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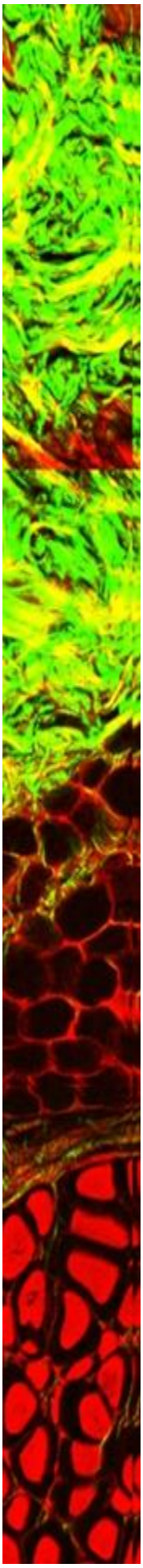
Tissue Preparation Guidelines for 2D Imaging on Genesis non-Linear Microscope



1. Tissue sample preparation for paraffin-embedding

Tissue fixation

For animals, cardiac perfusion with saline, followed by a 10% formalin was performed to flush out blood cells. If biochemical studies need to be performed on the tissue, a 10% formalin flush should not be used as it may interfere with subsequent analysis. For routine stains where perfusion is not required, tissue is sectioned and drop-fixed in a 10% formalin solution. Fixative volume should be 20 times that of tissue on a weight per volume; use 2 ml of formalin per 100 mg of tissue. Due to the slow rate of diffusion of formalin (0.5 mm hr), tissue should be sectioned into 3 mm slices on cooled brain before transfer into formalin. This will ensure the best possible preservation of tissue and offers rapid uniform penetration and fixation of tissue within 3 hours. Tissue should be fixed for a minimum 48 hours at room temperature.

Parrafin infiltration

Tissue should have a thickness of not more than 3 mm thick with an area of 20 mm x 30 mm before it is embedded into parrafin blocks.

Sectioning tissues to bond to glass slide

Tissues are sectioned to a thickness of 50 um (for 3D imaging) and 5 um (for 2D imaging). Paraffin sections are placed on the warming block in a 65°C oven for 20 minutes to bond the tissue to the glass without coverslip & deparaffin before imaging.

http://protocolsonline.com/histology/sample-preparation/paraffin-processing-of-tissue/

2. Tissue sample preparation for cryosectioning

Tissues are fixed in fresh 4% paraformaldehyde on ice for 5-10 minutes and washed repeatedly with 1X PBS for 5 minutes. They are then transferred to 30% sucrose, until the tissue sinks before the tissue is transferred through a 1:1 mixture of OCT:sucrose and the into OCT. The tissue is then placed in the cryomold, overlayed with OCT before it is oriented and frozen quickly on dry ice. Once the tissue is in the mold with OCT, it should be oriented and frozen quickly because a film can form on the top of the mold and make moving the tissue difficult. If the mold is small enough, place each block into an eppendorf tube and store at -80°C. Cut 25 um sections (For multiphoton imaging) and place on silinized superfrost slides.

http://www.protocol-online.org/cgi-bin/prot/view_cache.cgi?ID=613

3. Tissue sample preparation for fresh tissue

Specimen was fixed in a 3:1 mixture of 100% ethanol and glacial acetic acid for 60 minutes at room temperature before it was washed with 70% ethanol for 15 minutes and rinsed with deionized water. It is then dehydrated through graded alcohols and cleared in toluene, prior to mounting with microscope slides with Permount. For deep-tissue imaging, tissue are cut in 20 um sections.

Non-paraffinized specimens

The non-paraffinized specimens were fixed in formalin (10%), dehydrated through alcohol passages, and directly immersed in a 1:1 mixture of mineral oil and glycerol, under vacuum (to degas the tissue and aid penetration) for 2 hours at room temperature, without having undergone paraffin embedding. (The organs were imaged with their outer surface in contact with the coverslip—no cutting or sectioning was performed)

Paraffinized-Deparaffinized specimens

The DP specimens were first processed as normal for histopathology, being fixed in formalin, dehydrated, then embedded in paraffin wax. Then the block was deparaffinized by first melting the block at 65° C, then using xylene and alcohol passages, and infiltrated with a 1:1 mineral oil and glycerol mixture under vacuum for 2 hours at room temperature. Lungs were inflated before fixation to preserve alveolar structure. (The organs were imaged with their outer surface in contact with the coverslip—no cutting or sectioning was performed)

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3750743/
http://www.sciencedirect.com/science/article/pii/S0006349507713565
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