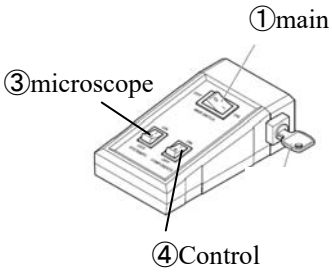
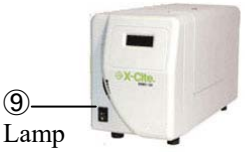
LSM780 (More) Simple manual

【1】 How to start up

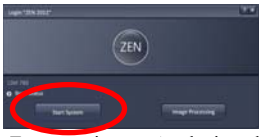
Turn on the power in order. ①③④⑤⑥
Turn on the reflected light power supply. ⑨
Start the PC. ⑩ (Wait about 3 minutes.)
　　Log in the "LSM User".
　　Double-click the "ZEN" icon.
　　Click the "Start System" button。
When ZEN starts up, ⑦ set to Run (upper).
⑧ set to 9 o'clock position.

（The green LED will light up after a while.
If the red LED lights up, turn the knob back slightly.）





**If an error is displayed on the lower left of the screen or the startup process does not proceed,Something is not working properly.
Follow the steps below to restart.
Close the "ZEN" software. Turn off ④ and ③. Turn on ③ and ④. Restart the PC.**



For scanning　　Analysis only

【2】 How to turn on the laser … It lights up automatically

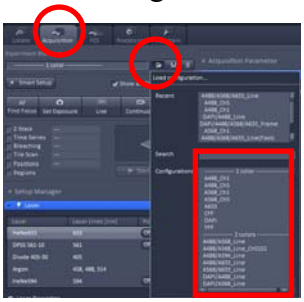
【3】 How to optical path setting of laser microscope

Click the "Acquisition" button on the main tab.
Set up the light path using Experiment Manager or Smart Setup.

Looking for colocalization, use Smart Setup.
Experiment manager is easy
if there is no problem even
if the optical section thickness is different.

＜Setting with Experiment Manager＞　Line sequential

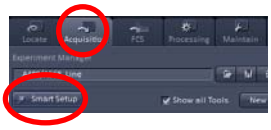
Click on the OPEN icon
that is located to the right of the Experiment Manager.
Select a setting from the list.

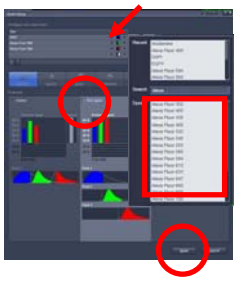


Settings containing the word "Fast" are not recommended
as they are prone to fluorescence leaks.

＜Setting with Smart Setup＞　Frame sequential

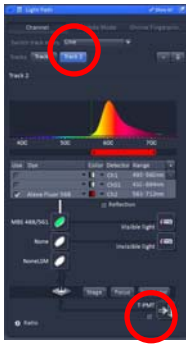
Click the "Smart Setup" button
below the Experiment Manager.
Click the pull-down of Dye, choose a dye from Dyes list.
To register all the dyes being used.
Select the "Best Signal" and click the "Apply" button.





＜Common settings for Experiment Manager, Smart Setup＞
(When taking a 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.

Adjust the contrast referring to
"Adjusting differential interference" below.



【4】 How to visual observation with 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（Select a condenser that matches the objective lens and turn the DIC slider knob to adjust the contrast.）

Click the objective lens icon to select a lens.
Adjust the focus where you want to shoot.

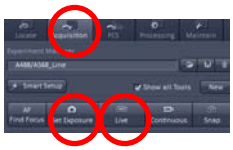
When using oil immersion or water immersion, apply the liquid according to the separate method.

Adjusting differential interference

Match the condenser
x10～x20 … DIC Ⅱ
x40 , x63w , x63oil …DIC Ⅲ

【5】 How to acquisition of image

Click the “Acquisition” button on the main tab.



