


High Throughput Crystallization System Development at Bristol-Myers Squibb

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Contents

- Overview of High Throughput Crystallization (HTC) System
 - Integration of Raman Spectroscopy
 - Bruker XRD Evaluation
- 

Importance of API Form in Pharmaceutical industry

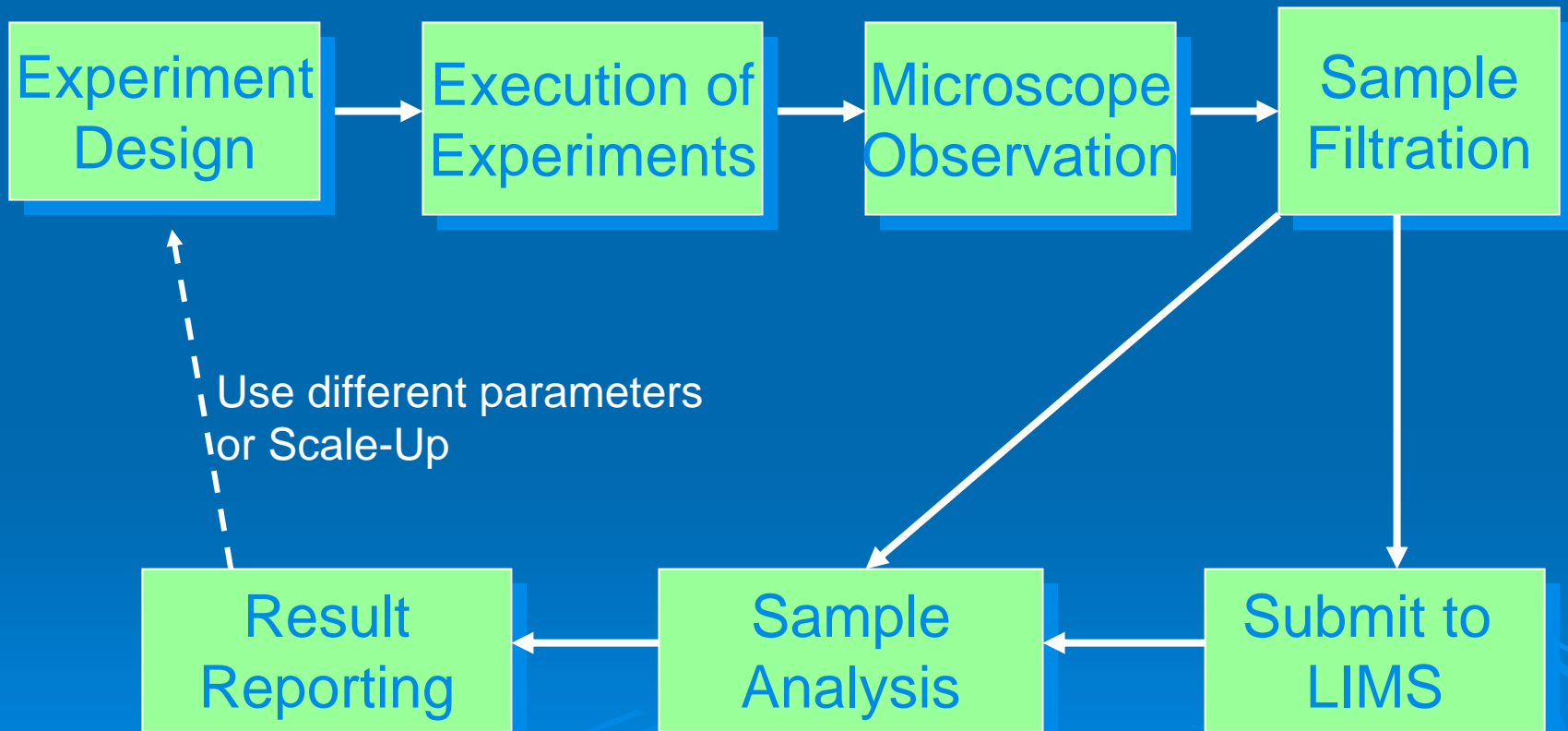
(API = Active Pharmaceutical Ingredient)

