Integrating Informatics and High Content Screening to Find a Cure for Spinal Cord Injury

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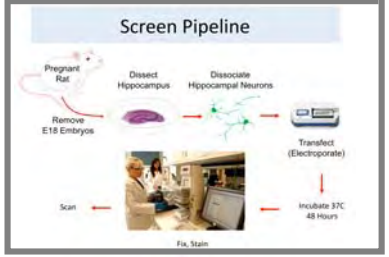
Introduction

The Miami Project to Cure Paralysis, part of the University of Miami Miller School of Medicine, includes a laboratory devoted to High Content Screening (HCS) of neurons. The goal of the laboratory is to uncover signaling pathways, genes, compounds or drugs that can be used to promote nerve growth. HCS of various libraries on primary neurons requires a team-based approach, a variety of process steps and complex manipulations of cells and libraries to obtain meaningful results. The approach of HCS itself also produces huge amounts of data in the form of images, well and cell based data. A single experiment can generate data from 300,000 neurons with 140 parameters per cell. Managing sample workflow and library data, along with the vast amounts of experimental results is challenging. The laboratory is currently implementing an informatics solution to meet the ever-growing data deluge… turning data into knowledge.

FIGURE 1. Graphical depiction of Scientific Workflow. Chain of custody, sample status/location and experimental data must be managed throughout the laboratory workflow



Project to Cure Paralysis Scientific Workflow (Fig. 1):

Typical screening pipeline includes the following steps:

•Brain regions are harvested from embryos or post-natal pups.

•Brain pieces are then dissociated and transfected in 96 well plates with DNA.

•Neurons are then allowed to grow in 96 wells for 2-3 days before being fixed and stained for imaging.

•Sometimes, the neurons are treated with chemicals or drugs instead or in addition to the DNA

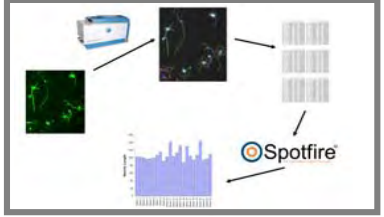
Additional information on lab workflow and capabilities can be found at www.vlemmonlab.com.

FIGURE 2. Thermo Fisher Cellomics VTI High Content Microscope with plate handling robot is the central player in the LemBix Lab High Content Screening facility. But extensive work with compounds, plasmids coding for cDNAs or shRNAs, and primary neuronal cultures must be tracked. The 16,000 cDNA collection from Open Biosystems is shown on the right.

Challenges for HCS of Primary Neurons

•Media Variability
•Plate and substrate variability
•Prep-to-Prep variability of cells
•Many individuals in work flow
•Variability in transfection
•Liquid handling - working with loosely adherent cells
•Rapid assessment of assay quality

FIGURE 3. Automated image analysis: the data from the HCS microscope is exported to Spotfire. The images from outliers checked. The data is normalized and pooled across experiments

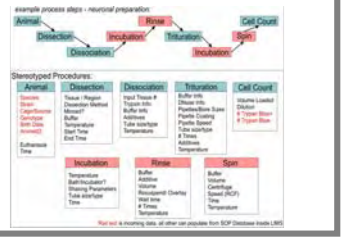


The LemBix Lab turned to Thermo Scientific Nautilus LIMS (Laboratory Information Management System) to manage its workflows and operations.

Goals of the project

- Improved Tracking Of Data
  - ✓ Plate management and work flow documentation
  - ✓ "Real Time" Reporting - Lab members and supervisors have immediate access to information on collaborative projects
  - ✓ Ease tracking reagents in multiwell plates
  - ✓ Link digital information with work flow
  - ✓ Document stocks and lots used in experiments
- Improved Quality Of Data
  - ✓ Less manual data entry = fewer transcription errors
- Enhanced Productivity
  - ✓ Reduced Turnaround Times
- Improved Forward Planning
- Enforced Business Rules
  - ✓ Ensure SOPs adhered to
- Capture details about workflow to identify problems or optimal conditions

FIGURE 4. Process steps in neuronal preparation that need to be documented in the LIMS



Workshop Process – Project Approach

In order to minimize costs and engage the LemBix laboratory staff, a workshop approach was recommended to create a solution that fits the needs of the end-users.
This style of implementation relies on the close interaction of Thermo Scientific personnel and LemBix researchers to develop a novel configuration of Nautilus workflows to track the progress of work through the lab, improve efficiency of data collection, and enhance the traceability of reagents and solutions used in experiments.
Highlights of this approach include:

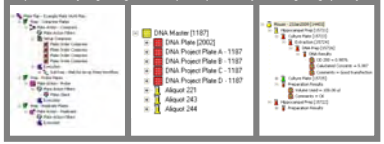
- Gathering and Documenting Requirements
- Setting up the LIMS environment
- Developing documentation for management and expansion of the LIMS system
- Training lab personnel via "Workshops" including:
  - •Configuration of Nautilus workflows
  - •Integration of instruments
  - •Development of reports
  - •Coordination with external systems

Benefits of the "Workshop" approach

- Reduced cost of implementation
- User acceptance
- Client ownership of the system
- Flexibility to expand/modify functionality as needs change

The close interaction of Thermo Fisher and LemBix labs gives both parties insight into the desired needs and best practices in this line of research. It allows each to stay on the leading edge of scientific and technological breakthroughs.

FIGURE 5. Screenshots depict the patented graphical workflow utility in Nautilus LIMS and some examples of integrated data hierarchies created from the workflow. The middle image is an plate hierarchy. Right image is an example sample hierarchy. All configured graphically.



Conclusion

High Content Screening is being counted on to uncover signaling pathways, genes, compounds or drugs that can be used to promote nerve growth. The process produces huge amounts of data. The screening pipeline includes solid experimental techniques combined with instrumentation and analytical tools. Implementation of Nautilus LIMS is underway to better organize data resulting in increased productivity and accuracy of data.

Acknowledgements

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