

# CellVoyager (CV7000) User Guide



*High-throughput Cytological Discovery System*

**Wako** Wako Automation



Cell  
Voyager

NIH NCI  
September 19–20, 2016

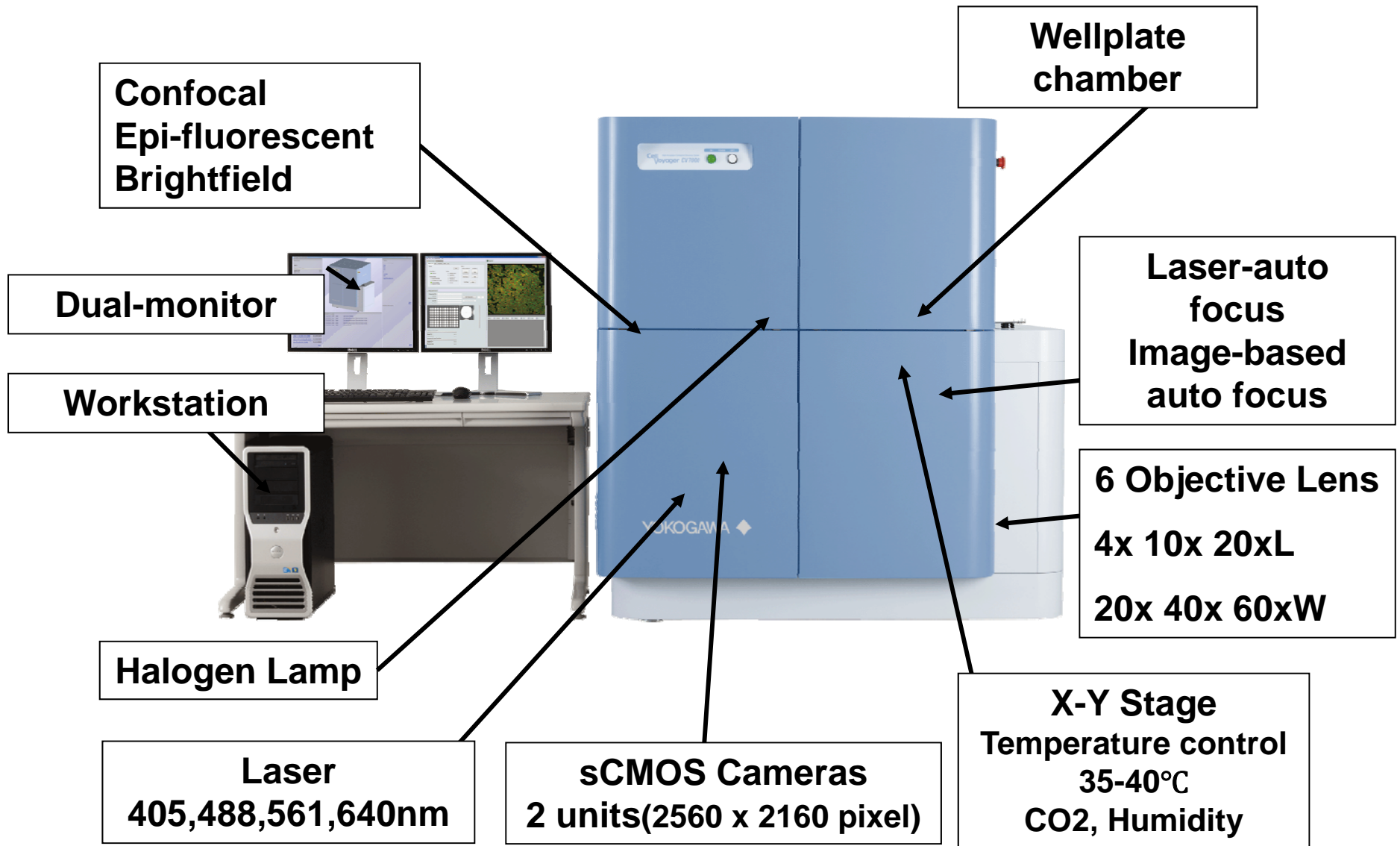
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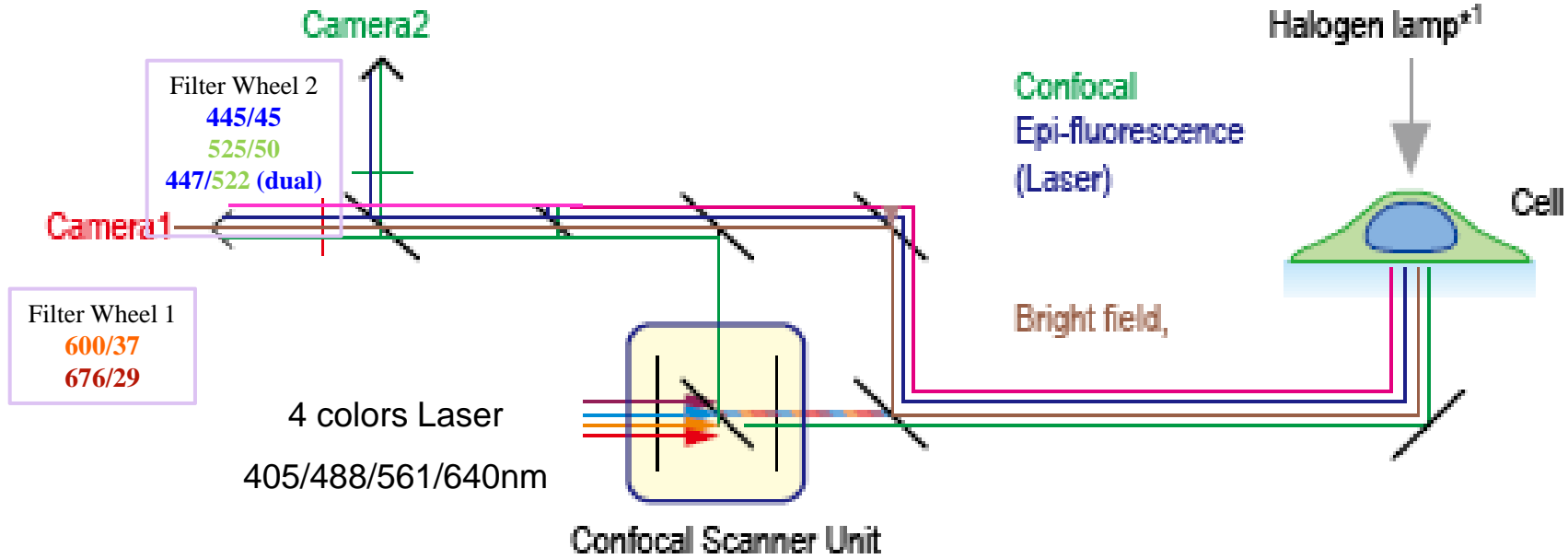
See Instruction Manual for more detailed information.

# 1. CellVoyager CV7000



# 1. CellVoyager CV7000

- Filter information



Confocal Fluorescence 405/488/561nm	Confocal image acquisition of 405/488/561nm
Confocal Fluorescence 405/488/640nm	Confocal image acquisition of 405/488/640nm
Epifluorescence 405/488/561/640 nm	Epifluorescence of 405/488/561/640 nm
Bright field	Bright field imaging

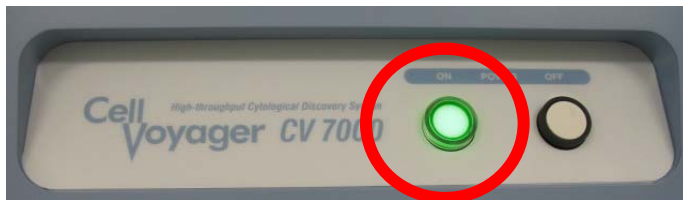
## 2.1 Starting and Shutting down of the CV7000

### Starting the system

1. Turn on the MAIN POWER breaker on the rear side of the equipment.



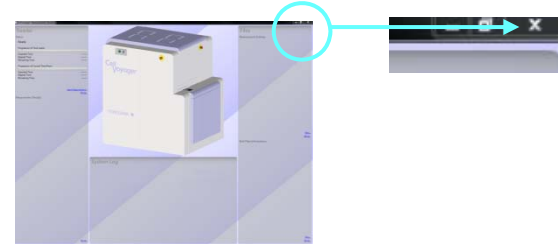
2. Press the POWER ON button on the front side of the equipment.



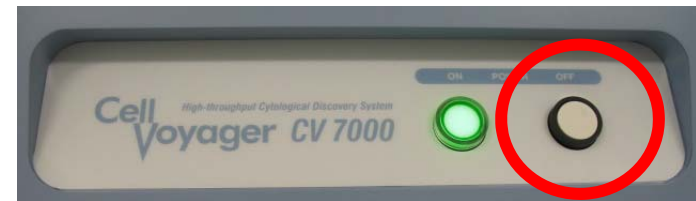
4. Start the Work Station.
5. Start the application software.

