

凍結切片・パラフィン切片の作製方法
Preparation of tissue sections from frozen and paraffin blocks
(Practice in English/Japanese)

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この実習では、組織の凍結標本からクリオスタットを用いて凍結切片を、パラフィン標本からミクロトームを使ってパラフィン切片を作成する方法を習得する。また、ヘマトキシリン・エオシンにより切片を染色する方法を学ぶ。

We will guide you how to make sections from frozen or paraffin-embedded tissues using a cryostat or microtome, respectively. We will introduce how to perform Hematoxylin-Eosin staining.

Tissue sectioning

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Practice (17:00-18:20)

Sectioning: room 401, Central Research Laboratory
Microscope: room 404, Central Research Laboratory

Split the attendees into three groups

Group A (English): Experiment 1→2→3
Group B (Japanese): Experiment 2→3→1
Group C (Japanese): Experiment 3→1→2

Attention

Xylene should be handled inside the fume hood.
The section blade is very sharp.

Materials

Mouse liver frozen blocks

The adult normal mouse liver was fixed with 4% paraformaldehyde at 4°C for overnight. After fixation, the tissue was incubated in 15% sucrose / PBS for 4 hours and then 30% sucrose / PBS for overnight at 4°C. The tissue was embedded in the O.C.T. freezing medium, frozen with liquid nitrogen, and stored at -80°C.

Pig liver paraffin blocks

The adult pig liver was fixed with 10% formaldehyde. After fixation, the tissue was incubated in a series of ethanol from 70% to 100% and xylene and embedded in paraffin. The paraffin block has been kept in room temperature.

Pig liver paraffin sections

Paraffin sections were made from the aforementioned block.

Pig liver paraffin sections stained with Hematoxylin & Eosin (H&E)

The pig paraffin liver section was stained with H&E.

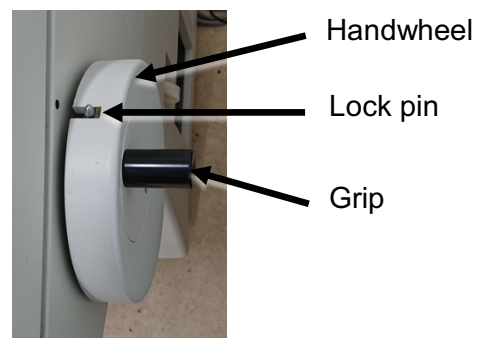
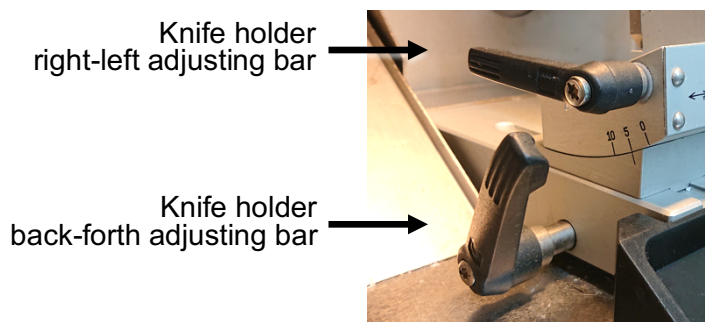
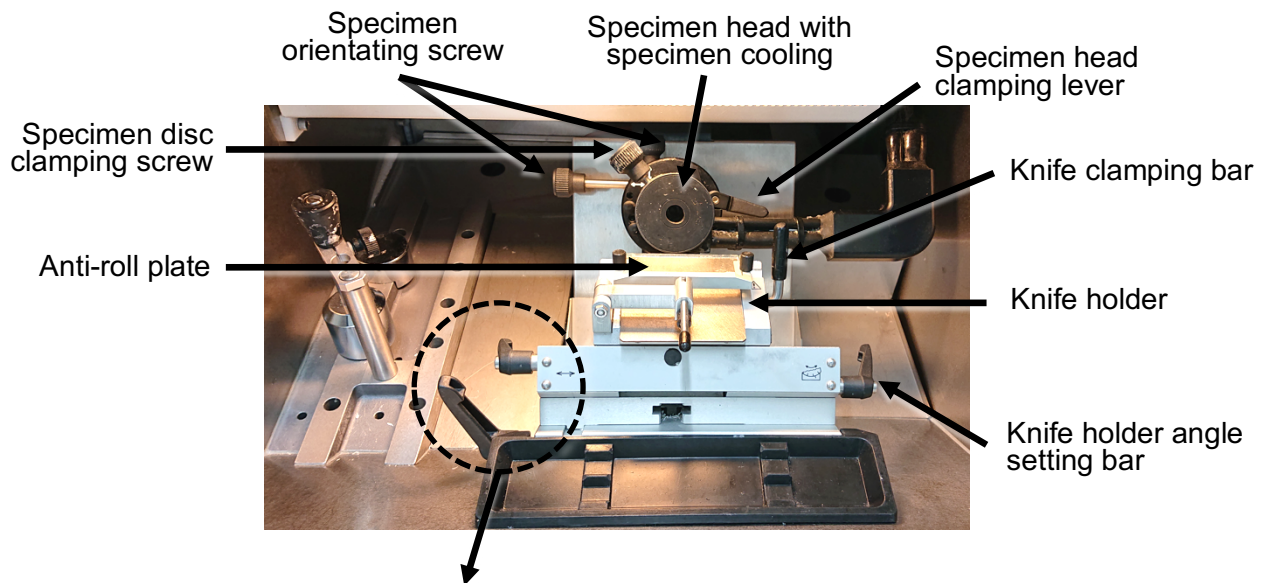
Experiment 1

