レーザー蛍光顕微鏡を用いた組織切片観察
Observation of tissue sections using microscopes
(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。

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

September 9, 2026, 17:00-18:20

Laser scanning fluorescence microscope

Kinji Asahina, Futoshi Toyoda, Takefumi Yamamoto, Yasuhiro Mori, Tokio Terado,
Kumi Okamoto, Akari Uno (Central Research Laboratory)

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)
Group A (English: room 408)
Group B (Japanese: room 407)

Leica Stellaris 8 (ST8): room 408 (Leica staff, Okamoto)

Leica SP8: room 407 (Yamamoto, Uno)

- Observe the stained section using Leica ST8 (**Supplement 1**) or SP8 (**Supplement 2**) confocal fluorescence microscope.
- Using the white light laser, we can detect different fluorescence signals.
- After using ST8 or SP8, attendants may experience other confocal microscopies such as Nikon AX (room 410) or Dragonfly201 (412).

Note. Fluorescence dyes and their characters

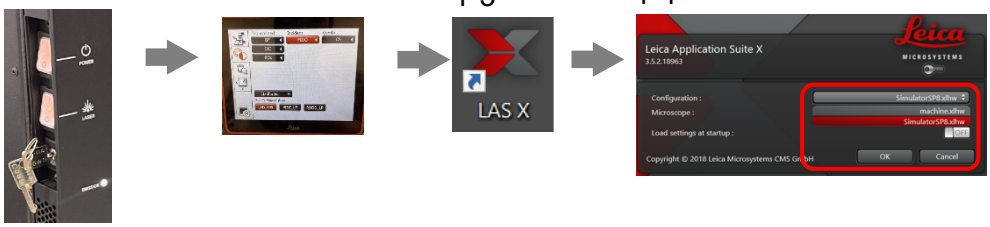
	Excitation	Emission	Color
DAPI	358 nm	461 nm	Blue
GFP	488 nm	509 nm	Green
AlexaFluor 488	495 nm	519 nm	Green
BODIPY 558/568	558 nm	569 nm	Red
tdTomato (RFP)	554 nm	581 nm	Red
Propidium iodide	537 nm	618 nm	Red
AlexaFluor 647	650 nm	671 nm	Purple

Supplement 1

Basic operation of Leica ST8

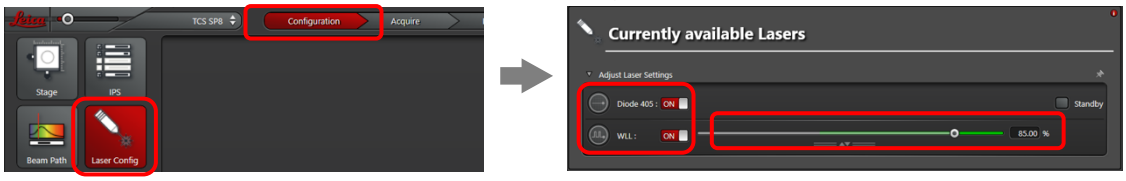
1. Start up
    1. Turn on Power, Laser, and Emission
    2. Confirm the LCD panel appears on microscope
    3. Start the **LAS X** software on the desktop
    4. Select the **machine.xlhw** from the Configuration list and **DMi8** from the Microscope list

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



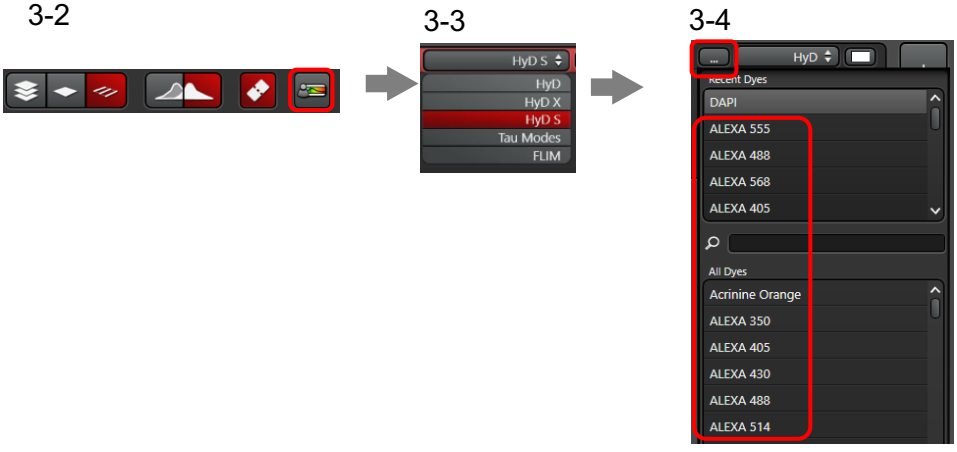
2. Set up lasers
    1. Open the **Configuration** tab to open the Configuration menu
    2. Click the **Laser Config** to open the **Currently available lasers** window
    3. Start the **Diode 405** and **WLL** (White light laser)
    4. Check WLL output is at 85%

