

# LSM780 Simple manual [for 2D imaging]

**Correct me if i'm wrong**

## 【1】 How to start

Switch on the main switch. ①~④

(If the Ar laser is required)

Switch on the power supply and turn the key. ⑤ ⑥

Wait about 5 minutes.

Set the switch to "run". Wait about 30 seconds. ⑦

(after the green LED lights) Adjust the power control knob. ⑧

(Position to 9 o'clock) That the red LED is not lit.

Turn on the reflected light power supply. ⑨

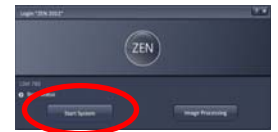
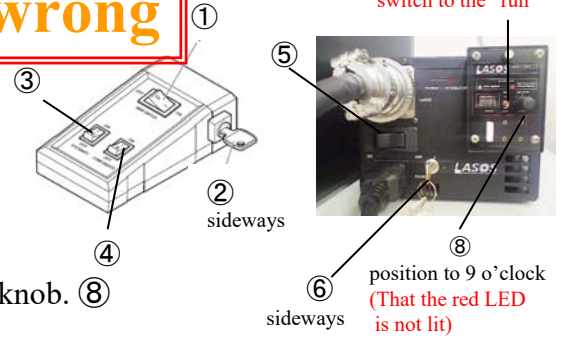
(When the display is flashing, the light does not go out.)

Start the PC. ⑩ (Wait about 3 minutes.)

log in the "LSM User".

Double-click the "ZEN" icon.

Click the "Start System" button.



for imaging for analysis

### Control area of the machine

When you click on the bar, items are displayed.  
Can be separated by dragging the bar.

This area has many operations manual.

The microscope operation in Locate button.

The LSM operation in Acquisition button.

Button for scanning is arranged below the main tab.

Two columns in the Acquisition tool group.

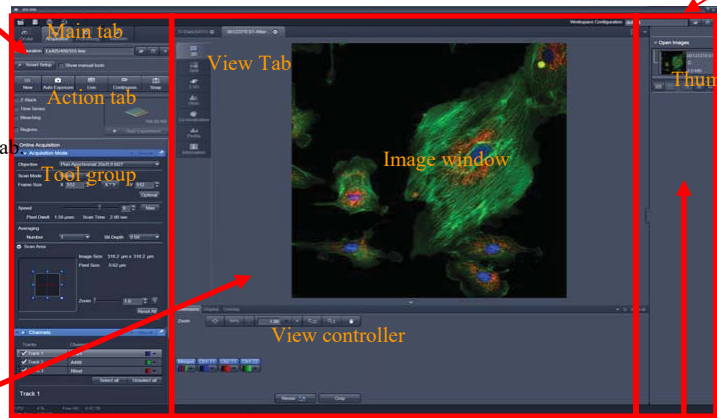
Left column optical path setting.

Right column scan settings.

Additional configuration(experiment setting) is added to the lower side of the right column.

### Main window

Window size changes (Workspace zoom)



### Image window and view control area

Display switching button is disposed on the left side. View controller are arranged on the lower side.

Tool will change the display state. Tool is divided by a tab.

### Image area

! Mark has not been saved yet.

View image by double-clicking.

Put a star on the good image.

## 【2】 How to turn on the laser

Click the "Acquisition" button on the main tab.

Click the "Laser" bar. Turn on the necessary laser.

(There is no control in the argon and diode.)

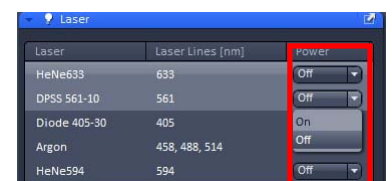
Diode 405...for example, DAPI, Hoechst

Ar 458, 488, 514...for example, FITC, Axela488, GFP, CFP, YFP

DPSS 561...for example, Rhodamine, Cy3, Alexa568

HeNe594...for example Alexa594

HeNe 633... for example, Cy5, Alexa633



【Confirmation】

Did you set the Ar power?

There are two ways Page 2. Please choose one or the other.

"Setting loading method"(Line Sequential) or "Smart setup method"(Frame Sequential)

< Setting loading method (Experiment Manager) >

**[3]** Optical path setting of laser microscope

Click the "Acquisition" button on the main tab.

Click on the OPEN icon that is located to the right of the Experiment Manager.

Select a setting from the list.

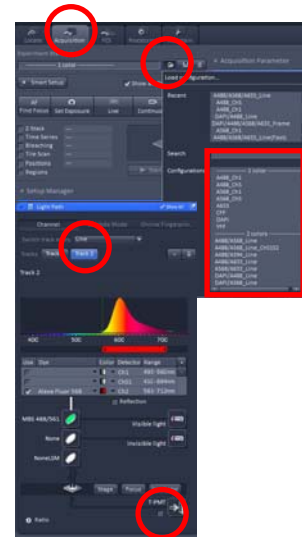
(When using differential interference image)

Click the "Light Path" bar. Click the Track button.

(Do not chosen DAPI, Hoechst track.)

Put a check in the T-PMT check box.

Correctly set the condenser of the microscope.



**[4]** Viewed under a microscope

Click the "Locate" button on the main tab.

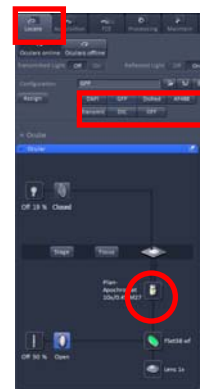
Click the Dye button.

- DAPI...for blue dye(band path)
- GFP...for green dye(band path)
- DsRed... for red dye(band path)
- AF488... for green dye(long path)
- Transmit...bright field
- DIC...differential interference

Choose a lens with an icon of the objective lens.

