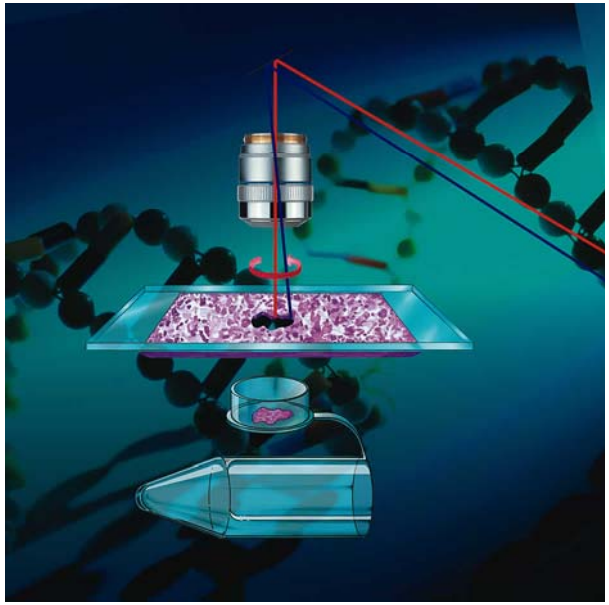
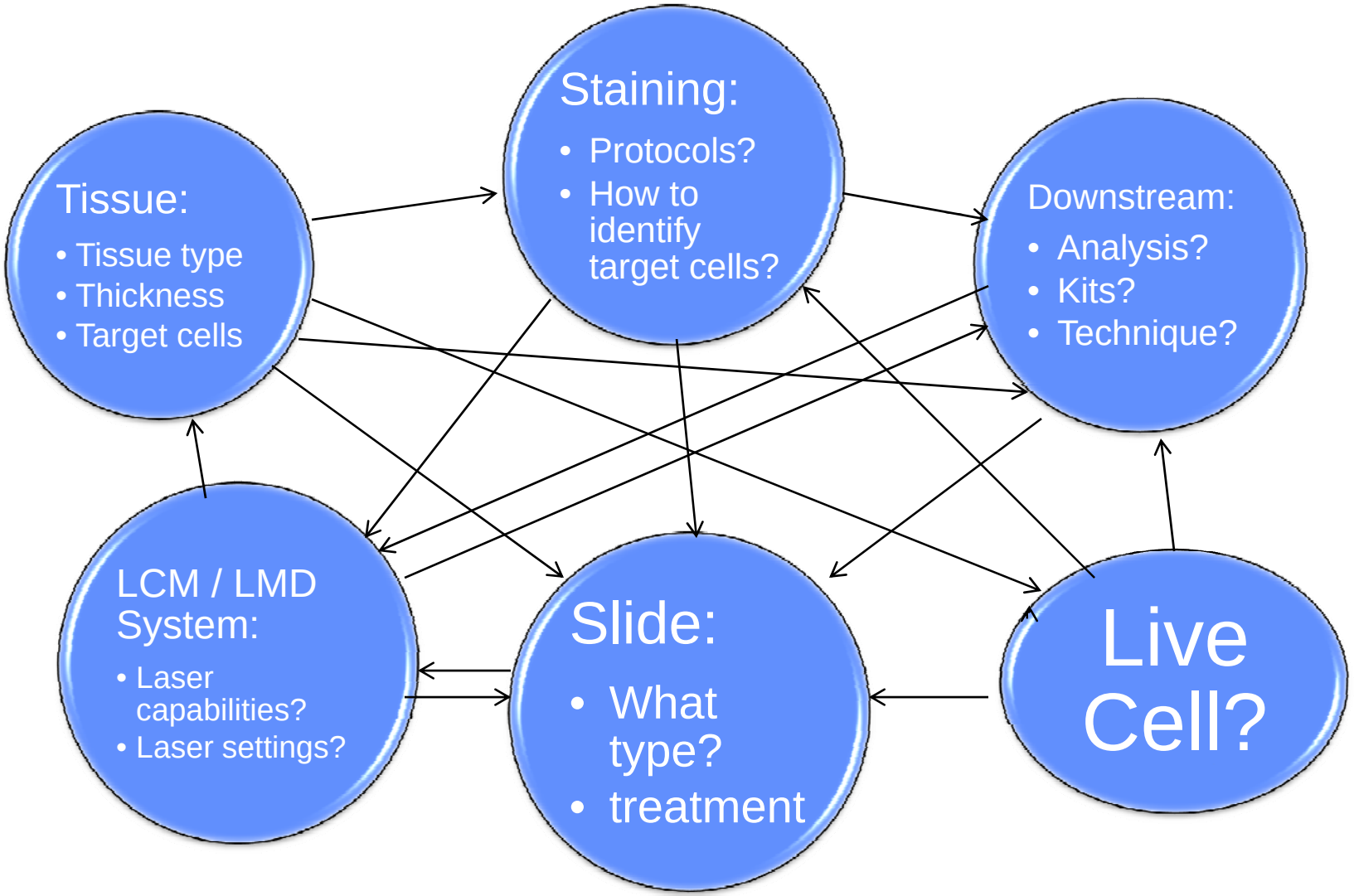
Sample Preparation Considerations for Laser MicroDissection



