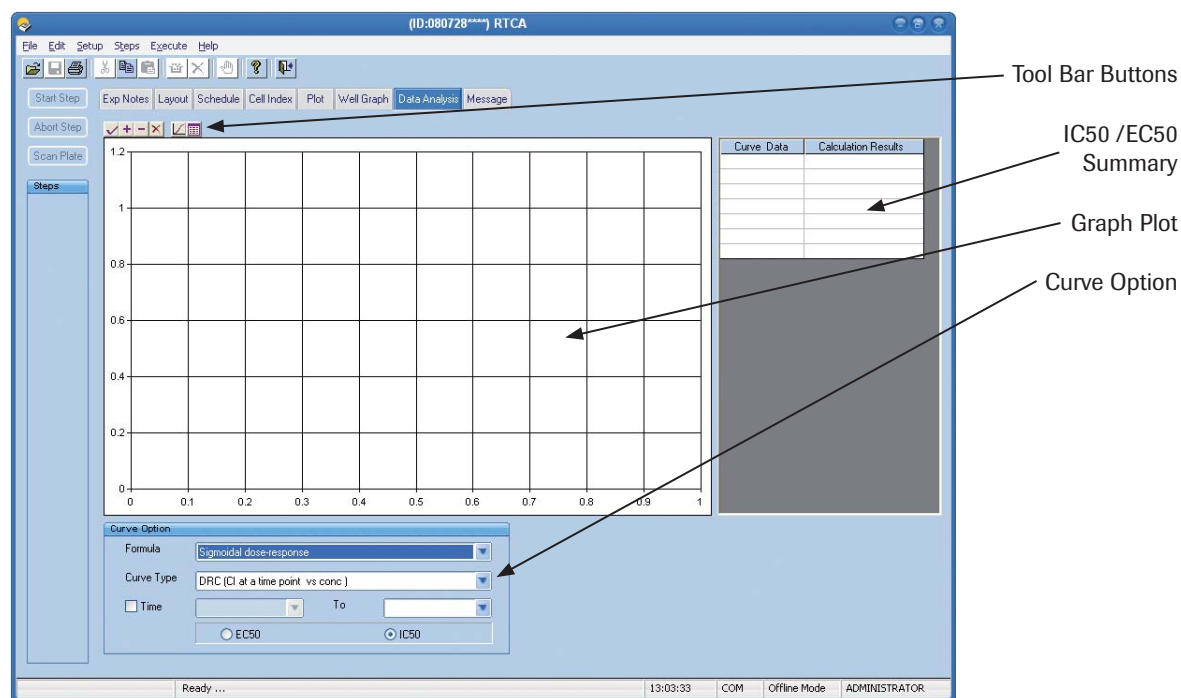
## 10. Data Analysis

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

- ▶ Calculate and display  $IC_{50}/EC_{50}$  values for experimental data where 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.

### 10.1 User Interface

The *Data Analysis* Page is divided into four main areas: Tool Bar Buttons, Graph Plot, Curve Option and  $IC_{50}/EC_{50}$  Summary Table.



## 10.2 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 the experiment, data analysis may be performed while the experiment is running and the Software is acquiring data in the background:



*If data acquisition is infrequent (for example, time interval is 15 minutes or more), then data analysis can be performed on an on-going experiment.*



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

## 10.3 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.*

B

## 10.4 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 one time point, select the following items:

- 1 Select *DRC (CI at a time point vs conc)* from the *Curve Type* drop-down list.
- 2 Choose *Sigmoidal dose-response* from the *Formula* drop-down list.
- 3 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, then calculates  $IC_{50}/EC_{50}$  values and values for other parameters in the equation.  $IC_{50}/EC_{50}$  values are shown in the Table on the right side of the Page.



The number in a yellow box next to each curve stands for 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 table due to limited space. There are tooltips available for each cell. If you want to see more information on each cell, just click on the respective cell. Then, a pop-up window containing information on the corresponding cell will open.



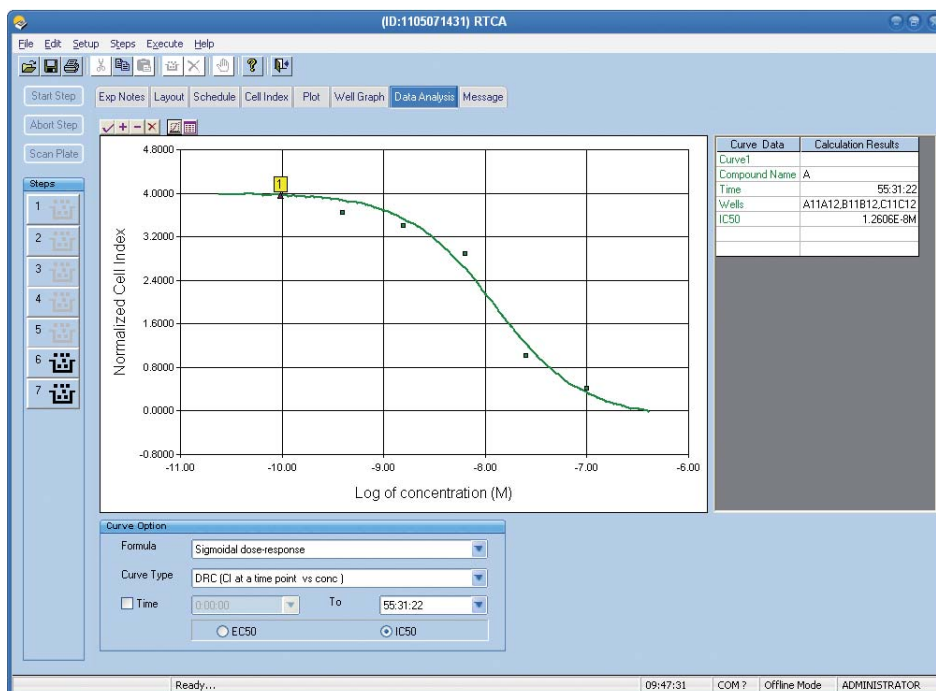
If you select a user defined unit (“-define-”) it is not possible to perform certain calculations on the *Data Analysis* page, e.g. DRCs. An error message will appear.

There is a checkbox on the lower left part of the *Data Analysis* page. By default, this box is unchecked. If a checkmark is placed in it, a blue line (start time) and a red line (end time) will be shown on the *Plot* page; these 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.



Curve Types and time period can be preselected in Settings for Data Analysis on the *Plot* page. See section 7.15.

To show curve-fitting details, move cursor onto the curve. The formula, curve type, time point, and fitted parameters will be shown in a box.





## 10.5 Curve Types and Curve-Fit Formula

### 10.5.1 Curve Types

There are eight curve types available:

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

 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. See section 7.15.

**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, then uses the chosen formula to perform curve-fitting and calculate  $IC_{50}/EC_{50}$  values at the chosen time point.

