
FDSS7000EX/uCELL Software Instruction Manual

Version 2.9.5

Thank you for your purchase.

 **CAUTION**

- Follow the safety precautions in order to avoid personal injury and damage to property when using this system. The manual describes the correct handling method of the system and provides cautions in order to avoid accidents. **Read this manual carefully** beforehand use the system correctly. After reading the manual, store it in a location where you can refer to it at any time.

Note

FDSS7000EX is an upper compatible model of FDSS7000 and this software can be used for both FDSS7000 and FDSS7000EX.

Hamamatsu Photonics K.K.



HAMAMATSU





(This page is blank.)


SAFETY PRECAUTIONS

CLASSIFICATION OF WARNING

We have classified the warnings symbols that appear in this instruction manual and on the system as follows for your convenience. Make sure that you fully understand them and obey the instructions they contain.

 WARNING	Improper handling of the system without observing these warnings could lead to serious injury to the user and even death.
 CAUTION	Improper handling of the system without observing these cautions could lead to personal injury to the user or damage to property.

 Note	This symbol indicates a note to help you get the best performance from the system. Read the contents of the note carefully to ensure correct and safe use. Failure to observe one of these notes might impair the performance of the system.
	This symbol indicates a cautionary item that should be obeyed when handling the system. Read the contents carefully to ensure correct and safe use.
	This symbol indicates an action that is forbidden. Read the contents carefully and be sure to obey them.
	This symbol indicates a compulsory action or instruction. Read the contents carefully and be sure to obey them.

 Note	In the software, a character of 'μ' is not indicate. So, in the software window, a character of 'u' is used instead of 'μ'. For example, ul is used in stead of μl.
---	---

SOFTWARE USER AGREEMENT

In order to use the software that comes with this product (indicates entire product if software is a single product item; hereafter called "this software"), the user must agree to the following terms and conditions. Always be sure to read these terms before use.

Copyrights

All ownership rights, intellectual property rights and all other rights relating to this software and instruction manual are the property of Hamamatsu Photonics K. K. (hereafter called "Hamamatsu"). Except for those items expressly permitted in this user agreement, Hamamatsu does not assign or grant any rights to the user, and Hamamatsu retains all rights relating to this software and instruction manual.

Scope of usage rights

Hamamatsu grants the user non-exclusive rights to use of this software subject to the following conditions.

- (1) User may install and use this software on a single computer.
- (2) User may copy this software only for the purpose of making a backup.
- (3) User may not analyze, change or modify this software.
- (4) User may transfer this software to a third party provided that all of the following conditions are fulfilled.
 - User transfers all software products including this software and related materials and copies of this software, and retains absolutely none of the materials or copies of this software.
 - The third party who will receive this software agrees to the terms in this software user agreement

Scope of warranty Limited Warranty

- (1) If the storage media containing this software is found to be defective during the warranty period and this software does not properly function, then this software will be replaced free of charge or a substitute product given to the user.
- (2) Hamamatsu accepts absolutely no liability in any case whatsoever, for damages or losses arising directly or indirectly relating to or incidental to use of this software.
- (3) If an error (bug) is discovered in this software and is corrected, then the corrected software or software for making the correction (hereafter written as "correction software") or information relating to making the correction

will be provided. However, the necessity, the time, and the period for providing the correction software or information relating to the correction will be decided at the discretion of Hamamatsu.

Disclaimer of liability for damages

The legal liability of Hamamatsu including warranty against defects relating to this software and its usage is limited to the contents of this software user agreement. Hamamatsu shall not be liable to provide compensation for any damage or loss resulting from using this software, including direct, indirect or incidental damages or losses to the user. This holds true even if Hamamatsu or its suppliers have been advised of the possibility of such damages or losses.

Governing law

This software user agreement shall be governed by the laws of Japan.

Others

If a conflict or doubt should arise regarding this user license agreement or items not established within this user license agreement, then that matter shall be resolved by mutual consultation carried out in good faith by both parties. In the event a lawsuit should arise, it shall be resolved by a court of law having jurisdiction in the area where the head office of Hamamatsu Photonics is then located.

CONTACT INFORMATION

HAMAMATSU PHOTONICS K. K., Systems Division

812 Joko-cho, Higashi-ku, Hamamatsu City, 431-3196, Japan
Telephone (81) 53-431-0124, Fax: (81) 53-435-1574
E-mail: export@sys.hpk.co.jp

U.S.A. and Canada:**Hamamatsu Corporation**

360 Foothill Road, Bridgewater, N.J. 08807-0910, U.S.A.
Telephone: (1)908-231-0960, Fax: (1)908-231-0852
E-mail: usa@hamamatsu.com

Germany:**Hamamatsu Photonics Deutschland GmbH**

Arzbergerstr. 10, D-82211 Herrsching am Ammersee, Germany
Telephone: (49)8152-375-0, Fax: (49)8152-265-8
E-mail: info@hamamatsu.de

France:**Hamamatsu Photonics France S.A.R.L.**

19, Rue du Saule Trapu, Parc du Moulin de Massy, 91882 Massy Cedex, France
Telephone: (33)1 69 53 71 00, Fax: (33)1 69 53 71 10
E-mail: infos@hamamatsu.fr

United Kingdom:**Hamamatsu Photonics UK Limited**

2 Howard Court, 10 Tewin Road, Welwyn Garden City
Hertfordshire AL7 1BW, United Kingdom
Telephone: (44)1707-294888, Fax: (44)1707-325777
E-mail: info@hamamatsu.co.uk

North Europe:**Hamamatsu Photonics Norden AB**

Smidesvågän 12, SE-171 41 Solna, Sweden
Telephone: (46)8-509-031-00, Fax: (46)8-509-031-01
E-mail: info@hamamatsu.se

Italy:**Hamamatsu Photonics Italia S.R.L.**

Strada della Moia, 1/E 20020 Arese (Milano), Italy
Telephone: (39)02-935 81 733, Fax: (39)02-935 81 741
E-mail: info@hamamatsu.it

China:**Hamamatsu Photonics (China) Co., Ltd.**

1201 Tower B, Jiaming Center, 27 Dongsanhuan Beilu, Chaoyang District, Beijing 100020, China
Telephone: (86)10-6586-6006, Fax: (86)10-6586-2866
E-mail: hpc@hamamatsu.com.cn

- The contents of this manual are subject to change without notice.
- The unauthorized reproduction or distribution of part or all of this manual is prohibited.
- If one of the following problems occurs, contact Hamamatsu Photonics. (See the Contact Information.) We will deal with the problem immediately.
 - Some contents of the manual are dubious, incorrect or missing.
 - Some pages of the manual are missing or in the wrong order.
 - The manual is missing or dirty.

Table of Contents

1. FDSS software installation	
1) Before FDSS software installation	7
2) FDSS software installation	7
3) Microsoft Windows operation	8
4) About a power supply option setup of Windows.	8
2. How to operate FDSS (Easy Manual)	
1) Starting system	9
2) System exiting	12
3) Workflow of FDSS application software	15
4) Measurement example	16
5) Loading data file	21
6) Dispensing head / tip / ligand plate exchanging.....	22
7) Data display with correction	26
8) Correction data creation	27
9) Auto fluorescence correction data (at fluorescence) / Dark correction data (at luminescence[Analog][EMCCD]) creation.	29
10) Shading correction data creation	31
3. How to operate FDSS software	
1) Starting the software.....	35
2) Measurement method choice.....	36
3) Combination between dispensing head and tip (FDSS7000EX)	37
4) Combination between dispensing head (uCELL).....	38
5) Main Window	39
6) Running a command	40
7) Toolbar.....	41
8) Plate choice.....	43
9) Quitting software	44
4. Protocol Menu	
1) Assay protocol setting (Setup & Acquire command).....	46
2) External Control (Start External Control command)<Option>	69
5. Measurement	
1) Workflow for measurement	72
2) Confirmation using plate,filter,tip	73
3) Aspirating ligand from loading line (Only at the configuration which has just one loading line.).....	73
4) Assay Plate In	74
5) Dispensing head tip/Ligand plate exchanging	75
6) Dispensing/Aspirating before measurement	76
7) Pre Incubation	76
8) Camera sensitivity setting at fluorescence and luminescence [Analog][EMCCD] measurement.....	77
9) Data acquisition start.....	78
10) Measurement finish	79
6. File menu	
1) FDSS Plate Data loading (Open Plate Data command)	82
2) Text output (Text Out Data command)	84
3) Graphs printout (Print Out command).....	89
4) Save the display (Save by Bitmap command)	90

5) The graph is displayed to new window (Display Graph in new window command).....	91
6) The graph is copied to the clipboard(Copy graph to clipboard command)	92
7) Output of two or more data (Batch Export command)	93
8) FDSS software quitting (Exit command)	98
7. View menu	
1) Toolbar (Tool Bar command)	100
2) Status bar (Status Bar command)	100
3) Zoom/Unzoom Graph (Zoom/Unzoom Graph command).....	101
8. Setp menu	
1) Supervisor Password (Supervisor Password command).....	104
2) Microplate Setup (Microplate setup command)	105
3) Microplate Mapping (Microplate mapping command).....	106
4) Auto Fluorescence / Dark (Auto Fluorescence / Dark command)	112
5) Shading (Shading command).....	116
6) Filter Combination (Filter Combination command)	124
7) I/F Port (I/F Port command)	126
9. Help menu	
1) About FDSS (About FDSS command).....	128
10. Plate Data display and analysis	
1) Over View	129
2) All wells graph display	130
3) Remark graph display	131
4) Correlation diagram display	132
5) Micro plate pseudo color display	134
6) Calculation table display	135
7) Data Control box	142
11. Filter Control (at fluorescence measurement)	
1) Filter Control box	157
12. Dispenser Control box	
1) Dispenser Control box (In the case of FDSS3000/6000/7000EX).....	159
2) Dispenser Control box (In the case of uCELL)	163
3) Height setting on Trituration	169
4) Exchange of dispensing head & tip and ligand plate (In the case of FDSS7000EX)	170
5) Exchange of dispensing head and ligand plate (In the case of FDSS3000/6000).....	174
6) Exchange of dispensing head and ligand plate (In the case of uCELL).....	175
13. Camera Control box	
1) Camera Control box (at fluorescence & luminescence [Analog][EMCCD] measurement)	180
2) Camera Control box (at luminescence [Photon Counting] measurement)	181
3) Image Window ,Microplate Window	182
4) Live Toolbar	182
5) Color setup box	183
6) Enhance setup box	184

1. FDSS software installation

Note

Hamamatsu engineer installs software basically.

1) Before FDSS software installation

Before installing the FDSS application software, check that Microsoft Windows 7 or XP is installed in the computer. If not, install it according to the Microsoft Windows Reference Manual.

All the FDSS software is supplied on CD-ROM. The setup program contained on the disk allows easy installation of the software.

The setup program performs the following tasks.

1. Creating the FDSS directory
When the FDSS directory is not created, the setup program automatically creates it.
2. Copying files
The setup program copies the FDSS source module run files and all other necessary files into the FDSS directory.

2) FDSS software installation

If other application software is installed, check that it is not running.

1. Insert Install CD-ROM into the CD-ROM drive of the computer.
2. Select "Run..." from the Start menu.
3. The dialog box then appears, so enter "<CD-ROM Drive>:\FDSSXXXX Ver.2.9.5\Disk1\setup" and click "OK". (Enter "E:\FDSS7000EX Ver.2.9.5\Disk1\setup" when installing the software from drive E. The splash screen and the first setup dialog box soon appear.
 - 3-1) In the case of FDSS7000EX
(CD-ROM Drive):\FDSS7000EX Ver.2.9.5\Disk1\setup
 - 3-2) In the case of uCELL
(CD-ROM Drive):\uCELL Ver.2.9.5\Disk1\setup
 - 3-3) In the case of FDSS Offline
(CD-ROM Drive):\FDSS Offline Ver.2.9.5\Disk1\setup
4. Follow the instructions displayed on the screen and proceed to the subsequent steps.

3) Microsoft-Windows operation

This manual does not include a description of how to operate Microsoft Windows. If you are not familiar with Microsoft Windows, you should first read the Microsoft Windows User's Guide and make yourself understood it

4) About a power supply option setup of Windows

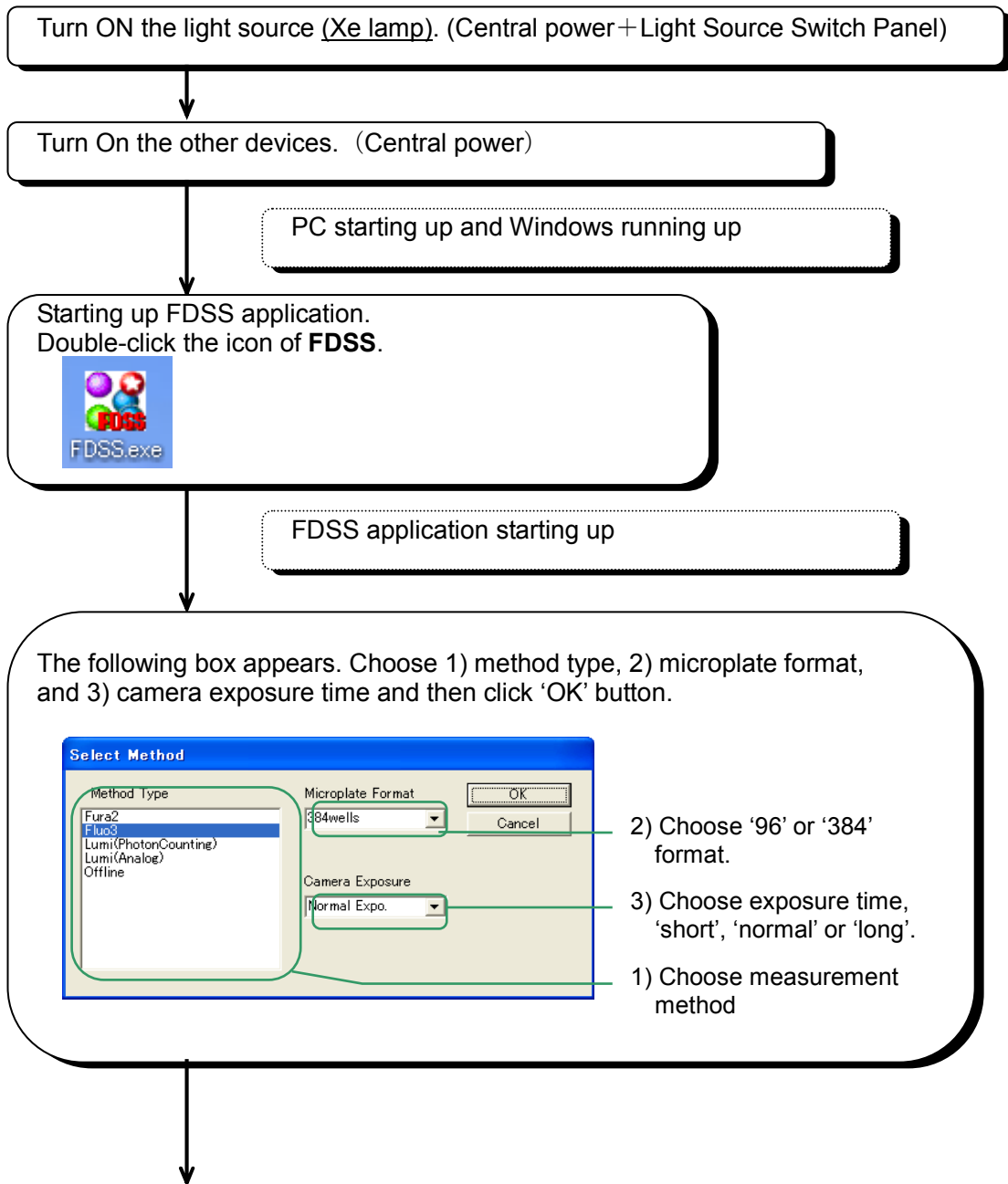
Please set a sleep setup of a power supply option as OFF.

A problem may arise in connection of a camera after a sleep return.

2. How to operate FDSS (EASY MANUAL)

1) Starting system

(1-1) In the case of FDSS3000/6000/7000EX

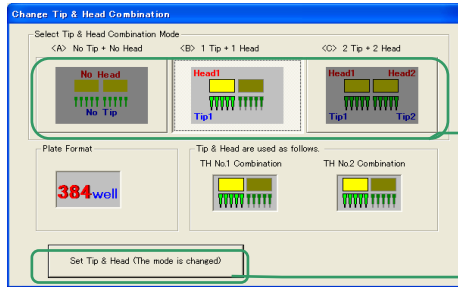


< In the case of FDSS7000EX >

After door of dispenser open, the following box appears.

First of all, Place the 1) Head, 2) Tip used

Choose the combination between 'Head' and 'Tip' used, and then click 'Set Tip & Head' button



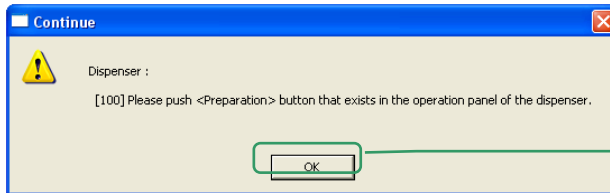
Chose the combination between 'Head' and 'Tip'

Click 'Set Tip & Head' button

After door of dispenser close, the following box appears.

Click 'OK' button after push 'Preparation button' of front panel of dispenser.

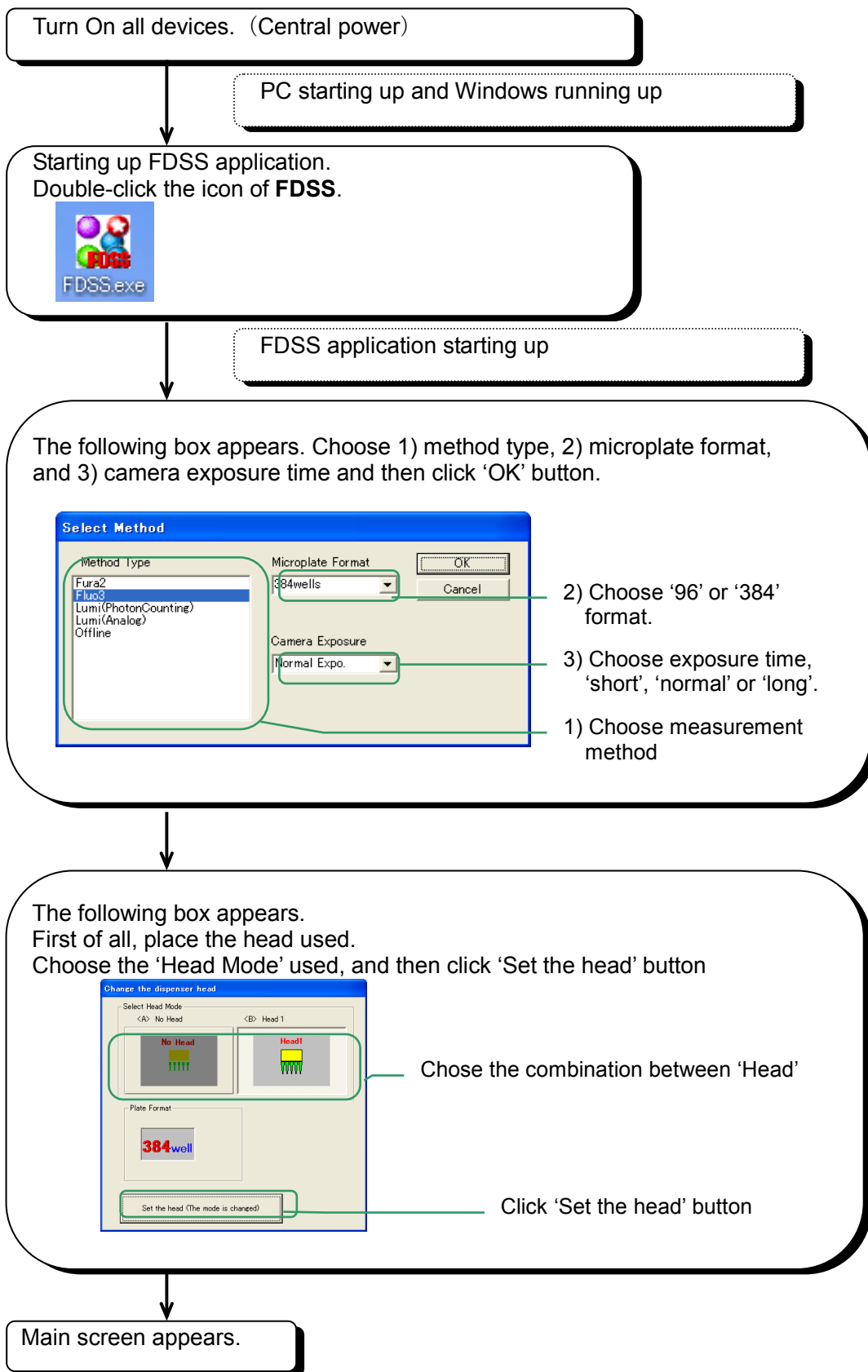
(It is not displayed according to the system, and Preparation is done by the automatic operation.)



Click 'OK' button

Main screen appears.

(1-2) In the case of uCELL

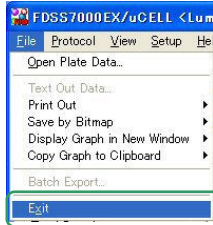


2) System exiting

(2-1) In the case of FDSS3000/6000/7000EX

Exiting FDSS application software

Choose Exit command in File menu.

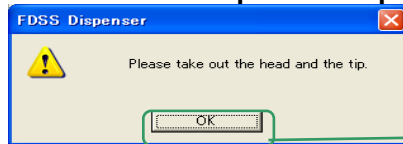


Choose 'Exit'.

< In the case of FDSS7000EX >

After door of dispenser open, the following box appears.

Bring out 'Head' and 'Tip' from dispenser and then Click OK button.



Click 'OK' button.

After door of dispenser close, the following box appears.

Click 'OK' button after push 'Preparation button' of front panel of dispenser. (It is not displayed according to the system, and Preparation is done by the automatic operation.)



Click 'OK' button.

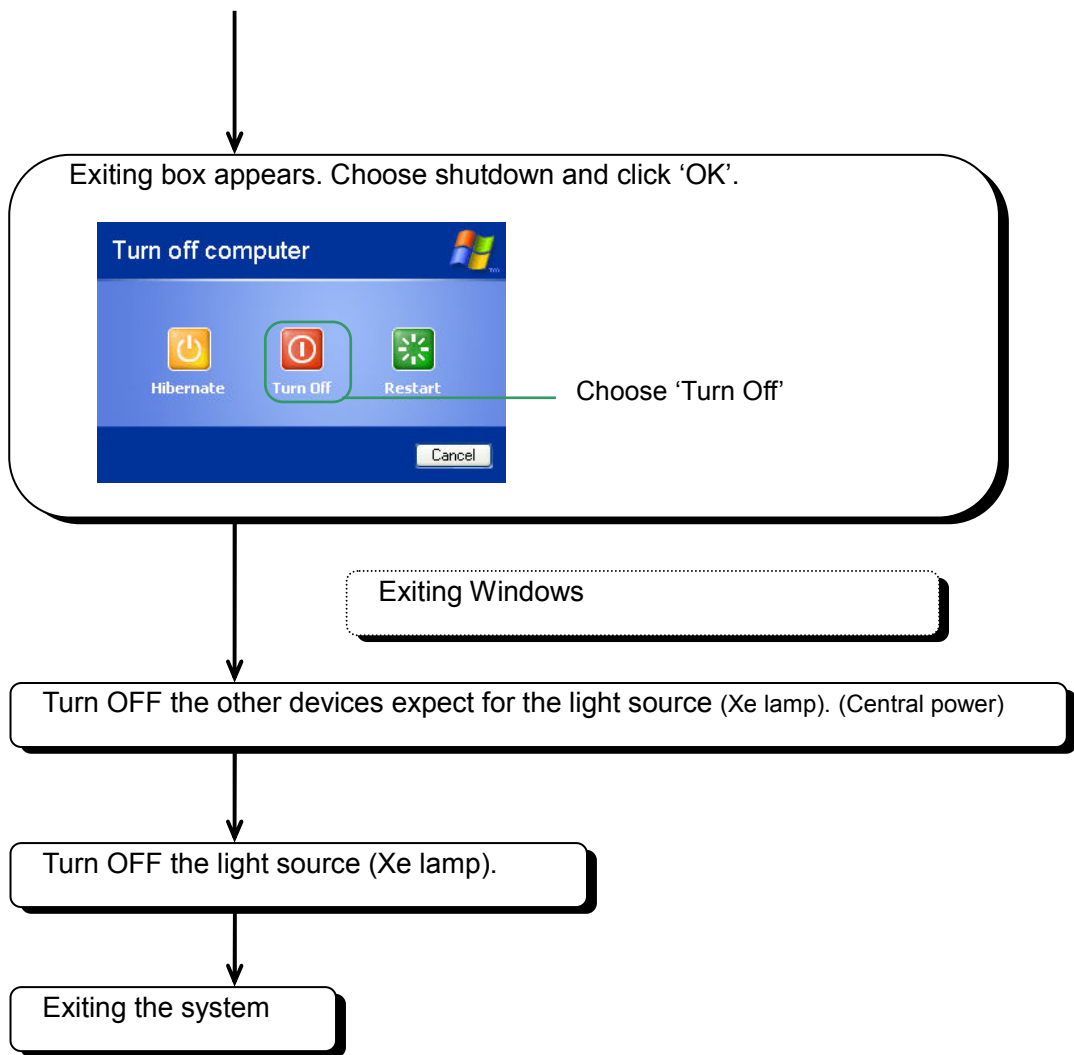
FDSS application software exiting

Exiting Windows

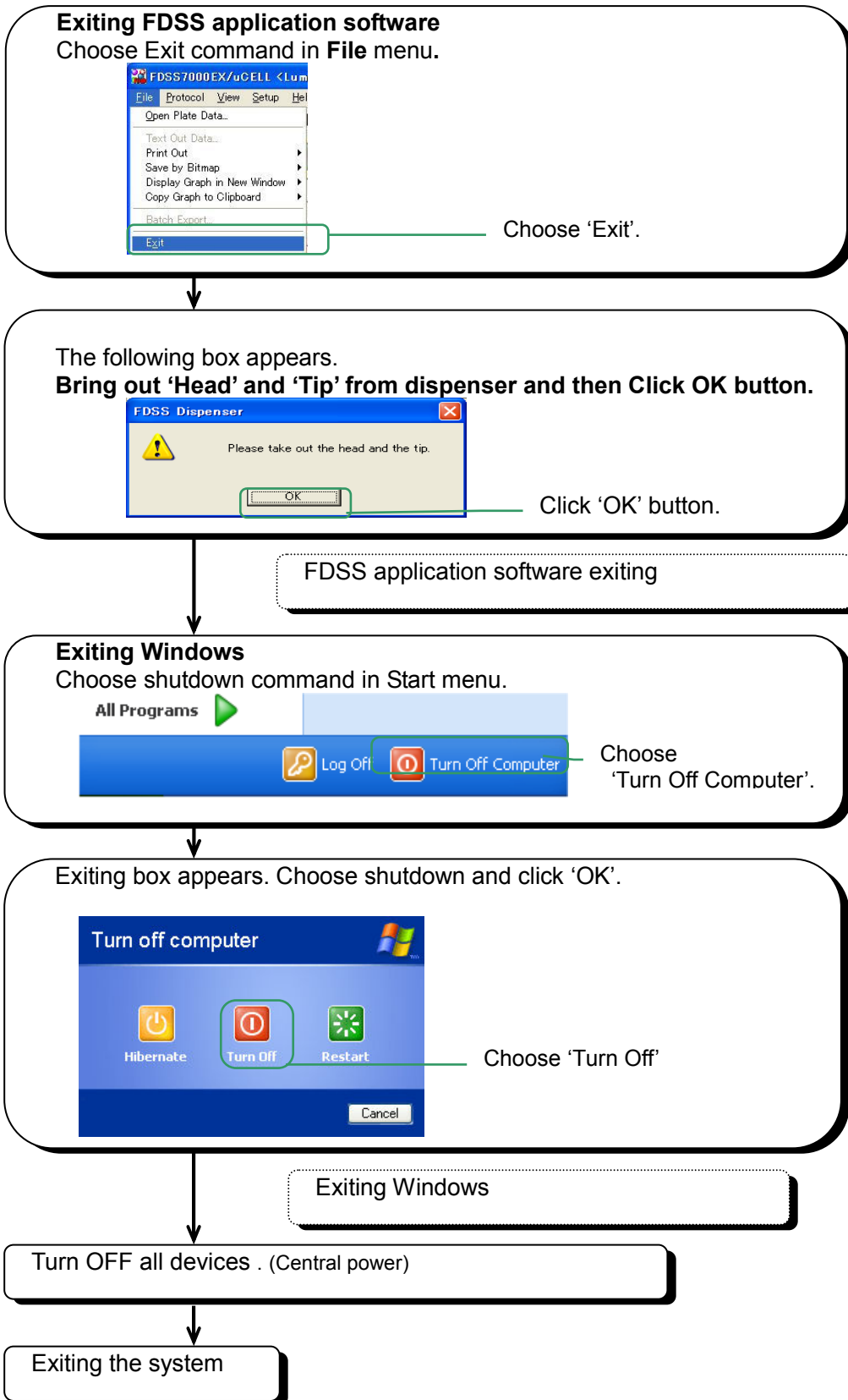
Choose shutdown command in Start menu.



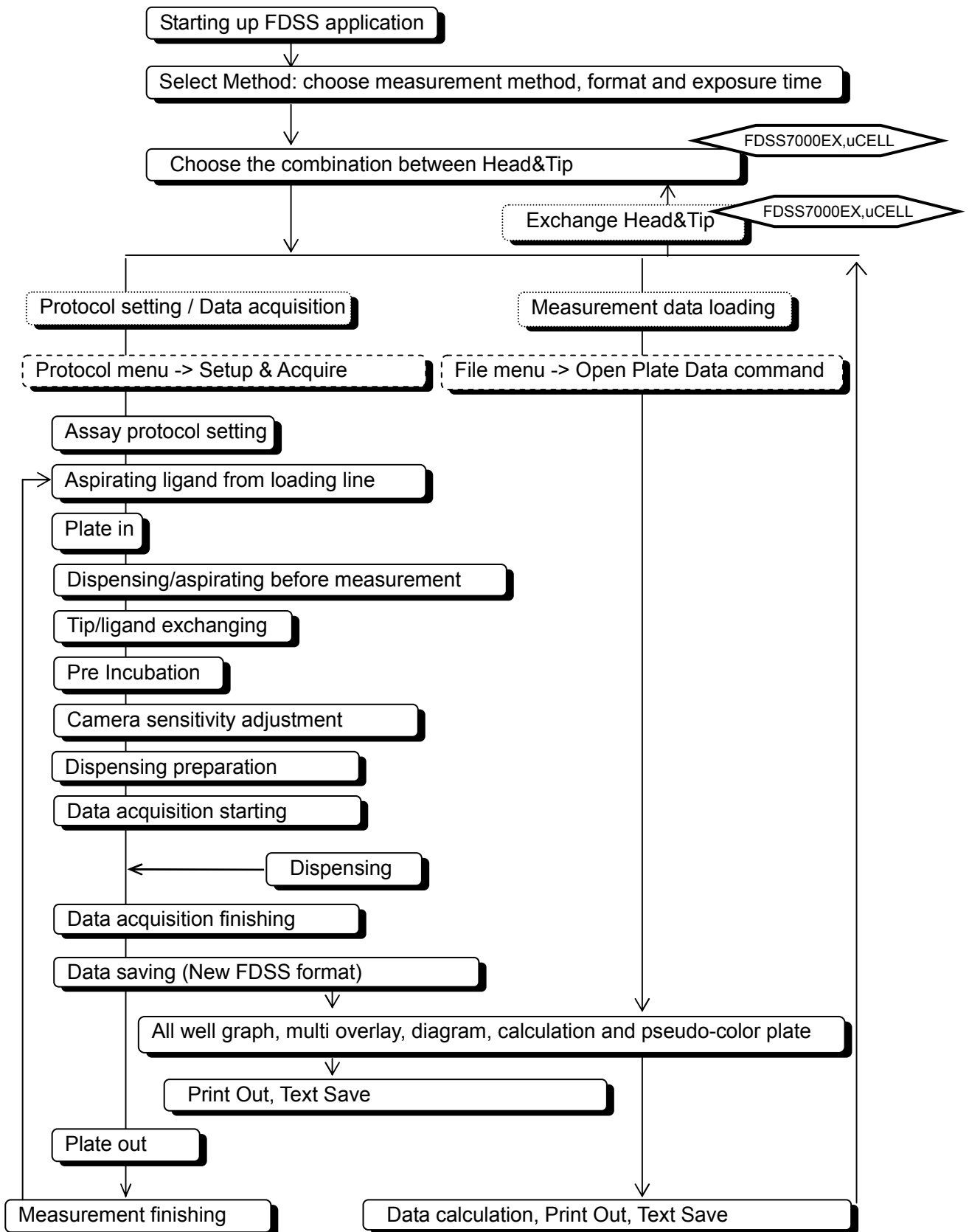
Choose 'Turn Off Computer'.



(2-2) In the case of uCELL



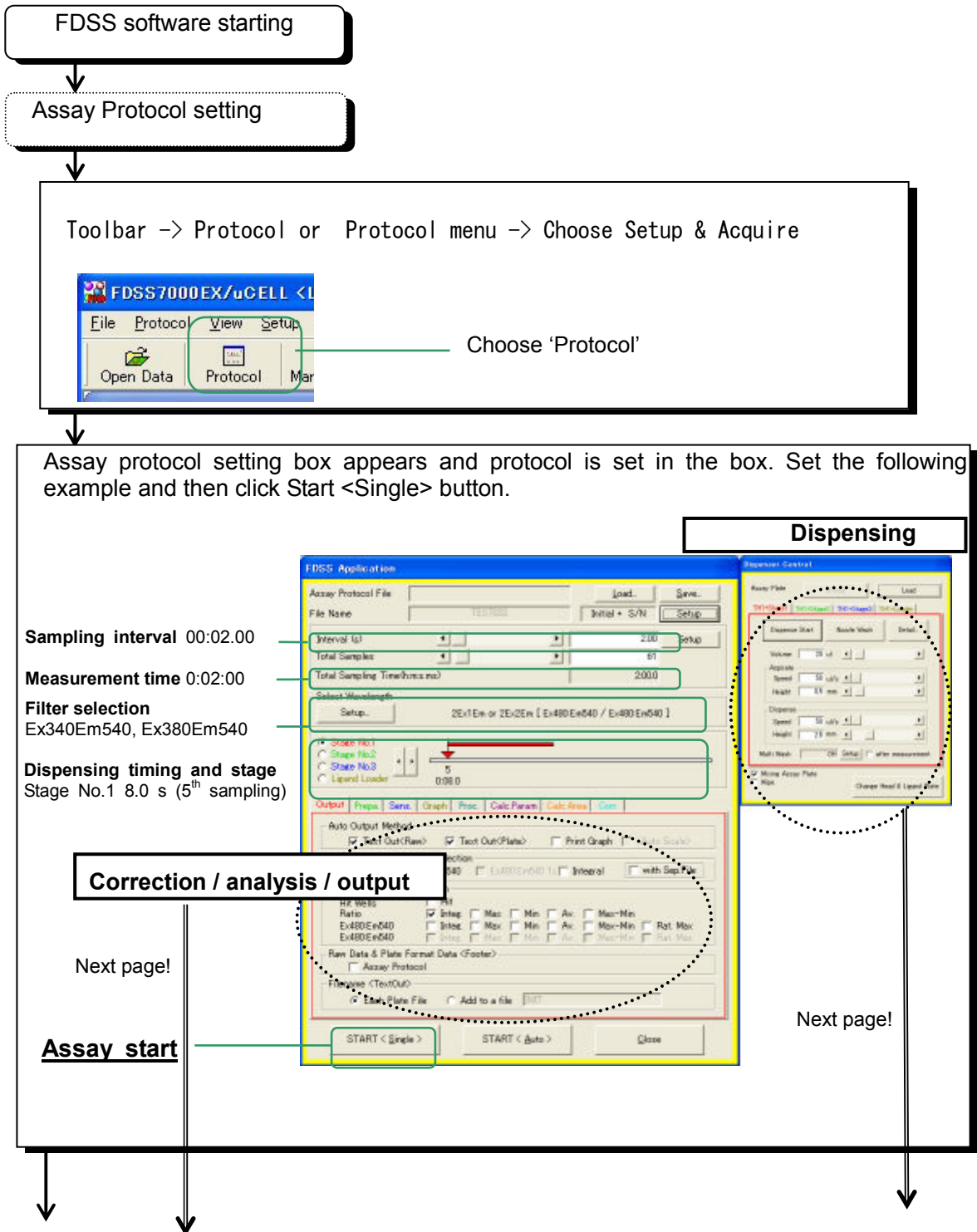
3) Workflow of FDSS application software



4) Measurement example

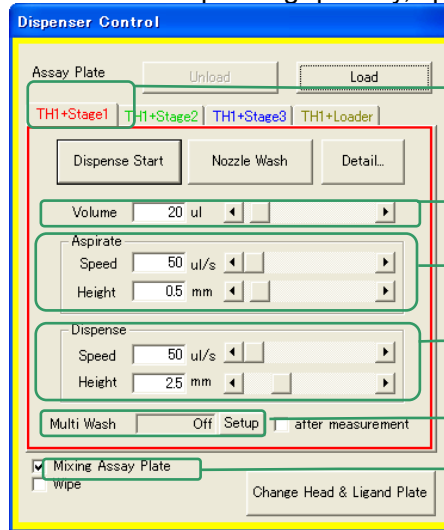
<Experiment to get data and save text data automatically>

Here one example is shown to explain measurement flow. The condition is fluorescence measurement, 2-excitation switching, 1 emission, 20 µl dispensing quantity, 1 time dispensing, 2 seconds interval, and 2 minutes measurement time. And then the data is saved with text format. (File name is 'TESTXXXX')



**Dispensing parameter
<In the case of FDSS3000/6000/7000EX>**

Set parameter for dispensing quantity, speed and height.

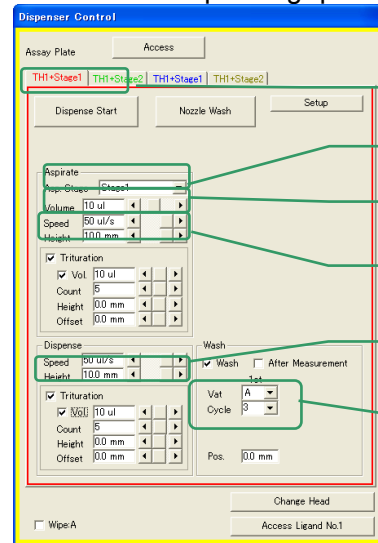


- TH1+Stage1 tab**
Or Head1 + Stage1 tab
- Dispensing quantity**
20 μ l
- Aspirating speed / height**
50 μ l/s · 0.5 mm
- Dispensing speed / height**
50 μ l/s · 2.5 mm
- Washing setting:** Off or 0
- Mixing setting**
Enabler (check)

* Setup for the washing differ by FDSS configuration

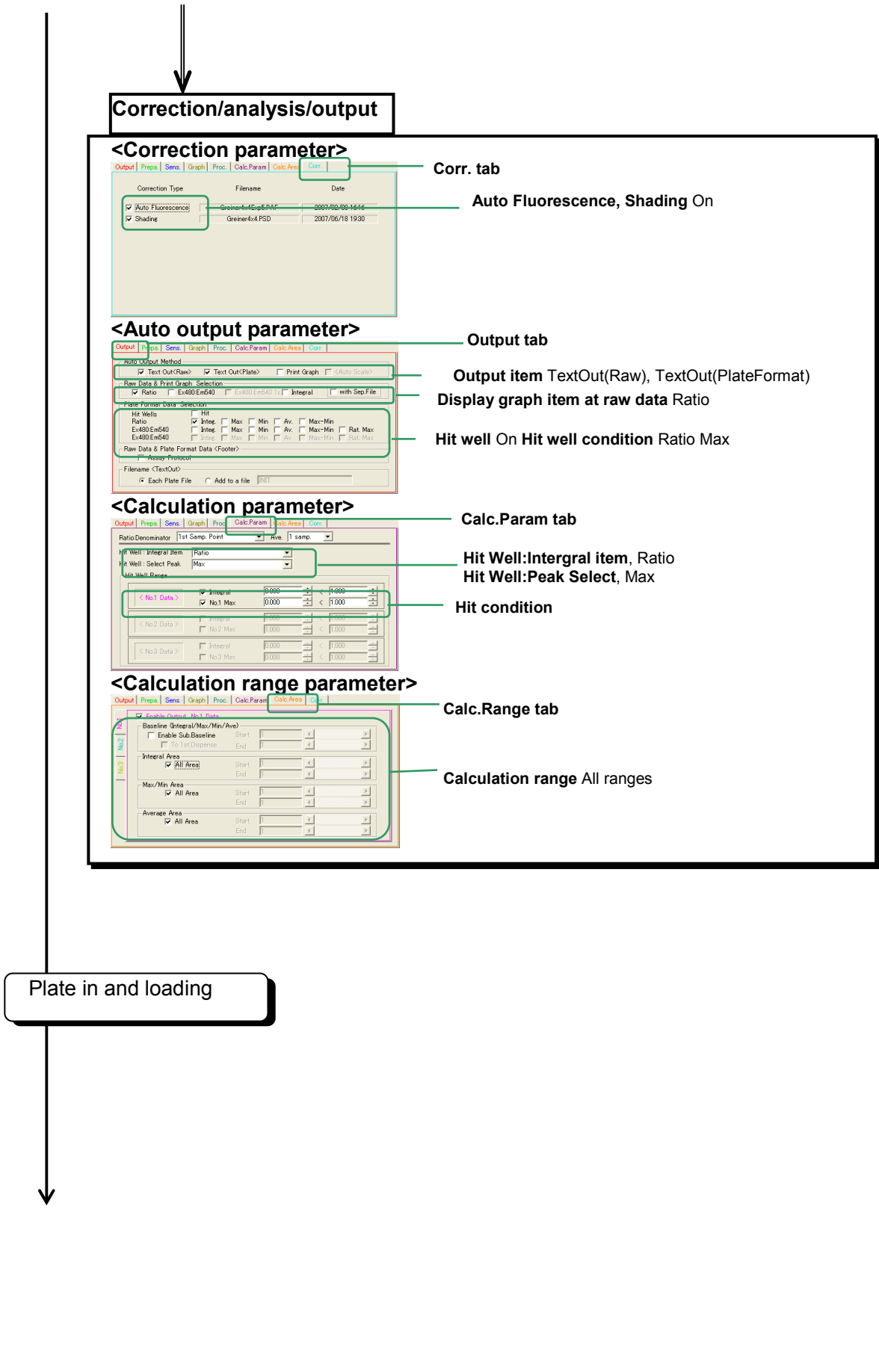
**Dispensing parameter
<In the case of uCELL>**

Set parameter for dispensing quantity, speed and height.



- TH1+Stage1 tab**
- Aspirate from Stage1**
- Dispensing quantity**
20 μ l
- Aspirating speed / height**
50 μ l/s · 0.5 mm
- Dispensing speed / height**
50 μ l/s · 2.5 mm
- Washing setting:** Off or 0

* Setup for the washing differ by FDSS configuration



Camera auto sensitivity adjustment

Auto sensitivity adjustment is executed and the message box appears for confirmation. Click 'OK' if sensitivity is set well.

Change sensitivity

Confirm the sensitivity level

Data acquisition starting

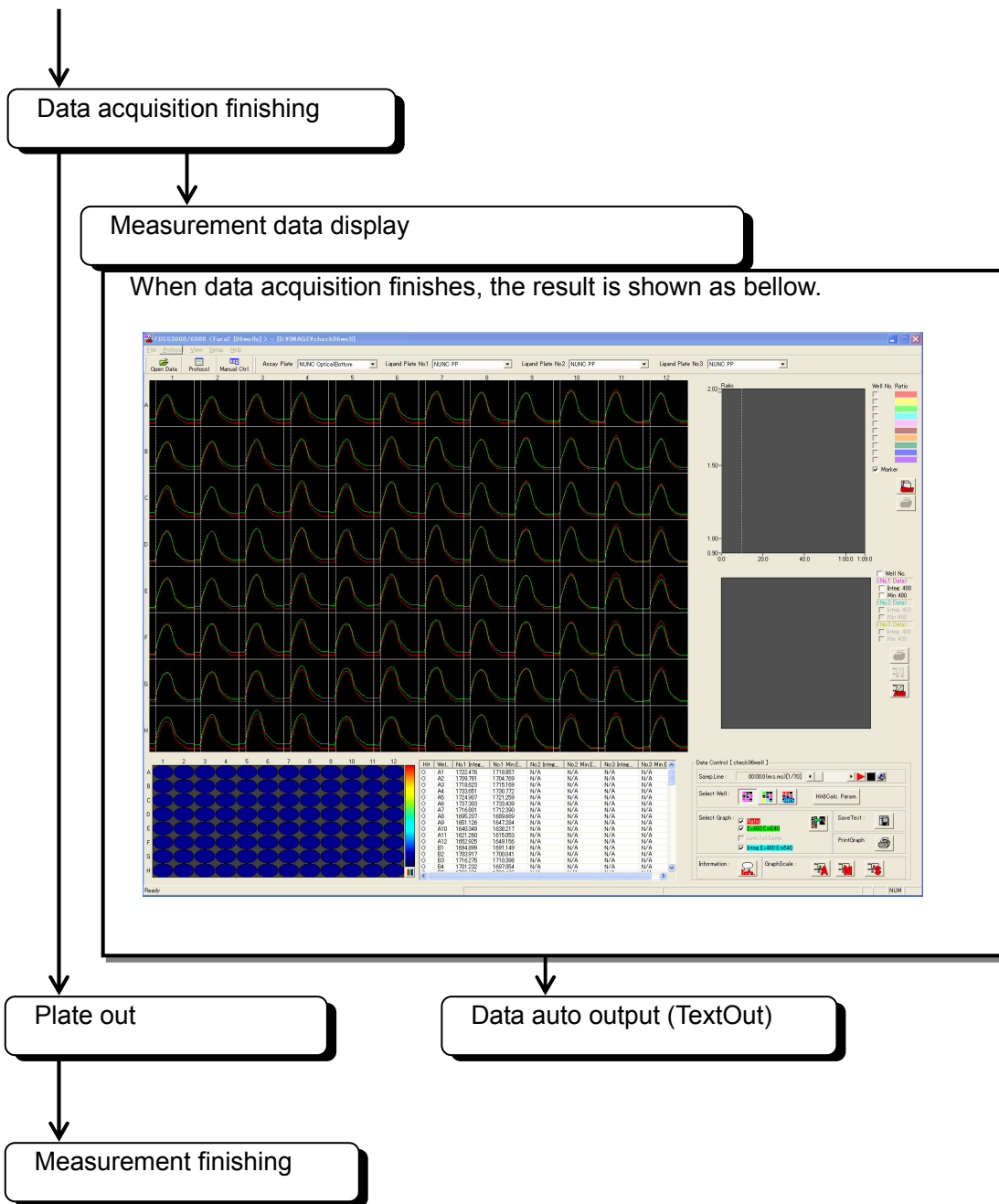
The following is shown during data acquisition.