### Shutting the system

1. Close the screen of the application software.



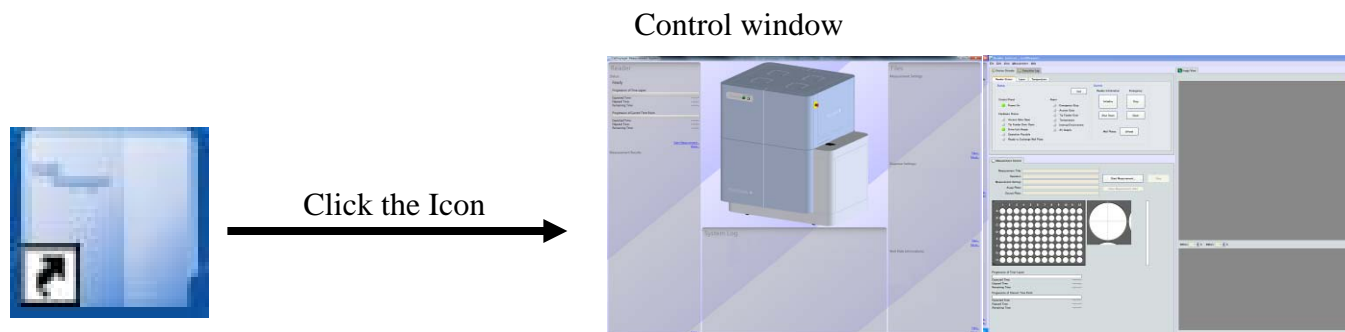
2. Turn off the power of the measurement PC.
3. Press the POWER OFF button.



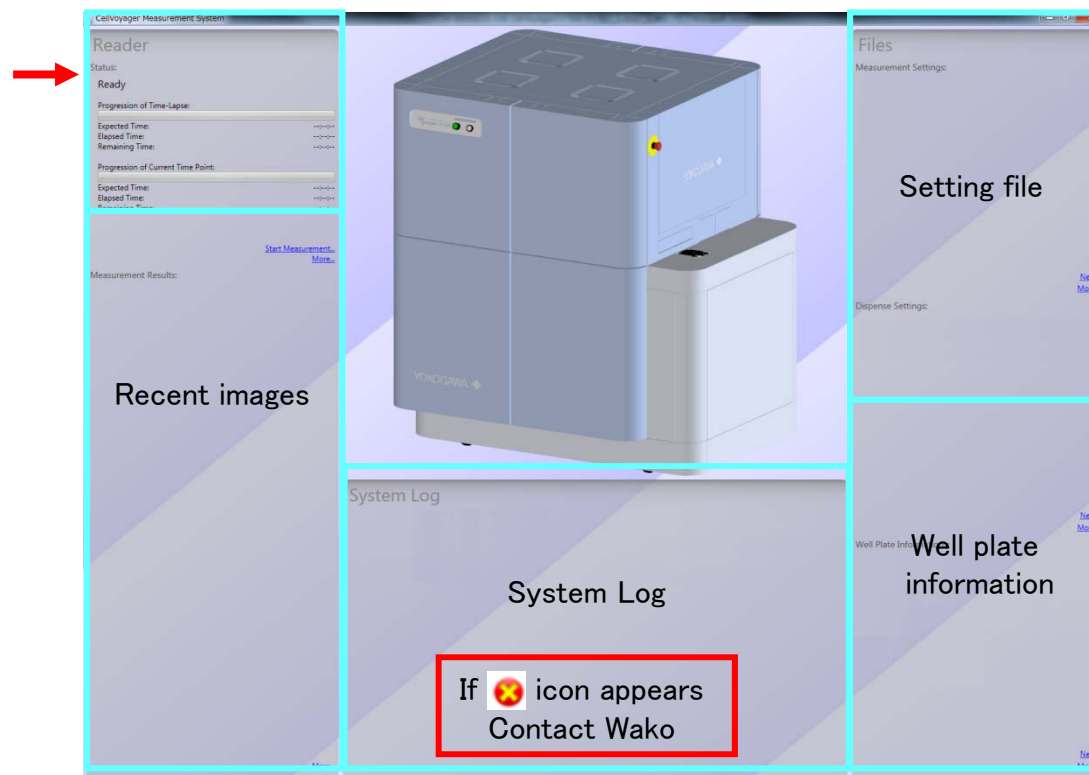
4. Turn off the MAIN POWER breaker on the rear side of the equipment.



## 2.1 Main window of the application software

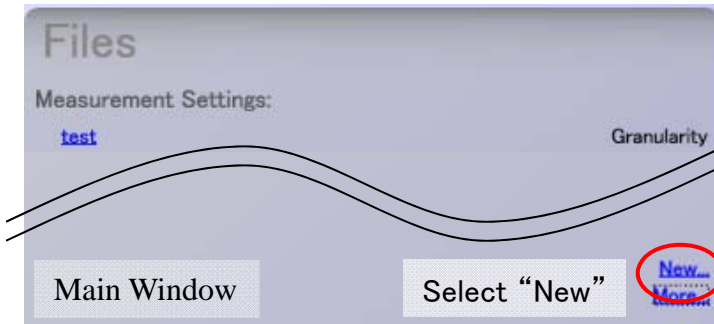


Wait a while till  
"Ready" status appears



## 2.2 Select the Plate and Acquisition setting

### Select Plate



Well Plate Type List - CellVoyager

Select a well plate type :

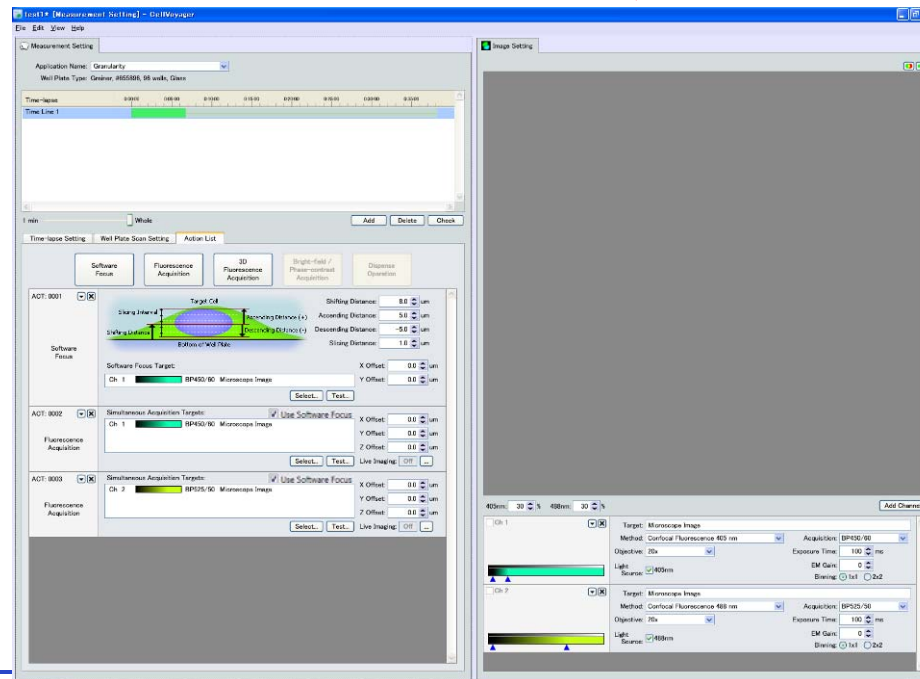
Vendor	Name	Well Number	Shape	Bottom Material	Bottom Thickness
NUNC	#164588	96 wells	Round	Glass	190 um
NUNC	#165305	96 wells	Round	Plastic	250 um
BD	#353948	96 wells	Round	Plastic	880 um
Greiner	#655090	96 wells	Round	Plastic	190 um
Greiner	#655896	96 wells	Round	Glass	175 um
Greiner	#781896	384 wells	Rectangle	Glass	175 um
IWAKI	MT4940-010	96 wells	Round	Plastic	1000 um

OK Cancel



Select plate and Click "OK"

