CF Advanced Microscopy and Imaging - Paris Lodron Universität Salzburg

Histology Introduction and Training

The introduction includes instructions on safety measures, how to operate the machines to embed your sample in Paraplast and how to cut Paraplast blocks and OCT-Tissue Tek blocks. You will then undergo an appointment-based training session, after which you will be able to create slides to visualize specific data in the cells using staining and dyeing methods.

Once you have been through the **Introduction** with a Core Facility manager, either Gerti Achatz or Daniela da Costa Santos (ext.# 5977) you will have *access to* the instruments, the online booking system (Clustermarket), and the Teams CF group.

Read the following instructions carefully!

Always wear lab coat! Use gloves when you are handling chemicals and cleaning the machines!

❖ Room E-2.053 (Incubators and Embedding Station)

All Manuals are in the labeled drawer beside the Embedding Station. The online versions (in English) are in the CF website. PLEASE read the necessary ones so you will handle the machines correctly!

Note: use the Clustermarket booking system (online) to Reserve the machines beforehand and stick to the time you have booked.

Protocol Suggestion for end of dehydration and embedding the samples in Paraplast (PP):

- You can store your Xylol bottle in the **cabinet for the chemicals** which is always locked.
- The key for it is on a plastic box on the shelf beside the Fume hood.
- Transfer your sample cassettes carefully to the first Xylol (I) glass Cuvette and avoid contamination of the 100% EtOH; if it happens you should discard the Ethanol. After Xylol incubation do not forget to *lock* the chemical cabinet again.

The heavy doors of the cabinet for the chemicals close automatically! Be careful so they don´t knock the glasses out of your hands!

- 100% Xylol I 40 min
- 100% Xylol II 40 min

} under the Fume hood, at room temperature.

➢ PP/Xylol glass Cuvette and 100% PP(I) glass Cuvette are *stored* directly in the <u>top</u> incubator with exhaust fan.

- 1:1 PP/Xylol 60 min 60°C <u>top</u> incubator
- 100% PP I 60 min 60°C <u>top</u> incubator
- **From here onwards there is no more Xylol in the sample.**
- 100% PP II ON 60°C *bottom* incubator

Embedding Station

DO NOT CHANGE THE **MACHINE'S** SETTINGS!

- **Bring** *Paraplast Plus* (Carl Roth, Cat.# X881.1) for the machine.
- Fill the information required on the notebook on the left side of the embedding machine. You will be charged based on the number of embedded samples.
- *Switch on* the machine (big black switch and the green light ON) before starting the embedding with time enough to melt the Paraplast:
  - 30 min before in case you have up to 20 samples. It would need ¼ of Paraplast in the reservoir.
  - 1 hour if you have around 50 samples, which needs a full reservoir of Paraplast.
  - There is also an option to set an automatic start, for an early schedule or when you are the *only* user at the day. Talk to us if you want to use this option.
- *Switch on* the cooling plate – it takes approximately 10 min to be cold.
- Place the glass with your samples in PP into the right-side reservoir (it has melted PP inside) to keep the PP in the glass melted.
- Open the Paraplast dispenser valve turning it to the right until you have the desirable amount of PP coming out of it.
- Take a plastic mold from the big box next to the machine or bring your own. (The CF has metal mold to lend to you if preferred.)
- Pour PP on it enough to cover the sample space and leave it on the heated plate of the machine.
- Take your sample with warm tweezers from the cassette and place it in the mold in the position required for the sample, applying light pressure, then transfer the mold to the *cooled area*. Throw away the cassette lid.
- Put some more PP on top of it.
- Press the cassette's labeled grid on top of the mold and fill it up completely with PP.
- Place the mold-cassette slowly on the cooling plate and wait for it to harden – it takes about 15-20 minutes.
- Remove the block (PP-sample-grid) from the mold using tweezers; clean the mold and place it back in its box.

  Note: You can use the heating plates at both sides of the embedding machine to melt the PP excess off the paraplast-sample block. Storage the paraplast blocks at room temperature.

- When you finished, *switch off*: embedding machine and cooling plate.
- **Clean** both machines well with paper towel; scrape off the PP with the big PLASTIC spatula from the heating plate, the cooled area, and the bench; empty the two PP dispenser drawers under the heating plates; place two sheets of paper towel on the cooling machine.

❖ Room E-2.003 (Microtome, Cooling Plate and Cryotome)

All Manuals (in German) are in the labeled drawer beside the Microtome. The online versions (in English) are in the CF website. PLEASE read the necessary ones so you will handle the machines correctly!

Microtome

DO NOT CHANGE THE SETINGS OF THE WATER BATH AND THE SLIDE DRYER!

