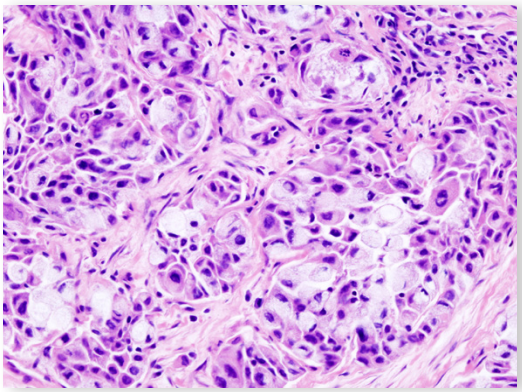
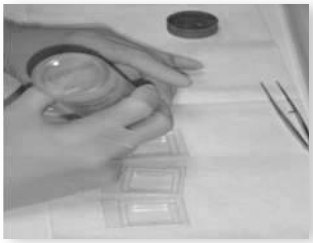
Protocol ♦ Sample Embedding & Freezing

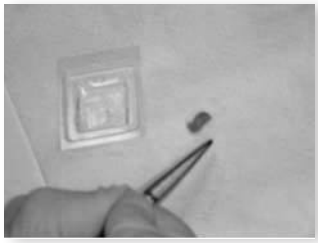
The preparation of sample slides for microdissection begins with the acquisition of tissue. The most common methods used are tissue biopsy, needle aspirations and cell smears. This protocol is an overview of how to process tissue samples (e.g. tumor biopsies) for easy and efficient laser microdissection. At the same time, it helps you to optimally preserve tissue quality for downstream molecular analysis of DNA, RNA, or proteins in order to achieve high quality data. Please read this protocol carefully and then follow these steps quickly and accurately.



Materials:

- Cryomold
- O.C.T. Compound (Tissue-Tek)
- 70 % Ethanol to sterilize work area
- RNase free PBS
- Dry ice
- Liquid nitrogen
- Isopentane
- Disposable gloves
- Kimwipes (or other lint-free towels)
- - 80 °C Freezer
- Sterile forceps and scalpel
- Cryogenics vials and storage box





Method:

For tumor biopsy using OCT embedding media:

Note: Tissue will have inherent RNases, DNases and Proteases. To minimize their activity it is important to process sample as soon as possible after biopsy removal from the animal. Delay in processing may compromise biomolecule integrity.

- Wipe down work area and employ RNase-free technique
- After excision of biopsy, rinse with RNase-free PBS
- Limit biopsy size to 0.5 x 0.5 x 1 cm (reduce size if needed according to desired orientation for optimal anatomical morphology)
- Pre-label cryomolds and coat bottom surface with OCT (tissue freezing media)
- Add biopsy in proper orientation and cover with additional OCT
- Freeze immdiately by one of the following methods:
  1. Floating in an iso-pentante bath chilled with dry ice (preferred)
  2. Placing in a perti dish floating in liquid nitrogen
  3. Placing in powdered dry ice

Note: Do not plunge directly into liquid nitrogen, this negatively affects morphology

- Transfer to dry ice for transport and store at - 80 °C