### Setting window of the Acquisition



Time-lapse Setting tab

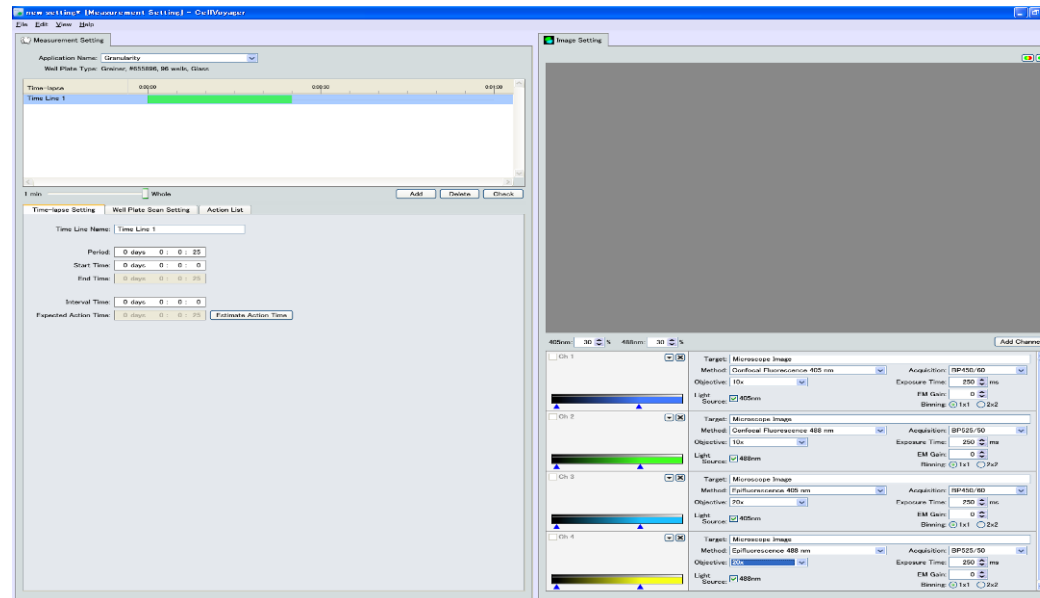
Well Plate Scan Setting tab

Action List tab

Channel Setting

## 2.3 Setting for Time-lapse Acquisition

- Setting for Time-lapse Acquisition.



- No need to set for the single acquisition.



## 2.4 Set the Acquisition point

Time-lapse Setting | **Well Plate Scan Setting** | Action List

Target Wells:  
 Scan All Wells  
 Scan Selected Wells: ← Arbitrarily-Selectable the Well

Select Well

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

Select All  
Deselect All

- Press LEFT mouse button to select wells  
- Press RIGHT mouse button to deselect wells

Acquisition Points:  
 Cell Count  
 Cell Search  
Target Cell Number: 0 Search Maximum  
Repeat: 0 Saving Images: 0  
Set Cell Recognition Parameters...

Tile  
Overlapping Pixel: 0 Acquire Whole Well

Fixed Position Add Points... Clear Points  
- Press LEFT mouse button to add point  
- Press RIGHT mouse button to remove point

FOV Preview: 10x Apply to Channel Settings

Notice: Acquisition point setting is IGNORED when action list of time line contains DISPENSE OPERATION.

Tile acquisition of entire well  
(50 is recommended for Overlapping Pixels)

Use this to arbitrarily select the FOV.

Add Acquisition Points

Rectangular 4 Points  
 Rectangular 9 Points  
 Rectangular 16 Points  
 Round 6 Points  
 Round 8 Points  
 Round 12 Points  
 Tiled Points

Pitch: 2000 um Clearance  
Rotation: 0 Degree of the angle

FOV Preview: 10x

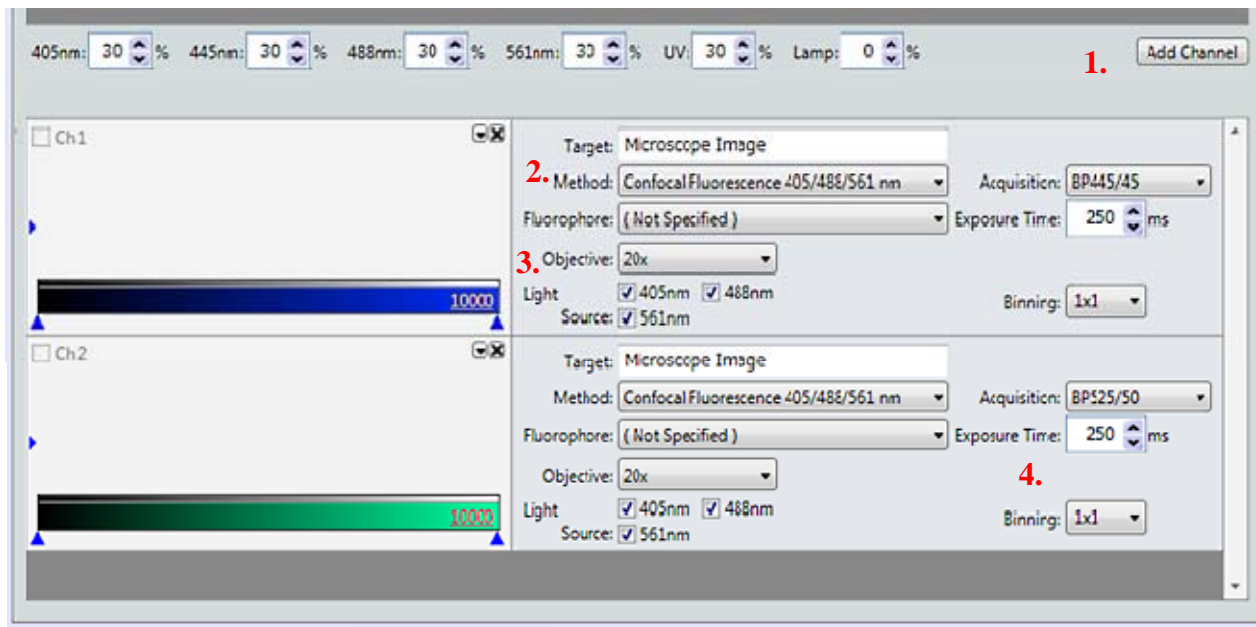
Pattern and the Number

Add Points Cancel

## 2.5 Select the Wave Length and Magnification

1. Create new channel by the "Add Channel" button
2. Select Optical path and wave length by "Method"
3. Select Magnification by "Objective" pull down menu
4. Set "Exposure Time" and "Binning"

Create new Channel



•Method –Combination of the optics–

Confocal Fluorescence 405/488/561nm	Confocal image acquisition of 405/488/561nm
Confocal Fluorescence 405/488/640nm	Confocal image acquisition of 405/488/640nm
Epifluorescence 405/488/561nm	Epifluorescence of 405/488/561nm
Epifluorescence 405/488/640nm	Epifluorescence of 405/488/640nm
Epifluorescence UV Lamp	UV light source
Bright field	Bright field imaging
Digital Phase Contrast	Digital Phase Contrast imaging

•Lens –Objective lens–

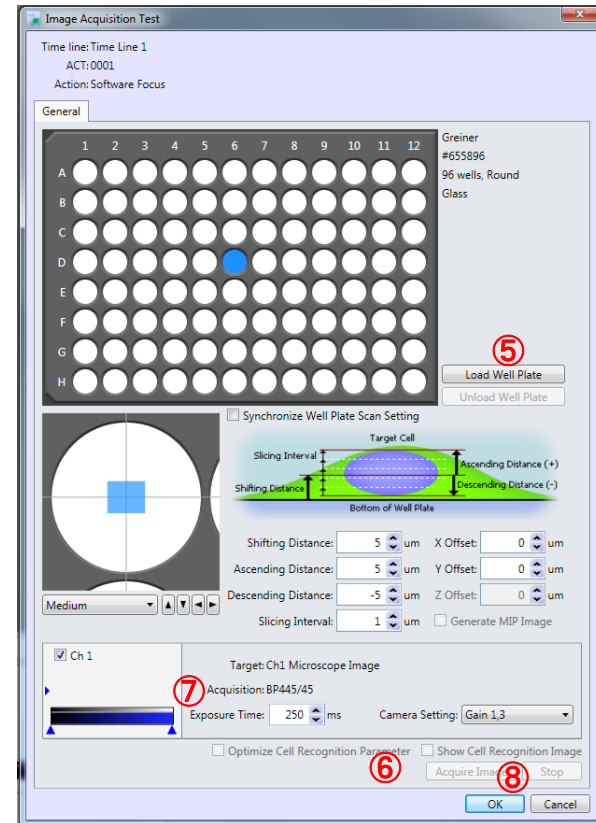
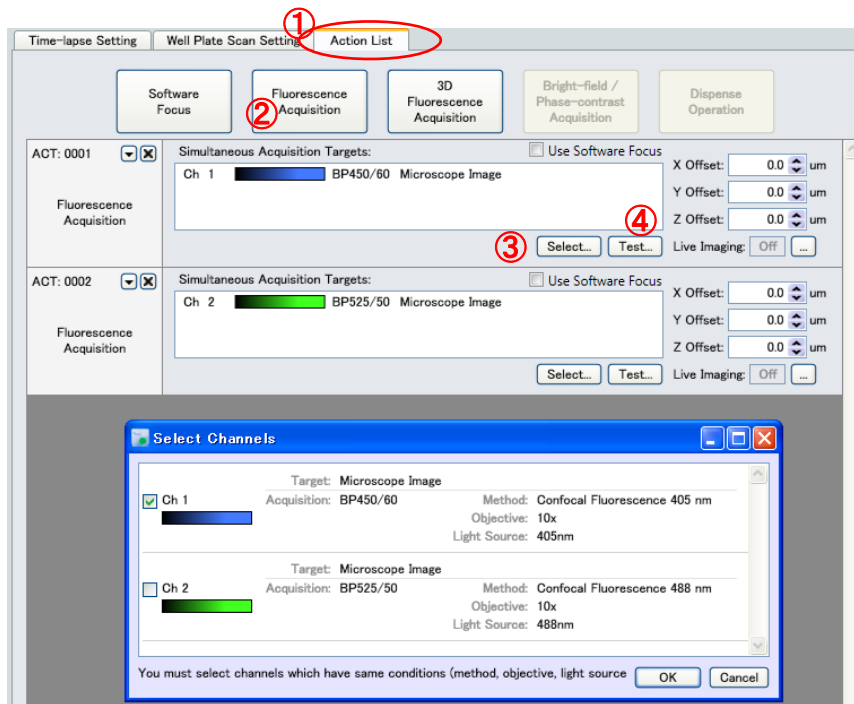
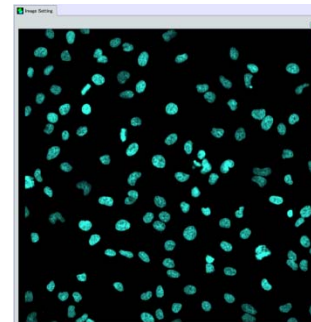
4X	N.A.=0.16
10x	N.A.=0.4
20x	N.A.=0.75
20X LWD	N.A.=0.45
40x	N.A.=0.95
60xW	N.A.=1.2