<Adjustment in the Experiment Manager>

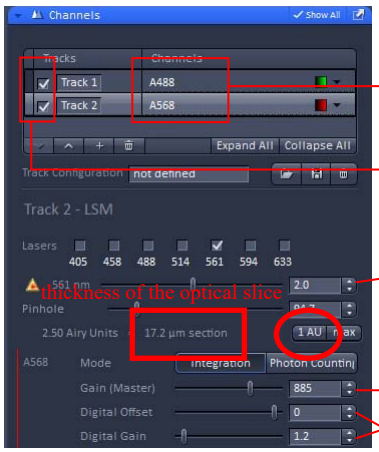
Click the “Channels” bar.
Click the “1AU” button of long wavelength side.

When changing the objective lens, be sure to check 1 AU.

Click "Set Exposure" at the top to adjust the brightness automatically.

Scan continuously with the “Live” to focus.
Adjust the brightness with Gain (Master) and the contrast with Digital Offset.
Select the Channels name to adjust other channels.

Click “Stop” after adjustment.



Change channel setting (Click on the name part)
Only one check on when adjusting in smart setup
thickness of the optical slice
Laser power (2% is default)
brightness
contrast

<Adjustment in the Smart Setup>

Click the “Channels” bar.
Click the “1AU” button of long wavelength side.
Check the thickness of the optical slice and match the thickness of the other channels.

Click "Set Exposure" at the top to adjust the brightness automatically.

Set the Tracks check to one and adjust one color at a time.
(Select a color that is easy to focus on.)

Scan continuously with the “Live” to focus.
Adjust the brightness with Gain (Master) and the contrast with Digital Offset.
Select the Channels name to adjust other channels.
Click “Stop” after adjustment.

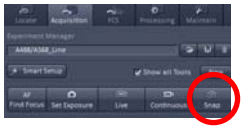
Check on the another track and click the channel name.
Adjust with Live scan.
Once all Tracks have been adjusted, check on all Tracks.

＜common operation＞

Scan for finishing with the following settings.

Open the Acquisition Mode bar.
Change averaging number to 4.
(Use 8 or 16 when there is a lot of noise.) Click “Snap”.

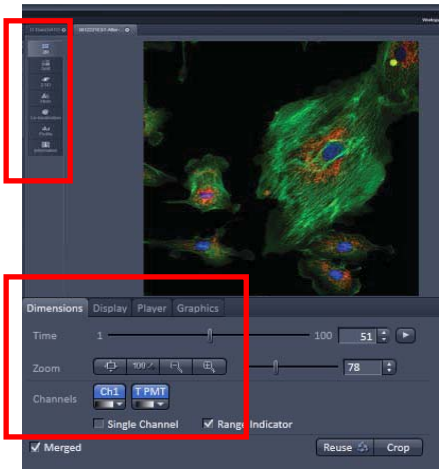
Frame size recommended 1024x1024



【6】 How to save the image

Select File-Save as on menu.
Specify the save location and name, and save in czi format.
Return to【4】and find for the next field.

【7】About other functions（Relatively used functions）



In image window

2D…merged display (default)

Split…split display

Info…Display of conditions

Dimensions…Change channel display and color

Ch name part…Change show/hide

Ch pull-down…Change color

Single Channel…Change single/multi color

Range Indicator…Confirmation of saturation

Reuse…Reproduce the settings when the image was acquired

Display…Adjust Brightness and Contrast（Image processing）

Graphics…Write annotations and scales

Insert the scale→Select the ruler icon and drag in the image

Display of length and area→Write a shape with the shape tool and check on “M” in the list

Partially cut out→Write a shape with the shape tool and click “Cut Region”

Crop (enlarge)

Click “Crop” at the bottom of the image.

Specify the place you want to take with the frame.