Sectioning frozen mouse liver blocks using cryostat (Yamamoto, Okamoto)

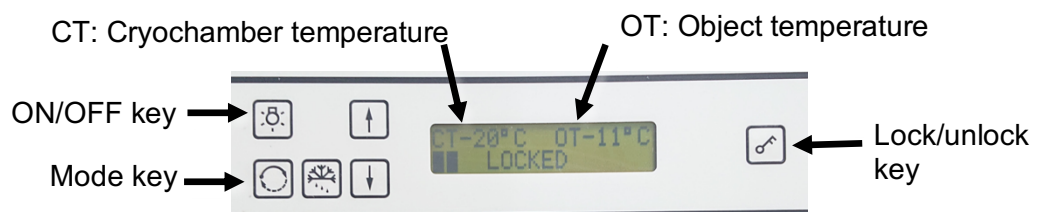
1. Set up Leica CM3050S Cryostat (See the detail in **Supplement 1**)
Confirm the cryochamber temperature (CT) is around -20°C
Set the object temperature (OT) at -20°C
Take the frozen sample from -80°C freezer
Put the sample into the cryochamber
Place the cutting blade into the cryochamber
2. Place the specimen disc on the left side hole inside the Cryostat
3. Add the O.C.T. medium on the specimen disc
4. Put the frozen tissue on the medium immediately
5. Gently push the sample from the top with a metal block
6. Wait until the medium freezes and becomes white
7. Place the specimen disc to the specimen head
8. Adjust its place with the screws and clamping lever
9. Make sure the specimen disc is firmly fixed
10. Adjust the cut angle around 5 on the left side of the knife holder
11. Insert a new blade into the knife holder
12. Slowly move the handwheel and adjust the cutting surface is close to the blade
13. Adjust the position and angle of the block against the blade
14. Cut the block around at 10-20 μm until the liver is being exposed
15. Slide the knife holder to the unused area that faces to the sample
16. Cut the block at 7 μm
17. Make better sections without wrinkles
(If the section does not look good, you may need to change the temperature, the cut angle, and change the blade position, etc...)
18. Mount the section onto the coated-slide glass
19. Dry sections at least several hours
20. Clean up the Cryostat
21. Store sections and frozen samples at -80°C

