

From Compound Management to Assay Platform: The Use of the Hamilton Star To Support InVitro Pharmacology

Christopher Napolitano

Research Associate

Merck & Co., Inc.

InVitro Pharmacology Department, Discovery PreClinical Sciences

Merck Research Laboratories

Rahway, NJ 07065

Christopher.napolitano@merck.com

Agenda

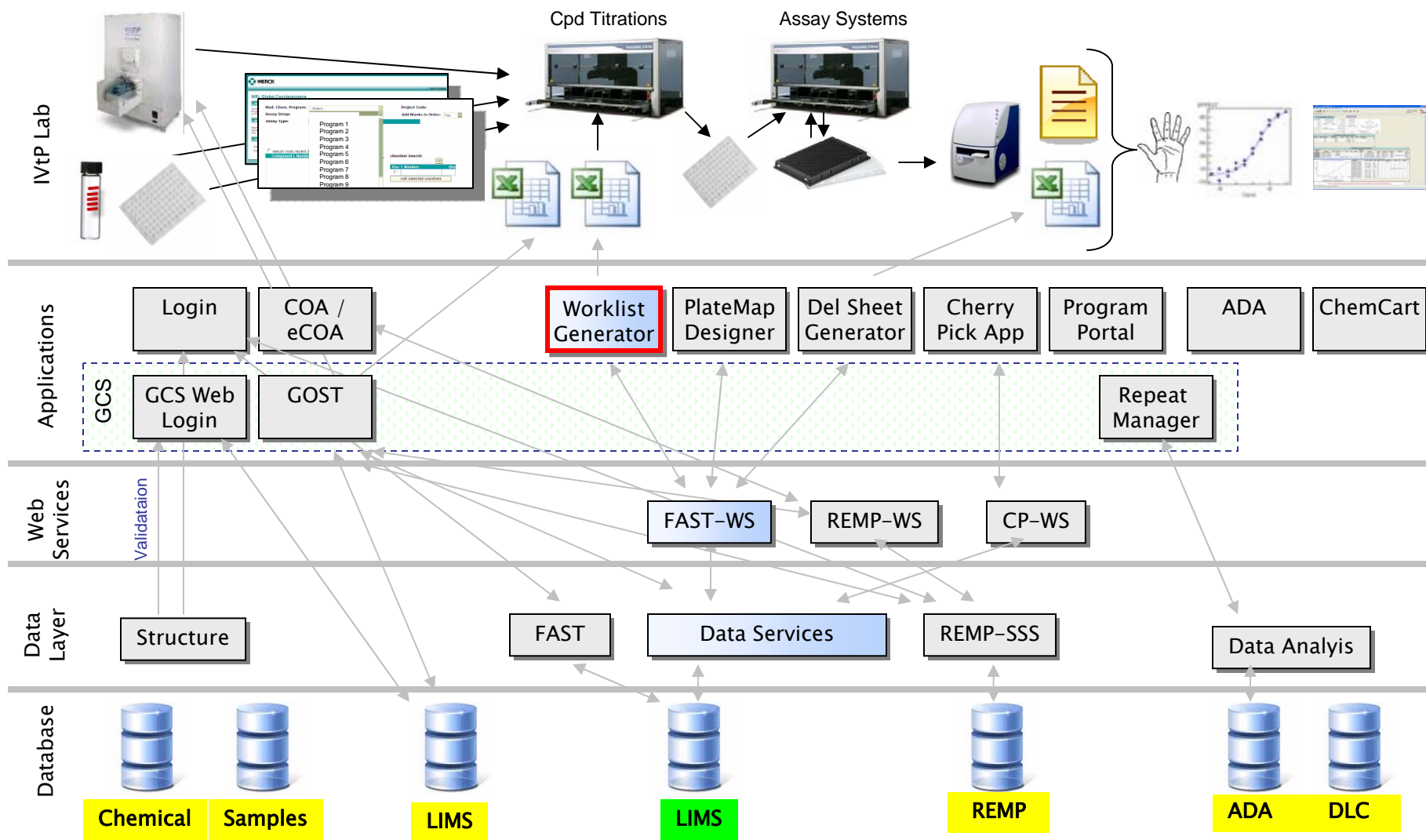
- Introduction to InVitro Pharmacology
- Hamilton Landscape within InVitro Pharmacology
- Worklist Utilization for Compound Management
- Customizations
- Maintenance
- Challenges Faced

InVitro Pharmacology

Team dedicated to the development and execution of *in vitro* assays necessary to characterize compounds during hit to lead identification and lead optimization

- Develop and execute in vitro assays for hit to lead identification and lead optimization
- In vitro follow-up of HTS screening hits to identify those most suitable Chemistry follow-up
- Innovative profiling, counter-screening, and biophysical characterization
- Rapid screening of specialized sub-libraries
- Identify POC compounds to support target validation
- Recombinant protein expression, purification and characterization for in-scope activities
- Cell line development, maintenance and production for in-scope activities
- Investigate and Integrate new assay technologies

Department Workflow



Hamiltons Supporting Workflow

Landscape includes 16 Liquid Handlers:

– Compound Management:

- 3 x Star Platforms
 - single arm: 16 Channel + iSwap
 - Velocity11 PlateLoc Heat Sealer
 - Custom REMP capping/decapping system
- 1 x Star Platform
 - single arm: 16 Channel + iSwap
 - Velocity11 PlateLoc Heat Sealer
 - Custom REMP capping/decapping system
- 3 x Star Plus
 - Dual arm: 8 channel + 384 head; iSwap + 96 head
 - Velocity11 PlateLoc Heat Sealer
 - REMP Reatrix 2D barcode scanner
 - REMP ACD96 decapper

– HighRes Engineering System

- 2 x StarLet Platforms
 - Single arm: 2 channel; 384 head

– Assay Ready Plate Creation

- 2 x iSwap Systems
 - Stand along iSwap
 - 4 Stackers
 - TTP Mosquito
 - Velocity11 PlateLocSealer

– Screening Operations

- 1 x StarPlus + StarLet(CYPRI)
 - 2 Systems integrated together
 - StarPlus: Dual arm: 8 Channel + 384 head; iSwap
 - StarLet: Single arm: 8 Channel + 384 + iSwap;
 - » Velocity11 PlateLoc Heat Sealer
 - » Liconic Incubator
- 1 x StarPlus(2022)
 - Single arm: 8 Channel, 384 head
 - Velocity 11 PlateLoc heat sealer
 - Liconic Incubator
- 1 x StarPlus(PXR)
 - Single arm: 8 Channel, 384 head
 - Liconic Incubator
 - PerkinElmer Envision
 - Autofilling tip wash station
- 1 x Star(CYPTDI)
 - Single Arm: 8 Channel, 96 head, iSwap
 - Temperature controlled carriers
- 1 x Star (3212)
 - Dual arm: 384 head, iSwap; 16 channel
 - (PerkinElmer Envision)
- 1 x StarLet
 - Single arm: 8 Channel + 384 head