All well graph

Remark graph

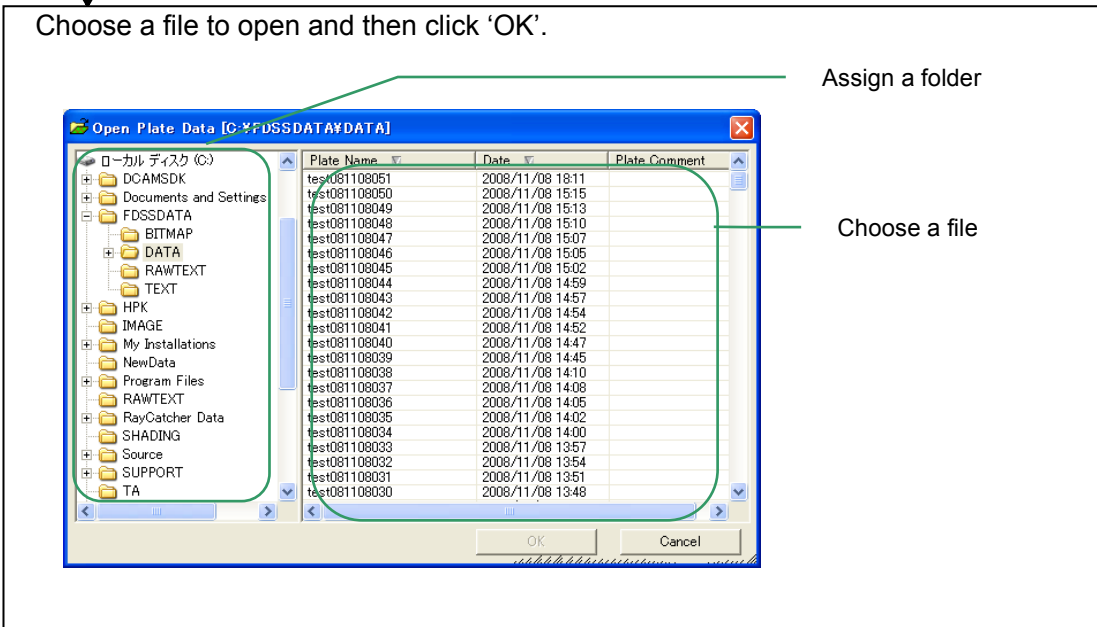
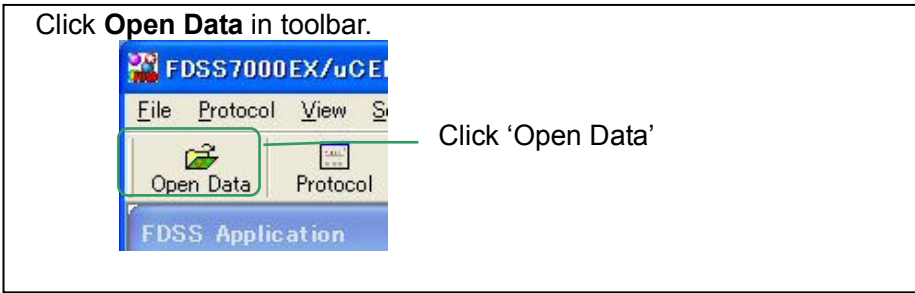
Raw image

Time flow



5) Loading data file

Plate data (New FDSS format) can be loaded with 'Open Plate Data' command in File menu.



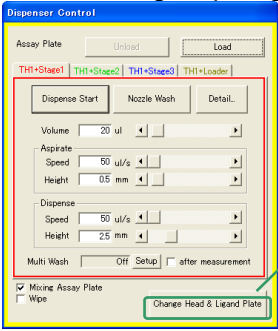
Loading data

6) Dispensing head / tip / ligand plate exchanging

(6-1) In the case of FDSS7000EX

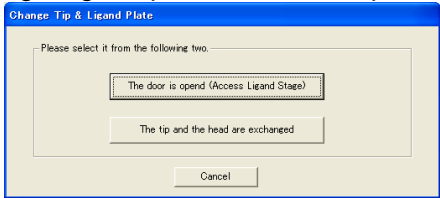
In order to exchange dispensing head / tip or ligand plate before / after measurement, the procedure is taken from Dispenser Control box.

Choose 'Change Tip&Ligand Plate' button in the Dispenser Control Box



Change Tip & Ligand Plate button

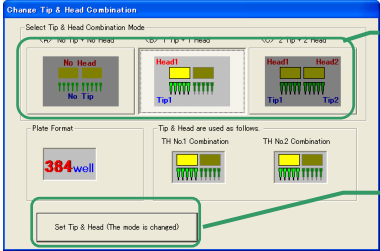
In the case of exchange head and Tip, Choose 'Exchange the tip and the head'.
In the case of exchange Ligand plate, Choose 'Open the door'.



Head and Tip and Ligand plate can be exchanged

Ligand plate can be exchanged

Place the 1) Head, 2) Tip used
Choose the combination between 'Head' and 'Tip' used,
and then click 'Set Tip & Head' button

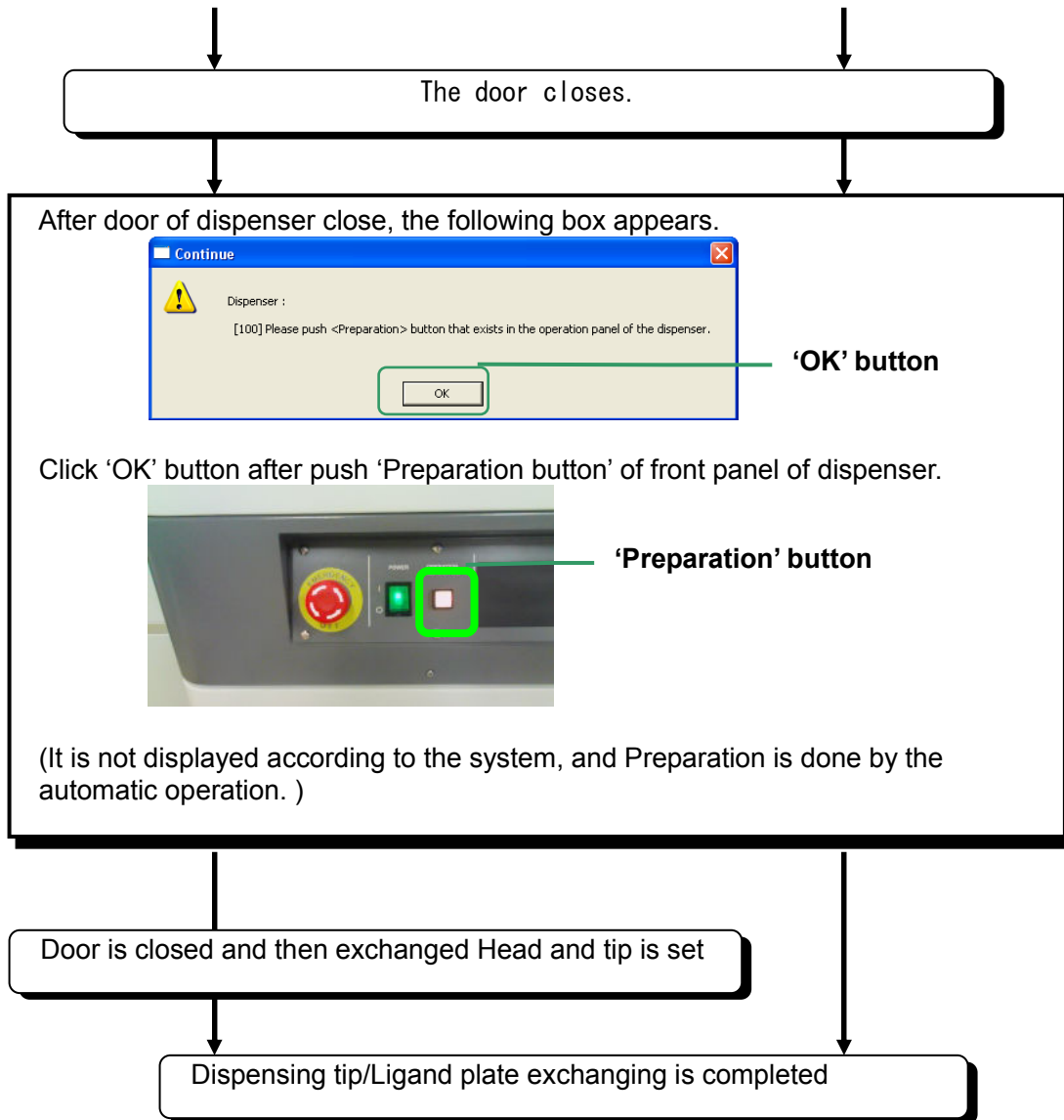


Choose the combination between Head and Tip

SetTip&Head button

Exchange Ligand plate and then click OK button





(6-2) In the case of uCELL

In order to exchange dispensing head or ligand plate before / after measurement, the procedure is taken from Dispenser Control box.

In the case of exchange head, Choose 'Change Head' button.
 In the case of exchange Ligand plate, Choose 'Access Ligand No.X' button.

Choose the tab in stages to exchanged

Change Head button

Access Ligand No.X button

Head can be exchanged

Ligand plate can be exchanged

Place the Head used
 Choose the 'Head Mode' used, and then click 'Set the head' button

Choose the Head Mode

Set the head button

Exchange Ligand plate and then click OK button

Dispensing tip/Ligand plate exchanging is completed

(6-3) In the case of FDSS3000/6000

In order to exchange dispensing tip or ligand plate before / after measurement, the procedure is taken from Dispenser Control box.

Choose dispensing head / stage tab in **Dispenser Control** and then click **'Change Tip&Ligand Plate'** button.

Chosen dispensing head and round table moves to door place and the tip or ligand plate can be exchanged.

The following message appears. After exchanging tip or ligand plate, click 'OK' button.

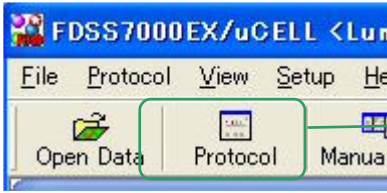
The door is closed and dispensing head and round table returns to the initial position.

7) Data display with correction

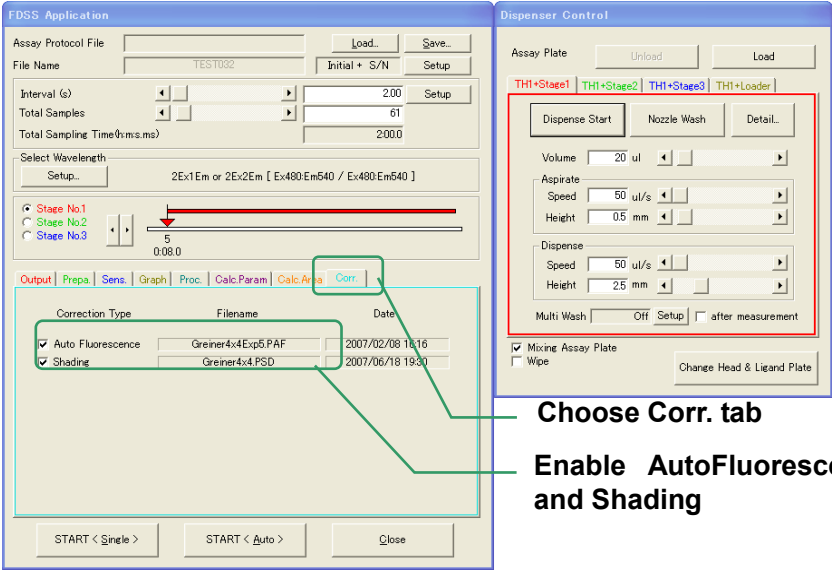
FDSS can calculate correction data for raw data and show the graph with corrected data. Correction data can be set at assay protocol setting.

Note Beforehand correction data is taken and registered. Refer to next page.

Click **Protocol** in toolbar.



Assay protocol setting box appears. Choose **Corr.** tab and enable correction parameter 'Auto Fluorescence' and 'Shading'.



Correction Type	Filename	Date
<input checked="" type="checkbox"/> Auto Fluorescence	Greiner4x4Exp5.PAF	2007/02/08 18:16
<input checked="" type="checkbox"/> Shading	Greiner4x4.PSD	2007/06/18 19:30

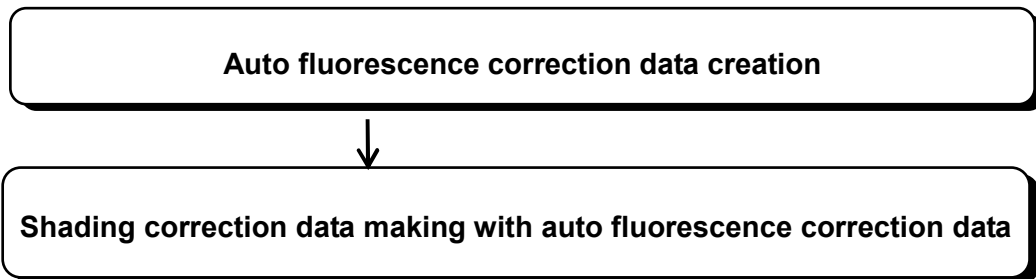
Choose Corr. tab
Enable AutoFluorescence and Shading

* No 'Auto Fluorescence' in luminescence measurement.

Correction is set.

8) Correction data creation

(8-1)At fluorescence measurement



Note Auto fluorescence correction is used only for fluorescence measurement

Correction data can be obtained by the following equations:

<Autofluorescence correction data>

= Fluorescent intensity - Dark signal

< Shading correction data >

= Fluorescent intensity - Dark signal - Autofluorescence correction data

< Experiment data

(after running autofluorescence correction and shading correction >

= (Fluorescent intensity - Autofluorescence correction data - Dark signal)
× Shading correction coefficient

< Shading correction coefficient >

= Average value of shading correction data / Shading correction data for target well

(8-2) At luminescence [Photon Counting] measurement**Shading correction data creation**

Correction data can be obtained by the following equations:

<Shading correction data>

= Photon Counting value

<Experiment data (after running shading correction)>

= Photon Counting value × Shading correction coefficient

<Shading correction coefficient>

= Average value of shading correction data /
Shading correction data for target well

(8-3) At luminescence [Analog][EMCCD] measurement**Dark correction data creation****Shading correction data creation****Note**

Dark correction is used for only luminescence measurement [Analog][EMCCD]

Correction data can be obtained by the following equations:.

<Dark correction data (Luminescence [Analog][EMCCD] measurement)>

= Luminance – Camera offset

<Shading correction data>

= Luminance – Camera offset – Dark correction data

<Experiment data

(after running Dark current correction and shading correction)>

= (Luminance – Camera offset – Dark correction data) ×
Shading correction coefficient

<Shading correction coefficient>

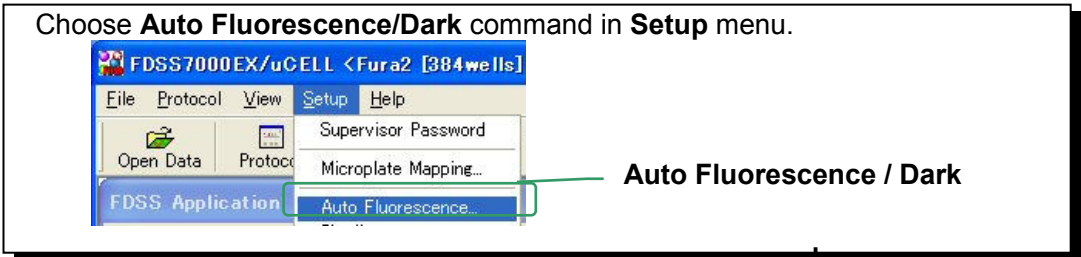
= Average value of shading correction data /
Shading correction data for target well

**9) Auto fluorescence correction data(at fluorescence)
/ Dark correction data(at luminescence[Analog][EMCCD]) creation**

< Sample for auto fluorescence correction data >

The sample for auto fluorescence (dark) correction should be ideally prepared with the actual measurement condition. It needs to prepare the same plate, filter and buffer quantity/type.

Choose **Auto Fluorescence/Dark** command in **Setup** menu.



Set sample plate for correction

<In the case of FDSS3000/6000/7000EX>

Sample plate for correction loaded in.
Set the plate onto the stacker and click **Load** button in **Dispenser Control** box.

The 'Dispenser Control' window shows the 'Load' button highlighted with a green box and arrow, labeled 'Load button'. The interface includes 'Assay Plate', 'Unload', 'Load', 'TH1+Stage1', 'TH1+Stage2', 'TH1+Stage3', 'TH1+Loader', 'Dispense Start', 'Nozzle Wash', and 'Detail...' buttons.

<In the case of uCELL>

Sample plate for correction loaded in.
Click **Access** button in **Dispenser Control** box and set sample plate.

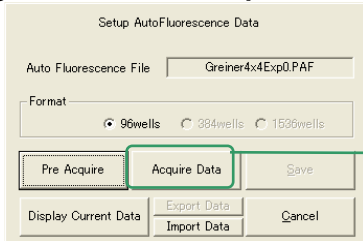
The 'Dispenser Control' window shows the 'Access' button highlighted with a green box and arrow, labeled 'Access button'. The interface includes 'Assay Plate', 'Access', 'TH1+Stage1', 'TH1+Stage2', 'TH1+Stage1', 'TH1+Stage2', 'Dispense Start', and 'Nozzle Wash' buttons.

Check auto fluorescence image of the sample plate
Set Sensitivity Max in **Camera Control** box. Click '**Pre Acquire**' button in **Setup Auto Fluorescence Data** box and get image data.

The 'Camera Control' window shows the 'Sensitivity' slider set to 10, highlighted with a green box and arrow, labeled 'Set sensitivity max'. The 'Live' section has 'Live On' and 'Live Off' buttons, and a 'Live Image Window...' button.

The 'Setup AutoFluorescence Data' dialog box shows 'Auto Fluorescence File' set to 'Greiner4x4Exp0.PAF', 'Format' set to '96wells', and the 'Pre Acquire' button highlighted with a green box and arrow, labeled 'Click 'Pre Acquire' to check an image.' Other buttons include 'Acquire Data', 'Save', 'Display Current Data', 'Export Data', 'Import Data', and 'Cancel'.

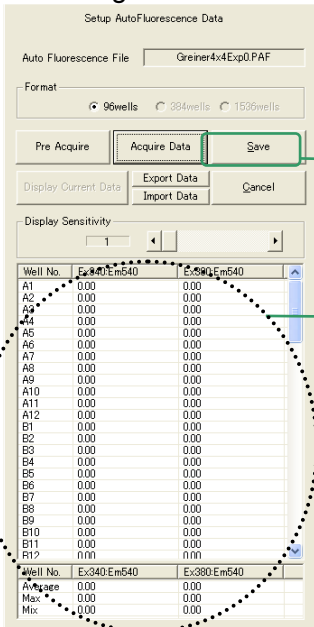
The following box appears after getting an image. Check whether it has unnecessary bright spot by dust. If it has, load the plate out and remove the dusts. And then try it again. If not, click **Acquire Data** button in **Setup Auto Fluorescence(Dark) Data** box.



Click 'Acquire Data'.

Acquiring correction data.

The following box appears after acquiring correction data. Click **'Save'** and register the data. It is registered automatically.

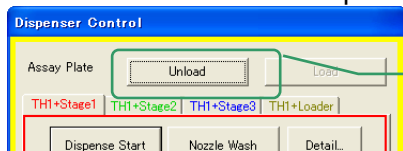


Click 'Save' to register.

Display the result

<In the case of FDSS3000/6000/7000EX>

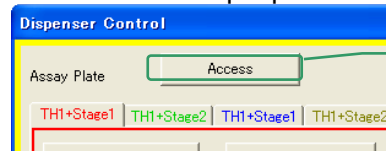
Sample plate loaded out
Click **Unload** button in Dispenser Control box.



Unload button

<In the case of uCELL>

Sample plate loaded out
Click **Unload** button in Dispenser Control box and take out sample plate.



Access button

Finish

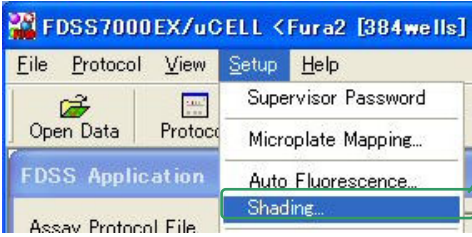
10) Shading correction data creation

The sample data that has homogeneous fluorescence can be used to correct nonuniformity on hardware and optics.

<Sample for shading correction data>

The sample for shading correction should be ideally prepared with the actual measurement condition. It needs to prepare the same plate, filter and buffer quantity/type.

Click **Shading** command in **Setup** menu.

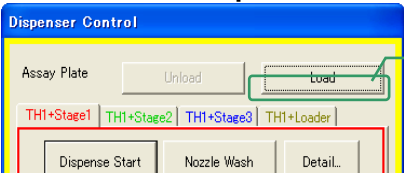


The screenshot shows the 'Setup' menu with 'Shading...' selected. A green line points to this option with the label 'Shading button'.

Set the sample for shading correction.

<In the case of FDSS3000/6000/7000EX>


Sample plate for correction loaded in.
Set the plate onto the stacker and click **Load** button in **Dispenser Control** box.



The screenshot shows the 'Dispenser Control' window with the 'Load' button highlighted by a green box and a green line pointing to it with the label 'Load button'.

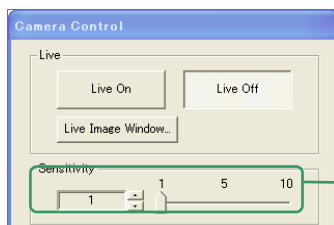
<In the case of uCELL>

Sample plate for correction loaded in.
Click **Access** button in **Dispenser Control** box and set sample plate.

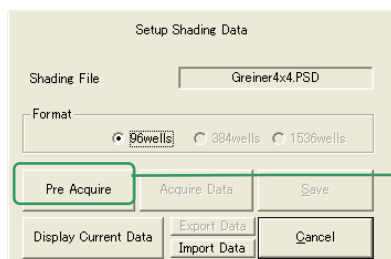


The screenshot shows the 'Dispenser Control' window with the 'Access' button highlighted by a green box and a green line pointing to it with the label 'Access button'.

<At fluorescence measurement and luminescence[Analog][EMCCD] measurement>
 To check shading image of the sample plate. **Set Sensitivity** a certain value in **Camera Control** box. Click '**Pre Acquire**' button in **Setup Shading Data** box to acquire image data.



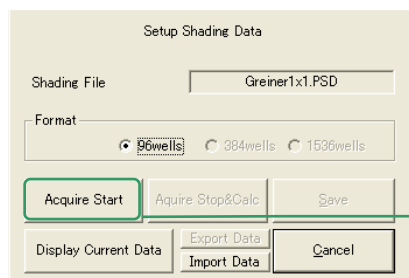
Set Sensitivity



Click 'Pre Acquire' to acquire fluorescence image.

<At luminescence [Photon Counting] measurement>

To acquire shading image of the sample plate. Click '**Acquire Start**' in **Setup Shading Data** box to start acquiring photon counting image data. Integration images are observed then.



Click 'Acquire Start' to start acquire photon counting image data.

<Fluorescence measurement>

Acquiring fluorescence image for checking

<Luminescence [Analog] [EMCCD]measurement>

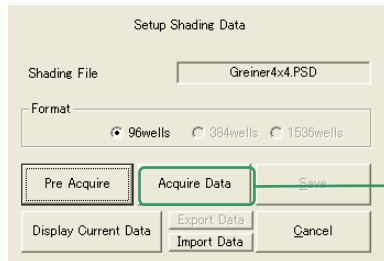
Acquiring luminescence image for checking

<Luminescence [Photon Counting] measurement>

Acquiring Photon Counting image

<At fluorescence measurement and luminescence[Analog][EMCCD] measurement>

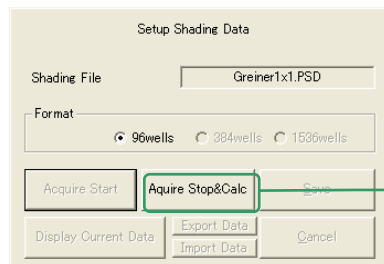
To Check after getting an image whether it has unnecessary bright spots by dusts or not. If it has, load plate out and remove dusts. And then try again. If not, click **Acquire Data**.



Click 'Acquire data'.

<At luminescence [Photon Counting] measurement >

Click 'Acquire Stop&Calc.' to stop acquisition and to create shading data when total image is homogeneous and doesn't have unnecessary bright spots.



Click 'Acquire Stop&Calc.'.

The following box appears after acquiring correction data. Click 'Save' and register the data. It is registered automatically.

Click 'Save' to register.

Sensitivity changes

Display the result

Display the result

<Fluorescence measurement> <Luminescence[Analog][EMCCD]measurement>
 <Luminescence[Photon Counting]measurement>

Fluorescence/Luminescence [Photon Counting] measurement
 The relation is not in sensitivity, and common correction data is used.

Luminescence[Analog][EMCCD] measurement
 It has the correction data of each sensitivity. Sensitivity is changed and all the sensitivity correction data is acquired.

<In the case of FDSS3000/6000/7000EX>

Sample plate loaded out
 Click **Unload** button in Dispenser Control box.

Dispenser Control

Assay Plate Unload Load

TH1+Stage1 TH1+Stage2 TH1+Stage3 TH1+Loader

Dispense Start Nozzle Wash Detail.

<In the case of uCELL>

Sample plate loaded out
 Click **Access** button in Dispenser Control box and take out sample plate.

Dispenser Control

Assay Plate Access

TH1+Stage1 TH1+Stage2 TH1+Stage1 TH1+Stage2

Finish

3. How to operate FDSS software

1) Starting the software

Start up the program as outlined below.

- 1) Check each cable connection.
- 2) Turn on the excitation light source (at fluorescence)

Note

Noise usually generates at the instant that the light source is turned on or off. Make a habit to first turn on the light source before turning on other peripheral units, and last turn off the light source after turning off other units. It will take time for the light source to become stabilized. So turn on the light source about 30 minutes before beginning experiments.

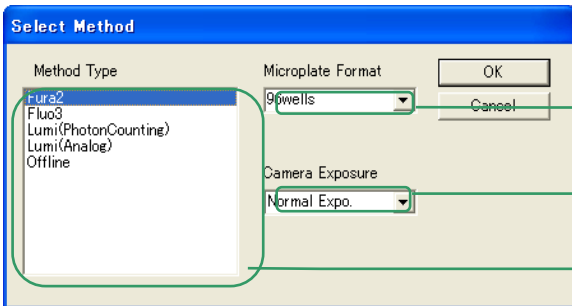
- 3) Turn on the peripheral equipment other than the excitation light source.
- 4) Start up the FDSS software. **Double-click FDSS icon or choose Program => HAMAMATSU => FDSS** from start menu.

Note

When starting the FDSS software and in the case the anti-virus software is installed, there will be possibility that the time of acquiring data takes longer than the set-up time. In the case, set the monitoring function of anti-virus software off or other set-up which might cause longer time acquisition off. And since starting screen saver function and set-up of the electric power saving during the measurement might cause problem, set these function off (during the measurement.)

2) Measurement method choice

When the FDSS software starts up, the following dialog box appears for selecting the measurement method, plate format and camera exposure time.



< Method Type > Choice of measurement method

- Fura2** : Fura2 (2 wavelength excitation) fluorescence measurement
- Fluo3** : Fluo3 (1 wavelength excitation) fluorescence measurement
- Lumi[Photon Counting]**: Photon counting luminescence measurement
- Lumi[Analog]** : Analog luminescence measurement
- Lumi[EMCCD]** : Analog luminescence measurement by EMCCD camera

< Microplate Format > Choice of microplate format

- 96wells** : 96 well format plate usage
- 384wells** : 384 well format plate usage
- 1536wells** : 1536 well format plate usage

< Camera Expo. > Choice of camera exposure time

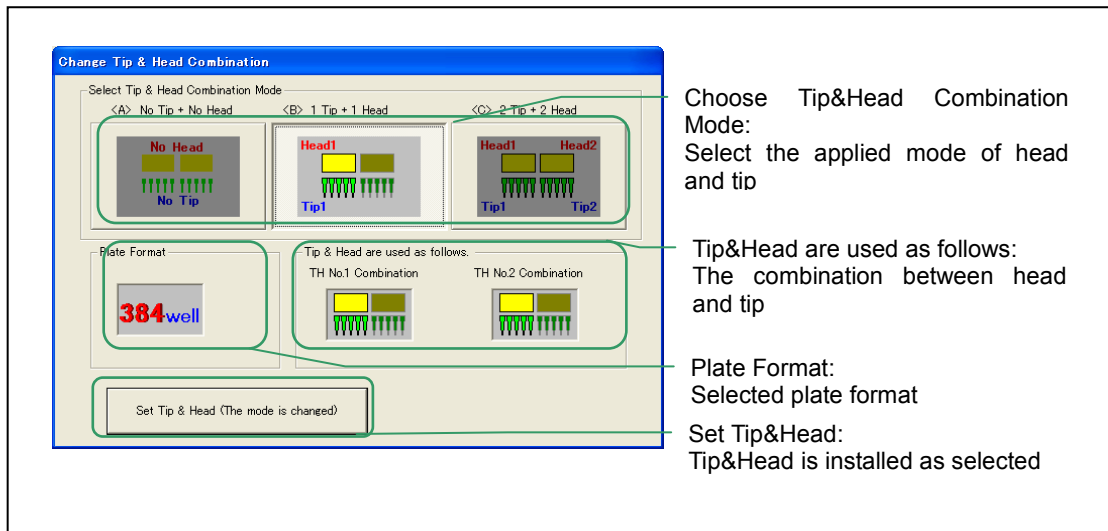
- Normal Expo.** : Standard exposure time
- Long Expo.** : Long exposure time used when the sample image is too much dark
- Short Expo.** : Short exposure time used when the sample image is too much bright.

Note The measurement method list depends on the system configuration.

3) Combination between dispensing head and tip **(FDSS7000EX)**

At FDSS7000EX, after selecting how to measurement and then clicking OK button (as described pre-page.) if dispensing unit keep the head and tip installed, after the head and tip was installed, the door of FDSS7000EX is opened.

When the door is opened, such box as follows is displayed.



Exchange of the dispensing unit and tip or ligand plate is performed.

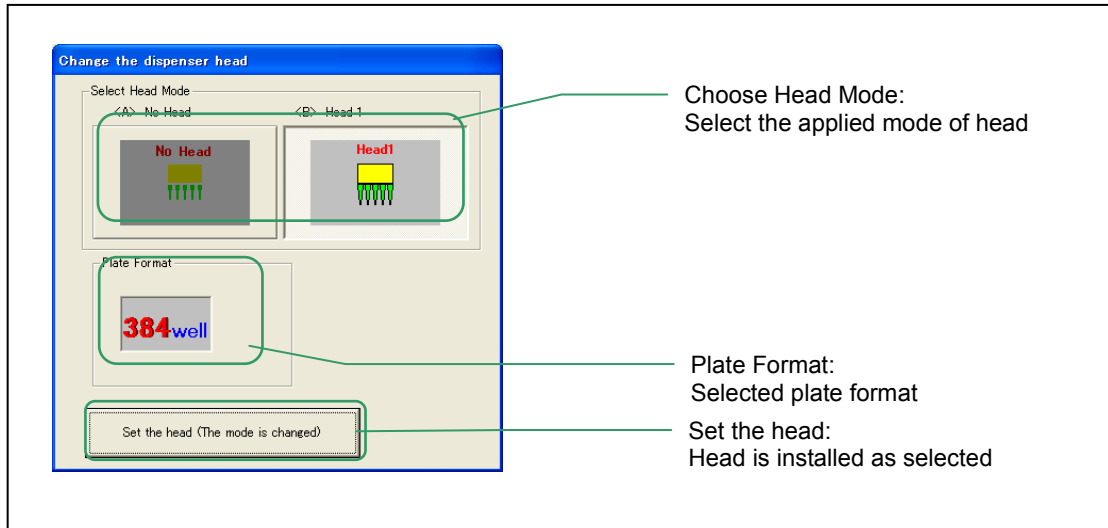
In the above box, select the combination between head and tip used and then click the Set tip & Head button.

After that, the door of FDSS7000EX is closed and then the head and tip are loaded as being set.

For details, Please refer to Chapter 12 4) As for exchange of dispensing head&tip and ligand plate.

4) Combination between dispensing head (uCELL)

At uCELL, after selecting how to measurement and then clicking OK button (as described pre-page.) if dispensing unit keep the head installed, after the head was uninstalled, such box as follows is displayed.



Exchange of the dispensing head is performed.

In the above box, select the combination between head used and then click the Set the head button.

For details, Please refer to Chapter 12 6) As for exchange of dispensing head and ligand plate.

5) Main Window

After selecting the way of measurement and then clicking 'OK' button as described at 2), or after clicking 'Set Tip&Head' button as described at 3), the window as follows is displayed. The main window starts up with the assay protocol setting box. The operation mode and commands can be set with Toolbar or menu command.

<Main Window>

(1) Menu : To choose the menu such as File and Setup and to execute each command from the menu.

(2) Operation mode : To choose the operation mode such as the data opening, protocol setting and manual control.

(3) Plate select bar : To set the plate type to be used at the measurement.

6) Running a command

This section explains brief description of each menu. For more details on how to run use menu, refer to the corresponding chapter.



- (1) **File** : Used to load recorded measurement data as necessary. And the graphs can be printed out and data can also be saved as text format.
- (2) **Protocol** : Used to set parameters for data acquisition and create various types of correction data.
- (3) **View** : Used this menu to switch display for tool bar and status bar.
- (4) **Setup** : Used this menu to get correction data, to set data folders and to make setting file. Supervisor password is needed for this menu.
- (5) **Help** : It shows software version information.

Running a command

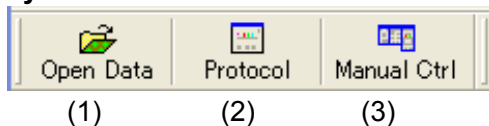
Each command can be run in the main window that is displayed as shown below. Clicking the menu name opens the command list and then clicking command name make it running.



7) Toolbar

The frequently used functions can be chosen by clicking the icons.

Frequently used functions



- (1) **Open Data** : Loading data
- (2) **Protocol** : Setting assay protocol and start measurement
- (3) **Manual Ctrl** : Display Camera Control Box, Filter Control Box and Dispenser Control Box.

(7-1) Open Data

'Open Plate Data' box appears and the plate data can be chosen for loading. It is the same as to choose 'Open Plate Data' command from File menu.

<Open Data>

Plate Name	Date	Plate Comment
test081108051	2008/11/08 18:11	
test081108050	2008/11/08 15:15	
test081108049	2008/11/08 15:13	
test081108048	2008/11/08 15:10	
test081108047	2008/11/08 15:07	
test081108046	2008/11/08 15:06	
test081108045	2008/11/08 15:02	
test081108044	2008/11/08 14:59	
test081108043	2008/11/08 14:57	
test081108042	2008/11/08 14:54	
test081108041	2008/11/08 14:52	
test081108040	2008/11/08 14:47	
test081108039	2008/11/08 14:46	
test081108038	2008/11/08 14:10	
test081108037	2008/11/08 14:08	
test081108036	2008/11/08 14:05	
test081108035	2008/11/08 14:02	
test081108034	2008/11/08 14:00	
test081108033	2008/11/08 13:57	
test081108032	2008/11/08 13:54	
test081108031	2008/11/08 13:51	
test081108030	2008/11/08 13:48	
test081108029	2008/11/08 11:39	

(1) **Open Plate Data box** : To choose the Plate Data for loading.

(7-2) Protocol

Assay protocol setting box appears and the protocol for the measurement can be set in it. It is the same as to choose Setup&Acquire command from Protocol menu.

<Protocol>

(1) **FDSS Application box**
: To set the assay protocol. The measurement starts with the box.

(2) **Dispenser Control box**
: To set the parameter of the dispensing on the assay protocol.

(7-3) Manual Control

Manual Control boxes appear and the devices can be controlled manually with them.

<Manual Control>

(1) **Camera Control box**
: To control the camera manually. It can control High Voltage (HV) as well.

(2) **Filter Control box (At the fluorescence measurement)**
: To control the shutter, excitation and fluorescence wavelength manually.

(3) **Dispenser Control box**
: To control the dispensing system manually.

8) Plate choice

To choose an assay plate type and ligand plate type to be used at measurement from the registered plate information selection in Plate Selectbar. It leads parameters to be automatically set here such as ROI (Region Of Interest), correction data and dispensing initial position according to the chosen plate.

<Assay plate setting>

Assay Plate NUNC OpticalBottom 1)

<Ligand plate> 2)

Assay Plate NUNC OpticalBottom | Ligand Plate No.1 NUNC PP | Ligand Plate No.2 NUNC PP | Ligand Plate No.3 NUNC PP | Ligand Plate Loader NUNC PP

1) Assay Plate
: To choose a plate to be used for measurement. It automatically sets ROI, correction data file, dispensing initial position and luminescence sensor position.

2) Ligand Plate No.1,2,3,Ligand Plate Loader
: To choose a ligand plate to be used for measurement. It automatically sets aspirating initial position. No1, 2, 3 corresponds to Stage1, 2, 3 respectively at 'Dispenser Control'.
Ligand Plate Loader corresponds to the plate of the ligand loader line.

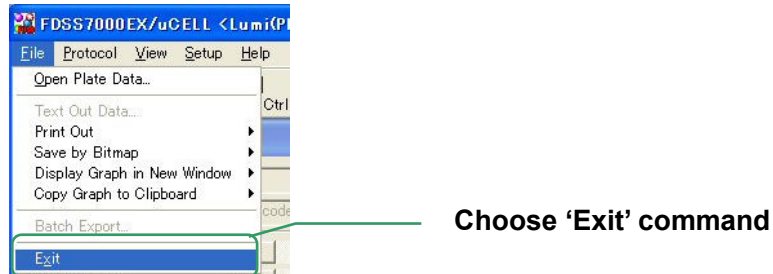
Note

This plate choice setting is linked with Assay Protocol.
(This is loaded/saved with AssayProtocol.)

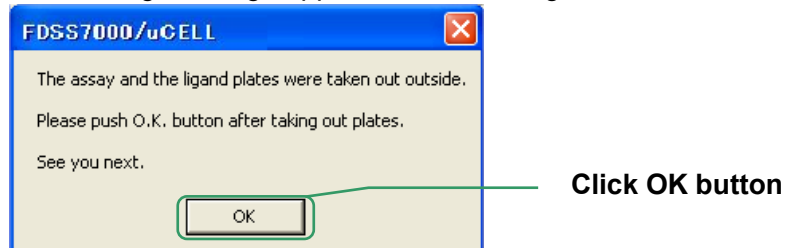
9) Quitting software

To quit the program, follow the procedure below.

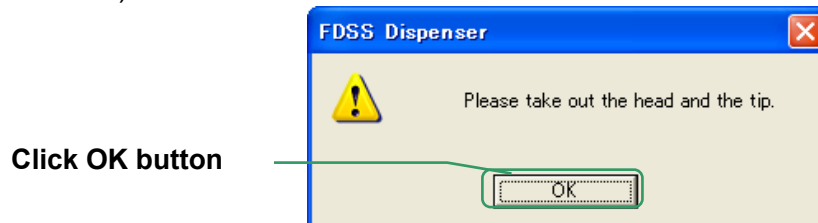
- (1) Select "Exit" from the File menu. Or, double-click the left top of the main window.



- (2) The main unit loads a plate out if it is set inside and then finish its behavior. Click 'OK', when the following message appears after finishing.



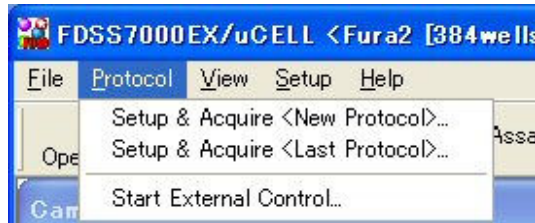
- (3) If head and tip is loaded, after unloading them the door is opened. Message as below is displayed. After unloading head and tip, click 'OK button' (FDSS7000)



- (4) FDSS software closes and then quite Windows.

4. Protocol Menu

This menu is used to acquire data and to set an assay protocol for data acquisition. When you choose Protocol menu, the command list appears as below.



Setup & Acquire<New Protocol>

: This command displays an assay protocol setting box by an initial value.
Protocol can be set and data acquisition can be started in this box.

Setup & Acquire<Last Protocol>

: This command displays an assay protocol setting box by the last value.
Protocol can be set and data acquisition can be started in this box.

Start External Control

: This command starts the external control mode. (Optional Function)

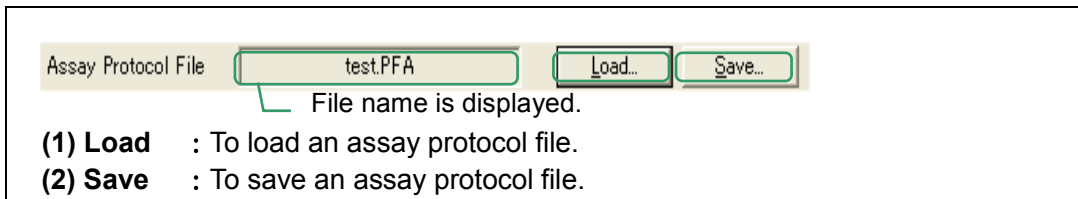
1) Assay protocol setting (Setup & Acquire command)

The following box appears with clicking 'Setup & Acquire' command. The protocol can be set and data acquisition can start in this box.

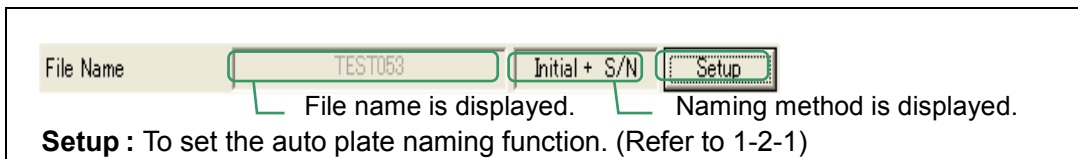
(1) Assay Protocol File : To save and load an assay protocol.
 (2) File Name : To name the measurement data.
 (3) Measurement time : To set a sampling interval and total samples.
 (4) Select Wavelength : To set wavelength for measurement
 (5) Dispense Timing : To set dispensing timing.
 (6) Dispenser Control : To set dispensing parameter at each stage.
 (7) Setting Tab : To set correction, calculation, output and so on.
 (8) START <Single> : To start single measurement with only one plate.
 (9) START <Auto> : To start sequential measurement with plural plates.
 (10) Close : To finish an assay protocol setting and close the box.

(1-1) Assay Protocol File

Used to save and load an assay protocol. Clicking 'Open' and 'Save' displays the loading box and saving box respectively. The file format is 'assay protocol file (*.PFA).'

**(1-2) File Name**

Used to set how to name the measurement data file.



(1-2-1) Auto plate-naming function

Clicking 'Setup' leads a setting box 'Setup Plate name' for auto plate-naming function.

The screenshot shows the 'Setup File Name' dialog box with the following settings and callouts:

- (1) Automatic File Name: On (Automatic) Off (Manual)
- (2) Automatic Parameter: Method: Serial Num.(Count up); Initial Name: TEST; Serial number: 1; Digit of Serial number: 3
- (3) Barcode-Option: Reflected in a File Name (same name is permitted); Reflected in a Comment
- (4) Data Folder: Plate Data: C:\FDSSDATA\#DATA#; Text Data (Table: Plate Format): C:\FDSSDATA\#TEXT#; Text Data (Raw: Data Array): C:\FDSSDATA\#RAWTEXT#; Bitmap Data (Screen Image): D:\FDSSDATA\#BITMAP#
- (5) Plate Read: Back Loader Front Loader
- (6) Saved in Date Folder: Saved in Date Folder; ".1.TXT" is added
- (7) 2 barcode is written in a comment: 2 barcode is written in a comment

(1) Automatic File Name
Automatic Plate Name
 : Choose the automatic naming function, On, or manual naming at each measurement, Off, at single measurement. Auto naming function is automatically applied at sequential measurement with plural plates.

(2) Automatic Parameter
Method : Choose the plate-naming method from the following options.
Serial Number = Named with auto count up as Serial Number (S/N). Initial count number is set at 'Serial number'.
Initial + S/N = Named with combination of Initial Name and S/N.
Initial + Date + S/N = Named with combination of Initial Name, Date and S/N.
Date + S/N = Named with combination of Date(yymmdd) and S/N
Barcode = Named with the read barcode data (Option)
Initial Name : To set header characters when 'Initial+S/N' method is chosen.
Serial number : To set initial numbers for auto count up.
Digit of Serial number: To set digit numbers at auto count up.

(3) Barcode Reader (Option)
Reflected in a File Name
 : When checked, the barcode data reflects onto File Name.
Reflected in a Comment
 : When checked, the barcode data reflects onto Comment field.
Plate Read
 : Choose which barcode is employed from an assay plate or ligand plate

(4) Data Folder
Plate Data
 : To specify the data folder for Plate Data
Text Data (Table: Plate Format)
 : To specify the data folder for Text Data with a plate format.
Text Data (Raw: Data Array)
 : To specify the data folder for Text Data with a data array format.
Bitmap Data (Screen Image)
 : To specify the data folder for Bitmap Image.

(5) Saved in Date Folder
 : The date subfolder is made and the plate data is saved.

- (6) “_1.TXT” is added <Please refer to 2-1 in Chapter 6>
: “_1.TXT” is put on the end of the file name of the No.1 data.
- (7) 2barcode is written in a comment
: The barcode data of front and back is reflected in the comment.