- Physical properties (play a dominant role in drug product performance)
 - Solubility (bioavailability) – related to patient experience
 - Stability, Particle size, Flow properties
- Legal requirements
 - Intellectual Property (IP) Protection
- Regulatory requirements
 - “Orangebook” Listing for polymorph patent required as of 2003
 - New Drug Application (NDA) holder must have evidence that claimed form is “same” as drug substance approved in NDA

Role of HTC system in Form Selection

- Tool for discovering form candidates
 - Design – what experimental space to investigate
 - Synthesis - Polymorphs, salts, co-crystals and solvates
 - Analysis – of candidates
 - Selection – based on data

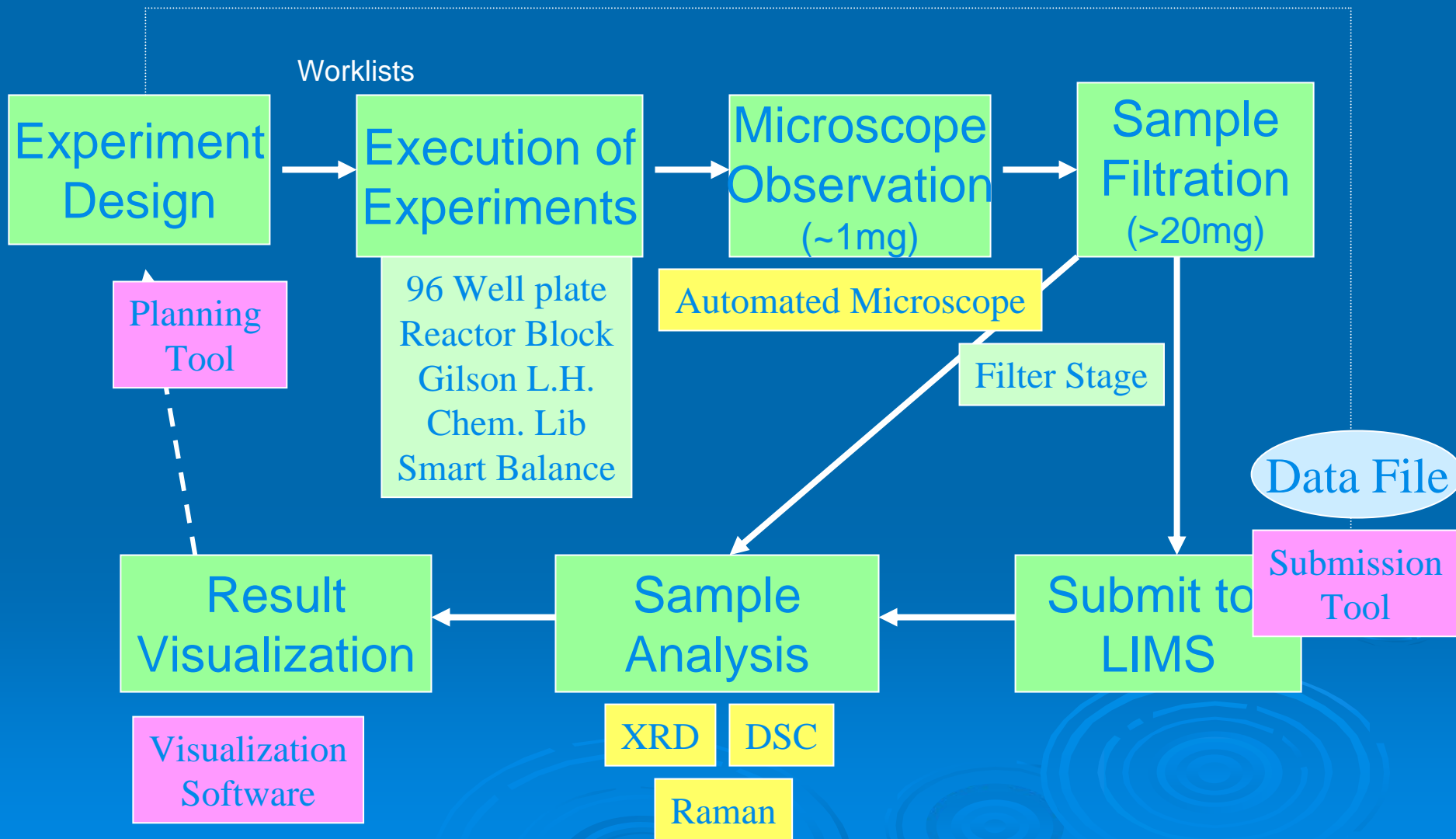
Manual Form Team Workflow in 90's



Goals

- Increase experimental space covered
(Increase chances of finding optimal API form)
- Increase throughput using limited resources
- Increase analytical palette
- Improve decision making
- Shorten timeline and increase quality of investigation