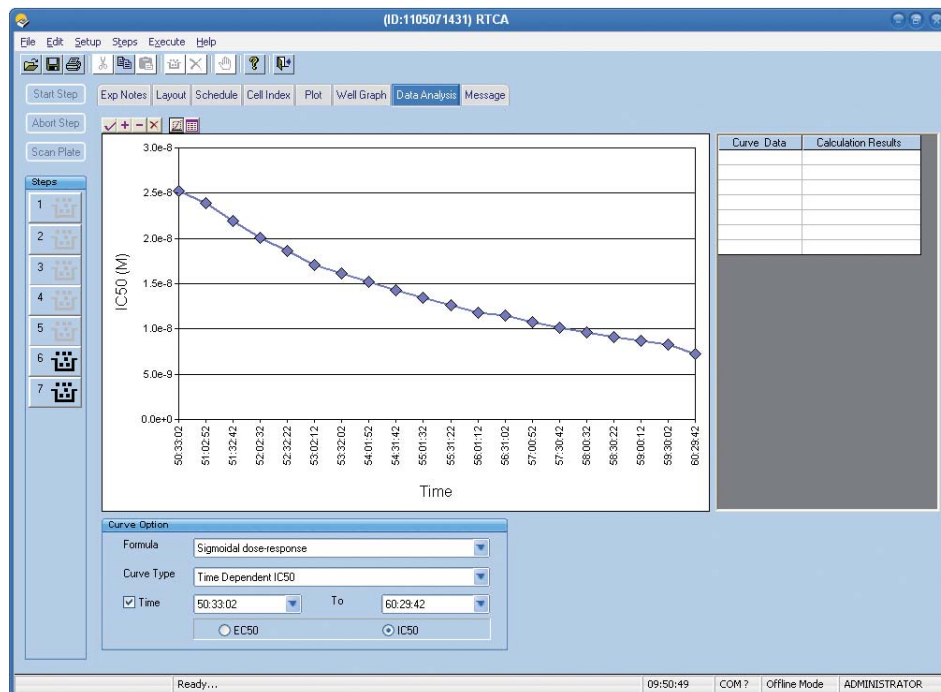
**DRC (max CI within a time period vs conc):** Shows dose-response curve (X-axis: compound concentration; Y-axis: maximum of Cell Index or normalized Cell Index in the chosen time period) for all selected wells, then uses the chosen formula to perform curve-fitting and calculate  $IC_{50}/EC_{50}$  values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

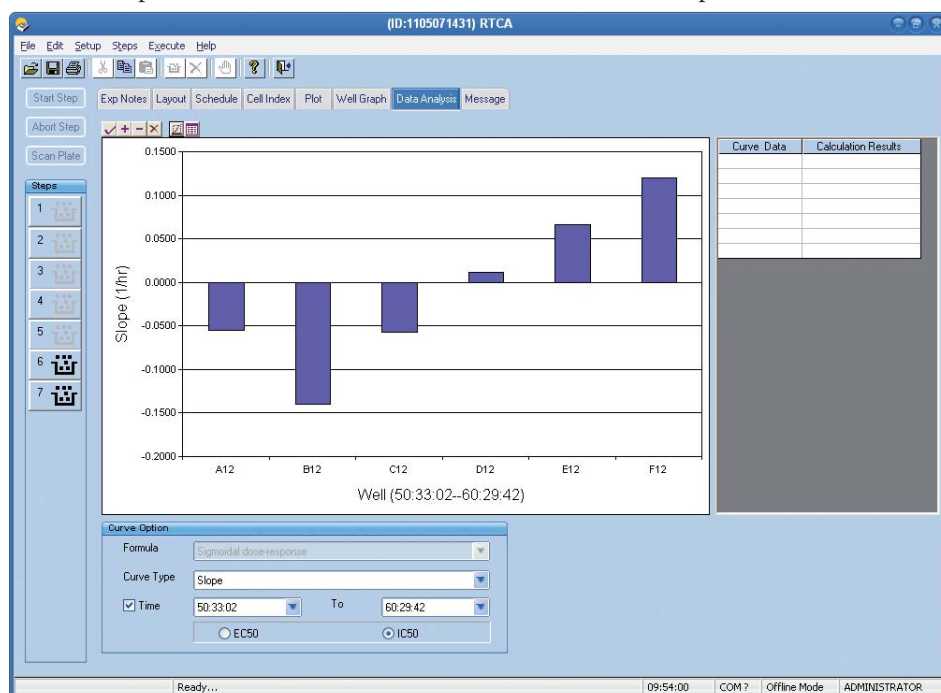
**DRC ((max - min) CI in a time period vs conc):** Shows dose-response curve (X-axis: compound concentration; Y-axis: (maximum minus minimum) of Cell Index or normalized Cell Index in the chosen time period) for all selected wells, then uses the chosen formula to perform curve-fitting and calculate 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

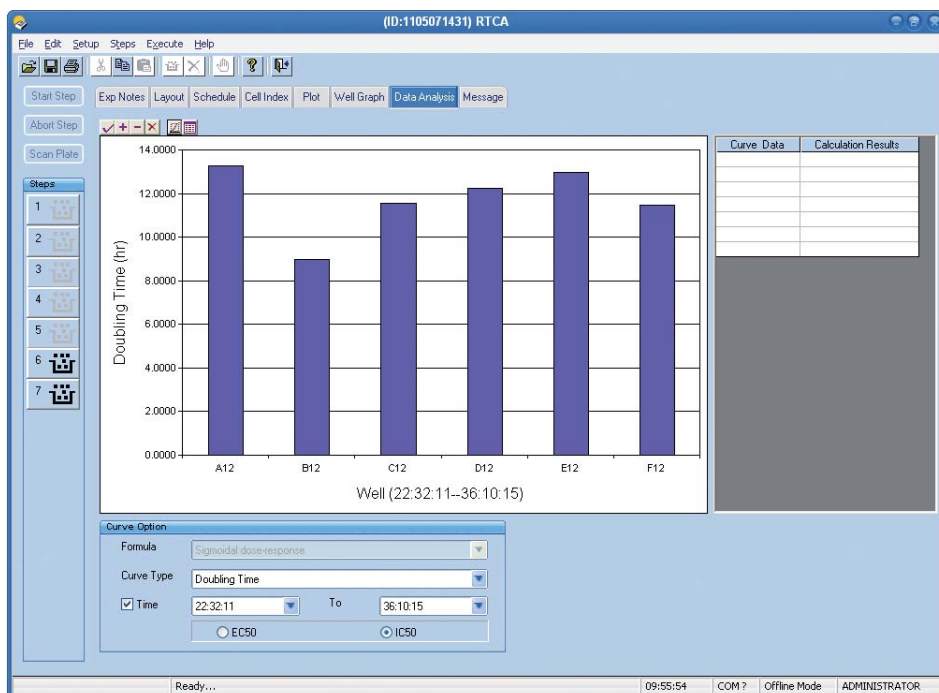
**Time Dependent  $IC_{50}$ :** Automatically extracts 20 time points within the chosen time period, calculates  $IC_{50}/EC_{50}$  values at each of these points, and draws a curve showing the time-dependence of  $IC_{50}$  or  $EC_{50}$  values (X-axis: time point; Y-axis:  $IC_{50}/EC_{50}$  values).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



**Slope:** For each selected well, the Software calculates the slope of the Cell Index (or Normalized 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 example illustrated below:



**Doubling Time:** 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 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 example illustrated below:



### 10.5.2 Curve Fit Formula

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

- 1 Sigmoidal dose-response  

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

$$Y = Bottom + (Top - Bottom) / (1 + 10^{-(\log EC_{50} - X) * HillSlope})$$

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  $EC_{50}$  would be used in the formulas and the calculated results would be labeled  $EC_{50}$  (for example, the sixth curve type would be called Time Dependent  $EC_{50}$ ).



