レーザー蛍光顕微鏡を用いた組織切片観察（実習・英語/日本語）
Observation of Tissue Sections Using Microscope (Practice in English/Japanese)

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HE 染色した切片を顕微鏡観察する。また、共焦点レーザー蛍光顕微鏡を使い、蛍光標識プローブで染色した組織切片の観察方法を学ぶ。

We will show how to monitor and capture images from HE-stained sections. Using confocal laser fluorescence microscopes, we will show you how to visualize localization of proteins/nucleic acids stained with fluorescent probes on sections.

September 11, 2024, 17:00-18:20

Laser scanning fluorescence microscope

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Toshiyuki Watanabe (Andor Technology)

Practice
Staining (17:00-17:20)
Room 416, Central Research Laboratory

Experiment 1 (Staining)
Room 309 (Asahina)

Materials

Sections
We have 2 cryosections prepared from the mouse liver.

Reagents
DAPI solution (1 mg/ml)
DAPI (4',6-diamidino-2-phenylindole) binds to DNA
DAPI has an absorption maximum at 358 nm (ultraviolet)
Emission maximum is 461 nm (blue)
Working solution: 100-fold dilution in PBS

BODIPY 558/568 Phalloidin solution
Phalloidin is a toxin isolated from the deadly mushroom
Phalloidin binds to F-actin
BODIPY 558/568: excitation 558 nm, emission 569 nm
Working solution: 50-fold dilution in PBS

Mounting medium
0.5% n-propyl gallate
90% glycerol
1xPBS

Procedure
1. Incubate the slide in PBS for 3 min
2. Repeat this washing procedure total 3 times
3. Dry the slide glass
4. Draw a circle around each section with a PAP pen
5. Wait until the PAP pen dries
6. Wash with PBS
7. Apply 50 μl of the DAPI and Rhodamine-Phalloidin solution to the section
8. Incubate in this solution for 5 min
9. Wash with PBS 3 times
10. Drop the mounting medium to the section
11. Cover the cover glass without making bubbles

Experiment 2

Confocal laser scanning microscope (17:20-18:20)

Room 407 (Leica TSC SP8 X)

Room 412 (Andor Dragonfly201 and Nikon Ti2-E microscope)

Split the attendees into two groups

Group A (English: Leica→Andor)

Group B (Japanese: Andor→Leica)

Leica SP8: room 407 (Yamamoto, Okamoto)

Observe the stained section using Leica SP8 confocal fluorescence microscope.
Using the white light laser, we can detect different fluorescence signals.
Please see the detail in **Supplement 1**.

Andor Dragonfly 201: room 412 (Watanabe, Fukunaga)

Observe the stained section using Dragonfly confocal fluorescence microscope.
Please see the detail in **Supplement 2**.

Note. Fluorescence dyes and their characters

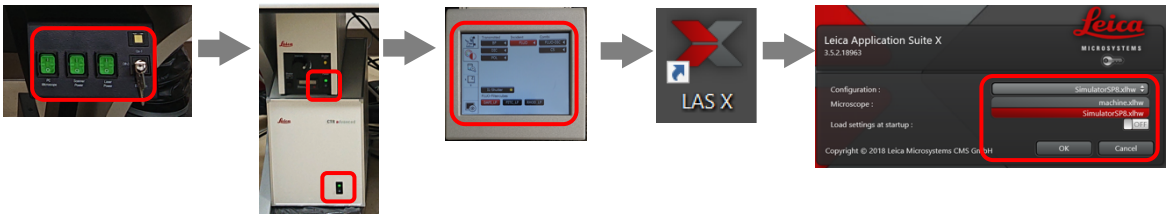
	Excitation	Emission	Color
DAPI	358 nm	461 nm	Blue
GFP	488 nm	509 nm	Green
AlexaFluor 488	495 nm	519 nm	Green
BODIPY 558/568	558 nm	569 nm	Red
tdTomato (RFP)	554 nm	581 nm	Red
Propidium iodide	537 nm	618 nm	Red
AlexaFluor 647	650 nm	671 nm	Purple