Modules for First HTC System



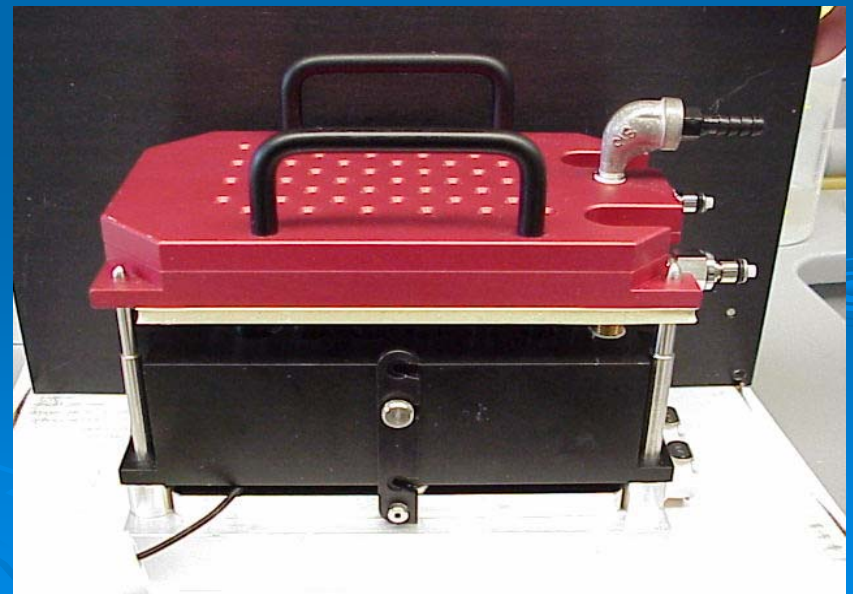
EXECUTION OF EXPERIMENTS

- **96 Well Glass Plates**

- Good for small scale (1mg) screen
- Compatible with Raman, Microscope, and Liquid Handler
- Direct examination through Microscope and Raman

- **Reactor Blocks / Test Tubes**

- Facilitate larger scale parallel reactions (> 20 mg)
- Temperature control capability (-20 to +120 ° C)
- Reflux condenser or inert gas manifold capability



AUTOMATED POLARIZED MICROSCOPE

- Fully automated image acquisition
- 4 plate capacity
~1 Hour per run
15 min / plate
- 32 to 540x
Magnification



Output from Microscope

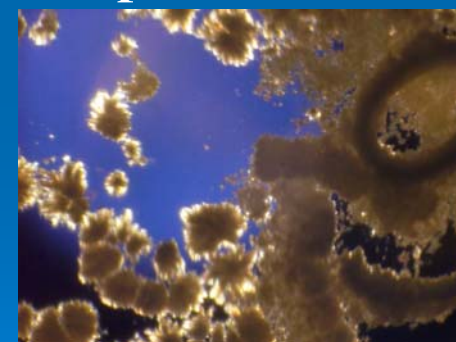
Solvents

	Toluene	EtOAc	THF	DCM	CH3CN	IPA	Acetone	EtOH	MeOH	BuOH	MTBE	MIBK
HCl												
MSA												
H3PO4												
H2SO4												
Benzene SA												
HBr												
HNO3												
Camphor SA												
Ethane SA												
D(+)-CSA												
Dichloro AA												
Dihydroxyl BA												
Oxalic A												
Toluene SA												
Adipic Acid												
Camphoric Acid												

Counter ions



Adipate, Toluene



Tosylate, EtOH

SAMPLE FILTRATION (> 20 mg scale)

- **Multiple Direct Filtration**

- Up to 48 crystallization slurries can be directly filtered onto X-ray analysis device.