(1-3) Measurement time

Used to set a sampling interval and choose the single or double interval. Two different sampling intervals can be set as one part of the protocol and then be switched during measurement accordingly with Double interval function.

- (1) Interval (s) : To set a sampling interval between data points.
- (2) Total Samples : To set the total sampling number.
- (3) Total sampling Time (h:m:s): To display the total measurement time.
- (4) Setup : To set the double interval (Refer to 1-3-1).

(1-3-1) Double Interval

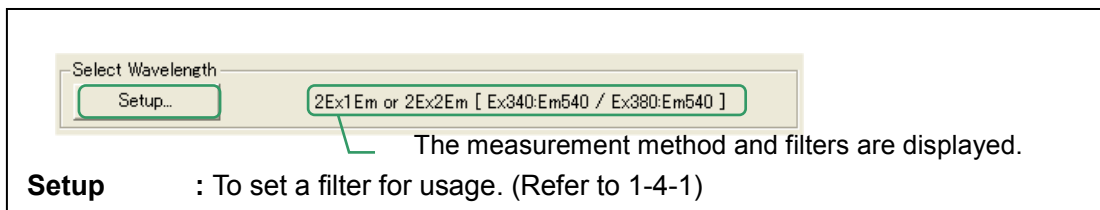
Clicking 'Setup' in Interval leads a setting box 'Setup Double Interval' for double interval setting.

Interval	Section Samples	Section Time	Total Time (Samp.No.)
1st / 1.0(s)	10	00:09.0	00:00.0 (1)
2nd / 5.0(s)	10	00:50.0	00:09.0 (10)
3rd / 1.0(s)	10	00:10.0	00:59.0 (20)
4th / 5.0(s)	10	00:50.0	01:09.0 (30)
5th / 1.0(s)	10	00:10.0	01:59.0 (40)
6th / 5.0(s)	71	05:55.0	02:09.0 (50)
			08:04.0 (121)

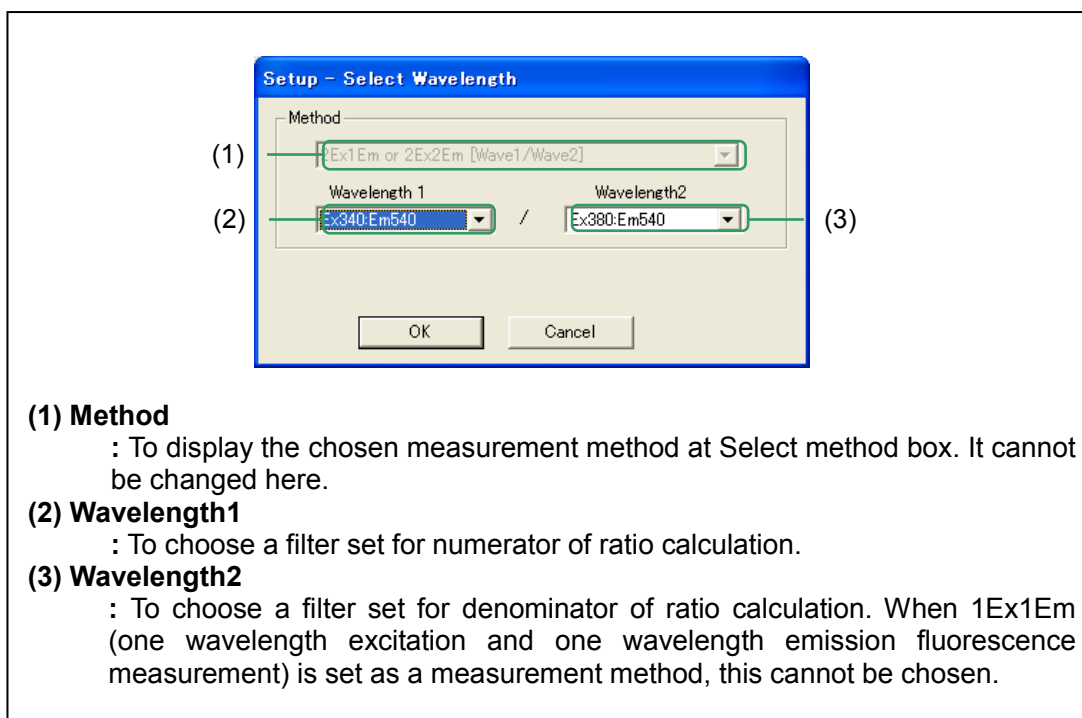
- (1) **Select Double** : Choose the interval method, single or double.
Single : to acquire data with a certain amount of interval.
Double : to acquire data with switching 2 kinds of sampling interval at specified timing
- (2) **Interval** : To set a sampling interval.
- (3) **Sampling Number** : To set total sampling number.
- (4) **Sequence** : To set timing for switching 1st interval and 2nd interval at Double Interval.
Interval : To display the set interval.
Section Samples: To set the sampling number by specified interval.
Section Time : To display period of the specified interval
Total Time : To display the total period from measurement starting.

(1-4) Select Wavelength

Use to set and display filters for measurement. The measurement method cannot be changed here.

**(1-4-1) Select Wavelength**

Clicking 'Setup' leads a setting box 'Select Wavelength' for filter setting.



(1-5) Dispense Timing

Use to set dispensing timing with the specified stage.

Disp.	6	48	95	139	215
Incu.					
ChgOut	0:05.0	0:47.0	1:34.0	2:18.0	3:34.0

(1) Stage selection
: Choose one stage from four and then set timing with the specified stage. The color of characters corresponds to the color of timing arrow bar.

(2) Dispensing timing arrow bar
: Arrows with the colored bars show dispensing timing. And the length of the bar means mechanical moving period of dispenser, and next dispensing timing cannot be set among the period.

(3) Dispensing timing frame bar
: The white bar means total measurement time and timing arrow bars are relatively changed accordingly. Downside number shows a sampling number and time.

(4) The presence of the Preparation setting is displayed
: When various settings are done in “Prepa.” Tab of the protocol, the display that shows the presence of the setting is done.
Disp. : It is displayed that the setting that does “Preparation of Dispenser” is done.
Incu. : It is displayed that the setting that does “Pre Incubation” is done.
ChgOut : It is displayed that the setting that does “Change Out” is done.

<How to set dispensing timing>
Choose a stage and then move timing bar or left-click at dispensing setting area.

<How to cancel dispensing timing>
Choose a stage and then right-click at arrow-head part of timing bar.

<How to set dispensing parameter>
Parameters such as dispensing volume, speed, height and washing number can be set at the separate **Dispenser Control box**.

Note

Dispensing timing might be not in time though the interval between dispensing and dispensing can be set at executable intervals according to the parameter of dispensing set.
Please confirm there is no problem by trying before it measures it actual.

(1-6) Dispenser Control box <In the case of FDSS3000/6000/7000EX>

Use to control a dispenser manually and set dispensing parameter. Setting can be done as a Stage unit, and the dispensing head corresponding to the stage is set at shipping according to the configuration.

(1) Assay Plate

(2) Stage selection tag

(3) Dispensing parameters

(4) Mixing Assay Plate

(5) Auto Cell Tank

(6) Change Head & Ligand Plate

(7) On/Off of tip wiping function at the washing.

(1) Assay Plate
: To load an assay plate in or out when manually controlling the dispenser.
Load : To load an assay plate in.
Unload : To load an assay plate out.

(2) Stage selection tag
: To display combination between dispensing heads and ligand plate stages and to set dispensing parameter here.

(3) Dispensing parameters
: To set dispensing parameter with a Stage unit, choosing a Stage selection tag (Refer to 1-6-1)

(4) Mixing Assay Plate
: To choose ON or OFF of mixing (vibration) function for an assay plate. It is available only at fluorescence measurement.

(5) Auto Ligand Tank
: To choose ON or OFF of auto ligand feeding function. (Option)

(6) Change Head & Ligand Plate
: To exchange dispensing head, tips or ligand plates, specifying which tips and plates with Stage selection tag.

(7) On/Off of tip wiping function at the washing.

Note Click the tag into active and then click this command.

(1-6-1) Dispensing parameter

Used to set dispensing parameter. Setting can be done as a Stage unit, and the dispensing head corresponding to the stage is set at shipping according to configuration.

The screenshot shows a software interface for setting dispensing parameters. At the top, there are four tabs: 'TH1+Stage1' (red), 'TH1+Stage2' (green), 'TH1+Stage3' (blue), and 'TH1+Loader' (yellow). Below the tabs are three main buttons: 'Dispense Start', 'Nozzle Wash', and 'Detail...'. Under 'Dispense Start' is a 'Volume' field set to '20 ul'. Below that is an 'Aspirate' section with 'Speed' set to '50 ul/s' and 'Height' set to '0.5 mm'. Below that is a 'Dispense' section with 'Speed' set to '50 ul/s' and 'Height' set to '2.5 mm'. At the bottom is a 'Multi Wash' section with a dropdown set to 'Off', a 'Setup' button, and a checkbox for 'after measurement'. Numbered callouts (1-9) point to these elements: (1) Stage selection tag, (2) Dispense Start button, (3) Active tab, (4) Nozzle Wash button, (5) Volume field, (6) Aspirate Speed field, (7) Dispense Speed field, (8) Multi Wash dropdown, and (9) Setup button.

(1) Stage selection tag : Choose at which combination dispensing parameter is set.

(2) Dispense Start : To start dispensing operation when operated manually. It dispenses liquid with the active tab head/stage and set parameters in it.

(3) Nozzle Wash : To wash a dispensing head when operated manually. It washes with the active tag head and washing times set into 'Wash Cycles'.

(4) Details : To set the detail parameter such as aspirated place and mixing in/out function (Refer to 1-6-2).

(5) Volume : To set dispensing quantity (unit: μl) with the active tag head.

(6) Aspirate : To set aspirating parameter with the active tag head.
Speed : To set aspirating speed (unit: $\mu\text{l/s}$).
Height : To set aspirating height from the bottom of plate, (unit: mm).

(7) Dispense : To set dispensing parameter with the active tag head.
Speed : To set dispensing speed (unit: $\mu\text{l/s}$).
Height : To set dispensing height from the bottom of plate, (unit: mm).

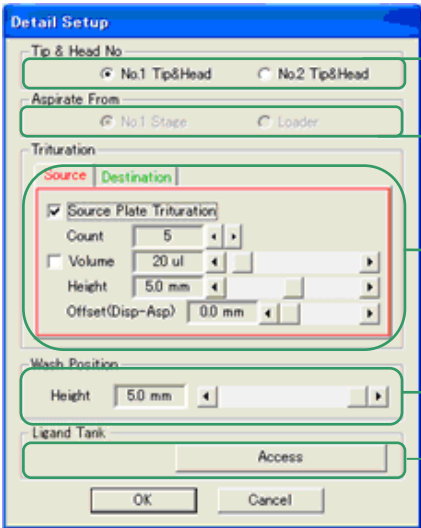
(8) Multi wash (In the case there is Multi wash function)
 : By clicking set up button, to set how many times the dispensing head is washed
Wash Cycles (In the case there is no Multi wash function)
 : To set how many times the dispensing head is washed.

(9) after measurement : To choose at which timing the dispensing head is washed.
Check : To wash a dispensing head after measurement. It works at data acquisition measurement, not at manual control.
No Check
 : To wash a dispensing head after dispensing operation.

(1-6-2) Dispensing detail parameter setting

Used to set dispensing parameter. Setting can be done as a Stage unit, and the dispensing head corresponding to the stage is set at shipping according to configuration.

<FDSS7000EX>



(1) Tip & Head No

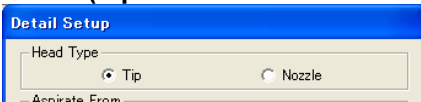
(2) Aspirate From

(3) Trituration

(4) Wash Position

(5) Ligand Tank

<FDSS6000> (Tip&Head No of FDSS7000EX change as follows)



(1) **Tip & Head No:** To choose the combination of dispensing head and tip
There are No.1 and No.2 in the combination, it can be set when starting or changing the head and tip

Head Type : To choose the dispensing head type, tip or nozzle.

(2) **Aspirate From:** To choose from which place liquid is aspirated and then dispensed.
Stage : Aspirate from a ligand plate on the round table.
Loader : Aspirate from a ligand plate on the loading line.

(3) **Trituration** : To set mix-in/out at aspirating and/or dispensing
<Please refer to 2-1 in Chapter 12>

Source Plate : To run mix-in/out at aspirating
Destination Plate: To run mix-in/out at dispensing
Count : To set how many times mix-in/out is done (Common at Aspirate and Dispense).
Volume : To set how much quantity is used at mix-in/out.
Height : To set the height at mix-in/out.
Offset : To set the difference of the height when mix-in and mix-out.

(4) **Wash Position:** To set the washing position
Height : To set the height at washing. (Unit: mm, from the default value where the edge of tip a bit entrances into the washing nozzle.)

(5) **Ligand Tank** : To access the ligand tank for the auto ligand feeder. (Option)
Access : To turn the position of the cell tank for the auto cell feeder to the door position and then to open the door so that it can be accessed for exchanging it

(1-6-3) Parameter setup for Multi Wash Function

In the case there are Multi wash function in your FDSS7000EX, parameter setup is performed on the following box. The setup is performed by Stage

Multi Wash : On/Off

Setup of Parameter

Multi Wash : Setup of On/Off
Parameter : Setup of washing parameter

It is possible to use the washing bat up to 3 bats
The washing is performed in order of 1st Washing -> 2nd Washing -> 3rd Washing
Same washing bat can not be set more than once.

Vat No : Setup for the number of washing unit used
Wash Cycles : Setup for how many times washing is performed
(In the case '0' is set, washing is not performed.)
Ultra Sonic : Setup for On/Off of ultrasonic function
Wash Method : Setup for how to wash
Replace : After filling the washing bat by washing solvent, aspirating and draining washing is performed.
Overflow : while flowing the washing solvent, washing is performed

Note

Wash method depends on setup state of washing bat
Please use this function after confirming the using dispensing setup

(1-7) Dispenser Control box <In the case of uCELL>

Use to control a dispenser manually and set dispensing parameter. Setting can be done as a tab unit.

(For details, Please refer to Chapter 12.)


The screenshot shows the 'Dispenser Control' window. At the top, there are two tabs: 'Assay Plate' and 'Access'. Below the tabs is a row of four tabs labeled 'TH1+Stage1', 'TH1+Stage2', 'TH1+Stage1', and 'TH1+Stage2'. Below these are three buttons: 'Dispense Start', 'Nozzle Wash', and 'Setup'. The main area contains three parameter setting boxes: 'Aspirate', 'Dispense', and 'Wash'. The 'Aspirate' box has fields for 'Asp. Stage' (Stage1), 'Volume' (10 ul), 'Speed' (50 ul/s), and 'Height' (10.0 mm), with a checked 'Trituration' section containing 'Vol.' (10 ul), 'Count' (5), 'Height' (0.0 mm), and 'Offset' (0.0 mm). The 'Dispense' box has fields for 'Speed' (50 ul/s) and 'Height' (10.0 mm), with a checked 'Trituration' section containing 'Vol.' (10 ul), 'Count' (5), 'Height' (0.0 mm), and 'Offset' (0.0 mm). The 'Wash' box has a checked 'Wash' checkbox, an unchecked 'After Measurement' checkbox, 'Vat' (A), 'Cycle' (3), and 'Pos.' (0.0 mm). At the bottom, there is a 'Wipe:A' checkbox, a 'Change Head' button, and an 'Access Ligand No.1' button.

(1) Assay Plate Access : Loading and unloading of assay plate
 (2) TH1+StageX tag : Switching of dispensing parameter combination
 (3) Dispense Start : Execution of dispensing
 (4) Setup : Setting of various setting box
 (5) Nozzle Wash : Washing of tip nozzle
 (6) Aspirate : Setting of aspiration parameter
 (7) Dispense : Setting of dispensing parameter
 (8) Wash : Setting of washing parameter
 (9) Change Head : Exchange of dispensing head
 (10) Access Ligand No.X : Exchange of ligand plate
 (11) Wipe : Setting of On/Off of wiping after washing

Note Click the tag into active and then click this command.

(1-8) Setting tab

Used to set correction, auto sensitivity adjustment, graph display, auto data output, graph calculation parameter and preparation, specifying a tab to be set.

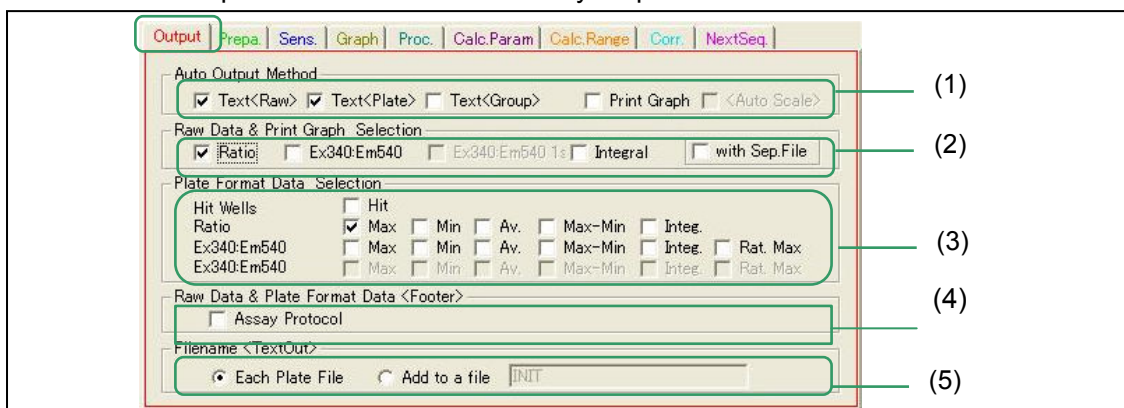


Click a tab to de displayed and to be set

Output	: To set auto data output (Refer to 1-8-1).
Prepa.	: To set preparation parameter such as pre-incubation and plate number. (Refer to 1-8-2)
Sens.	: To set the auto sensitivity adjustment function. (Refer to 1-8-3)
Graph	: To set parameter to be displayed in a graph. (Refer to 1-8-4)
Proc.	: To set parameter of group and data processing. (Refer to 1-8-5)
Calc.Param	: To set calculation parameter in graph data such as Hits Well and Baseline. (Refer to 1-8-6)
Calc.Range	: To set the calculation area. (Refer to 1-8-7)
Corr.	: To set correction such as auto fluorescence and shading. (Refer to 1-8-8)

(1-8-1) Output setting tab

Used to set parameter to be automatically output with TextOut and PrintOut.



- (1) Auto Output Method:** To choose the output method from the following.
- Text<Raw>** : To output graph data of all wells with the text format. Output items are set at Raw data & Print Graph Selection.
 - Text<Plate>** : To output the calculation result of all wells with text format. Output items are set at Plate Format Data Selection.
 - Text<Group>** : To output group calculation results (EC/IC Calculation). No Output data configuration window.
 - PrintGraph** : To printout all wells graph. Output items are set at Raw data & Print Graph Selection.
 - <AutoScale>** : To set the auto scale at PrintGraph.
- (2) Raw Data & Print Graph Selection:** To set items to be output at 'TextOut<Raw>' and 'PrintOut'.
- Ratio** : Ratio value dividing Fluorescence No1 intensity by Fluorescence No2 intensity at 2-wavelength excitation or emission measurement. Or, Ratio value dividing Fluorescence No1 intensity by initial value of it.
 - Fluorescence No1 (Luminescence)** : Intensity of Fluorescence No1.
 - Fluorescence No2** : Intensity of Fluorescence No2.
 - Integral** : Integrated value of intensity or ratio, which is set at 'Calc. Param'. (Refer to 1-7-6)
 - With Sep. File** : Raw data of each item is output with another file. (Refer to 2-1 in Chap.6)
- (3) Plate Format Data Selection:** To set items to be output at TextOut<Plate>.
- Hit** : Hit data with Hit well analysis function for Peak and Integral parameter.
 - Ratio** : Ratio value dividing Fluorescence No1 intensity by Fluorescence No2 intensity at 2-wavelength excitation or emission measurement. Or, Ratio value dividing Fluorescence No1 intensity by initial value of it.
 - Fluorescence No1 (Luminescence)** : Intensity of Fluorescence No1.
 - Fluorescence No2** : Intensity of Fluorescence No2.
 - Integ.** : Integrated value
 - Max** : Maximum value during measurement
 - Min** : Minimum value during measurement
 - Ave** : Average value during measurement
 - Max-Min** : Maximum value- Minimum value
 - Rat.Max** : Intensity value at the maximum value point of Ratio at 2 excitation or 2 emission measurement.
- (4)Raw Data & Plate Format Data <Footer>**
- Assay Protocol** : To output Assay Protocol information at footer of Text data.
- (5) Filename:** To set a file name at TextOut.
- Each Plate File** : To create Text file at each measurement. TextOut file name is named by Plate File name.
 - Add to a file** : To output plural measurement data with the text format into one file. TextOut name is named by input character in the field.

(1-8-2) Preparation setting tab

Used to set preparation such as Pre Incubation and Change Out, and dispensing operation before starting measurement, to set the plate number at sequential measurement (Auto measurement mode).

<In the case of FDSS3000/6000/7000EX>

<In the case of uCELL>

(1) Number of plates
: To set how many plates are measured at Auto measurement mode. When plates run out on the way to the specified number, the measurement finishes.

(2) Exchange cmpd plate Every X plates (Auto measurement)
: The cmpd plate are exchanged every X plates of the measurement plate.

(3) Pre Incubation(min)
: To run Pre incubation before starting measurement. Choose the number to wait for how many minutes or 'Off' when not necessary.
The plate stands by after being loaded in and waits ready for the specified time (Unit: min).

(4) Change Out : Tip and ligand plate can be exchanged before measurement.
1~4(FDSS6000) : The combination of head and stage of a specified number are moved forward and the door is opened.
Tip&Head(FDSS7000EX) : Tip·Head can be exchanged and the door is opened.
OpenDoor(FDSS7000EX) : The door is opened.
Every X plates : To set Change Out every X plates. (Auto measurement)

(5) Change Ligand Plate
: Ligand plate can be exchanged before measurement.
1~4(uCELL) : The ligand stage of a specified number are moved forward.
Every X plates : To set Change Ligand Plate every X plates. (Auto measurement)

(6) Change Head : Head can be exchanged before measurement.
On(uCELL) : Head can be exchanged.
Off(uCELL) : Head can not be exchanged.
Every X plates : To set Change Head every X plates. (Auto measurement)

(7) Preparation of Dispenser

: To set the aspirating and/or dispensing before starting measurement. Is it not available for 'Change Out' and 'Preparation of Dispenser' to work simultaneously.

Before Loading Assay Plate Step1/2

: Dispensing operation before a assay plate loaded in.
(Useful at configuration, which has only one loading line.)

Dilute Ligand Loader From

: To load a ligand plate in, aspirate from a ligand plate on a stage and then dispense a ligand plate on the ligand loader. (If there is no setup of the following "Before Loading Assay Plate", load a ligand plate out.)
Choose from which stage the dispensing head aspirates.

Disp.From Ligand Loader To

: To load a ligand plate in, aspirate from a ligand plate on the ligand loader and then dispense a ligand plate on a stage. (If there is no setup of the following "Before Loading Assay Plate", load a ligand plate out.)
Choose from which stage the dispensing head aspirates.

Aspirate From Loader

: To load a ligand plate in, aspirate ligand from it and then load it out.
Choose into which stage dispensing heads dispense over amount aspirated liquid.

After Loading Assay Plate Step1/2

: Dispensing operation after a assay plate loaded in.

Dispense Buffer From

: To aspirate from a ligand plate on the round stage and then dispense into an assay plate. Choose from which stage the dispensing head aspirates.

Aspirate Medium To

: To aspirate from an assay plate and then dispense into a ligand plate on a stage. Choose into which stage dispensing head dispense.

Disp. To Stage1 From

: To aspirate from a ligand plate on a stage and then dispense into a ligand plate on the stage1.
Choose from which stage the dispensing head aspirates.

Disp. To Loader From

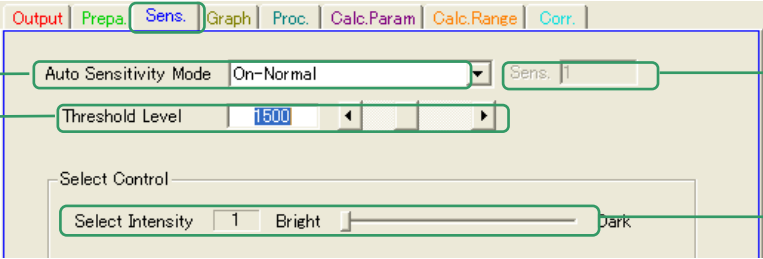
: To aspirate from a ligand plate on a stage and then dispense into a ligand plate on the ligand loader.
Choose from which stage the dispensing head aspirates.

Dilute Ligand Loader From <Option Function>

: To aspirate from a ligand plate on a stage and then dispense into a ligand plate on the ligand loader.
Choose from which stage the dispensing head aspirates.

(1-8-3) Sensitivity setting tab

Used to set parameter for Auto sensitivity adjustment function. Auto sensitivity adjustment function is useful to set proper detector gain and to keep reasonable dynamic range according to sample brightness.



(1) Auto Sensitivity Mode
: To choose the setting mode for Auto sensitivity adjustment function.
Off : Not to use Auto sensitivity adjustment function. It doesn't see intensity before measurement. It starts measurement with the fixed initial camera gain at opening the assay protocol box.
Manual-1st.Plate Only
: Not to use Auto sensitivity adjustment function.
 (To see intensity and set gain at the first plate by manual at Auto measurement mode.)
On-Normal
: To see intensity and set camera gain at each plate.
Off-Fix : To see intensity with manually set the fixed gain in 'Sens'.
On-1stPlate Only
: To see intensity and set gain at the first plate. And then the sensitivity adjustment setting is employed for the rest plates at Auto measurement mode. It sees and sets at each Single measurement mode.

(2) Threshold Level
: To set Threshold level for sensitivity adjustment standard.

(3) Sens : To set Sensitivity at the fixed sensitivity setting.

(4) Select Intensity
: To choose bright range for the sensitivity setting. (Refer to 1-8-3-1)
Range No 1 to 11
: Brightness comes from average value of 8 well at Auto sensitivity adjustment setting. All wells are ranked based on high intensity and divided them into 10 groups. And brightest 8 wells at each group are picked up and the average is used for sensitivity setting. Darkest 8 wells are used for Range 11.

(1-8-3-1) Auto sensitivity setting method

Threshold level is a standard value to set camera gain (Sensitivity) at the sensitivity adjustment. Threshold level depends on assays but it is basically set as bellow.

Case1: Big intensity change is observed like Fluo3. :

Threshold Level = 1,500 (In the case of ORCA)

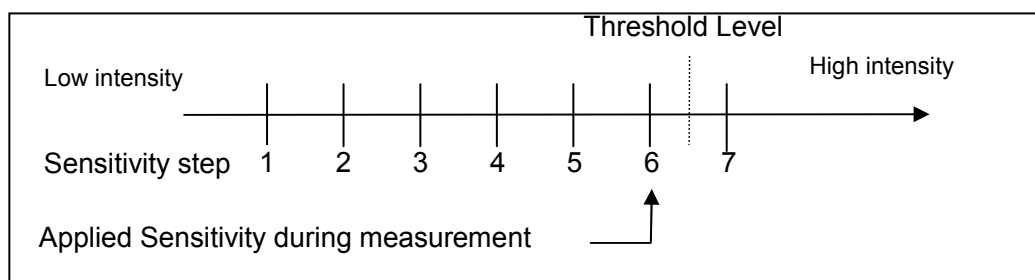
Threshold Level = 20,000 (In the case of EMCCD)

Case2: Ratio value is acquired like Fura2 :

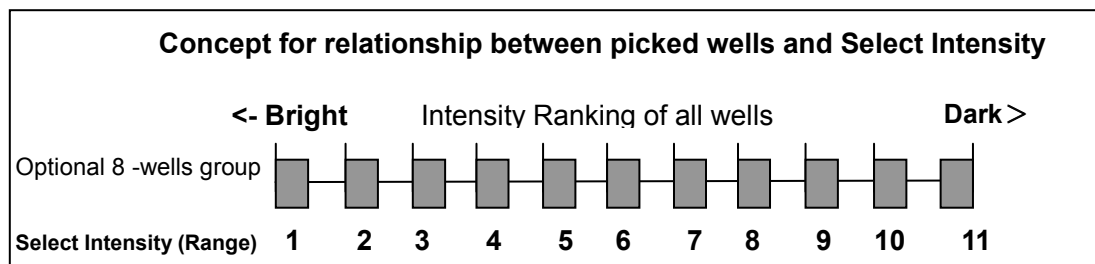
Threshold Level = 2,000 (In the case of ORCA)

Threshold Level = 30,000 (In the case of EMCCD)

In Sensitivity adjustment, 8-wells average value which is set at 'Select Intensity' (Refer to 1-8-3-2) gets up camera gain (Sensitivity step) by one-steps through to Threshold level. When 8-wells average intensity reaches Threshold Level, the gain setting goes back to set the last Sensitivity step of Threshold Level and then start measurement with the Sensitivity step.

**(1-8-3-2) Select Intensity**

Intensity values of a certain wells are picked up as standard for Auto sensitivity adjustment setting and these values are set with Select Intensity parameter. The following figure shows the concept of Select Intensity. All wells are ranked based on high intensity and divided them into 10 groups and the brightest 8 wells at each group are picked up and the average value of the 8 wells is used for auto sensitivity setting. Darkest 8 wells are used for Range 11.



Select intensity is to set wells for judging the above average intensity. When the set value is bigger, darker wells are picked up as standard for auto sensitive adjustment. For example:

Case1: All wells have the same cells with the same quantity (Basically the intensity values are the same.)

: Select Well Level = Any value is OK

Case2: There are several wells which have much brighter intensity and it would be better to ignore the bright wells

: Select Well Level = 2 to 3 (Clear off 20 % to 30 % bright wells)

Case3: Originally there are compounds which have auto florescence in well and around 50 % of total number and it would be better to ignore the bright wells

: Select Well Level = 5 to 6 (Clear off 50 % to 60 % bright wells)

Relationship between Threshold Level parameter and Intensity Level parameters as shown when manually adjusting sensitivity

**Target intensity used as standard for auto sensitivity adjustment
= Threshold Level**

**Average intensity of 8 wells is used for auto sensitivity adjustment
= Select Intensity**

(1-8-4) Display Graph setting tab

Used to set which kind of graph and graph scale (y-axis) to be displayed at measurement.

(1) **Display Graph** : To set which kind of graph to be displayed.

(2) **Ratio Range** : To set graph scale of ratio value.

(3) **Intensity Range** : To set graph of ratio value of
fluorescence/luminescence intensity

(4) **Integral Range** : To set graph scale of integral value.

(5) **Display Marker** : On/Off of marker display during measurement
Real Time Auto Scale
: On/Off of Auto-scale of Intensity and Ratio during the measurement
Real Time Time Scale Expand
: On/Off of Auto-expand display of time axis during the measurement

(1-8-5) Processing Setup Tab

Setup for the data processing, data processing is performed when the data is read after getting data

Setup for ON/OFF of data processing

Display of the plate well: Setup & Display of control well

Setup for parameter of each data processing

Setup for select of group well

Processing

- : Set up for On/Off of the each data processing
- S.U. = Spatial Uniformity Correction
- N.C. = Negative Control Correction
- S.B. = Subtract Bias
- P.C. = Positive Control Scaling

Select Group : Select well group and setup

Group List = Selectable group is shown here.
Select the group and setup well configuration on the plate well display

Setup Group = Add,delete,edit Group

Spatial Uniformity

- : Setup of reference range for use of S.U. (Set up of sampling number)

Negative Control

- : Setup of basis area for use of N.C. (Set up of sampling number)

Subtract Bias

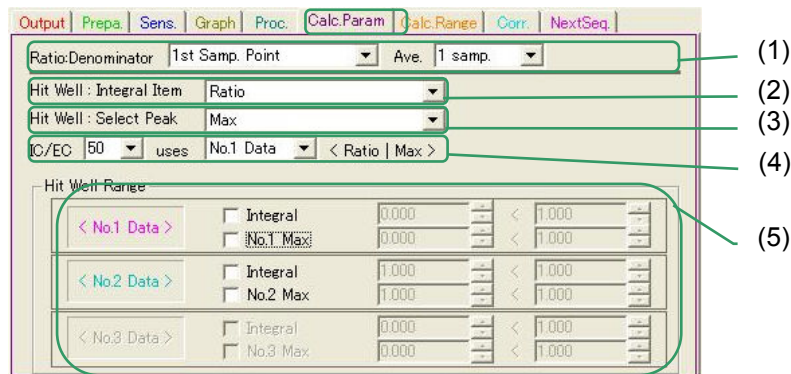
- : Setup of Bias range (Set up of sampling number)
- Minus Clip can calculate '0' if the value is minus after being subtracted

Positive Control

- : Setup of calculating area for used in P.C. (Set up of sampling number)

(1-8-6) Calc.Param setting tab

Use to set parameter for calculation proceeding about measurement data such as Hit well analysis.

**(1) Ratio / Denominator**

: To set the denominator item of ratio (refer to 1-8-6-1)

<when two wavelength measurement>**Wavelength2 Intensity**

: Intensity value of wavelength2 of each sampling point

<when one wavelength measurement>**1st.Samp.Point**

: Intensity value in which 1st. sampling point is assumed to be a reference point

Ave. : Average value of the set sampling from the 1st. sampling point

Delay : The reference point is moved behind the set sampling

<Option Function>**X Disp. Point <Option Function>**

: Intensity value in which sampling point before No.X dispensing point is assumed to be a reference point

Ave. : Average value before the set sampling from the reference point

Delay : The reference point is moved behind the set sampling

(2) Hit Well/Integral Item

: To set item for Hit well analysis, Ratio or Florescence(Luminescence) intensity.

Normally ratio value at fluorescence and Intensity at luminescence measurement are recommended.

(3) Hit Well/Select Peak

: To set peak for Hit well analysis, Max or Min. Normally Max value at the measurement where the change goes up.

(4) IC/EC X uses No.X Data**IC/EC X**

: Set IC/EC Percentage

Percentage value can be configured from 10 to 90, 10 value step.

Uses No.X Data

: Set the data number using for IC/EC calculation.

The number should be selected from the data number 1 to 3 in Calc.Range Setup Tab.

(5) Hit Well Range

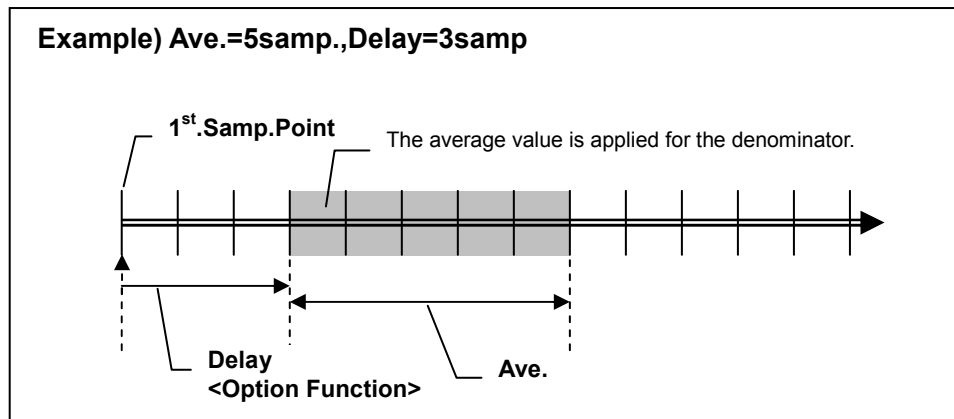
: To set parameter range at integral and at max at for condition of Hit well analysis. Hit well analysis function is to automatically pick up applicable well to the set condition at Integral and Max.

(1-8-6-1) Ratio/Denominator setting

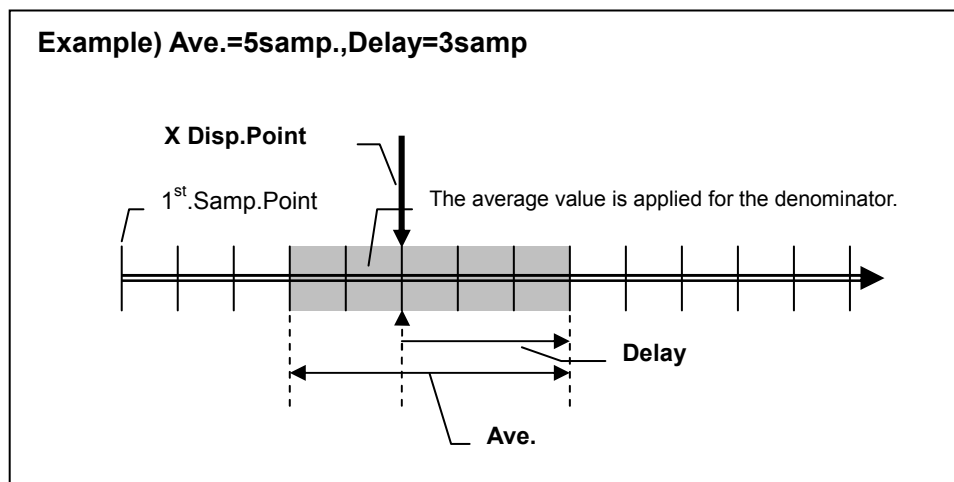
It can be set which value is used at the denominator of the ratio for the ratio calculation on one wavelength measurement, using Ratio/Denominator setting.

<1st.Samp.Point setting>

The average value of the set number in 'Ave.' is applied for the denominator of the ratio, being shifted from the set number in 'Delay' from 1st.Samp.Point.

**<X Disp.Point> (Option Function)**

The average value of the set number in 'Ave.' is applied for the denominator of the ratio, being shifted from the set number in 'Delay' from X Samp.Point.



(1-8-7) Calc.Area setting tab

Use to set calculation area at calculation proceeding about measurement data.

(1) No.1,2,3 Tab
 : To select the area which is separated at the dispensing timing.

(2) **Enable Output No.X Data**
 : To enable the calculation of the data with check.

(3) **Baseline(Integral:Max;Min:Av:Max-Min)**
 : To set the baseline for the calculation. When enabled, the average value of the baseline range is subtracted at the result. Integral value reflects on real time graph at measurement.

Enable Sub.Baseline
 : To enable the baseline finite difference function with check.

To 1st.Dispense
 : To set range to be recognized from measurement start to the first dispensing timing.

Start/End
 : To set range manually to be recognized as a baseline.

(4) **Integral Range**
 : To set range for the integration (Integral)

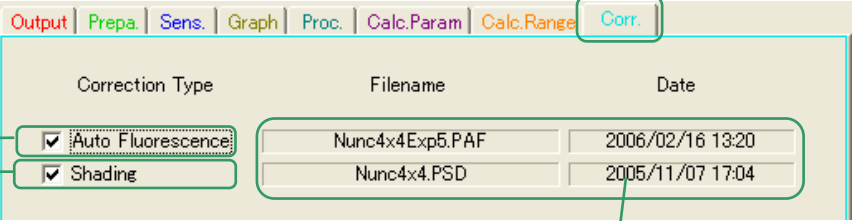
(5) **Max:Min:Max-Min Range**
 : To set range for Max (maximum intensity) ,Min (minimum intensity) and Max-Min value.

(6) **Average Range**
 : To set range for Average (average intensity) value.

All ranges : To set all ranges from the first sampling to final one.
Start/End : To set a certain range with sampling number for Start/End.

(1-8-8) Correction setting tab

Used to set ON/OFF for auto fluorescence correction data or shading correction data which are created at each command in Setup menu (Refer to 3 and 4 in Chapter 8).



Correction Type	Filename	Date
<input checked="" type="checkbox"/> Auto Fluorescence	Nunc4x4Exp5.PAF	2006/02/16 13:20
<input checked="" type="checkbox"/> Shading	Nunc4x4.PSD	2005/11/07 17:04

It displays correction data information. The data files are created with plate information and they are automatically chosen from the reiterated data here.

(1) Auto fluorescence correction On/Off
: To employ auto fluorescence correction data with check (Not necessary at luminescence measurement).

(2) Shading correction On/Off
: To employ shading correction data with check.

(1-8) Start <Single>

To start single measurement with only one plate. The result is displayed after measurement.

(1-9) Start <Auto>

To start sequential measurement with plural plates. 'Number of Plates' in Preparation setting tab can set how many plates. File name is named by auto naming function or bar code reader (optional). In order to finish the easement with fewer plates than the specified number, click 'Stop' during measurement.

(1-10) Close

To close the assay protocol setting box.

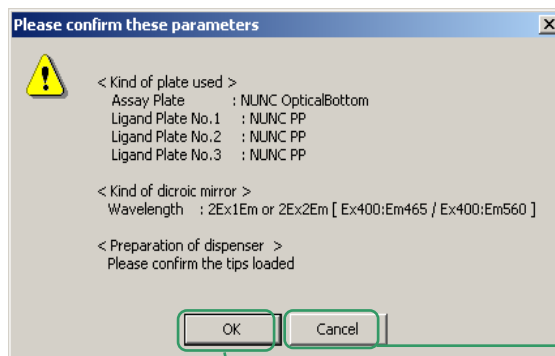
2) External Control (Start External Control command)

< Option >

This command is used when FDSS is controlled by the scheduling software at the robot integration. Clicking 'Start External Control' displays the following message box and FDSS turns to the external control mode. Users cannot operate FDSS directly under the external control mode.

<Confirmation message for the plate, filter and dispensing head>

Confirm the plate, filter and dispensing head are properly set, and then click 'OK'.

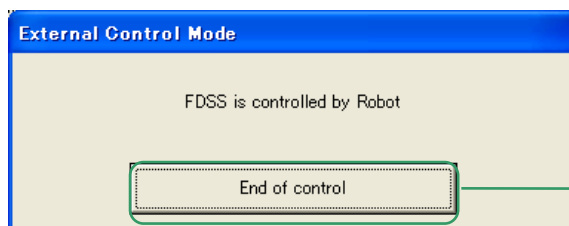


Cancel the external control operation

Start the external control operation

<External control mode window>

Under the external control operation, the following box appears. Click 'End of Control'. Users cannot operate FDSS directly under the external control mode.



Finish the external control operation

(This page is blank.)

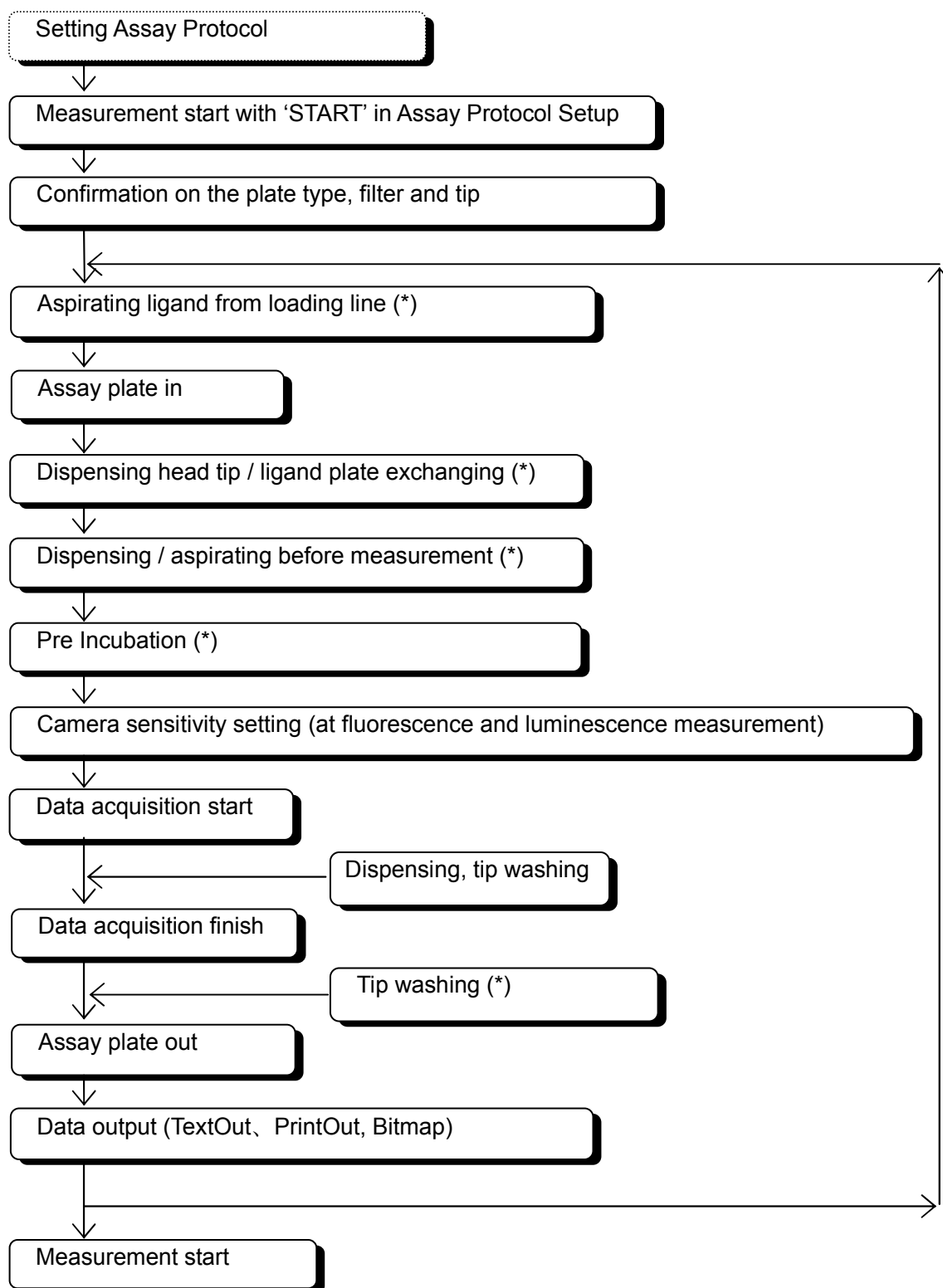
5. Measurement

This chapter describes to start measurement and to acquire data. It has several measurement methods such as 1 wavelength excitation measurement (Fluo3/4), 2 wavelengths excitation measurement (Fura2) and Photon Counting measurement (Luminescence). The measurement method is chosen at starting up software.

<Start measurement>

Clicking 'Start <Single>' or 'Start <Auto>' in Assay Protocol Setup box starts measurement based on the set protocol.