2-1,2 2-3,4



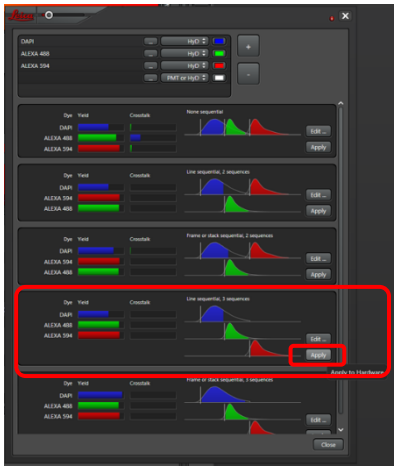
3. Set up fluorescent filter
    1. Open the **Acquire** tab at the upper part of the screen
    2. Click the **Configure the beam path with the Dye Assistant** to open the **fluorescent filter setting** window
    3. Select **HyD** (Hybrid detector), HyD S or HyD X from the detector list (When HyD is selected, the appropriate one of HyD S or HyD X is automatically selected)
    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

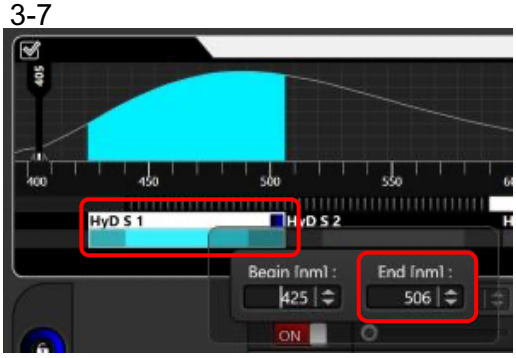


6. Click **Apply** on the **Line sequential (N) sequences** (N: number of fluorophores) pattern of the image acquisition patterns
7. Double click the bar indicating dynamic range of each fluorophore and enter the **End** value so that dynamic range is set to 50 nm for all excitation
8. Select the **Analog** (in case of HyD S) or **Digital** (in case of HyD X) from the Operating Mode list

3-6



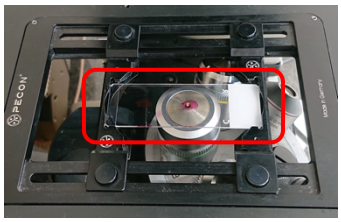
3-7



3-8

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

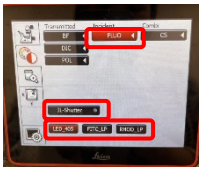
4-1



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

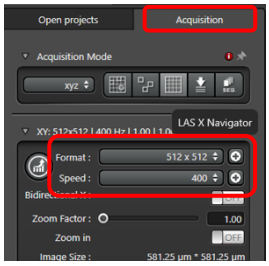
4-5



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 microscope, XY-dial or right side of the control panel)

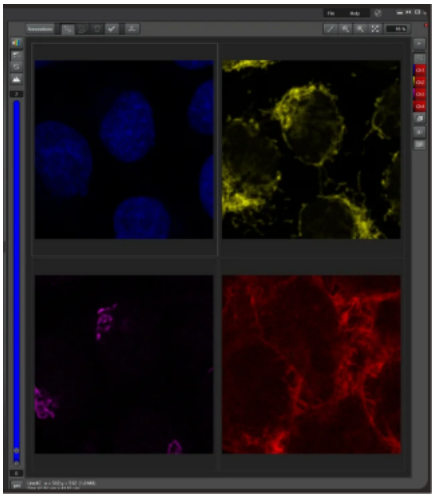
4-7



4-8

10. Select the image of the fluorophore for brightness adjustment on the **Viewer** window
11. Adjust the brightness by using the **Smart Gain** and **Smart Intensity** dial of the control panel

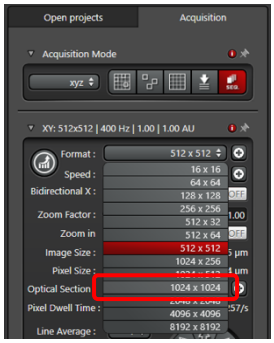
4-10



4-11

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

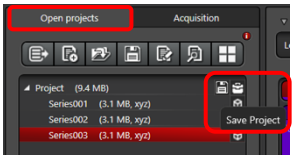
4-13



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.