- **Direct mount on X-ray Diffractometer**

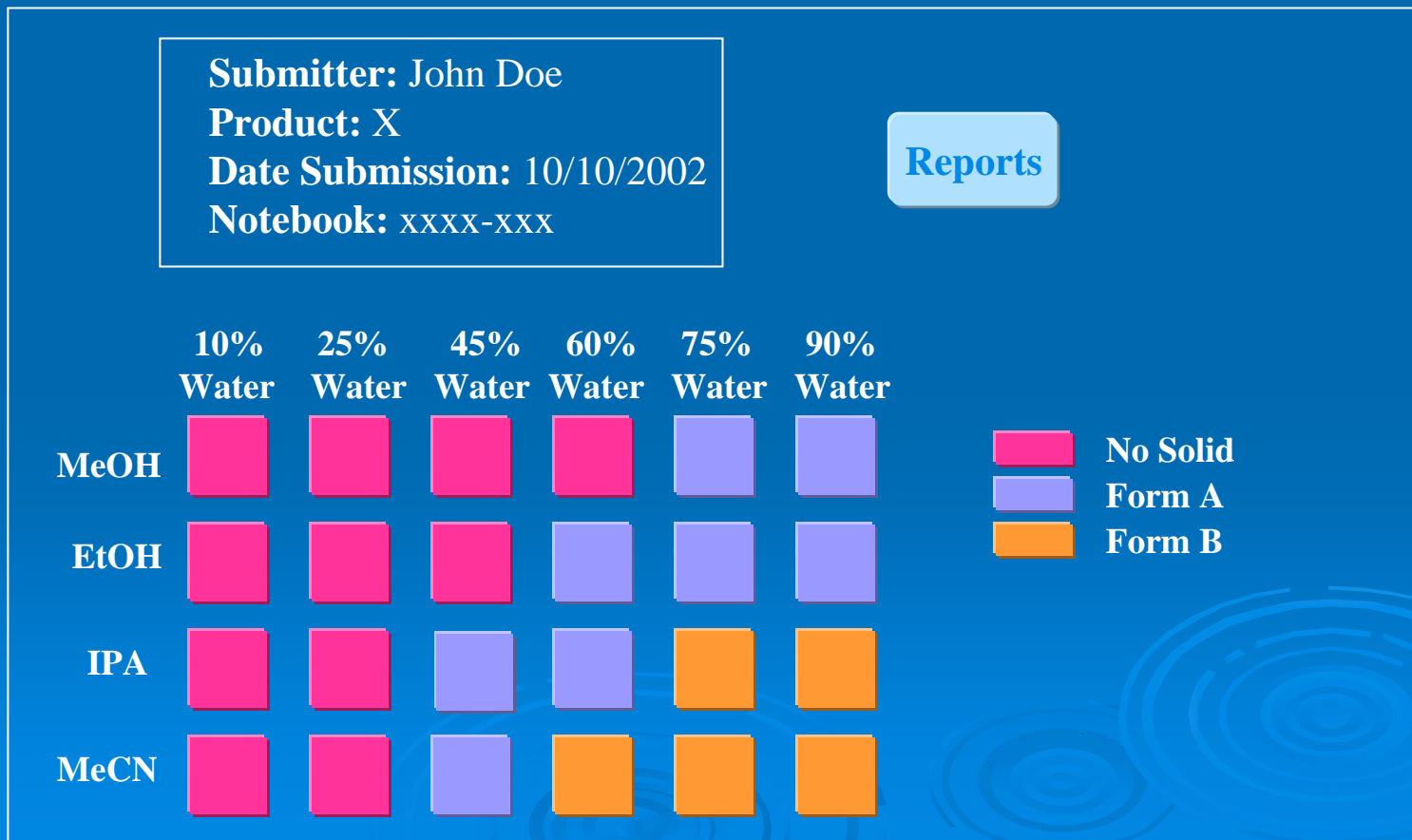
- Eliminate manual sample preparation
 - Minimize the sample size to 5 mg



X-ray Filter Stage and Holder (patented)