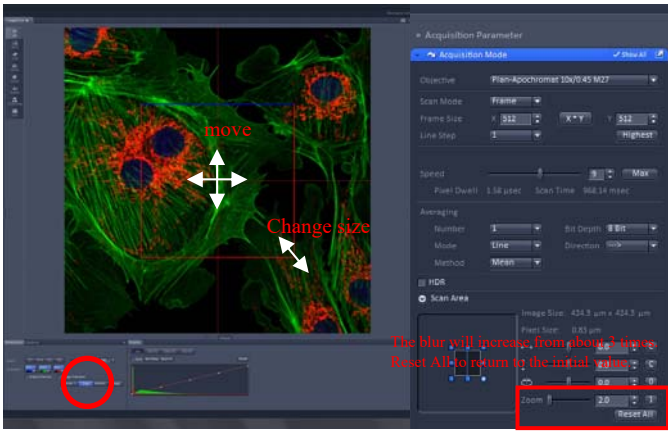
（Drag in the frame to move.

Drag the corner to change the size of the frame.）

“Live” scan to enlarge the specified location.

(If necessary, set Average and acquire images with Snap.)

“Reset All” to return to the initial value.



Stage (Move the stage to the specified location)

Click “Stage” at the bottom of the image.

Click where you want to center in the image. (Move the stage)

Range Indicator (Confirmation of saturation)

Click “Split”.

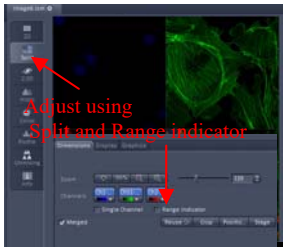
Click “Live” and check on “Range Indicator”.

The saturation part is red. Intensity zero is blue.

If you adjust Offset so that the background part becomes blue, it looks beautiful.

When using images for quantification, be careful not to have red and blue parts.

If you uncheck the “Range Indicator”, the original color will be restored.



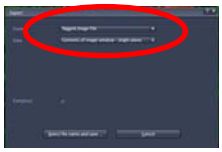
Export（Save the image in a general format）

Select File-Export on menu.

Select format. (Normally TIFF format)

Select data type. (Normally “Contents of image window single plane”)

Click “Select file name and save” button to save.



Raw Data=for analysis

Contents of image window=Screen snapshot

(You can save beautifully if the screen resolution is high.)

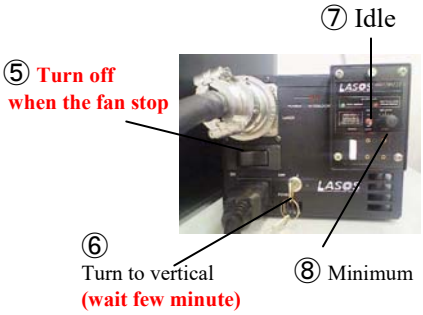
Full resolution image window=Keep original resolution

(Font size may change)

single plane=Only the displayed plane

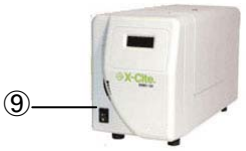
Series=All stack image

【8】 How to shutdown



Turn the knob ⑧ to the minimum state.
Switch ⑦ to Idle.
Turn the key ⑥ to vertical.
(Cooling starts. Do the following operation, but **do not turn off switch ⑤ until the fan stop**.)

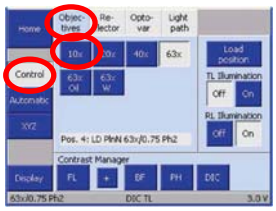
Close the ZEN software.
Turn off the reflected light power supply. ⑨



Save the data to USB.
Shutdown the Windows.

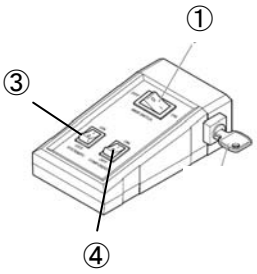
Cleaning the objective lens.

Change the objective lens using the touch panel.
(Microscope – Control – Objective)
After cleaning, set x10 objective.
Move stage to center.