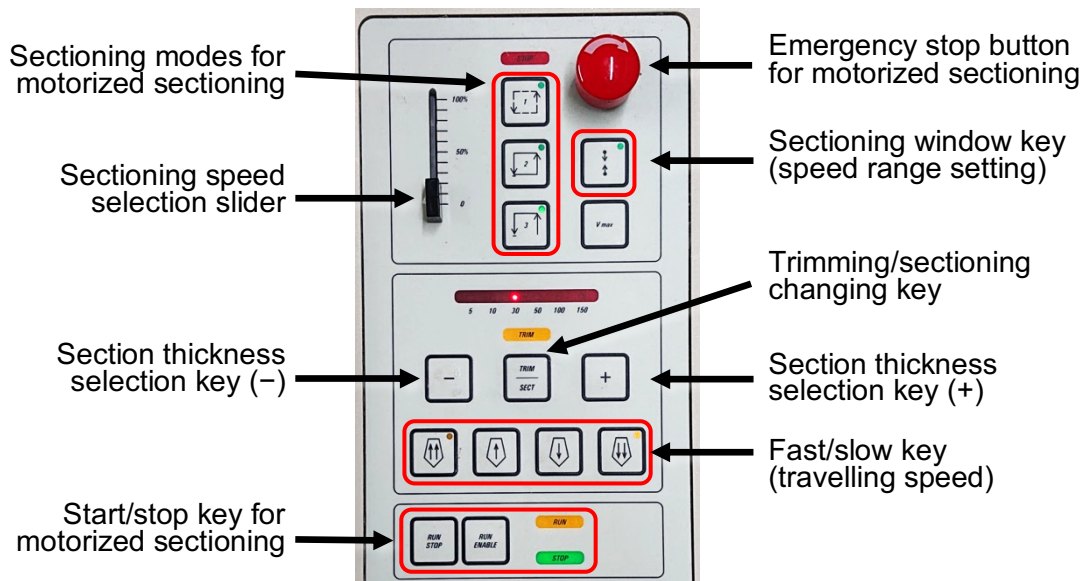
Supplement 1-1

Basic operation of Leica cryostat



Cryochamber illumination





Need to prepare in advance

- Microtome knives/blades: feather®C35, feather®A35
- Compound (tissue freezing medium): OCT compound, Leica FSC22
- Embedding well: cryomold (#1-3)
- Glass slides: MAS-coated slide glass is recommended



Checking the angle at knife holder

Select appropriate clearance angle around 5°C at the left side of knife holder

Start-up of the refrigeration system of specimen head

1. Display 『SET TEMP OT---°』 by pressing  once in control panel
 2. Start-up the refrigeration system by pressing  in control panel
- Note: Prior to loading specimen, make sure the specimen head temperature reaches the set value.

Inserting the knife

Insert a disposable blade into the knife holder



Trimming/sectioning

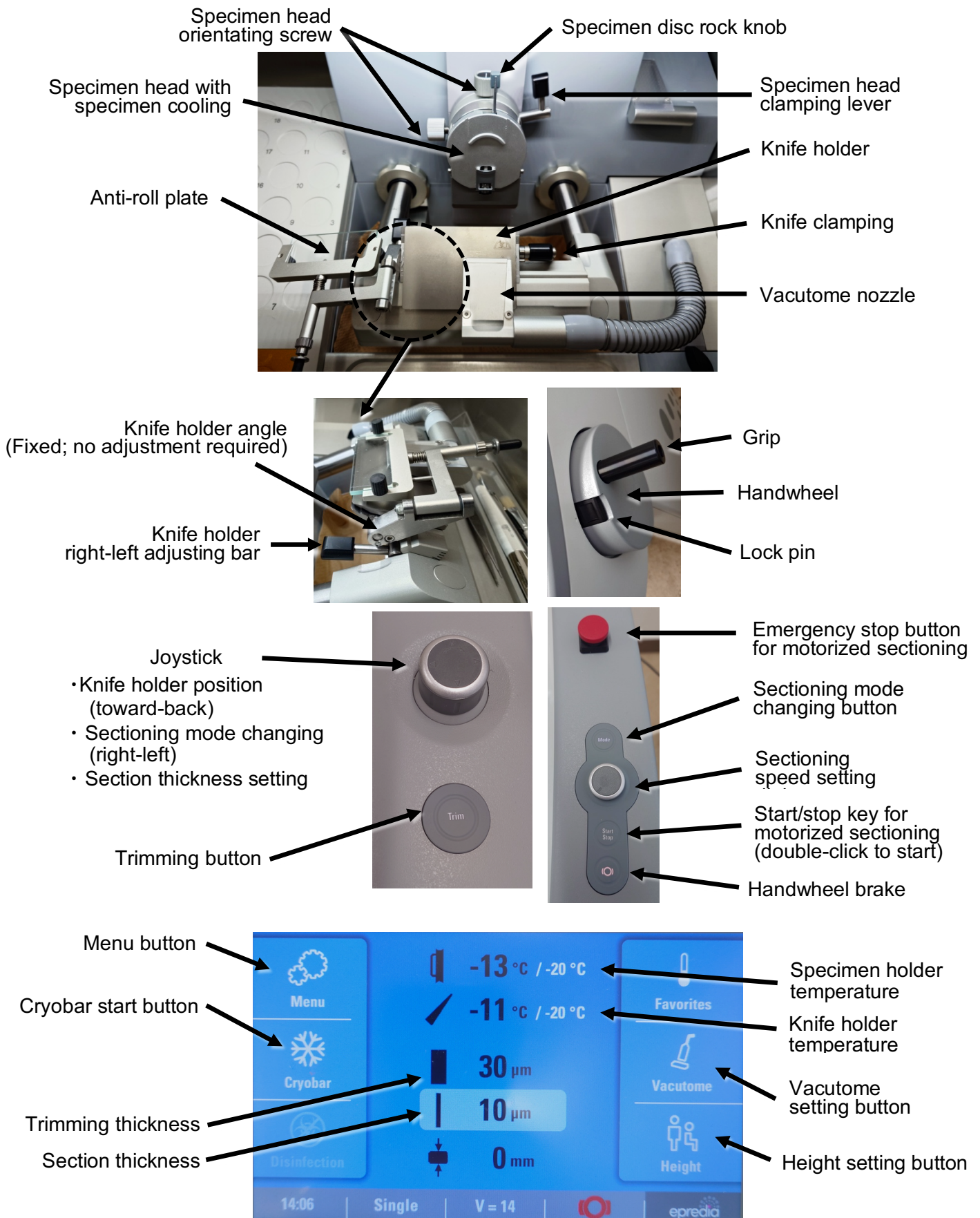
1. Place the specimen disc on the left cooling area
2. Put enough amount of the tissue freezing medium on the specimen disc
3. Quickly place the frozen sample specimen onto the specimen disc
4. Immediately push the sample with a metal bar and wait until the sample freezes
5. Place the specimen disc onto the specimen head
6. Adjust the angle and Z position of the sample against the blade
7. Activate trimming mode by pressing "Trimming" in control panel and trim specimen down to desired sectioning plane
8. Deactivate trimming mode and start trial sectioning (2 to 3 sections)
9. Place the anti-roll plate onto knife and start sectioning
10. Mount the sections on a glass slide