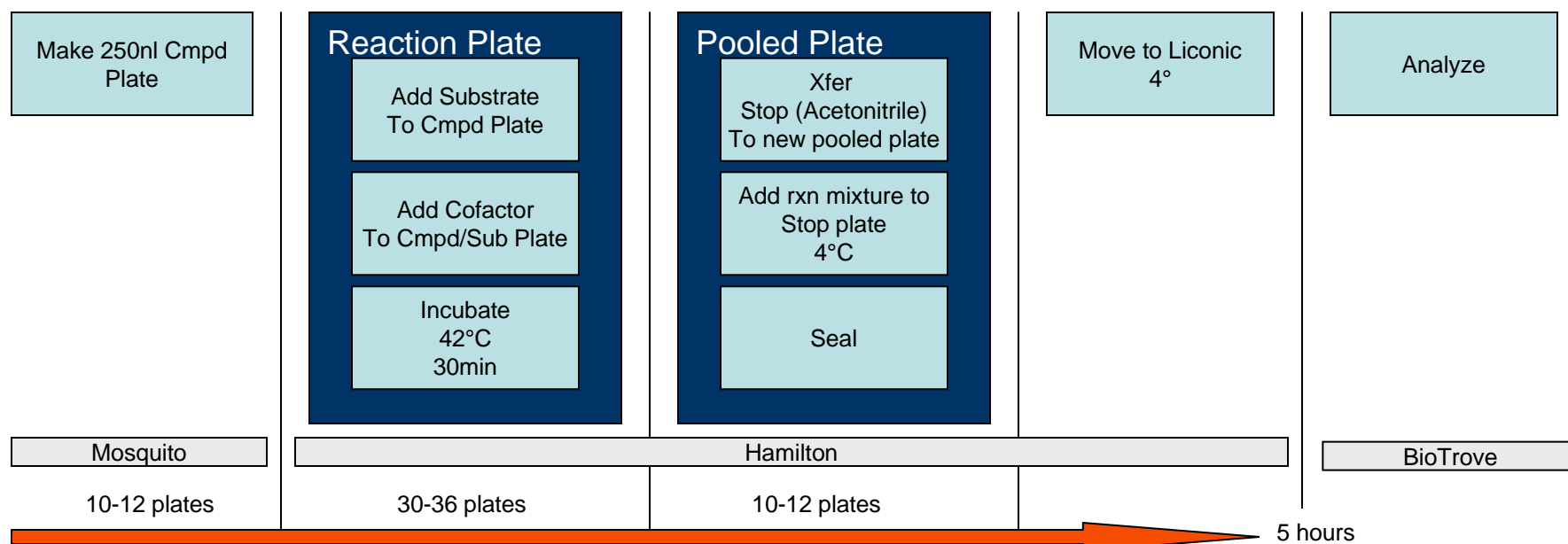


Types of Assay Systems

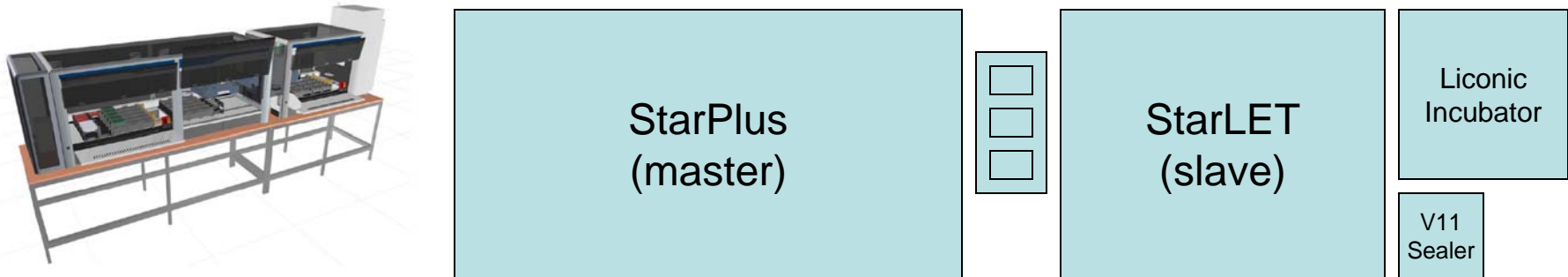
- Cell Based Assays
- Enzyme Assays
- Radioactive Filter-binding assays
- Mass Spec Detection Assays

Cytochrome P450 (CYP) Assays

- Cytochrome P450 represent a superfamily of enzymes (>3000 members) that catalyze the metabolism of a large number of endogenous substrates and xenobiotics
- Total 57 human cytochrome P450s have been identified; families CYP1, CYP2, CYP3 are primarily associated with the metabolism of drugs