When laser cooling fan stop , turn off the switch ⑤.

Turn off the switch. ④ , ③ , ①



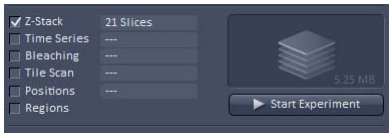
It is the end when you fill in the use record.
We look forward to seeing you again.

About viewer software

We recommend ZEN2012x64. You can not download from the Internet. Please contact my laboratory.

Zen2009…for 32bit OS (older version) Not compatible with CZI format.
Zen2012x64…for 64bit OS (recommend version)
ZEN 2.3 or later…newest version (Not recommended due to low compatibility)

Introduction of special function



I will skip about how to use.

Z-Stack…Acquire continuous images and create 3D images.
Time Series…Acquire images regularly and observe them. (Time lapse)
Bleaching…Irradiate part of sample with a high power laser to fade the fluorescence. (FRAP , Photo activation)
Tile Scan…Connect the image to create a wide range of images.
Positions…Record coordinates and observe at multiple points. (Multi point)
Regions…Make a region of interest (ROI) and use to scan or bleach.
Lambda scan…By utilizing the spectral function, separates multi fluorescence or autofluorescence.
HDR…Acquire High Dynamic Range image.

You can combine multiple functions.
Ex.） Z＋Time＝4D Imaging , Time＋Positions＝Multi point time lapse , Time＋Bleach＋Regions＝FRAP

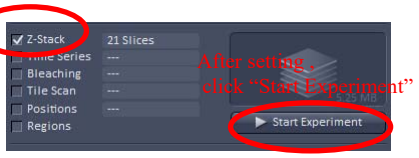
It also supports FSC measurement, but its operation has not been confirmed . Please contact Zeiss when using.

LSM780 (More) Simple manual [Z-Stack]

The image acquisition procedure is omitted. Please refer to the (more) simple manual for the basic operation.
Z-Stack is a function to acquire continuous section images while changing the focus.

<How to set of Z-Stack>

Check the Z-Stack.



Open the Z-Stack bar. (lower of right row)
Click the "Live" button to scan continuously.
Turn the focus knob towards you. Click "Set first" at the start position of Z stack.
Turn the focus knob towards the back. Click "Set Last" at the end position of Z stack.
Click "Stop" to stop the scan.
Click the Smallest number button.
(The number of scans is automatically set in steps of half the optical section thickness.)
Set the Averaging Number and
click the "Start Experiment" button to start the scan.
Save the image in czi format when scanning is completed.

Cover glass side is First

Turn up clockwise to go up.

Items to be added or changed in the Z stack image

Left side of image

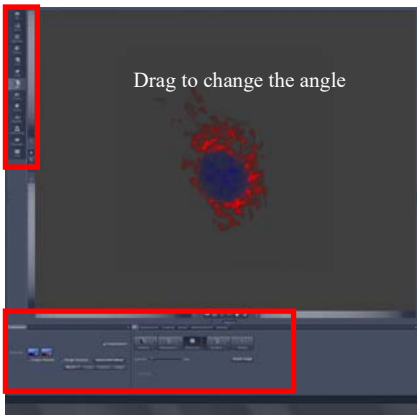
Gallery…List display
Ortho…orthogonal display
3D…Create 3D image. Details of the parameters will be described later.

bottom of image (Left)

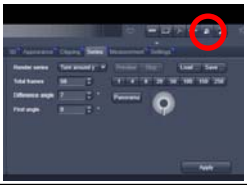
Dimensions…Z-Position bar is displayed. Used to move the position.

bottom of image (Right) (3D parameter)

Drag the 3D image to change the angle. Click the home icon to return to the home position.