Supplement 1

Basic operation of Leica SP8

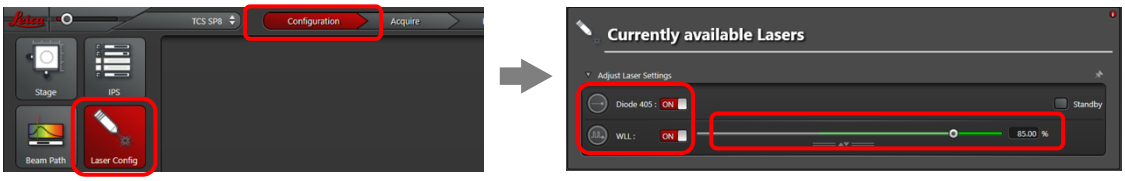
1. Start up
    1. Turn on PC Microscope, Scanner Power, Laser Power, and Emission
    2. Turn on the system and the mercury lamp for fluorescence
    3. Confirm the LCD panel appears on microscope
    4. Start the **LAS X** software on the desktop
    5. Select the **machine.xlhw** from the Configuration list and **DMi8** from the Microscope list

1-1 1-2 1-3 1-4 1-5



2. Set up lasers
    1. Open the **Configuration** tab to open the Configuration menu
    2. Click the **Laser Config** to open the **Currently available lasers** window
    3. Start the **Diode 405** and **WLL** (White light laser)
    4. Check WLL output is at 85%

2-1,2 2-3,4

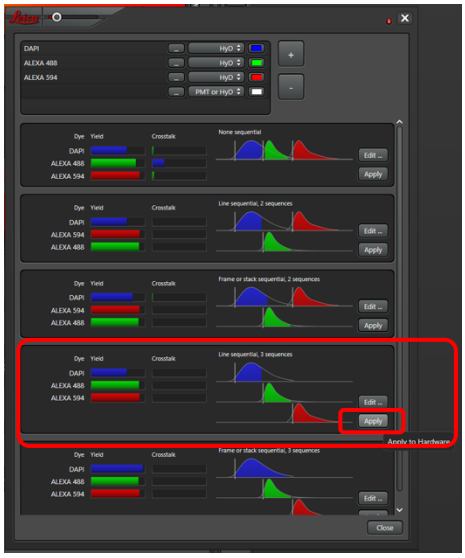


3. Set up fluorescent filter
    1. Open the **Acquire** tab at the upper part of the screen
    2. Click the **Configure the beam path with the Dye Assistant** to open the **fluorescent filter setting** window
    3. Select **HyD** (Hybrid detector) from the detector list
    4. Click the 『…』 button to open the Selecting Dye window and select the dyes to be used
    5. Repeat steps 3 to 4 for each dye selected

3-2 3-3 3-4

6. Click **Apply** on the **Line sequential (N) sequences** (N: number of fluorophores) pattern of the image acquisition patterns
7. Double click the bar indicating dynamic range of each fluorophore and enter the **End** value so that dynamic range is set to 50 nm for all excitation

3-6



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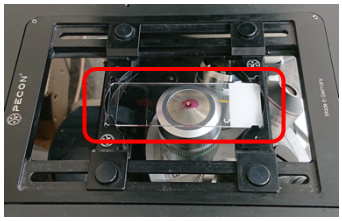
4. Image acquisition
   1. Place the specimen with the cover glass surface down on the stage
   2. Select the objective required from the **Objective** list

4-1



4-2

   3. Tap the **Contrast-Method** on the touch panel
   4. For visible light observation, select **BF** from the **Transmitted** list and tap **TL-Shutter** to open the shutter
   5. For fluorescence observation, select **FLUO** from the **Incident** list, select the desired fluorochrome (DAPI_LP, FITC_LP, RHOD_LP) from the **FLUO-Filtercubes** and tap **IL-Shutter** to open the shutter
   6. Bring the target area of the specimen into focus by using the XY-dial of the Smart Move controller while observing through eyepiece

