Mohs Surgery: Frozen Section Techniques

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Goals of this Talk
- educate the listener about purposes of mohs surgery
- discuss the fundamentals of mohs histology and its application to the surgery.
- explain the frozen section techniques utilized in mohs histology.
- review processing complex tissues for mohs histology.

What is Mohs Surgery?
Mohs Surgery is a skin cancer removal technique in which pre-diagnosed surgical margins are removed and mapped by a surgeon, and is processed using frozen sectioning. The outer circumference and deep margin are viewed in the same plane histologically.

The surgeon acts as pathologist and microscopically examines the specimen slides, mapping and removing any remaining cancerous tissue.

The surgery/frozen sectioning/pathology is repeated until the site location is free of tumor. Proper techniques with mohs surgery yield a 98% Non-recurrence rate.

Brief History of Mohs Surgery:
- Dr. Frederic Mohs performed medical student research using zinc chloride and bloodroot paste.
- Zinc chloride paste was used to remove skin cancers on patients starting in June 23, 1936.
- 1936 Dr. Mohs coined the term chemosurgery.
- 1944-1956 Dr. Mohs is sole advocate for the “fixed tissue technique” zinc chloride fixed itself to the cancerous tissue which allowed for complete removal.
- Early 1950’s he used fresh tissue techniques for removal of BCC’s in areas where zinc chloride paste would be dangerous (eyelids, lips, etc.).
- 1970’s Perry Robbins, M.D. modified the “Mohs Technique” using fresh tissue frozen histology.

Overview of the Mohs Frozen Section Techniques:
1. specimen orientation and mapping
2. prepping and inking the tissue
3. embedding the tissue
4. sectioning the tissue in the cryostat
5. staining the slides with Hematoxylin and Eosin
6. Coverslipping the slides

Specimen Orientation and Mapping:
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- each specimen should have a distinct 12 O’clock marker
- mapped there should be distinct 12, 3, 6, and 9.
- prepping to lay flat is best completed prior to inking.
- inks properly applied show full representation & orientation

Prepping to Lay Flat:

- bisected specimens should be cut in two with one clean swipe
- outer margin is prepped to lay flat by angling the scalpel
- most effective relaxing incisions are parallel with the epidermal rim
- incisions are made 1/3 way from outer circumference
- care must be taken not to cut through the specimen

Outer Rim and Sides of the Specimen must be prepped to lay in the same plane as the deep margin.
Inking/Chromacoating the Specimen:

- cut edges or epidermal rims must be inked completely
- take care to avoid inking the underside of the specimen
- remember: true margin=either full ep. or full ink present
- different ink colors should meet to avoid un-inked gaps

Embedding the Tissue:

- specimen must be placed on the mounting slide or cryo-mold so that the full deep and out margin are in contact with no bubbles or gaps
- look at the underside of the slide or mold to verify complete margin contact

Do Not Distribute or Duplicate
Embedding the Tissue:

- using a warm chuck/button cover both chuck/button and specimen with tissue freezing medium
- combine both so liquid tfm melds and freeze together
- prepping to lay flat is best completed prior to inking
- gently freeze to avoid epidermal/tfm separation

Sectioning in the Cryostat:

- remove the mounting slide/cryomold be warming with your hand and gently pulling.
- angle specimen block so it is equidistant from the microtome blade

Staining Protocol:

- Fixation
- TFM Dissolving
- Nuclear Stain-Hematoxylin
- Differentiation**
- Blueing
- Cytoplasmic Stain-Eosin
- Washing and Dehydration
- Clearing
- 99% Alcohol
- Tap Water
- Gill's 3 Hematoxylin
- Acidified Water
- Tap Water/Blueing
- Eosin Y 1% Alcoholic
- 99% Alcohol
- Xylene Substitute

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Staining and Coverslipping

- run slides through the staining protocol
- agitate slides in each station
- blot slides between each station to minimize cross-over carry contamination
- coverslip with appropriate cover slipping medium

Critical Concepts for Mohs Histology

- specimen must be prepped to lay flat without cutting through from superficial to the deep margin.
- once embedded, the specimen must be cut as shallow as possible while attaining 100% representation of the mohs specimen histologically.
- Specimen is known to have cancer present superficially, we are trying to stay underneath the "known superficial cancer" to determine if the surgeon has gotten underneath & around the cancer site to "clear margins".
- any areas of the margin found to contain tumor/cancer will be removed with an additional "stage" of mohs (tissue removed /processed/ slides read).

Mohs Truisms

- **High Noon:** 12'Oclock Orientation must always be maintained
- **1/3 at 30°:** cut 1/3 in from outer circumference at 30° from the horizon to prep to lay flat effectively
- **Specimen Exposed:** Embed specimen so true margin is exposed in the block
- **Equidistant:** Adjust block so face is Equidistant to the microtome blade.
- **Deep to Shallow:** cut deep enough to get complete margin, then stay shallow

Complex Tissues:
Processing

- Suture in the Specimen
  - remove suture whenever possible
  - suture present will niche blades repeatedly
  - thick cuts may be the only way to get tissue represented
  - suture specimens are typically from recurrent locations
**Fatty Specimens**
- Can take up to 15 min. to completely freeze
- Complex tissue composition may result in epi folding
- Can be frozen colder with liquid nitrogen/freeze spray
- May require thicker cuts

**Cartilage Specimens**
- Requires positively charged/poly-l-lysine slides
- Wants to retain its original shape
- May require squash/freeze technique to get flat
- Must be run through the stains very gently

**Lip Tissue**
- May contain oral mucosa/Lip Vermilion/Epidermis
- Has very little fat
- Muscle/connective tissue present very often
- Complex tissue specimens require extra care determining rim margin.
Periosteum
- can contain scalp fat and deep hair follicles
- very vascular (blood clots must be blotted away
- extremely elastic and easy to mis-orient.
- thin layer of elastic tissue lining the skull
- take very few discards between sections

Eyelid Tissue
- can have epidermal and conjunctival epithelium borders
- may contain eyelashes in specimen
- aggressive cases can involve the lacrimal duct
- may involve wedge tissue processing.

Processing an Eyelid Wedge

In Summary:
- Mohs surgery is a microscopically controlled skin cancer removal technique in which the Mohs histotechnician is a critical component producing slides for margin evaluation.
- Specimen prepping involves mapping, chromacoating, and applying relaxing incisions to the specimen.
- Specimen embedding is critical to ensure that all deep and circumferential margins are represented early in sectioning.
- Cryostat sectioning must be performed so as to stay as shallow in the block as possible while getting 100% of the specimen margin represented.
- Mohs surgery has a "leap frog" approach where more tissue is removed/processed/pathologically until all margins are "clear or free of tumor".
- Complex specimens can be challenging for the Mohs technician but must be represented fully for accurate removal of the skin cancer present.