A Novel Platform to Improve HTS Compound Management Operations

Pierre Baillargeon
HTS/Lead Identification
I. Introduction to Lead Identification and Compound Management at Scripps Florida

II. Review of issues with DMSO solvated compounds in HTS libraries and methods for addressing these issues

III. An introduction to the High-resolution Image Acquisition and Processing Instrument for Compound Management applications (HIAPI-CM)

IV. Practical applications of HIAPI-CM
The Lead Identification Laboratories at Scripps

Where robotics, chemistry and biology join forces to help discover new drugs

Integrated Assay Development, uHTS and Compound Management

- Located in Jupiter, FL
- >115 targets screened in over 145 primary campaigns to generate more than 35 million data points for academic & industrial collaborators
- S1P1 drug candidate entered phase 1 clinical trials in January 2011

- >982K Compound Screening Collection
- >622K Proprietary (largest in academia, ~30k unique compounds, focused sub-libraries, professionally curated)
- >360K Public Domain (NIH)
- Integrated Compound QC (LC-MS, HIAPI-CM)
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What are your primary concerns in your current role or your department?

- Sample quality: 66.70%
- New automation technology: 33.30%
- Integration of automation technology: 33.30%
- Sample data management: 66.70%
- Building a library to meet new research requirements: 66.70%
- Budget cuts limiting technology investment: 16.70%
- Job cuts: 16.70%

“The findings seem to illustrate the growing challenge of increasing levels of data and how to process these into understandable usable nuggets of information”

Graphs and quote reproduced from the 2012 IQPC ‘Setting up Compound Management Globally’ Compound Management report

What role does CM play at Scripps?

- HTS Library Procurement
- HTS Library Stewardship
- HTS Library QC (via LC-MS)
- Cherry pick for HTS Core
- Compound re-synthesis & restock
- Receive & reformat samples from external collaborators
- Ship samples to external collaborators
- Medicinal Chemistry support
What operational challenges are encountered during these interactions?

- Verifying quality of external samples & sample data
- Degradation of samples over time
- Enforcing internal QA/QC standards
- Tracking sample properties & genealogy in a way that can be easily audited in the future
- Managing large sample libraries which are constantly in flux (multiple copies, samples becoming depleted, new acquisitions, etc.)
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How do we currently address these issues and what are the limitations?

- LC-MS (time consuming)
- Acoustic auditing (time consuming, labware dependent)
- Manual visual inspection (time consuming, subjective)
COMPOUND SUBMISSION
• Has the correct sample been put into the labware?
• Is the volume and concentration of the sample accurately recorded?

COMPOUND PROCESSING
• Has the sample store delivered the correct samples?
• Has the liquid handling automation pipetted the samples properly?
• Has the plate been registered correctly?

COMPOUND DELIVERY
• Do we have a record of what we are delivering?

Room for improvement! How can we QC samples more efficiently?
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Why Do We Need HIAPI-CM?
Why Do We Need HIAPI-CM?

- Are empty/partially filled wells in Quadrant 1?
- Do these wells contain precipitate?
- My db says this well is empty. Does it actually have sample in it?
- My HTS detection format is sensitive to purple compounds. Where are they?
- Are these wells partially filled?
HIAPI-CM Applications & Features

• Instant detection of errors from compound reformatting
  • Detect missed/partially filled wells, quadrant effects

• “Pre-screen” compound plates for HTS assay interferents
  • Detect colored compounds, suspended materials

• Identify database entry errors in corporate LIMS db
  • Determine which full/empty wells in plate differ from the full/empty well assignments in corporate db

• Periodic check-ups on the “health” of stored compounds
  • Detect ppt. formation, evaporation

• Detection of compound solubility
  • Identify HTS library samples that have “crashed out” of solution
What is HIAPI-CM?

• A custom HTS plate reader, incorporating recent advances in machine vision, image analysis, & the spectroscopy sciences

• It saves labor by *automatically* identifying & annotating issues specific to compound libraries:
  • Colored compounds
  • Precipitate / Crystallization
  • Low volume / Full wells

• It can be used in “stand-alone” mode, or integrated with automation

• Works with microtiter plates

• Fast: reads a 384 well plate in <1 minute

• All measurements are non-contact

• User-friendly, intuitive analysis software
HIAPI-CM Features: Instrument Design

- Completely automated & intuitive to operate
- Classification Results shown in real-time
- Simple interface only requires user to select plate type to perform analysis
- Analysis results exported as text file or compared to existing records in corporate LIMS database
HIAPI-CM Plate Gallery provides an easy to navigate interface to edit, export or browse analysis results.

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From the Plate Gallery, a user can click on a plate to load the Plate Detail View. The user can inspect different artifact analysis results or overwrite classifications made by HIAPI-CM.
HIAPI-CM Features: Color Recognition & Classification

- HIAPI-CM detects and classifies colored compounds

- HIAPI-CM identifies the “standard” colors listed below:

<table>
<thead>
<tr>
<th>Color Detected</th>
<th>Dye Used</th>
<th>Limit of Detection (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>Allura Red</td>
<td>35.1</td>
</tr>
<tr>
<td>Green</td>
<td>Brilliant Green</td>
<td>9.0</td>
</tr>
<tr>
<td>Blue</td>
<td>Euroglaucine</td>
<td>0.6</td>
</tr>
<tr>
<td>Yellow</td>
<td>Tartrazine</td>
<td>12.7</td>
</tr>
<tr>
<td>Violet</td>
<td>Gentian violet</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Before Processing

After Processing
HIAPI-CM Features: Precipitate Detection

Compound plates contain precipitates which are masked by colors in the wells:

HIAPI-CM Precipitate analysis is:
- Insensitive to color
- Able to detect ppt. beyond “naked eye” inspection
HIAPI-CM Feature: Low Volume Detection

What can cause a well to have a low volume?
- Incorrectly reported volume in LIMS
- Oversampled by cherry picking operations
- Evaporation
- Liquid handling errors (clogged tips, samples transferred to incorrect wells/quadrants, etc.)

