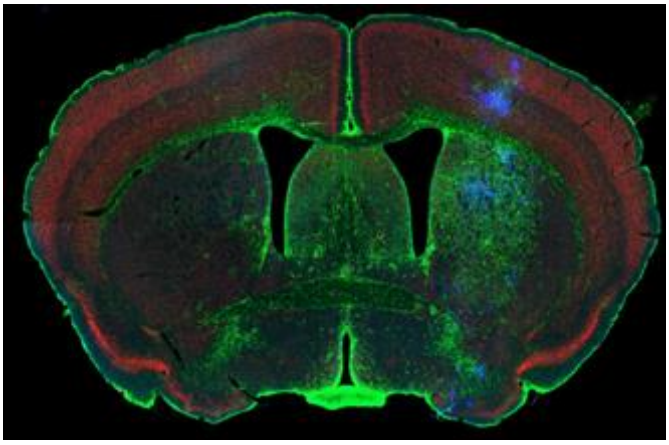
Application Note: Advancements in Rodent Brain Imaging

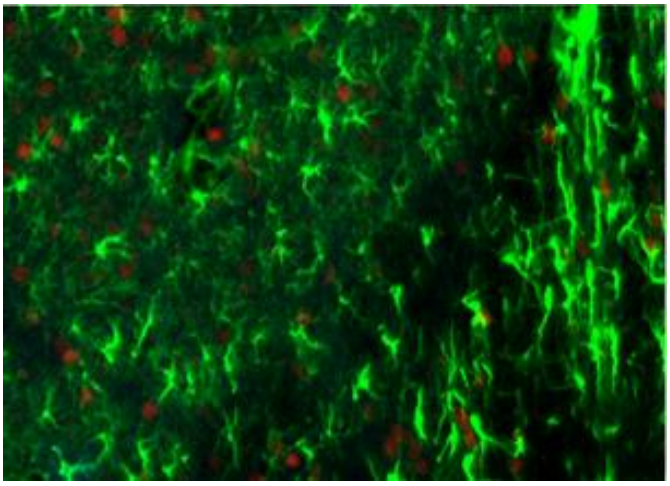
Introduction

Rodent brain imaging has opened the door to a more profound understanding of how the brain works and serves as a basis to identify the causes of many neurodegenerative diseases. Recent success has involved the mapping of full neural-network diagrams depicting all input and output of every region in a rodent brain. This advancement provides great benefits through the identification and location of specific neuronal circuits involved in cognitive, emotional and motor processes. Resulting maps offer more enhanced opportunities to study diseases (*e.g.,* schizophrenia) caused by abnormal interactions between two different cerebral structures. Imaged models have also become invaluable in better understanding the causation and associated processes of different neurodegenerative diseases (*e.g.,* Alzheimer's and Huntington's). Models show how specific diseases may be associated with the loss of neurons and synapses in various regions. They can also outline possible treatments that can be investigated involving the blockage of pathways leading to neuron death as well as those which can lead to the disappearance of synapses between cells and the loss of their associated functions. Furthermore, such modeling has resulted in great advances in sourcing gene mutations leading to abnormal protein expression resulting in motor-neuron degeneration.

Several technological advancements have made rodent brain imaging possible by making it easier to produce more accurate, high quality brain images. As an example, fluorescent tracer injections (viral or classical) help indicate neural and axonal pathways in and out of the brain. Ultrasound methods open the blood/brain barrier, thus allowing for the targeted diffusion of microtubules containing immunotherapies to specific regions of the brain. Microscopic advancements enable imaging deeper into tissue (dependent on the opacity of the sample and the working distance of the objective lens) as well as providing enhanced optical sections at various depths from thick tissue samples (approximately 10 times sample thickness compared to an optical slice). Optical sectioning techniques offer many advantages such as preparation time savings, increased accuracy, precision of spatial positioning for sections with reduced deformation and the reduction of the use of thin sectioning. Optical sections enable the development of 3-D micrometer scale tomography used in the production of high-resolution atlases of small sized stained rodent brains, including the depiction of associated neurons and corresponding processes as well.

Clear images with better optical resolution, particularly in the depth direction, can also be produced using confocal fluorescence techniques where out-of-focus light from the specimen that is outside the focal plane is eliminated by a pinhole.