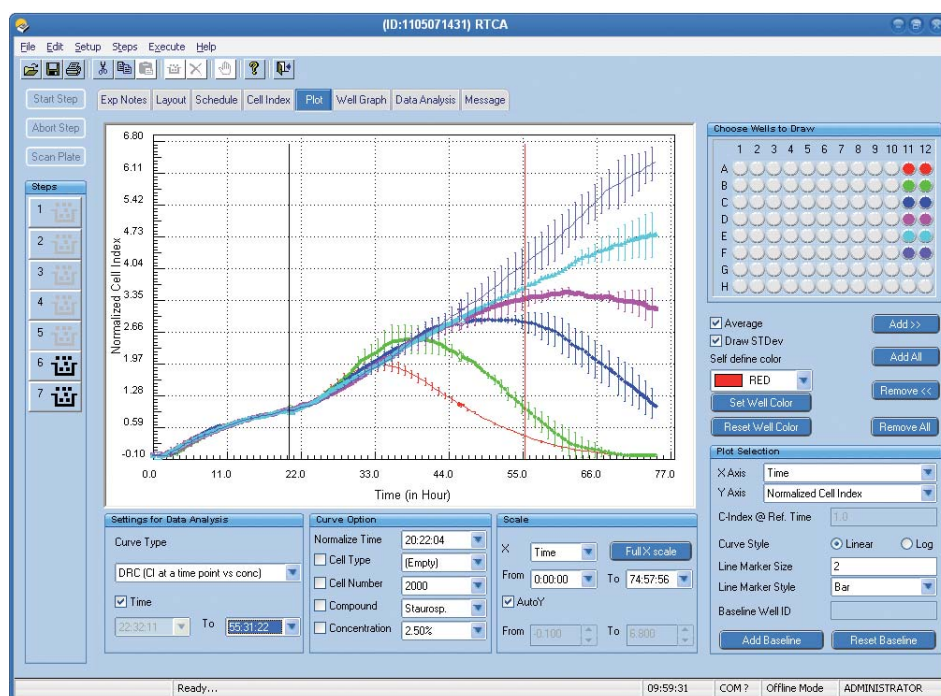
*The choice of  $EC_{50}$  or  $IC_{50}$  simply affects how the results are labeled and shown, but does not affect the results themselves.*



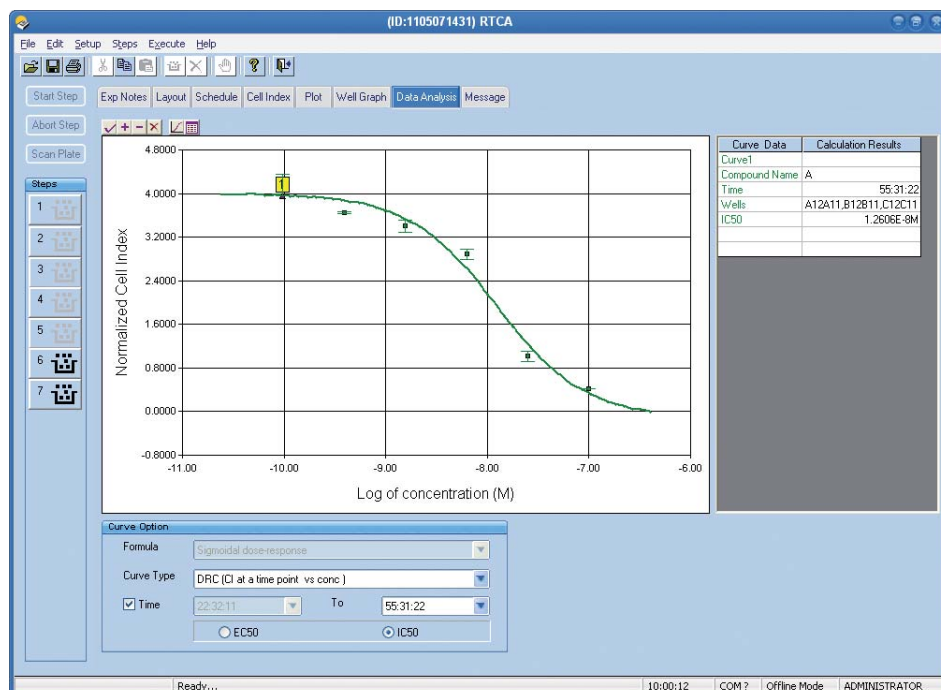
## 10.6 Data Analysis on Averaged Data for Replicate Wells

The *Data Analysis* page can process averaged Cell Index or averaged Normalized Cell Index for replicate wells. On the *Plot* page, select wells that have same *cell type* and same *compound name* (as listed on the *Layout* page). In addition, some of the selected wells should have exactly the same *concentration*. Put a checkmark in the *STDev* 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 at each time point, for corresponding wells in well column 11 (wells A11 to F11) and well column 12 (A12 to F12).





Switch to the *Data Analysis* page (by clicking the corresponding tab) and then select (or add) *DRC at a time point*. The software will plot a dose-response curve with standard deviation for each concentration point.

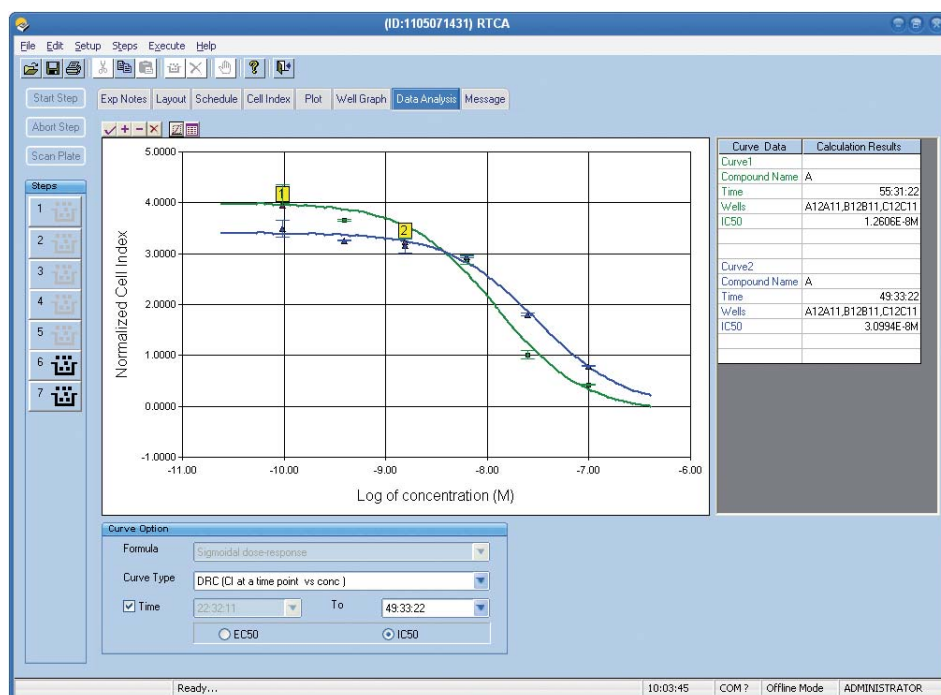


## 10.7 Compare Dose-Response Curves and Corresponding $EC_{50}/IC_{50}$ Values for Selected Wells at Different Time Points

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 at a time point* for the Curve Type, 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 Page. Please see the example illustrated below:

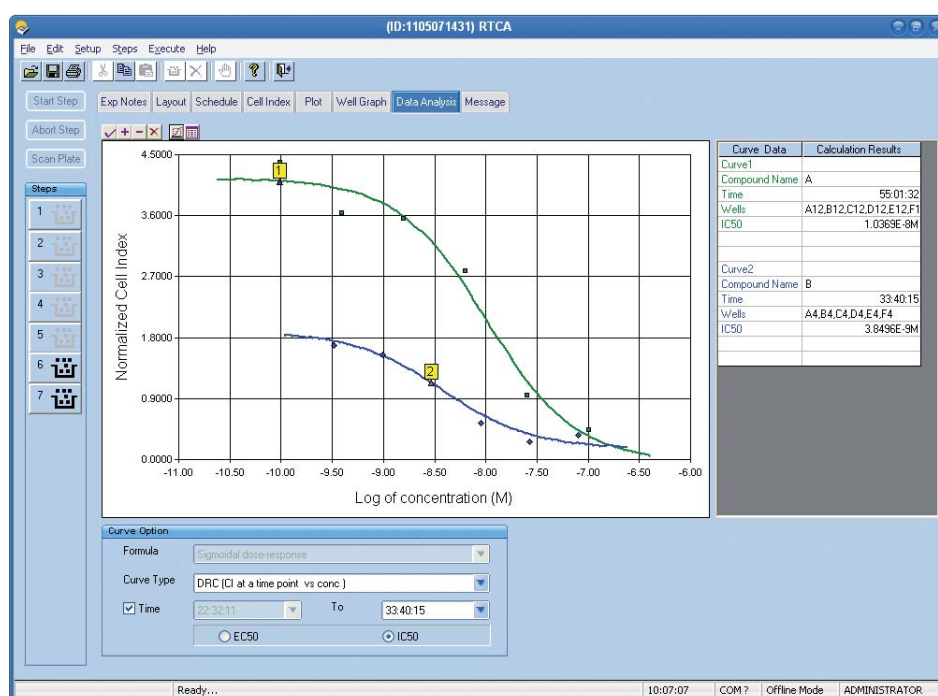


## 10.8 Compare Dose-Response Curves and Corresponding EC<sub>50</sub>/IC<sub>50</sub> Values for Different Wells at the Same Time Point

To compare DRC and corresponding EC<sub>50</sub>/IC<sub>50</sub> 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<sub>50</sub>/EC<sub>50</sub> data are shown in the table on the right side of the Page. Please see the example illustrated below:

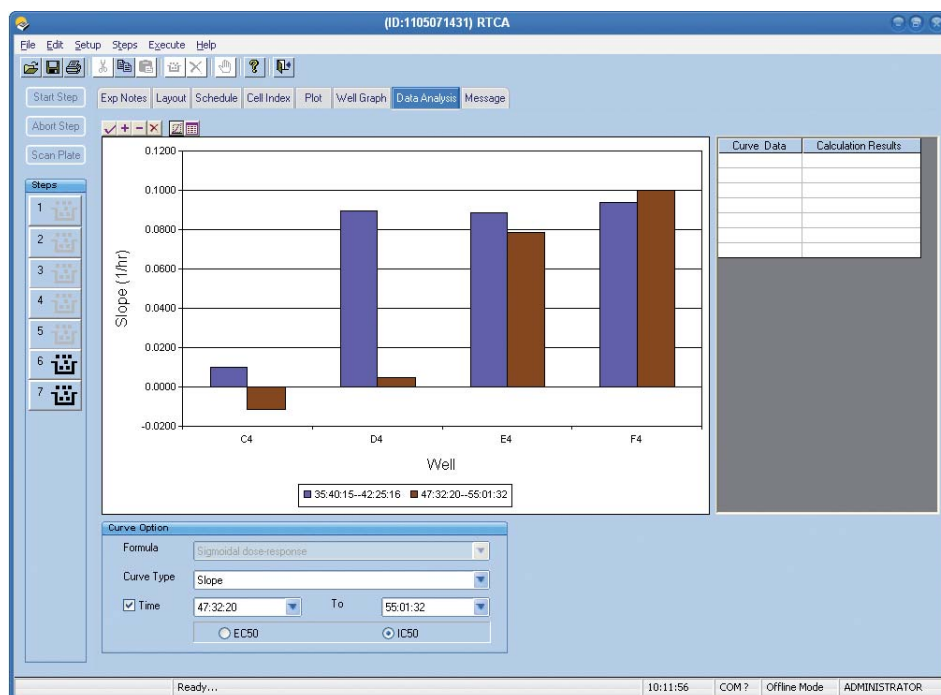


## 10.9 Compare Cell Index Slopes (or Cell Index Doubling Times)

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 that shows a comparison of slopes:



## 10.10 Tool Bar Button Definitions





### 10.10.1 Draw Curve, Add Curve and Clear Curve

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

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

### 10.10.2 Copy Chart and Copy Data on Chart

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

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

B

## 11. Message Page

The *Message* page provides two kinds of information:

### 11.1 Messages about Experiments

The top message box contains messages about issues and problems in RTCA experiments.

Two types of messages are included: One is a message from the Scan Plate function, which will read either *Plate scanned. Connections ok* or *Please check connections on the positions: well ID X:Y*.



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

The other message appears when the experiment is started. It lists the wells which may have background problems. This message is provided to help the user in planning the layout for the experiment. If you have problem wells, please check the connection of the E-Plate 96 and RTCA SP Station and follow recommendations for cleaning in the RTCA SP Instrument Operator's Manual. If problems persist, contact your local Roche representative. Please also send appropriate data files (please refer to the RTCA SP Instrument Operator's Manual and section 12 below).

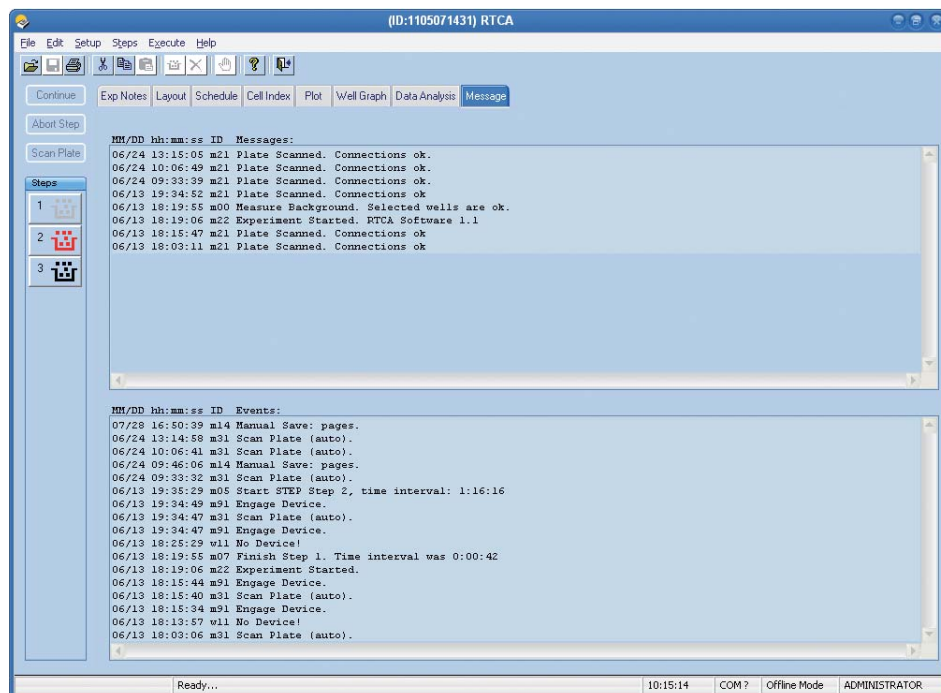
### 11.2 Experiment Monitoring Events

In the bottom message box, events that occur during each experiment are summarized. The recorded information includes the start and stop time of each step, the time the scan plate was performed, time for resuming the experiment, etc.