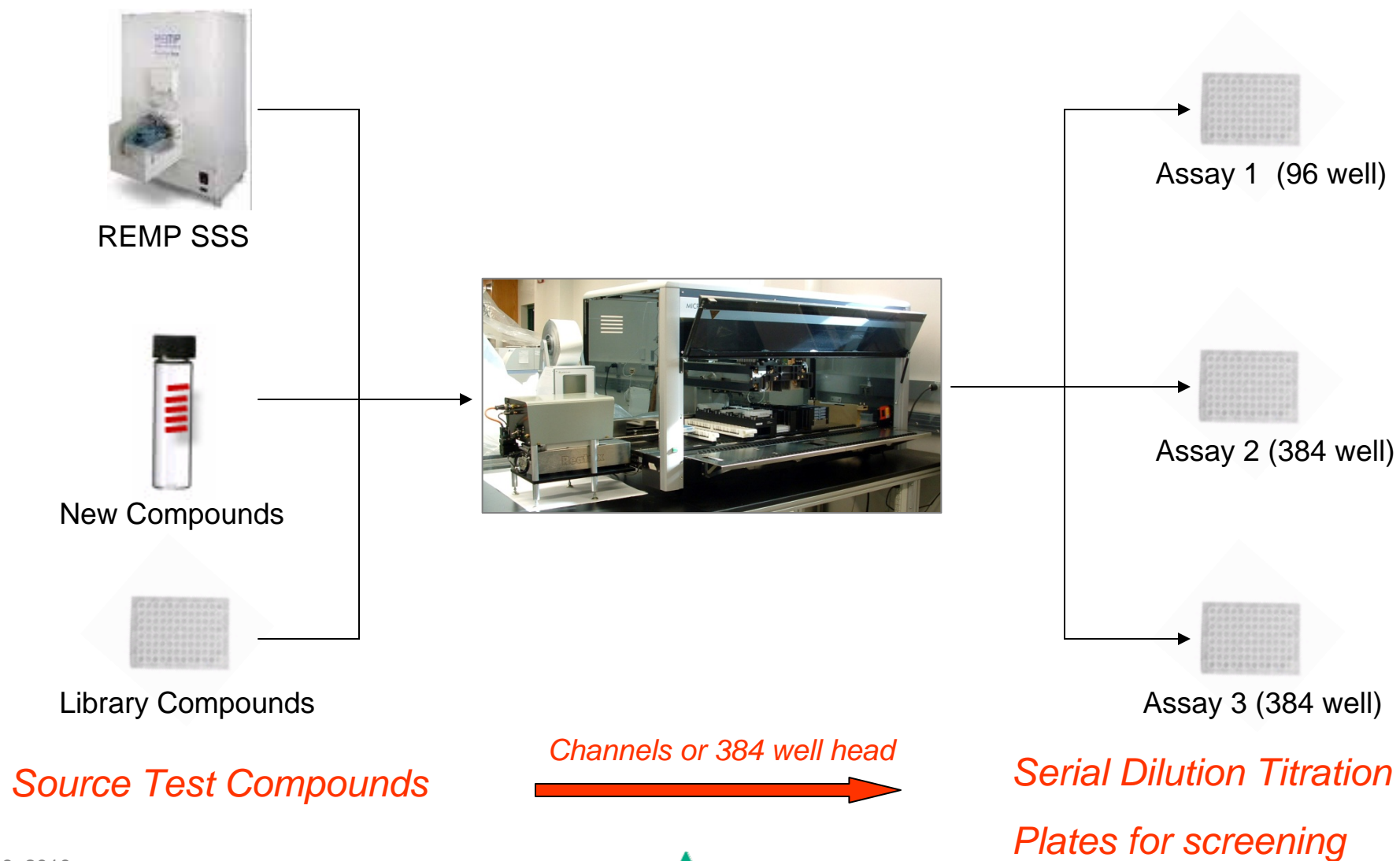
CYP Assay System



- Two independent systems controlled through one parent method computer
 - StarPlus used for reaction plate set up
 - StarLET used for pooling plate creation
- Plates transferred between systems by middle hand-off location
- Communication between computers over TCP/IP
- Assay detection performed on Mass Spec (BioTrove) System
 - Ratio of product:internal standard
 - 6 reads/well (2 reads for each CYP isoform tested)

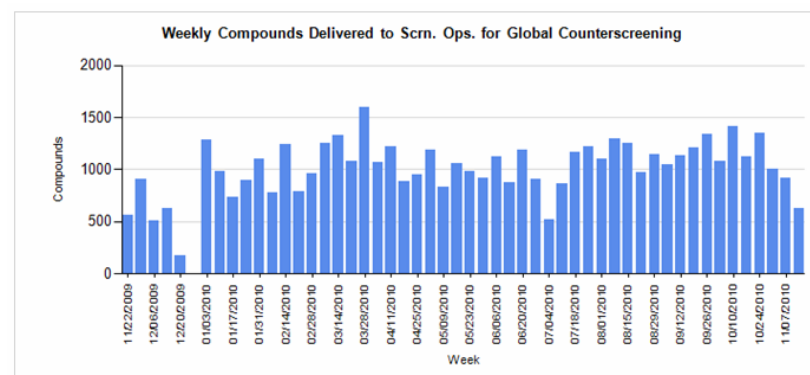
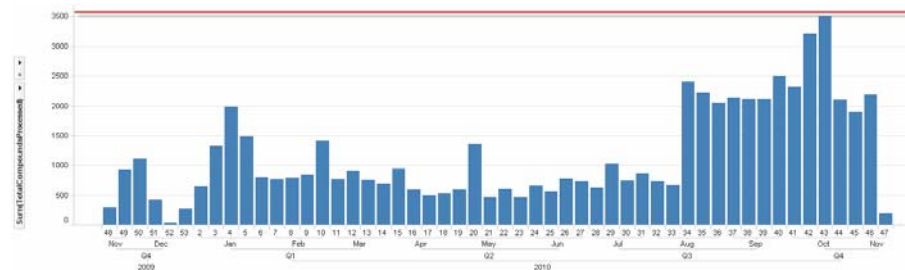
Compound Management

Compound Management



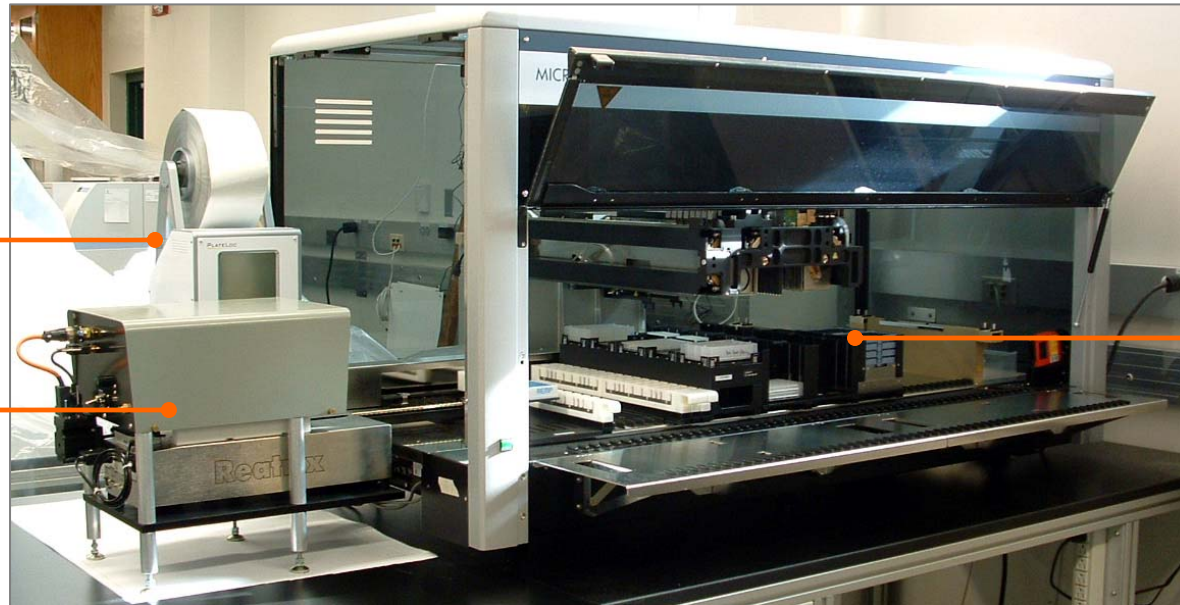
Compound Management

- Lead Identification / Lead Optimization
 - Dozens of compounds for dozens of programs / assays
- Global Counterscreening (GCS)
 - Hundreds of compounds from dozens of programs for few assays