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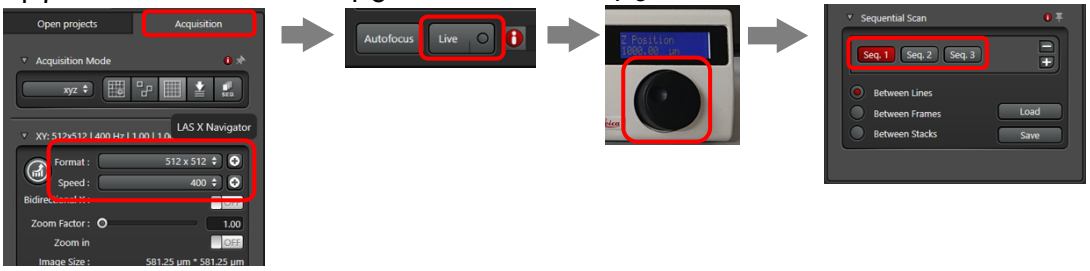
4-6

7. Select “512×512” or “256×256” from **Format** (pixel numbers of the acquired image) list and “400” from **Speed** (scan speed) list on the **Acquisition** window
8. Click **Live** at the lower left on the screen to start laser scanning and observe the live imaging on the screen
9. Adjust the focus with the focus dial on the right side of the control panel
10. Select the **cSeq.n** (n: the number of fluorophores) of the fluorophore for brightness adjustment on the **Sequential Scan** window

4-7 4-8 4-9 4-10



11. Adjust the brightness by using the laser output adjusting slider
12. Click **Live** to stop laser scanning
13. Select “1024×1024” from **Format** list and “400” from **Speed** list
14. Click **Start** to acquire image data
15 Repeat steps 1 to 10 for image data acquisition

4-11 4-13 4-14

5. Save images
   1. Open the **Open projects** tab at the upper left on the screen
   2. Click the **Save Project** on the right side of the *Project*
   3. Select the **DATA(E:)** HDD
   4. Enter a file name in the **File name** field
   5. Select **Leica Image File(LIF)(*.lif)** from the **Save as type** list
   6. Click **Save** to save image data
   7. LIF file can be processed with LAS X free software (Windows)
   8. If need, export the original file as TIFF, JPG, AVI, or MOV format.

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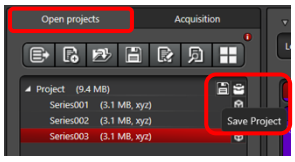
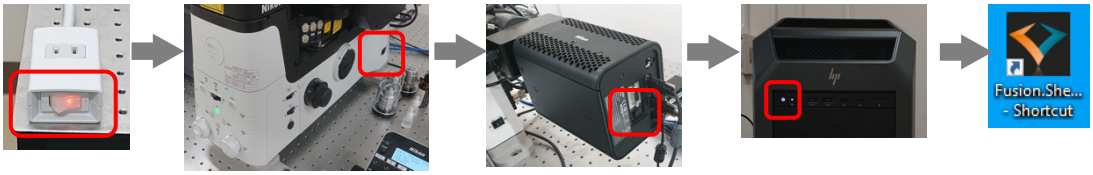


6. Shut down
   1. Open the **Configuration** tab on the upper part of the monitor
   2. Click **Laser Config** to open the **Currently available Lasers** window
   3. Deactivate the **Diode 405** and **WLL** lasers on the **Currently available Lasers** window
   4. Click the “X” at the upper right of the screen to close the **LAS X** software
   5. Shut down the PC and then Microscope, Scanner Power, Laser Power, and Emission
   6. Turn off the Laser power supply and mercury lamp
   7. Fill out the user record sheet

Supplement 2
Basic operation of Andor Dragonfly 201

1. Start up
    1. Turn on the main switch and wait 10 sec until hearing the alarm sounds twice
    2. Tun on the microscope, the LED lamp, and the PC
    3. On the screen, select your account and enter your password to log in
    4. Start the Fusion software