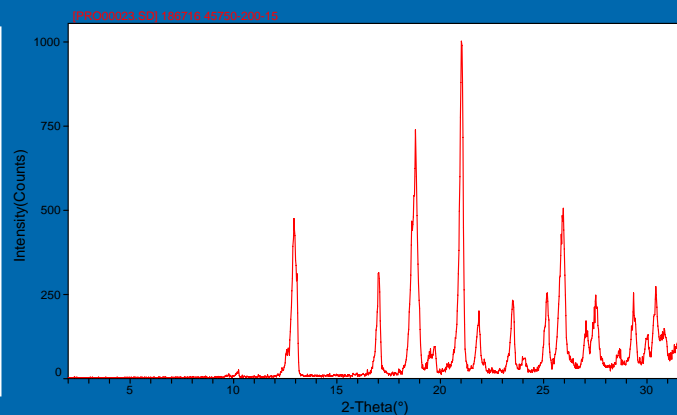
DATA VISUALIZATION

- Present Analysis Results graphically (as Colored Buttons)
- Represent Experiment as Performed (rather than tabular)
- Visualize the Effect of Varied Parameters
- Share Analytical Data and Experimental Conditions Among Scientists : Colored Road Map for Future Investigation

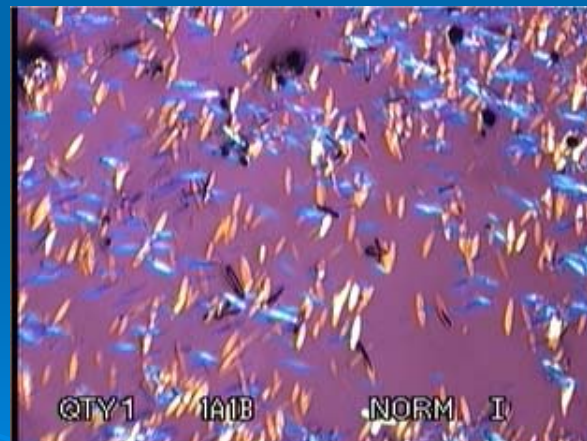


Sample of Single Experiment Results

Drug Amt: 10 mg
Solvent: IPA
Solvent Vol.: 2 ml
Aging Time: 40C/4h
Antisolvent: Water
Antisolvent. Vol: 6 ml
Agitation: Magnetic Stirrer.



Result: Form B
New peak at 3.45 and 5.46

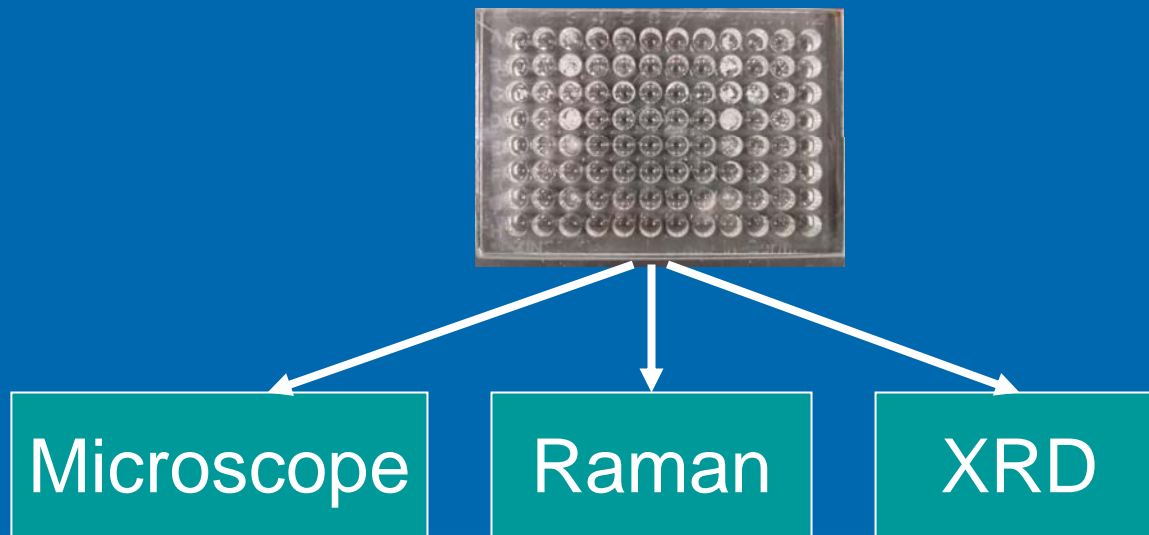


HTC Workflow Gap Analysis

- Difficult to identify “hits” with Microscope alone
- Impossible to sort the “hits”
- Scale-up Crystallization was required for Raman and XRD analyses
- Delayed identification of potential “leads”