**** Need a single, simple to use method to handle 1000's of titration curves per month ****

Plate Creation Workstation



Decapping/2D Barcode Scanning Unit

- (De)Capping, Reading 2D barcode of REMP storage tubes
- Custom integration

Velocity11 PlateLoc

- Heat sealer
- Small footprint, reliable and fast

Hamilton Star:

- 16 fully independent tips- uses disposable tips
- No system liquid, syringes, valves, tubing, waste,...
- Dual liquid level detection (capacitive and/or pressure)
- Auto-loading with full barcode reading capabilities

Compound Management

- Single Venus Method to:
 - Use multiple sources
 - REMP tubes
 - 1 dram high recovery vials
 - 96- or 384- well source plates
 - Use multiple destination plates
 - Max 5 distinct titration layouts in 96 and/or 384 well format
 - Max 8 separate plates of each titration layout
 - Add standards / controls to titration plates
 - Use disposable tips
 - Label created titration plates

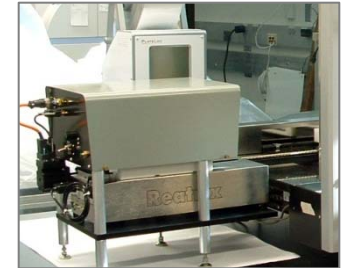
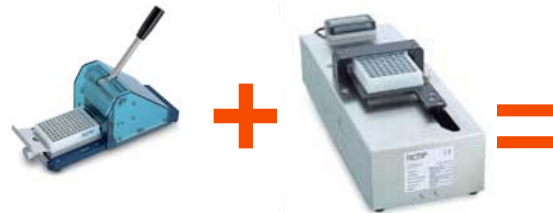
Enabling Technologies

- Software
 - Worklist Generator
 - Automated Capper/Decapper Vector library (.hsl)
- Hardware
 - Automated Capper/Decapper
 - Merck custom integrated workstation
 - Customized 1 dram high recovery vial racks
 - Common Deck Layout



Custom REMP Plate (De)Capper

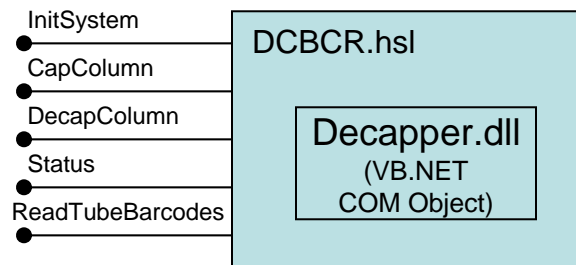
- Custom integration of:
 - REMP manual (de)capper
 - REMP Reatrix Barcode Reader
 - Smart Motors



- Joint effort between RY-Bioelectronics (hardware) and RY IVtP (software)
- Purpose:
 - Avoid duplicate moves on the Hamilton Star
 - Automated (de)capping a single column of REMP tubes at a time
- Capabilities
 - (De)capping
 - Barcode reading
 - Inert atmosphere dispensing
 - Cap sensing

REMP Plate (De)Capper Library

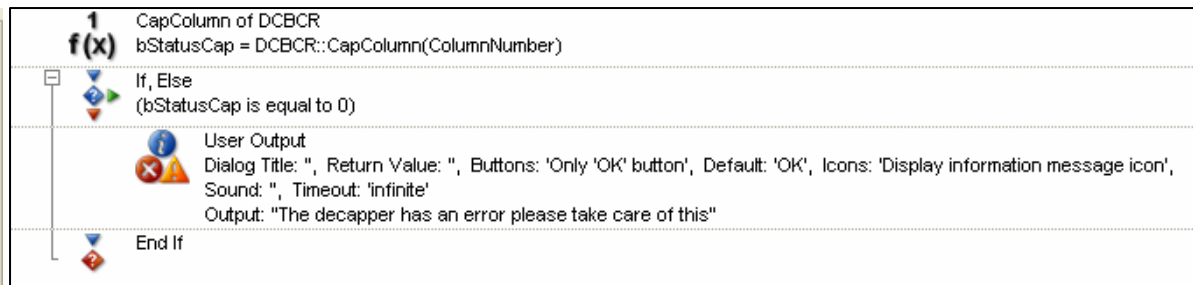
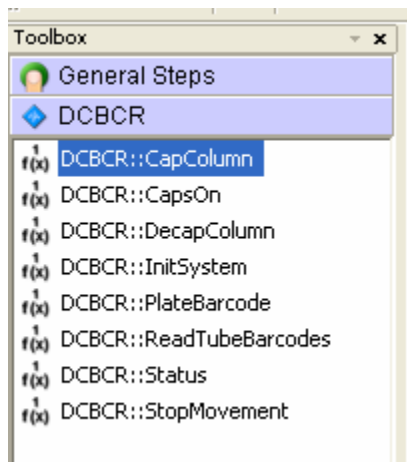
- Custom .hsl library to control the decapper



```
function InitSystem(variable comport, variable simulate)
{
    variable tmp; //must declare these first!
    variable tmp2;
    _simulate = simulate;

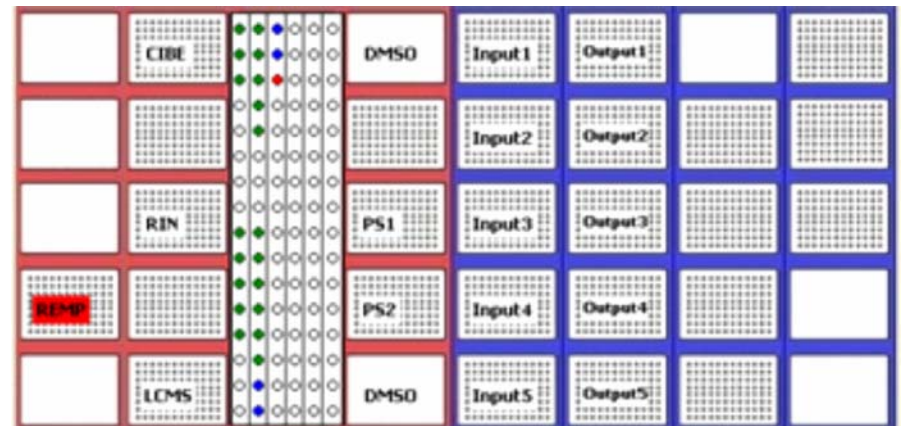
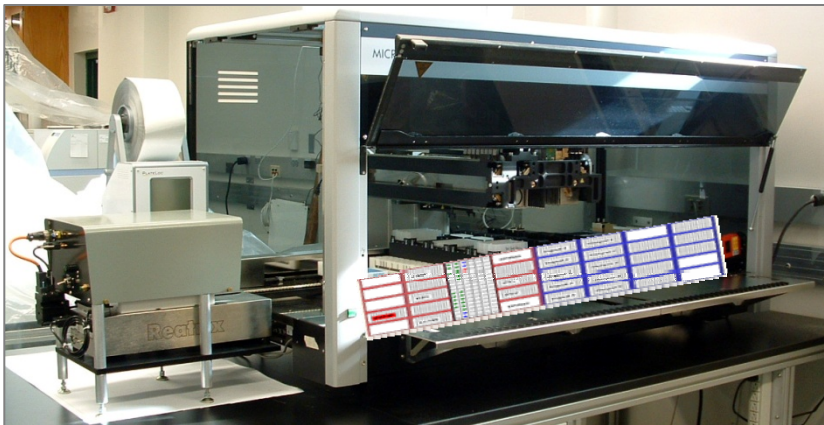
    // create the COM object interface to the dll
    _dcbcrObj.CreateObject("arkDecapper.nsDecapper.Decapper");
    //_VBTestCOM.CreateObject("VB6TestCOMdll.VB6ThisIsOnlyATest", withEvents);
    Trace("Decapper COM Object Created ");

    // will return 0 if not init; 1 if init
    Trace("Initializing Decapper ... ");
    tmp = _dcbcrObj.InitializeSystem("COM1", simulate);
    Trace("System Initialized ");
    return (tmp);
}
```



