

Laboratory for Advanced Electron and Light Optical Methods

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January 17, 2012

Cutting in Samples For Cryostat Sectioning and Light Microscopy

I. Purpose: To cut samples in so that they will be properly cryofixed and section well with a cryostat.

II. Materials Needed:

Stainless steel 100 ml beaker
Ring stand with ring and wires from which to suspend beaker
Small plastic soup thermos
New carbon-steel razor blades
3 X 5" card stock or something similar
Forceps for handling the card with attached sample
Dewar for holding liquid nitrogen and frozen samples
Liquid nitrogen

III. Procedure:

A. General: Pieces of tissue to be frozen should not be overly deep (2-3 mm deep is the recommended maximum depth) and should be of the minimum surface area needed for the study to lessen sectioning artifacts. The specimen planchets to which they will be attached are approximately 2.5 cm in diameter, so samples in the 1 cm diameter size range are good. Since freezing does not extend to great depths from the surface of the sample, it is wise to use a razor blade to cut the surface of the sample to a smooth surface prior to freezing. **Do not cut numerous sections from a sample to smooth the face and to produce a full-face section. You will quickly go beyond the depth of good freezing and have poor sample preservation in evidence.**

B. Freezing samples:

1. Preparing the Sample for Freezing: After trimming the sample to an appropriate height and overall size as described above, place the sample on a small piece of card stock with a corner turned up so that the card with the attached sample can be easily grasped for plunging into the cryogen. The card can be labeled with a pencil to identify it when stored.

Freezing the sample by putting it into OCT on a planchet and then placing the cryostat anvil on top of the specimen in a -20°C cryostat cabinet can be done with some samples, but is not the recommended choice for good sample freezing because of the relatively low freezing rate.

2. **Concept of Freezing using Liquid Nitrogen-Cooled Isopentane:** Liquid nitrogen is a relatively poor cryogen because it has a boiling point of approximately -196°C . It drops the sample temperature at only $16,000^{\circ}\text{C}/\text{sec}$, and the sample instantly becomes surrounded by an insulating bubble of nitrogen gas. This is because the heat of the sample causes the liquid nitrogen to boil. A superior freezing method for cryostat work uses isopentane cooled in liquid nitrogen to freeze the sample. The melting point of isopentane is -160°C , but drops the sample temperature $45,000^{\circ}\text{C}/\text{sec}$ because the boiling point of isopentane is $+28^{\circ}\text{C}$.
3. **Procedure:** After affixing the samples to card stock, fill the soup thermos with liquid nitrogen and put it on the base of the ring stand supporting the stainless steel beaker. Fill the beaker with 25-30 ml of isopentane and lower the beaker into the liquid nitrogen. As the liquid nitrogen boils off, replenish the soup thermos. When the bottom of the isopentane-containing beaker begins to frost up internally, quickly plunge the card stock with affixed sample into the isopentane. Hold under the surface for about 30-45 seconds. The sample should generally change color (becoming lighter) as it freezes. After it has frozen, quickly transfer the card and sample to a dewar containing liquid nitrogen for storage, transport to a storage area, or immediate transport to the cryostat.
4. **Sample Storage:** If samples need to be stored, it is best to put them in a low-temperature freezer (-80°C). They should be stored in the smallest containers possible in the freezer to minimize sublimation into the surrounding air, leading to freezer burn. If stored at -20°C , they will probably show evidence of freezer burn within a week or two. Samples stored at -80°C will generally be fine for months.
5. **Attaching Samples to Cryostat Planchets:** Quickly remove the samples from the liquid nitrogen and put into the -20°C cabinet of the cryostat. Quickly add a drop of OCT to the surface of a planchet, place the card stock with sample card-stock side down onto the OCT and place the cryostat anvil on top of the sample. This will press the card down into the near room-temperature OCT while keeping the sample cooled from the top (the anvil) while being somewhat insulated from the room-temperature OCT by the card stock.
6. **Cutting Sections:** Follow the instruction booklet for the cryostat.