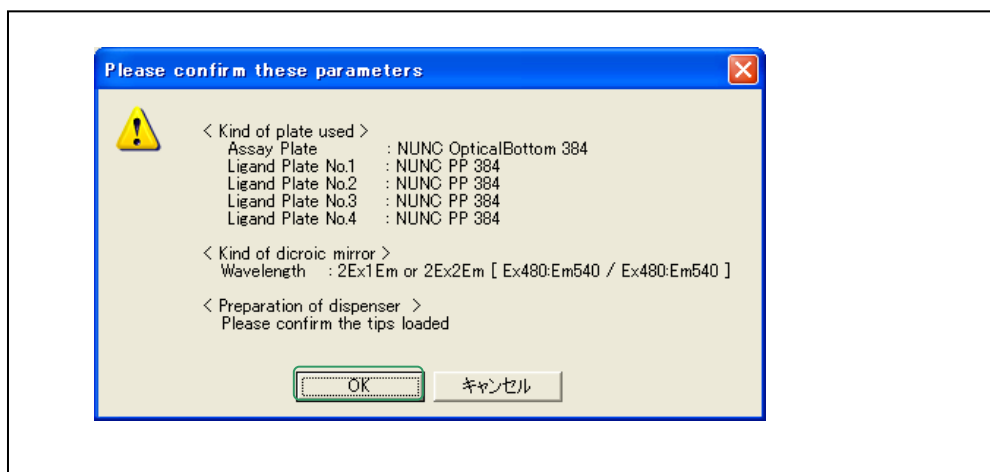
1) Workflow for measurement



(*) : When setting it with assay protocol

2) Confirmation using plate, filter, tip

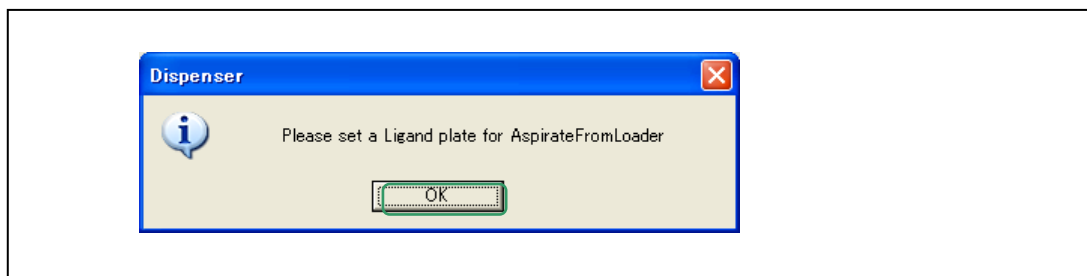
When clicking 'START' in Assay Protocol Setup to start measurement, the following message appears. Confirm the plate type, filter and tip are properly set and then click 'OK'.



3) Aspirating ligand from the loading line **(Only at the configuration which has just one loading line.)**

It can load a ligand plate through the loading line and aspirate ligand before measurement. This is used effectively only at the configuration which has just one loading line.

Enable 'Aspirate From Loader' at Preparation tab in Assay Protocol Setup box and then click 'Start'. The following box appears at no stackers. Set a ligand plate on the loading arm and click 'OK'. The plate is loaded in for ligand to be aspirated and then loaded out. **Automatically done at the configuration with stackers.**

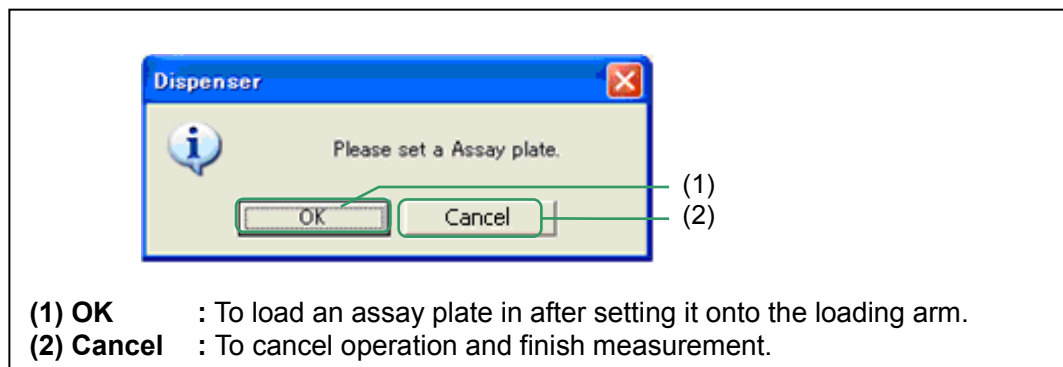


4) Assay plate In

The following box appears when an assay plate is manually set and then loaded in, after clicking 'START' <Single>. Set a plate onto the loading arm and also click 'OK' to load it in.

(For uCELL, please put an assay plate on the assay plate folder and click 'OK' button.)

Automatically done at the configuration with stackers.



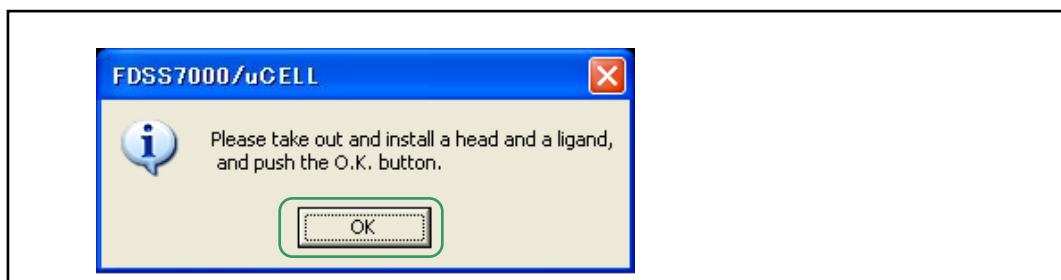
Note

When an assay plate starts to be loaded in, if the loading fails, the following messages appear. Confirm the plate is set well at the loading arm or stacker and then click 'OK'.



5) Dispensing head tip/ Ligand plate exchanging

Dispensing heads and/or ligand plates can be exchanged after the plate loaded and before starting measurement when setting enable 'Change Out' at Preparation tab in Assay Protocol Setup box. The following box appears. Clicking 'OK' proceeds to next step'.



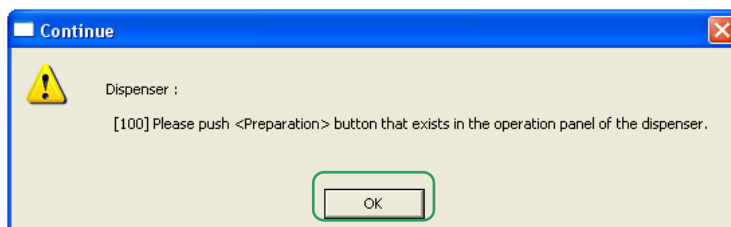
Note

For FDSS7000EX.

The door of the dispenser closes when OK button of the above-mentioned message box is pushed.

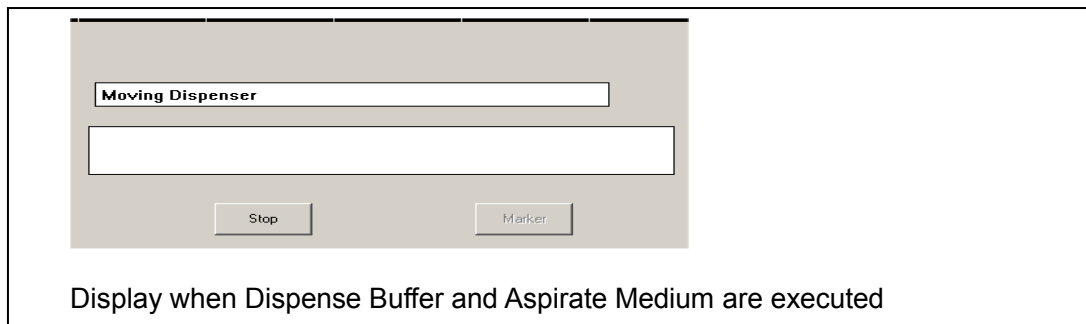
After the door of the dispenser closes, the following message box is displayed. Please push the OK button after pushing the 'Preparation' button of the front panel of the dispenser.

(It is not displayed according to the system, and Preparation is done by the automatic operation.)



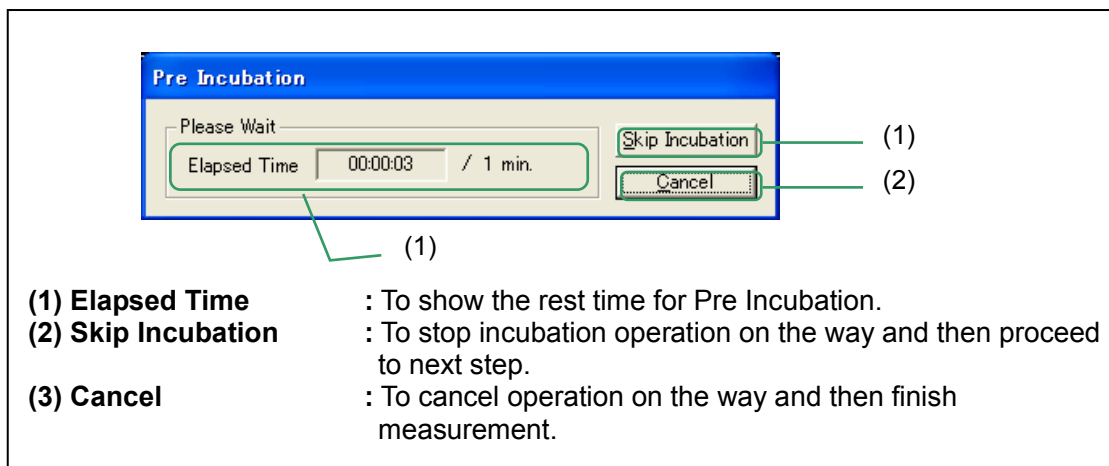
6) Dispensing/Aspirating before measurement

Dispensing ligand and/or aspirating medium runs before starting measurement when setting enable 'Dispense Buffer' and/or 'Aspirate Medium' at Preparation tab in Assay Protocol Setup box. The status message shows 'moving dispenser'.



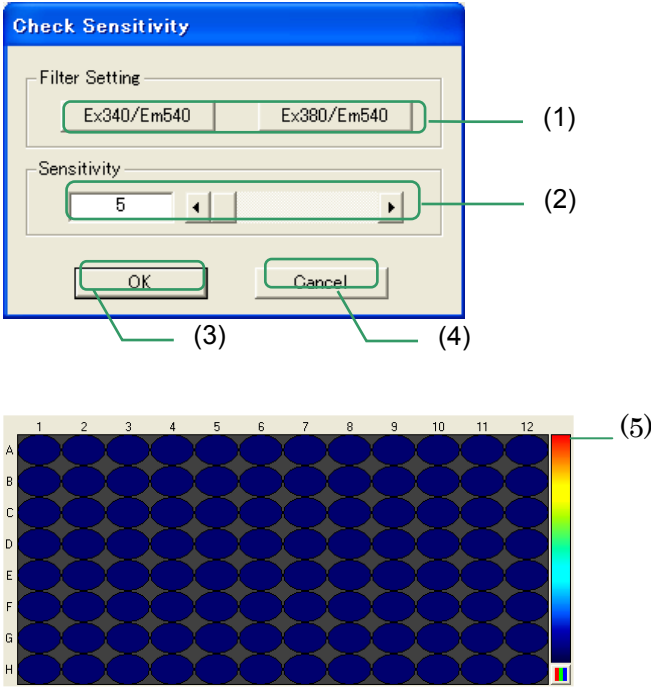
7) Pre Incubation

Pre Incubation runs before starting measurement when setting enable at Preparation tab in Assay Protocol Setup box. The plate stands by after being loaded in and waits ready for the specified time. The following box is displayed during Pre Incubation and it shows the rest time. Clicking 'skip' stops incubation operation even on the way and then proceeds to next step.



8) Camera sensitivity setting at fluorescence and luminescence [Analog] [EMCCD] measurement

Sensitivity adjustment automatically starts after the plate in and preparation operation and shows the following setting box. Clicking 'OK' after confirming image simulate data at 'Microplate Display'.



(1) Filter Setting : To choose a filter to be used for acquiring image when checking sensitivity.

(2) Sensitivity : To set manually sensitivity to be used for measurement.

(3) OK : To proceed to next step, data acquisition.

(4) Cancel : To cancel operation on the way and finish measurement.

(5) Microplate Display : To display Ratio/Intensity/Integral on the specified sampling line with pseudo color.

Note

Sensitivity can be changed manually. In case auto sensitivity adjustment (Auto, Fix) is ON, it skips the following box and proceeds to next step.

9) Data acquisition start

It automatically starts data acquisition after auto sensitivity adjustment. The following is displayed during acquisition.

(1) (2) (3) (4)

(1) Real time graph display
: To display all wells graph data. The items and scale to be displayed are set at 'Display Graph' tab in assay protocol.

(2) Process status and operation
: To display the process status and how many samplings remains, and to operate commands during data acquisition. (Refer to 9-1).

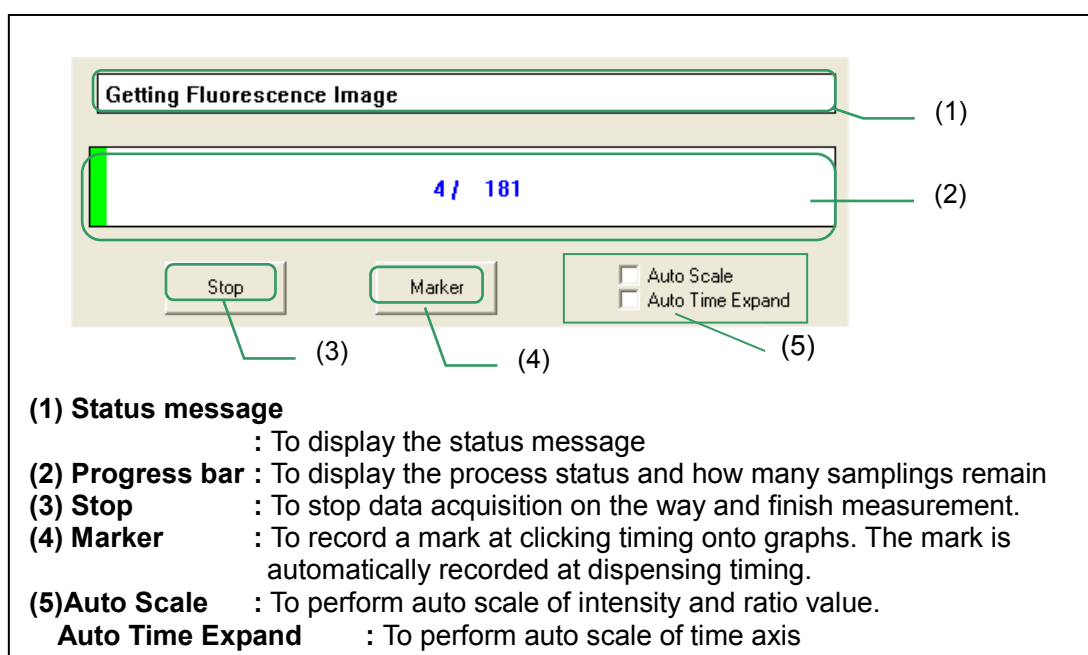
(3) Single graph display
: To display single graph data. It can show maximum 10 wells data with overlay. Left- clicking one graph on (1) Real time graph display shows it at this display.

(4) Live image : To display camera live images.

(9-1) Operation during data acquisition

Acquisition status is shown as below during data acquisition. Clicking 'Stop' stops acquisition and finish measurement. Clicking 'Marker' records a marker at clicking timing onto graphs. The marker is automatically recorded at dispensing timing.

It can show single graph and also show maximum 10 wells data with overlay. Left-clicking one graph on Real time graph display shows it at Single graph display.



10) Measurement finish

It shows measurement result just after completing measurement. When running sequential measurement with plural plates with **START <Auto>**, the plate loader comes back to the initial position and then loads a plate in for next measurement.

Note

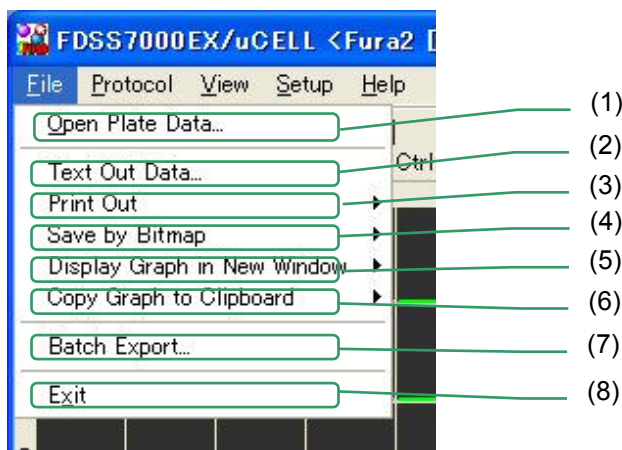
Measurement data can be processed at Data control box.

(This page is blank.)

6. File menu

Used to load Plate Data (measurement data), print out graphs and output data with a text format.

<Command> Clicking File menu leads commands as bellow.



- (1) **Open Plate Data...** : To load FDSS plate data (measurement data).
- (2) **Text Out Data...** : Export data with a text format.
- (3) **Print Out...** : To print out graphs with some methods.
- (4) **Save by Bitmap...** : To save the display by a bitmap format.
- (5) **Display Graph in New Window...**
: To display the graph in new window.
- (6) **Copy Graph to Clipboard...**
: To copy the graph to clipboard.
- (7) **Batch Export...** : Text out and calculation are done by plural data.
- (8) **Exit** : Quitting FDSS application software.

1) FDSS Plate Data loading (Open Plate Data command)

Clicking 'Open Plate Data' command from File menu leads the following box. Specify Plate Data to be loaded with a mouse or key and click 'OK' to loads Plate Data which is specified. Clicking 'Cancel' cancels operation and goes back to the original screen.

Plate Name	Date	Plate Comment
test081108051	2008/11/08 10:11	
test081108050	2008/11/08 15:15	
test081108049	2008/11/08 15:13	
test081108048	2008/11/08 15:10	
test081108047	2008/11/08 15:07	
test081108046	2008/11/08 15:05	
test081108045	2008/11/08 15:02	
test081108044	2008/11/08 14:59	
test081108043	2008/11/08 14:57	
test081108042	2008/11/08 14:54	
test081108041	2008/11/08 14:52	
test081108040	2008/11/08 14:47	
test081108039	2008/11/08 14:45	
test081108038	2008/11/08 14:10	
test081108037	2008/11/08 14:08	
test081108036	2008/11/08 14:05	
test081108035	2008/11/08 14:02	
test081108034	2008/11/08 14:00	
test081108033	2008/11/08 13:57	
test081108032	2008/11/08 13:54	
test081108031	2008/11/08 13:51	
test081108030	2008/11/08 13:48	
test081108029	2008/11/08 11:39	

(1) Folder Name : To specify a folder where Plate Data files are stocked.
 (2) Plate Name : To display a file name of Plate Data.
 (3) Date : To display a date when it is created.
 (4) Plate Comment : To display a comment which is recorded in Plate Data.
 (5) OK : To load Plate Data which is specified.
 (6) Cancel : To cancel loading data.

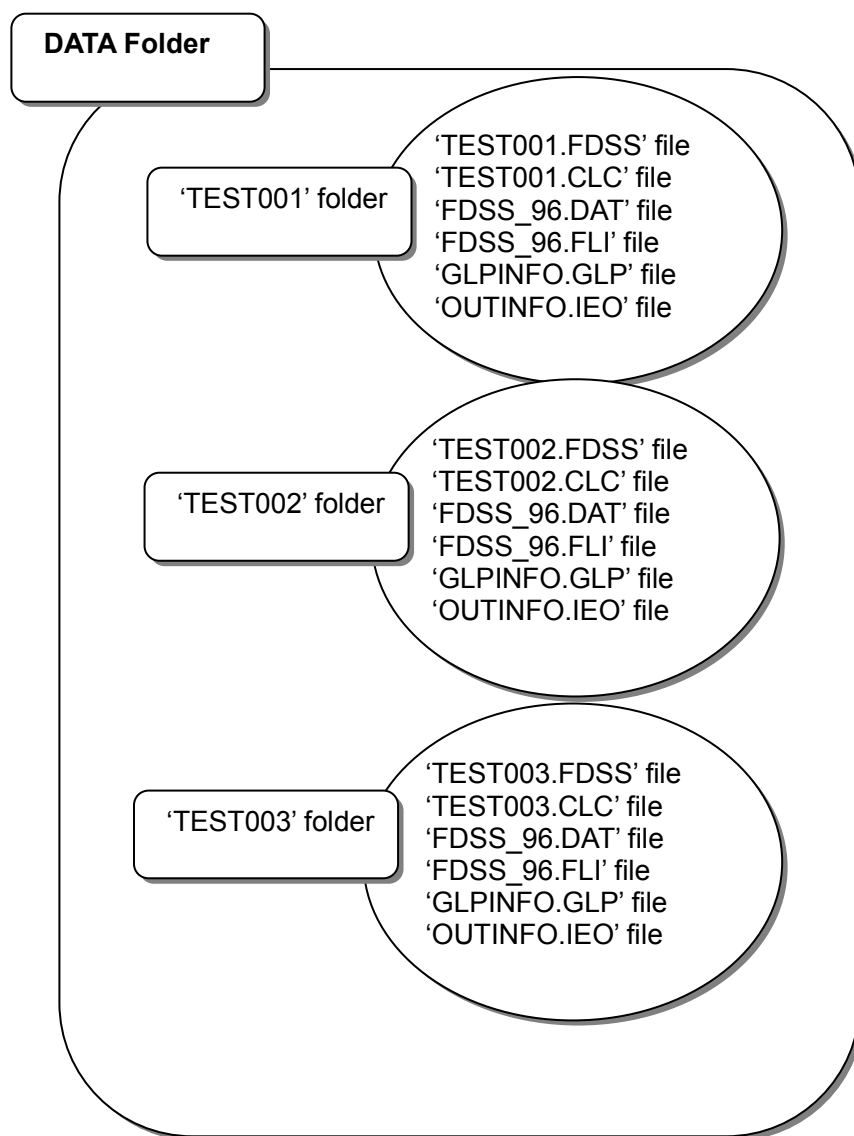
(1-1) Plate data

Plate Data consists of six different format data files and the files are managed in one folder.

<Example: Plate Data name 'TEST001' (with 96well format)>

'TEST001' folder
'TEST001.FDSS' file
'TEST001.CLC' file
'FDSS_96.DAT' file
'FDSS_96.FLI' file
'GLPINFO.GLP' file
'OUTINFO.IEO' file

Copy or delete Plate Data as folder unit.



2) Text output (Text Out Data command)

Clicking 'Text Out Data' command from File menu leads the setup box to save a file. Name the file and set Output items, then click 'OK' to save data

(1) **Data Name** : To set a file name of data.

(2) **Filename** : To display a file name of actual output.

(3) **Raw Data** : To set output items with a text format at Raw data.
With Sep.File : Raw data of each item is output with another file.

(4) **Use Assay Protocol**: To choose items which are set at an assay protocol setting box.

(5) **Plate Format Data**: To set items to be output at TextOut<Plate>.
Hit : Hit data with Hit well analysis function for Max and Integral parameter.
Ratio : Ratio value dividing Fluorescence No1 intensity by Fluorescence No2 intensity at 2-wavelength excitation or emission measurement. Or, Ratio value dividing Fluorescence No1 intensity by initial value of it.
Fluorescence No1 (Luminescence) : Intensity of Fluorescence No1.
Fluorescence No2 : Intensity of Fluorescence No2.
Integral : Integrated value of intensity or ratio, which is set at 'Calc. Param'.
Integ. : Integrated value.
Max : Maximum value.
Min : Minimum value.
Av. : Average value.
Max-Min : Maximum - Minimum value.
Rat.Max : Intensity value at maximum value point of Ratio at 2 excitation or 2 emission measurement.

(6) **Group Data**
: Check "Group Data" when you need group data output.

(7) **Raw Data & Plate Format Data <Footer>**
: To set the assay protocol information at the footer part of text data.

(8) **OK** : To set output parameter and to proceed to next step.
Cancel : To cancel operation

(2-1) File name of Text output

Text output can output text data with Raw and/or Plate format (max. 3 files for 3 data areas).

Plate Format data can output max 3 files according to the number of the data area.

Setup Text Out Parameter

Filename: [111018003]

Data Name: [111018003]

Raw Data: C:\FDSSDATA\RAW\TEXT\111018003_RAW.TXT

Plate Format Data No.1: C:\FDSSDATA\TEXT\111018003_1.TXT

No.2: C:\FDSSDATA\TEXT\111018003_2.TXT

No.3: N/A

Group Data: C:\FDSSDATA\TEXT\111018003_GRP.TXT

Output Item

Raw Data: Ratio Ex480Em540 Integ. with Sep. File Use Assay Protocol

Plate Format Data

Hit Wells: Hit

Ratio: Max Min Av. Max-Min Integ. Rat. Max

Ex480Em540: Max Min Av. Max-Min Integ. Rat. Max

Ex480Em540: Max Min Av. Max-Min Integ. Rat. Max

Group Data

Group Data

Raw Data & Plate Format Data (Footer)

Assay Protocol

Annotations:

- 'with Sep.File' Option (points to the checked checkbox)
- Group Data: Group data: (points to the Group Data section)
- Group Data Filename: TEST001_GRP.TXT (in an oval)
- Raw Data: Ratio data: Ex480Em540 data: (points to the Raw Data section)
- Raw Data Filename: TEST001_RAW.TXT (in an oval)
- 'with Sep.File' is checked (points to the checked checkbox)
- Raw Data Filename: TEST001_RAW_Ratio.TXT TEST001_RAW_Ex480Em540.TXT (in an oval)
- Plate Format Data: No.1 data: No.2 data: No.3 data: (points to the Plate Format Data section)
- Plate Format Data Filename TEST001.TXT TEST001_2.TXT TEST001_3.TXT (in an oval)
- “_1.TXT” is added’ is checked (points to the checked checkbox)
- Plate Format Data Filename TEST001_1.TXT TEST001_2.TXT TEST001_3.TXT (in an oval)

Plate Format Data
: To output with the added characters ‘_2’, ‘_3’ at the tail end of the named ones. To output with the added characters ‘_1’, ‘_2’, ‘_3’ at the tail end of named ones when “_1.TXT” is added’ parameter is checked.

Raw Data
: To output with the added characters ‘_RAW’ at the tail end of the named ones. To output with the added characters ‘_RAW_”Item name”’ at the tail end of the named ones when ‘with Sep.File’ parameter is checked.

Group Data
: Outputs of group data have ‘_GLP’ on the tail of file name.

Note

The place and “_1.TXT” is added’ parameter that saves data can be set in ‘Setup Filename’ box in Assay Protocol setting box.

(2-2) Text output format

Text output format is tab-delimited base. And it consists of the header part and well data.

<Raw data format>

It is tab-delimited base text data. (x , y) = (time , well) format is saved at bellow header part.

	A	B	C	D	E	F	G
1							
2							
3							
4							
5							
6							
7							
8	No.	Comment	Type	0	1001	2002	
9	A1		Ratio	0	46.433	46.059	
10	A2		Ratio	0	45.108	45.977	
11	A3		Ratio	0	46.911	46.222	
12	A4		Ratio	0	46.512	46.93	
103	H11		Ratio	1	1.002	0.999	
104	H12		Ratio	1	0.999	0.999	
105							
106	[Assay Protocol]						
107	Item	Parameter	Value1	Value2	Value3	Value4	
108	<Measurement Parameter>						
109		Protocol Name					
110		Measurement1 Ex1 Em					
111		Measurement Ex480:Em540 / Ex480:Em540 1st Data					
112		Samples	121				
113		Interval Mode	Single				
114		Interval 1st F	1				

(1) Header
 (2) Plate Data File Name : To describe a file name of data.
 (3) Comment : To describe an input comment.
 (4) Date : To describe data when data is acquired.
 (5) Time : To describe time when data is acquired at the date.
 (6) Mark1 : To describe when marking is put during measurement.
 (7) Sampling Time : To describe sampling time (Unit: ms) and shows time axis.
 (8) Data : To describe measurement data.
 (9) Type : To describe data item.
 (10) No. : To describe Well number.
 (11) Footer : To describe the assay protocol information.
 (12) Processing: To describe data processing information.

Note

When the number of markers increases, the record is added below 'Mark1'.
 Note that the below rows are shifted down.

<Plate Format data format>

It is tab-delimited base. Data is saved with a plate format at below header part.

	A	B	C	D	E	F	G
1	Data File NC:\#FDSSDATA\#DATA\#check96well						
2	Comment : fdasasfdasfdaf						
3	Date : 2004/9/27						
4	Time : 21:13:13						
5	ProcessingSU,NC,SB,PC						
6	Marker : 00:10:0						
7							
8	Type :	No.1	Integ.Ratio	Calc.Range : 1 < 15			
9	No.	1	2	3	4	5	
10	A	2647.51	2770.841	2973.37	4113.032	4216.775	
11	B	2218.843	2741.672	3345.949	3898.046	3926.792	
12	C	2618.715	2972.754	3578.778	4521.052	4625.994	
13	D	2779.149	3213.551	4077.591	4534.912	4531.332	
14	E	2502.151	3049.19	3513.521	3989.792	4102.212	
15	F	2837.454	3684.3	4266.668	4404.845	4315.759	
16	G	3333.756	3842.954	4181.989	5026.196	4439.751	
17	H	3332.824	3542.027	4303.06	4897.184	4747.884	
18							
19	[Assay Protocol]						
20	Item	Parameter	Value1	Value2	Value3	Value4	
21	<Measurem						
22		Protocol Name					
23		Measurement Mo1 Ex1 Em					

(1) Header

(2) Plate Data File Name : To describe a file name of data.

(3) Comment : To describe an input comment.

(4) Date : To describe data when data is acquired.

(5) Time : To describe time when data is acquired at the date.

(6) Mark1 : To describe when marking is put during measurement.

(7) Type : To describe data item.

(8) Column : To describe column number of well

(9) Data : To describe measurement data.

(10) Row : To describe row number of well.

(11) Footer : To describe the assay protocol information.

(12) Data processing : To describe the data processing information

(13) Calc.Range : To describe range of calculation

Note

When the number of markers increases, the record is added on right side of 'Mark1'.

<Group Data Format>

Group data format is TAB separated text data.

Consists of Header part, Group data parts, IC/EC value, and well group information part showing in plate format.

	A	B	C	D	E	F
1		Data File Name	C:\FDSSDATA\DATA\Check96well			
2		Comment :	TEST Comment			
3		Date :	2011/10/18			
4		Time :	16:48:01			
5		Processing :				
6		Marker :	0020.0			
7						
8		Type :	No.1 Integ Ratio	Calc.Range : All ranges		
9		Group No.	Concentration	Units	No.1 Max.Ratio	Standard Deviation
10		Negative Control	0.1	uM	0.044	0.005
11		Group02	10	uM	0.047	0.006
12		Group03	100	uM	0.335	0.165
13		Group04	1	mM	0.69	0.155
14		Group05	10	mM	0.906	0.168
15		Group06	100	mM	1.082	0.157
16		Group07	1	M	1.338	0.271
17		Group08	10	M	1.265	0.162
18						
19		IC/EC50	4314.132	uM		
20						
21		Group Map				
22		No.	1	2	3	4
23		A	N/A	N/A	N/A	NegativeControl
24		B	N/A	N/A	N/A	Group08
25		C	N/A	N/A	N/A	Group07

(1) Header

(2) Plate Data File Name

: To describe a file name of data.

(3) Comment : To describe an input comment.

(4) Date : To describe data when data is acquired.

(5) Time : To describe time when data is acquired at the date.

(6) Data processing

: To describe the data processing information

(7) Marker : To describe when marking is put during measurement.

(8) Type : To describe data item.

(9) Calc.Range : To describe range of calculation.

(10) Column

: To describe type of calculation.

(11) Calculation value

: To describe calculation value.

(12) Row : To describe row number of group.

(13) IC/EC Value

: To describe value of IC/EC.

(14) Group Map

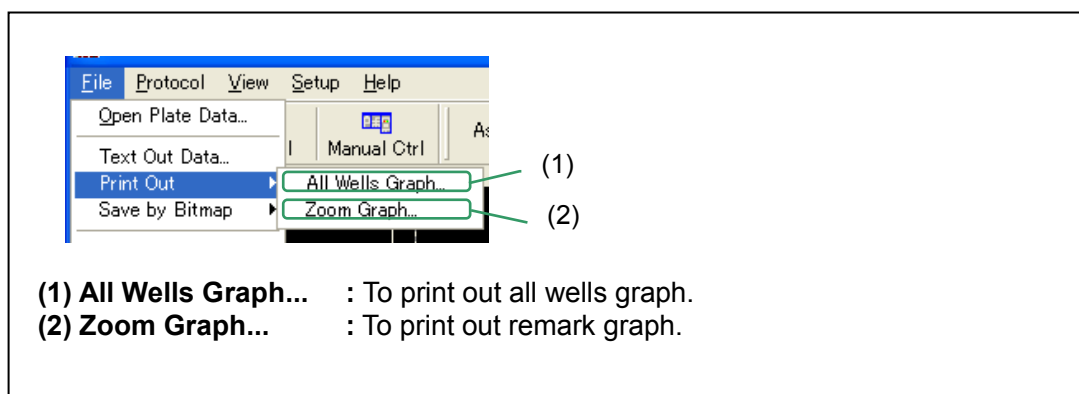
: Well group in plate format.

Note

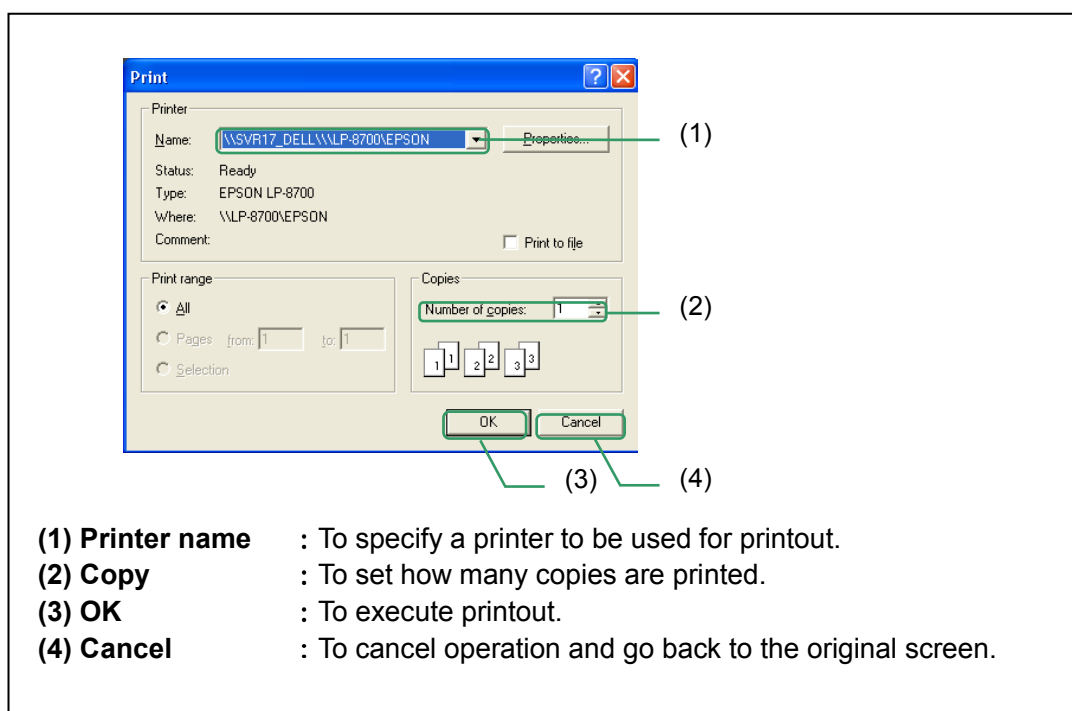
When the number of markers increases, the record is added on right side of 'Marker'.

3) Graphs printout (Print Out command)

Clicking 'Print Out' command from File menu leads the following pop up menu when Plate data is loaded.

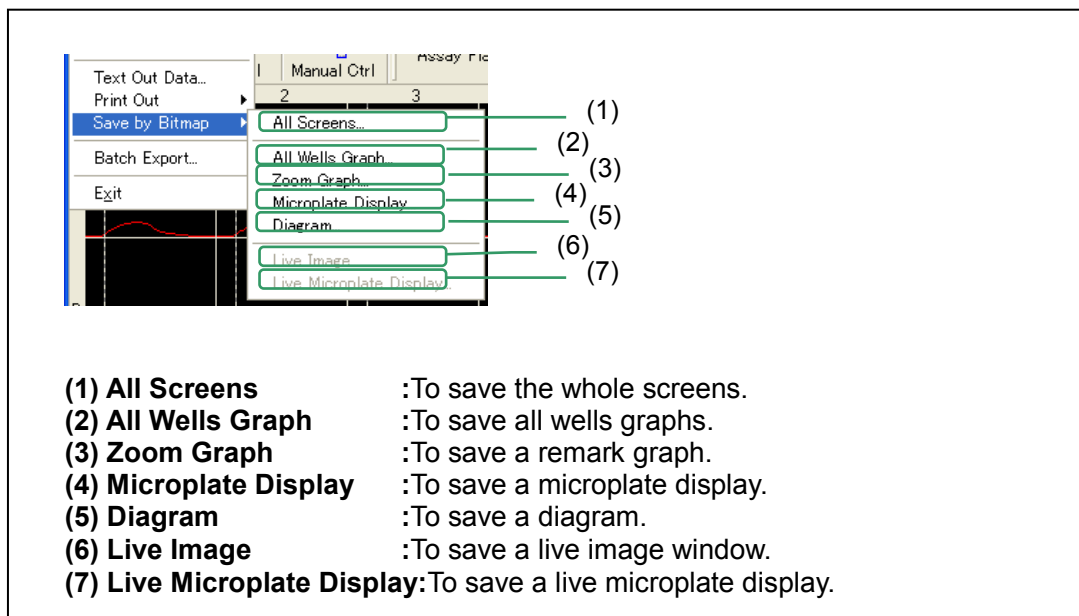


Clicking 'All Wells Graph' command or 'Zoom Graph' command leads the following box (Windows printer box) . Specify a printer type to be used and click OK to execute printout. Graph items which are set at 'Select Graph' are printout. Clicking 'Cancel' cancels operation and goes back to the original screen.

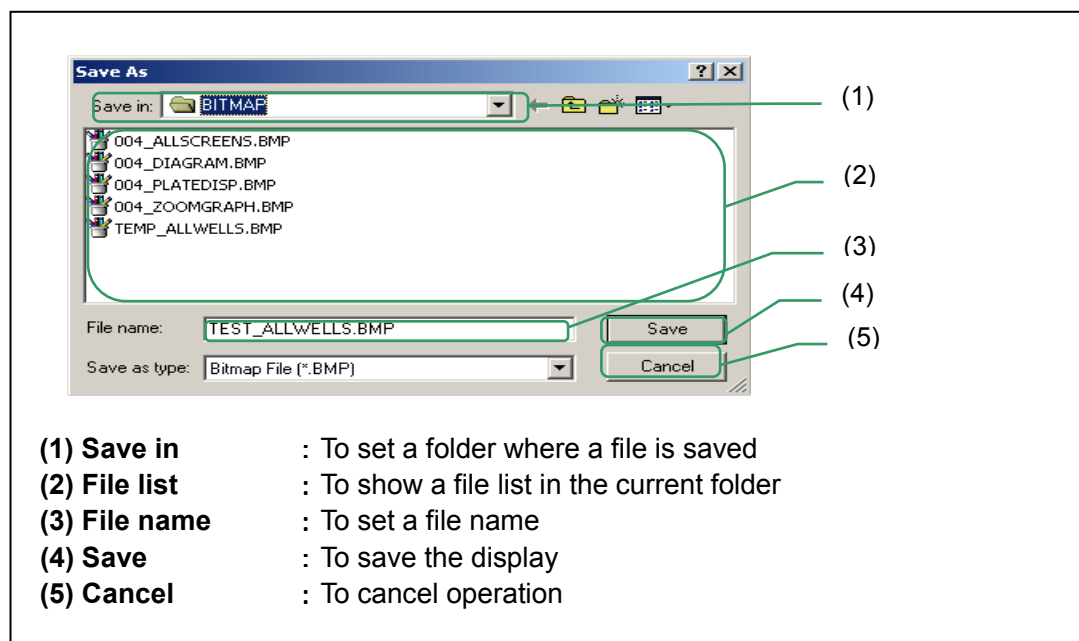


4) Save the display (Save by Bitmap command)

Clicking 'Save by Bitmap' command from File menu leads the following pop up menu.



Clicking each command from the pop up menu leads the following box. Set Save in and a file name and click OK to execute saving.



5) The graph is displayed to new Window (Display Graph in new window command)

The whole screen, all wells graphs, a remark wells graph, a microplate display, a diagram, a live image window and a live microplate display are displayed in a new window.

Clicking 'Display Graph in New Window' command from File menu displays the following pop up menu.

(1) All Screens
 (2) All Wells Graph
 (3) Zoom Graph
 (4) Microplate Display
 (5) Diagram
 (6) Live Image
 (7) Live Microplate Display

(1) **All Screens** :To display the whole screens in new window.
 (2) **All Wells Graph** :To display all wells graphs in new window.
 (3) **Zoom Graph** :To display a remark graph in new window.
 (4) **Microplate Display** :To display a microplate display in new window.
 (5) **Diagram** :To display a diagram in new window.
 (6) **Live Image** :To display a live image window in new window.
 (7) **Live Microplate Display**:To display a live microplate display in new window.

Clicking each command from pop up menu displays the following new window. This window is always displayed in top most.

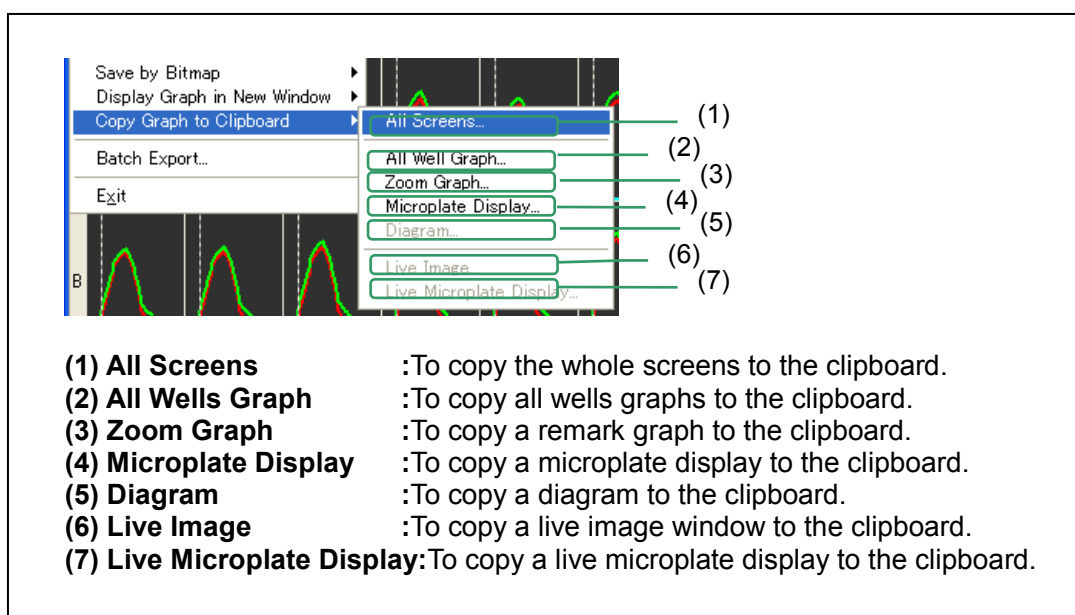
New Window

Usual graph display

6) The graph is copied to the clipboard **(Copy graph to clipboard command)**

The whole screen, all wells graphs, a remark wells graph, a microplate display, a diagram, a live image window and a live microplate display are copied to the clipboard of Windows.

Clicking 'Copy Graph to clipboard' command from File menu displays the following pop up menu.



Clicking each command from pop up menu copies the selected graph to clipboard of Windows.

The copied graph image can be used by doing the paste with other software.

7) Output of two or more data(Batch Export command)

Clicking 'Batch Export' command from File menu leads the following window when Plate data is loaded.

<Setup Batch Export Box>

Plate Name	Date	Plate Comment
051021013	2005/10/21 14:47	
051021014	2005/10/21 15:25	
051021015	2005/10/21 16:31	
051021016	2005/10/21 17:54	
051024000	2005/10/24 09:49	
051024001	2005/10/24 09:53	
051024002	2005/10/24 11:09	
051024003	2005/10/24 11:25	
051024004	2005/10/24 11:34	
051024005	2005/10/24 17:35	
051024006	2005/10/24 17:40	
051024007	2005/10/24 17:45	
051024008	2005/10/24 17:50	
051024009	2005/10/24 17:55	
051025000	2005/10/25 10:05	
051025001	2005/10/25 10:23	
051025002	2005/10/25 10:26	
051025002	2005/10/25 10:26	

(1) Folder Name : To set a folder where a file is saved
 (2) File list : To select a exporting file
 (3) Processing : To set On/Off of the data processing
 (If 'Setup Processing' is done, it becomes effective)
 (4) Setup Processing : To set parameter of the data processing
 (5) Change Hit&Calc.Parameter : To set On/Off of the recalculation when data is output
 (If 'Setup Setup Hit&Calc.Param' is done, it becomes effective)
 (6) Setup Hit&Calc.Param. : To set parameter of hit well analysis and calculation
 (7) Output(TextOut) : To set On/Off of a text output
 (If 'Setup Text Out' is done, it becomes effective)
 (8) Setup Text Out : To set parameter of text out
 (9) Output(Graph) : To set On/Off of a graph output
 (If 'Setup Graph Out' is done, it becomes effective)
 (10) Setup Graph Out : To set parameter of graph out
 (11) Start : To start to output data
 (12) Cancel : To cancel operation

(7-1) About setup Processing

In the setup processing, Setup for On/Off of each data processing and re-setup of parameter can be performed. In the case Setup processing is selected, the following box is displayed. In order to reflect this setup, put check in Processing in Setup Butch Export and then start.

