Easy usage of confocal laser scanning microscope LSM510
Basic operation   Version 3.2

① *Turn on the system*
・Turn on <u>main switch</u>. (under the laser unit.)

・Turn on microscope <u>light switch</u>. (under the laser unit.)

・Turn on computer power switch. (under the desk.)
    － Wait for the LSM510 program to start. (automatic start)－

Click [Scan New Images] and [<u>Start Expert Mode</u>] button.

② *Turn on the laser*
Click on the button of main menu. [<u>Acquire</u>] → [<u>Laser</u>]

Click on the laser featuring the appropriate wavelength to excite the dye labeling your specimen.

・In the case of the <u>Argon laser</u>.

    Click on the **[Standby]** button and **OUTPUT** scrollbar to set the intensity to **45%**.

・ In the case of the <u>HeNe laser</u>.

    Click on the [<u>ON</u>] button.

Click on the [Close].

> Relation between the dye labeling and laser
> Argon/2…FITC,Cy2,GFP,Alexa488,Fluo3
> HeNe1…Rhodamine,Cy3,PE,PI

③ *Setting the microscope*
Click on the button of main menu. [<u>Acquire</u>] → [<u>Micro</u>]

Move the Slider on the side of the microscope to the VIS position.

・Click picture of objective and select an <u>objective</u>.

    (×10 , ×20 , ×40oil , ×63oil , ×63W , ×40)

・The filter is set clicking these buttons. (The up side of window)

    (UV , FITC LP , Rhodamine , FITC BP , FITC/Rhod , Transmission , OFF)

Put the specimen on the stage and <u>adjust focus</u>. Move the target to the center of view.

> The lighting of the room can be turned on and off with the hand switch.

Move the Slider on the side of the microscope to the LSM position.

    (The filter changes into None in the automatic operation.)

Click on the [Close] button.

> Relation between the dye labeling and filter
> Fset01(UV) …   Hoechst.DAPI
>   (Only the observation)
> Fset09(FITC LP) …   FITC,Cy2,GFP,Alexa488,Fluo3
> Fset15(Rhodamine) …   Rhodamine,Cy3,PE,PI
> Fset17(FITC BP) …   FITC,Cy2,GFP,Alexa488,Fluo3
> Fset23(FITC/Rhod) …   FITC&Rhodamine
> None(Transmission) …   Transmit(DIC),Laser Scan

④ *Setting the beam path*
Click on the button of main menu. [<u>Acquire</u>] → [<u>Config</u>]

    Usually you select [Single track].In the case of double stain you select [Multi track].

    Click [<u>Config</u>] button (The right side of window) and select dye name.

    Click **[Apply]** button to apply setting.

Click on the [Close]

> Single Track
>   FITC(Cy2) …   505nm Long Pass
>   FITC(Narrow) …   505-550nm Band pass
>   Rhodamine(Cy3) …   560nm Long pass
>
> Multi Track
>   Rhodamine/FITC …   505-530nm Band Pass
>   560nm Long pass

⑤ *Scan an image*
Click on the button of main menu. [<u>Acquire</u>] → [<u>Scan</u>]

Click on the [<u>Channels</u>] tab of Scan control window.

    "PinholeΦ=1.00AiryUnits" is good value to obtain a confocal image.

    ・Move the pinhole slider, and set the pinhole diameter to become "<u>PinholeΦ=1.00</u>".

        In the case of two channels use.

            ch1…Set pinhole diameter to become "PinholeΦ=1.00AiryUnits"

            ch2…Pinhole diameter set to become <u>the same value as the optical slice of ch1</u>.

    ・Click [<u>Find</u>] button for automatic optimization of image brightness and contrast.

        (In the case of multitrack use , execute it with both channels.)

        Do not do Find when you do not want to change the condition.

Click [Cont] button for continuas scan.

　　　・The focus is adjust on the screen..

　　　・Sensitivity is changed by "Detector Gain" and it adjusts brightness.

　　　・The contrast is adjust by "Ampl Offset".

　　　・"ampl gain" does more sensitizations, but the noise increases, too.

　　　・When the adjustment finished, click [Stop] button.

　　　(If it wants to expand more, click [Crop] button.

　　　　Enclose the target place with the square. And click [Single] button.)