•Exposure Time

•Binning

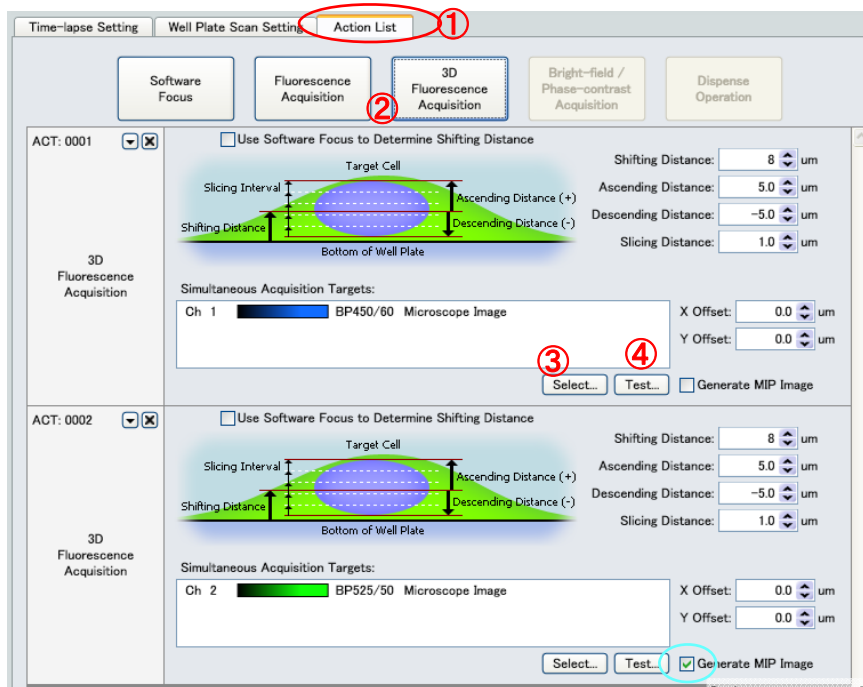
## 2.6 Setting of Acquisition –Single Z Slice Acquisition–

1. Select “Action List” tab
2. Click ”Fluorescence Acquisition” to show setting window
3. Click ”Select” button and select the “Channel”
4. Click ”Test” and show up the “Image Acquisition Test” window
5. Click ”Load Well Plate” and set the Well Plate
6. Click ”Acquire Image” and arrange the Z Offset
7. Arrange the Exposure Time
8. Stop Preview by the ”Stop” button and click ”OK” to close the window.

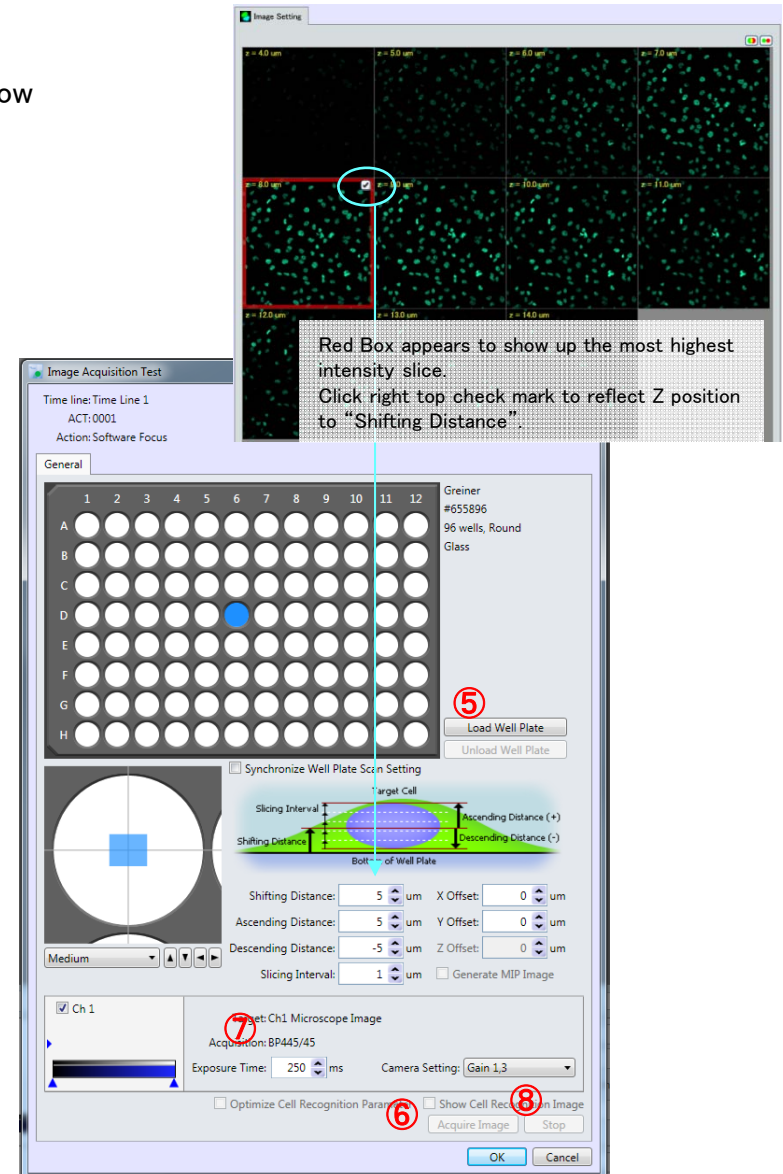


## 2.7 Setting of Acquisition-3D Acquisition-

1. Select "Action List" tab
2. Click "3D Fluorescence Acquisition" button to show up setting window
3. Click "Select" button and select Channel
4. Click "Test" and show up Image Acquisition Test window
5. Click "Load Well Plate" and set the plate
6. Click "Acquire Image" and find the focusing position
7. Adjust exposure time and Binning.
8. Click "Stop" and click "OK" to finish the test window.