<Setup Processing Box>

(1) Processing : Set up for On/Off of the each data processing
 S.U. = Spatial Uniformity Correction
 N.C. = Negative Control Correction
 S.B. = Subtract Bias
 P.C. = Positive Control Scaling

(2) Select Group : Select well group and set up
 Group List = Selectable group is shown here.
 Select the group and setup well configuration on the plate well display.
 Setup Group = Add, delete, edit Group

(3) Spatial Uniformity : Setup of reference range for use of S.U. (Set up of sampling number)

(4) Negative Control : Setup of basis area for use of N.C. (Set up of sampling number)

(5) Subtract Bias : Setup of Bias range (Set up of sampling number)
 Minus Clip can calculate '0' if the value is minus after being subtracted

(6) Positive Control : Setup of calculating area for used in P.C.
 (Set up of sampling number)

(7) Selection display : Selection display of well.

(8) OK button : After reflecting the setup, to finish

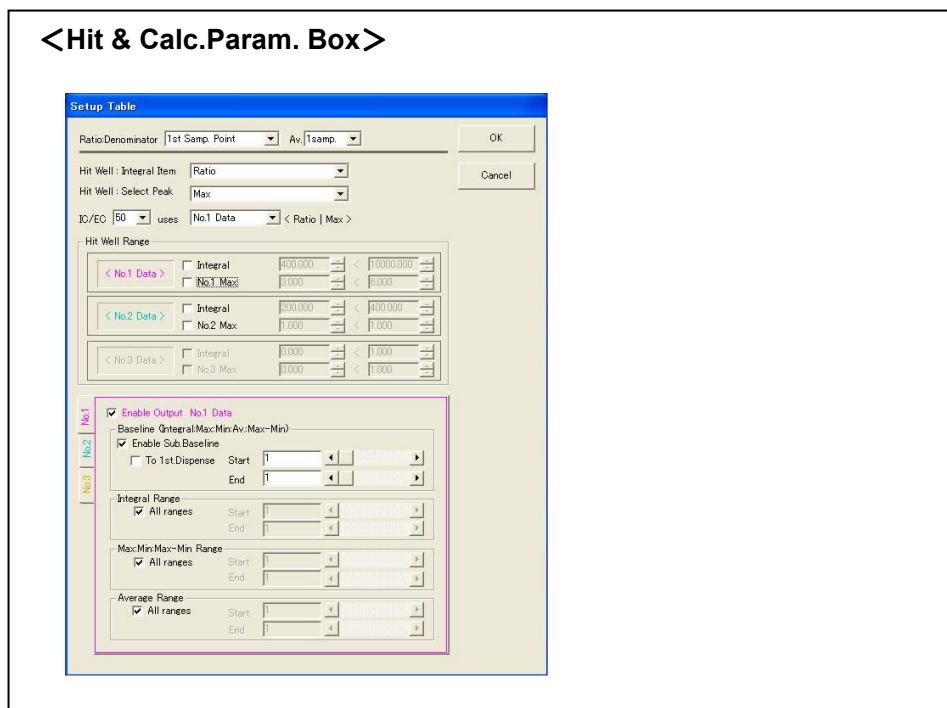
(9) Cancel button : After deleting the setup, to finish

(7-2) About Setup Hit&Calc.Param.

To set condition parameter for Hit well function and the area for Integral value and Max value., then click 'OK' to turn effective.

(Refer to 7-2-1 in Chapter10)

To reflect this setting, it is necessary to put the check in Hit&Calc.Parameter of Setup Batch Export box. And then click OK to execute Start.



(7-3) About Setup Text Out

Name a file and set Output items, then click 'OK' to turn effective.

To reflect the setting, it is necessary to put the check in Output(TextOut) of Setup Batch Export box. Then click OK to execute Start.

<Setup Text Out Parameter Box>

(1) **Filename:**
Each Plate File : To create Text file at each data. TextOut file is named by Plate File name.
Add to a file : To output plural data with a text format into one file. TextOut file is named by input characters in the field.

(2) **Raw Data:** To set output items with a text format at Raw data format.
With Sep.File : Raw data of each item is output with another file.

(3) **Use Assay Protocol:** To choose items which are set at assay protocol setting box.

(4) **Plate Format Data** : To set items to be output at TextOut<Plate>.
Hit : Hit data with Hit well analysis function for Max and Integral parameter.
Ratio : Ratio value dividing Fluorescence No1 intensity by Fluorescence No2 Intensity at 2-wavelength excitation or emission measurement.
Or, Ratio value dividing Fluorescence No1 intensity by initial value of it.
Fluorescence No1 (Luminescence): Intensity of Fluorescence No1.
Fluorescence No2: Intensity of Fluorescence No2.
Integral : Integrated value of intensity or ratio, which is set at 'Calc. Param'.
Integ. : Integrated value.
Max : Maximum value.
Min : Minimum value.
Av. : Average value.
Max-Min : Maximum-Minimum value.
Rat.Max : Intensity value at maximum value point of Ratio at 2 excitation or 2 emission measurement.

(5) **Group Data**
: Check "Group Data" to output group data IC/EC Calculation.

(6) **Raw Data & Plate Format Data <Footer>**
: To set the assay protocol information at the footer part of text data.

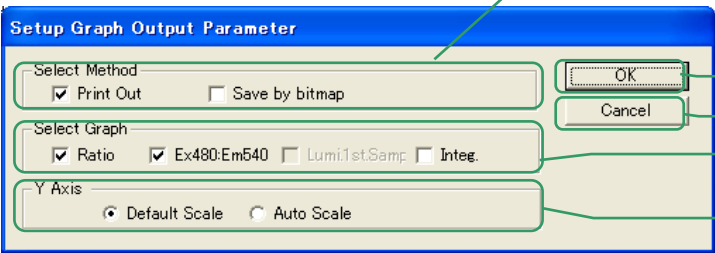
(7) **OK** : To set output parameters and to proceed to next step.

(8) **Cancel** : To cancel operation

(7-4) About Setup Graph Out

In the Setup Graph Out, how to output all of graph can be performed. When Setup Graph Out is selected, the following box is displayed. In order to reflect this setup, put check in Output(Graph) in Setup Batch Export and then start.

<Setup Graph Output Parameter Box>



(1) Select Method : To select how to out-put graph from print-out and Bitmap image.

(2) Select Graph : To select the graph to print-out

(3) Y Axis : To select how to display Y axis when printing out

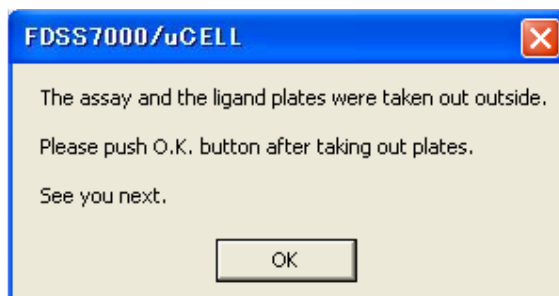
(4) OK button : After reflecting the setup, setup finish

(5) Cancel button : After deleting the setup, setup finish

8) FDSS software quitting (Exit command)

Click 'Exit' command from File menu to quit FDSS application software. The system starts moving for the termination.

When a plate is set inside a dark box, automatically it is loaded out and then the following message box appears. Take out the plate and click 'OK' to close the software.

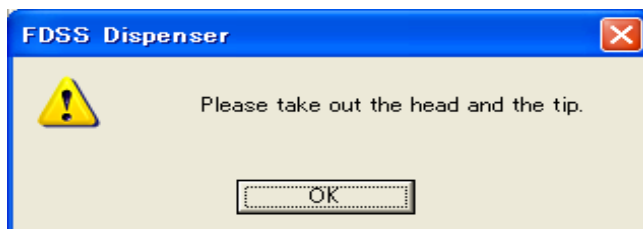


For FDSS7000EX.

When the head and tip is installed, the head and tip is removed, the door of the dispenser opens, and the following message is displayed.

The head and the chip are removed. The OK button is pushed.

(This processing might not be done by the setting.)



7. View menu

To set ON/OFF for Tool bar and Status bar.

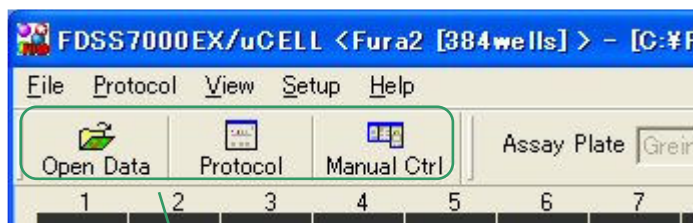
<Command> Clicking 'View menu' shows the following command list.



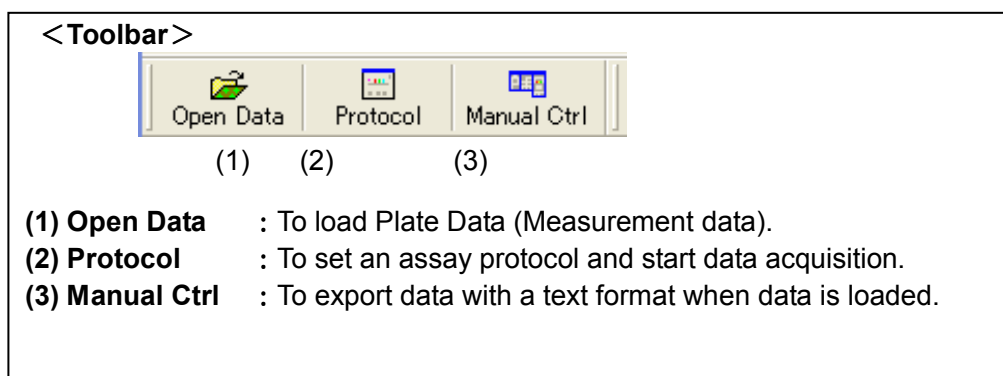
- | | |
|--------------------------|---------------------------------|
| Toolbar | : To set ON/OFF for Tool bar. |
| Status Bar | : To set ON/OFF for Status bar. |
| Zoom/Unzoom Graph | : To select Zoom/Unzoom Graph. |

1) Tool bar (Tool Bar command)

Tool bar is shown as below. The frequently used commands such as Open Plate Data and Assay Protocol setting box open can be easily chosen to execute. Clicking Tool Bar command in View menu can switch tool bar to be displayed or not displayed.

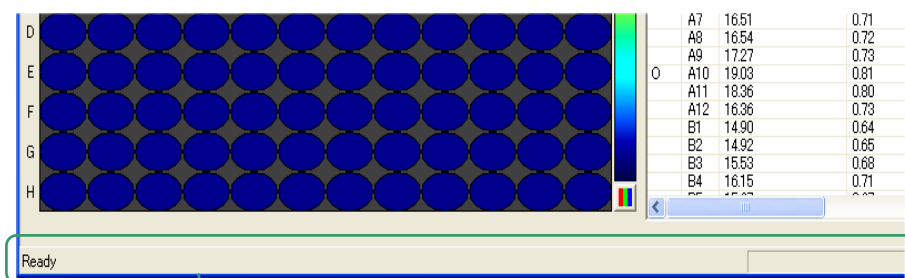


Tool bar



2) Status bar (Status Bar command)

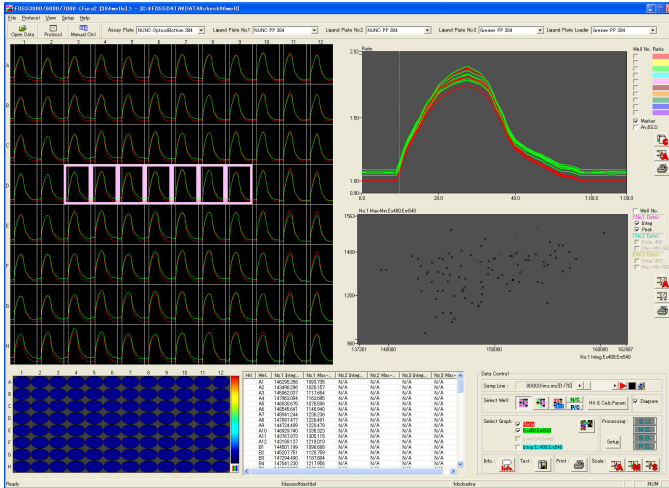
Status bar is shown as below. It shows the explanation of menu / command. Clicking Status Bar command in View menu can switch tool bar to be displayed or not displayed.



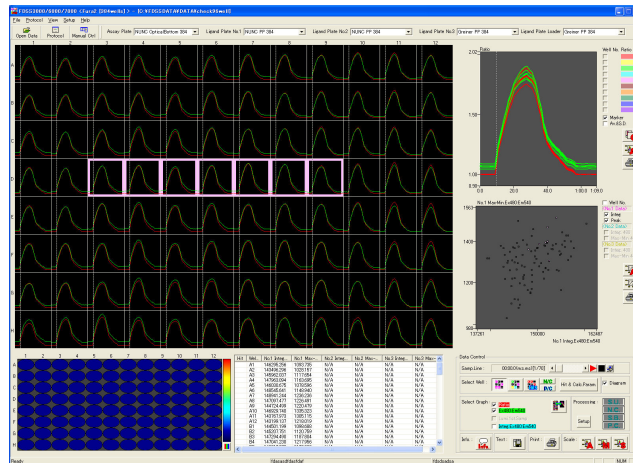
Status bar

3) Zoom/Unzoom Graph (Zoom/Unzoom Graph command)

Clicking Zoom/Unzoom Graph command from View menu leads switching the ratio of display between all wells graph display and remark graph / correlation chart.



Switch



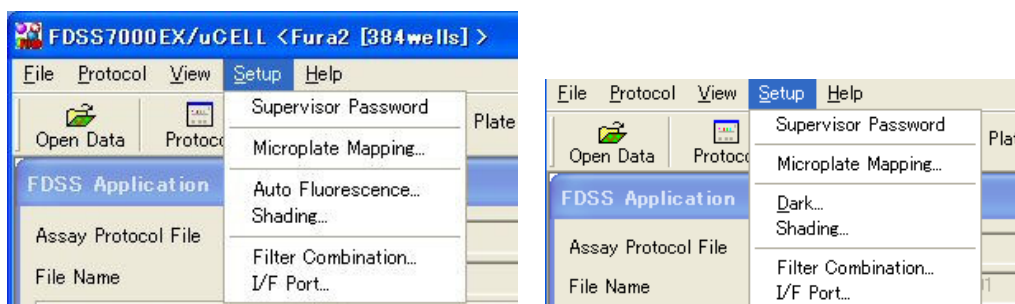
(This page is blank.)

8. Setup menu

Used to acquire correction data, plate PRI, filter combination position and data folder assignment. All commands are protected by a password. Please inquiry to management supervisors.

<Command>

Clicking Setup menu shows the following command list



<At luminescence [Analog][EMCCD] measurement>

Supervisor Password : To input a password to cancel restrictions for commands.

Microplate Mapping : To set ROI for a measurement plate.

Auto Fluorescence : To acquire auto fluorescence correction data.
(At fluorescence)

Dark : To acquire dark correction data.
(At luminescence [Analog][EMCCD])

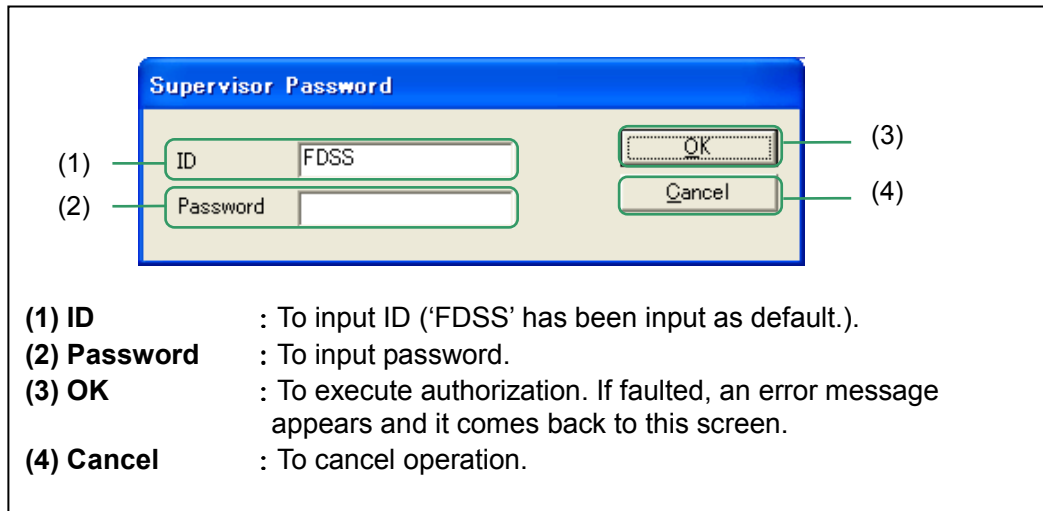
Shading : To get shading correction data.

Filter Combination : To set filter combinations.

I/F Port : To set control ports for related devices.

1) Supervisor Password (Supervisor Password command)

Clicking 'Supervisor Password' command in Setup menu leads Supervisor Password input box. Authorizing a password can enable commands available. Please inquiry a password to management supervisors.



2) Microplate Setup (Microplate setup command)

Below “Registration of Plate” window will show up when you select “Microplate Setup Command” from the Setup Menu.

This window box is to register the information of the plate which is going to be used in FDSS Software.

<Registration of Plate window>

(1) Select Plate : Choose the plate name to edit
Select “_NewPlate” when creating new plate name.

(2) Plate Name : Plate name will appear here. Input plate name when this is for new plate created by “_NewPlate”

(3) Param/Corr. Filename : Set the name of Parameter or Correction file used for this plate. Normally, the plate name related file name is used.

(4) Plate Map Name : Set the Plate Map Name used for this plate. Normally use “Standard” in common with other plates

(5) Parameter : Configure each parameter of the plate here.
Lid : Choose “Use lid” or “No use lid”
Thickness : Set the plate thickness in (1) and (2)
(1) Height on bottom : Distance between well bottom and the plate skirt bottom.
(2) Height under bottom for PhotonCounting : Distance between under the well bottom and the plate skirt to define the position of the Photon Counting sensor to be connected on the plate bottom.

(6) The place used : Check the position this plate being used.

(7) Registration Button : Register with currently shown configuration.

(8) Delete Button : Delete the current plate information and configuration.

(9) Cancel Button : Cancel the edit of the information of the plate.

(10) Close Button : Stop registration and close the window.

3) Microplate Mapping (Microplate mapping command)

The available area for each well is set by ROI setup.

Clicking 'Microplate Mapping' command in Setup menu leads Setup Microplate ROI box. It sets ROI (Region Of Interest) for wells at measurement.

<At fluorescence/ Luminescence[Analog][EMCCD] measurement>

<At luminescence [Photon Counting] measurement>

(1) Mapping File : To display a file name of ROI data to be created/ edited.
(2) Format : To display a plate format
(3) Form : To display ROI form (shape) corresponding to well shape.
(4) ROI Size : To set the size of ROI. Basically X and Y should be the same number.
(5) Real Well Size(At fluorescence measurement)
: To set actual well size. (Refer to 3-3)
(6) Position : To tune finely a ROI position. It is registered with Register button.
(7) Acquire Data (At fluorescence measurement)
: To acquire image of plate.
(8) Calculation (At fluorescence measurement)
: To execute calculation.
(9) Start Acquisition (At luminescence [Photon Counting] measurement)
: To acquire Photon Counting image of plate.
(10) Stop Acquisition (At luminescence [Photon Counting] measurement)
: To stop acquisition.
(11) Calculation (At luminescence measurement)
: To execute calculation.
(12) OK : To register created ROI data.
(13) Cancel : To cancel operation.
(14) Int. Threshold : Threshold level at binarization

(3-1) ROI data creation

<Sample for shading correction data>

The sample for ROI data should be ideally bright enough to distinguish wells easily and should not include unnecessary spots.

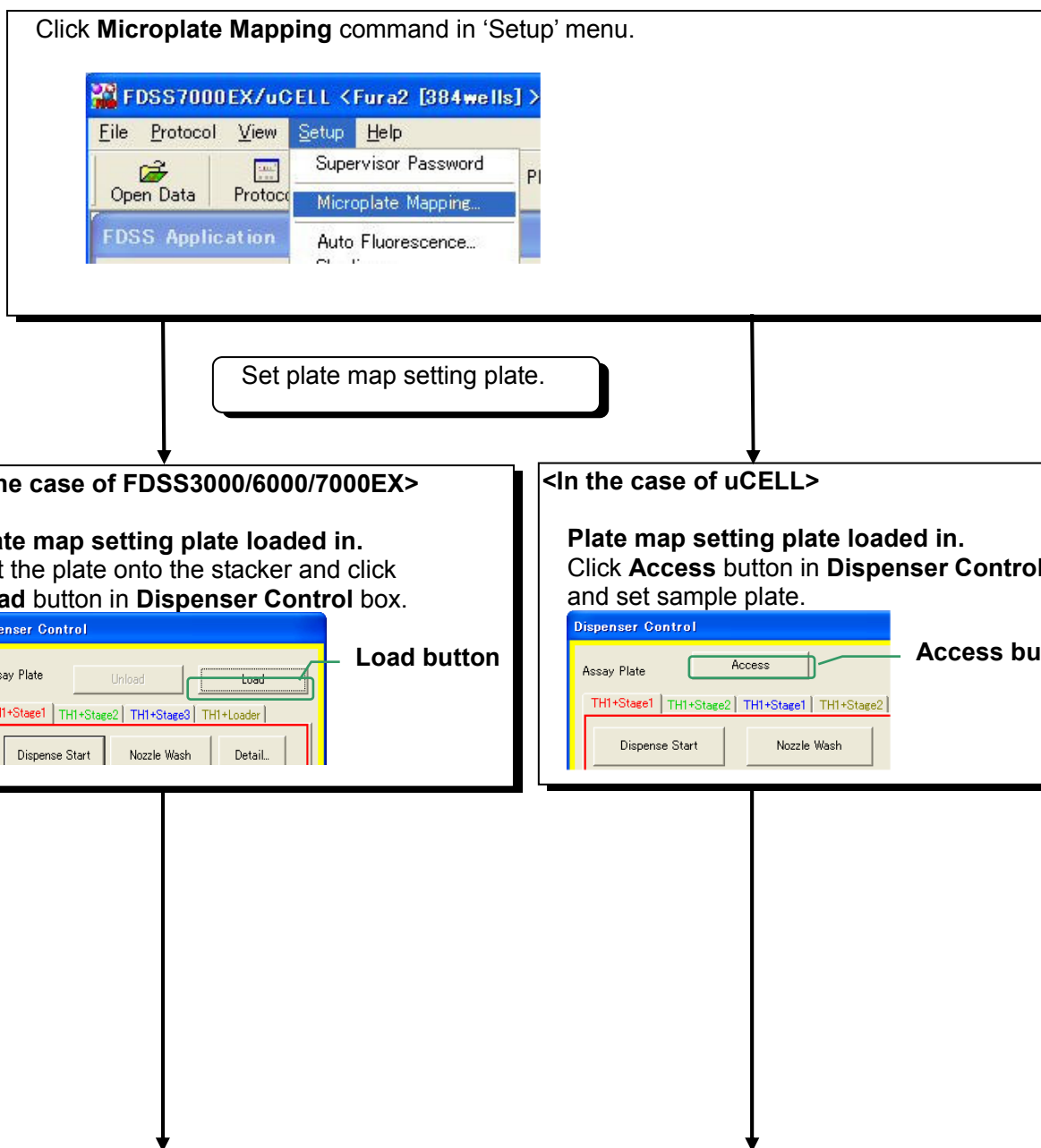
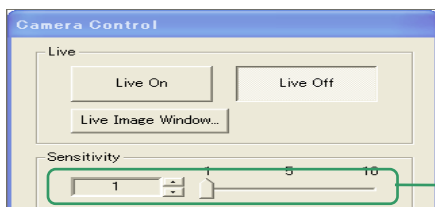


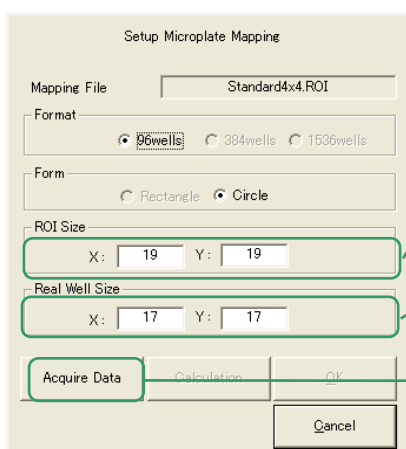
Plate image acquisition

<At fluorescence measurement & Luminescence [Analog][EMCCD] measurement>

Set **Sensitivity** a certain value in **Camera Control** and click 'Acquire Data' in **Setup Microplate ROI** to acquire an image for ROI setting.



Set Sensitivity a certain value.



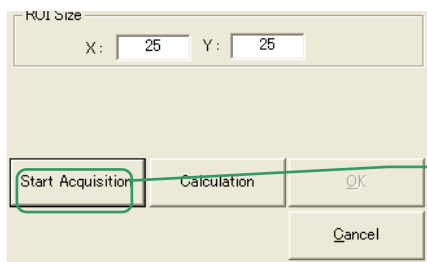
Set size of ROI so that the area can cover signal area. Refer to (3-3).

Set size of actual well. Refer to (3-3).

Click 'Acquire Data' to start acquiring fluorescence image for ROI setting.

<Luminescence [Photon counting] measurement>

Click 'Start Acquisition' in 'Setup' Microplate Mapping to acquire photon counting images for ROI setting.



Click 'Start Acquisition' to start acquiring photon counting images for ROI setting.

Acquiring Plate data

<Fluorescence measurement> Acquiring fluorescence images

<Luminescence[Analog] [EMCCD]measurement>

Acquiring luminescence images

<Luminescence[Photon counting]measurement>

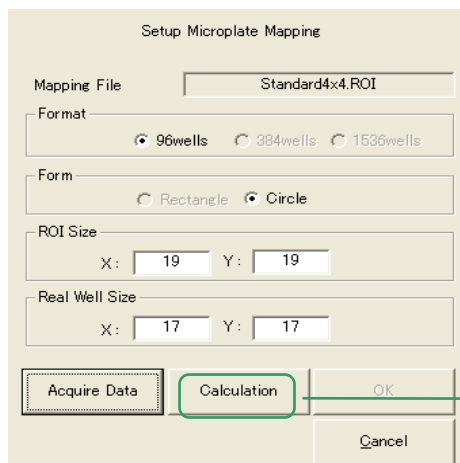
Acquiring Photon Counting images



ROI Calculation

<At fluorescence measurement & Luminescence [Analog][EMCCD] measurement>

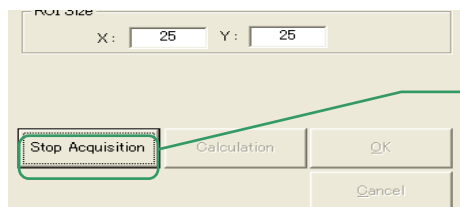
The following is shown after acquiring Plate data. Confirm it can distinguish visually wells and background enough, and then click **'Calculation'** after confirming the calculation is executed.



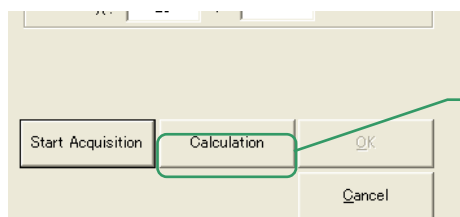
Click **'Calculation'** to execute ROI calculation.

<At luminescence [Photon counting] measurement >

The following is shown during Photon Counting image acquiring. Confirm it can distinguish visually well and background enough in Photon Counting image, and then click **'Stop Acquisition'**. And finally click **'Calculation'** after confirming the calculation is executed.



Click **'Stop Acquisition'** to stop photon counting acquisition.



Click **'Calculation'** to execute ROI calculation.

Creating ROI data.



<At fluorescence measurement>
 The following message might be displayed at two wavelength fluorescent measurement.
 In this case, please select the image of another wavelength, and make the Mapping data for two wavelength.

Message Box of 'Please create the plate map of another wavelength~'

Please select the another wavelength

The following appears after ROI setting calculation. Confirm on the images whether each ROI covers each well correctly and then click 'OK' to register ROI data.

Click 'OK' to register ROI data.

<In the case of FDSS3000/6000/7000EX>

Plate map setting plate loaded out
 Click **Unload** button in Dispenser Control box.

Unload button

<In the case of uCELL>

Plate map setting plate loaded out
 Click **Unload** button in Dispenser Control box and take out sample plate.

Access button

Finish

(3-2) File name of ROI data

A file name of ROI data is automatically named by a plate type which is chosen at Plate Select bar and camera parameter (binning) which is set to be used for measurement. Please confirm a plate type and camera condition before ROI data creation. At an assay protocol setting box, correction data is automatically picked up depending on a plate type and camera.

(3-3) Real Well Size (At fluorescence measurement & luminescence[EMCCD] measurement)

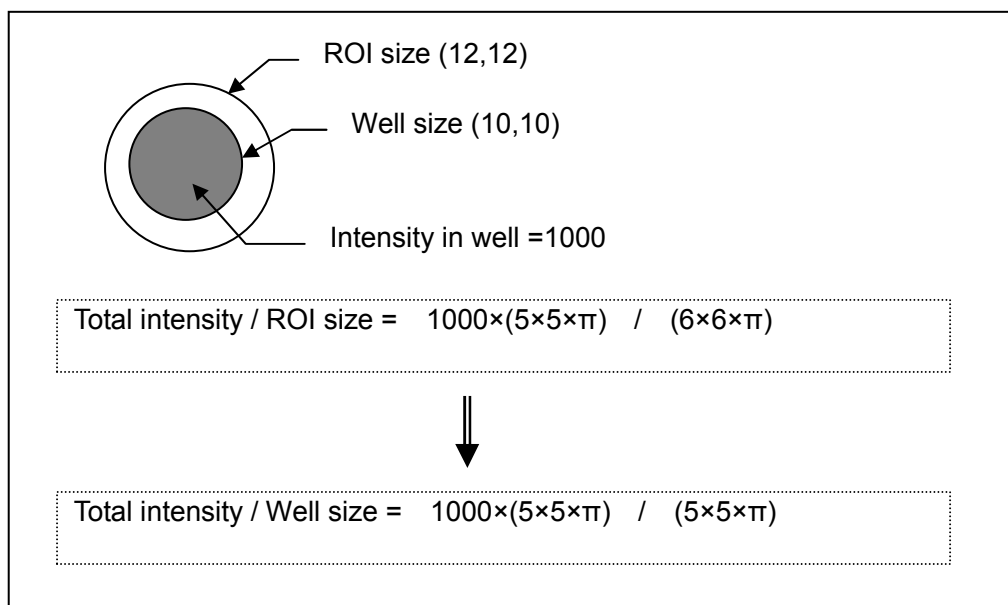
ROI size is sometime set bigger than actual well size because of vibration mixing function or device allowance. Output data of one well is an average value in one well and it is shown as below:

Intensity of one well = Total intensity within ROI / ROI size (Pixel number)

However, depending on measurement condition such as vibration mixing function, smaller intensity value may be output than actual one. So, FDSS application software has one parameter 'Real Well Size' to set ROI area flexibly.

“Real Well Size“ = Actual well size (Unit:Pixel)

For example, when set (X,Y)=(12,12) in case actual well size is (X,Y)=(10,10), “Real well Size“ is “10”.



4) Auto Fluorescence / Dark (Auto Fluorescence/ Dark command)

Plates themselves have auto fluorescence and the characteristic depends on plate types and manufacturers. FDSS application software can have correction data to eliminate the auto fluorescence which affects measurement.

Clicking 'Auto Fluorescence' command in Setup menu leads Setup Auto Fluorescence Data box. It creates, edits and displays auto fluorescence correction data. This command is available only at fluorescence measurement & luminescence measurement [Analog][EMCCD].

The screenshot shows the 'Setup AutoFluorescence Data' dialog box. It contains the following elements:

- (1)** Auto Fluorescence File: Greiner4x4Exp0.PAT
- (2)** Format: 96wells (selected), 384wells, 1536wells
- (3)** Pre Acquire, **(4)** Acquire Data, **(5)** Save
- (7)** Display Current Data, **(8)** Export Data, **(6)** Import Data, **(9)** Cancel
- (10)** Display Sensitivity: 1
- (11)** Correction data display table:

Well No.	Ex:340:Em540	Ex:380:Em540
A1	0.00	0.00
A2	0.00	0.00
A3	0.00	0.00
A4	0.00	0.00
A5	0.00	0.00
A6	0.00	0.00
A7	0.00	0.00
A8	0.00	0.00
A9	0.00	0.00
A10	0.00	0.00
A11	0.00	0.00
A12	0.00	0.00
B1	0.00	0.00
B2	0.00	0.00
B3	0.00	0.00
B4	0.00	0.00
B5	0.00	0.00
B6	0.00	0.00
B7	0.00	0.00
B8	0.00	0.00
B9	0.00	0.00
B10	0.00	0.00
B11	0.00	0.00
B12	0.00	0.00
R12	0.00	0.00
Well No.	Ex:340:Em540	Ex:380:Em540
Average	0.00	0.00
Max	0.00	0.00
Min	0.00	0.00

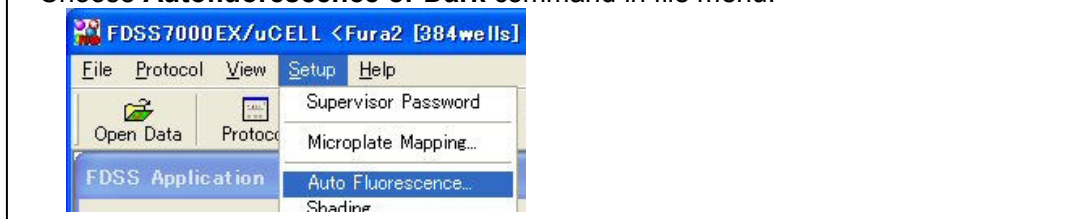
(1) Auto Fluorescence File : To display a file name of correction data to be created/ edited.
(2) Format : To display a plate format
(3) Pre Acquire : To check a fluorescence image before acquiring correction data.
(4) Acquire Data : To acquire an auto fluorescence image and create auto fluorescence correction data.
(5) Save : To register correction data which is displayed.
(6) Cancel : To cancel operation.
(7) Display Current Parameter: To display the original registered correction data.
(8) Export : To export correction data which is displayed to Excel file.
(9) Import : To import correction data which was exported and edited to/in Excel.
(10) Display Sensitivity : To switch Sensitivity to show correction data. Correction data is acquired at each Sensitivity.
(11) Correction data display : To display acquired correction data

(4-1) Auto fluorescence/ Dark correction data creation

<Sample for auto fluorescence/Dark correction data>

The sample for correction should be prepared ideally under the actual measurement conditions like the same plate, filter and buffer quantity/type.

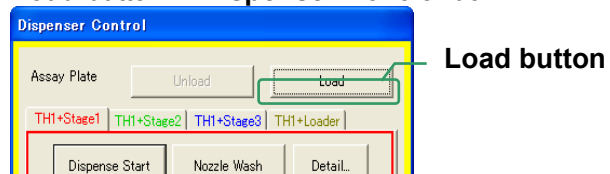
Choose **Autofluorescence or Dark** command in file menu.



Set sample plate for correction

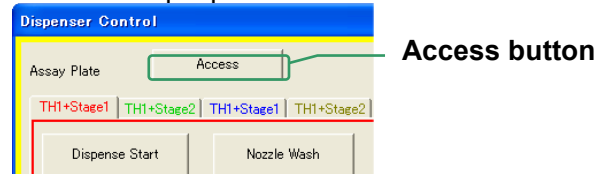
<In the case of FDSS3000/6000/7000EX>

Sample plate for correction loaded in.
Set the plate onto the stacker and click **Load** button in **Dispenser Control** box.

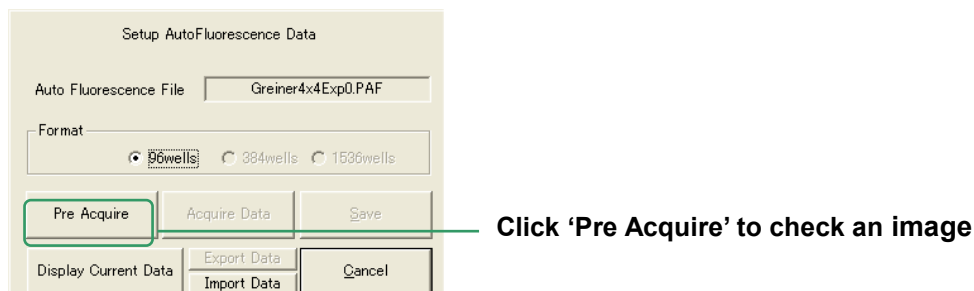
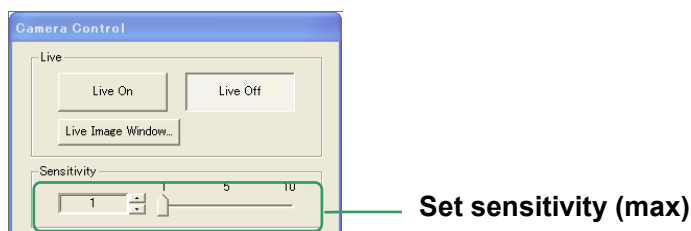


<In the case of uCELL>

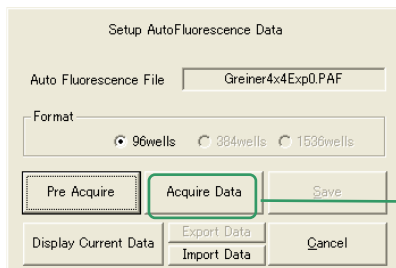
Sample plate for correction loaded in.
Click **Access** button in **Dispenser Control** box and set sample plate.



Check an auto fluorescence image of the sample plate
Set Sensitivity (Max) in **Camera Control** box. Click '**Pre Acquire**' button in **Setup Auto Fluorescence or Dark Data** box and get image data.



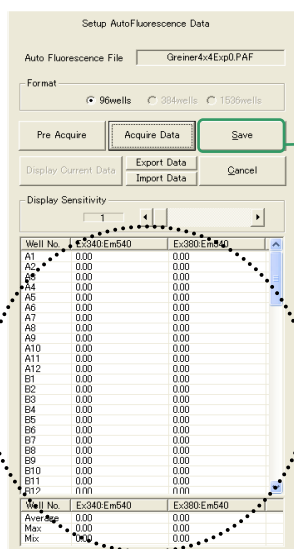
The following box appears after getting an image. Check whether it has unnecessary bright spots by dusts. If it has, load the plate out and remove dusts. And then try again. If not, click **Acquire Data** button in **Setup Auto Fluorescence Data** box.



Click 'Acquire Data'.

Acquiring correction data.

The following box appears after acquiring correction data. Click **'Save'** and register the data. It is registered automatically.

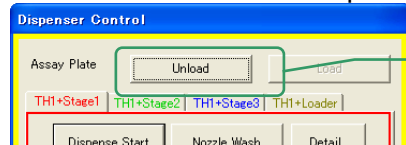


Click 'Save' to register.

Display the result

<In the case of FDSS3000/6000/7000EX>

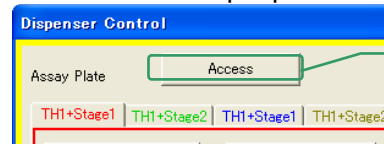
Sample plate loaded out
Click **Unload** button in Dispenser Control box.



Unload button

<In the case of uCELL>

Sample plate loaded out
Click **Unload** button in Dispenser Control box and take out sample plate.



Access button

Finish

(3-2) Import and Export for Auto fluorescence correction data

It can export auto fluorescence correction data to Excel and edit it there. And then it can import the edited one back to FDSS application software.

Note

Wells data position is fixed at Import and Export between Excel and FDSS application software. Please just edit numbers with the fixed position and don't move data position.

Note

FDSS application software can import active Excel data which is saved once with any name. Don't forget saving the data before 'Import' clicking.

(3-3) File name of auto fluorescence correction data

A file name of correction data is automatically named by a plate type which is chosen at Plate Select bar and camera parameter (exposure time and binning) which is set to be used for measurement. Please confirm a plate type and camera condition before correction creation. At an assay protocol setting box, correction data is automatically picked up depending on a plate type and camera condition when correction is set ON.

5) Shading (Shading command)

Plates themselves have shading and the characteristic depends on plate types and manufacturers. FDSS application software can have correction data to eliminate plate shading which affects measurement.

Clicking 'Shading' command in Setup menu leads Setup Shading Data box. It creates, edits and displays shading correction data.

<Sample for shading correction data>

The sample for shading correction should be ideally prepared under the actual measurement conditions like the same plate, filter and buffer quantity.

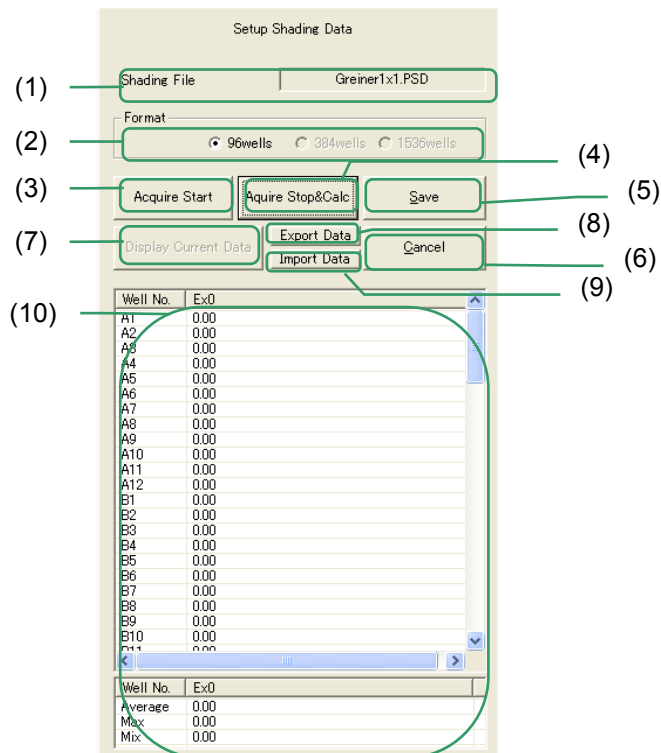
Setup Shading Data box <At fluorescence measurement>

The screenshot shows the 'Setup Shading Data' dialog box. It includes a text field for 'Shading File' (Greiner4x4.PSD), a 'Format' section with radio buttons for '96wells', '384wells', and '1536wells', and several action buttons: 'Pre Acquire', 'Acquire Data', 'Save', 'Display Current Data', 'Export Data', 'Import Data', and 'Cancel'. A table at the bottom displays correction data for wells A1 through B12, with columns for 'Well No.', 'Ex:340:Em:540', and 'Ex:380:Em:540'. The table also includes summary rows for 'Average', 'Max', and 'Min'.

Well No.	Ex:340:Em:540	Ex:380:Em:540
A1	0.00	0.00
A2	0.00	0.00
A3	0.00	0.00
A4	0.00	0.00
A5	0.00	0.00
A6	0.00	0.00
A7	0.00	0.00
A8	0.00	0.00
A9	0.00	0.00
A10	0.00	0.00
A11	0.00	0.00
A12	0.00	0.00
B1	0.00	0.00
B2	0.00	0.00
B3	0.00	0.00
B4	0.00	0.00
B5	0.00	0.00
B6	0.00	0.00
B7	0.00	0.00
B8	0.00	0.00
B9	0.00	0.00
B10	0.00	0.00
B11	0.00	0.00
B12	0.00	0.00
Average	0.00	0.00
Max	0.00	0.00
Min	0.00	0.00

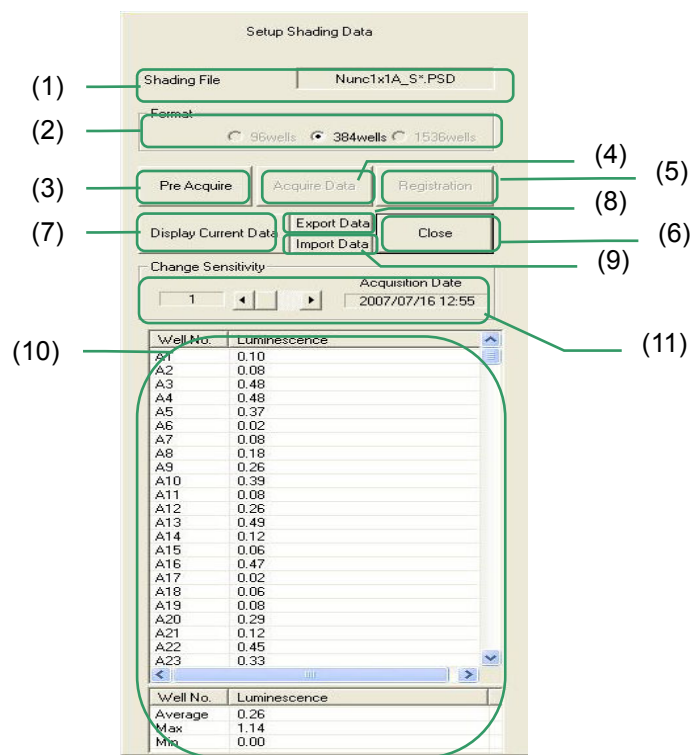
- (1) **Shading File** : To display a file name of correction data to be created/ edited.
- (2) **Format** : To display a plate format.
- (3) **Pre Acquire** : To check a fluorescence image before acquiring correction data.
- (4) **Acquire Data** : To acquire a fluorescence image and create shading correction data.
- (5) **Save** : To register correction data which is displayed.
- (6) **Cancel** : To cancel operation.
- (7) **Display Current Data**
: To display the original registered correction data.
- (8) **Export** : To export correction data which is displayed to Excel file.
- (9) **Import** : To import correction data which was exported and edited in Excel.
- (10) **Correction data display**
: To display the acquired correction data

<Setup Shading Data box : Luminescence [Photon counting] measurement>



- (1) **Shading File** : To display a file name of correction data to be created/ edited.
- (2) **Format** : To display a plate format.
- (3) **Acquire Start** : To check a luminescence image before acquiring correction data.
- (4) **Acquire Stop&Calc**
: To acquire a luminescence image and create shading correction data.
- (5) **Save** : To register correction data which is displayed.
- (6) **Cancel** : To cancel operation.
- (7) **Display Current Data**
: To display the original registered correction data.
- (8) **Export** : To export correction data which is displayed to Excel file.
- (9) **Import** : To import correction data which was exported and edited in Excel.
- (10) **Correction data display**
: To display the acquired correction data

<Setup Shading Data box : Luminescence [Analog][EMCCD] measurement>



- (1) **Shading File** : To display a file name of correction data to be created/ edited.
- (2) **Format** : To display a plate format.
- (3) **Pre Acquire Start**
: To check a luminescence image before acquiring correction data.
- (4) **Acquire Data**
: To acquire a luminescence image and create shading correction data.
- (5) **Registration** : To register correction data which is displayed.
- (6) **Close** : To close setup box.
- (7) **Display Current Data** : To display the original registered correction data.
- (8) **Export** : To export correction data which is displayed to Excel file.
- (9) **Import** : To import correction data which was exported and edited inExcel.
- (10) **Shading Correction Data**
: To display shading accruing value of shading correction data
- (11) **Change Sensitivity**
: To change sensitivity.