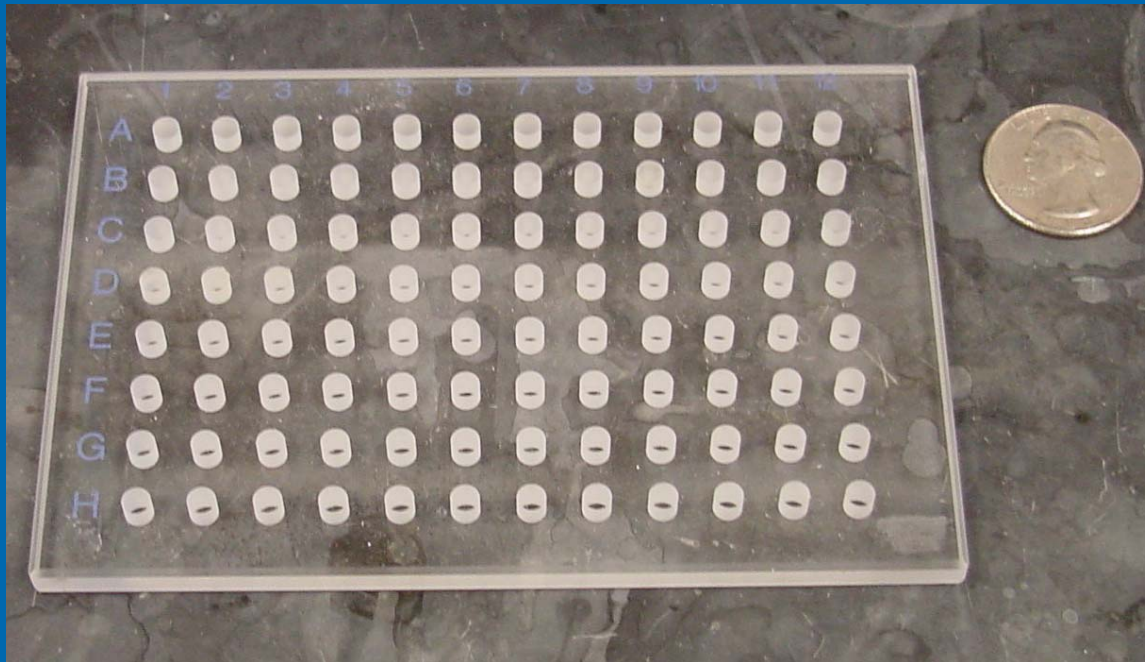
Workflow Enhancement

Single plate for three Analyses with no extra sample preparation or scale-up



- Borosilicate glass has properties which interfere with Raman and XRD signals
- Several 96 well **quartz** plates with thin bottoms were designed and fabricated for evaluation

BMS Designed 96 Well Quartz Plates



Bottom Thickness

- 0.18 mm (Small well)
- 0.25 mm (Medium well)
- 0.37 mm (Large well)

Well Diameter

- 3 mm
- 4 mm
- 6 mm

US Patent filed for the plate design
and use with HTC/XRD applications

Plate Preparation for Evaluation

- Raman algorithm and correlation tests as well as Bruker XRD and quartz plates.
- The plates were prepared with different amounts and composition of the N1 and H₀ forms of an API

Amount : 2 mg, 1mg, 0.5 mg, 0.25 mg, and 0.1 mg of N1

Composition of N1/H₀: 100%, 90%, 75%, 50%, 25%, 10%, 0%

2mg N1	1mg N1	0.5mg N1	0.25mg N1	0.1mg N1	100% N1	90% N1	75% N1	50% N1	25% N1	10% N1	0% N1
2mg N1	1mg N1	0.5mg N1	0.25mg N1	0.1mg N1	100% N1	90% N1	75% N1	50% N1	25% N1	10% N1	0% N1

First two rows of 96 well plates

Raman Spectroscopy Integration



Collaboration

- Began with evaluation of several vendors instruments
- Selection based on evaluation criteria
- Continued with the PO (purchase order)
 - Line item deliverables were included for implementation and customization for integration

Customization for Integration

➤ Data Communication

- Input and output requirements
 - Use of ASL file as input for experiment (generated by submission program (in-house))
 - Output group number following data analysis
- Naming conventions for data
 - Unique well qualified name generated from information in ASL file to ease data searching