(When change to a different type of lens, microscope to pause.)

After put the oil, press the "Done" button on the touch panel.)



Differential interference setting  
x10~x20 ... II  
x40, x63w, x63oil ... III

**[5]** Take the pictures with LSM

Click the "Acquisition" button on the main tab.

Click the "Channels" bar.

Click the "1AU" button of long wavelength side.

Click the "Set Exposure" button on the action tab.

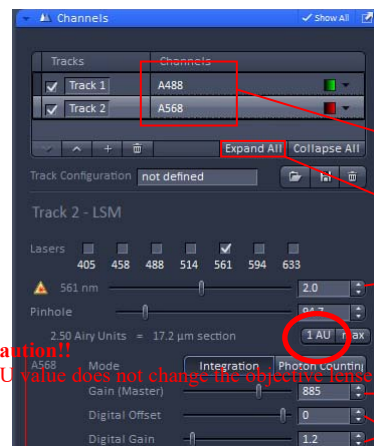
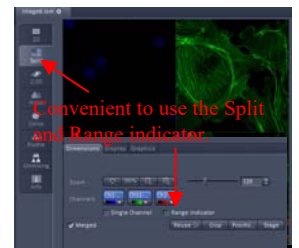
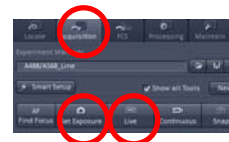
Click the "Live" button.

After adjusting the focus, adjust the contrast(Digital Offset) and brightness(Gain(Master)).

Adjust the other tracks by clicking on the name of Channels.

Click the Stop After adjusting.

When you press the center button of the joystick switch the fine / course of XY.



Display switching

Configuration listing

Laser power

Caution!!  
AU value does not change the objective lens after the change.  
brightness  
contrast

Lower the gain so as not to break the ChS.

## < Dye selection method (Smart setup) >

- [3]** Optical path setting of laser microscope  
Click the “Acquisition” button on the main tab.

Click the “Smart Setup” button below the Experiment Manager.  
Click the pull-down of Dye, choose a dye from Dyes list.

To register all the dyes being used.

Select the “Best Signal” and click the “Apply” button.

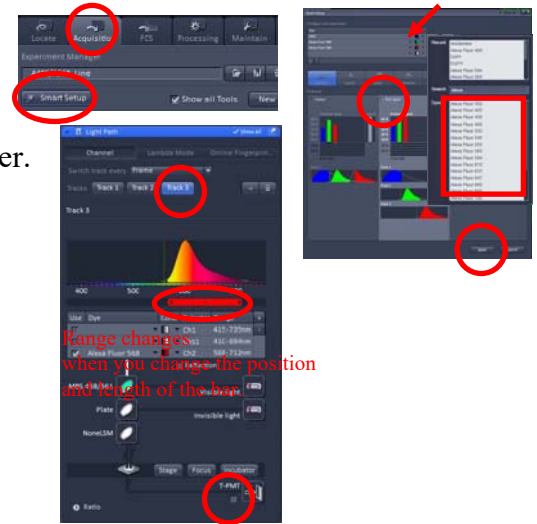
(When using differential interference image)

Click the “Light Path” bar. Click the Track button.

(Do not chosen DAPI, Hoechst track.)

Put a check in the T-PMT check box.

Correctly set the condenser of the microscope.



Range change is Set when you change the position and length of the bar.

- [4]** Viewed under a microscope

Click the “Locate” button on the main tab.

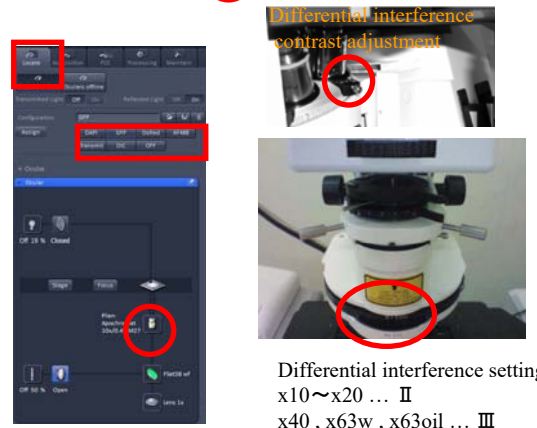
Click the Dye button.

- DAPI...for blue dye(band path)
- GFP...for green dye(band path)
- DsRed... for red dye(band path)
- AF488... for green dye(long path)
- Transmit...bright field
- DIC...differential interference

Choose a lens with an icon of the objective lens.

(When change to a different type of lens, microscope to pause.)

After put the oil, press the “Done” button on the touch panel.



Differential interference contrast adjustment

Differential interference setting  
x10~x20 ... II  
x40, x63w, x63oil ... III

- [5]** Take the pictures with LSM

Click the “Acquisition” button on the main tab.

Click the “Channels” bar.

Click the “1AU” button of long wavelength side.

(Other channels please match the optical slice.)

Check to any one the Tracks.

Click the “Set Exposure” button on the action tab.

Click the “Live” button.

After adjusting the focus, adjust the contrast(Digital Offset)

and brightness(Gain(Master)).

Click the Stop After adjusting.

Check to other any one the Tracks.

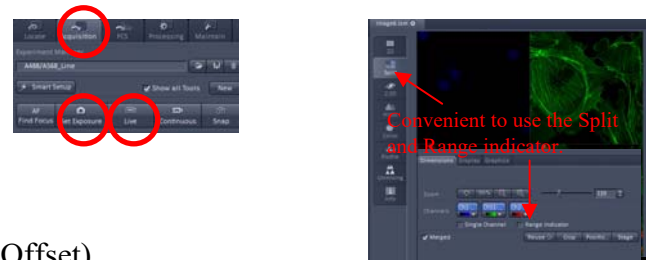
Click on the name of Channels.