Additionally, this box lists errors that occurred during an experiment and were detected by the RTCA SP Instrument, along with error codes. Some error codes are related to the use of the instrument; for example, removal of the E-Plate 96 from the RTCA SP Station 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 Roche representative.

## 11.3 Example of a Message Page

Following figure shows a typical *Message* page appearing at the end of an experiment:



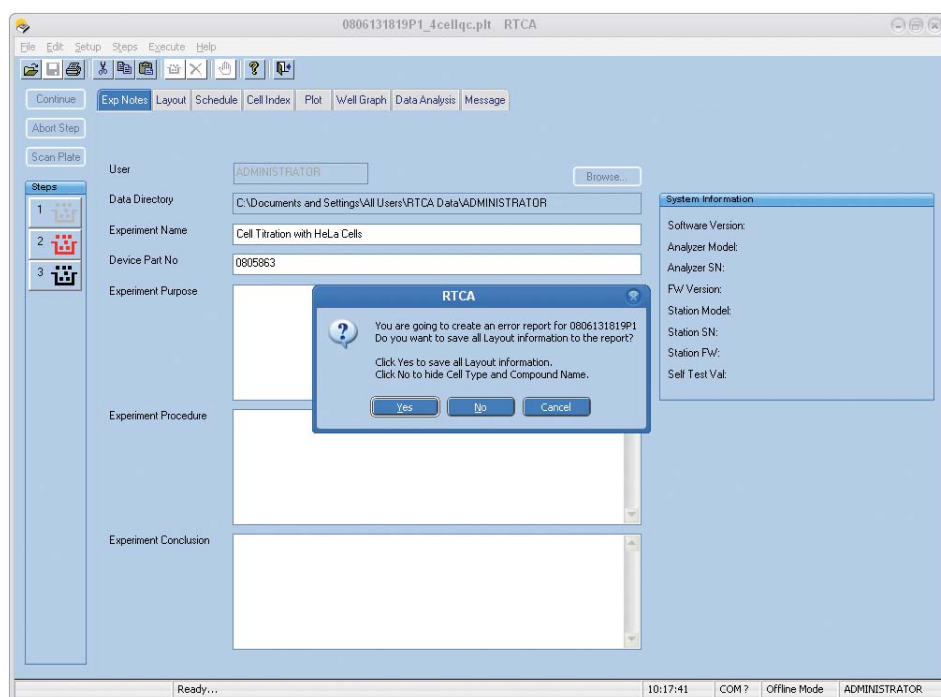


## 12. Create a Problem Report

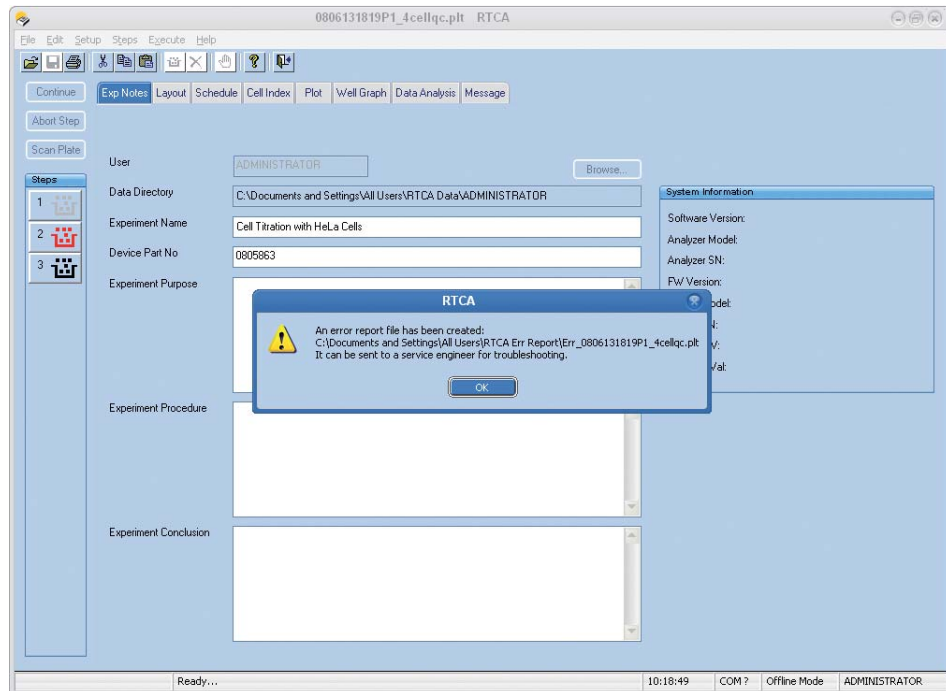
Whenever users want to contact a Roche representative for support (e.g., to solve recurring issues during an experiment), they must send a Problem Report file to allow precise assessment of the issue. To generate a Problem Report file, do the following steps:

- ▶ Launch the RTCA Software.
- ▶ Open the experiment file on which you want to report any issues.
- ▶ Open the *File* Menu and select *Create Problem Report*.
- ▶ A dialogue window pops up asking whether it is OK to save all the Layout information to the problem report file. Click *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.
- ▶ If you select either *Yes* or *No*, the RTCA Software will create a Problem Report file with the file name Err\_\*\*\*\*\*.plt.

Example: The dialogue window asks the user whether all Layout information can be sent to customer support.