　　（ Look at another item "Take a three-dimensional image" when you want to take a three-dimensional image.）

　Click on the [Mode] tab of Scan control window.

　　　・The averaging number chooses 4 usually. And click [Single] button.

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Crop
Fluorescence fade out early
when the zoom magnification
is high.
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⑥ *Save data*

　Insert the formatted MO disk for Windows. (The capacity that can be used is 128,230, and 640MB.)

　Look at the another item "How to save data in CD-R(RW)" if you want to save data in CD-R/DVD-R.

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USB was supported.
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　　　・Click on the [Save as] button in image window.

　　　　・Click on the [New MDB] button only once of the start. Select "Removable Disk M:".

　　　　　Name the database name, and click [Create] button.

　　　・Input the sample name to the "name" frame , and click [OK] button.

　　　　（The saved data cannot be used with other software. Look at another item "Change into the general format"

　　　　　when you want to convert the image into the general format. ）

　　　・Close the image window.

⑦ *To the next scanning*

　　・The [Mode] button of the scan control window is clicked.

　　"zoom", "rotation", "offset", and "average number".Return them to default.

　　・Repeat from ③ to ⑥.

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Default setting
Zoom=1
Rotation=0
Offset=Center
Average number=1
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　　　Memo for repetition

　　　　　Slider to VIS → Find the target → Slider to LSM → Confirm PinholeΦ value → [Cont]inuas scan

　　　　　→ Adjust focus → Set average number to 4 → [Single] scan → [Save as] data

⑧ *When finishing using*

　　・Click on the button of main menu. [Acquire] → [Laser]

　　　　・Select laser and turn [off] laser power.

　　・Click on the button of main menu. [Acquire] → [Micro]

　　　　・Change objective by x10. Clean the immersion lens.

　　　　　（Wipe it a few times with the cotton bud wet in the cleaning liquid. ）

　　・Click on the button of main menu. [File] → [Exit]

　　・Click on [Exit] button of the title screen.

　　・Eject MO

　　　　Close all windows. Right-click on the MO icon in desktop , and select "取り出し(eject)".

　　　　（First of all stop computer when MO doesn't eject.

　　　　　At this point , do not push the eject button. Data will delete if the button was pushed. ）

　　Shutdown computer

　　・〈Windows〉 START → Shut Down

　　　　ー Wait until shutting down ー

　　・When "再起動(restart)" button is displayed on the screen, turn off computer.

　　　　（Take it out here if MO does not eject. ）

　　・Turn off microscope light switch.

　　・Confirm the fan on the argon laser stops. Turn off main switch.

　　・Record in the note.

Other operations and functions

Image viewer can be obtained from the homepage of ZEISS.

<HP> http://www.zeiss.de/   <Software name> LSM5 Image Browser

Function of an Image browser
>   The image in the data base can be seen.
>   The separation of the channel can be done.
>   The condition when saving it can be seen.
>   Change into the general format. (Export)
>   Input of scale and measurement of distance.
>   Adjustment of brightness and contrast.
>   The brightness of one point can be displayed. (It is not possible to integrate.)
>   A part of three-dimensional construction can be made.
>   (A consecutive thing cannot be made. The thing made beforehand can be seen.)

How to draw scale (It is possible by LSM image browser)
>   ・Open the database. Click on the button of main menu. [File] → [Open]
>   Select database file and click [開く(open)] button.
>   Choose the image from the database and double-click it.
>   ・Click on [Overlay] button in image window. Click on scale icon.
>   ・Draw the line while calculating when it drags in the image.
>   (The place can be moved, and the color be changed.)

Change into the general format (TIFF) (It is possible by LSM image browser)
>   ・Open the database. Click on the button of main menu. [File]→[Open]
>   Select database file and click [開く(open)] button.
>   Choose the image from the data base and double-click it.
>   ・Click on the button of main menu. [File] → [Export]
>   Image type …   Contents of image window(Like the screen display)
>   Single Image With raw data (Raw data)
>   "保存する場所(Save in)" … Select MO.
>   "ファイルの種類(Save as type)" … TIF − Tagged Image File(*.tif)
>   "ファイル名(File name)" … Input file name and click [保存(Save)].