Click the “Live” button. Adjust the contrast and brightness.

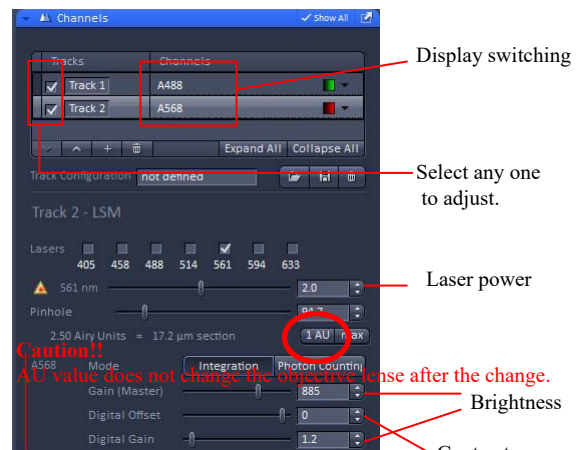
Click the Stop After adjusting.

When the adjustment of all tracks is finished,

check to all tracks.



Convenient to use the Split and Range indicator.



Display switching

Select any one to adjust.

Laser power

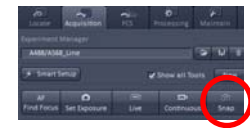
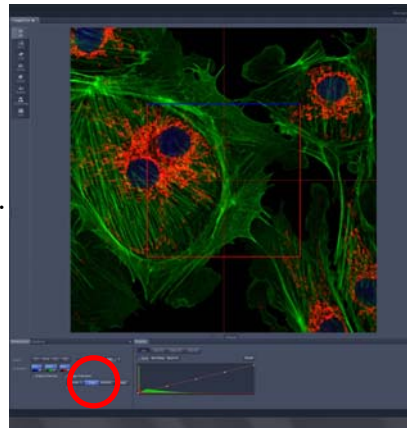
Brightness

Contrast

Caution!  
AU value does not change the objective lens after the change.

Lower the gain so as not to break the ChS.

(If you would like to enlarge the image)  
 Click the “Crop” button  
 on the lower side of the image.  
 Move the frame to the desired location.  
 Click the “Snap” button on the action tab.

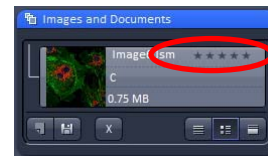


Click the “Acquisition Mode” bar.  
 Change to 4 Number of Averaging.  
 Click the “Snap” button.

## 【6】 Save the image

< Save a dedicated format. (Conditions remain) >

Choose “Save as” from the File menu.  
 Choose the **czi** format.  
 Specify the location and name.

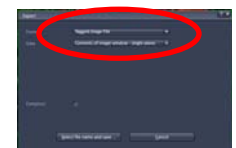
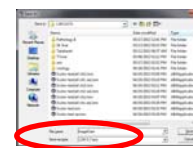


Evaluation is attached to the thumbnail.  
 (Correspondence only CZI format)

A bug has been found in LSM5 format saving.  
 If you need LSM5 format, save czi format just in case.

< Save a generic format. (Conditions does not remain) >

Choose “Export” from the File menu.  
 Choose the file format. (Select “TIF” usually.)  
 Choose the data format. (Select “Contents of image window single plane”)  
 Click the “Select file name and save” button and save the data.



Raw Data = For analysis  
 Contents of image window = Screen snapshot  
 Full resolution image window = To snap shots while holding the original resolution.  
 single plane = Output current data  
 series = Output all stack data

Click the “Acquisition Mode” bar.  
 Change to 4 Number of Averaging.  
 Click the “Reset All” button of the “Scan Area” field.  
 Repeated from 【4】 to look for the next target.



## 【7】 Commonly used functions and tools

The left side of the image window

2D...Display of merging or single color.

Split...Display on a separate color.

Info...Display conditions.

The bottom of the image window

Dimensions tab...Change of color

Part of channel name...Change to visible, invisible.

Part of color...Change the color.

Single Channel... Change to merging, single color.

Range Indicator...Display to confirm the saturation.

Reuse...Apply settings when you get the picture.

Display tab...Change the Brightness, Contrast.

Graphics tab...Writing of scale and annotation

I want to put the scale. → Click the scale icon and drag in the image.

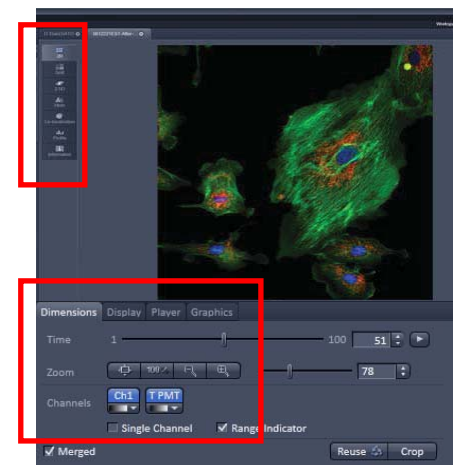
I want to show the length. → Click the any graphic tool and write in image.

Check to “M” in the list.

I want to cut the part. → Click the any graphic tool and write in image.

Click the “Cut region” button.

Object is written, you can load and save.



## 【8】 How to finish

Click the “Acquisition” button on the main tab.  
Click the Laser bar. Turn off the all laser.

Close the ZEN software.  
Shutdown the windows.

Power off in reverse order.

Switch off the reflected light power supply. ⑨

The position of the minimum power control knob. ⑧

Set the switch to “Idle”. ⑦

Turn to the key length. ⑥

(Start the cooling. Wait about 5 minutes until the fans stop.)

Do the cleaning of the objective lens.