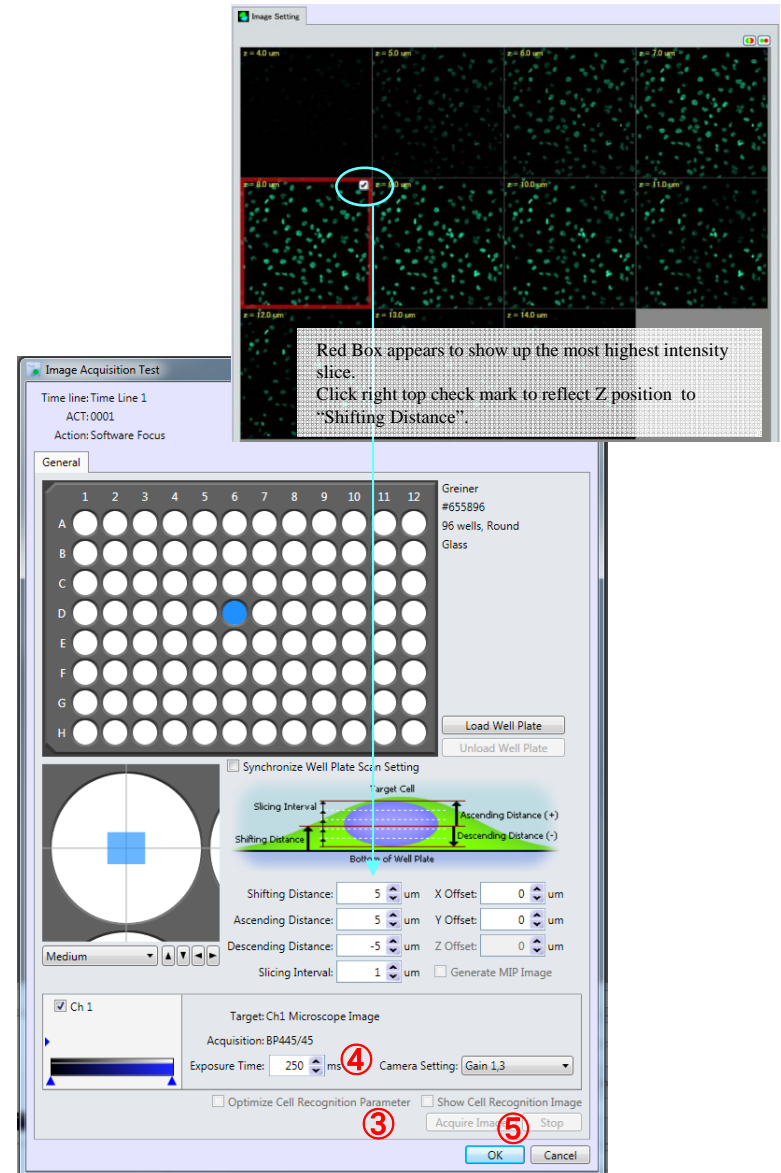
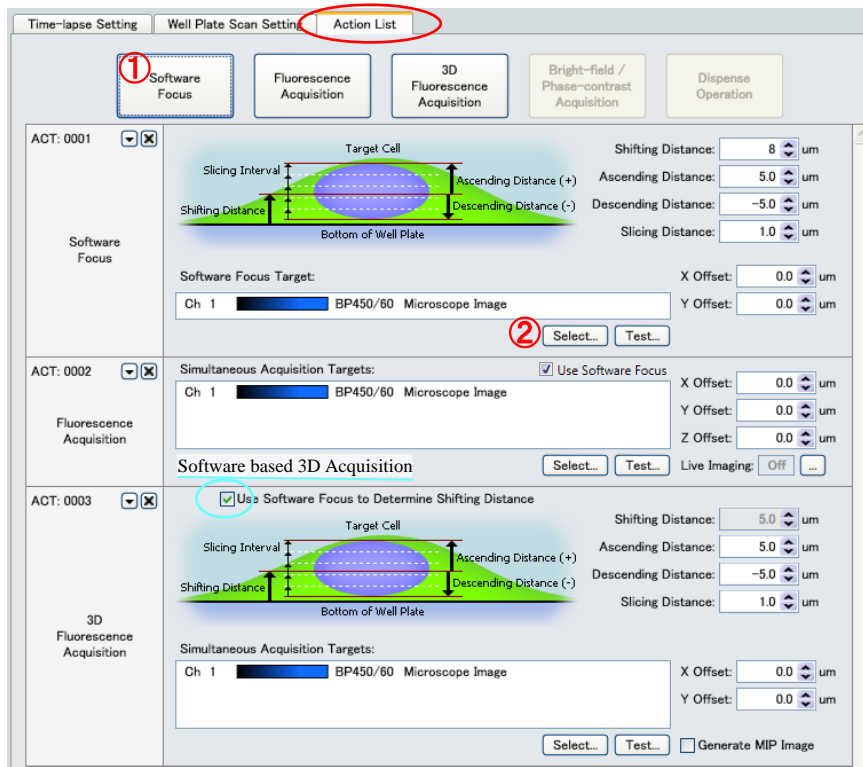
Select to save MIP image.



## 2.8 Setting of Acquisition–Use Software Focus–

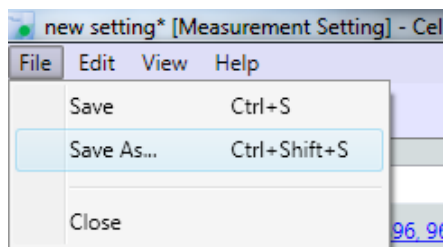
Make most brightest Z position to the Standard position.

1. Click “Software Focus” and Select the Wavelength from ”Select”.
2. Click ”Test” and Image Acquisition Test window is appears.
3. Set the plate and click ”Acquire Image” to select the Z position.
4. Select Binning and exposure time.
5. Click ”Stop” and click ”OK” to finish the test window.

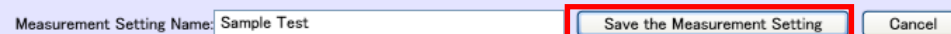
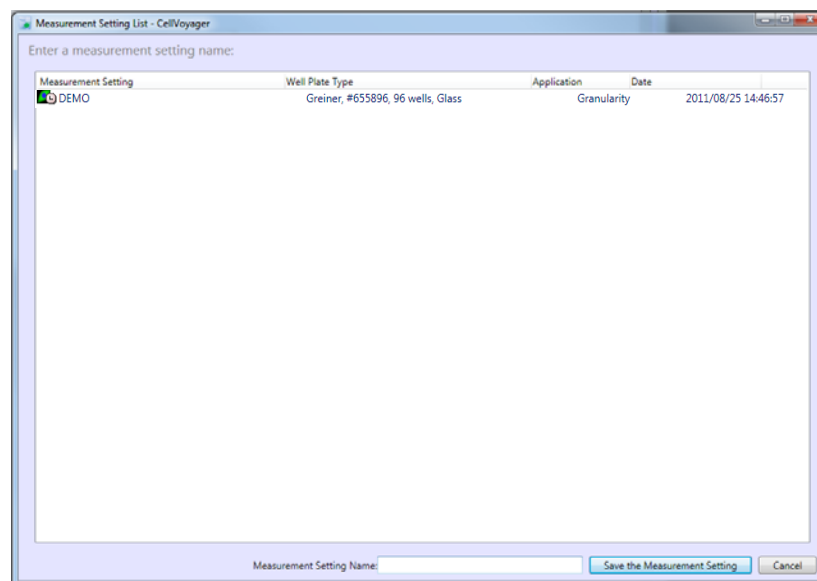


## 2.9 Save the Acquisition Setting


1. Select "Save" or "Save As" from "File".



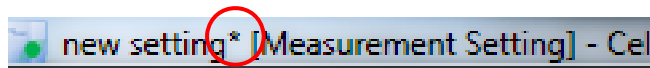
2. Enter the "Measurement Setting Name" then click "Save the Measurement Setting"



## 2.10 Start Acquisition

1. Click  at the right window.

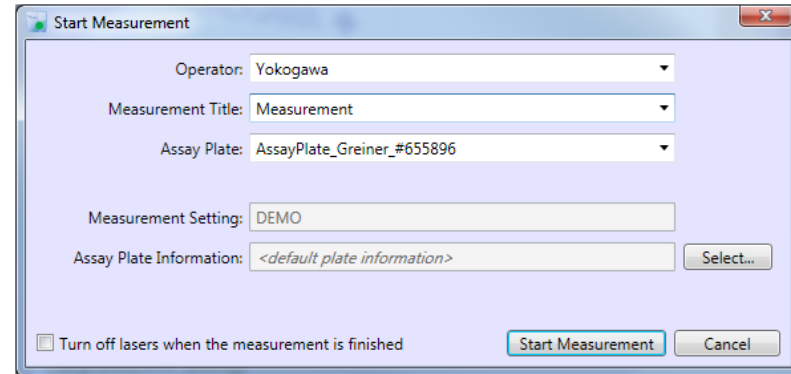
(!) \* mark appears when the measurement setting is un-saved.



2. Select Measurement Setting and click "OK"



3. Enter "Measurement Title", "Operator" and click "Start Measurement" to start acquisition.



4. Click "NO" when the plate already loaded at CV7000.



Start Acquisition.

## 2.11 After Acquisition –View Images–

Scroll the mouse wheel to zoom into/out of the image

Select the display format for channel images. Show overlaid images of multiple channels or select display for channel images.

Change the number of all images displayed on the display area. (Image number of the image being displayed/total number of images)

Time Point: 0001/0001  
Z Position: 01/01  
Acquisition Point: 01/04

Click the Arrow mark to show the next Time series, Z position or View field.

Row and column Images: Show images in the well plate view.  
Time Point Images: Show images along the time axis.  
Z Images: Show images for each Z slice.  
Acquisition Point Images: Show images for each acquisition point.



## 2.12 After Acquisition –Movie output–

① Click an image to display the sub screen.