Take a three-dimensional image (It is <u>not possible</u> by LSM image browser)
>   Operate to ⑤ of a basic operation.   (When brightness is too bright, 3D cannot be beautifully done.)
>   ・Click on the button of main menu. [Acquire] → [Scan] , [Z Stack]
>   ・The [Mark First/Last] tab is clicked.
>   ・Scan with the [XY cont] button.
>   Move focus to the starting point, and click [Mark First] button.
>   Move focus to the ending point, and click [Mark Last] button.
>   ・Click on [Stop] button.
>   ・Click on the button. [Z Slice] → [Optimal Interval]
>   (The number of the slice is set by the automatic operation. You may specify it with the manual.)
>   ・Click on the [Mode]. The averaging number chooses 4 and click [Start] button.
>   ・Save the image when the scanning finished.

Three-dimensional construction (It is possible by ImageBrowser version 4.0 or later)
>   ・Click on the button of main menu. [3D View] → [Projection]
>   ・Fast Angle 0
>   ・Number Projections 32
>   ・Difference Angle [Panorama]
>   ・Click [Transparency] tab. Confirm that max has been selected.
>   ・Click on [Apply] button.
>   It is rotated with the [Slice] or [Anim]button.
>   Save the image if it is necessary.

Setting to see brightness value
>   Execute an image browser.
>   Click on the button of main menu.
>   [Options] → [Options]
>   Click [Image Status Display] tab.
>   Check the "Pixel Intensity" check box
>   and click "OK".
>   Put the mouse pointer on the point
>   that wants to see brightness.
>   "Intensity" is displayed under the window.
Caution   When you have changed brightness
>   and the contrast ,
>   intensity has changed, too.

Image type keywords
Raw…Raw data
Contents… Like the screen display

Full Resolution… By full resolution

Single…Only the displayed image
Series…All stack images

If you use ImageBrowser version 3.5 or later…
When the file type is changed into AVI,
 the animation file can be made.

Loading of the previous condition (It is not possible by LSM image browser)
・Open the database. Click on the button of main menu. [File] → [Open]
Select database file and click [開く(open)] button.
Choose the image that wants to refer.
The condition is apply click on the [Reuse] button.
（Change objective by manual. ）

Take a difference interference contrast image (This is not confocal image)
Setting of microscope
・Click on the button of main menu. [Acquire] → [Micro]
・Move the Slider on the side of the microscope to the VIS position.
・Select an objective.
・Check the "Transmission" button.
・Insert the "analyzer".
・Setting the "condenser".
Using this position.（×10〜40 … II 、 ×40<u>oil</u> or later … III）
・Insert the "polarizer".
・Squeeze squeezing only a little.
・Adjust the "DIC slider" and apply the contrast.
・Move the Slider on the side of the microscope to the LSM position.
・<u>Pull out the "analyzer" only.</u>
Setting of LSM
・Click on the button of main menu. [Acquire] → [Config]
・The check box on the left side of chD is turned on. And select white.
（In the case of Multitrack , apply to the FITC side.）
・Click on the button of main menu. [Acquire] → [Scan] , [Channels]
Adjust the gain of chD.
Save the image if it is necessary.

analyzer

DIC slider

condenser
and squeeze

polarizer
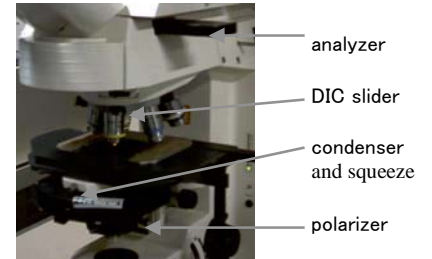
Operation when application error is generated.
Reboot the computer with the laser power on.

How to save data in CD-R(RW)
Click on the [Save as] button in image window.
Select the hard disk after clicking "NEW MDB".
Save data on the hard disk and keep working usual.
Do the following before shutdown the computer.
Start "B' s Recorder GOLD".
Drag the folder that wants to be written in the right side frame of B's Recorder.
Set CD-R(RW) media of enough capacity.
Click [書込み(Write)] button.
Start writing when you click the [OK] button.
Close the window when work ends.
Check the data written in CD-R.
Delete your data saved on the hard disk.