Mass Production Neurohistology and Contemporary Stains Accelerate AD Research

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Abstract:

Alzheimer's disease research has led to dramatic increases (over past methodology) in the number of neurological specimens to be processed and analyzed. Compounding the increased quantity requirements is the demand to reduce cycle times in the drug development process. The challenges to achieving these goals are the amount of time and expense required to analyze pathologic results. Traditional neurohistologic methodology entails processing each specimen individually, and many traditional stains require manual pathologic review. The advent of multiple tissue processing techniques and improved signal to noise stains that are amenable to automated interpretation offer solutions to these challenges.

The simultaneous processing of multiple specimens embedded into a matrix enables multiple specimens to be cut, stained and permanently grouped on each slide to facilitate comparisons. After multiple embedding, each subsequent step takes only as long as it would take to process a single specimen. In effect, this approach introduces mass production for neurohistology. An example of this approach is MultiBrain™ technology which has the capability to process as many as 40 brains simultaneously. MultiBrain™ technology eliminates the added time and expense required for neurohistological processing of the sections while providing numerous qualitative benefits.

An example of contemporary staining techniques is the Campbell Switzer Alzheimer's pathology stain that reveals three significant features of AD within a single, low cost staining protocol. This stain provides a high contrast signal that enables image analysis of the neuritic plaques and neurons with neurofibrillary tangles that are the hallmark pathologic features of Alzheimer's disease. Diffuse and amyloid core plaques are both displayed, however each has its own color making differentiation possible. Features are revealed in high contrast against a pale background, ideal for image analysis.

Rapid interpretation of results is a key enabler in reducing R&D cycle times and budgets so that resources can be utilized more effectively in Alzheimer's research. Automated analysis techniques are possible and should be leveraged, but require a high contrast staining capability such as the Campbell-Switzer method and an extremely high throughput neurohistology solution such as MultiBrain™ technology in order to reduce the analysis cycle time from months to days.

Challenge #1:

•Due to natural variations across animals, relatively large numbers of animals are required to assess disease models and treatment efficacy.
•Many compounds and approaches are desired to be tested in a parallel time frame.
•Traditional histology methods are an obstacle in drug development due to time and $$ requirements.
•Industry competition demands decreased cycle times in drug development to gain a competitive advantage.

Opportunity:

•Institute principles of mass production to neurohistology to lower costs, accelerate results and standardize quality.

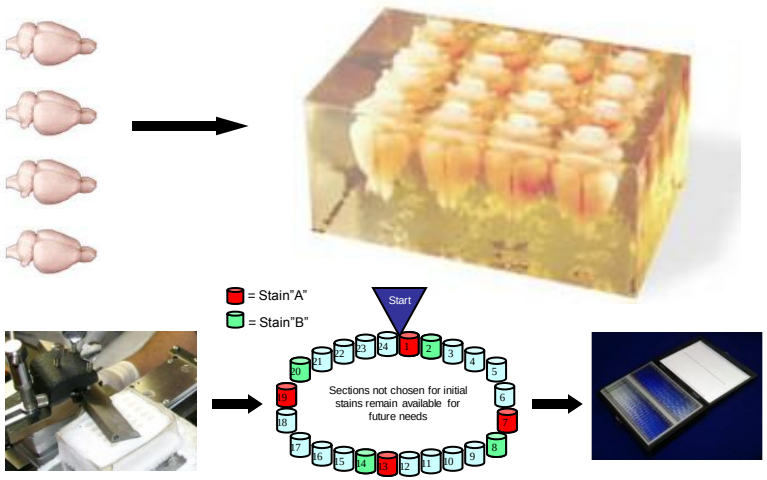
Challenge #2

•Histologic results must be quantifiable to enable meaningful, statistical analysis.
•Staining techniques must provide a distinct signal without background staining "noise".

Opportunity:

•Improve staining contrast to be more amenable to densitometric analysis.
•Generate more statistically meaningful results by analyzing MORE cases with the same resources

MultiBrain™ Technology



Sectioning yields 'sheets' containing each specimen

Each 'sheet' is placed in the next cup in sequence with every 24th section in each cup

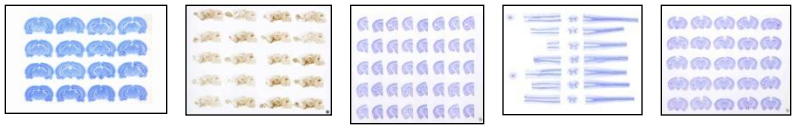
MultiBrain™ Features:

•Unified processing of multiple brains, spinal cords
•Uniform thickness of sections
•Uniform staining across all sections
•Variety of possible layouts, tissue types and orientation

MultiBrain™ Advantages:

•Accelerated histology up to 40X faster than traditional methods
•Simpler, more rapid comparative analysis
•Flexibility to retroactively stain adjacent sections
•Inherent Quality Control

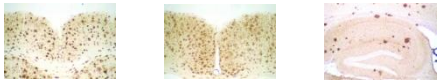
MultiBrain™ principles can be applied to a variety of tissues types, orientations and numbers



16 Nissl stained coronal rats | 20 sagittally oriented Campbell-Switzer stained mice | 40 coronal, Nissl stained mouse hemispheres | 7 Nissl stained monkey spinal cords, transverse/longitudinal orientation | 25 Nissl stained coronal mice

Alzheimer's Disease Neurohistology

Typical AD Stains:



Aβ-1-40 Mouse Cortex PS-1/APP Transgenic | Aβ-1-42 Mouse Cortex PS-1/APP Transgenic | 4G8 Mouse Brain Sagittal PS-1/APP Transgenic

Alternate Approaches:

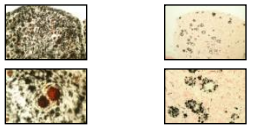
The Campbell-Switzer AD stain reveals diffuse and congophilic plaques as well as tangles in one step.

Sagittal cut view of AD model stained with 4G8 immuno (left) and Campbell-Switzer AD stain (right)

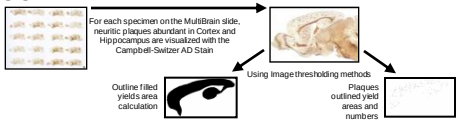
Coronal cut view of AD model stained with Aβ-1-40 immuno (left) and Campbell-Switzer AD stain (right)

The deOlmos Amino CuAg Degeneration Stain reveals the co-location of mature, dense, congophilic plaques with degeneration as shown on these adjacent sections

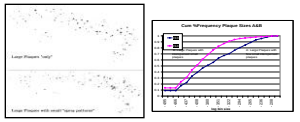
Campbell-Switzer AD Stain | **deOlmos Amino CuAg Degeneration Stain**



Analysis:



Analysis is then possible (Illustration is a comparison of 2 APP AD mice):



Conclusions:

High throughput techniques such as MultiBrain® enable the neurohistologic phase of drug discovery to be executed in weeks vs. months.

Mass production accelerates the processing and analysis of large numbers of tissues, allowing researchers to broaden their statistical sample base.

Contemporary neurohistologic techniques transform a burdensome, bottleneck activity into a rapidly executed, data generating, drug discovery acceleration engine.

•High contrast staining allows densitometric analysis to be used to create "index of effect" data across a broad sampling size

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v2