Finishing work

1. Remove the blade from the knife holder and clean up waste
 2. Display 『SET TEMP OT 20°』 by pressing  once in control panel
 3. Shut down the refrigeration system by pressing  in control panel
- Note: Do not shut down CT
4. Fill out the user record sheet

Supplement 1-2

Basic operation of PHC cryostat



Need to prepare in advance

Microtome knives/blades: feather®C35, feather®A35

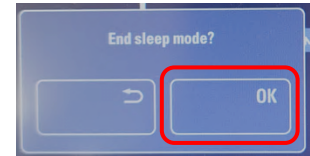
Compound (tissue freezing medium): OCT compound, Leica FSC22

Embedding well: cryomold (#1-3)

Glass slides: MAS-coated slide glass is recommended

Start-up of the refrigeration system of specimen head

1. Press the touch panel to wake it from sleep mode
2. Press OK of 『End sleep mode?』 on the touch panel to start-up the refrigeration system



Inserting the knife

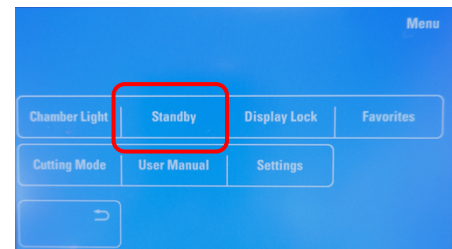
Insert a disposable blade into the knife holder

Trimming/sectioning

1. Place the specimen disc on the left cooling area
2. Put enough amount of the tissue freezing medium on the specimen disc
3. Quickly place the frozen sample specimen onto the specimen disc
4. Immediately push the sample with a metal bar and wait until the sample freezes
5. Place the specimen disc onto the specimen head
6. Press the Handwheel brake button on the left side of the unit to release the brake
7. Tilt the joystick either to the left or to the right to activate "Trimming" mode and trim specimen down to desired sectioning plane
8. Tilt the joystick either to the left or to the right to activate "Fine" mode and start trial sectioning (2 to 3 sections)
9. Place the anti-roll plate onto knife and start sectioning
10. Mount the sections on a glass slide