Acquisition Point: 2 / 3

② Select the field to create movie files.

③ Click "Generate movie" from the "File".

④ Specify the time point range to create movie files.

Time Point: from 1 to 51

Targets:

- Ch 1 BP450/60 Microscope Image
- Ch 2 BP525/50 Microscope Image
- Ch 3 BP675/30 Microscope Image

⑤ Select...

Multiple Z Image Processing: Maximum Intensity Projection

Movie Setting: Video Compressor: Uncompressed Frame Rate: 30 fps

Output Folder: C:\YBTSData\MeasurementData\Yokogawa\test\_2010090

⑥ To create movie files is started by clicking "Generate Movie."

Generate Movie Close

⑤ Select the channels to create the movie file.

Select Channels

Channel	Target	Acquisition	Method	Objective	Light Source
1	Microscope Image	BP450/60	Confocal Fluorescence 405/488/635 nm	40x	405nm
2	Microscope Image	BP525/50	Confocal Fluorescence 405/488/635 nm	40x	488nm
3	Microscope Image	BP675/30	Confocal Fluorescence 405/488/635 nm	40x	635nm

Output Folder: D:\YBTSData\MeasurementData

Progression of All Movie Files: AssayPlate\_Greiner\_#655896\_E4\_F001\_T0001\_T0003.avi 1 of 1 File

Progression of Current Movie: Time Point 2

## 2.13 After Acquisition –Shading and Registration Correction–

1. Click the icon  on the desktop of the measurement PC.

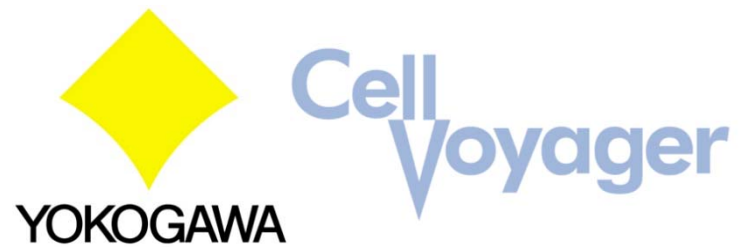
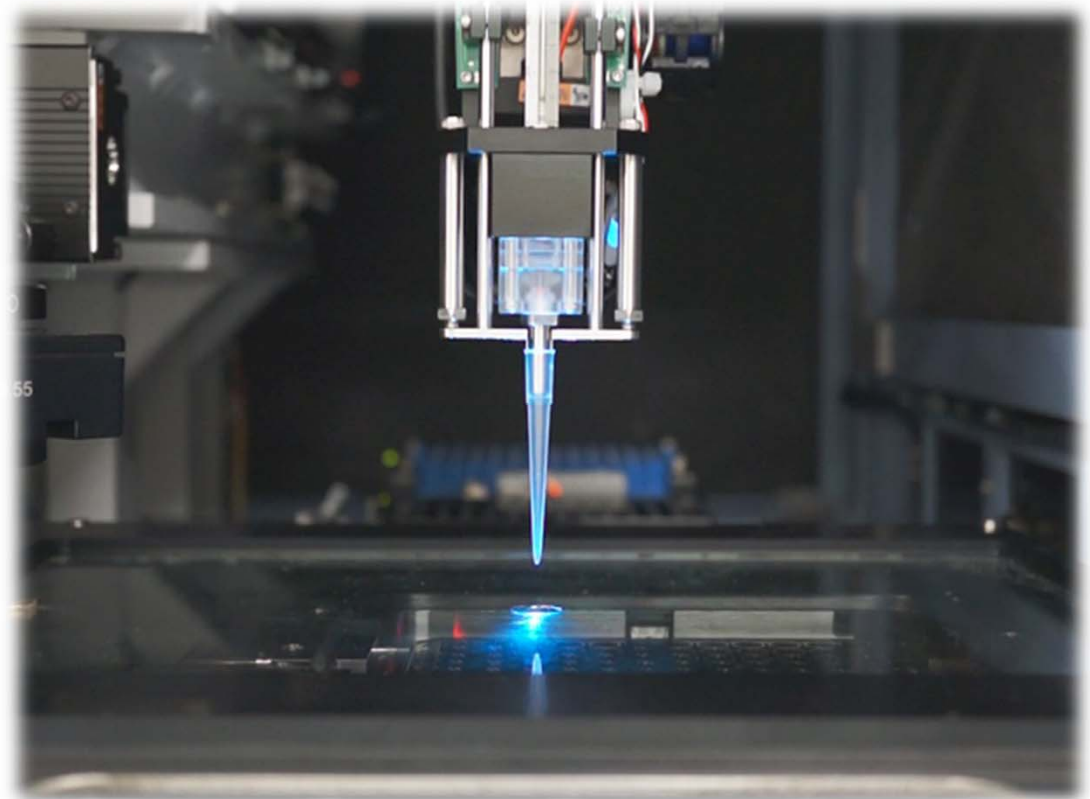
2. Select “MeasurementDetail.mrf” file and then click “Open”.

3. Select Output folder. \*Default setting of output folder is “BTSDData¥CorrecterMeasurementData”.

4. Select “Dark and shading” / ”Image registration” from Parameter and click “Start”.

The image shows a sequence of steps for using the CV Image Correction Tool. On the left, the tool's interface is shown with red boxes and circled numbers 1 through 4. Step 1 highlights the 'Select' button for the input folder. Step 2 highlights the 'Select' button for the output folder. Step 3 highlights the 'Image correction' section, specifically the 'Dark and shading' radio button and the 'Image registration' section with the 'Channel' checkbox. Step 4 highlights the 'Start' button in the 'Process' section. On the right, a file tree for 'D:\BTSDData\MeasurementData' shows a folder structure with 'MeasurementDetail.mrf' highlighted by a red box. Below this, a 'Select Measurement Result File' dialog box shows 'MeasurementDetail.mrf' selected in the file list, with the 'Open' button highlighted by a red box and circled number 2.

CellVoyager (CV7000)  
User Guide  
Dispenser



## Table of Content

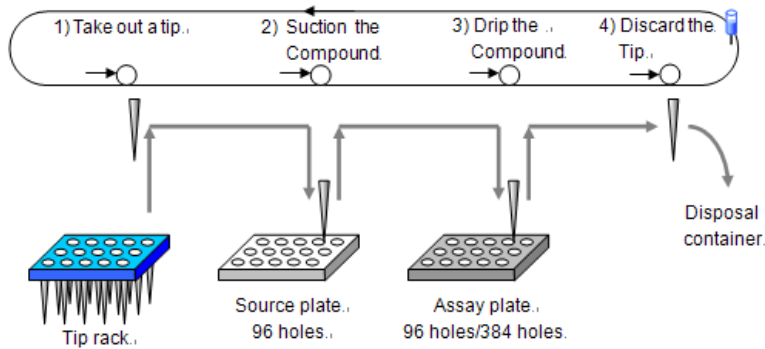
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1. Dispensing Function
2. Control Software
  - 2.1 Temperature control
  - 2.2 Well Plate Information
  - 2.3 Dispense Settings
  - 2.4 Setting of the Plate and Tip Rack
  - 2.5 High Speed Time Lapse

See Instruction Manual for more detailed information.

# 1. Dispensing Function

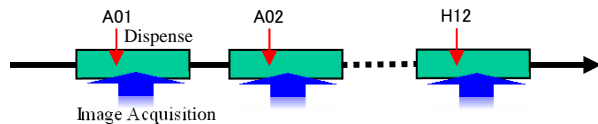
## Over View of Dispensing Mechanism.



## Types of the available Dispensing at CV7000

High speed time lapse (Acquire image after the dispensing.)

A01 (Image Acquisition → Dispensing → Image Acquisition) → A02 (Image Acquisition → Dispensing → Image Acquisition) → H12 (Image Acquisition → Dispensing → Image Acquisition)



Time Lapse (Dispense all the well then acquire image)

Image Acquisition (A01 → H12) → Dispensing (A01 → H12) → Image Acquisition (A01 → H12)

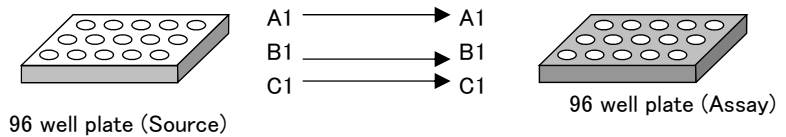


## Dispensing Pattern

The following settings are available.

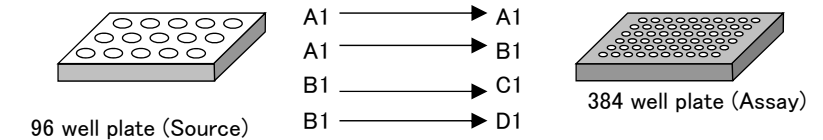
### 1) 96 well plate (Source) → 96 well plate (Assay)

the simplest pattern where the wells in one plate have a one-to-one association with the wells in the other plate.

