Microscopy Technical Guide  
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Summary: The goal of this guide is to provide a high level and technical overview of microscopy techniques, and terms found while encountering work for BRAIN Initiative and any other project work a member of RTI International may encounter. This document is not meant to be exhaustive or used as a citation, rather an informal guide to help staff better understand complicated microscopy principles. With that in mind, the tone will be casual and informative.

Fixation

* The chemical process by which tissue is “frozen in time”. Fresh tissue is often placed in a chemical fixative, such as an aldehyde, to stop the process of putrefaction and decay. The fixative’s job is to leave the tissue, cells, and environment in a “native-like” state for examination.

Clearing

* Tissue clearing is the process by which tissue is “cleared” to be sectioned or further examined via microscopic techniques. This means taking tissue and treating with chemicals to cause the tissue to become more transparent. Due to the need for “thicker sections” to fully scan or visualize 3D portions of tissue, it has become more important to render tissue transparent. This allows scanning and creation of 3D models due to optical sectioning.

Embedding

* The process by which tissue is embedded into a material, often plastic resin, or paraffin wax, to then be preserved for examination. The embedding process does multiple things: it ensures the tissue is placed into a stable form for cataloging and examining. Capable of being stored for years. It allows the tissue to then be sliced via microtomy for further microscopy techniques.

Ultramicrotomy

* Microtomy and ultramicrotomy is the mechanical process of using a microtome. A device/tool in which the user places an embedded piece of tissue in a chuck (holding cradle) to be sliced into very small sections.

Sectioning

* The process by which a block of tissue is “sectioned” into ultra-thin sections. These sections can then be viewed microscopically, often after being placed onto a glass slide. The sections are obtained by placed the block of tissue in a microtome and cut using a specialized knife. These knives often have a diamond or gemstone edge, for longevity and sharpness. Due to having to cut plastic. Paraffin wax is often cut with disposable steel blades, to help reduce chance of contamination. The knives often have a “boat” or area upon which water makes contact with the knife edge so that tissue sections can float. There they can be picked up or even imaged.

Tissue block

* A block of tissue that has be embedded. Most often in paraffin wax or Epon-plastic resin. These tissue blocks can be stored for many years and are sectioned in a microtome.

Dehydration

* A chemical process in which tissue has water molecules removed. This is often done through a series of graded alcohols or ethanols. Acetone can also be used. This is done as embedding materials such as wax or resin are hydrophobic and would not penetrate completely.

Refractive Index

* The ratio of the speed of light in a vacuum to its speed in a specific medium (material). Basically, the higher the RI, the “denser” something is. Or the harder it is for light to travel. This usually means the higher the RI the harder it is to see through. This is important for lasers and beams of light. Due to the nature and technique of much of microscopy.

Optical Sectioning

* Creating a “Z-stack”. Imaging a piece of tissue or a sample by scanning it from top to bottom. By sectioning several sections or creating a stack, a 3-D image can then be created from the often hundreds of smaller sections or images. Think of cutting an onion into a hundred horizontal slices and scanning from above, and then reassembling that onion. But, now you can completely view every part of that onion three dimensionally.

Fluorescence Microscopy

* The use of light emitted as specific wavelengths to illuminate specimens.

Confocal Microscopy

* These specimens are often dyed or stained with probes or fluorophores which will react to the specific wavelength of light. Thus causing the “glow” or fluorescence. Use a spatial pinhole to block out of focus light in image formation.

MOST

* Micro-optical sectioning tomography (MOST). Created to image an entire “fixed” mouse brain, specifically with a focus on circuitry + connectivity. Developed alongside advancements in tissue clearing, to render an entire brain “clear” or see-through. Individual neurons can be visualized at a 3D level.

fMOST

* Use of fluorescent dyes and molecular probes to enable tracing between structures, as well as connections between neurons.

Light-Sheet fluorescence microscopy (LSFM)

* An adaption from confocal microscopy that uses a sheet of light versus a point of light to give greater contrast and reduce background noise (signal). It is also faster at acquiring images than traditional confocal microscopy. Often used on chemically cleared organisms.

Transmission Electron Microscopy (TEM)

* A microscope that uses a power supply, tungsten filament, and a beam of electrons as its light source. It produces a black and white two-dimensional image at high resolutions. A specialty in showing cellular morphology.

Scanning Electron Microscopy (SEM)

* Similar to the TEM, the SEM produces three-dimensional images. SEM by default scans the surfaces of specimens and substances but can be enhanced to perform optical sectioning.

References

[Review of micro-optical sectioning tomography (MOST): technology and applications for whole-brain optical imaging [Invited] (nih.gov)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6701528/)

[Clarifying Tissue Clearing (nih.gov)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4537058/)

[Optical sectioning microscopy - PubMed (nih.gov)](https://pubmed.ncbi.nlm.nih.gov/16299477/)