Finishing work

1. Remove the blade from the knife holder and clean up waste
2. Display 『Standby』 by pressing 『Menu』 on the touch panel
3. Shut down the refrigeration system by pressing 『Standby』
4. Fill out the user record sheet



Experiment 2

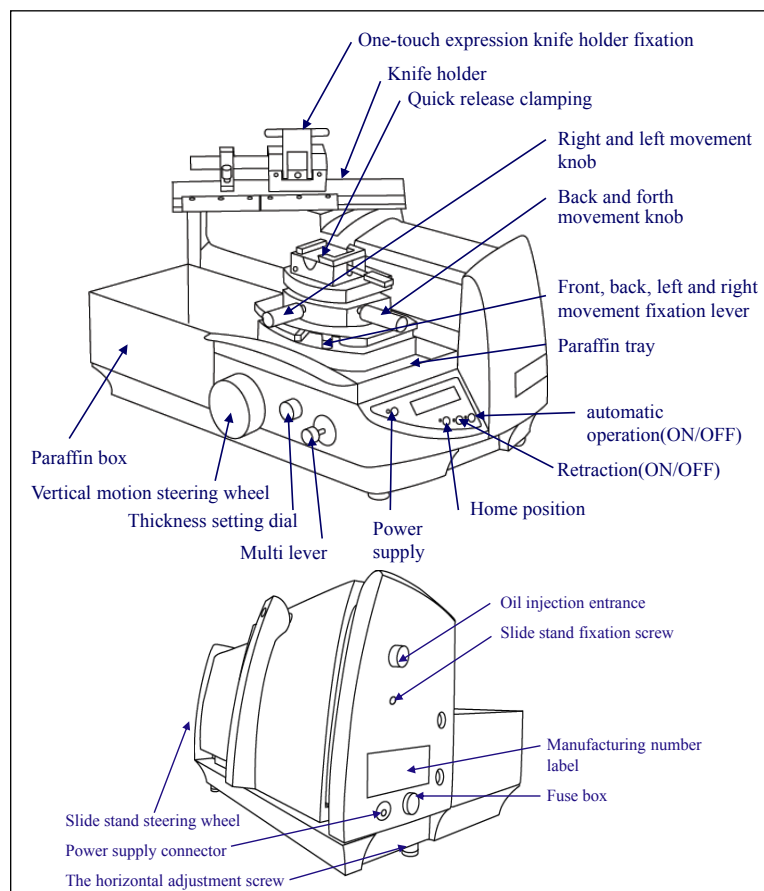
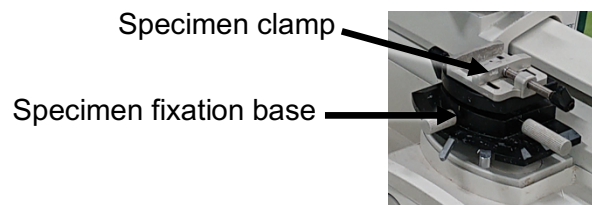
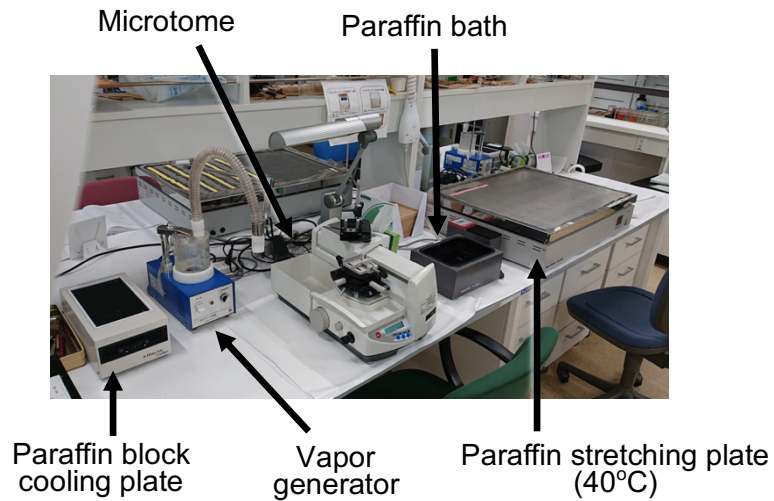
Sectioning pig liver paraffin blocks using microtome (Mori, Uno)