3D…Projection method
Transparent…Transparent display (Hard to see the internal structure)
Maximum…General display (No concept of front and back)
Appearance…Parameter of projection method
Transparency…If you make it transparent, you can see inside but not outside.
Threshold…Cut low brightness areas.
Ramp…Contrast
Maximum…Upper limit of brightness
Background…Background color (Easy-to-see color…Transparent=gray Maximum=black)
Light…brightness
Series…Animation source file setting (The position currently displayed is the rotation start position)
Render series…Axis of rotation
Total frames…Number of frames
Difference angle…Animation interval ("Panorama" is automatically set to one rotation)
First angle…Start position angle (Usually 0 °)
Click "Apply" to create an animated source image.

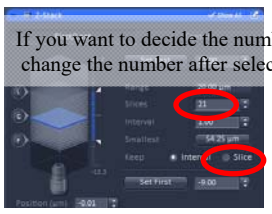


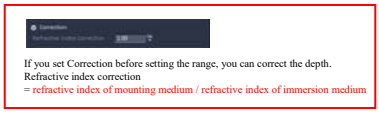
Note that the previous parameters remain

For continuous images, you will be able to select movie files such as AVI in Export.

General 3D fluorescence image settings
3D mode…Maximum
Appearance setting
Threshold…0
Background color…black
Light…1
Animation setting
Turn around Y
Total frames 100
First angle 0
panorama

- memo -



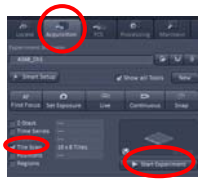


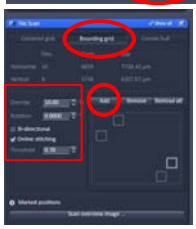
LSM780 (More) Simple manual [Tile Scan]

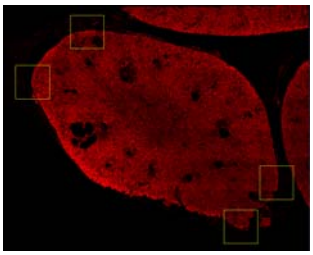
The image acquisition procedure is omitted. Please refer to the (more) simple manual for the basic operation.
Tile Scan is a function that shoots multiple places and joins them together to make one big image.

<Setting of Tile Scan>

Check on "Tile Scan".
Open the "Tile Scan bar".
Select the "Bounding grid" tab. Set Overlap to 10%.
Check on "Online stitching". Set Threshold to 0.7.
Click "Live" button to start the scan.
Move to the end point of the range you want to capture.
Click Add button in the Tile Scan window.
Repeat the same operation to enclose the entire area you want to capture.
Click "Live" button to stop the scan. Set the average if necessary.
Click "Start Experiment" button to start tile scan.
When the scan is finished,
the combining process will be performed automatically.



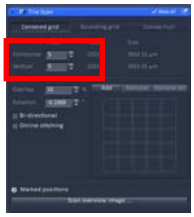




Find and register the outermost part
(end point) of the range
you want to capture.

<Setting of Tile Scan by specifying number of sheets>

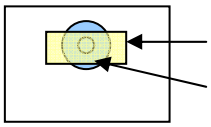
Check on "Tile Scan".
Open the "Tile Scan bar".
Select the " Centered grid" tab. Set Overlap to 10%.
Check on "Online stitching". Set Threshold to 0.7.
Click "Live" button to start the scan.
Move to the center of the range you want to capture.
Click "Live" button to stop the scan. Set the average if necessary.
Set the number to Horizontal and Vertical.
Click "Start Experiment" button to start tile scan.
When the scan is finished,
the combining process will be performed automatically.



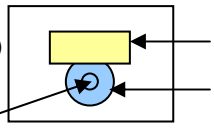
How to not change the location when applying immersion medium

Use the coordinate registration function of the microscope to register the observation position.