Deck Layout

- Ease of use paramount
 - All Sources go in one location
 - Vials, REMP Tubes, Library Plates
 - All Titration Plates Go Into One Location
 - All Tips are stacked
- Single deck layout to support numerous titration patterns



Worklist Generator

- Barcode and program driven



GCS Worklist Generator - 2.4.0.0

Confirm Deck Layout - Scan Yes or No

Welcome NAPOLITC

Sources Scanned: 4		Active Assays: 1 of 2				Standards Scanned: 4 of 4		
Position	Barcode	Active	Assay Name	Standards	Controls	Position	Barcode	
Wals16Pos1	IC002	<input checked="" type="checkbox"/>	Assay 1	4	1	<input checked="" type="checkbox"/>	Wals16Pos2-15	Standard 1
Wals16Pos1	IC003					<input checked="" type="checkbox"/>	Wals16Pos2-16	Standard 2
Wals16Pos2	IC004			0	0	<input checked="" type="checkbox"/>	Wals16Pos3-1	Standard 3
Wals16Pos2	IC005					<input checked="" type="checkbox"/>	Wals16Pos3-2	Standard 4

Controls Scanned: 1 of 1

Position	Barcode
<input checked="" type="checkbox"/> Wals16Pos3-3	Control 1

Destinations Scanned 1 of 1

Plate#	Barcode	Plate	Ass
Destination: 1	TEST1	384 Well	DP

Compounds: 19

Position	LNumber
Wals16Pos1-1	Compound 1
Wals16Pos1-2	Compound 2
Wals16Pos1-3	Compound 3
Wals16Pos1-9	Compound 4
Wals16Pos1-10	Compound 5
Wals16Pos1-11	Compound 6
Wals16Pos1-12	Compound 7
Wals16Pos1-13	Compound 8
Wals16Pos2-1	Compound 9
Wals16Pos2-2	Compound 10
Wals16Pos2-3	Compound 11
Wals16Pos2-4	Compound 12
Wals16Pos2-5	Compound 13
Wals16Pos2-9	Compound 14
Wals16Pos2-10	Compound 15
Wals16Pos2-11	Compound 16
Wals16Pos2-12	Compound 17
Wals16Pos2-13	Compound 18
Wals16Pos2-14	Compound 19

Reset Confirm Exit



Worklist Format

- .xls file providing instructions to a *single* method
- Worklist consists of a series of “Tasks” and “Task Details”
 - “Tasks” give instructions
 - Cap, Decap, Pipette, Seal, etc..
 - “Task Details” give specifics on the task
 - How many plates to seal
 - How many rows to cap or decap
 - How many tips to use
- Vector / Venus method written to interpret the tasks and task details