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

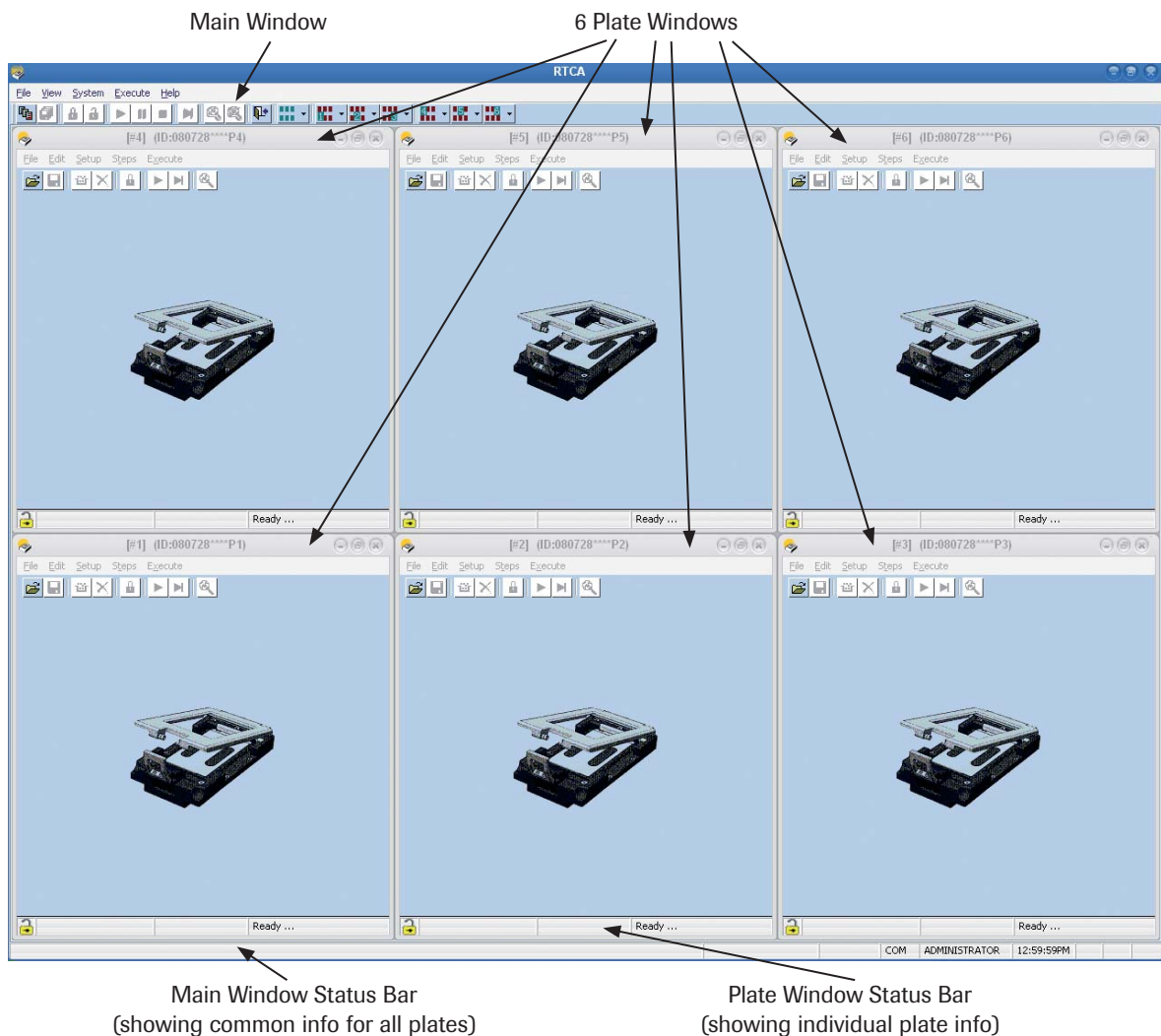


## 13. RTCA Software Functions Unique To The RTCA MP Instrument

This section is for users of the RTCA MP Instrument only. It describes the user graphic interfaces for the RTCA MP Instrument, followed by a description of RTCA Software functions that are common to both the RTCA SP Instrument and the RTCA MP Instrument. Finally, it discusses functions and operations unique to RTCA MP Instrument.

### 13.1 MP User Graphic Interface

The MP user graphic interface consists of a Main Window and six Plate Windows.



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 other Plate Windows.

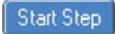





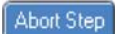



## 13.2 The Functions and Controls Common to both RTCA MP and RTCA SP Instruments

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 appearance and /or locations of the functions on an individual Plate Window may be different from those on the SP Window.*



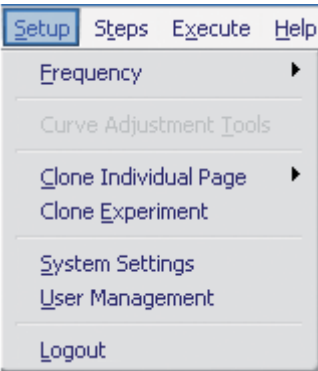
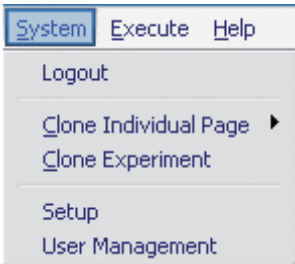
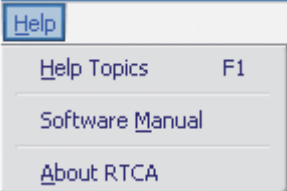
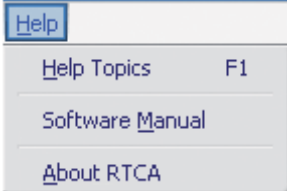
The Table below shows controls found in both the SP Window and the MP individual Plate Windows. However, either the appearances or locations of these functions are different.

SP	MP	Function	Comments
		Start a Step	One button that carries different labels according to experiment status
		Pause the running experiment	
		Continue a paused experiment	
		Abort a running Step	
		Scan Plate	

The Main Window contains the common functions that apply to all Plate Windows. Some of the Main Window functions have corresponding functions in the SP Window, such as *System Settings, User Management, Logout, and Help*. However, the MP Main Window also contains several new functions, such as *Start All, Pause All, Continue All, Abort All and Scan All*, which will be discussed in the next section.

B

The Table below shows the functions on the MP Main Window that have corresponding functions on the SP Window.


SP	MP	Function	Comments
		Logout	Function applies to all Plate Windows.
 <ul style="list-style-type: none"> <li>► [Setup]</li> <li>► Logout</li> <li>► System Settings</li> <li>► User Management</li> </ul>	 <ul style="list-style-type: none"> <li>► [System]</li> <li>► Logout</li> <li>► Setup</li> <li>► User Management</li> </ul>	Logout Set parameters Manage users	Functions apply to all plate windows.
		Help information	Function applies to all plate windows.

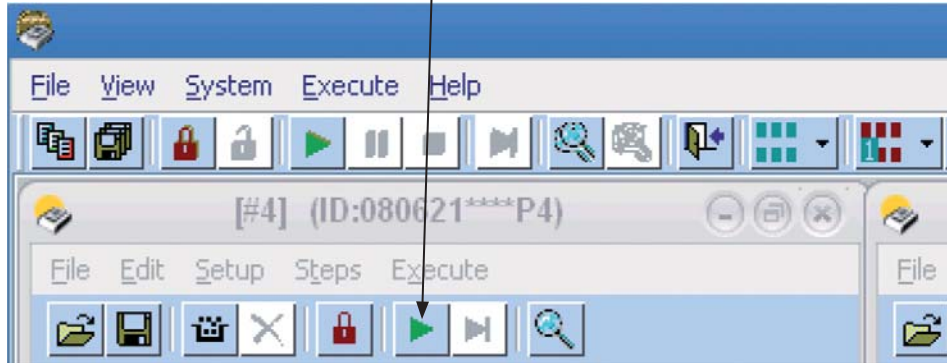
### 13.3 Overview of Functions and Controls Unique to the RTCA MP Instrument

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



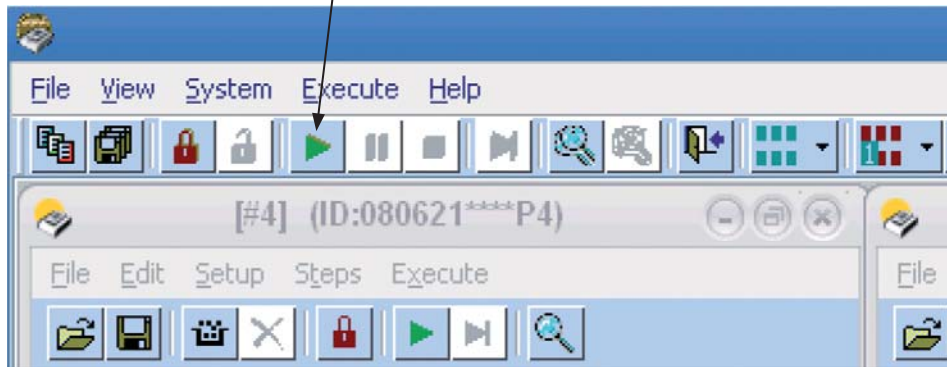
*Actions taken on each Plate Window are applied to that individual Plate Window only.*