➤ Automation of analysis

- Processing of data and grouping of results
 - Custom macro developed for data processing

Thermo Electron's Nicolet Almega Raman Spectrometer

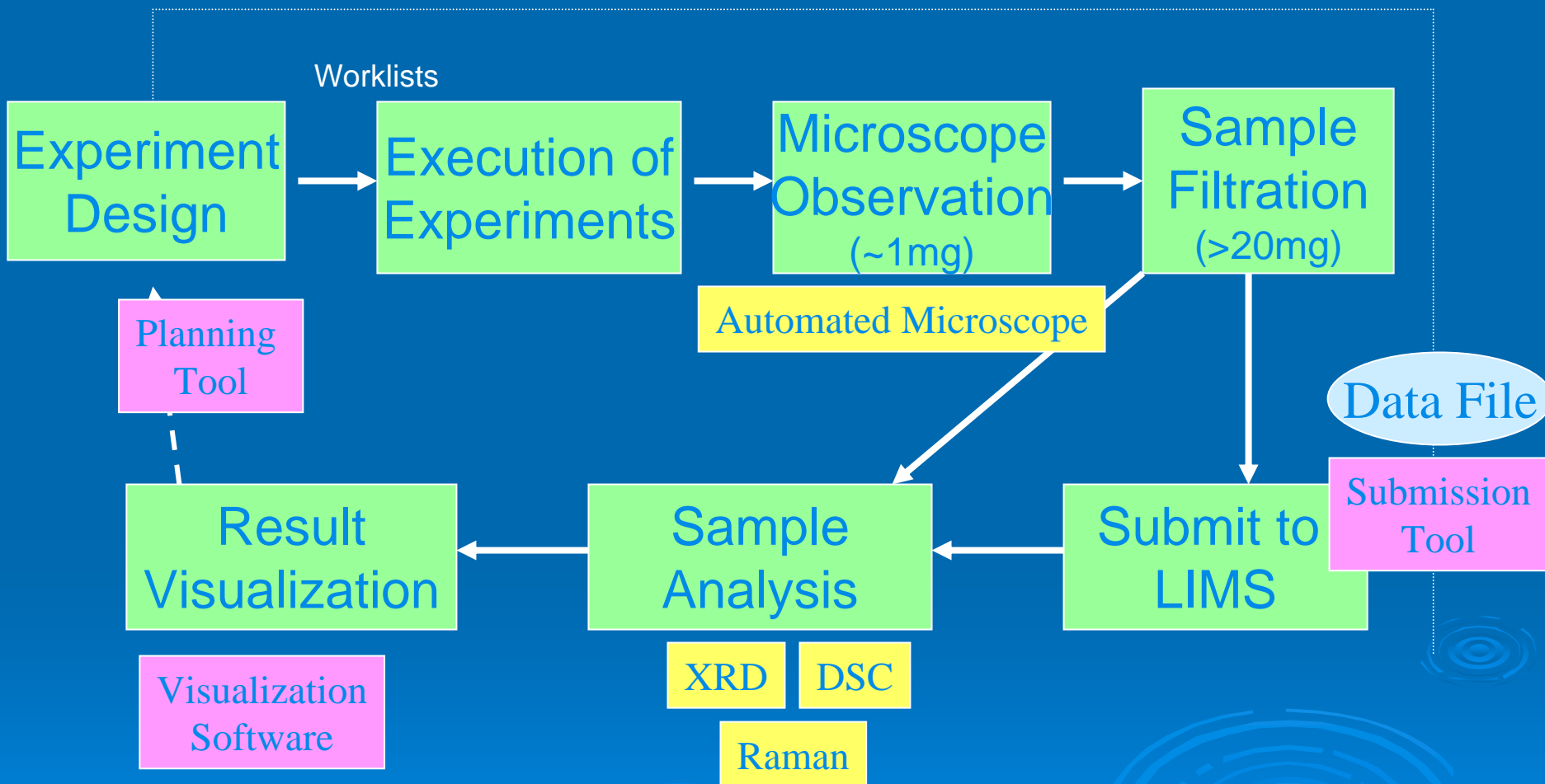


- Dual laser (785 nm and 633 nm)
- Dispersive
- Multiple gratings for desired resolution
- Motorized stage (various plate size)
- Easy array setup*
- Algorithm for easy analysis*
- Custom Software*