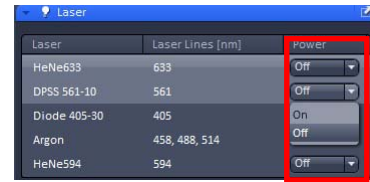
Use the touch panel. Touch in order.

Microscope-Control-Objective

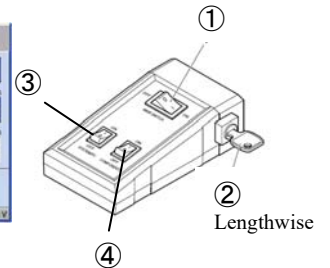
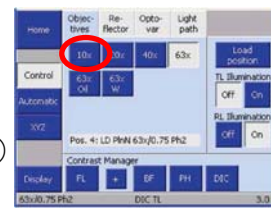
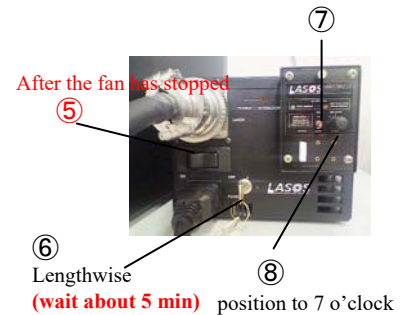
When you have finished cleaning, choose x10 lens.

(After the fan has stopped) Turn off the Ar laser power. ⑤

Turn off the main switch. ④→③→②→①



Reverse order



Cheers for good work.

Write in the record of laser microscopy at the end.

## Do you need a viewer?

Offers the viewer of three in our laboratory.

Choose a match for your PC. (Support Windows XP or later. Windows 8 is unconfirmed.)

Zen2009...for all PC. Old version. CZI format not supported.

Zen2012x86...for 32bit PC. 3D not supported. Support LSM5 format but not for LSM.

Zen2012x64...for 64bit PC. Same as the LSM system. We recommend.

Zen 2.3 or later...Not recommended due to many problems.

## Introduction of other functions

Z-Stack...Get serial section image and re-build.

Time Series...Time lapse scanning.

Bleaching...Bleaching the part of sample by high power laser.

Tile Scan...By connecting the image, obtain wide range high-resolution images.

Positions...Store the coordinates. To use for the auto scan or move.

Regions...Scan a specific area.

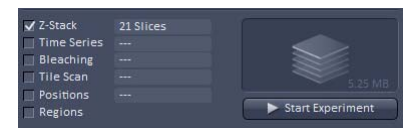
Lambda scan...Fluorescence spectroscopy. Separate the close wavelength dyes.

HDR...High dynamic range imaging.

These function work in complex.

Example... Z + Time = 4D Imaging, Time + Positions = Multi point time lapses, Time + Bleach + Regions = FRAP

FCS(fluorescence correlation spectroscopy) Please contact ZEISS when using.



# LSM780 Simple manual [for 3D imaging]

Use the 2D imaging manual, get the image.

This manual is the operation of 2D imaging later.

Check the “Z-Stack” in the multidimensional tool area.

Click the “Z-Stack” bar. Click the “Live” button.

Go to the start position by changing the focus. Click the “Set First” button.

Go to the end position by changing the focus. Click the “Set Last” button.

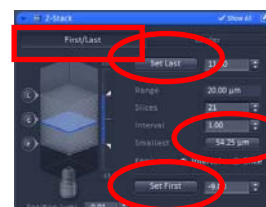
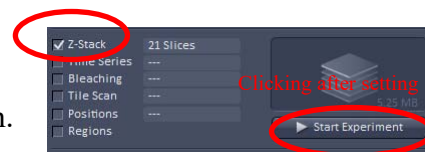
Click the “Stop” button.

Click the **number buttons** on the Smallest item.

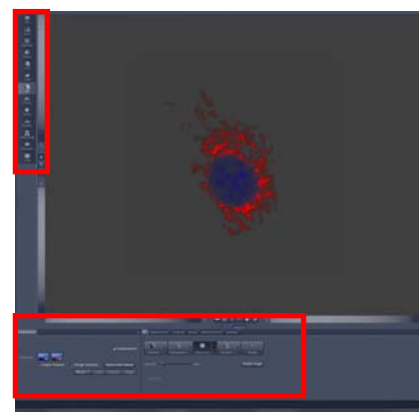
(Scan number is set automatically.)

Set **Averaging Number** and click the “Start Experiment” button.

Save the image at **LSM5 format**.



Cover glass side is First.



## Additional functions of the stack image

### *The left side of the image window*

**Gallery**...Tile display

**Ortho**...Cross-section view

**Cut**...Arbitrary cut view

**3D**...Re-build stereoimage

### *The bottom of the image window*

**Dimensions**...Changing the image by moving the Z-Slice Bar.

**Player**... Change the image automatically.

### *The bottom of the image window (additional right tab)*

If you select 3D, re-build tool is added.

When you drag an image and can change the angle.

(To return to the initial position when you click the house icon.)

**3D**...Display method

**Transparent**...Display in consideration of the transparency. (Internal hardly appears)

**Maximum**...Built with information of the maximum intensity. (Internal structure is visible)

Shadow, Surface, Mixed...Not use.

**Appearance**...Detailed configuration

**Transparency**...When increase the transparency, during the visible.

**Surface**...Surface setting (not use)

**Background**...Background color setting (Maximum = black , Transparent = gray)

**Light**...Surface lightness

**Special**...Color-coded in depth information

**Series**...Animation setting

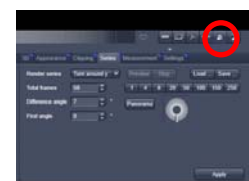
**Render series**...Setting the rotation axis

**Total frames**...Setting the number of frames

**Difference angle**...Interval (Panorama = Go round)

**First angle**...0° = Position that is currently displayed

Create a rotating image when you click “Apply” button.