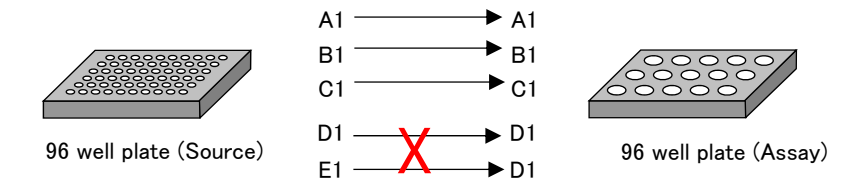


### 2) 96 well plate (Source) → 384 well plate (Assay)

One well in the source plate is associated with multiple wells in the assay plate.



### 3) 384 well plate (Source) → 96 well plate (Assay)



Caution) Dispensing from the multiple wells in the source plate to one well in the assay plate cannot be performed.

## 1. Dispensing Function

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- Before using the dispenser...

- Please make sure the following points.

- Turn on the Environmental control

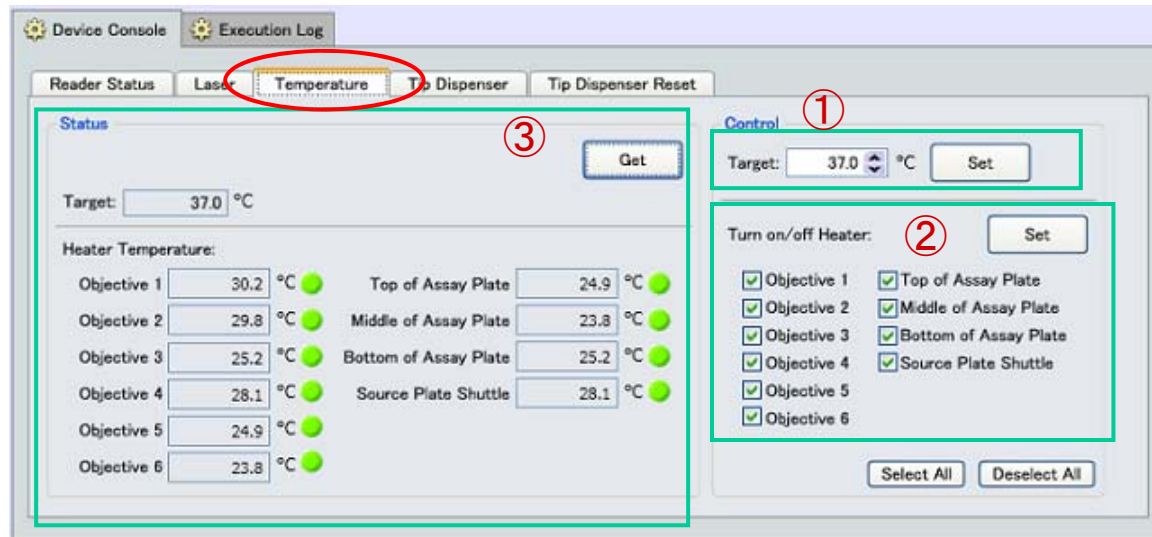
Temperature, CO2, Water

- Uncover the lid of both plates.

It may damage the system

## 2.1 Temperature control

- Click the “Temperature” tab of Device Console at Recorder Control window. (Right monitor)



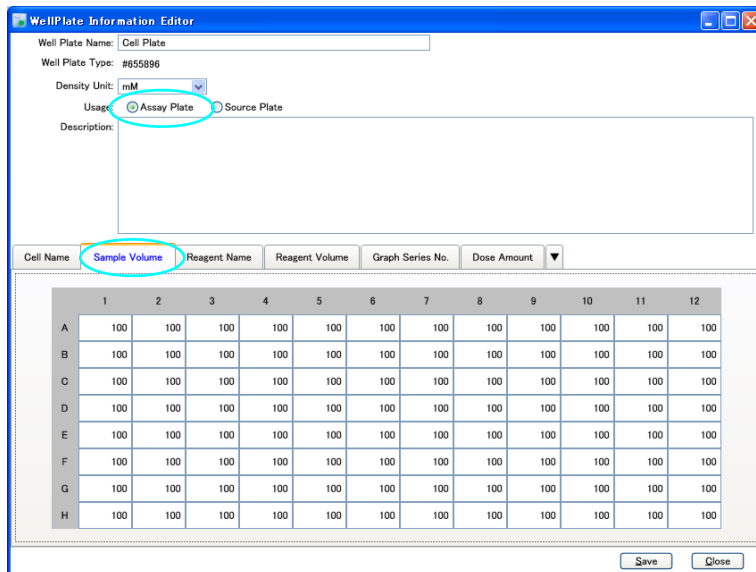
- Enter the temperature to be set.
- Select all of the check, and then click "Set".
- Click "Get" and check the current status information.

## 2.2 Well Plate Information

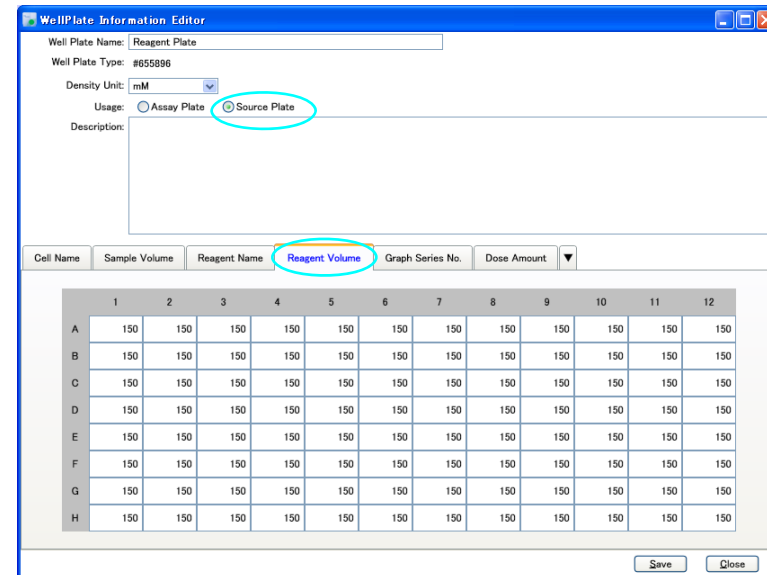
- Click “New” at the bottom of the Well Plate Information area



- Enter the Sample Volume tab at Assay Plate(Cell plate)
  - Enter the volume of the assay plate (e.g.100ul)



- Enter the Reagent Volume tab at Source Plate(Reagent plate)
  - Enter the volume of the reagent at each well (e.g.200ul)



**ATTENTION)** Please add 100  $\mu$ l for each reagent volume since dispenser needs 100ul extra volume for safety. Don't use much more than the working volume of each well plate.



## 2.3 Dispense Settings

- Select “New” from the Dispense Settings at right side of the Main window.



分注設定 [ Dispense Setting ] - CellVoyager

Dispense Mapping Dispense Simulation

Source Plate 8 Wells 12 Wells 24 Wells 48 Wells 96 Wells 384 Wells

Assay Plate 8 Wells 12 Wells 24 Wells 48 Wells 96 Wells 384 Wells

① Select the types of Source Plate and Assay Plate.

② Select the wells from Source plate, and associate with each well Assay plate.

Basic Dispense Setting

- 1  Liquid Surface
- 2  Prewet
- 3  AspirateStir
- 4  Airgap
- 5  Aspirate
- 6  AspirateTiptouch
- 7  Dispense
- 8  DispenseStir
- 9  DispenseTiptouch

## 2.3 Dispense Settings

**Dispense Setting**

SourceWell	AssayWell	Se
Plate1 / A11	Plate1 / A11	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / A12	Plate1 / A12	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B01	Plate1 / B01	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B02	Plate1 / B02	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B03	Plate1 / B03	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B04	Plate1 / B04	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B05	Plate1 / B05	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B06	Plate1 / B06	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B07	Plate1 / B07	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B08	Plate1 / B08	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B09	Plate1 / B09	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B10	Plate1 / B10	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um
Plate1 / B11	Plate1 / B11	50ul,33ul/sec,LiquidLevel[ 1000um ],LiquidTracking,0um

**Basic Dispense Setting**

- Liquid Surface
- Prewet
- AspirateStir
- Airgap
- Aspirate
  - Volume: 50 ul
  - Speed: 33 ul/sec
  - PostDelay: 0 ms
- AspirateTiptouch
- Dispense
  - Volume: 50 ul
  - Speed: 33 ul/sec
  - Z Position:
    - Liquid Level: 1000 um
    - Liquid Level Tracking
    - End Position
    - Start Position
    - Plate Top: 1000 um
  - PostDelay: 0 ms
- DispenseStir
- DispenseTiptouch

**Dispense Mapping**

Source Plate: WellPlateInformation File: Reagent.Plate

Assay Plate: WellPlateInformation File: Cell.Plate

Left tab shows the dispense setting of the selected well.