HIAPI-CM distinguishes normal wells (“full”) from empty/partially filled wells (“low volume”)
RESULTS OF COMPOUND PLATE QUERY IN CORPORATE DATABASE:

• Scripps database (MDL Symyx/Plate Manager) displays several wells as empty (white rectangles in figure on left)

RESULTS OF THE SAME COMPOUND PLATE QUERY IN HIAPI-CM DATABASE:

• HIAPI-CM compares instrument results to records archived in corporate db

• HIAPI-CM identifies discrepancies with corporate db records (red “x’s” in figure on left):
  • Colored compounds (upper left)
  • Full wells that corporate db assigns as “empty”

HIAPI-CM CAN BE USED TO AUDIT/UPDATE CORPORATE DB RECORDS
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What new information do we obtain with HIAPI-CM in the process?
- We know which wells have been filled and which have low volume
- We know which wells contain colored samples
- We know which wells contain compounds that have precipitated

What can we do with this information?
- Compare filled & low volume wells against LIMS to verify proper liquid handling & plate registration
- Compare ‘color fingerprint’ of samples against database of known samples to identify issues with incorrect concentration & samples
- Take measures to put precipitated samples back into solution (sonication, heating, etc.)
- Keep a historical record of the health of the plate at the point when it was delivered (a receipt of transaction)
To visually inspect **three** 384 well plates:
- Plate #1 - 3 minutes 12 seconds
- Plate #2 - 1 minute 46 seconds
- Plate #3 - 1 minute 40 seconds

In the same amount of time, HIAPI-CM can automatically inspect **fifteen** 384 well plates!
Practical Examples: HTS Library Analysis

To date, Scripps has run over 1,000 plates through HIAPI-CM resulting in over 1,000,000 wells which have been analyzed for color, precipitate and low volume artifacts.

This includes Scripps Florida’s internal 622k Drug Discovery library and the NIH’s Molecular Libraries-Small Molecule Repository (MLSMR) 362k library.

How can we leverage all of this new data to improve our workflow?

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Practical Examples: Low volume detection

Did my pipettor transfer all compounds properly?

HIAPI-CM enables just-in-time QA of compound management liquid handling
Practical Examples: Color Analysis

HIAPI-CM data can be used to monitor consistency of sample color over time...

A sample provided by an external source in 2009 vs. the same sample provided by the same source in 2011.

With HIAPI-CM, we can automatically query and identify inconsistencies across entire compound collections.

Is this the same sample? Is the concentration recorded correctly?
Why should we care about sample color?
### Practical Examples: Color Analysis

Running color comparison reveals color inconsistencies...

<table>
<thead>
<tr>
<th>Sample</th>
<th>Distance</th>
<th>Plate Barcode</th>
<th>Concentration (mM)</th>
<th>Plate Scan Date</th>
<th>Well # [name]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>00505482</td>
<td>2.5</td>
<td>2011-05-25 14:28:19</td>
<td>268 [L4]</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>00505482</td>
<td>2.5</td>
<td>2011-05-25 14:28:19</td>
<td>300 [M12]</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>00505482</td>
<td>2.5</td>
<td>2011-05-25 14:28:19</td>
<td>315 [O3]</td>
</tr>
<tr>
<td>5</td>
<td>130</td>
<td>00505482</td>
<td>2.5</td>
<td>2011-05-25 14:28:19</td>
<td>317 [N5]</td>
</tr>
</tbody>
</table>

Two copies of plate prepared by external vendor reveal inconsistencies...
Practical Examples: LIMS Comparison

When checking reformatted plates, CM team noticed high # of LIMS disagreement wells...

... examining the plate detail view reveals a pattern in quadrant 1...

... checking the LIMS record reveals samples in quadrant 1 are not registered ...
... CM team updates LIMS record and runs plate through HIAPI-CM to confirm the corrected LIMS record matches the physical locations of samples within the plate.
Practical Examples: Precipitate Analysis

Precipitate filled screening plate

“Normal” screening plate
Practical Examples: Precipitate Analysis

“Normal” screening plate

Precipitate filled screening plate

HIAPI-CM automatically identifies plates with high levels of precipitate
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- Received samples are quickly QC’d upon arrival; HIAPI-CM results are then compared to the vendor’s plate map.
- HIAPI-CM periodically audits compound library over its lifecycle
- Automated QA/QC of samples stops subjectivity and prevents CM staff burnout
- HIAPI-CM plate “snapshots” allow us to easily & visually track sample genealogy
- Routine QC of large libraries now possible by automating labor intensive visual inspections
Scripps has licensed the HIAPI-CM technology to Brooks Life Science Systems for commercialization & sale. The Brooks Plate Auditor will be part of the Brooks sample analysis line of instrumentation. Stop by booth 918 in the exhibition hall for more information!

Additional information, including HIAPI-CM Pilot Screen results, can be found in “Monitoring of HTS compound library quality via a high-resolution image acquisition and processing instrument”. Baillargeon P, Scampavia L, Einsteder R, Hodder P; J. Lab Automation, June 2011
One Final Thought…

“We now have the technical ability to get the wrong answers with unprecedented speed. If you put the wrong stuff into the front end of our analytical pipeline, we will not only lose the war on cancer, we’ll pollute the scientific literature with incorrect data that will take us a long time to sort out. This is a crisis that requires disruptive innovation.”

Carolyn Compton - Director, Office of Biorepositories and Biospecimen Research at National Cancer Institute
Wired Magazine article on Biobanking, May 2010
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