Example Worklist

Name Box	B	C	D	E	F	H	I	J	K	L	M	N	AssayName
1 Task	TaskDetails	RunID	SrcName	SrcPos	SrcBarcode	SrcVolume	DilFactor	DilVolume	DestName	DestPos	DestBarcode	MixVolume	AssayName
2 ChangePlates	1												Assay 1
3 TipsToUse	8												Assay 1
4 Pipette	LoadCompounds	21382	SourcePlatePos384_1	F3	Compound 1	45	1	0	PipettingStation384_1	A3	TEST1	0	Assay 1
5 Pipette	LoadCompounds	21382	SourcePlatePos384_1	G3	Compound 2	45	1	0	PipettingStation384_1	C3	TEST1	0	Assay 1
6 Pipette	LoadCompounds	21382	SourcePlatePos384_1	H3	Compound 3	45	1	0	PipettingStation384_1	E3	TEST1	0	Assay 1
7 Pipette	LoadCompounds	21382	SourcePlatePos384_1	L3	Compound 4	45	1	0	PipettingStation384_1	G3	TEST1	0	Assay 1
8 Pipette	LoadCompounds	21382	SourcePlatePos384_1	O3	Compound 5	45	1	0	PipettingStation384_1	I3	TEST1	0	Assay 1
9 Pipette	LoadCompounds	21382	SourcePlatePos384_1	P3	Compound 6	45	1	0	PipettingStation384_1	K3	TEST1	0	Assay 1
10 Pipette	LoadCompounds	21382	SourcePlatePos384_1	B4	Compound 7	45	1	0	PipettingStation384_1	M3	TEST1	0	Assay 1
11 Pipette	LoadCompounds	21382	SourcePlatePos384_1	F4	Compound 8	45	1	0	PipettingStation384_1	O3	TEST1	0	Assay 1
12 TipsToUse	8												Assay 1
13 Pipette	LoadCompounds	21382	SourcePlatePos384_1	H4	Compound 9	45	1	0	PipettingStation384_1	B3	TEST1	0	Assay 1
14 Pipette	LoadCompounds	21382	SourcePlatePos384_1	O4	Compound 10	45	1	0	PipettingStation384_1	D3	TEST1	0	Assay 1
15 Pipette	LoadCompounds	21382	SourcePlatePos384_2	C4	Compound 11	45	1	0	PipettingStation384_1	F3	TEST1	0	Assay 1
16 Pipette	LoadCompounds	21382	SourcePlatePos384_2	O4	Compound 12	45	1	0	PipettingStation384_1	H3	TEST1	0	Assay 1
17 Pipette	LoadCompounds	21382	SourcePlatePos384_3	K3	Compound 13	45	1	0	PipettingStation384_1	J3	TEST1	0	Assay 1
18 Pipette	LoadCompounds	21382	SourcePlatePos384_3	B4	Compound 14	45	1	0	PipettingStation384_1	L3	TEST1	0	Assay 1
19 Pipette	LoadCompounds	21382	SourcePlatePos384_3	E4	Compound 15	45	1	0	PipettingStation384_1	N3	TEST1	0	Assay 1
20 Pipette	LoadCompounds	21382	SourcePlatePos384_3	F4	Compound 16	45	1	0	PipettingStation384_1	P3	TEST1	0	Assay 1
21 TipsToUse	3												Assay 1
22 Pipette	LoadCompounds	21382	SourcePlatePos384_3	M4	Compound 17	45	1	0	PipettingStation384_1	A4	TEST1	0	Assay 1
23 Pipette	LoadCompounds	21382	SourcePlatePos384_3	O4	Compound 18	45	1	0	PipettingStation384_1	C4	TEST1	0	Assay 1
24 Pipette	LoadCompounds	21382	SourcePlatePos384_4	B4	Compound 19	45	1	0	PipettingStation384_1	E4	TEST1	0	Assay 1
25 TipsToUse	4												Assay 1
26 Pipette	LoadStandards	21382	SourcePlatePos384_6	E3	Standard 1	45	1	0	PipettingStation384_1	B4	TEST1	0	Assay 1
27 Pipette	LoadStandards	21382	SourcePlatePos384_6	G3	Standard 2	45	1	0	PipettingStation384_1	D4	TEST1	0	Assay 1
28 Pipette	LoadStandards	21382	SourcePlatePos384_6	L3	Standard 3	45	1	0	PipettingStation384_1	F4	TEST1	0	Assay 1
29 Pipette	LoadStandards	21382	SourcePlatePos384_6	K4	Standard 4	45	1	0	PipettingStation384_1	H4	TEST1	0	Assay 1
30 TipsToUse	1												Assay 1
31 Pipette	LoadControls	21382	Vials16Pos1	1	Control 1	3.6	12.6	41.4	PipettingStation384_1	N4	TEST1	33.8	Assay 1
32 TipsToUse	16												Assay 1
33 Pipette	LoadDMSO	21382	BackReservoir	1	DMSO	30	1	0	PipettingStation384_1	A24	TEST1	0	Assay 1
34 Pipette	LoadDMSO	21382	BackReservoir	2	DMSO	30	1	0	PipettingStation384_1	C24	TEST1	0	Assay 1
35 Pipette	LoadDMSO	21382	BackReservoir	3	DMSO	30	1	0	PipettingStation384_1	E24	TEST1	0	Assay 1
36 Pipette	LoadDMSO	21382	BackReservoir	4	DMSO	30	1	0	PipettingStation384_1	G24	TEST1	0	Assay 1
37 Pipette	LoadDMSO	21382	BackReservoir	5	DMSO	30	1	0	PipettingStation384_1	I24	TEST1	0	Assay 1
38 Pipette	LoadDMSO	21382	BackReservoir	6	DMSO	30	1	0	PipettingStation384_1	K24	TEST1	0	Assay 1
39 Pipette	LoadDMSO	21382	BackReservoir	7	DMSO	30	1	0	PipettingStation384_1	M24	TEST1	0	Assay 1
40 Pipette	LoadDMSO	21382	BackReservoir	8	DMSO	30	1	0	PipettingStation384_1	O24	TEST1	0	Assay 1
41 Pipette	LoadDMSO	21382	FrontReservoir	1	DMSO	30	1	0	PipettingStation384_1	B24	TEST1	0	Assay 1

Venus Method Example

Method: AssignTipSequence CapREMPColumn CreatePipettingSequences CreatePlateStackArray CreatePlateStackArrayOld DecapREMPColumn DiluentCHpattern DisposeOfTipBoxes EmptyTipArrays

106 Loop over following files:
- ParentWorklist
'loopCounter1' used as loop counter variable

107 **X=i+1** Assignment with Calculation
'LineCounter' = 'LineCounter' + '1'

108 **X=i+1** Assignment with Calculation
'LinesRead' = 'LinesRead' + '1'

109 TrcTrace of HSLTrcLib
TrcTrace("LinesRead=", ILinesRead)

110 File: Read
Read from file 'ParentWorklist'

111 Array: Set At
Set 'arrLineRead' within the array

112 TrcTrace12 of HSLTrcLib
TrcTrace12("Task = ", Task, "; TaskDetails = ", TaskDetails, "; Read = ", Read)

113 If, Else
(Task is equal to "ChangePlates")

117 If, Else
(Task is equal to "TipsToUse")

128 If, Else
(Task is equal to "Decap")

135 If, Else
(Task is equal to "Cap")

140 If, Else
(Task is equal to "ChangePreDilutionPlate")

143 If, Else
(Task is equal to "Pipette")

380 Comment
<Reset the variables for the next set of plates>

381 **X=0** Assignment
'DilutionPointNumber' = '0'

382 If, Else
(Task is equal to "SealPlates")

399 If, Else
(Task is equal to "EndOfFile")

405 **X=0** Assignment
'EjectTips' = '1'

406 End Loop

A	B	R
Task	TaskDetails	Read
Cap	12	

1 f(x) CapColumn of DCBCR
bStatusCap = DCBCR::CapColumn(ColumnNumber)