**Encouraged setting. 50–100  $\mu$ l would be minimize the variation of the aspirate volume.**

**Detailed information of each reaction**

- ① Liquid Surface: Detect the liquid surface of the Source plate
- ② Prewet: Get inside of the tip wet by the liquid of Source plate.
- ③ AspirateStir: Aspirate/discharge the liquid of Source plate.
- ④ Airgap: To extrude the drip at the tip, aspirate air before the liquid.
- ⑤ Aspirate: Aspirate the liquid from Source plate.
- ⑥ AspirateTiptouch: Contact tip to wall of the well at Source plate to drip the drop.
- ⑦ Dispense: Dispense the solution.
- ⑧ DispenseStir: Aspirate/discharge the liquid of Assay plate.
- ⑨ DispenseChiptouch: Contact tip to wall of the well at Assay plate to drip the drop.

## 2.3 Dispense Settings

The screenshot displays the Cell Voyager software interface for setting up a dispense simulation. The main window is titled "new file\* [ Dispense Setting ] - CellVoyager".

**Dispense Setting Panel:** Shows a table of SourceWell and AssayWell configurations. The "Dispense" checkbox is checked. Under "Dispense", "Liquid Level" is selected with a volume of 50 ul and a speed of 33 ul/sec. "DispenseTiptouch" is also checked.

**Dispense Mapping Panel:** Contains two tabs: "Dispense Mapping" and "Dispense Simulation". The "Dispense Simulation" tab is selected. It shows a grid for the "Source Plate" and "Assay Plate".

**Well Plate Information Dialogs:** Two dialog boxes are open, one for the Source Plate (Well Plate Type: Greiner, #855896, 96 wells, Glass) and one for the Assay Plate (Well Plate Type: Greiner, #855896, 96 wells, Plastic). Both have "Start Simulation" buttons circled in red.

**Simulation Log:** A log window shows the simulation results. It starts with "Simulation Finish..." and "\*\*\*\* Dispense Simulation Completed !!! \*\*\*\*". It lists error counts for various wells, all showing 0 errors. The log ends with "Simulation Start..." and a circled "Start Simulation" button.

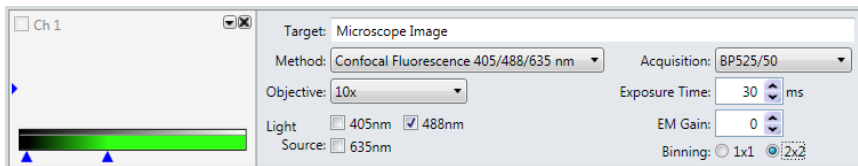
- ① Select the “Dispense simulation” tab
  - ② Select the Well Plate Information from “...” botten of the Source plate, Assay plate
  - ③ Select the well which to simulate the ability.
  - ④ Click “Start Simulation” and show up the result.
- ※ Confirm No error has happened at this step.

## 2.4 Setting of the Plate and Tip Rack

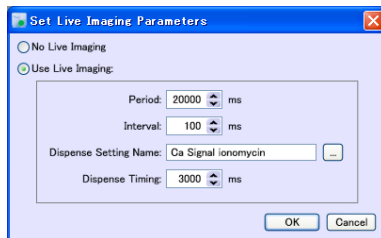
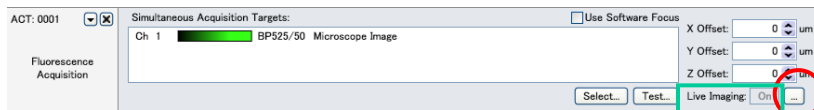
- Click “New ” Button of the Measurement setting area.

Select the products name of the Assay plate. Click ”New” and select the product name of the Assay plate. Display the measurement setting file .

- Setting of the acquisition channel.



- Select Action List and display the high speed timelapse setting window from clicking ”...”.



Live Imaging  
ON: Use high speed time lapse  
OFF: Only the usual time lapse

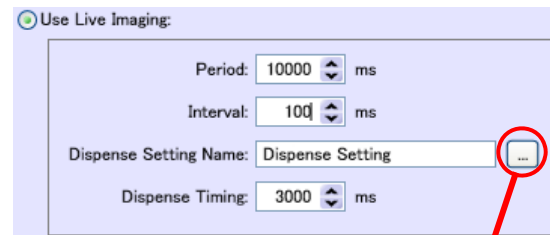
- Select the “Use Live Imaging” and enter the condition.

Period: Period of the fast time lapse.

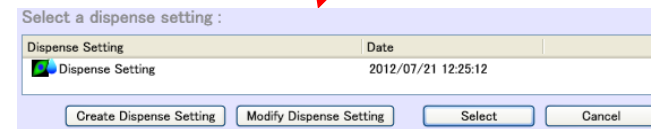
Interval: Interval of the high speed time lapse.

Dispense Setting Name: Dispenser setting file

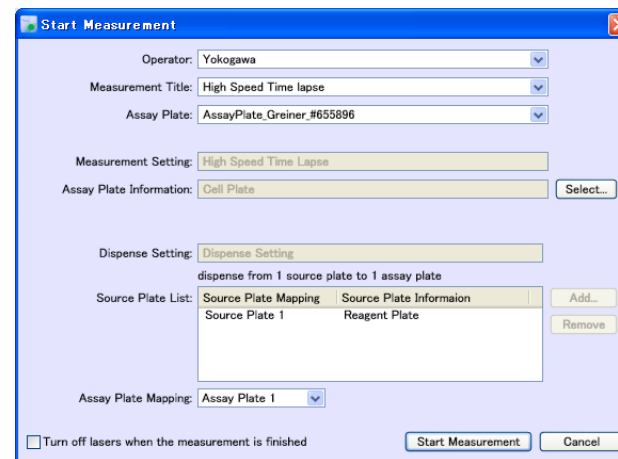
Dispense Timing: Time point which start dispensing.



This setting acquire every 50ms for 10 sec. The dispense timing is 3 sec and move to the next well after the 10 sec of image acquisition.

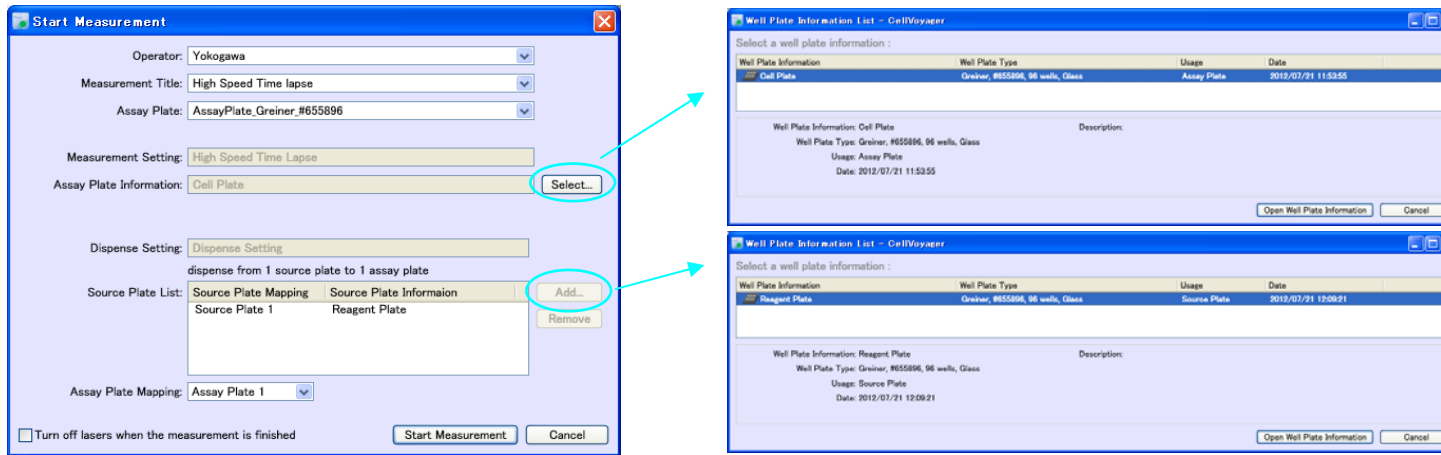


- Sever the setting file and start image acquisition.



## 2.5 High Speed Time Lapse

- Click "Start Measurement" of the Start Measurement and select the Assay plate and the Source plate.



**CAUTION** Please make sure the lid of the Assay / Source plate were certainly removed. It may break the dispenser if the image acquisition were preformed by using the lid.

- Set the tip rack. Click "Load" and the rack move to the position. The click "Get Status" to confirm the condition.