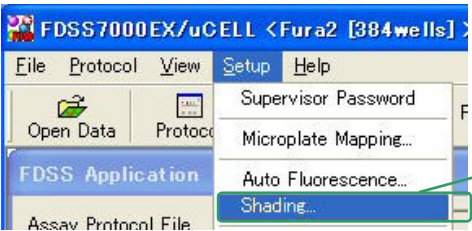
(5-1) Shading correction data creation

The sample data that has homogeneous fluorescence can be used to correct nonuniformity on hardware and optics.

<Sample for shading correction data>

The sample for shading correction data should be ideally prepared under the actual measurement conditions like the same plate, filter and buffer quantity.

Click **Shading** command in **Setup** menu.

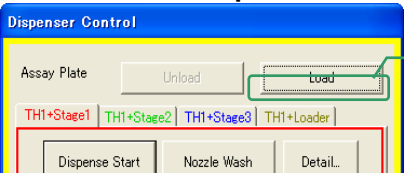


Shading button

Set the sample for shading correction.

<In the case of FDSS3000/6000/7000EX>


Sample plate for correction loaded in.
Set the plate onto the stacker and click **Load** button in **Dispenser Control** box.



Load button

<In the case of uCELL>

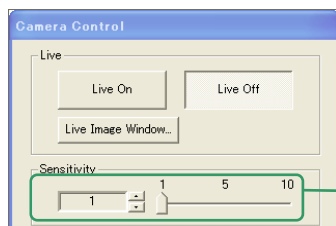
Sample plate for correction loaded in.
Click **Access** button in **Dispenser Control** box and set sample plate.



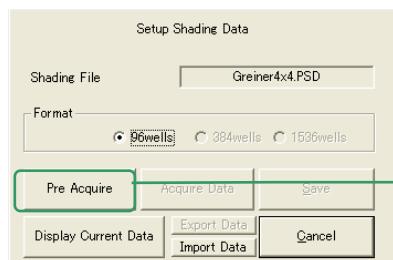
Access button

<At fluorescence measurement and luminescence [Analog][EMCCD] measurement >

To check shading image of the sample plate. **Set Sensitivity** a certain value in **Camera Control** box. Click **'Pre Acquire'** button in **Setup Shading Data** box to acquire image data.



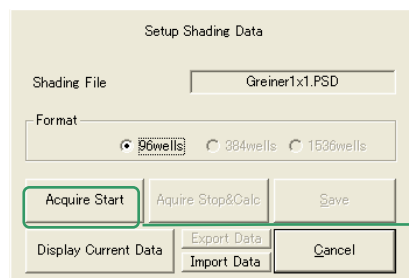
Set Sensitivity



Click 'Pre Acquire' to acquire fluorescence image.

<At luminescence [Photon Counting] measurement>

To acquire shading image of the sample plate. Click **'Acquire Start'** in **Setup Shading Data** box to start acquiring photon counting image data. Integration images are observed then.

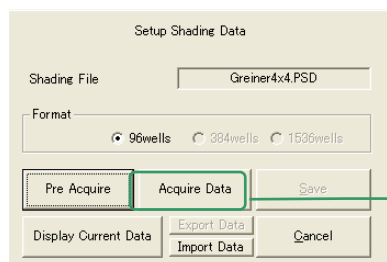


Click 'Acquire Start' to start acquire photon counting image data.

- <Fluorescence measurement>**
Acquiring fluorescence image for checking
- <Luminescence [Analog][EMCCD] measurement>**
Acquiring luminescence image for checking
- <Luminescence [Photon Counting] measurement>**
Acquiring Photon Counting image

<At fluorescence measurement and luminescence [Analog][EMCCD] measurement>

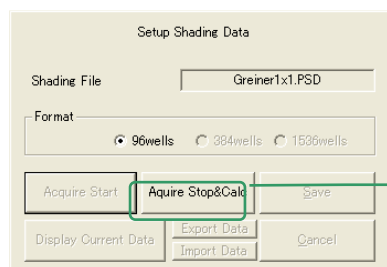
To Check after getting an image whether it has unnecessary bright spots by dusts or not. If it has, load plate out and remove dusts. And then try again. If not, click **Acquire Data**.



Click 'Acquire data'.

<At luminescence [Photon Counting] measurement >

Click 'Acquire Stop&Calc.' to stop acquisition and to create shading data when total image is homogeneous and doesn't have unnecessary bright spots.



Click 'Acquire Stop&Calc.'.

The following box appears after acquiring correction data. Click 'Save' and register the data. It is registered automatically.

Click 'Save' to register.

Sensitivity changes

Display the result

Well No.	Ex:340:Em540	Ex:380:Em540
A1	0.00	0.00
A2	0.00	0.00
A3	0.00	0.00
A4	0.00	0.00
A5	0.00	0.00
A6	0.00	0.00
A7	0.00	0.00
A8	0.00	0.00
A9	0.00	0.00
A10	0.00	0.00
A11	0.00	0.00
A12	0.00	0.00
B1	0.00	0.00
B2	0.00	0.00
B3	0.00	0.00
B4	0.00	0.00
B5	0.00	0.00
B6	0.00	0.00
B7	0.00	0.00
B8	0.00	0.00
B9	0.00	0.00
B10	0.00	0.00
B11	0.00	0.00
B12	0.00	0.00
Average	0.00	0.00
Max	0.00	0.00
Min	0.00	0.00

<Fluorescence measurement> <Luminescence[Analog][EMCCD]measurement>
 <Luminescence[Photon Counting]measurement>

Fluorescence/Luminescence[Photon Counting]measurement
 The relation is not in sensitivity, and common correction data is used.

Luminescence[Analog][EMCCD] measurement
 It has the correction data of each sensitivity. Sensitivity is changed and all the sensitivity correction data is acquired.

<In the case of FDSS3000/6000/7000EX>

Sample plate loaded out
 Click **Unload** button in Dispenser Control box.

Unload button

<In the case of uCELL>

Sample plate loaded out
 Click **Access** button in Dispenser Control box and take out sample plate.

Access button

Finish

(5-2) Import and Export for shading correction data

It can export auto shading correction data to Excel and edit it there. And then it can import the edited one back to FDSS application software.

Note

Wells data position is fixed at Import and Export between Excel and FDSS application software. Please just edit numbers with the fixed position and don't move data position.

Note

FDSS application software can import active Excel data which is saved once with any name. Don't forget saving the data before 'Import' clicking.

(5-3) File name of shading correction data

A file name of correction data is automatically named by a plate type which is chosen at Plate Select bar and camera parameter (exposure time and binning) which is set to be used for measurement. Please confirm a plate type and camera condition before correction creation. At an assay protocol setting box, correction data is automatically picked up depending on a plate type and camera condition when correction is set ON.

6) Filter Combination (Filter Combination command)

It sets filters and dichroic mirror combination and which combination should be shown on Filter Control box. 10 combinations are set here and 4 of them can be operated in Filter Control box.

Clicking 'Filter Combination' command in Setup menu leads Setup Filter Combination box.

Note This setting is saved and managed at each Measurement Method.

'Display Filter Set'

Display	Ex Filter	Em / DM Filter
<input checked="" type="checkbox"/>	Shutter	Em:540/DM:UV
<input checked="" type="checkbox"/>	340	Em:540/DM:UV
<input checked="" type="checkbox"/>	380	Em:540/DM:UV
<input type="checkbox"/>	Shutter	Em:540/DM:UV
<input type="checkbox"/>	Shutter	Em:540/DM:UV
<input checked="" type="checkbox"/>	480	Em:540/DM:B
<input type="checkbox"/>	Shutter	Em:540/DM:UV
<input type="checkbox"/>	Shutter	Em:540/DM:UV
<input type="checkbox"/>	Shutter	Em:540/DM:UV
<input type="checkbox"/>	Shutter	Em:540/DM:UV

'Ex Filter Exchanger'

No.	Wavelength
No.1	Shutter nm
No.2	340 nm
No.3	380 nm
No.4	Shutter nm
No.5	Shutter nm
No.6	480 nm

'Em/DM Filter Exchanger'

No.	Combination
No.1	Em:540/DM:UV
No.2	Em:565/DM:B
No.3	Em:0/DM:B
No.4	Em:0/DM:B
No.5	Em:540/DM:B
No.6	Em:565/DM:B
No.7	Em:0/DM:B
No.8	Em:0/DM:B

'Em Filter Wavelength'

No.	Em Filter Wavelength
No.1	540 nm
No.2	565 nm
No.3	0 nm
No.4	0 nm

'DM Filter Type'

No.	DM Filter Type
No.1	UV
No.2	B

'Ex Filter Exchanger'

A filter set in an excitation filter is set.

(4)Wavelength : To input wavelength of an excitation filter which is employed in filter switching devices (999=Shutter).

'Em/DM Filter Exchanger'

shows filter setting when a filter exchanging device at an emission side.

(5)Combination : To display combinations between emission filters and dichroic mirrors(DM) which are automatically combined.

(6)Em Filter Wavelength : To input wavelength of an emission filter which is employed in filter exchanging devices.

(7)DM Filter Type : To input a dichroic mirror type which is employed in filter exchanging devices.

(6-1) Filter setting

Setting is needed in Setup Filter Combination command when a new filter is added or changed.

Procedure

a) Excitation filter setting

Set to input wavelength of a new excitation filter into 'Wavelength' on '**Ex Filter Exchanger**'.

b) Emission filter setting

Set to input wavelength of a new emission filter into 'Em Filter Wavelength' on '**Em/DM Filter Exchanger**'.

c) DM (Dichroich Mirror) setting

Set to input wavelength of a new emission filter into DM Filter Type on '**Em/DM Filter Exchanger**'.

d) Filter combination setting

Set the combination between excitation filters, emission filters and dichroich mirrors at 'Ex Filter' and 'Em/Dm Filter' in 'Display Filter Set'. And specify at 'Display' maximum 4 combinations to be operated in Filter Control box.

e) Setup Filter Combination command finish

Click 'OK' to set and finish 'Setup Filter Combination' command.

f) Assay Protocol change

Set again filters to be used at Assay protocol for new filter setting to reflect.

Note

The filter combination setting effects on all Assay Protocol on the Method. Be sure of Assay Protocol setting after any filter is added or changed.

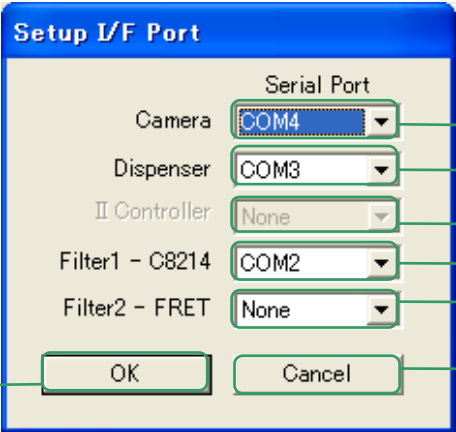
7) I/F Port (I/F Port command)

FDSS application software controls several devices and it can set serial ports for each device.

Clicking 'I/F Port' command in Setup menu leads 'Device Setup box'.

Note

This setting is saved and managed at each Measurement Method.



(1) Camera : To set a port for a fluorescence detector (Fluorescence configuration).

(2) Dispenser : To set a port for a dispenser.

(3) II Controller : To set a port for an I.I. (Image Intensifier) controller (At luminescence configuration).

(4) Filter1 : To set a port for an excitation filter switching device

(5) Filter2 : To set a port for FRET filter switching device

(6) OK : To renew the setting.

(7) Cancel : To cancel operation.

9. Help menu

Help menu shows version information of FDSS application software.

<Command>

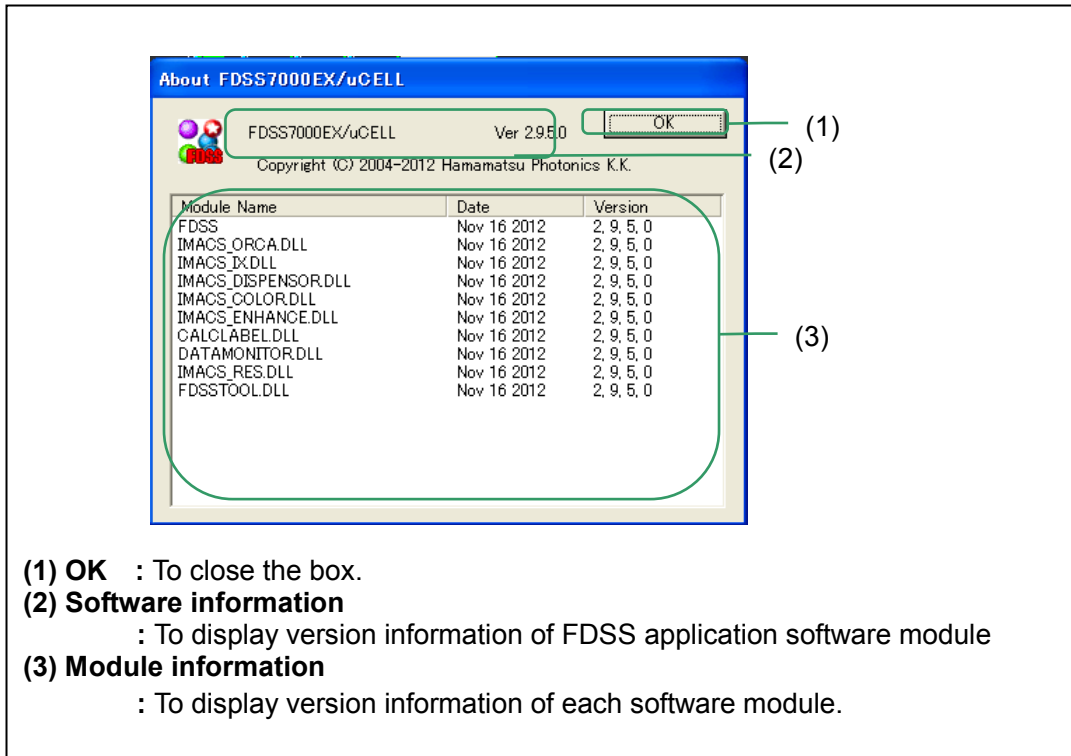
Clicking 'Help' leads the following command.



About FDSS : To show version information of FDSS application software version.

1) About FDSS (About FDSS command)

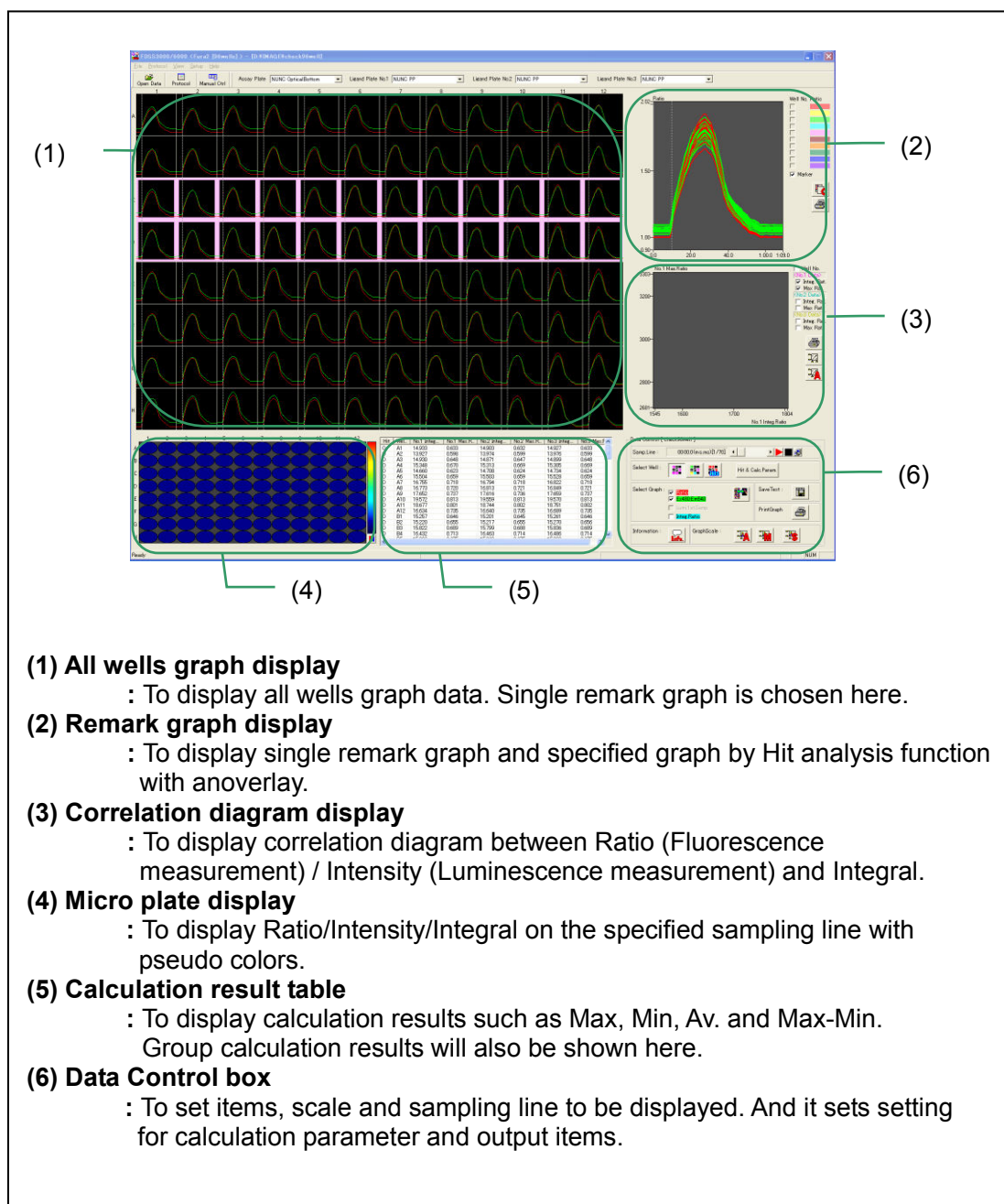
It shows version information of FDSS application software. Clicking 'About FDSS' in 'Help' menu shows the following box.



10. Plate Data display and analysis

1) Over View

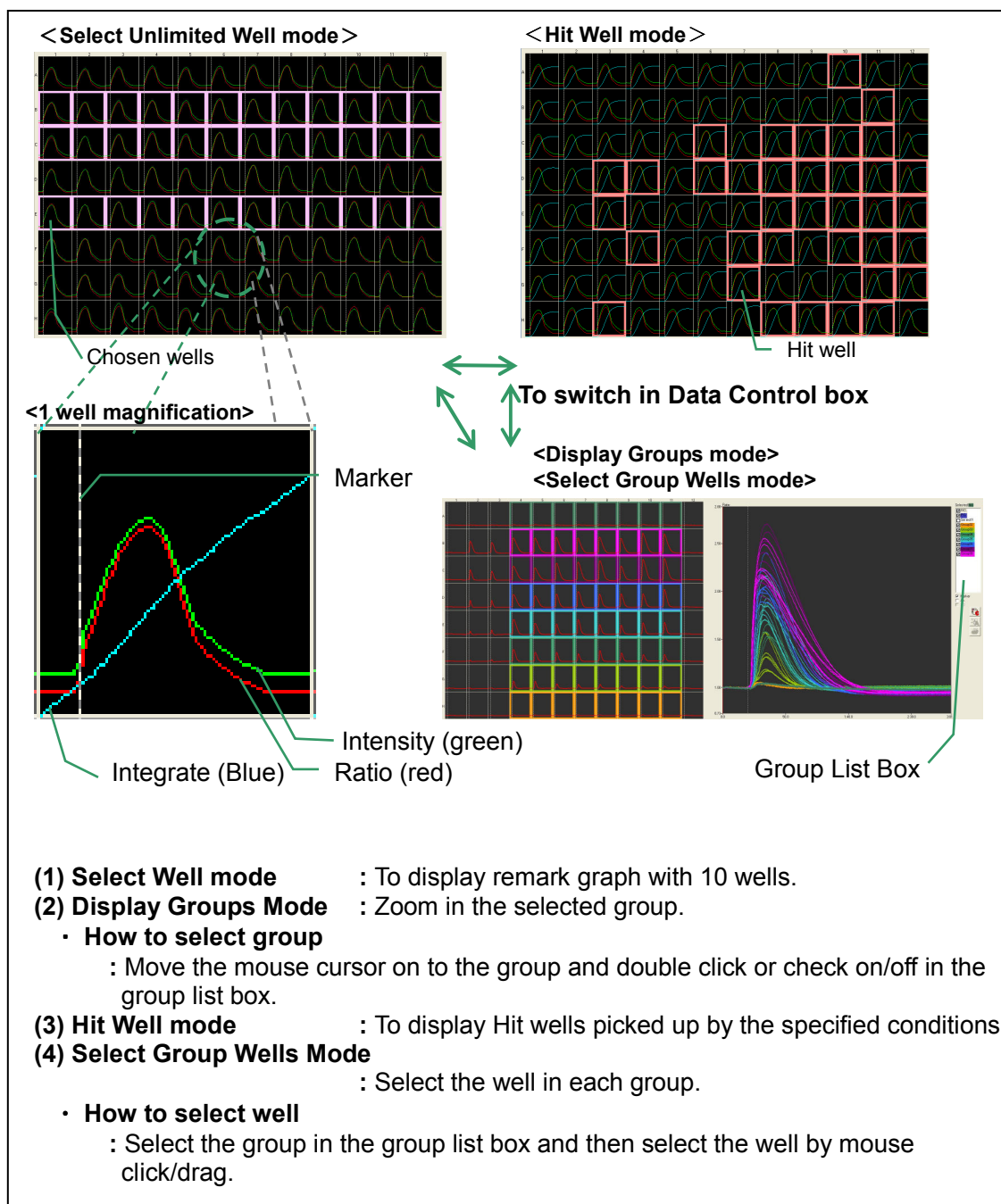
The following screen appears when measurement is completed. And it is also shown at Plate Data is loaded. It can display, output and analysis Plate data.



2) All wells graph display

All wells graph display has the following functions, and it has 2 modes such as Select Well mode and Hit Well mode for functions.

- All well graph display such as Ratio, Intensity (Photon Count) and Integral
- Mark display
- Well choice for remark graph
- Hit well display
- Select group well (including negative/positive control well)

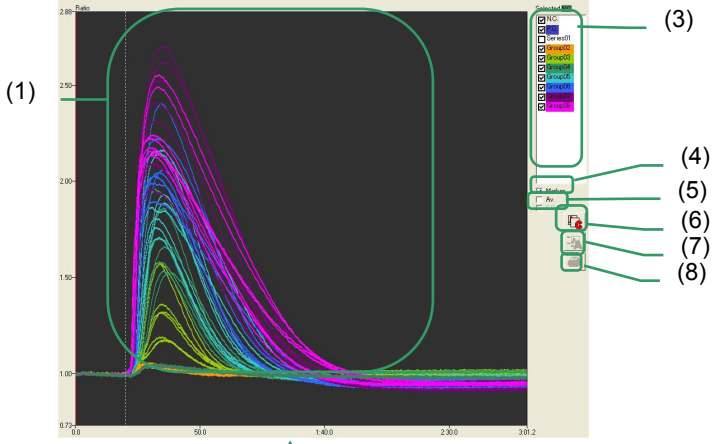


3) Remark graph display

Remark graph display has the following functions.

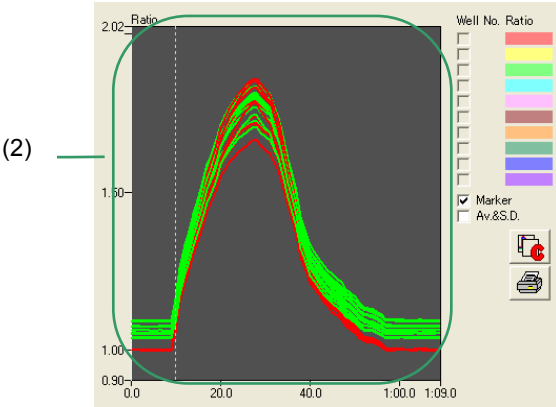
- Overlay display for chosen wells or groups
- Overlay display for Hit wells
- Marker display and sampling line display
- Overlay display for Av. and S.D graph

< Display Groups/Select Group Wells Mode >



↕ To switch in Data Control box ↕

<Select unlimited mode, Hit Well mode >



(1) Display Groups/Select Group Wells
: Shows all selected group data overlaid.
Each group graph color can be configured as desired.

(2) Hit Well mode
: To display graphs of Hit wells with an overlay. Each item is distinguished by colors.

(3) Group List Box
: Group list which can be configured will show up here.
Select and check the group to show details.

(4) Marker : To set a marker On/Off to be displayed.

(5) Clear : To all-clear the chosen wells.

(6) Print Out : To printout the remark display.

(7) Av.&S.D. : To overlay Av.and S.D. of the selected graph.

(8) Auto Scal : To auto-scale of selected well

4) Correlation diagram display

Correlation diagram display has the following function. (Correlation diagram is not available in Display Groups · Select Group Wells Mode.)

- a) Correlation display between maximum and integral value of Ratio/Intensity.
- b) Histogram display of maximum value of Ratio/Intensity
- c) Histogram display of integral value of Ratio/intensity

<Correlation diagram display>

↕ To switch with display item choice ↕

<Histogram display>

(1) Correlation diagram display
: To display correlation diagram between maximum and integral value of Ratio/Intensity.

(2) Histogram display
: To display Histogram between maximum and integral value of Ratio/Intensity.

(3) Display item
: To choose Well No., Max value and Integration value with max 3 areas. When 2 items are chosen, the correlation chart appears. When 1 item is done, the histogram display shows up.

(4) Print Out : Printout correlation diagram and histogram.

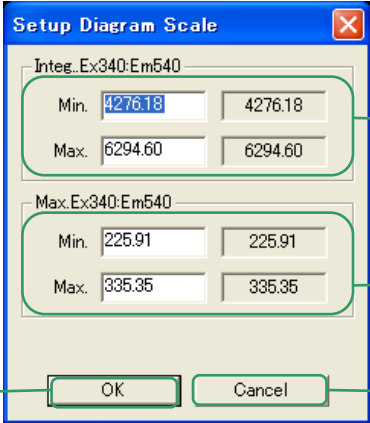
(5) Scale : Set scale of correlation diagram and histogram manually. (Refer to 4-1)

(6) Auto : Set scale of correlation diagram and histogram automatically.

(4-1) Scale setting for correlation diagram

(4-1) Scale Setup

Clicking 'Scale' in correlation diagram display, scale can be set manually in the following box.



(1) (2) (3) (4)

(2) Integ.Ratio item
: To set scale of Integral value. Input number on left boxes, referring to a right box which shows minimum and maximum value within sample data.

(2) Max.Ratio item
: To set scale of Maximum value. Input number on left boxes, referring to a right box which shows minimum and maximum value within sample data.

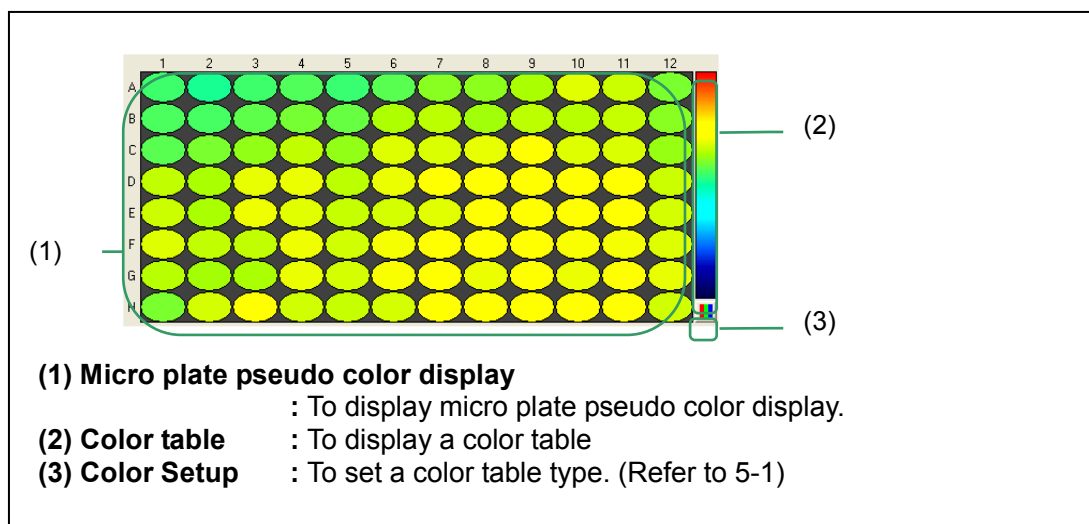
(3) OK : To renew graph with the set scale value

(4) Cancel : To cancel operation.

5) Micro plate pseudo color display

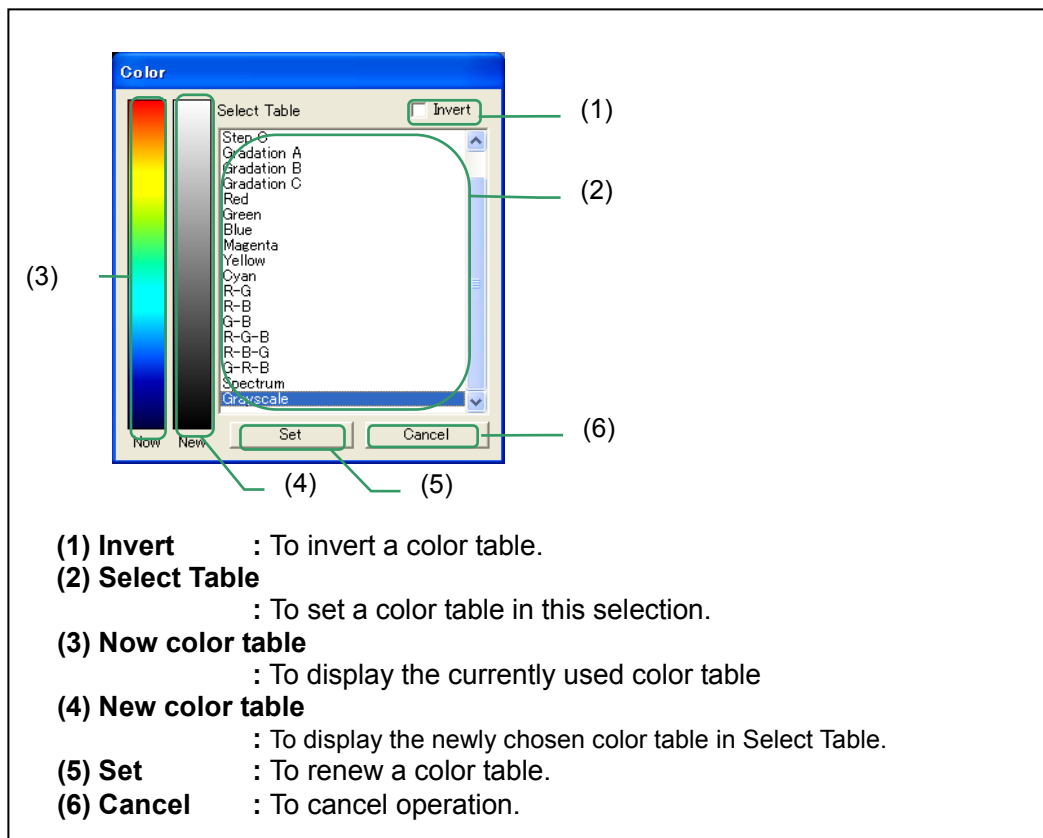
Micro plate pseudo color display has the following function.

a) Data kinetic display with specified item and time.



(5-1) Color Setup

Clicking 'Color Setup' in the pseudo color display leads Color Setting box. The color table can be chosen in it.



6) Calculation table display

(6-1) Unlimited Wells/Hit Well Display

Calculation table display has the following functions.

- 1) To display the result data of each well.
- 2) Calculation parameter is set at Hit&Calc.Param in Data Control box.

The item for calculation is as below:

- a) Hit well
- b) Number well
- c) Integral value : maximum 3 data (To set at Hit&Calc.Param)
- d) Peak value : maximum 3 data (To set at Hit&Calc.Param)

Hit	Well	No.1 Integ...	No.1 Max.R.	No.2 Integ...	No.2 Max.R.	No.3 Integ...	No.3 Max.R.
O	A1	14.933	0.633	14.903	0.632	14.927	0.632
O	A2	13.927	0.598	13.974	0.599	13.976	0.599
O	A3	14.930	0.648	14.871	0.647	14.899	0.648
O	A4	15.348	0.670	15.313	0.669	15.305	0.669
O	A5	14.660	0.623	14.708	0.624	14.734	0.624
O	A6	15.504	0.659	15.503	0.659	15.528	0.659
O	A7	16.755	0.718	16.794	0.718	16.822	0.718
O	A8	16.773	0.720	16.813	0.721	16.849	0.721
O	A9	17.652	0.737	17.616	0.736	17.659	0.737
O	A10	19.572	0.813	19.559	0.813	19.570	0.813
O	A11	18.677	0.801	18.744	0.802	18.751	0.802
O	A12	16.634	0.735	16.640	0.735	16.689	0.735
O	B1	15.257	0.646	15.201	0.645	15.261	0.646
O	B2	15.220	0.655	15.217	0.655	15.270	0.656
O	B3	15.822	0.689	15.799	0.688	15.836	0.689
O	B4	16.432	0.713	16.463	0.714	16.486	0.714

(1) Calculation item : To display calculation item. Clicking an item sorts the data line.

(2) Calculation table : To display calculation table view. Clicking well data reflects on all wells graph display and remark graph display.

(6-1-1) Setting change for calculation parameter

Calculation parameter can be changed or re-set in 'Param' at 'Hit&Calc.Param' in 'Data Control' box. (Refer to 7) It sets item for Integral, Hit well condition, calculation area at Max,Min,Average,Max-Min,Integral and baseline setting.

(6-1-2) Text out of calculation table

The calculation table can be output as a text format. 'Text Out' in 'Data Control' box executes a text output (Refer to 7)

(6-2) Display Groups Display

When 'Display Groups' is selected, calculation results table display indicate following data with some function shown below.

- Shows the calculation data of each group.
- Calculation parameters can be changed at the 'Hit&Calc.Param' TAB in Data Control box.
- Calculated items are
 - a) Group No. : Group number
 - b) Concentration : Group concentration
 - c) Calculation value : Calculation value which configured at Hit&Calc.Param TAB
 - d) Standard Deviation : Standard deviation of each group
 - e) Z Factor : Z factor of each group using Min/Max data
 - f) Z Factor with N.C. : Z factor of each group using N.C.data
 - g) Comment : Comment of each group

<Calculation results table>

Group No.	Concentration	U.	No.1 Max:Ratio	Standard Devi.	Z Factor	Z Factor with N.C.	Comment
Group02	1000	uM	0.047	0.006	0.592	-12.352	TEST02
Group03	1000	uM	0.335	0.165	-0.480	-0.754	
Group04	1000	mM	0.690	0.155	0.327	0.257	
Group05	1000	mM	0.905	0.159	0.443	0.395	
Group06	1000	mM	1.032	0.157	0.564	0.532	
Group07	1000	M	1.532	0.211	0.552	0.361	
Group08	1000	M	1.265	0.162	0.616	0.590	Group8

IC/EC X No.1 Max:Ratio <Change Param> Display IC/EC Graph

(1) Calculation item : Show calculated items
 (2) Calculation Table : Show calculation results
 (3) IC/EC X No.1 Max:Ratio <Change Param> button : Set parameter for IC/EC Calculation
 Setup Table Box will appear. Refer to (7-2-1) for detail
 (4) Display IC/EC Graph button : IC/EC Graph Box will appear. Refer to (6-2-3) for detail

(6-2-1) How to change IC/EC calculation parameter

Parameters can be changed and re-calculate IC/EC value.

In order to re-calculate, select IC/EC X No.1 Max:Ratio <Change Param> button or Hit&Calc.Param button on the select well part in data control box. Refer to 7) Data Control box.

(6-2-2) How to output calculation result table in text file data.

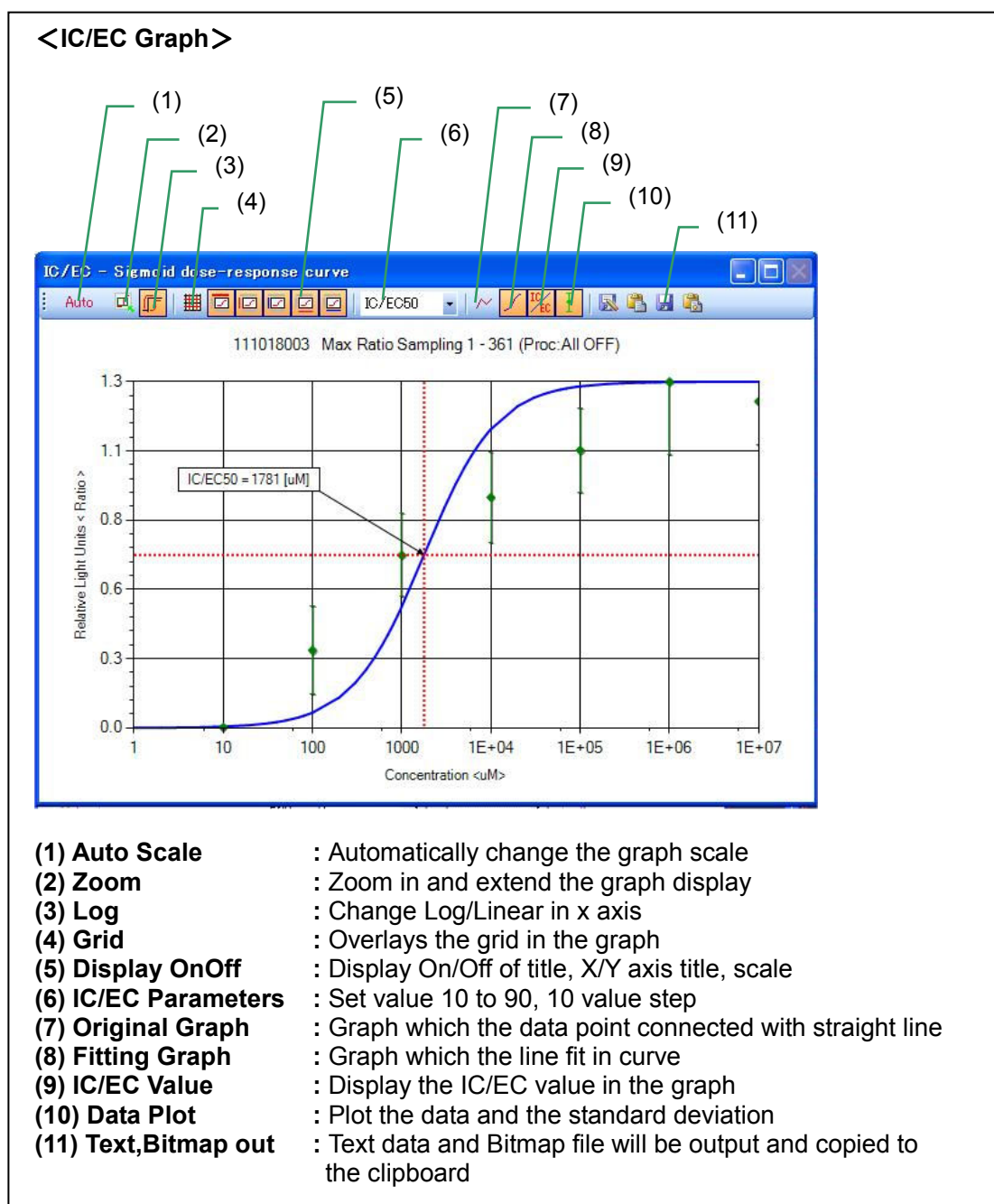
Results can be output as text file data. In order to output in text file, Please refer to 7) Data Control Box part for detail.

(6-2-3) IC/EC Graph Display

By clicking Display IC/EC Graph button below IC/EC graph will be displayed.

This button will be changed to Hide IC/EC button when IC/EC Graph is displayed.

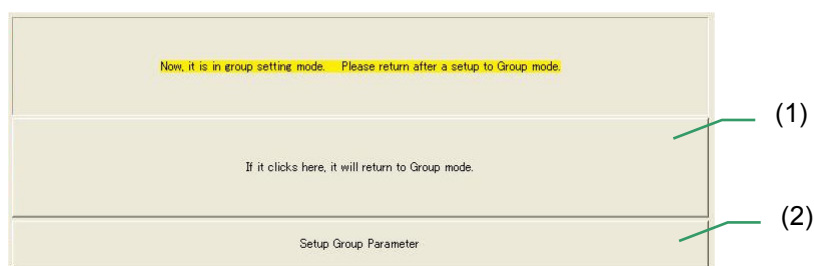
To hide the IC/EC Graph, push Hide IC/EC Graph button.



(6-3) Select Group Wells Display

When Select Group Wells is selected in Select Well, group configuration item will be displayed have below function.

- Displays group parameter configuration box
- Returning to Display Groups

<Group parameter configuration window>**(1) If it clicks here, it will return to Group Mode button**

: Switches between Select Well and Display Groups

(2) Setup Group Parameter Button

: Displays Setup Group Box Refer to (6-3-1)

(6-3-1) Setup Group Box

Setup Group Box will show up when Setup Group Parameter button is clicked. Group can be added, deleted and edited from this box.

<Setup Group Parameter Box>

The screenshot shows the 'Setup Group Parameter' dialog box. It is divided into two main sections: 'Group List' and 'Group Parameter'. The 'Group List' section contains a list of groups with their respective concentrations and units, such as 'Group02 : 10.000 mM' and 'Group03 : 1.000 mM'. The 'Group Parameter' section is currently set to the 'Group' tab, showing fields for 'Group Name' (Group03), 'Concentration' (1.000), and 'Color' (mM). There are also 'Update' and 'Cancel' buttons. A 'Comment' field is located at the bottom. On the right side of the dialog, there are several control buttons: 'OK', 'Cancel', 'Save Group', 'Load Group', 'Add', 'Delete', and 'Delete All'. Green lines with numbers (1) through (7) point to these elements: (1) points to the 'Group List' area, (2) to 'Save Group', (3) to 'Load Group', (4) to 'Add', (5) to 'Delete', (6) to 'Delete All', and (7) to the 'Group Parameter' section.

<Group Tab>

This close-up shows the 'Group' tab of the 'Group Parameter' section. It includes fields for 'Group Name' (Group03), 'Concentration' (1.000), and 'Color' (mM). There are 'Update' and 'Cancel' buttons. A 'Comment' field is also present. Green lines with numbers (8) through (13) point to these elements: (8) to the 'Group' tab, (9) to the 'Group Name' field, (10) to the 'Color' dropdown, (11) to the 'Concentration' field, (12) to the 'Update' button, (13) to the 'Comment' field.

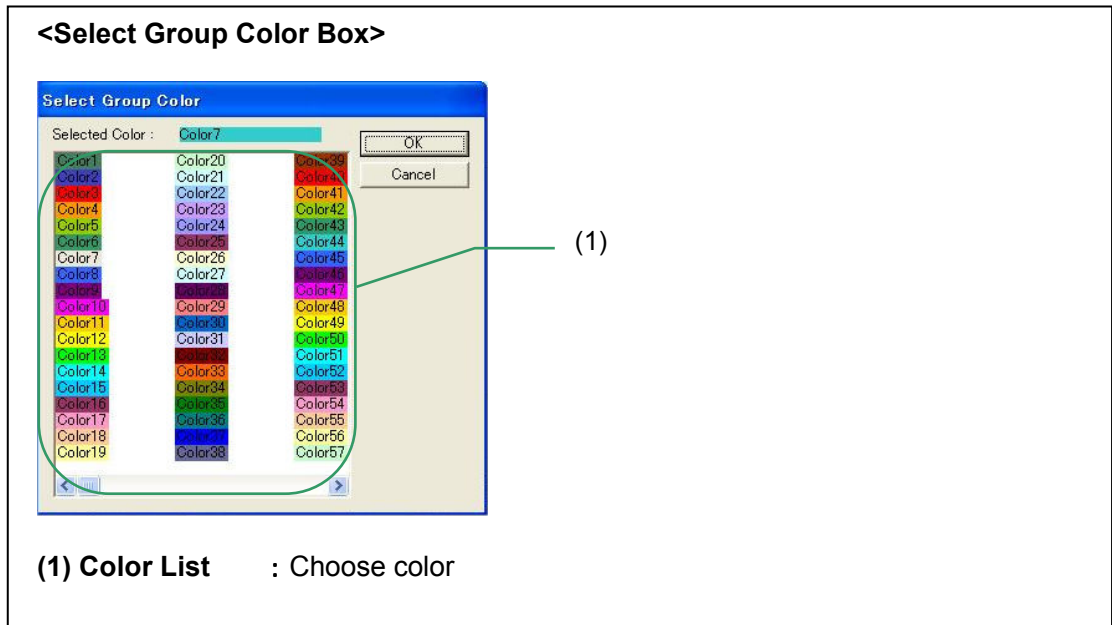
<Series Tab>

This close-up shows the 'Series' tab of the 'Group Parameter' section. It includes fields for 'Group Name' (Series05), 'Start Conc.' (10.000), 'Step Conc.' (10.000), and 'Replicates' (None). There are also dropdown menus for 'Color' (uM), '+Plts' (+Plts), and a numeric field for '1'. There are 'Update' and 'Cancel' buttons. A 'Comment' field is also present. Green lines with numbers (14) through (16) point to these elements: (14) to the 'Series' tab, (15) to the 'Group Name' field, (16) to the 'Start Conc.' field.