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 login user.*












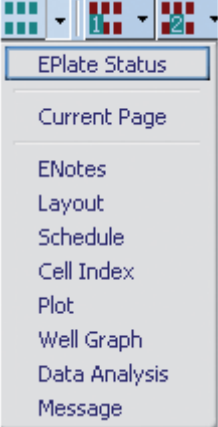
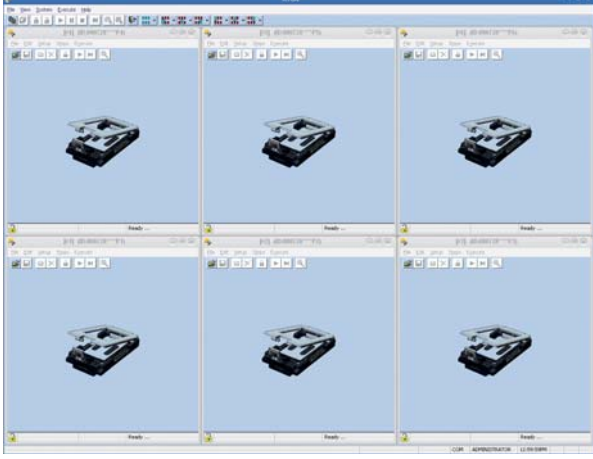
Clicking the *Start All* button  on a Main Window will start experiments on all valid plates.



*“Valid Plates” means:*

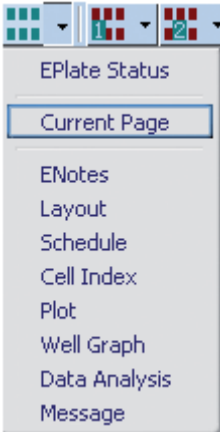
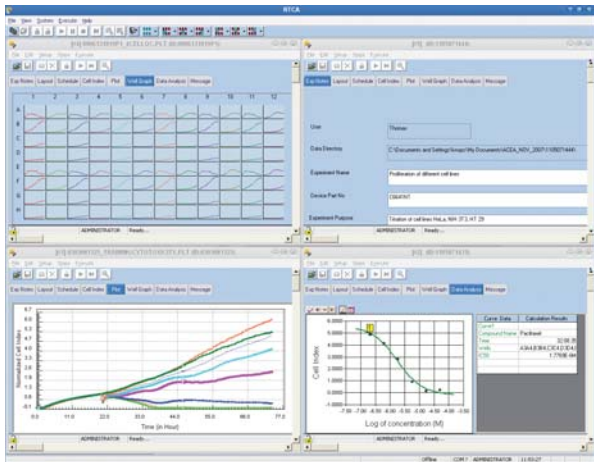
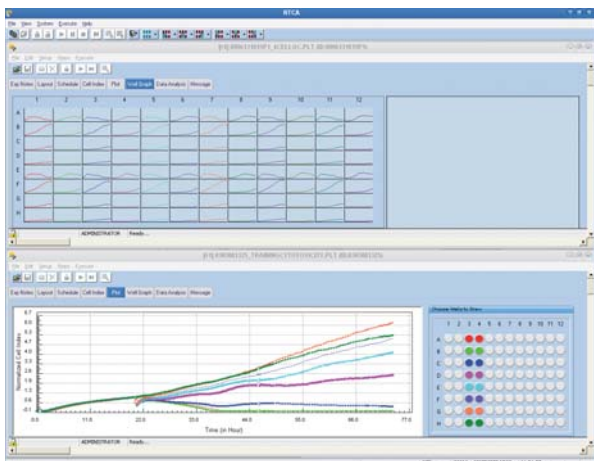

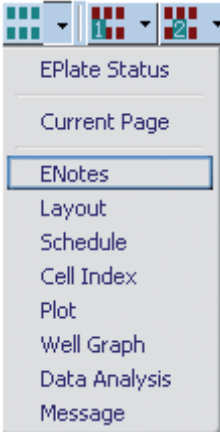
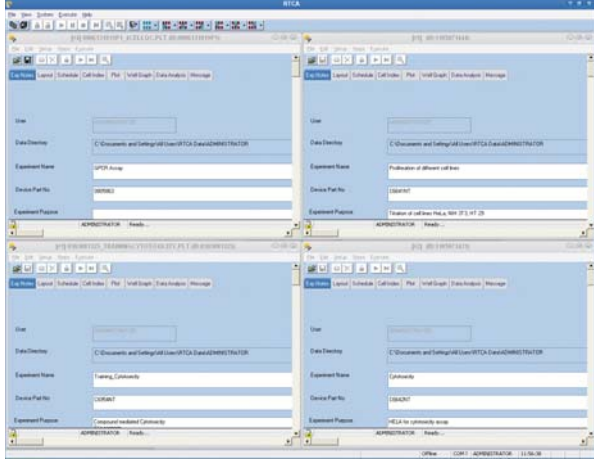
- 1) The E-Plate 96 is owned by the login user.
- 2) The E-Plate 96 experiment is ready to be started.

The Table below describes all the functions and controls unique to the RTCA MP Instrument.



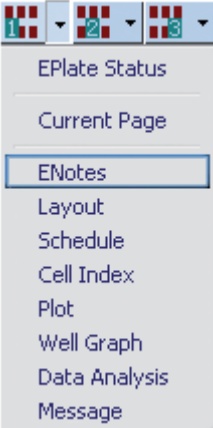

Controls	Function / Example	Comment
	Reopen the most recently opened experiment(s)	Any reserved plate window ignores this command.
	Save changes for plate window(s)	
	Lock cradle(s)	A plate window ignores this if it is locked already.
	Unlock cradle(s)	A plate window ignores this if it is unlocked already.
	Start / Continue experiment(s)	
	Pause experiment(s)	
	Cancel pending action(s)	Pending action: action issued, but has not been executed because analyzer is busy with another plate window
	Abort current running Step for plate window(s)	
	Scan plate window(s)	
	Stop Scan for plate window(s)	
	Restore all 6 plate windows to fit within the main window, and show cradle status for each plate window (see next 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)





Controls	Function / Example	Comment
 <p>EPlate Status</p> <p>Current Page</p> <p>ENotes</p> <p>Layout</p> <p>Schedule</p> <p>Cell Index</p> <p>Plot</p> <p>Well Graph</p> <p>Data Analysis</p> <p>Message</p>	<p>Resize plate window(s) owned by the login user within the main window, and keep current page displayed in plate window(s).</p>  	<p>The example shows:</p> <p>The logged-in user is using 4 plate windows, but the 4 plate windows are on different pages.</p> <p>Click “Current Page” under  to resize the 4 plate windows to fit within the main window, and keep all current pages displayed.</p> <p>If you do not want to show all of the plate windows you are using, you can minimize those plate windows, then click “Current Page”, to show only unminimized windows.</p> <p>(In example at left, the user has 4 plate windows, but two of them were minimized and are not shown)</p>
 <p>EPlate Status</p> <p>Current Page</p> <p>ENotes</p> <p>Layout</p> <p>Schedule</p> <p>Cell Index</p> <p>Plot</p> <p>Well Graph</p> <p>Data Analysis</p> <p>Message</p>		<p>Show <i>ExpNotes</i> Page(s) for plate window(s) owned by logged-in user.</p> <p>Similarly, if you chose one of the other pages (e.g., <i>Plot</i> page), the respective page is shown in plate window(s) owned by the logged-in user.</p>



Controls	Function / Example	Comment
	Maximize individual plate (P1 ~ P6) window. 	After plate window is maximized, 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 "ENotes" under  ) will maximize Plate 3 window, and show its <i>Exp Notes</i> page.	There are 6 numbered drop-down menus on the main window toolbar. Each controls its corresponding plate window.