5-1, 2



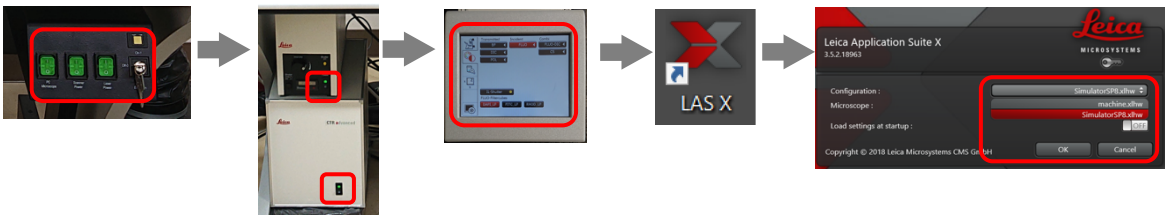
6. Shut down
    1. Open the **Configuration** tab on the upper part of the monitor
    2. Click **Laser Config** to open the **Currently available Lasers** window
    3. Deactivate the **Diode 405** and **WLL** lasers on the **Currently available Lasers** window
    4. Click the “X” at the upper right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

Supplement 2

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



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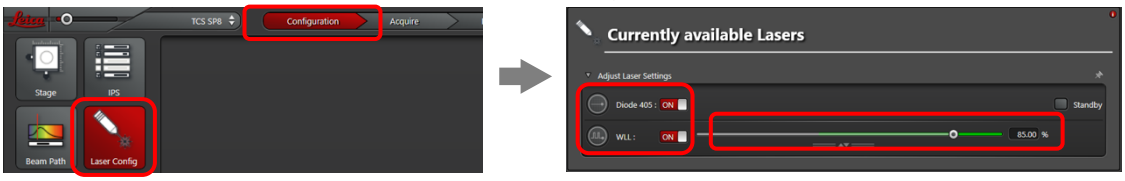

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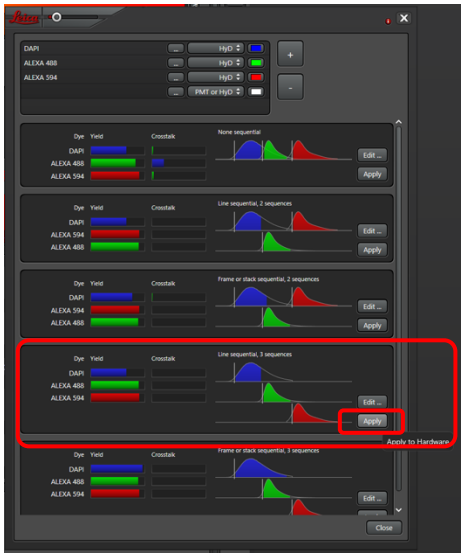

3-7

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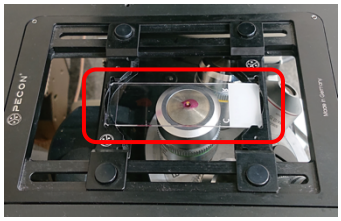

4-2

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4-3, 4

4-5

4-6

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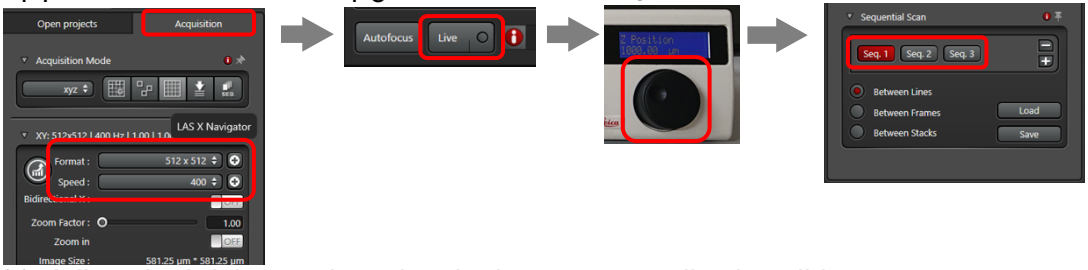
8. Click **Live** at the lower left on the screen to start laser scanning and observe the live imaging on the screen
9. Adjust the focus with the focus dial on the right side of the control panel
10. Select the **cSeq.n** (n: the number of fluorophores) of the fluorophore for brightness adjustment on 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

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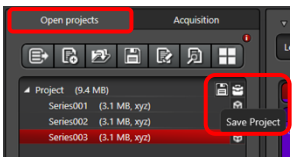

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   3. Deactivate the **Diode 405** and **WLL** lasers on the **Currently available Lasers** window
   4. Click the “X” at the upper right of the screen to close the **LAS X** software
   5. Shut down the PC and then Microscope, Scanner Power, Laser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