- | | |
|---|--|
| (1) Group List | : Show current group/series in the list.
Select the group/series to edit group parameter |
| (2) Save Group | : Save the parameter configured to the file |
| (3) Load Group | : Load the parameter from the file |
| (4) Add | : Add new group/series in the list |
| (5) Delete | : Delete group/series from the list |
| (6) Delete All | : Delete all group/series from the list
(Negative/Positive Control will not be deleted) |
| (7) Group Parameter / Group-Series TAB | : Switch group setup and series setup |
| Group TAB | : Setup each group one by one |
| Series TAB | : Setup in batch row/column when selecting the well |
| (8) Group Name | : Show/edit name of the group and series |
| (9) Color | : Set group color
Push Color to set color of the group in Select Group Color Box
Refer to (6-3-2) for detail |
| (10) Update | : Register the set value |
| (11) Cancel | : Return to original value before edit |
| (12) Concentration | : Setup concentration |
| (13) Comment | : Input comment if needed |
| (14) Start Conc. | : Start value for batch setup |
| (15) Step Conc. | : Step value for batch setup and calculation method |
| | <ul style="list-style-type: none"> ▪ + : Plus (Addition) ▪ - : Minus (Subtraction) ▪ * : Multiply (Multiplication) ▪ / : Divide (Division) |
| (16) Replicates | : Setup for replicating row column and replicating number |
| | <ul style="list-style-type: none"> ▪ None : No setup replication in column/row ▪ Columns : Replicate setup in columns ▪ Rows : Replicate setup in rows |

(6-3-2) Select Group Color Box

When Color button is clicked, below Select Group Color Box will show up.
Select the color for each group.



7) Data Control box

Data Control has the following function.

- To set display item, scale and sampling line for all wells and remark graph and pseudo color display.
- To output data with a text format or printout.
- To set calculation parameter.
- To set data processing

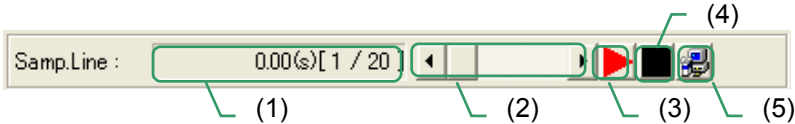
The screenshot shows the 'Data Control' window with the following elements and callouts:

- (1) Samp.Line: 00:00.0(ms.ms)[1/361]
- (2) Select Well: Grid icons, Hit & Calc.Param., Diagram
- (3) Select Graph: Ratio, Ex480:Em540, Ex480:Em540.1st.Samp, Integ.Ratio
- (4) Info: Info, Text, Print
- (5) Scale: A, M, S
- (6) Info: Info
- (7) Scale: A, M, S
- (8) Diagram: Diagram
- (9) Processing: SU, NC, SB, PC, Setup

(1) **Samp.Line** : To set and display sampling line.
 (2) **Select Well** : To switch display mode, Select Well mode or Hit well mode.
 (3) **Select Graph** : To display items to be displayed in graph.
 (4) **Save Text** : To set items to be output at Text Out.
 (5) **Print Out** : To print out all graphs.
 (6) **Info.** : To display and output plate data.
 (7) **Graph Scale** : To switch a scale method and set manual scaling.
 (8) **Diagram** : To show Diagram.
 (9) **Processing** : To set On/off of data processing

(7-1) Samp.Line

It sets sampling line and display the setting. And it sets and excuses Playback function with kinetic change. Playback function shows kinetic change of micro plate data with pseudo colors.



(1) Sampling line : To display sampling time (unit; s) and sampling number [sampling number / total sampling number].

(2) Scroll bar : To set sampling number to display micro plate pseudo color data.

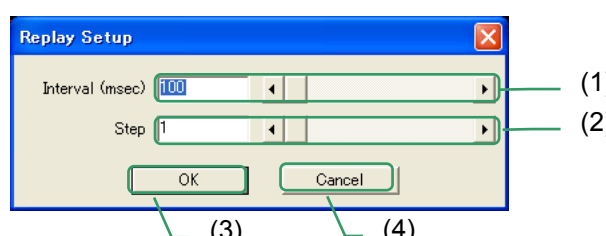
(3) Playback : To execute Playback function to show kinetic change of micro plate data with pseudo colors.

(4) Stop : To stop Play back function.

(5) Setup : To set parameter for Playback function.

(7-1-1) Playback setup

It sets playback speed and step reading number for Playback function.



(1) Interval : To set playback speed with a millisecond unit. The number shows an interval period between samples.


(2) Step : To set step reading number. The number shows how many samples are skipped between displayed samples.

(3) OK : To apply and renew the setting.

(4) Cancel : To cancel operation.

(7-2) Select Well

It switches display mode, Select Well mode or Hit well mode. And it sets parameter of conditions for Hit well function.



The screenshot shows a menu titled 'Select Well :'. It contains five icons, each with a callout number below it:

- (1) Select Unlimited Well: Icon with a grid of pink squares.
- (2) Display Groups: Icon with a grid of green squares.
- (3) Hit Well: Icon with a grid of red squares.
- (4) Hit & Calc.Param.: Icon with a grid of blue squares.
- (5) Select Group Wells: Icon with a grid of yellow squares.

(1) Select Unlimited Well
: To display graphs with Select Well mode. It can choose unlimited number of well and display them on remark graph display. Wells can be chosen by left-clicking on all wells graph display, calculation table or correlation diagram display. The frame of the graph is enclosed with pink at each chosen well.

(2) Display Groups
: Display graph of the wells in the selected group
Group graph display will be on/off by double clicking the well in the group
Calculation table shows the group calculation data
(Group setup can be configured by Select Group Wells Button)

(3) Hit Well
: To display Hit wells which are resulted by Hit well conditions. The frames of the graphs are enclosed with a red color at the chosen wells and they are displayed on a remark display. A correlation diagram display shows wells with red dots.

(4) Hit&Calc.Param
: To set condition parameter for Hit well function. It sets area for Integral value and Max value. (Refer to 7-2-1)

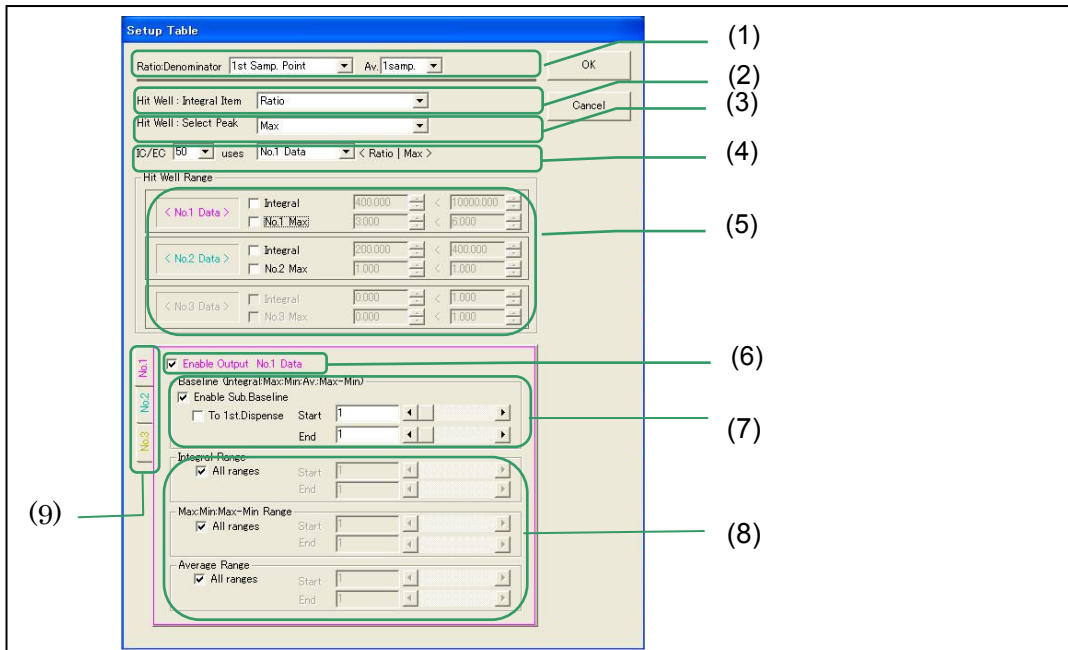
(5) Select Group Wells
: Select the well in the group
Well selection can be done by left click on the mouse
Group will should be chosen in group list box before selecting the well

Note

At select of Select Group Wells button, after select of control well, click Select Unlimited Well/Display Groups/Hit Wells button in order to set the select. (When selecting Select Group Wells button, operation is restricted.)

(7-2-1) Hit & Calc.Param

It sets conditions for Hit well and Calculation functions.



(1) Ratio / Denominator : To define the denominator item of ratio
<when two wavelength measurement>

Wavelength2 Intensity

: Intensity value of wavelength2 of each sampling point is used.

<when one wavelength measurement> (Refer to 1-8-6-1 Chap.4)

1st.Samp.Point: To use Intensity value in which 1st. sampling point is assumed to be a reference point

Ave. : To use Average value of Intensity from the 1st. sampling point to the set sampling

Delay (Option Function)

: The reference point is moved behind the set sampling

X Disp. Point (Option Function)

: To use Intensity value in which sampling point before No.X dispensing point is assumed to be a reference point

Ave. : To define Average value of Intensity from a reference point to the set sampling

Delay : The reference point is moved behind the set sampling

(2) Hit Well/Integral Item : To choose an item to be applied for Hit well function, whether Ratio or Ex1/Lumi

(3) Hit Well/Select Peak : To choose a peak to be applied for Hit well function, whether Max , Min , Av. or Max-Min.

(4) IC/EC X uses No.Y Data

: Setup parameters for IC/EC calculation

IC/EC X : Set value from 10 to 90, 10 value step

No.Y Data : Select which data number to be used for group calculation
 Calculation use the item set in Integral Item and Select Peak in HIT WELL, use the range value set in the data number tab.

(5) Hit Well Range : It sets conditions for Hit well function. The parameter is Integral value and Peak value. The area is set here and the correspond wells are picked up automatically.

All Area : To apply all ranges for calculation.

Start/End : To set a calculation range manually.

(6) Enable Output No.X Data

: To set On/Off of No.X data.

(7) Baseline

: To set baseline subtraction.

To 1st Dispense

: To set a range from measurement start to the first dispensing timing as baseline.

Start/End : To set a range as baseline manually.**(8) Integral/Max/Min/Max-Min/Average Range**

: To set a calculation range.

(9) No.1,2,3 Tab

: To switch data number by Tab.

(7-3) Select Graph

It sets items to be displayed at all wells graph display and remark graph display. And the top item within the checked ones is applied for micro plate pseudo color display.

The screenshot shows a 'Select Graph' dialog box with a list of options and two graph preview icons. The options are:

- Ratio (1)
- Ex340:Em540 (2)
- Ex380:Em540 (3)
- Integ.Ratio (4)

Two graph preview icons are shown on the right:

- Icon (5) is associated with the 'Ratio' option.
- Icon (6) is associated with the 'Ex340:Em540' and 'Ex380:Em540' options.

The following definitions are provided for each option:

(1) Ratio
: To display Ratio value. It is not available on luminescence.

(2) Fluorescence 1/Luminescence
: To display fluorescence or luminescence. A numerator is applied here at two-wavelength excitation or emission fluorescence measurement.

(3) Fluorescence 2
: To display fluorescence No2 at two-wavelength excitation or emission fluorescence measurement. A denominator is applied here. It is not available at luminescence or one wavelength excitation and emission measurement.

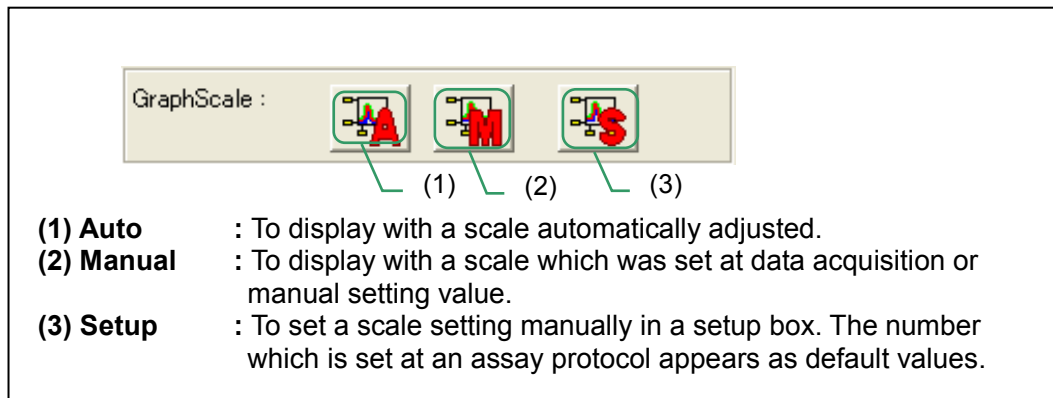
(4) Integral
: To display Intergral value. It is set at Hit Well/Intergral Item in table setup whether ratio or Ex1/Lumi is applied for Intergral.

(5) Display Ratio
: The display ratio of all wells graph and remark graph is switched.

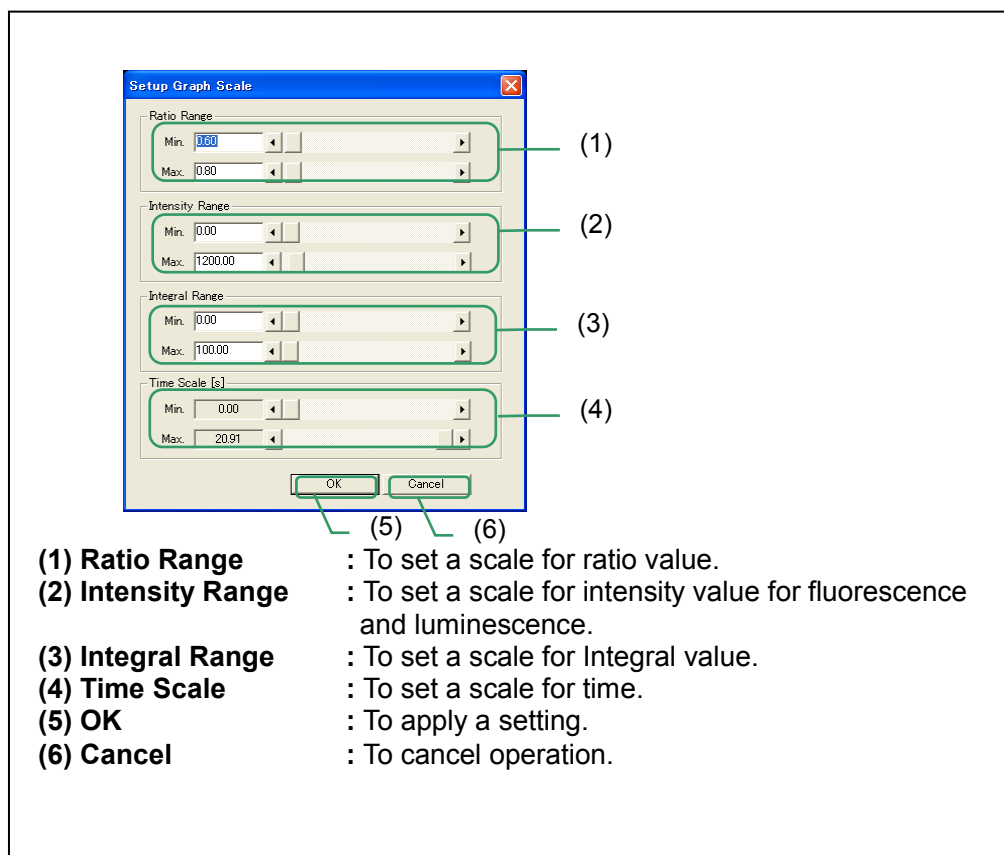
(6) Background color of graph
: The background color of the graph is switched to the black and the white.

(7-4) Graph Scale

It sets a scale type and switch it for all wells graph display, remark well display and micro plate pseudo color display.

**(7-4-1) Setup**

Clicking 'Setup' leads Graph Scale Setup box and a scale for each item is set in it.



(7-5) Info.

It displays Plate Data information such as a plate type and assay protocol. It exports data with a text format.



Information : To display Plate Data information.

(7-5-1) Info.

Clicking 'Info.' leads Plate Information box and it displays the information of Plate Data. Comment input and text output is capable here.

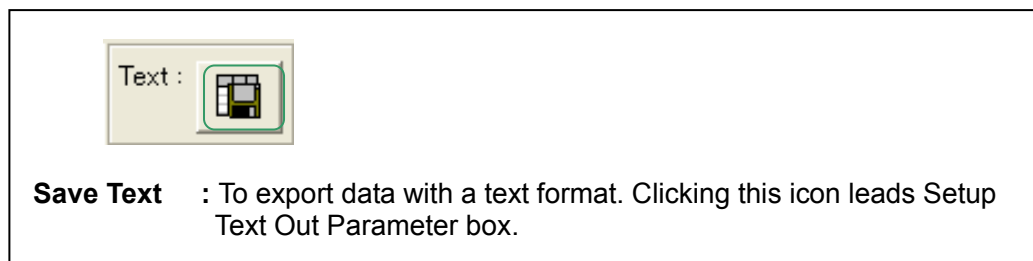
Serial No.	Date	Time	Hit Well	Total Well	Hit/Total	Comment
699000427	09/27/04	21:13:13	40	96	41.667%	

Item	Parameter	Value1	Value2	Value3	Value4
<Measurement Para.	Protocol Name	1Ex1Em			
	Measurement Mode	Ex480Em640 / Ex4.			
	Samples	121			
	Interval Mode	Single			
	Interval 1st [sec]	1.00			
	Interval 2nd [sec]	0.60			
	Double Sequence	---	---	---	---
	Dispense Timing	---	---	---	---
	Preparation Dispens..	---	---	---	---
	Pre Incubation [min]	None			
<Correction Paramet..	Autofluorescence C.	Nunc4x4Exp4 PAF			
	Shading Correction	Nunc4x4 PSD			
	Calibration Correcti..	None			
<Camera Parameter>	Exposure Time [sec]	0.207 sec			
	Sensitivity	1			
	Binning	4 x 4			
<Dispenser Paramete..	Volume [ul]	<Ligand1>	<Ligand2>	<Ligand3>	
	Aspirate Speed [ul/..	50	50	50	
	Aspirate Height [mm]	0.5	0.5	0.5	
	Dispense Speed [ul..	50	50	50	
	Dispense Height [mm]	2.5	5.0	5.0	
	Wash Count	5	5	5	
	Aspirate Pipet	0	0	0	
	Dispense Pipet	0	0	0	
	Aspirate From	Stage	Stage	Stage	
	Mixing	Off			
<Calouration Parame..	Hit Well / Integral It..	Ratio			
	Hit Well Integral Ra..	15.00	100.00		
	Hit Well Ratio Rang..	0.80	2.00		
	Baseline Sub.	Off			
	Baseline Start/End	---	---	---	
	Integral Area Start/..	All Area	All Area	All Area	
	Peak Area Start/End	All Area	All Area	All Area	
	Average Area Start..	All Area	All Area	All Area	
<Plate Parameter>	Assay Plate	NUNC OpticalBottom			
	Ligand1/2/3 Plate	NUNC PP	NUNC PP	NUNC PP	

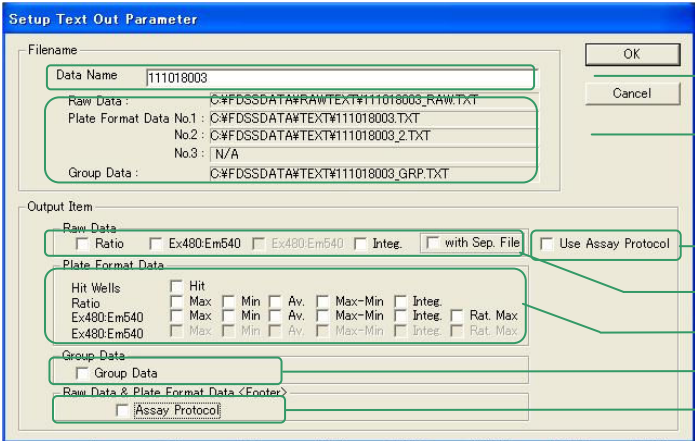
- (1) **Plate Name** : To display a file name of Plate Data.
 (2) **Comment** : To display, input and edit comments
 (3) **Basic and parameter information** : To display information of Plate data.
 (4) **Save as Text** : To export data with a text format.
 (5) **OK** : To apply comments.
 (6) **Cancel** : To cancel operation.

(7-6) Save Text

It sets items for exporting data with a text format in Save Text.

**(7-6-1) Text Out**

Clicking Save Text leads Setup Text Out Parameter box to set a filename and to choose items to be exported. <refer to 2)Text Output in Chapter 6>



(1) OK button

(2) Cancel button

(3) Use Assay Protocol checkbox

(4) Raw Data section

(5) Plate Format Data section

(6) Group Data section

(7) Raw Data & Plate Format Data <Footer> section

(1) Data Name : To set a file name of data

(2) File Name : To appear an actual file name

(3) Use Assay Protocol
: To employ output items which are set in Assay protocol.

(4) Raw Data : To choose items to be output at Raw data.
With Sep. File : Raw data of each item is output with another file.

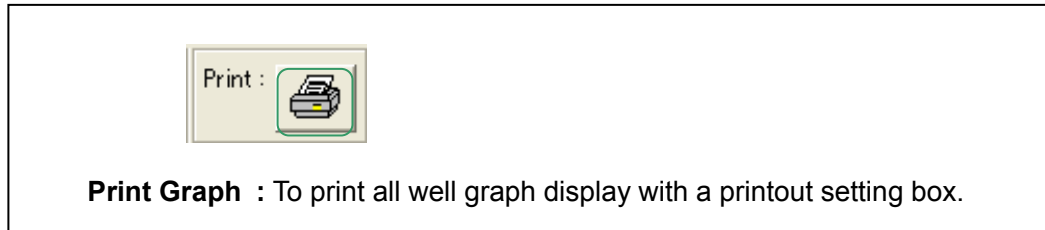
(5) Plate Format Data
: To choose items to be output at Plate Format data.

(6) Group Data
: Check "Group Data" to output group data.

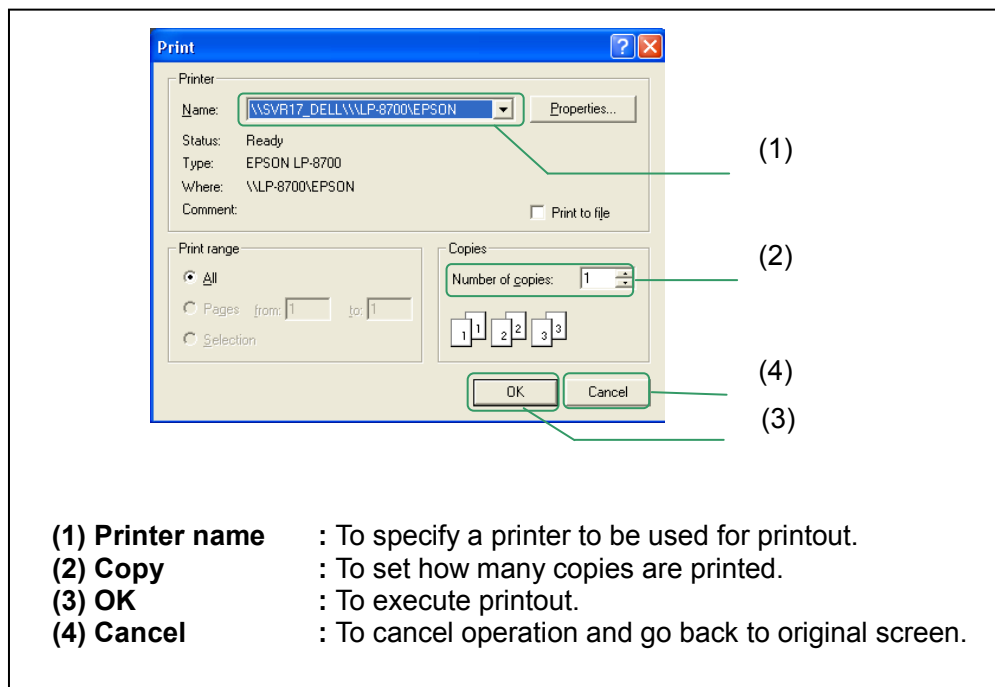
(7) Raw Data & Plate Format Data <Footer>
: To set to output the assay protocol information at the footer part of text data.

(7-7) Print Graph

It prints out all well graph display at Print Graph.

**(7-7-1) Setup box**


The printout setting box appears all wells graph display to set the printout.



(7-8) Diagram

Display/non-display of the correlation diagram display is switched.

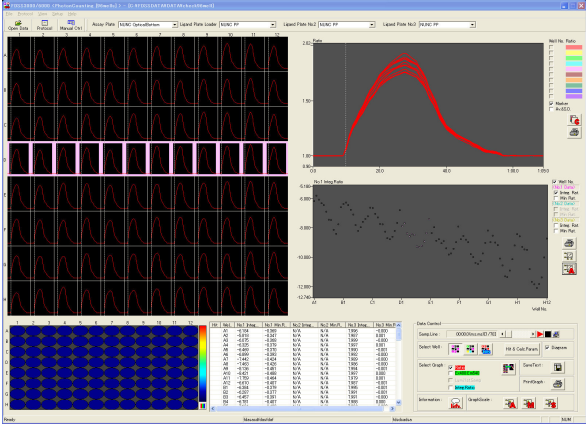
<Diagram Item>



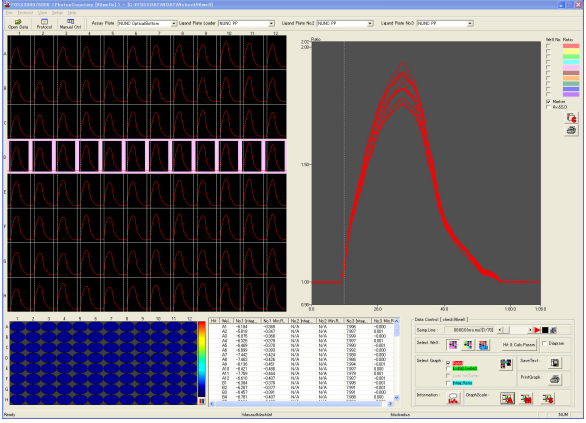
(1)

(1) Diagram :
 Display/non-display of the correlation diagram display is switched.
 When the button is put into the state of the check, the correlation diagram is displayed. The correlation diagram becomes non-display when putting it into the state of the uncheck.

<Checked>



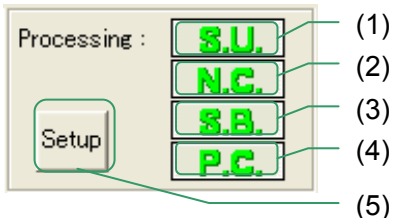
<Unchecked>



(7-9) About Processing

In the Processing, On/Off of 4 kinds of data processing and parameter setup is performed

<Processing>



(1) S.U.(Spatial Uniformity Correction) : Select of ON/OFF(OFF: Grey)
Spatial Uniformity Correction is performed by using specified range.

(2) N.C.(Negative Control Correction) : Select of ON/OFF(OFF: Grey)
Canceling Negative Control Correction is performed by dividing changed portion of specified control well.

(3) S.B.(Subtract Bias) : Select of ON/OFF(OFF: Grey)
To subtract the data of specified range

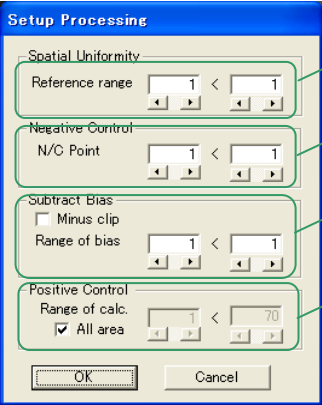
(4) P.C.(Positive Control Scaling) : Select of ON/OFF(OFF: Grey)
To perform correction on the basis (100) of intensity of specified control well

(5) Setup :
To setup parameter of above 4 data processing (For detail: Refer to 7-9-1)

(7-9-1) About Setup

After clicking Setup button, the following box is displayed.
In here, Parameter setup of 4 data processing is performed.

<Setup Processing>



(1) Spatial Uniformity
Reference range (By Sampling No.) :
Specify the reference range act as a standard for spatial uniformity correction
Specify the range which is considered it has most uniformity at the measurement data (e.g. the first sampling)

(2) Negative Control
N/C Point (By Sampling No.) :
Specify the reference range act as a standard of time axis used for Negative Control Correction is performed by using the value of reference range specified as a standard.

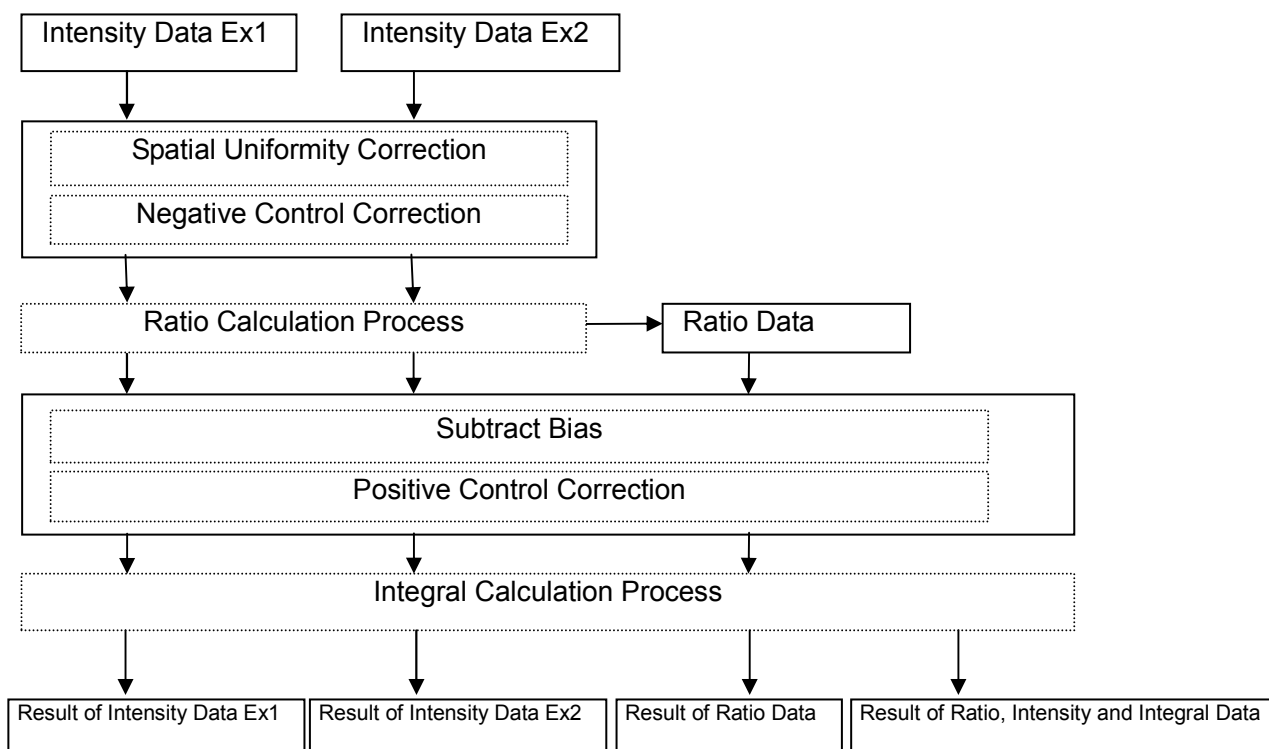
(3) Subtract Bias
Minus clip :
To select whether to null the minus value which might occur by being subtracted or not.
Range of bias (By Sampling No.) :
Specify the range used as Bias. (The mean value in the range is used as Bias.)

(4) Positive Control
Range of calc. (By Sampling No.) :
Specify the calculate range used for scaling.
To perform scaling on the basis (100) of maximum value in the specified range
All ranges :
To set all of range as the range to calculate

(7-9-2) About the order of data processing

When a data is loaded on FDSS, data processing is run as below.
Doing On/OFF is possible on each processes, and each processes are run by such flow as described below.

1. Spatial Uniformity Correction (On/Off is possible)
2. Negative Control Correction (On/Off is possible)
3. Calculate Ratio (Constantly On)
4. Subtract Bias (On/Off is possible)
5. Positive Control Scaling (On/Off is possible)
6. Calculate Integral (Constantly On)

<Flow of calculation>

(7-9-3)About the calculation method of data processing**1. Spatial Uniformity Correction**

It spatial uniform corrects it by using data within the range set by Ref.range.

<Parameter>

·reference range : Start -> End (Ref.range)

<Calculation method>

1. The correction factor of each well is requested by dividing the mean value within the range set by Ref.range of all wells by the mean value within the range set by Ref.range of each wells.
2. It corrects it by multiplying the correction factor of each well by all data of each well.

<Calculation type>

Correction Factor:

$$\text{Corr.Factor}[\text{well}] = \text{Av.}[\text{all well}][t=\text{Ref.range}] / \text{Intensity}[\text{well}][t=\text{Ref.range}]$$

Corrected Intensity:

$$\text{Value}[\text{well}][t] = \text{Intensity}[\text{well}][t] * \text{Corr.Factor}[\text{well}]$$

2. Negative Control Correction

The time direction is uniformly corrected by using set Negative control well.

<Parameter>

·Negative Control point : Start -> End (NC point)

·Negative Control well (NC well)

<Calculation method>

1. The correction factor of each sampling is requested by dividing the mean value of NC well within the range set with NC point by the mean value of NC well at the time of each sampling.
2. It corrects it by multiplying the correction factor of each sampling by all data of each sampling.

<Calculation type>

Correction Factor :

$$\text{Corr.Factor}[t] = \text{Av.}[\text{NC well}][t=\text{NC point}] / \text{Av.}[\text{NC well}][t]$$

Corrected Intensity :

$$\text{Value}[t] = \text{Intensity}[t] * \text{Corr.Factor}[t]$$

3. Calculate Ratio

The ratio is calculated from the intensity data. The calculation method is different according to measurement Method.

<Calculation type>

2ex1em,1ex2em and 2ex2em(Fura2 , FRET etc.)

$$\text{Ratio}[t] = \text{Ex1}[t]/\text{Ex2}[t]$$

1ex1em(Fluo4 etc.)

$$\text{Ratio}[t] = \text{Ex1}[t]/\text{Ex1}[t=0]$$

4. Subtract Bias

The mean value within the set range of Bias is used and subtracted.

<Parameter>

· range of bias : Start -> End (BiasRange)

<Calculation method>

1. The mean value within the range set with BiasRange of each well is assumed to be Bias value of each well.
2. It corrects it by subtracting the Bias value of each well from all sampling of each well.

<Calculation type>

Bias Value :

$$\text{Bias}[\text{well}] = \text{Intensity}[\text{well}][t=\text{BiasRange}]$$

Corrected Intensity :

$$\text{Value}[\text{well}][t] = \text{Intensity}[\text{well}][t] - \text{Bias}[\text{well}]$$

5. Positive Control Scaling

Scaling that uses set Positive control well is done.

<Parameter>

· range of calculation (range)

· Positive Control well (PC well)

<Calculation method>

1. The PC well mean value of the first sampling set with range is subtracted from the mean value of PC well of each sampling set with range. (Dif[t])
2. The maximum value of Dif[t] is divided from 100 and it is assumed the correction factor.
3. It scales it by multiplying the correction factor by all intensity data.

<Calculation type>

$$\text{Dif}[t] = \text{Av.}[\text{PC well}][t] - \text{Av.}[\text{PC well}][t=\text{Start}]$$

Correct Factor :

$$\text{Corr.Factor} = 100 / \text{Max}(\text{Dif}[t])$$

Corrected Scale :

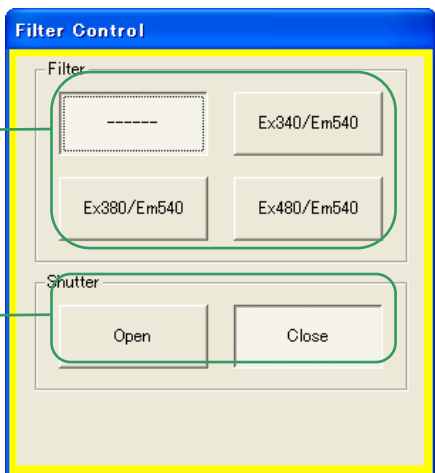
$$\text{Value}[\text{well}][t] = \text{Intensity}[\text{well}][t] * \text{Corr.Factor}$$

11. Filter Control (at fluorescence measurement)

Fluorescence optics such as a filter switching and shutter operation can be manually controlled by FDSS application software. At measurement they are automatically controlled by FDSS software.

1) Filter Control box

Filter Control box is shown as below and it stands by on screen.



(1) Filter : To switch 4 kinds of filter combination.

(2) Shutter : To switch shutters at excitation light sources.

Note
Filter combination can be set at 'FilterCombination Setup' command in 'Setup' menu.

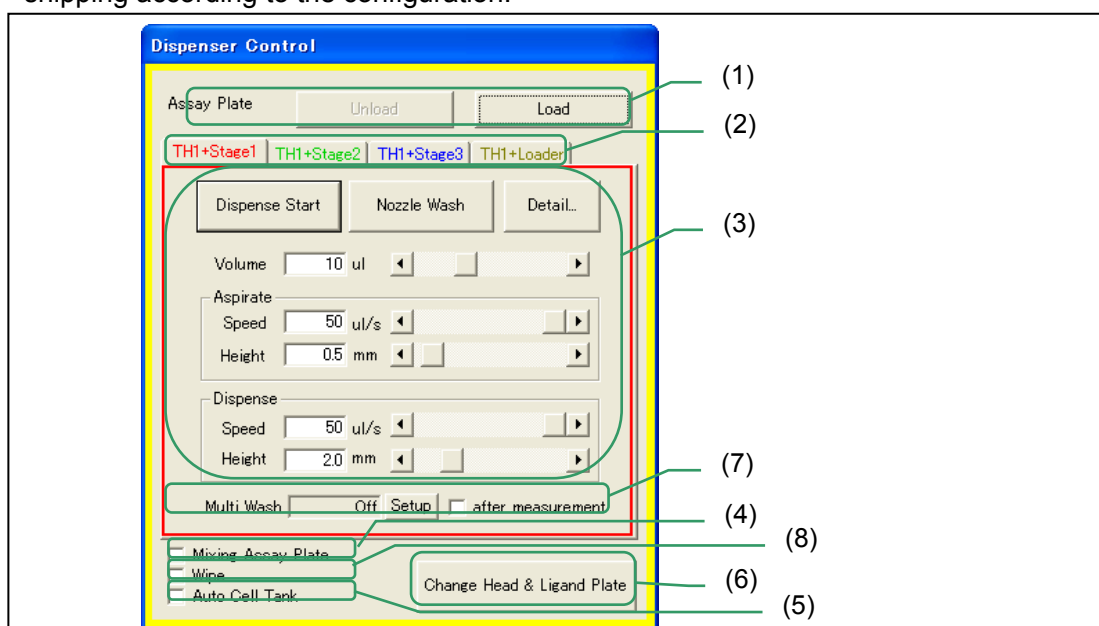
(This page is blank.)

12. Dispenser Control box

1) Dispenser Control box

(In the case of FDSS3000/6000/7000EX)

Used to control a dispenser manually and set dispensing parameter. Setting can be done as a Stage unit, and the dispensing heads corresponding to the stages are set at shipping according to the configuration.



- (1) **Assay Plate:** To load an assay plate in or out when manually controlling the dispenser.
Load : To load an assay plate in.
Unload : To load an assay plate out.
- (2) **Stage selection tag**
: To display combination between dispensing heads and ligand plate stages and to set dispensing parameter here.
- (3) **Dispensing parameters**
: To set dispensing parameter with a Stage unit, choosing Stage selection tag (Refer to 1-1)
- (4) **Mixing Assay Plate**
: To choose ON or OFF of the mixing (vibration) function for an assay plate. It is available only at fluorescence measurement.
- (5) **Auto Ligand Tank:**To choose ON or OFF of auto ligand feeding function. (Option)
- (6) **Change Tip & Ligand Plate**
: To exchange dispensing head tips or ligand plates, specifying which tips and plates with Stage selection tag.
- (7) **Multi Wash/Wash Cycles:** To set the number of times to wash
- (8) **Wipe** : To choose ON or OFF of the wiping function after washing.

Note

Click the tag into active and then click this command.

Note

Dispensing parameter set here reflects on Assay Protocol. And when Assay Protocol is loaded after setting here, the loaded parameter is valid.

(1-1) Dispensing parameter

Used to set dispensing parameter. Setting can be done as a Stage unit, and the dispensing heads corresponding to the stages are set at shipping according to the configuration.

(1) ——— TH1+Stage1 | TH1+Stage2 | TH1+Stage3 | TH1+Loader ——— (3)

(2) ——— Dispense Start ——— (4)

————— Nozzle Wash ——— (4)

————— Detail.. ——— (4)

————— Volume 10 ul ——— (5)

————— Aspirate ——— (6)

————— Speed 50 ul/s ——— (6)

————— Height 0.5 mm ——— (6)

————— Dispense ——— (7)

————— Speed 50 ul/s ——— (7)

————— Height 2.0 mm ——— (7)

————— Multi Wash Off Setup ——— (9)

————— after measurement ——— (8)

(1) Stage selection tag
: Choose at which combination dispensing parameter is set.

(2) Dispense Start
: To start dispensing operation when operated manually. It dispenses liquid with the set parameter in an active tab head/stage.

(3) Nozzle Wash
: To wash dispensing head when operated manually. It washes with the set washing times at 'Multi Wash' in an active tag head.

(4) Details: To set detail parameter such as aspirated place, mixing in/out function (Refer to 1-2).

(5) Volume
: To set dispensing quantity (unit: μl) with an active tag head.

(6) Aspirate
: To set aspirating parameter with an active tag head.
Speed : To set aspirating speed (unit: $\mu\text{l/s}$).
Height : To set aspirating position, height from the bottom of a plate. (unit: mm).

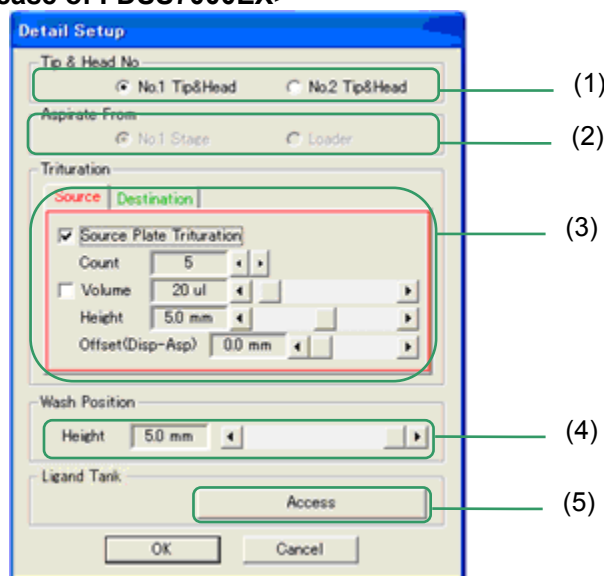
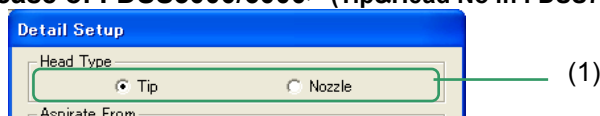
(7) Dispense
: To set dispensing parameter with an active tag head.
Speed : To set dispensing speed (unit: $\mu\text{l/s}$).
Height : To set dispensing position, height from the bottom of a plate. (unit: mm).

(8) Multi Wash or Wash Cycle
: To set how many times the dispensing head is washed.

(9) after measurement
: To choose at which timing the dispensing head is washed.
Check : To wash dispensing head after measurement.
No Check : To wash dispensing head after each dispensing operation.

(1-2) Dispensing detail parameter setting

Used to set dispensing parameter. Setting can be done as a Stage unit, and the dispensing heads corresponding to the stages are set at shipping according to the configuration.

<In the case of FDSS7000EX>**<In the case of FDSS3000/6000> (Tip&Head No in FDSS7000EX is different as follows.)**

- (1) **Tip&Head No** : To choose a combination of dispensing head and tip.
Head Type : To choose a dispensing head type, tip or nozzle.
- (2) **Aspirate From**: To choose from which place liquid is aspirated and then dispensed.
Stage : Aspirate from a ligand plate on the round table.
Loader : Aspirate from a ligand plate on a loading line.
- (3) **Trituration** : To set mix-in/out at aspirating and/or dispensing.
Source Plate : To run mix-in/out at aspirating.
Destination Plate: To run mix-in/out at dispensing.
Count : To set how many times mix-in/out is done.
Volume : To set how much quantity is used at mix-in/out.
Height : To set the height at mix-in/out from Aspirate or Dispense height.
Offset : To set the difference of the height when mix-in and mix-out
- (4) **Wash Position**: To set washing position
Height : To set washing height at washing.
(Unit: mm, from the default value where the edge of tip a bit entrances into the washing nozzle.)
- (5) **Ligand Tank (Option)**
Access : To turn the position of the cell tank for the auto ligand feeder to the door position and then to open the door so that it can be accessed for exchanging it.

(1-3) Dispenser basic working

1) To load assay plate into measurement position.

Set an assay plate onto a stacker and click 'Loading'. When 'Loading' is disabling and 'Unloading' is enabling, the plate is already set at the measurement position.

2) To load assay plate out

Click 'Unloading'. When 'Loading' is enabling and 'Unloading' is disabling, the plate is already not set at the measurement position.

3) To dispense liquid

Choose 'TH+Stage' tab or 'Head+Stage' tab for the head and stage combination to be used when an assay plate is set at the measurement position and then click 'Dispense Start'

4) To change dispensing parameter

Choose 'Head+Stage' tab for head and stage combination to be used and then change dispensing parameter. And click 'Detail' and set Trituration function.

5) To set ON/OFF for assay plate vibration function

Choose check (ON) or not on 'Mixing Assay Plate'.

6) To exchange tip of dispensing head and ligand plate on round table

Click 'Change Tip&Ligand Plate'

In the case of FDSS7000EX, A Box to select exchange mode is displayed and then can select either the mode to exchange only ligand plate on the ligand stage or the mode to exchange not only the ligand plate but also dispensing head and tip. (About in detail please refer to 3-2)

In the case of FDSS3000/6000, the ligand stage and dispensing head move a position to be able to change them and then the door of FDSS open.

7) To exchange applied mode of dispensing head and tip (only FDSS7000EX)

Click 'Change Tip&Ligand Plate'

As a box to select exchange mode is displayed, select the mode to exchange not only the ligand plate but also dispensing head and tip. It can be changed at the time of the setup of dispensing head and tip. (refer to 3-3)

8) To wash dispensing tip (At configuration which has washing function)

Click 'Nozzle Wash' to wash dispensing tip with the set number in Wash Cycles parameter.