Rodent brain (top) depicting simultaneous acquisition of fluorophores Cy2, Cy3 and Cy5; Brain image (bottom) magnified to 0.5 µm

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Limitations of Traditional Microscopy in Rodent Brain Imaging

Rodent brain imaging studies typically involve large amounts of tissue sections. Using traditional microscopy techniques to image such volumes suffers from significant limitations:

1. Reduced Depth Penetration for Optical Sectioning: Current optical sectioning techniques do not provide sufficient imaging depth into tissue to obtain higher numbers of optical sections. Limited by small working distances and increased light dispersion with increased depth, most commercially available systems can only image tissue sections that are less than 20µm thick. This further inhibits the ability to produce precise 3-D data sets.

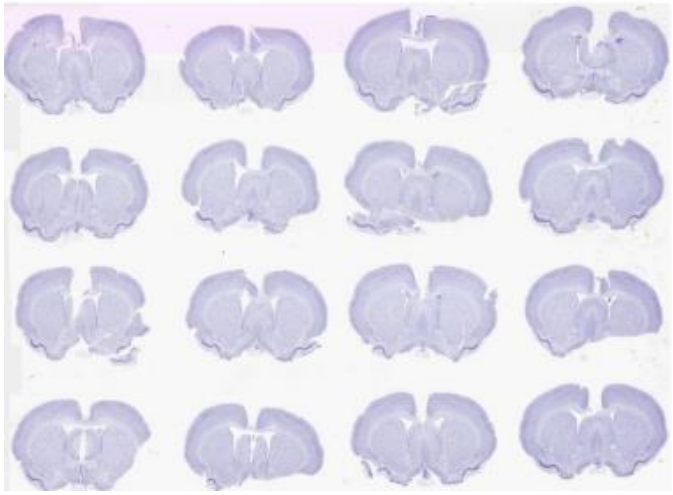
2. Light Interference Using Traditional Microscopes: Optical sectioning using traditional non-confocal microscopes are subject to interference as systems are very sensitive to light. Thus other light sources interfere with the images being produced. Furthermore, fluorescence images may contain large unfocused backgrounds overshadowing weak features in regions of interest.

3. Increased Processing Time and Photo-Bleaching: Frame-by-frame excitation in fluorescence micro scopy over-exposes and photo-bleaches combined fluorophores which are exposed to needless repeated illumination from sequential channel acquisition.

TissueScope™ – Huron Digital Pathology's Rodent Brain Imaging Solution

The TissueScope digital slide scanner provides key advantages for imaging rodent brain specimens. These include:

- Imaging thicker tissue sections, up to **100µm**, due to a large (3mm) proprietary laser-scan lens working distance which also facilitates optical sectioning and confocal Z-stacks. Optical sections can be as small as **1µm** in thickness. Increased penetration depth and optical sections translate into significant savings in preparation time and enhanced spatial registration accuracy and precision alignment.
- In combination with clearing methods, such as Scale, thick tissue sections, up to **500µm**, can be imaged without reducing the intensity of fluorescent proteins. This is particularly useful for viewing neural networks of the cerebral cortex, hippocampus and white matter.
- A proprietary laser-scan lens and optical configuration allows for a wide (5-10mm) field-of-view and the subsequent imaging of more rodent brain sections per unit area (up to 48 square inches area).
- Software solutions to assemble optical sections into 3-D models from acquired and stored images. Server and storage solutions house and share voluminous image sections acquired from samples.
- Enhanced focusing algorithms facilitate the imaging of thicker tissue topography while facilitating optical sectioning and Z-stacks.
- Only confocal fluorescence digital slide scanner that allows for the simultaneous acquisition of up to 3 fluorophores spanning the entire visible spectrum from 400nm-850nm providing significant time-savings and reduced photo-bleaching thanks to the unique flying-spot laser platform.
- Use of highly sensitive photomultiplier tubes for image detection allows for the acquisition of weak signals from samples otherwise missed on traditional microscopes.



Brightfield scan of 16 Nissil stained rodent brains on a 2" x 3" slide at 0.5 µm

Contact Huron Digital Pathology for your Rodent Brain section imaging applications!

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