- **Bring**: slides, one pair of tweezers, one paint brush, 2l of distilled water in plastic bottle, and blades.
- Put up to 1.5 l in the water bath and turn it on. Its temperature is set to 40°C.
- *Switch on* the light, it will help you to see your sample much better.
- *Switch on* the cooling plate and place your samples.
- *Switch on* the slide dryer machine. Its temperature is set to 40°C.
- *Switch on* the Microtome.
- Wait for about 15 min to cut your blocks; time for sample to harden. During this time, you can place the blade and label the slides (hint: use pencil 2H); you could also bring them ready. Suggestions for label: sample's name, slide number, date, µm, something specific about this sample, etc.
- Be careful when placing the blade on its holder.
- Make sure that there is no excess PP around the cassettes before placing them in the metal holder in a vertical direction.
- Before cutting, slowly move the block holder/sample closer to the blade. Check that the sample is behind the blade, which would prevent the block from breaking!
- Use the left side of the blade for trimming using 10-15 µm. Use the middle and right positions for sections with 3-6 µm.
- Cut 4-5 connected cuts and transfer them to the water bath where the cuts will float and stretch.
- Thereafter fish the cuts with Superfrost or Ultrafrost slides out of the water bath.
- Place it on the slide dryer.

Note: if you have difficulty to have a strait PP slice, carefully clean the blade with hand paper sliding it from the bottom to the top of the blade holder. If it does not help, place your sample again on the cooling plate.

For your own safety: when you are not cutting, place the safety guard (red) on the blade and activate the locking mechanism.

- ➢ When you finished, *switch off*: Microtome, Cool plate (dry it), Slide Dryer (unplug it) and Water bath (disconnect the cable and unplug it).
- ➢ **Remove the blade** and either keep it with your stuff or place it in the slide trash.
- ➢ **Clean** all machines with paper towel.
- ➢ Bring the **water bath** to the Room E-2.053 (embedding room) and pour the water into the sink. Dry it with paper towel and bring it back.

Cryotome

- **Bring**: *OCT-Tissue Teck* gel bottle, slides, one pair of tweezers, and blades.
- The Cryotome is **always on**.
- To keep its temperature low the Air conditioner is always on therefore leave the door closed.
- The OCT-Tissue-Tek samples should be either on dry-ice or normal ice in a closed styrofoam box.
- Bring a slide holder to let the slides drying slightly at RT and a slide box to keep them after ~15 min inside the ice box.
- Set the Chamber and Block holder Temperatures. On the wall above the machine there is a list of Temperatures suggested to some kind of tissues.
- Leave the slides prepared with the information you want, outside the chamber; you can leave some on its glass lid.
- Place your OCT-sample blocks (max. 10) inside the cold chamber.
- There are two metal specimen disks already in the chamber, cold and ready for you to attach your samples to it, using the OCT gel. If you have more than two samples pay attention if your third sample is well fixed before cutting it.
- Place one sample on the block holder and move the block holder towards you (Fig.1 #2).
- Position the block at the same angle as the blade (Fig.1 #15).
- Trim the block (remove the excess of OCT) using 15-30 µm (Fig.1 #2).
- Cut the block once you have reached your specimen using 10 µm turning the handwheel (on the right side) slowly forward. Cutting using less than 10 µm may damage your sample.

  Note: if you have difficulty to get a flat slice for your slide, clean the blade with hand paper and close the glass lid for around 5 minutes.
- Use a paint brush to stretch the slice. Open the glass lid only enough to place your hands inside; the temperature inside the chamber can increase very fast.
- Place one slide facing down close to the slice on the desired position and touch the slice with it. The slice will attach to the slide immediately.
- Place this slide on the slide holder and after 10-15 min into the slide box.

➢ When you *finished* use the paintbrush to gather the slices and pieces on the waste tray (Fig.1 #16) and suck all the pieces inside the chamber with the vacuum cleaner (Fig.1 #1 and #14). Clean the blade holder and the chamber with hand paper.

5.1 Control panel fields and cryostat chamber

1 Control panel field 1: Extraction, temperature and time control, illumination, UV disinfection
2 Control panel field 2: Electric coarse feed (sectioning and trimming thickness adjustment)
3 Control panel field 3: Motorized sectioning, optional (adjustment of stroke type, cutting speed etc.)

Fig. 11

4 Heat extractor, stationary (optional)
5 Peltier element (with 2 stations)
6 Freeze shelf, 15 positions
7 Position holder on freeze shelf
8 Heat and cold extractor, mobile (opt.)
9 Shelf, movable (optional)
10 Blade holder CE with blade ejector (a)
10b Finger rest on the blade holder CE
10c Knife guard on the blade holder CE
11 Extraction nozzle on the extraction hose
12 Extraction hose for section waste
13 Brush shelf (optional)
14 Adapter piece for extraction hose (the coarse filter insert is behind it)
15 Object head, directional
16 Waste tray

Fig.1

- **Acknowledge the** Core Facility **at:**
  https://www.plus.ac.at/biosciences/facilities/core-facilities/cf-advanced-microscopy-and-imaging/?lang=en

- **Machines' Manuals at:**

  - Embedding Station Leica EG1150H User Manual
    https://www.plus.ac.at/wp-content/uploads/2023/07/1_Embedding-station-Leica-EG1150H-user-manual.pdf
  - HistoCore ArcadiaC User Manual
    https://www.plus.ac.at/wp-content/uploads/2023/07/2-HistoCore_ArcadiaC_User-Manual.pdf
  - HistoCore AUTOCUT Rotary Microtome User Manual
    https://www.plus.ac.at/wp-content/uploads/2023/07/3_-HistoCore_AUTOCUT_-Rotary-Microtome_User-Manual.pdf
  - Cryotome CM1950 User Manual
    https://www.plus.ac.at/wp-content/uploads/2023/07/4_Cryotome_CM1950_User-manual.pdf