## 13.4 Launch RTCA MP Software

Double-click RTCA 1.1 icon on the RTCA 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 RTCA MP Instrument and display.*

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

# B

### 13.4.1 RTCA Software in MP Offline Mode

When RTCA Software enters the MP offline mode, all hardware-related controls such as *Lock / Unlock*, *Scan Plate*, *Start / Abort Step* are disabled. You can still use the RTCA Software to analyze a previously run experiment, or design experiments for later use.



*Please check Section 4.8 and 4.9 for details on designing an experiment template.*

### 13.4.2 RTCA Software in MP Real-Time Mode

When 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 Station. 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 for details of the Scan Plate function.*



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

After Scanning 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 *ExpNotes* (optional), *Layout*, and *Schedule* pages



*Please check Sections 2.4 – 2.6 to learn how to set up ENotes page.*



*Please check Section 3 to learn how to set up Layout page.*



*Please check Section 4 to learn how to set up Schedule page.*

► Or Cloning individual page or experiments from:

► Saved templates

► Existing experiments



*Please check Sections 4.8 – 4.9 for details on Cloning of Experimental Settings.*

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

### 13.5 Example of Multiple Users Running Experiments on the RTCA MP Instrument










Suppose that three users use the RTCA MP Instrument to perform their own experiments, with each user running two E-Plates 96. The three users are: **User1**, **User2**, and **Administrator**.

The sequence of such experiments may occur according to the following steps:




#### User1, User2, and Administrator prepare E-Plates 96 plates

- 1 All three users prepare two E-Plates 96 each. 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 and User2 inserts two prepared E-Plates 96 into P3 and P4 cradles.

#### User1 starts experiment with E-Plates 96 on P1 and P2 cradles










- 3 Launch RTCA Software to enter MP Real-Time mode
- 4 Login to RTCA Software with username "**User1**"
- 5 Maximize P1 Plate Window
- 6 Setup ENotes Page, Layout Page, and Schedule Page
  -  Please see Sections 2.4 – 2.6 on setting up ENotes page.
  -  Please see Section 3 on setting up Layout page.
  -  Please see Section 4 on setting up Schedule page.
- 7 Click  on 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 E-Plate 96 in cradle 2.
- 9 Remove the E-Plates 96 from cradle 1 and cradle 2, then add cells to the E-Plate Wells.
  -  Leave the E-Plate 96 in the tissue culture hood for 30 minutes at room temperature, so the cells settle to the bottom of the well.
- 10 Replace the E-Plates 96 on cradle 1 and cradle2 and start Step 2 for the two E-Plates by clicking  on Main Window.
  -  Cradles 1 and 2 will be locked automatically when Step 2 starts.
- 11 **User1**  logout.

#### User2 starts experiment with E-Plates 96 on P3 and P4 cradles




- 12 Login to RTCA Software with username "**User2**".
  -  User2 cannot access the P1 Plate Window and P2 Plate Window.
- 13 **User2** works with P3 Plate Window and P4 Plate Window, following 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** login again.
- 
- 16 Click  "Abort All" from the Main Window. This aborts the current step for experiments on 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 Click  "Unlock All" from Main Window. 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** removes E-Plates 96s from cradle 1 and cradle 2 and adds compounds to wells of E-Plates 96.
- 
- 19 **User1** replaces E-Plates 96 on cradle 1 and 2 respectively. RTCA Software detects the presence of E-Plates 96 on cradle 1 and 2 and starts an auto-scan of E-Plates 96 on cradle 1 and 2.
-  *The RTCA Software auto-scans the plates in the order they were re-inserted into the cradle pockets.*
- 
- 20 **User1** Start Step 3 by click  on Main Window for the two E-Plates.
- 
- 21 **User1**  logout.

**Administrator logs in to RTCA Software to work with all the E-Plates 96 on RTCA MP Station.**

- 22 **Administrator** inserts two E-Plates 96 into P5 and P6 cradles respectively.
- 
- 23 **Administrator** works with P5 and P6 Plate Windows by repeating Step 5 to Step 9 to start experiments for E-Plates on P5 and P6 cradles.
- 
- 24 **Administrator** checks the data on P1 to P4 Plate Windows by plotting the Cell Index curves for selected wells on the plot page of each Plate Window.
-  *Administrator has the right to access all other users' experiments running on the RTCA MP Instrument.*
- 
- 25 **Administrator**  logout.
- 
- 26 **Experiments for all six E-Plates on RTCA MP Station continue and RTCA Software is ready for User1, User2 or Administrator to login.**
-  *All six E-Plates 96 are locked so that no one can accidentally remove an E-Plate 96 without logging onto the RTCA Software.*

B

## C Appendix

### 1. Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and information, please visit and bookmark our home page, [www.roche-applied-science.com](http://www.roche-applied-science.com), and visit our special interest sites for

- ▶ The xCELLigence System:  
[www.roche-applied-science.com/sis/xcelligence](http://www.roche-applied-science.com/sis/xcelligence)
- ▶ Apoptosis and Cell Proliferation:  
[www.roche-applied-science.com/sis/apoptosis](http://www.roche-applied-science.com/sis/apoptosis)
- ▶ Cutting-Edge Transfection Reagents:  
[www.roche-applied-science.com/sis/transfection](http://www.roche-applied-science.com/sis/transfection)

#### Roche Applied Science Service and Support

At Roche Applied Science we are committed to providing innovative, high-quality instruments and reagents combined with excellent customer service – offering powerful tools to address the evolving needs of life science researchers worldwide.

Whether you need expert technical support, online access to comprehensive product information, convenient on-site product supply service and online ordering, or outstanding customer service to ensure accurate and timely product delivery, we provide a wealth of resources to help you achieve your research goals.

For more information, visit [www.roche-applied-science.com](http://www.roche-applied-science.com) to explore our products and services or to find a local representative.

<b>RTCA Analyzer</b>	<b>Cat. No. 05 228 972 001</b>
<b>RTCA SP Station</b>	<b>Cat. No. 05 229 057 001</b>
<b>RTCA MP Station</b>	<b>Cat. No. 05 331 625 001</b>
<b>RTCA Control Unit</b>	<b>Cat. No. 05 229 014 001</b>
<b>RTCA Protector Shield 96</b>	<b>Cat. No. 05 288 967 001</b>
<b>RTCA Resistor Plate 96</b>	<b>Cat. No. 05 232 350 001</b>
<b>RTCA Frame 96</b>	<b>Cat. No. 05 232 392 001</b>
<b>E-Plate 96</b> 6 Units	<b>Cat. No. 05 232 368 001</b>
6 × 6 Units	<b>Cat. No. 05 232 376 001</b>
<b>RTCA Contact Pins 96 (20 units)</b>	<b>Cat. No. 05 232 384 001</b>
<b>RTCA Software Package 1.1</b>	<b>Cat. No. 05 358 078 001</b>









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