Best Practices: Section-based Atlas Construction

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Tissue Protection Against Freezing

To limit tissue damage during subsequent tissue freezing step, brain tissue must be cryoprotected. Tissue is immersed in an increasing concentration (10%, 20%, and 30%) of sucrose in fixative. For each solution, the tissue is left in solution until it sinks. Graduated series reduces osmotic shock.

Tissue Freezing

Tissue is frozen using a double bath: methanol with a dry ice slurry is place in the outer bath and isopentane is placed in the inner one. The tissue is immersed in the inner chamber. This approach avoids $C0_2$ gas (generated by the dry ice sublimation) from limiting heat transfer from the tissue to the solution.

To limit ice crystal formation, tissue freezing must be rapid. Yet, for tissue as large as a mouse brain, if freezing is too rapid, the outer tissue will freeze before the inner core and this will lead to cracking of the brain. An effective compromise is at a rate of immersion into the cold isopentane solution at a rate of about a cm/min.

To minimize gross distortion, the tissue is frozen with the ventral surface resting on a plate. An effective freezing apparatus, and one we utilizes, is constructed of a lab jack to elevate the solution while the tissue is placed on a stationary Saran wrapped (to reduce adhesion of frozen tissue) metal plate suspended from a support stand.

Tissue Cutting

Cryosectioning typically relies on an anti-roll plate to aid in section collection. This approach, however, greatly distorts the section. Instead, commercial tape support system (Instrumedics) can be utilized which can yield high quality sections with limited distortion (Nissanov et al., 2001). This system relies on tape that is adhered to the tissue block prior to each cutting stroke. It supports the cut section. Once cut, the tissue can be transferred onto a polymer coated glass slide. The section is then

bonded onto the slide using a UV flash to cure the polymer. Once bonded, the tape can be removed and the tissue treated in the same manner as conventionally collected section.

Blockface Imaging

To aid in image registration, the tissue is imaged prior to each cutting stroke. To do so, the tissue must be accurately repositioned under the imaging camera prior to each stroke. To avoid perspective distortion, the imaging plane and blockface must be parallel. Only modest resolution is required for an effective subsequent registration.

Our approach employs a large horizontal cryostat, the Leica CM3500. Ours has been extensively modified to include an accurate photostop and facilities for imaging (Nissanov et al., 2006). The tissue is reposition with submicron accuracy under the camera following each stroke. To image we employ a Canon 5D digital SLR equipped with a Canon 180mm Macro lens to yield a pixel pitch of 9.9μm.

Section Staining

Shown in Table 1 is an effective Nissl stain protocol. To mix the 0.5% cresyl violet solution, dissolve 2.5g cresyl violet acetate powder in a 500 ml solution of distilled water. Stir the mix on overnight on low heat. The next day filter and add 2 drops of acetic acid. To mix the 95% ethanol with acetic acid solution, add 10 drops of acetic acid per 100ml of 95% ethanol.

Table 1. Cresyl violet staining.

Solution	Immersion Duration
CitriSol, Xylene, or other clearing agents	2 min
100% ethanol	3 min
95% ethanol	3 min
70% ethanol	3 min
distilled water	3 min
0.5% cresyl violet	5 min
distilled water	brief rinse (5 dips)
70% ethanol	10 dips
95% ethanol with acetic acid	45 sec (dip/sec; if necessary increase for higher contrast)
95% ethanol	3 min
100% ethanol	3 min
100% ethanol	3 min
CitriSol, Xylene, or other clearing agents	3 min
CitriSol, Xylene, or other clearing agents	3 min minimum (kept in solution while coverslipping)

Section Imaging

While there is a range of imaging devices that can be employed to capture the section image, our approach employs an SLR with a macro lens. Specifically, a Canon 1ds Mark III digital SLR equipped with canon 65mm 1-5x macro is used. Capture is set to yield a pixel pitch of 3.1µm. At this magnification, spatial distortion is minimal. The section is transilluminated using a Northern Light lightbox which is designed for high spatial uniformity and temporal stability.

Registration

A multistep registration process is employed to first accurately reconstruct the sectional volumes and then to align the reconstructed volume to its corresponding MR volume.

The first step is a coarse reconstruction. It employs moments-based alignment of section to blockface registration. The blockface and corresponding sectional images are masked from background manually and the rigid-body parameters necessary to align the later to the former are computed. The translation and rotational parameters are used to define the starting position for the second, refining step in reconstruction: section-to-section alignment using Automated Image Registration (AIR) software (Woods et al., 1998). AIR is an intensity-based alignment approach, and the input to it is the sectional gray value images following subtraction of background and convolution with an 11 by 11 median filter. AIR settings used is 2D rigid-body registration. Satisfactory results typically require multiple runs. The initial registration yields blocks of well-aligned sequential sections interrupted by abrupt misalignment steps. To correct for those, the initial position of the section following the misalignment is altered and the two adjacent sections interrupted by misalignment realigned. Once visual inspection determines that acceptable registration is attained, the resulting registration parameters from all the steps are applied to the original images.

The reconstructed volumes were aligned to their corresponding MR using a two step approach: a) the volumes (Nissl/systemic stain channels) were aligned using a multi-resolution, non-deformable process composed of a quaternion transform followed by an affine transform. This registration step brings both volumes into the same scale and compensate for any possible translations or rotations between them, and b) the registration is refined using a multi-resolution FEM based dipheomorphic registration algorithm to align homeomorphic structures through deformation. Mutual information is the metric of choice both for the non-deformable and the deformable steps. The needed routines for this approach are implemented in the NLM funded Insight Toolkit (www.itk.org).

References

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