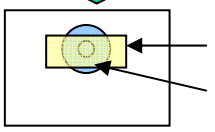
Find the target location with dry lenses.
Touch Microscope, XYZ, Save Position and Pos1 in this order on the touch panel.
Manually move to the position where the immersion medium can be applied.
(Use a x5 lens for movement to prevent collision with the frame.)
Touch Microscope , Control and immersion objective in this order on the touch panel.
Apply the immersion medium to the lens.
Touch Back on the touch panel. (Change to x5 dry lens)
Touch Microscope, XYZ and Pos1 in this order on the touch panel. (Move to observation position)
Touch Microscope , Control and immersion objective in this order on the touch panel.
Touch Done on the touch panel.
Fine-tune the focus.



Register the observation position on Pos1.



Apply the immersion medium at the opening.

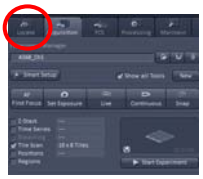


Return to observation position and change the lens.

※When returning from the immersion lens to the dry lens,
wipe off the immersion medium of the slide glass and lens.
※Save Pos1 again when the observation position changes.
※The registration information disappears when the microscope is turned off.
※If the immersion medium and the mounting medium are mixed, the focus will not be achieved.
If it becomes difficult to see on the way, replace the immersion medium.

Diopter adjustment

Bring the observer's focal point closer to the focal point of the laser microscope.

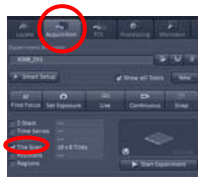


Display the image with a laser microscope and adjust focus.
Stop the scan and click “Locate” to switch to observation mode.
Select a filter that allows you to observe the image taken with a laser.
Do the following without touching the focus.
Look into the right eyepiece with your right eye. Rotate the right diopter adjustment ring to focus.
Look into the left eyepiece with your left eye. Rotate the left diopter adjustment ring to focus.

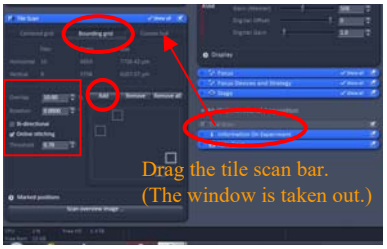

Diopter adjustment ring

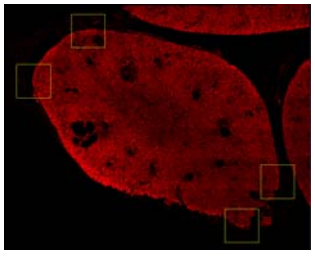
How to set while observing the tiling range

Set the range without using live scan.

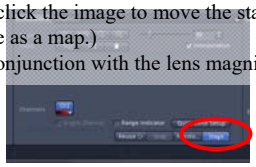


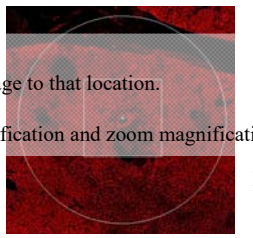
Click “Acquisition” button to change to acquisition mode.
Check on “Tile Scan”.
Drag the tile scan bar.
Select “Bounding grid”. Set “Overlap” to 10.
Check on “Online stitching”. Set “Threshold” to 0.7.
Click “Locate” button to change to observation mode.
Prepare for observation and find the end of the range you want to capture.
（In the case of the section of the picture, the yellow frame part.）
Move so that the end point is in the center of observation. Click “Add” in the Tile Scan window.
Move the location and register other end points.
(If there are many registered points, the outermost range is valid.)
After registering all the end points, Click “Acquisition” button to change to acquisition mode.
Click "Live" button and adjust the brightness and focus.
Set Average and start Tile Scan with “Start Experiment” button.





Memo
Click the “Stage” button and click the image to move the stage to that location.
(You can use a tile scan image as a map.)
(The square size changes in conjunction with the lens magnification and zoom magnification.)





Reference : When the scan zoom is 1x.
○= Range of observation
□= Range of laser scan

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			
Type	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

Usable container			
Slide glass	Multi well plate		
35mm, 60mm petri dishes(Can be used in CO2 incubator)			
The maximum magnification that can be used on the bottom of the plastic is 10 times.