1. Set the paraffin block to the specimen holder (See the detail in **Supplement 2**)
2. Adjust its position and angle
3. Insert the blade to the knife holder
4. Place the knife holder to the microtome
5. Make sure the sample and knife holder are fixed firmly
6. Slowly move the steering wheel and adjust the cutting surface is close to the blade
7. Adjust the position and angle of the block against the blade
8. Cut the block around at 10-20 μm until the sample is being exposed
9. Slide the blade holder to the unused area that faces to the sample
10. Cut the block at 4-5 μm
11. Make better sections without wrinkles
(If the section does not look good, you might need to breath on the block to give moisture, change the block position, and change the blade position, etc...)
12. Float the section on the hot water in the bath at 40-43°C
13. Wait until the paraffin section becomes flat on the hot water
14. Mount on the regular slide glass
15. Dry sections on the paraffin stretching plate overnight
16. Clean up the microtome and associated equipment
17. Store sections at room temperature

Supplement 2

Basic operation of microtome

1. Install the specimen clamp on the specimen fixation base
2. Turn the vertical motion steering wheel counterclockwise or operate the multi lever down to move the specimen fixation base down
3. Set a paraffin block on the specimen clamp firmly
4. Insert a knife into the knife holder
5. Fix the knife holder to the knife holder fixation
6. Slide the slide stand steering wheel to front so that the knife's cutting edge is moved over the embedded block
7. Turn the vertical motion steering wheel clockwise or operate the multi lever up to move the embedded block close to the knife's cutting edge
8. Release clamping of the specimen fixation base by pulling the front-back-left-right movement fixation lever backward and adjust the inclination of the specimen fixation base so that it is parallel to the knife's cutting edge
9. Clamp the specimen fixation base
10. While sliding the slide stand back and forth, raise the specimen fixation base by gradually turning the vertical motion steering wheel clockwise
11. Cut the embedded block roughly
12. Adjust the thickness setting with the thickness setting dial
13. Breathe (steam) on the specimen to eliminate static electricity from the surface
14. Slide the slide stand slowly forward to make a thin section
15. Float thin sections in the paraffin bath for stretching
16. Put floating sections on glass slides and place them on the paraffin stretching plate
17. Clean the microtome and surrounding area after use
18. Fill out the user record sheet



Experiment 3

H&E staining of pig liver paraffin sections (Terado)

1. We have paraffin sections prepared from the pig liver sample
2. Place the slide into the slide holder
3. Turn on the power of the fume hood
4. Make sure there are enough reagents for staining
(Xylene, ethanol, Hematoxylin solution, Eosin solution, water)
5. Put the slide with slide holder into the glass coplin jar containing Xylene
6. Incubate the section in this order
 - 100% Xylene 1, 10 min (remove paraffin)
 - 100% Xylene 2, 10 min
 - 100% Xylene 3, 10 min
 - 100% Ethanol 1, 5 min (gradually replace Xylene to ethanol)
 - 100% Ethanol 2, 5 min
 - 100% Ethanol 3, 5 min
 - 90% Ethanol, 5 min (gradually replace ethanol to water)
 - 70% Ethanol, 5 min
 - Running tap water, 5 min
 - Deionized water, 2 min
 - Mayer's Hematoxylin solution, 5 min
(Hematoxylin stains nuclei and membrane in purple. Need to change this solution every 2 weeks)
 - Running tap water, 15 min
 - Deionized water, 5 min
 - Eosin solution, 5 min (Eosin stains cytoplasm in orange. Eosin is water soluble)
 - Deionized water, 5 sec (a few seconds are sufficient to remove excess Eosin)
 - 70% Ethanol, 5 sec
 - 80% Ethanol, 5 sec
 - 90% Ethanol, 5 sec
 - 100% Ethanol 1, 3 min
 - 100% Ethanol 2, 3 min
 - 100% Ethanol 3, 3 min
 - 100% Xylene 1, 3 min
 - 100% Xylene 2, 3 min
 - 100% Xylene 3, 3 min
7. After the last Xylene incubation, put the slide on the paper inside the fume hood
8. Drop the mounting medium on the section
9. Slowly cover the slide with a cover glass without trapping bubbles
10. Leave the slide inside the fume hood overnight
11. We have the pig liver section that was stained with H&E
12. Go to room 404 to check the slide
13. Observe how the section looks using Nikon ECLIPSE Ni-E
14. Take pictures using the Nikon Digital Sight 10 camera
(see the detail in **Supplement 3**)
 - Turn on the digital camera, microscope, and computer
 - Select Digital Sight 10 and passive mode and Start NIS-Element AR
 - Change the light path either Eye piece or Qi2 (camera)
 - Check the slide using a 4X or 10X objective lens
 - In the Acquisition tab, select Live and find the best position
 - Change the objective lens to 20X or 40X if necessary
 - Click Capture to take a picture and save the file

