Reprinted from *American Laboratory* January 2010

Application Note

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Integrating Informatics and High-Content Screening to Find a Cure for Spinal Cord Injury: Selecting a LIMS for CNS Regeneration

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 laboratory is run by Vance Lemmon and John Bixby, hence, the LemBix laboratory. 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 produces huge amounts of data in the form of images, well-based and cell-based phenotypic measures. A single experiment can generate data from 300,000 neurons with 120 parameters per cell. Managing sample work flow and library data, along with the vast amount of experimental results, is challenging. The laboratory is currently implementing an informatics solution to meet the ever-growing data deluge and to turn data into knowledge.

Background

Traumatic injury to the central nervous system (CNS) usually results in irreversible loss of function. There are two main reasons for this: one is due to the death of nerve cells and their support cells; the second is due to the severing of very long processes (called axons) that transmit electrical signals from one nerve cell to others. Transected CNS axons do not regrow or reestablish connections with their targets. Consequently, individuals with injured spinal cords can be irreversibly paralyzed.

High-content screening offers an ideal way to identify genes; molecular pathways; and, ultimately, drugs that can promote the regeneration of CNS axons. HCS permits the quantification of cell morphology, including the lengths and numbers of axons, of neurons in culture. The LemBix laboratory can screen 3000 different genes in primary neurons and measure 120 different parameters in over 8 million neurons in the span of a few months.

Screening campaigns using primary neurons are uncommon due to the expense of culturing cells that require complex defined media and the variability between different preparations of nerve cells. The LemBix laboratory tackles the variability between preparations, in part, by including a number of control treatments that are used for normalizing data across experiments. But it is clear that much of the variability between different experiments is due to variability in reagents and cell preparation. In order to rapidly identify sources of variability, it is essential to have a LIMS that tracks supplies, reagents, and work flows.

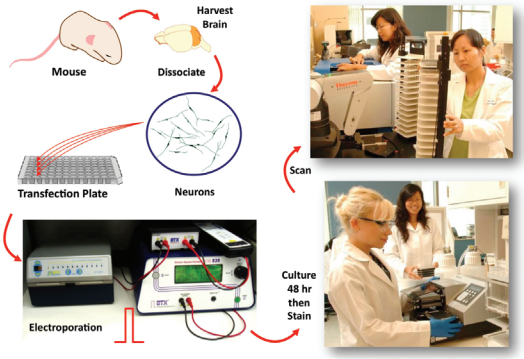

Figure 1 HCS work flow: CNS neurons are isolated as single cells before being cotransfected with expression vectors coding for potential regeneration-associated genes and green fluorescent protein. Then, neurons are plated into 96-well plates and cultured for 48 hr before being fixed and stained to permit visualization of nuclei and neuronal morphology. The plates are then imaged and analyzed using a ***Thermo Scientific*** *Cellomics™ VTI ArrayScan and Cellomics BioApplications.*

The challenge

The Miami Project to Cure Paralysis sought a LIMS for the LemBix laboratory that would facilitate its laboratory work flows and automate its previously manual data management processes. With the increasing workload in the high-throughput laboratory, a single experiment can generate data from 300,000 neurons with 120 parameters per cell, and managers required an informatics solution that could manage the flow of data and enhance productivity. Furthermore, laboratory managers needed to be able to readily access and analyze the data to provide them with knowledge that was previously difficult to acquire. The laboratory implemented an informatics solution to manage the enormous volumes of data in order to ease the administrative tasks of scientists so that they could focus on the laboratory's goal of finding a cure.

The solution

LIMS are widely used in industry, but have scarcely penetrated the academic research environment for a number of reasons. First, a complete installation and validation schedule for a LIMS capable of serving this academic research environment would be quite costly, approaching figures larger than a typical NIH research grant. Even the cost of a basic LIMS, including servers, database licenses, and any necessary customization, is high. Private benefactors of medical research, often the source of seed money for new research endeavors in academic research institutions, prefer to fund buildings and equipment and are rarely willing to fund software projects that can appear intangible and have unknown outcomes or time lines. Thus, obtaining sufficient resources to purchase a LIMS for a single university laboratory can be a daunting task. In addition, LIMS are designed with GLP/GMP in mind, and some industry LIMS can lack the kind of flexibility and ease of modification required in a basic research laboratory, where projects, procedures, and even staff and students change frequently.

Having concluded that a LIMS was essential to the success of its HCS campaigns,

scientists at the LemBix laboratory quickly realized they were unprepared for the process of selecting and purchasing a LIMS. Not only were the products varied, but the vendors spoke a language not understood in the research laboratory: the language of project management and industrial process control. To provide translation for this part of the project, researchers at the laboratory found a mentor in the university research office with years of big pharma project management experience. He guided the team through the development of the initial User Requirements Specifications and assisted with discussions and negotiations with vendors. The laboratory had constant input from the university IT group, purchasing department, and the newly established Center for Computational Sciences to ensure that selection processes met university guidelines as well as research needs.

After narrowing the field to three LIMS vendors, the LemBix laboratory requested detailed demonstrations and trial installations to better determine the most appropriate LIMS choice for its specific research needs. One note of hindsight is that the laboratory had a difficult time developing criteria for differentiating the diverse LIMS. A more efficient method may have included the use of an independent LIMS consultant for this phase of the project.

Implementation

The laboratory selected the Thermo Scientific Nautilus LIMS (**Thermo Fisher Scientific**, Philadelphia, PA) to manage its work flows and operations. Once the laboratory opted for the Nautilus LIMS, based on its proven plate handling capability, a scientific work flow was developed.