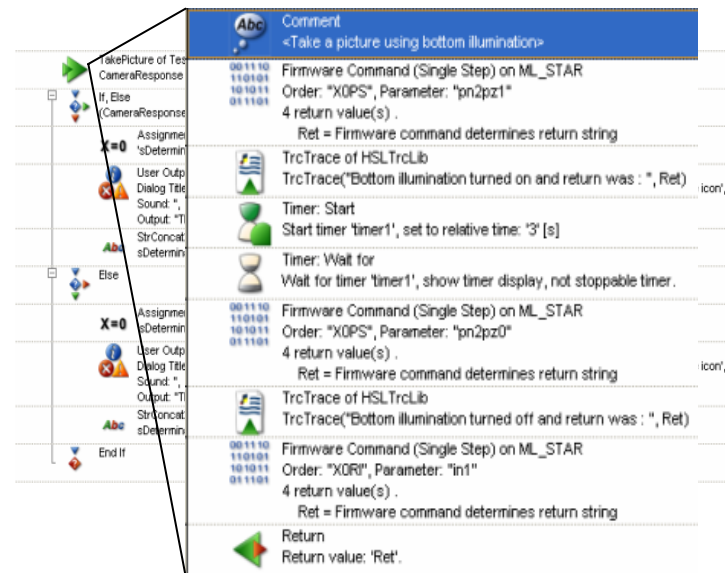
If, Else
(bStatusCap is equal to 0)

User Output
Dialog Title: ", Return Value: ", Buttons: 'Only 'OK' button', Default: 'OK', Icons: 'Display information message icon',
Sound: ", Timeout: 'infinite'
Output: "The decapper has an error please take care of this"

End If

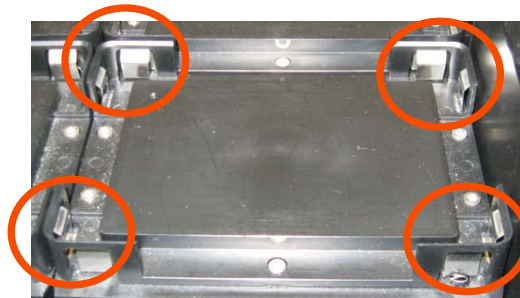
GCS REMP Cap Detection

- Integrated with a REMP ACD96
- Controlled via the I/O ports on the side of the Hamilton
- Commands sent directly to the camera via Firmware Command of Venus
 - Take picture setting output high/low (“X0PS”)
 - Read data via digital input (“X0RI”)



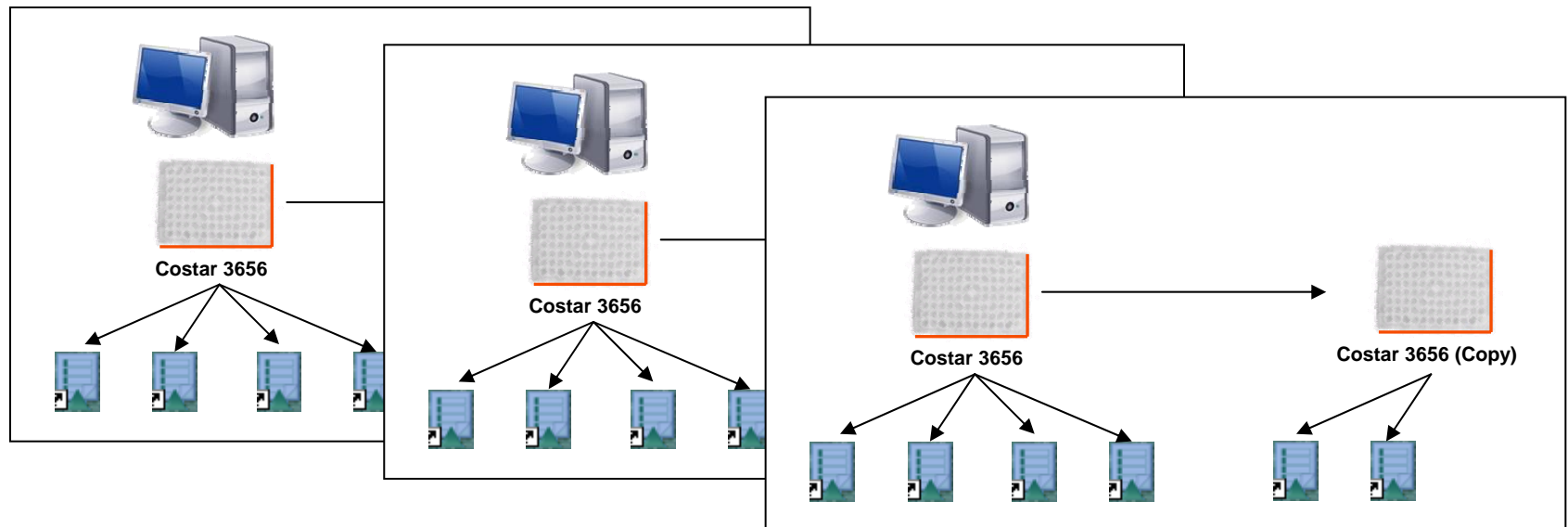
Ongoing Challenges

- Naming of labware
 - ? Cos_384_Sq = Costar _3656_Landscape ?
 - Proposal: <Manufacturer>_<Catalog#>_<Orientation>
- Carrier Nests
 - Standard nest wears out over time leading to positional problems