Note the previous value remain.

When use to export, you can create a video file.

## Making projection image

Display the original image.

Click the “Processing” button on the main tab.

Select “Maximum Intensity Projection”. Click “Select” button. Click “Apply” button.

# LSM780 Simple manual [for motorized stage]

Check the 2D imaging manual for basic operation.

Tile Scan... This is the function to get the big picture by connecting.

## <Setting for tile scan>

Check the "Tile Scan" in the multidimensional tool area.

Click the "Tile Scan" bar.

Set the number you want to include with the horizontal and vertical item.  
(Current position is center.)

For a successful connection, to create an overlap.

Usually, set to 10%.

Online Stitching item performs Stitch process automatically, when scanning is completed.

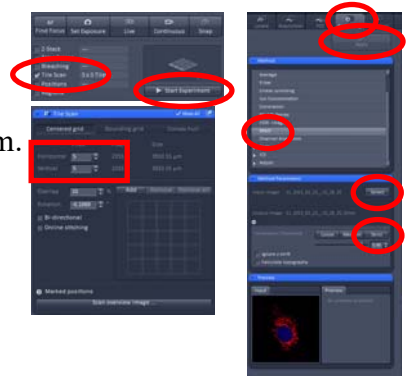
However, it does not return to its original state before treatment.

Start tiling when the "Start Experiment" button is clicked.

Performs Stitch process, when scanning is completed.

Display stack image. Click the "processing" button on the main tab.

Select "Stitch" item. Click the "Select" → "Strict" → "Apply" button.



(With the Bounding grid, you can set the range simply by specifying the required place.)

Positions... Register the coordinates and let scanned automatically.

Can be used simultaneously with other functions.

## <Setting for Positions>

Check the "Positions" in the multidimensional tool area.

Click the snap button, take a picture.

Click the "Positions" button on the lower side of the image.

Click the image, register the coordinates.

(You can also use the image of Tile scan for registration.)

Coordinates that are registered will be at the center.

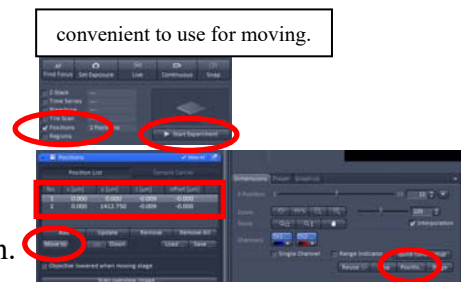
Click the "Positions" bar. You can check the registration information.

If you want to add the coordinates and repeat the operation.

If you want to move to coordinate registration, select the coordinates, and click the "Move to" button.

If you want to remove the coordinates, select the coordinates, and click the "Remove" button.

Shoot automatically to the location where you registered when the "Start Experiment" button is clicked.



When you switch to sample career tab, you will be able to register the coordinates like a plate.

Click the Property button, set the interval and the number of wells.

Set the plate on the stage. Move position to A1 well.

Click the "Calibrate" button. Click on the A1 position in plate image.

(Linked the image and plate.)

When click on the other position in plate image, move to there.

Click the "Select" button. Click on the shooting location in plate image.

The chosen location will change to blue.

Shoot automatically to the location where you registered when the "Start Experiment" button is clicked.

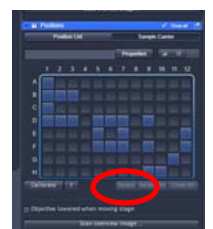
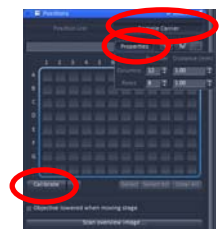


Plate	Columns	Rows	Distance
6well	3	2	40
12well	4	3	27
24well	6	4	19.5
48well	8	6	13
96well	12	8	9

# LSM780 Simple manual [for Lambda scan]

Check the 2D imaging manual for basic operation.

Click the “Acquisition” button on the main tab.

Click on the **OPEN icon** that is located to the right of the **Experiment Manager**.  
Select a “Lambda \*\*\*” from the list. (\*\*\*) is excitation wavelength.)

Click the “Light Path” bar. Set the Resolution and Range.  
(Usually, the range should be set to the excitation wavelength or longer.)  
(Usually, resolution using 8.7nm.)

<Take a picture>  
Get the image in order to avoid saturation.  
(If you want to use multiple lasers, take the balance at laser power.)

<Image windows area>  
2D...Display only one. Select the dimension tab when you want to display another.  
Lambda Coded...Display in false color.  
**Gallery**...Gallery view.  
**Unmixing**...Separate the colors that mix. When you have selected this function, a new tab is added.  
Unmixing...Configuration for separating the color. Choose a location to be reference.

<Automatic recognition methods>

Click the “Auto find / ACE” button.  
**Enter the number of the dye** in the “Number Components” frame.  
(Location is automatically selected.)

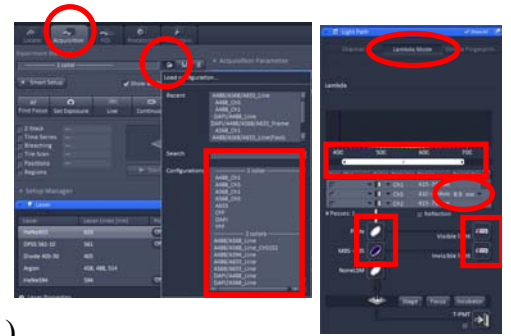
<Manual recognition methods>

Select a location using the ROI tool.  
Choose the location to look to monochromatic.  
Click the “Linear Unmixing” button when you select a location.  
(New image that separates the color is created.)