1 -1 1 -2 1 -4



2. Select fluorescence filters
    1. Click ◁ on the Remote Panel on the right of the screen to open the **Expander Panel**
    2. Open the **Protocol Manager** tab ( ) on the Expander Panel
    3. Click at the right side of the **Select Protocol** list to copy the pre-saved protocol
    4. Enter the name for the fluorescent filter setting in the **Name** field
    5. Click the **"+" (Add channel to the current protocol)** to select the fluorescent dyes
    Note: The fluorescent dye ending with "CF40_Sona2" should be selected.
    6. For color modification, open the **Channel Manager** tab on the Expander Panel and click **CHANNEL SETTING**
    7. Set **Auto Color** to **OFF** and click **Channel Color** to select the color to be modified and select a custom color from the color palette

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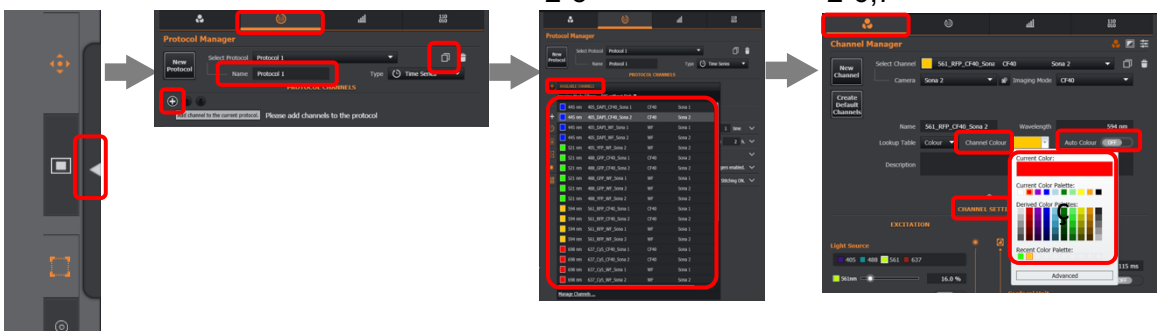
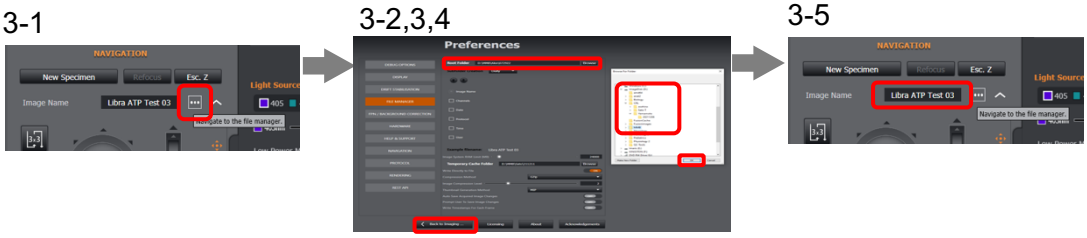


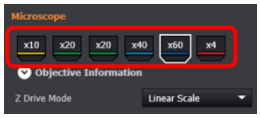
3. Setting the file location to be saved
    1. Open the **Acquisition Control** tab on the Remote Panel and click **"…" (Navigate to the file Manager)** on the **NAVIGATION** window
    2. Click **Browse** of **FILE MANAGER** on the Preferences window to open the **Browse For Folder** window
    3. Select the **Image Disk(D:)** HDD and create your folder
    4. Click **Back to Imaging…** to go back to the previous screen
    5. Enter a file name in the **Image Name** field on the Acquisition Control tab

3-1 3-2,3,4 3-5

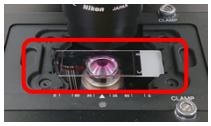


4. Observation of your slide before image capturing
    1. Select the objective required on the **Microscope** window
    2. Place the specimen on the XY-stage with the cover glass surface down.
    3. Select fluorescence filter on the XYZ controller
    4. Adjust the transmit light power if necessary
    5. Press the volume dial on the LED lamp controller to turn on the excitation laser light and adjust the brightness with that dial
    6. Turn off the excitation laser light by pressing the volume dial after the end of fluorescence observation