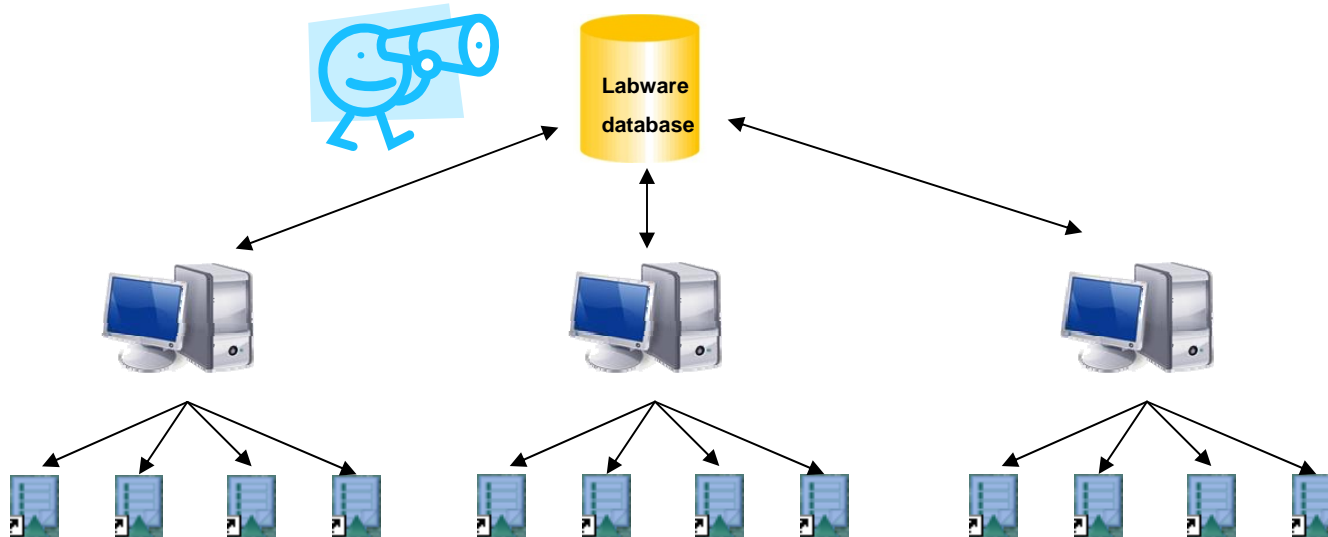
Ongoing Challenges

- Tracking labware in use and changes
 - If a change is made, how many methods are impacted?
 - Leads to creating another piece of labware
 - Same computer
 - Multiple computers



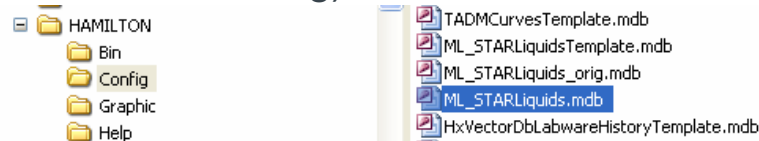
Ongoing Challenges

- Tracking labware in use and changes
 - If a change is made, how many methods are impacted?
 - Leads to creating another piece of labware
 - Same computer
 - Multiple computers
 - Proposed Solution: Centralized database with labware and what methods use the labware

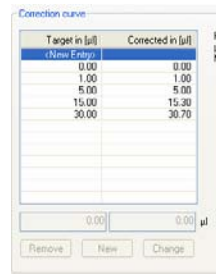


Ongoing Challenges

- Liquid classes are stored in the ML_StarLiquids.mdb access database (pathway = C:\Program Files\HAMILTON\Config)



- The correction curve information is stored in the CorrectionCurve field of the LiquidClass data table



CorrectionCurve	
5	Long binary data

- CorrectionCurve stores a data type of OLE Object

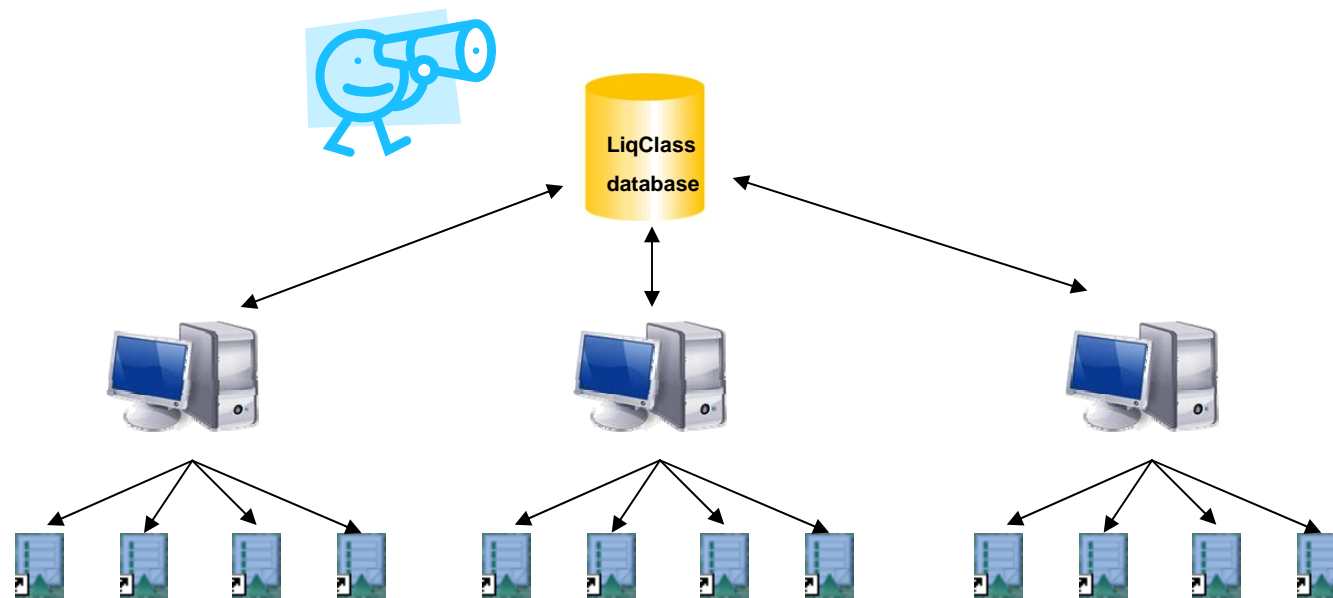
Field Name	Data Type
CorrectionCurve	OLE Object
PressureLIDSensitivity	Number

- No way to read results of a SQL query to obtain the information (binary data returned)
 - (for example – `SELECT * FROM LiquidClass WHERE LiquidClassName = '<Variable>'`)

LiquidClassID	LiquidClassName	LiquidName	LiquidVersion	LiquidDevices	LiquidNotes	OriginalLiquid	DispenseMode	TipType	CorrectionCurve
153	HighVolumeNeed...	Verification Solu...	1.0	1;4	<Binary data>	1	0	8	<Binary data>

Ongoing Challenges

- Proposed Solution: Centralized database with liquid classes and what methods use the labware



Acknowledgments

J Lachowicz
M Wildey
J Lisnock
A Woods

C Dufresne
A Hahn
C Fishman
V Kanthan

A Vergnon
A Struck
D Barbey
K Ferguson
K Houle

M Arocho
G Galviz
J Gibson
M Kurtz
D Shah
L Sonatore
J Wiltsie
S Xu
J Yudkovitz
D Zink

G Kath
D Conway
E Furlano
G King
N Simpson
F Solomon

R Ross
C Picarillo
M Rybka
K Kramer
C Fourier
B Houchins

Thank You!