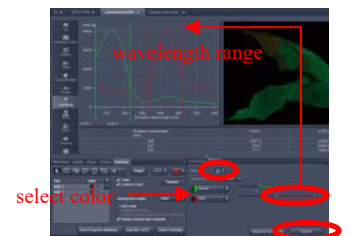
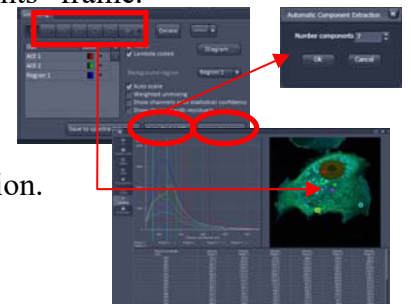
[Other method]

You can use it to save the data of reference from a sample of single staining.

**Extract**...Create a band-pass image.  
Bands...The number of colors you want to create.  
Intensity...Calculation method. Select any one.  
Sum (note the saturation) , Mean  
Automatic (automatically adjusts the intensity)  
Set the wavelength range.  
Create new image when click the “extract” button.



If the range set to from 410 to 690 nm and the resolution set 8.7 nm, image of 32 ch is taken.





### <Linear Unmixing with Offline>

- Displays images acquired in lambda mode.
- Click the **Processing** tab.
- Select **Linear unmixing** in method menu.
- Click **Select** button in method parameters menu.
- Enter the **number of fluorescent dyes** in the Components field.
- Click the **pseudo color button** under the Components field.
- Select the **reference spectrum** and **color** that you saved in advance.
- Click the **Apply** button. Dyes are separated.



### <Online Fingerprinting Mode>

This mode performs Linear unmixing in real time based on previously acquired reference spectrum.

- Open the **Light path** menu.
- Click the **Online Fingerprinting** button.
- Make the **optical path** setting the same as **when acquiring the reference spectrum**.
- Pull down the "dye" field. **Select the reference data**.
- Acquire images in normal usage.
- ※Master gain will change the sensitivity of all channel. Balance with digital gain.
- You have to adjust so as not to saturate.



# LSM780 Simple manual [Other functions]

Check the 2D imaging manual for basic operation. This function is used in addition to 2D imaging.

'Time series' is Time lapse function.

This function can also be used simultaneously with Z stack or position.

## <Setting for Time series>

Check the 'Time series' in tool area.

Open the 'Time series' bar.

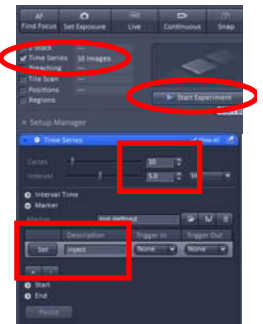
Make the following settings.

Cycle...Number of image to capture Interval...Interval

If the interval is shorter than the scan time, it will be continuous scan.

You can enter a marker by clicking the setting button during scanning.

Click the Start Experiment button in tool area to start time lapse.



Regions is a laser irradiation area designation This function is used for bleaching and scanning.

## <Setting for Regions>

Take an image for area designation.

Check the 'Regions' in tool area.

Open the 'Regions' bar.

Use draw tools to enclose arbitrary places. (Max 99 objects)

Select the check according to purpose of use.

Specified area scan...Check on the 'Acquisition'.

Click the 'Snap' button to scan the specified area.

Bleaching...Check the 'Bleach' and 'Analysis'.

Continue to set up bleaching.



## <Setting for Bleaching>

Check the 'Bleaching' in tool area. ('Time series' and 'Regions' are also automatically checked.)

Open the 'Bleaching' bar.

Check the 'Start Bleaching after # scans'. Specify timing to perform bleaching.

If you repeat the bleach, check the 'Repeat Bleach after # scans'.

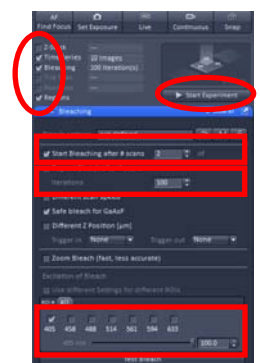
Specify the number of laser irradiation in the 'Iterations' field.

<Important> Check the 'Safe bleach for GaAsP'.

Set the laser and power to be irradiated in 'Excitation of Bleach' item.

Continue to set up 'Time series'.

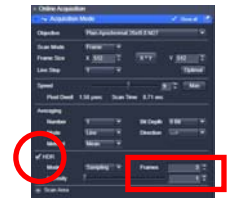
After setting, click the 'Start Experiment' button in tool area to start bleaching.



HDR (High Dynamic Range) is a technique for fitting bright and dark parts into one frame.

### <Setting for HDR>

- Open the 'Acquisition Mode' bar. Check the 'HDR'.
- Adjust sensitivity so that images do not saturate.
- Set 'Frames' and 'Intensity'. Click 'Snap' to take HDR image.
- (If you increase the number of frames, you can shoot those with luminance differences.)

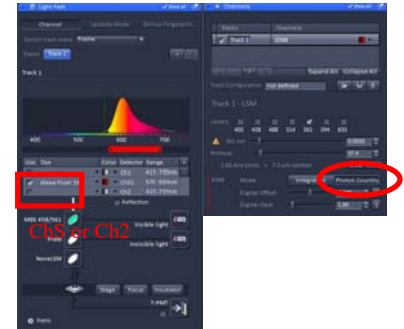


Use the 'Photon counting mode' when you want to shoot with a weaker laser power. Image quality is inferior to normal mode. Scan slowly with weak laser power.

※Ch 1 does not correspond to Photon Counting Mode.