Supplement 3

Operation of the inverted microscope and digital camera

1. Start up

1. Switch on two power boxes, the CCD camera, and the PC in sequence
2. Double-click the 『NIS-Elements AR』 icon on the desk top
3. When the *Setup and Configuration* window comes up, select either 『Acquisition』 or 『Offline Analysis』 mode and click **OK**

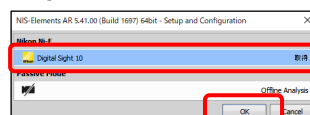
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1-2



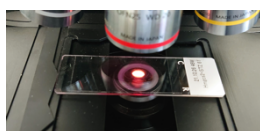
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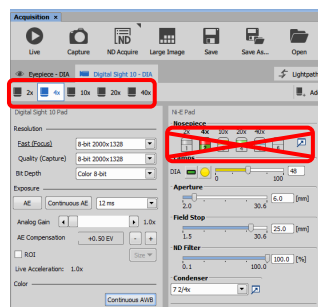
2. Observation through eyepiece

1. Place your slide glass on the motorized XY-stage
2. Select the objective on the *Acquisition* window of the right monitor
3. Switch the image display through eyepiece by selecting the 『Eyepiece - DIA』 tab
4. Find the area you are interested in at the center of the objective using the motorized XY-stage control rod
5. Focus on the image while observing through eyepiece

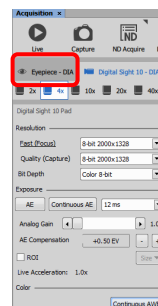
2-1



2-2



2-3



2-4

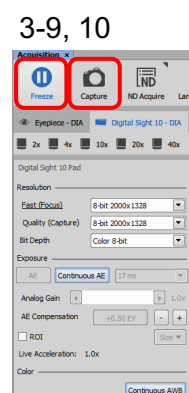
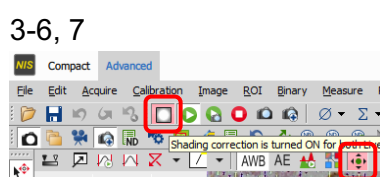
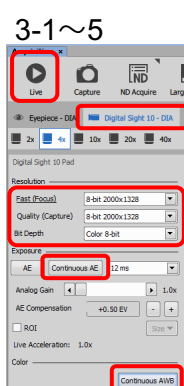


2-5



3. Image acquisition

1. Select the 『Digital Sight 10 - DIA』 tab on the *Acquisition* window
2. Select 『8-bit 2000×1328』 for Resolution from both the 『Fast (Focus)』 and 『Quality (Capture)』 list on the *Acquisition* window
3. Select 『Color 8-bit』 from the 『Bit Depth』 list
4. Activate 『Continuous AE』 and 『Continuous AWB』 with click
5. Click 『Live』 to monitor the live imaging on the display
6. Select the 『Shading Correction』 button at the top of the live imaging window with click and remove uneven light from the image
7. Select the 『Mouse XY』 button with click to allow using mouse scrolling
8. Center the target area of the specimen and focus on it
9. Click 『Live』 to stop the live imaging
10. Click 『Capture』 to capture image



4. Save images

1. Select 『Save As』 from *File* menu
2. Select the 『DATA DRIVE1(D:)』 HDD on the left side of the *ImageSaving* window
3. Create your folder with an affiliation name in the 『DATA DRIVE1(D:)』
4. Define the file name by entering a file name in the *File name* field
5. Select 『ND2 Image File Format(*.nd2)』 from the *File format* list and click *OK*
Note: nd2 format contains all the parameters with the image data.
6. You may save your image as TIFF or JPEG file

4. Shut down

1. Select 『Exit』 from *File* menu to close the NIS-Elements software
2. Export the image data saved on 『DATA DRIVE1(D:)』 HDD to the external USB or HDD
3. Shut down the PC for Ni-E
4. Switch off the CCD camera
5. Switch off the power boxes for Ni-E under the vibration isolated table
6. Fill out the user record sheet