9) To set ON/OFF for auto ligand feeder (Option)

Choose check (ON) or not on Auto Ligand Tank to feed ligand from a special tank to an assay plate.

10) To exchange the ligand tank of auto ligand feeder (Option)

Click 'Detail'

The detail setup box is displayed.

Click 'Access' button of the Ligand Tank item.

The tank moves to the exchange position and the door opens.

2) Dispenser Control box <In the case of uCELL>

Use to control a dispenser manually and set dispensing parameter. Setting can be done as a tab unit.

The screenshot shows the 'Dispenser Control' window. At the top is the title bar (1). Below it is the 'Assay Plate Access' button (2). A row of four tabs labeled 'TH1+Stage1', 'TH1+Stage2', 'TH1+Stage1', and 'TH1+Stage2' (3) is visible. Below the tabs are three buttons: 'Dispense Start' (4), 'Nozzle Wash' (5), and 'Setup'. The main area contains three sections: 'Aspirate' (6) with fields for Asp. Stage, Volume, Speed, Height, and Trituration; 'Dispense' (7) with fields for Speed, Height, and Trituration; and 'Wash' (8) with a checkbox for 'Wash After Measurement', 'Vat' (set to 'A'), 'Cycle' (set to '3'), and 'Pos.' (set to '0.0 mm'). At the bottom are 'Change Head' (9) and 'Access Ligand No.1' (10) buttons, and a 'Wipe:A' checkbox (11).

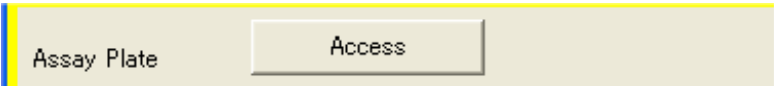
(2) **Assay Plate Access** : Loading and unloading of assay plate
 (2) **TH1+StageX tag** : Switching of dispensing parameter combination
 (3) **Dispense Start** : Execution of dispensing
 (4) **Setup** : Setting of various setting box
 (5) **Nozzle Wash** : Washing of tip nozzle
 (6) **Aspirate** : Setting of aspiration parameter
 (7) **Dispense** : Setting of dispensing parameter
 (8) **Wash** : Setting of washing parameter
 (9) **Change Head** : Exchange of dispensing head
 (10) **Access Ligand No.X** : Exchange of ligand plate
 (11) **Wipe** : Setting of On/Off of wiping after washing

Note

The dispensing parameters which were set here is used in Assay Protocol. And please care about when Assay Protocol file is loaded after parameter setup, loaded parameters are going to change.

(2-1) Assay Plate Access


Loading and unloading the plate are done.



Assay : Loading and unloading of assay plate
It becomes to access the assay plate folder when 'Access' button is clicked and the message box is displayed.
Please set up or take out the assay plate and click 'OK' button

(2-2) TH1+StageX Tab

Switch of dispensing parameter.

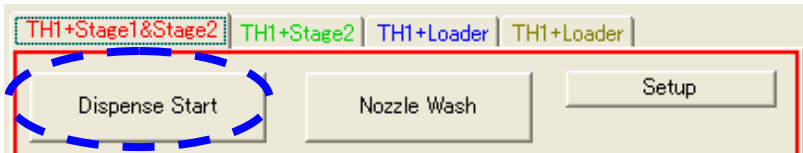


TH1+StageX : Switch of dispensing parameter
Up to 4 dispensing parameter setup is possible

If Preparation setup is performed at Prepa tab in Assay Protocol,
Available stage is limited

(2-3) Dispense Start

Dispensing is performed.

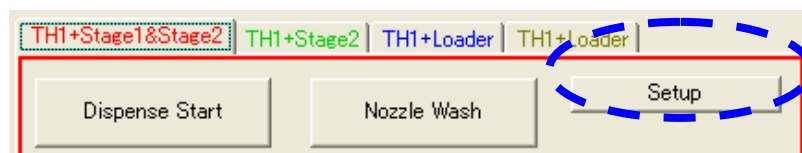


Dispense Start: According to the dispensing parameter set in TH1+StageX, dispensing is performed.

This can not be executed in AssayProtocol setup, execute it with Manual Mode.

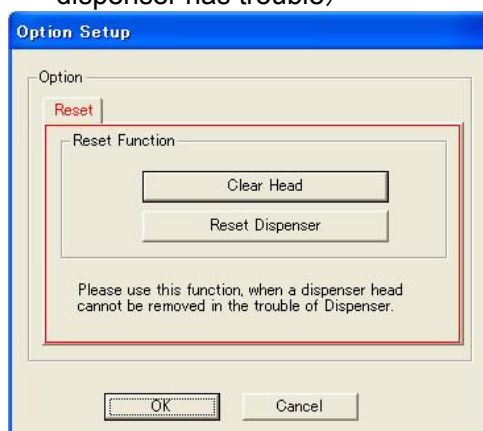
(2-4) Setup

The optional function setup is available. (This option only activated in supervisor mode which require password.)



Setup : The following option function setup is available.

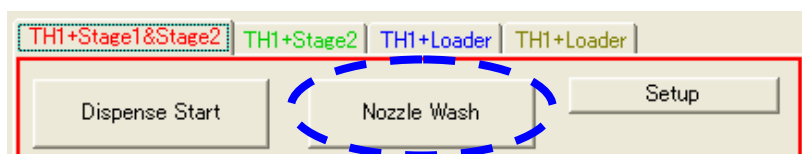
- 1) Setup of reset (This is the recovery command when the dispenser has trouble)



Clear Head : Remove the dispenser head
Reset Dispenser : Reset the dispenser

(2-5) Nozzle Wash

Washing is performed



Nozzle Wash : According to the dispensing parameter setup in TH1+Stage, washing is performed

This can not be executed in AssayProtocol setup, execute it with Manual Mode.

(2-6) Aspirate

The aspirate parameter is set.

<Tip head >

Aspirate

Asp. Stage Stage1 ▼

Volume 20 ul ◀ ▶

Speed 10 ul/s ◀ ▶

Height 2.0 mm ◀ ▶

Trituration

Vol. 20 ul ◀ ▶

Count 2 ◀ ▶

Height 5.0 mm ◀ ▶

Offset 0.0 mm ◀ ▶

Asp.Stage : Setup of where aspiration is performed
 Volume : Setup of aspiration volume
 Speed : Setup of aspiration speed
 Height : Setup of aspiration position (height from bottom of plate)
 Trituration : Setup of On/Off of trituration at aspiration
 Vol. : Setup of trituration volume
 Count : Setup of how many trituration is performed
 Height : Setup of height of trituration
 (height from aspiration position)
 Offset : Setup of offset of aspiration and dispensing at trituration

(2-7) Dispense

The dispense parameter is set.

<Tip head >

Dispense

Speed 20 ul/s ◀ ▶

Height 2.0 mm ◀ ▶

Trituration

Vol. 30 ul ◀ ▶

Count 5 ◀ ▶

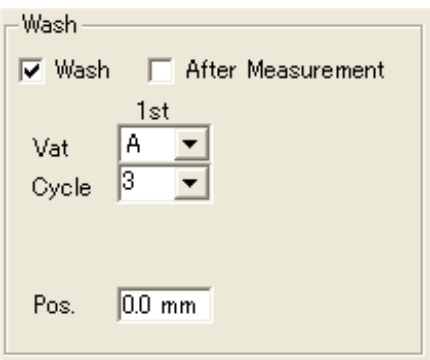
Height 5.0 mm ◀ ▶

Offset 0.0 mm ◀ ▶

Speed : Setup of speed of dispensing
 Height : Setup of dispensing position (height from bottom of plate)
 Trituration : Setup of On/Off of trituration at dispensing
 Vol. : Setup of volume of trituration
 Count : Setup of how many trituration is performed
 Height : Setup of trituration position (height from the dispensing)
 Offset : Setup of offset of aspiration and dispensing position at trituration

(2-8) Wash

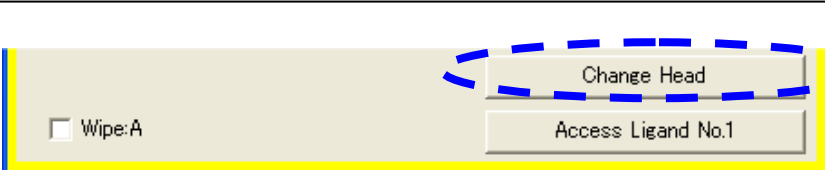
The washing parameter is set.



Wash : Setup of On/Off of washing
After Measurement : Setup of washing after measurement (On) or after dispensing (Off)
Vat : Setup of washing vat
The mounted washing bat A is selectable
Cycle : Setup of how many washing is performed
Pos. : Setup of height of washing (for fine adjustment)

(2-9) Change Head


The head are exchanged.



Change Head : Exchange of dispensing head
(For detail, refer to 7)

(2-10) Access Ligand No.X

The ligent plate are exchanged.




Access Ligand No.X : Loading and unloading of ligand plate
It becomes to access the ligand plate folder when 'Access' button is clicked and the message box is displayed.
Please set up or take out the ligand plate and click 'OK' button

(Ligand plate that can be accessed is ligand plate that has been selected in "(2-2)TH1+StageX" tab.)

(2-11) Wipe

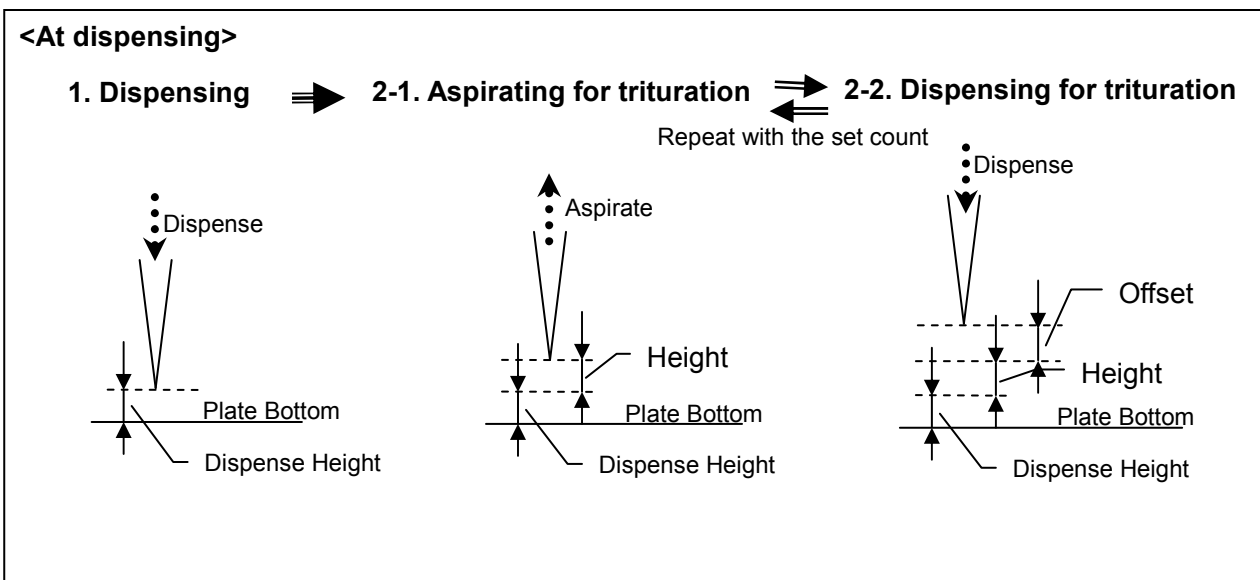
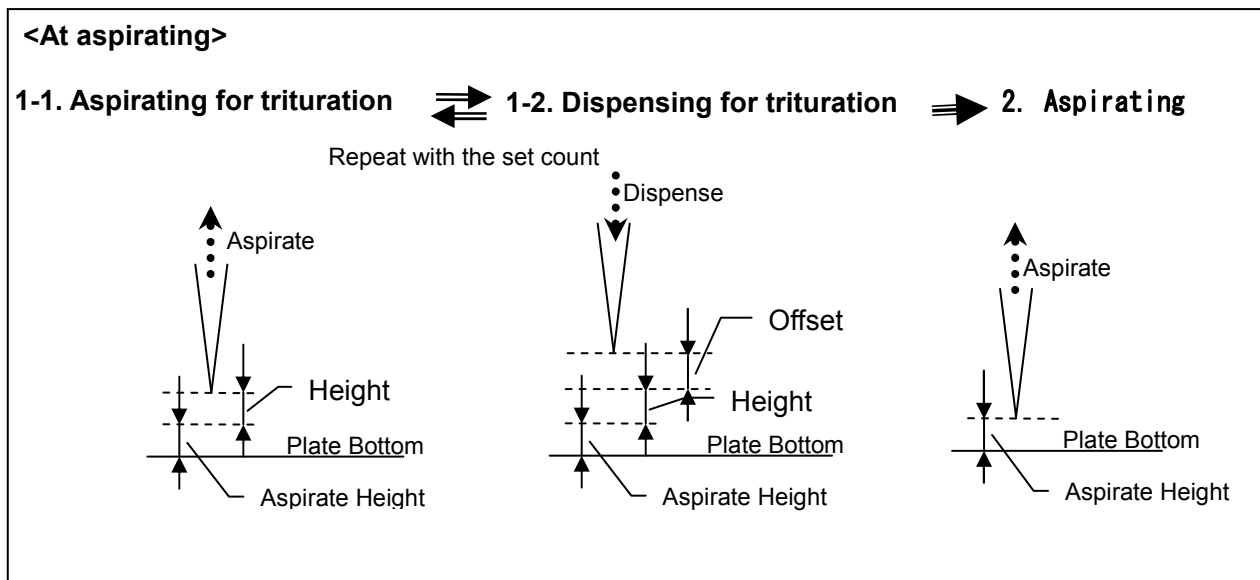
The wipe function is set.



Wipe:A : Setup of On/Off of wiping per washing unit
(If washing vat is not mounted, this is not displayed)

3) Height setting on Trituration

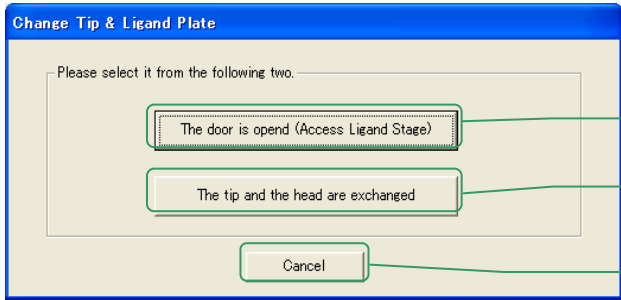
The relationship between the parameters and actual positions are shown as below. Trituration item in Detail Setup box includes Count, Height and Offset as parameters.



4) Exchange of dispensing head & tip and ligand plate **(In the case of FDSS7000EX)**

Click 'Change Tip & Ligand Plate' in the Dispenser Control box and the following box is displayed.

In this box, select of each exchange mode can be performed.



(1) The door is opened (Access Ligand Stage)

(2) The tip and the head are exchanged

(3) Cancel

(1) The door is opened : The door of dispenser opens
This is used for exchange of only ligand plate
The exchange of dispensing head & tip can not be performed.

(2) The tip and the head are exchanged
: After keeping dispensing head & tip to be enable to exchange, the door of dispenser opens.
This is used for exchange of dispensing head & tip
At the same time, ligand plate also can be exchanged.
At the time of the exchange, the applied mode of dispensing head and tip can be changed

(3) Cancel : The box is closed without performing the exchange

(4-1) Processes of 'the door is opened'

The door of dispenser opens.

After the door opens, the following box is displayed.

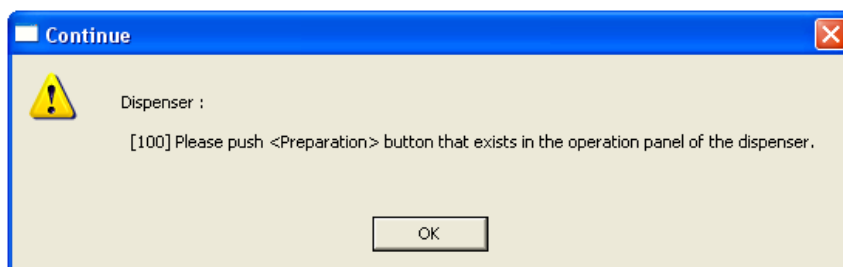


Exchange the ligand plate etc.... After it finishes, click 'OK' button.

The door closes.

When the door closes, the following box is displayed.

(It is not displayed according to the system, and Preparation is done by the automatic operation.)



Please push the OK button of the above-mentioned box after pushing 'Preparation' button of the front panel of following dispenser.

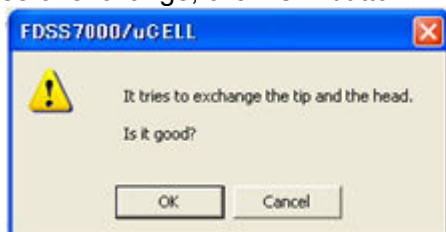
When the processing of the dispenser is completed, using software becomes possible.



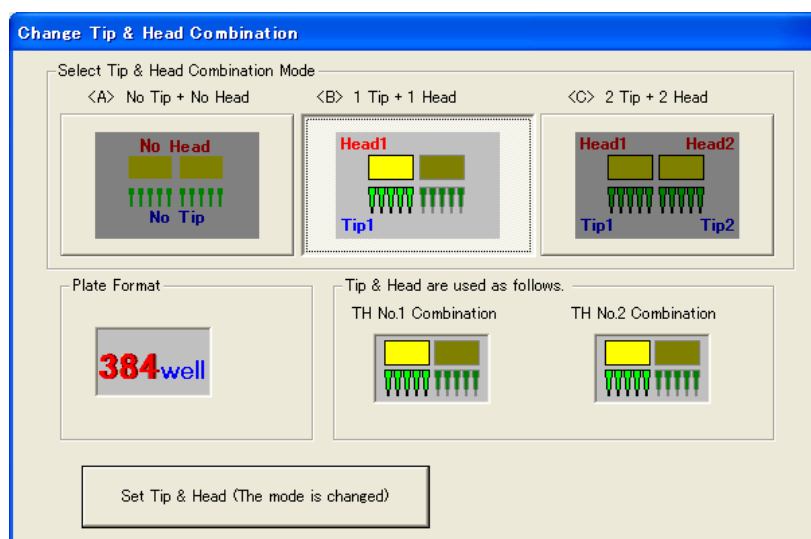
'Preparation' button

(4-2) Process of 'tip and head are exchanged'

The following confirmation message for operation of head and tip is displayed. In the case of exchange, click 'OK' button.



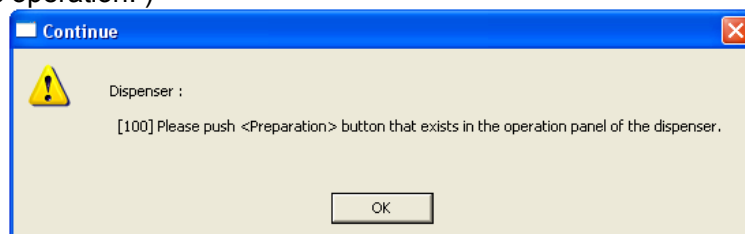
In the case the dispensing head and tip keep to be loaded, after they get unloaded, the door opens. When the door is opened, the following box is displayed.



The exchange of the dispensing head & tip and ligand plate are performed. Choose the combination between dispensing head and tip and then click 'Set Tip & Head' button. The door closes.

When the door closes, the following box is displayed.

(It is not displayed according to the system, and Preparation is done by the automatic operation.)



Please push the OK button of the above-mentioned box after pushing 'Preparation' button of the front panel of following dispenser.

When the processing of the dispenser is completed, using software becomes possible.

(4-3) Applicable modes of dispensing head & tip

In the FDSS7000EX, there are such 3 applicable modes as follows.

- A) The mode not to use any dispensing head and tip. (In this case, the dispensing operations are not performed at all.)
- B) Dispensing head: 1, Tip: 1
- C) Dispensing head: 2, Tip: 2

These 3 modes can be changed when the dispensing head and tip is exchanged.

It is set by the following box that the dispensing head and tip used in the case FDSS perform the dispensing.

That is set by specifying the combination between 2 dispensing heads and tips.

This specification can be performed in the 'Detail Setup Box'

The following shows the combination in the each usage mode

(1) Select Tip&Head Combination Mode
: Select the usage mode of dispensing head & tip

<A> No Tip + No Head : Any dispensing are not performed
 1 Tip + 1 Head : Dispensing head: 1, Tip: 1
<C> 2 Tip + 2 Head : Dispensing head: 2, Tip: 2

(2) Tip&Head are used as follows
: The combination between dispensing head and tip to be set in above

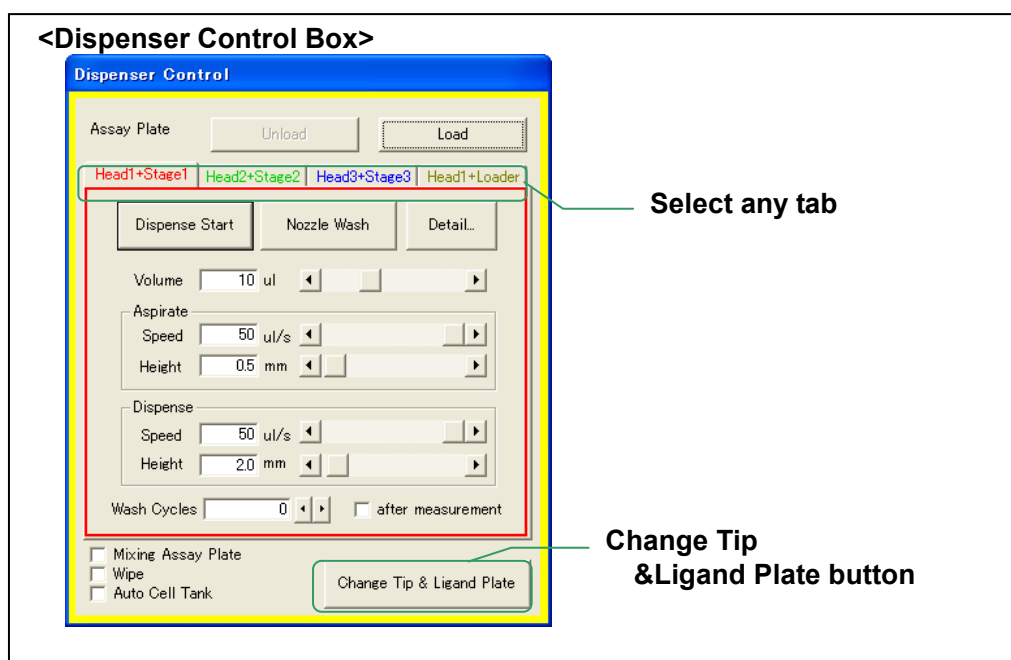
	TH No.1 Combination	TH No.2 Combination
<A> No Tip + No Head	Be not in use	Be not in use
 1 Tip + 1 Head	Tip 1 + Head 1	Tip 1 + Head 1
<C> 2 Tip + 2 Head	Tip 1 + Head 1	Tip 2 + Head 2

(3) Plate Format
: The plate format being selected at the starting

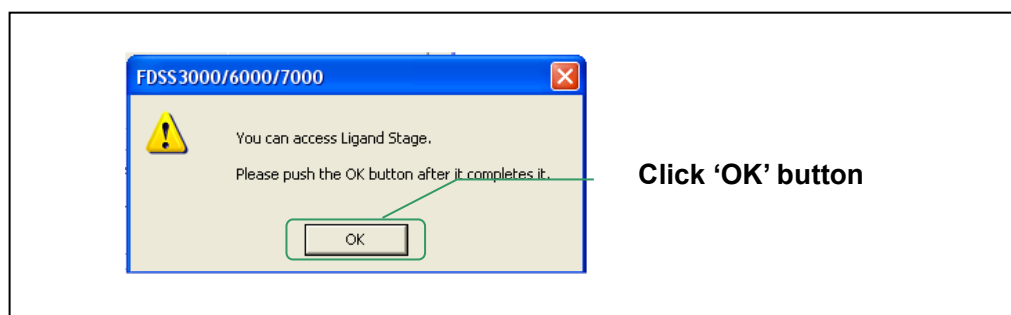
(4) Set Tip & Head
: To load the dispensing head and tip corresponding to the select

5) Exchange of dispensing head and ligand plate **(In the case of FDSS3000/6000)**

Select any tab which lists the name of the head & stage which you want to exchange.
It can be exchanged by clicking 'Change Tip & Ligand Plate' button.



When the 'Change Tip & Ligand Plate' button is clicked, after the selected dispensing head and ligand plate move to exchange position, the door opens.
And then as the following message is displayed, after finish of the exchange of dispensing head and ligand plate, click 'OK' button.
After that the door closes, the dispensing head and ligand plate is set.

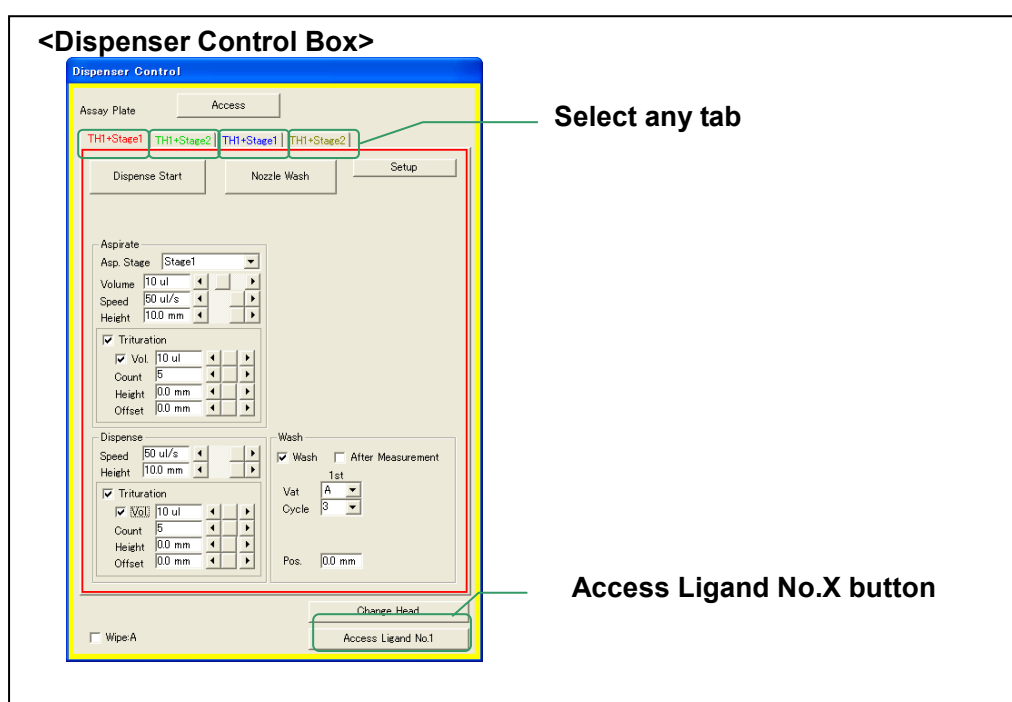


6) Exchange of dispensing head and ligand plate **(In the case of uCELL)**

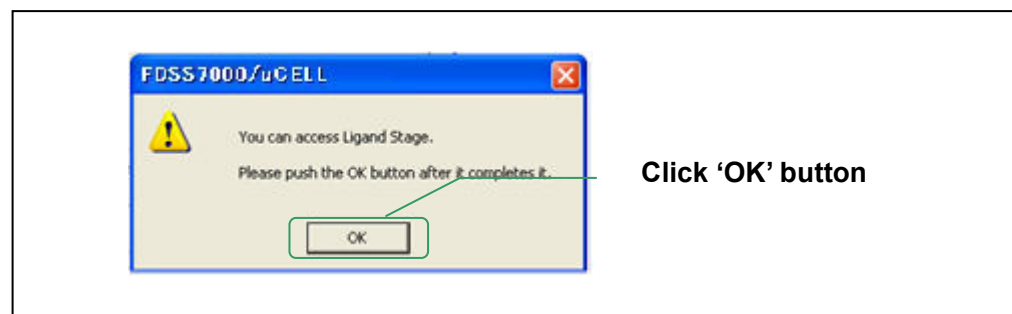
The dispensing head can be exchanged by clicking 'Change Head' button.
The ligand plate can be exchanged by clicking 'Access Ligand No.X' button.

(6-1) About the exchange of the ligand plate

Select any tab which lists the name of the stage which you want to exchange.
It can be exchanged by clicking 'Access Ligand No.X' button.

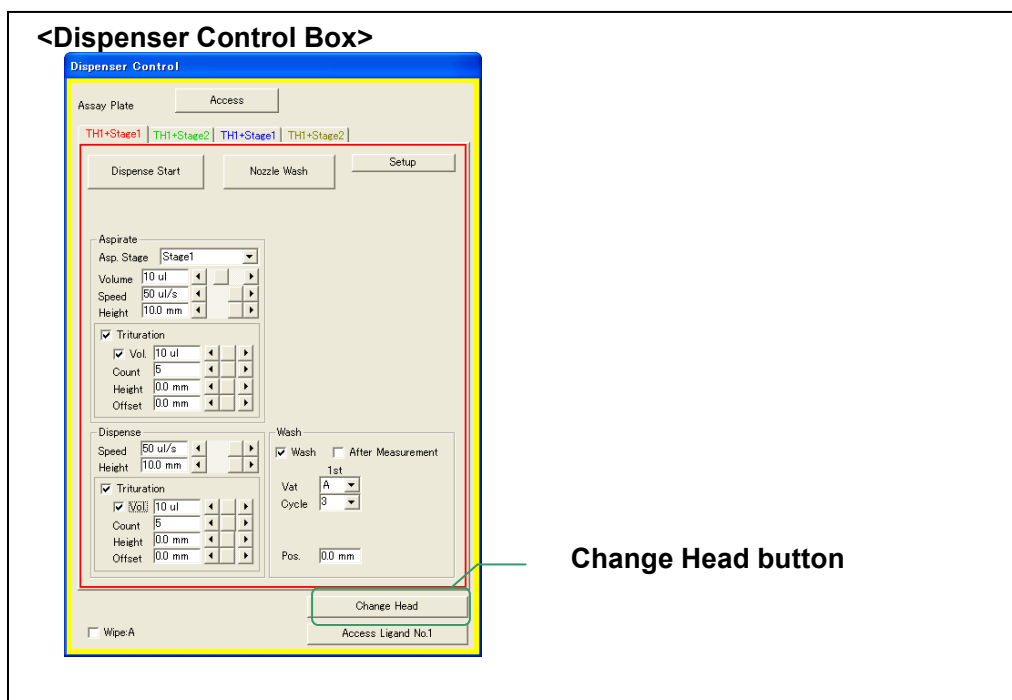


When the 'Access Ligand No.X' button is clicked, after the selected ligand plate move to exchange position, the following message is displayed.
After finish of the exchange of ligand plate, click 'OK' button.
The ligand plate is set.

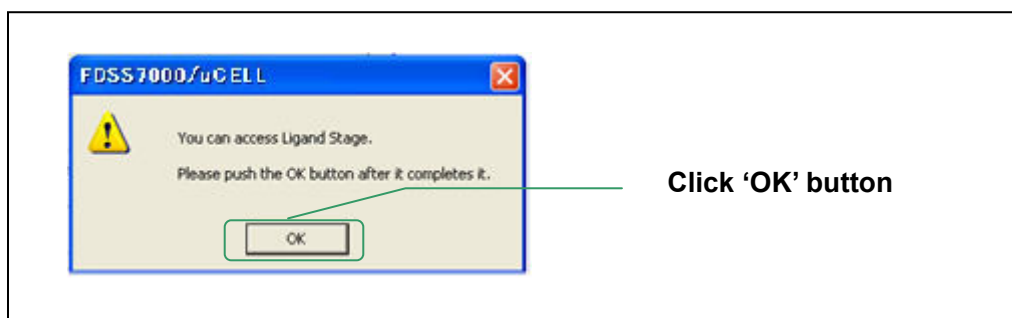


(6-2) About the exchange of the dispensing head

The dispensing head can be exchanged by clicking 'Change Head' button.

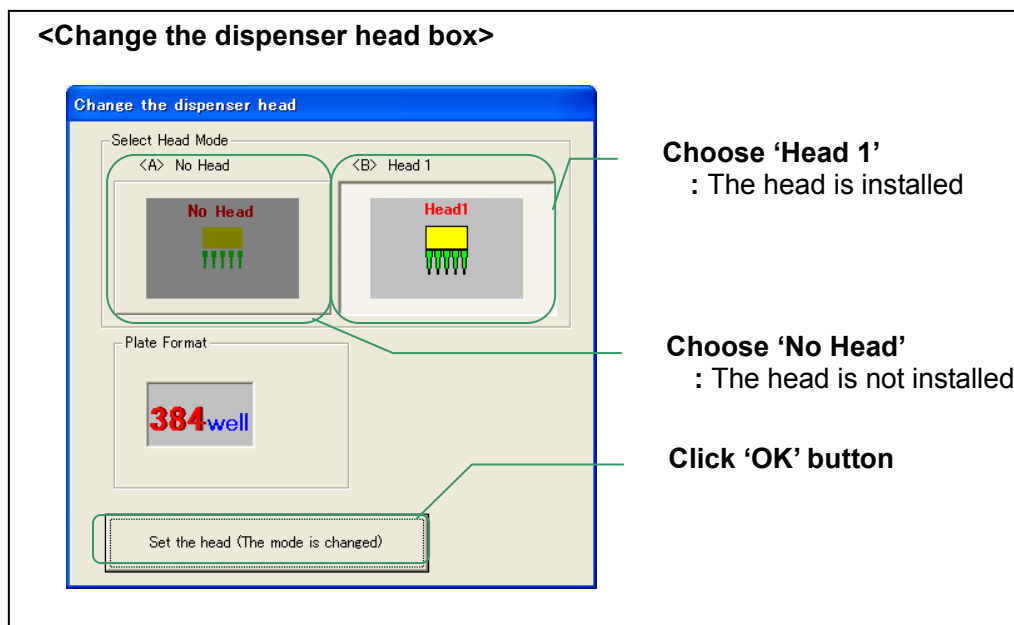


When the 'Change Head' button is clicked, the following message is displayed. Please click 'OK' button to exchange the dispenser head.



In the case the dispensing head keep to be loaded, after they get unloaded, the following box is displayed.

Please select 'Head Mode' used and click 'Set the head' button when the exchange of the dispenser head is finished.
The processing matched to 'Head Mode' is done.



(6-3) Applicable modes of dispensing head

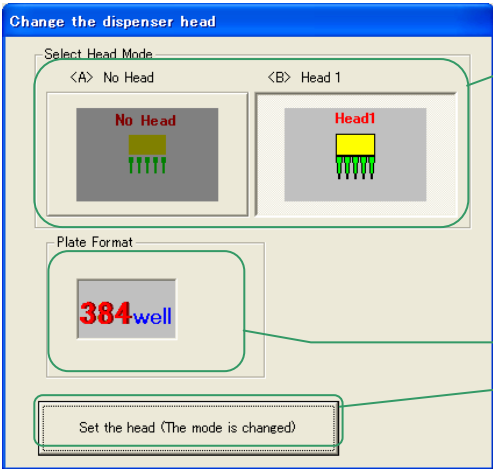
In the uCELL, there are such 2 applicable modes as follows.

- A) The mode not to use any dispensing head.
(In this case, the dispensing operations are not performed at all.)
- B) The mode to use 1 head.

These 2 modes can be changed when the dispensing head is exchanged.

It is set by the following box that the dispensing head used in the case FDSS perform the dispensing.

The following shows the combination in the each usage mode



(1) Select Head Mode
: Select the usage mode of dispensing head
<A> No Head : Any dispensing are not performed
 Head1 : One dispensing head is performed

(2) Plate Format
: The plate format being selected at the starting

(3) Set the head
: To load the dispensing head corresponding to the select

13. Camera Control box

Use to control a camera manually and it displays output from the camera.

Camera Control box controls a camera and peripheral devices and display output on Image Window and Microplate Window. It displays live images with Sensitivity 10 at fluorescence.

And it displays live images and photon counting images at luminescence [Photon counting] measurement.

And it displays live images with sensitivity 3 at luminescence [Analog] measurement.

And the pseudo colors display and enhance processing are available.

1) Camera Control box

(at fluorescence & luminescence [Analog][EMCCD] measurement)

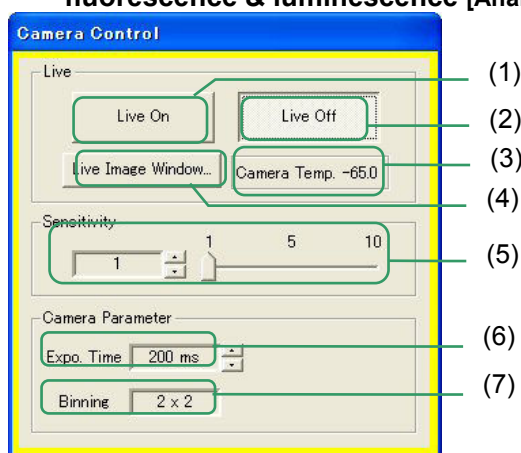
The following Camera Control box appears just after FDSS software starts up with a fluorescence or luminescence [Analog][EMCCD] method. Clicking 'Live On' displays Image Window and Microplate Window, and then fluorescence images are observed.

Note

Do not open the door of the dark box at Live ON.

<Camera Control box:

fluorescence & luminescence [Analog][EMCCD]measurement>



- (1) **Live On** : To acquire live images. It automatically turns 'Live Image Window' on.
- (2) **Live Off** : To stop acquiring live images.
- (3) **Camera Temp** : To display the cooling temperature of the camera.([EMCCD])
- (4) **Live Image Window** : To switch display ON/OFF for Image Window and Microplate Window.
- (5) **Sensitivity**
Sensitivity (Fluorescence) : To set sensitivity (camera gain) with 10 steps.
Sensitivity (Luminescence [Analog]) : To set sensitivity (I.I gain) with 3 steps
Sensitivity (Luminescence [EMCCD]) : To set sensitivity (EM gain) with 5 steps
- (6) **Expo. Time** : To display an exposure time which is currently set.
- (7) **Binning** : To display a binning which is currently set.

2) Camera Control box

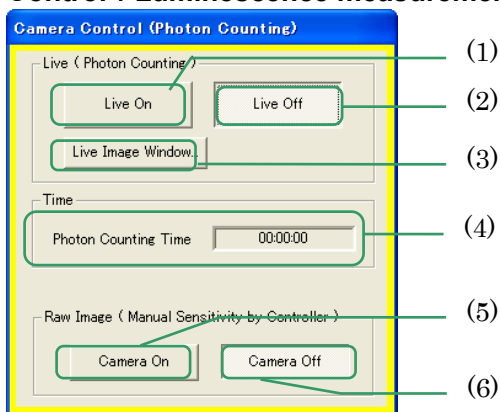
(at luminescence [Photon Counting] measurement)

The following Camera Control box appears just after FDSS software starts up with a luminescence [Photon counting] method. Clicking 'Live On' displays Image Window and Microplate Window, and then Photon counting images are observed.

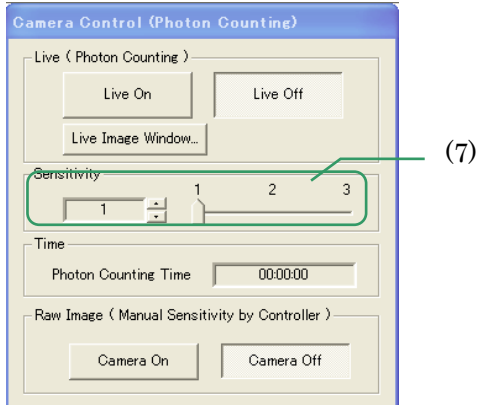
Note

Do not open the door of the dark box at Live ON.

<Camera Control : Luminescence measurement>



<Camera Control : Luminescence (with Gate function) measurement>

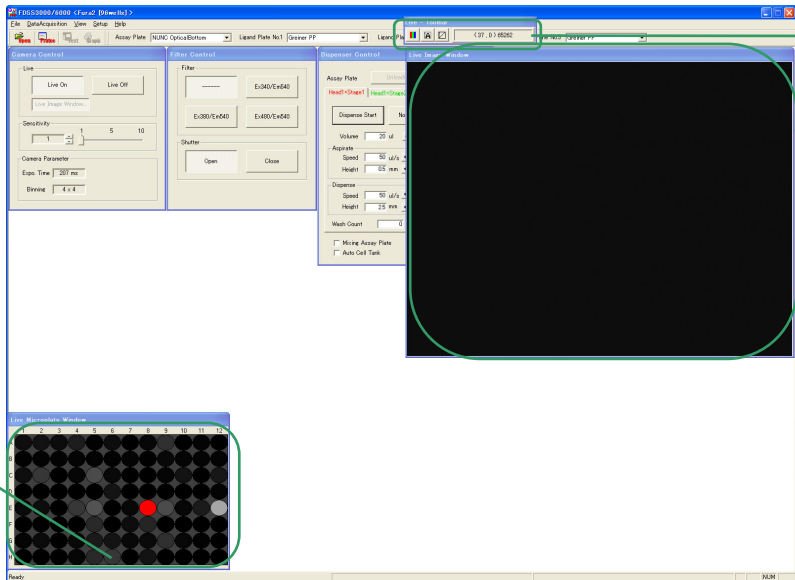


- (1) **Live On** : To start Photon Counting acquisition. It automatically turns 'Live Image Window' on.
- (2) **Live Off** : To stop Photon Counting acquisition.
- (3) **Live Image Window**
: To switch display ON/OFF for Image Window and Microplate Window.
- (4) **Photon Counting Time**
: To display the elapsed time of Photon Counting acquisition. The image is renewed each one second.
- (5) **Camera On** : To acquire and display live image for checking when sensitivity is controlled by an I.I. controller.
- (6) **Camera Off** : To finish acquiring and displaying live images.
- (7) **Sensitivity** : To set sensitivity (Gate Duty) with 3 steps.

3) Image Window , Microplate Window

Clicking 'Live On' or 'Live Image Window' in Camera Control box leads Image Window, Microplate Window and Tool Bar. Camera output images are displayed in Image Window and intensity data with a micro plate well in Microplate Window.

<At Live On>



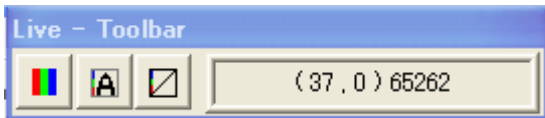
(1) **Image Window** : To display camera Live image / Photon Counting images.
And when mouse-arrow is located in a image window,
coordinate and intensity value are displayed.

(2) **Microplate Window**

(3) **Live Toolbar** : To display camera Live images / Photon Counting images.
: To set Color LUT and Enhance table.

4) Live Toolbar

Toolbar appears to set the pseudo color setting and enhance setting for Image Window and Microplate Window respectively.



(1) **Color LUT** : To set psuedo color LUT table. (refer to 5)

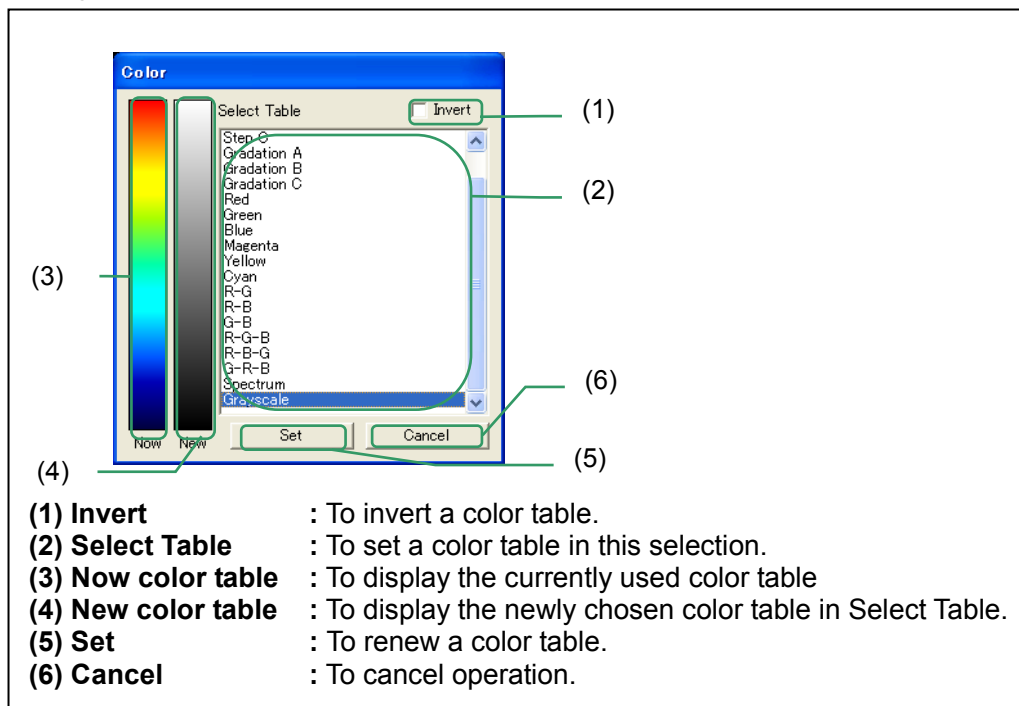
(2) **Auto enhance** : To display intensity with auto enhance.

(3) **Enhance table setup:** To set an enhance table for intensity.

(4) **Coordinate and intensity**
: To display coordinate and intensity value at the specified
point by a mouse-arrow in image Window.

5) Color setup box

Clicking 'Color LUT setup' in Toolbar leads Color setup box to set a psuedo color setting for Image Window and Microplate Window.



6) Enhance setup box

Clicking 'Enhance table setup' in Toolbar leads Enhance table setup box to set an enhance setting for Image Window and Microplate Window.

(1) Auto Enhance : To execute an auto enhance.

(2) Enhance lower limites : To set lower limite of an area for enhancement.
(Also available with a dragging graph.)

(3) Enhance higher limites :To set higher limite for an area for enhancement.
(Also available with a dragging graph.)

(4) Graph : To display graph with (X,Y)=(Intensity value, conveted value). It is changed by a dragging graph with a mouse.

(5) Histogram : To display a histogram of an image.
It refers to the set enhancement manually.

(6) Scale switching : To switch an intensity scale.

(7) Intensity scale : To display an intensity scale.