### <Setting for Photon Counting Mode>

- Open the 'Channels' bar. Click the 'Photon Counting' button.
- Change the laser power to 0.2 and shoot.
- Adjust image quality in the following way.
  - Changing Scan Speed will change the brightness and image quality. (Slow scan is better)
  - When still dark, increase the laser power and digital gain.
  - ChS may be more beautiful than ch2.



Smart Setup is a function that sets the optimal optical path by choosing a fluorescent dye.

### <Other proposal at smart setup>

- Fastest...Simultaneous excitation (occur crosstalk)
- Best Signal...Frame sequential (Recommend)
- Best Compromise...When wavelengths are sufficiently separated, they are excited simultaneously.
- Unmixing...Separate the fluorescence by calculation.

※Excitation wavelength may change with combination of fluorescence.

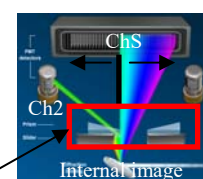


The LSM 780 can freely set the fluorescence wavelength.

### <How to change fluorescence wavelength>

- Click the track you want to change in the Light Path window.
- Click the channel you want to change.
- You can change the wavelength range by changing the length and position of the mirror.
- When Dye is selected, the spectrum is displayed.
- Set the wavelength range referring to the spectrum.

- ※When using line sequential, different wavelength ranges must be set for each detector.
- For example, when channel S is set from 400 nm to 500 nm, channel 2 can not be set to 500 nm or less. (Look at internal image)
- There is no restriction on the frame sequential.
- To set the frame sequential, change the 'Switch track every' item to 'Frame'.



When line sequential is used, this position is fixed.

# LSM780 Simple manual [for Incubator]

## <Panasonic CO 2 Incubator operation method>

Press SET twice.

Press the horizontal triangle key and the upper triangular key to set Co2 to 5%.

Press ENT twice.

(Leave incubator for about 1 hour to stabilize)

Panasonic CO 2 Incubator is for the sample that is waiting.  
If you do not need an incubator, operation is unnecessary.

If using an incubator, turn on the Temp Module S and CO2 Module before turning on the laser microscope.

## <How to operate incubator for microscope>

Replace the stage holder with a heating insert.

Attach an orange dummy cap.

Lid the heating insert.

Add sterile water to the bubbler and cap it.

Open the secondary pressure valve of the CO 2 cylinder. (Do not change pressure)

Turn on Temp Module S and CO2 Module. (It is normally ON)

Turn on the main switch and the COMPONENT switch of the centralized power supply.

Set the CO2 concentration and temperature by the following method.

Operate the touchscreen of the microscope.

Touch 'Microscope' - 'Incubation'.

Touch 'H Insert P' on the Incubation tab.

Set the temperature and change the control to ON. Touch OK.

Set Inc PM, H Dev, CO 2 in the same way.

(Leave incubator for about 1 hour to stabilize)

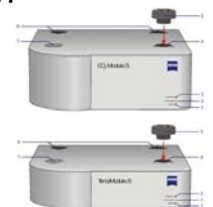
Dishes of 60 mm and 35 mm can be used.



Heating insert



Bubbler



## <Operation at image acquisition>

Adjust the adapter size when using a 35 mm dish. (Tighten the screws to fix the width)

Remove the orange dummy cap. Attach the adapter. (60 mm dish requires no adapter)

Place the dish and observe it.

## <After use>

Stop the incubator with the following method.

Operate the touchscreen of the microscope.

Touch 'Microscope' - 'Incubation'.

Touch 'H Insert P' on the Incubation tab.

Change the control to OFF. Touch OK.

Set Inc PM, H Dev, CO 2 in the same way.

Close the secondary pressure valve of the CO 2 cylinder.

Shut down the laser microscope.

Discard the water from the bubbler. Remove the cap and dry the bubbler.

Replace the heating insert with a stage holder.

## <Operation after using Panasonic CO 2 incubator>

Press SET twice.

Press the horizontal triangle key and the upper triangular key to set Co2 to 0%.

Press ENT twice.

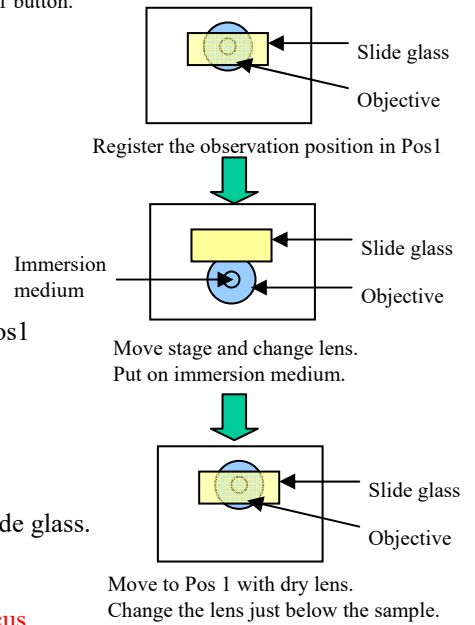
**Write in the records of the incubator.**

# How to put the immersion medium (oil , water)

Use the coordinate registration function of the microscope and register the observation position on the Pos 1 button.  
 (When repeating the same work, it is convenient to register the open position at pos 2.)  
 When moving the stage, using a low magnification objective lens will move faster.

Find the target place with dry objective lenses.  
 Operate the touch panel. (Microscope) , XYZ , Save Position , Pos1  
 Coordinates are displayed on the button of Pos1. (Coordinates have been registered.)  
 Move the stage to the open position. (Use 5x lens for movement)  
 Change the objective lens using the touch panel. (Microscope) , Control , target lens  
 Put the immersion medium on the lens.  
 Tap [Back] on the touch panel. (Change to dry lens)  
 Operate the touch panel to move to the observation position. (Microscope) , XYZ , Pos1  
 (Microscope) , Control , target lens  
 When the objective lens changes, tap Done on the touch panel.  
 Adjust the focus.