4-1



4-2



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5. Digital image acquisition
    1. Set **AUTO** to **ON** on the **CONTRAST** menu on the upper left of the **Application Bar**
    2. Open the **Acquisition Control** tab on the Remote Panel and click **Live** to start laser scanning and go to a live view of the current image
    3. Adjust the focus with the XYZ controller
    4. Click on the fluorophore set in **Protocol Channels** on the **Acquisition Control** tab and adjust the **Exposure Time** and **Laser** output for each fluorophore

    Note: The **Laser** output should be as low as possible and the **Exposure Time** should be used to adjust the brightness. If **Exposure Time** exceeds 1 second, the **Laser** output should be increased.

    5. Click  to display **Map Channels** and adjust the the image as required
    6. Click **Live** to stop laser scanning
    7. Enter a file name in the **Image Name** field
    8. Click **Acquire** to acquire image data
    9. Repeat steps 1 to 8 for image data acquisition

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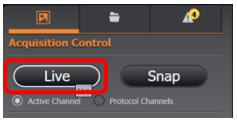
5-2



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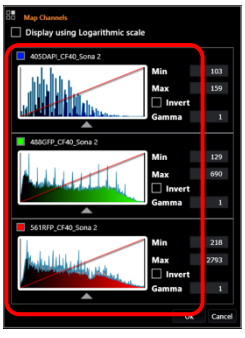
5-5



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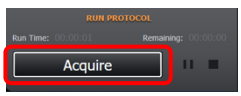

6. Export the image data
    1. Open the **Manage Files** tab and open the image to be exported from the RECENT IMAGES by double-clicking
    2. Click **Open in Imaris**
    3. Select **Save As** from the **File** menu
    4. Enter a file name in the **Image Name** field, select the file format (TIFF, JPEG etc.) from the **Save as type** list and click **Save** to save data
    5. Click the “X” at the upper right corner of the screen to close the **Imaris** software

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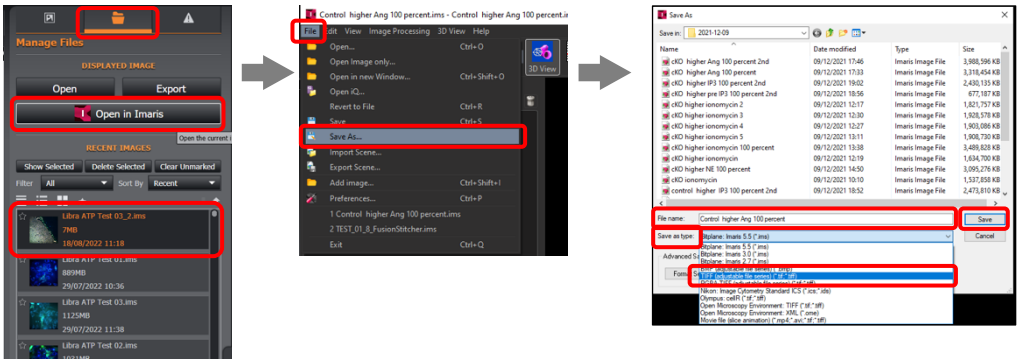

7. Shut down the system
    1. When the water/oil immersion objective is used, clean the tip of that objective using provided lens tissue
    2. Click the “X” at the upper right corner of the screen to close the Fusion software

    Note: Fusion saves all the parameter values while shutting down. No other PC activity should be performed until the **Shutting down** dialog box on the desktop disappears.
    3. Export the image data saved on Image Disk (D:) HDD to the external USB or HDD
    4. Shut down the PC
    5. Switch off the LED lamp
    6. Switch off the system on the right side of microscope
    7. Flip the main switch OFF on the left corner of the vibration isolated table
    8. Fill out the user record sheet

7-1 7-2 7-5 7-6 7-7