ABRF Meeting, March 2012

What are the Considerations for Sample Preparation for LCM / LMD?



Leica LMD Protocol Guide

Leica MICROSYSTEMS

Leica MICROSYSTEMS

Living up to Life

Sample Preparation For Laser Microdissection

Protocol Guide for Leica Microsystems Laser Microdissection Systems

Staining Protocols

- Less is More
- Adjust protocols for minimal stain to allow identification of target cells.
- Less stain can improve contrast in the image.
- Use no stain.
- Use stained sections on one slide as a navigational tool to identify areas of interest on unstained sections on another slide
- Use software module to transfer the regions of interest from a properly stained section on one slide to unstained serial sections on other slides.
- Fluorescent tags require special consideration.

Typical Sample Preparation for Laser Microdissection for Frozen Sections for Animal Cells

- Cut sections 4-20 um
- Mount on chosen slides
- Fixation with 70% EtOH for 30 sec. minimum
- Wash in DEPC water for 30 sec.
- Stain with Mayer's hematoxlin for 30 sec.
- Wash in DEPC water for 30 sec.
- Counterstain with eosin for 30 sec.
- Rinse with (95%) and (100%) EtOH for 30 sec.
- Air dry approximately 5 minutes

Fixation

Preserve the tissues; preserve the macromolecules; stop metabolism; allow visualization identification; better LMD.

Recommended

- Cryofixation, a good choice
- Fixation by ice-cold acetone, 30 – 45 seconds
- Fixation by graded alcohols up to 70%

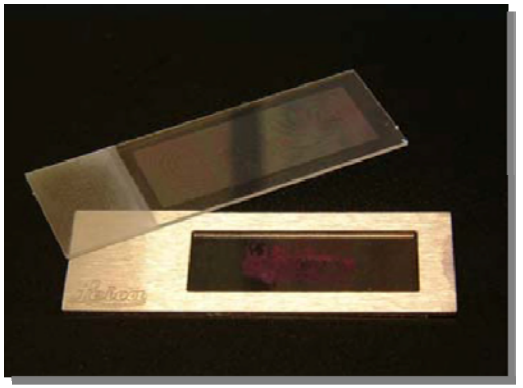
Other

- Xylene fixation for short times. (Xylene substitute not recommended.)
- Formalin fixation @ 4% will cross link RNA,
- Good fixation will allow for better LCM/LMD

Fluorescence Considerations

- Over-dehydration by Alcohols, or Xylene may cause both immunofluorescence and fluorescent proteins (e.g. GFP) to deactivate.
- Recommend series of alcohol fixation/dehydration up to 70% Ethanol.
- Recommend short time fixation/dehydration in Xylene.
- GFP is a common fluorescent protein tag; it varies widely by manufacturer. Contact with polymers may cause GFP to deactivate. Use a coating (e.g. poly-l-lysine) as a protectant.
- Choose a low auto-fluorescent slide (e.g. PPS, FLC, EP).

Choose the Right Slide



- Systems using foil slides:
- PET (polyethylene terephthalate) recommended for MALDI, SELDI ionization analysis.
- PEN (polyethylene naphthalate) not recommended for any ionization analysis.
- Expression Pathology EP Director slides recommended for proteomic analysis, for large areas; low auto fluorescence background.
- Low fluorescence background slides with PPS, FLC, EP.

Pre-treating Slides

- UV irradiation prior to mounting specimen has beneficial effects:
  - Makes all slides RNase, DNase, Protease free and sterile.
  - Makes foil slides more adherent for tissue.
  - Makes laser cutting of foil slides more efficient.

Know your LCM / LMD System

- What are the capabilities and limitations of the laser for cutting ROI's?
- What is the maximum sample thickness your system is capable of cutting/capturing?
- How can you optimize the system parameters for cutting/capturing your particular specimen?
- How can you save the laser parameters?

Some References

Citations:

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