※When returning from immersion to dry lens, wipe off the immersion medium of slide glass.  
 ※Save Pos1 again if the observation position changes.  
 ※The registered information disappears when the microscope is turned off.  
 ※If the immersion medium and the mounting medium are mixed, it will not be in focus.  
 If you can not see in the middle of the observation, wipe off the immersion medium and put it again.



# Diopter adjustment

Align the focus position of the observer with the focus position of the laser microscope.

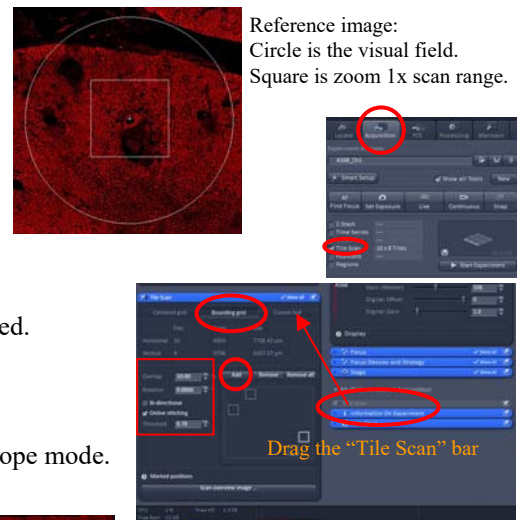
Scan the sample and focus with the laser microscope.  
 Click "Locate" button to switch to observation mode.  
 Set the microscope setting so that you can observe the image taken by laser microscope.  
 Do not touch the focus and do the following.  
 Look through the right eyepiece with your right eye. Turn the right diopter adjustment ring to focus.  
 Look through the left eyepiece with your left eye. Turn the left diopter adjustment ring to focus.  
 Look with both eyes and check that the subject is in focus.



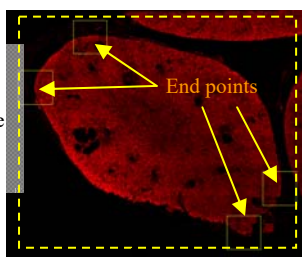
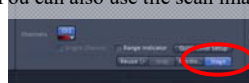
# How to set the tiling range visually

I will show you how to set tiling range by visual observation.

Click "Acquisition" button to set it to laser microscope mode.  
 Set to scan with a laser microscope.  
 Check "Tile Scan".  
 Pull out (drag the title bar) the Tile Scan bar at the bottom of the window.  
 Select "Bounding grid". Set Overlap to 10.  
 Check "Online stitching" and set Threshold to 0.7.  
 Click "Locate" button to switch to observation mode.  
 Find the end point of the range you want to capture in a state where it can be observed.  
 (For example, the yellow frame part of the figure.)  
 Move the end point to the center and click "Add" button in the Tile Scan window.  
 Move to another endpoint and register with "Add".  
 Once all points have been registered, click on Acquisition to switch to laser microscope mode.  
 Display the image with Live Scan, and adjust the brightness and focus.  
 Start Tile Scan with "Start Experiment" button.



appendix  
 Activate the stage button and click in the image to move the stage to that location.  
 (You can also use tile scan images)



Tile Scan range

Specification list of LSM780  
microscope: Inverted microscope AxioObserver.Z1

Filter set						
Band	No.	Name	Ex	DM	Em	Code
UV	49	DAPI	G365	FT395	BP445/50 (420-470)	488049-9901-000
B	38	GFP	BP470/40 (450-490)	FT495	BP525/50 (500-550)	000000-1031-346
G	43	DsRed	BP545/25 (533-558)	FT570	BP605/70 (570-640)	000000-1114-101
B	16	AF488	BP485/20 (475-495)	FT510	LP515	488016-9901-000

Objective							
Grade	Mag	Medium	NA	WD	Code	DIC	Code
Plan Apochromat	10	Air	0.45	2.1	420640-9900-000	II	000000-1045-073
Plan Apochromat	20	Air	0.8	0.55	420650-9901-000	II	426940-0000-000
Plan Apochromat	40	Air	0.95	0.25	420660-9970-000	III	426944-0000-000
C-Apochromat	63	Water	1.2	0.28	421787-9970-000	III	426946-0000-000
Plan Apochromat	63	Oil	1.4	0.19	420782-9900-799(000)	III	426957-0000-000
Plan Apochromat	5	Air	0.16	12.1	420630-9900-000	-	
APO Kalibrierobjekti		Air			420639-9000-700		

Lamp			
Name	Power	Life	Code
Metal halide	120W	1500h	000000-1313-162
Fiber	1.5m	3year	000000-1313-164
halogen	12V100W	2000h	000000-0518-961

Medium			
Name	Refractive index	Capacity	Code
Immersion oil	1.518	20ml	444960-0000-000
Immersion water	1.3339	20ml	444969-0000-000

Laser			
Name	Power	Life	Wavelength
Diode	30mW	5000h	405
Ar	25mW	5000h	488,458,514
DPSS	20mW	5000h	561
HeNe	2mW		594
HeNe	5mW	10000h	633

Detector			
Name	Type	Range	Resolution
Ch1	PMT	371-740nm	1nm
ChS	32chGaAsP-PMT	410-694nm	Highest 3nm Normal 8.7nm
Ch2	Cooled PMT	379-758nm	1nm

Beam splitter list	
MBS 458	Plate
MBS 458/514	MBS 355/445
MBS 458/561	MBS -405
MBS 458/514/594	MBS -445
MBS 488	MBS 690+
MBS 488/561	MBS 760+
MBS 488/594	MBS -405/760+
MBS 488/561/633	MBS -445/760+
T80/R20	T80/R20
Plate	None