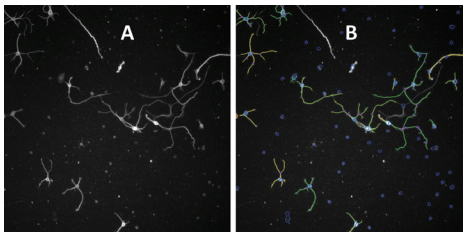

Figure 2 Representative images and tracing from the VTI ArrayScan and BioApplications. a) Hippocampal neurons were stained with anti-beta3 tubulin to visualize neurites. b) The BioApplication successfully traces and quantifies the processes emerging from the cell body and reports a variety of parameters including the length of the longest neurite, total neurite length, number of neurites emerging from the cell body, and number of branches from the neurites.

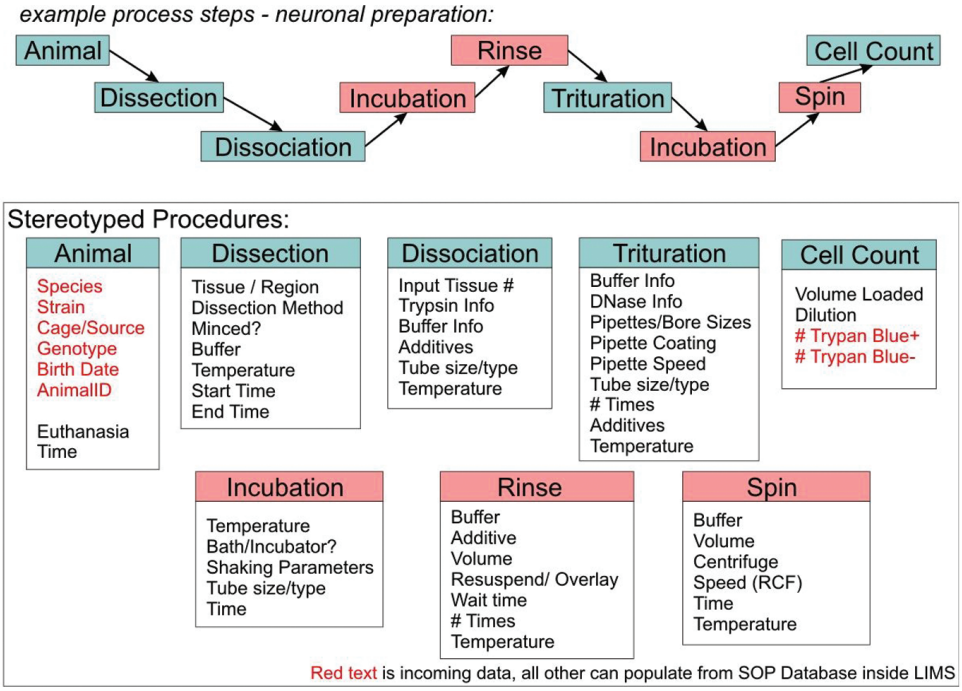

Figure 3 Process steps in the preparation of primary neurons for HCS. Each step has a number of parameters that need to be captured by the LIMS.

The typical screening pipeline includes many steps (see *Figure 1*). First, brain regions are harvested and these 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 (*Figure 2*). Sometimes, the neurons are treated with chemicals or drugs instead of or in addition to the DNA. Additional information on laboratory work flow and capabilities can be found at www.vlemmonlab.com.

Challenges for HCS of primary neurons

There are many challenges associated with HCS of primary neurons (see *Figure 3*). These include:

- 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.

Project goals

The goals of the project included improved tracking of data, plate management, and work flow documentation. The laboratory required real-time reporting in order for laboratory members and supervisors to have immediate access to information on collaborative projects. It was essential that the laboratory implemented a solution to ease tracking reagents in multiple-well plates, link digital information with work flows, and document stocks and lots used in experiments.

Overall, the laboratory specifically wanted to improve the quality of data, minimize manual data entry to avoid transcription error, and enhance productivity. For the LemBix laboratory, a key driver for implementing a LIMS was the chance to reduce turnaround times, improve forward planning, and ensure that standard operating procedures (SOPs) would be adhered to. It was important that a solution would ensure enforced business rules and be able to capture details about work flow to identify problems or optimal conditions.

Workshop process–project approach

In order to minimize costs and engage the laboratory staff, a workshop approach was recommended to create a solution that fit the needs of the end users. This style of implementation relies on the close interaction of **Thermo Fisher Scientific** personnel and LemBix researchers to develop a novel configuration of Nautilus LIMS work flows to track the progress of work through the laboratory, 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
- Training laboratory personnel via workshops, including Configuration of Nautilus Work Flows, Integration of Instruments, Development of Reports, and Coordination with External Systems.

Benefits of the workshop approach include reduced cost of implementation, user acceptance, client ownership of the system, and flexibility to expand/modify functionality as needs change.

The close interaction of **Thermo Fisher Scientific** LIMS consultants and the LemBix laboratory team gave both parties insight into the desired needs and best practices in this line of research. It allowed each to stay on the leading edge of scientific and technological breakthroughs.

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 enormous amounts of data. The screening pipeline includes solid experimental techniques combined with instrumentation and analytical tools.

Thermo Fisher Scientific introduced a workshop approach, in which a Thermo Fisher programming consultant worked alongside laboratory staff, simultaneously building the LIMS and teaching the staff, who then extended the LIMS. This method minimizes costs and engages staff while developing the best solution to integrate informatics in their high content screening environment. The LemBix laboratory implemented a Thermo Scientific Nautilus LIMS to improve productivity and organize and improve the accuracy of its collected data. Implementation of the LIMS is underway to better organize data, resulting in increased productivity and accuracy of data. The LIMS is helping to accelerate discovery for the Miami Project to Cure Paralysis in its high-content screening laboratories.

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