***opportunities for
collaboration**

HTC Workflow

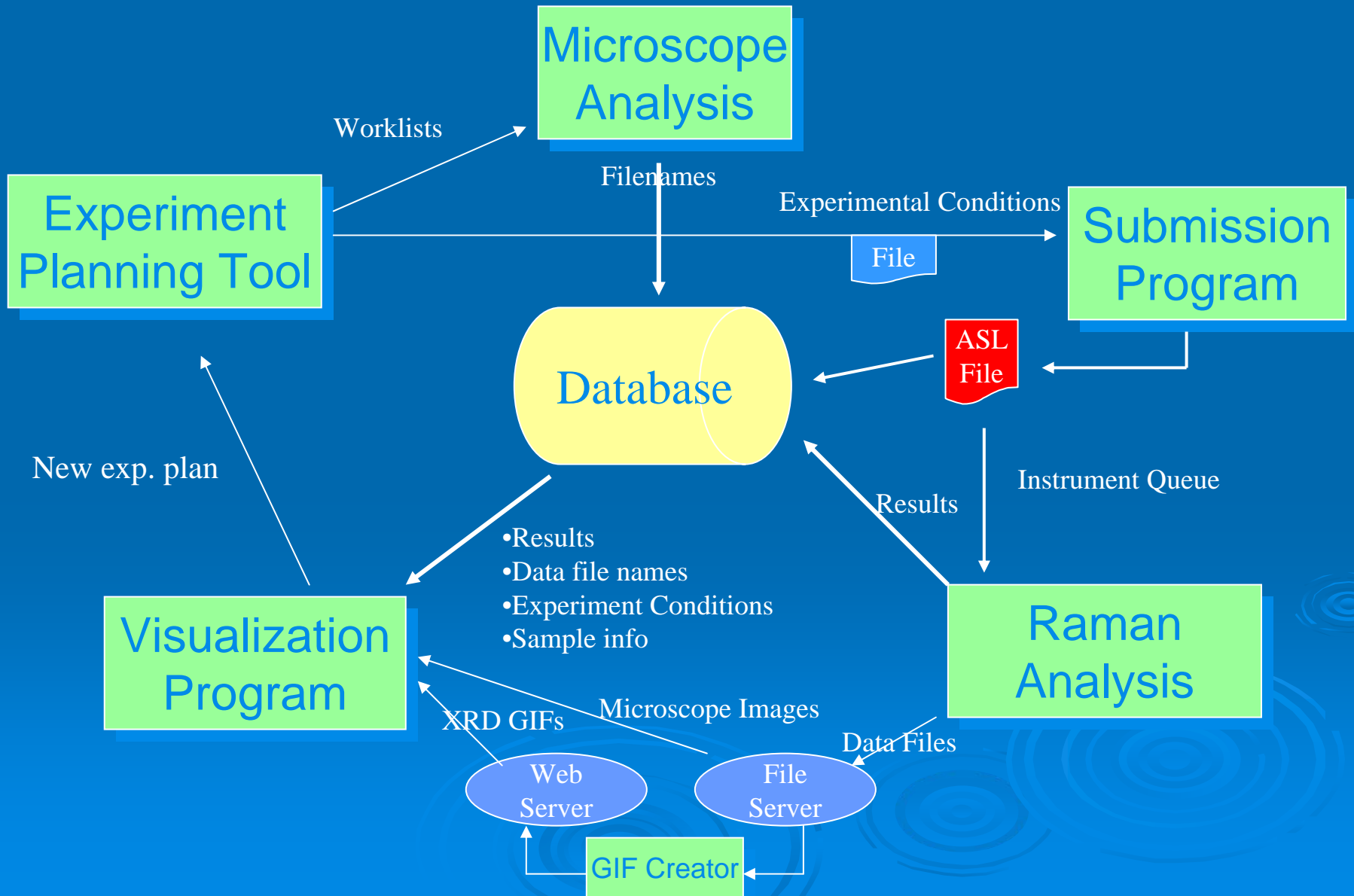
Software and instruments



All of these modules have to communicate effectively within the system

Data Flow

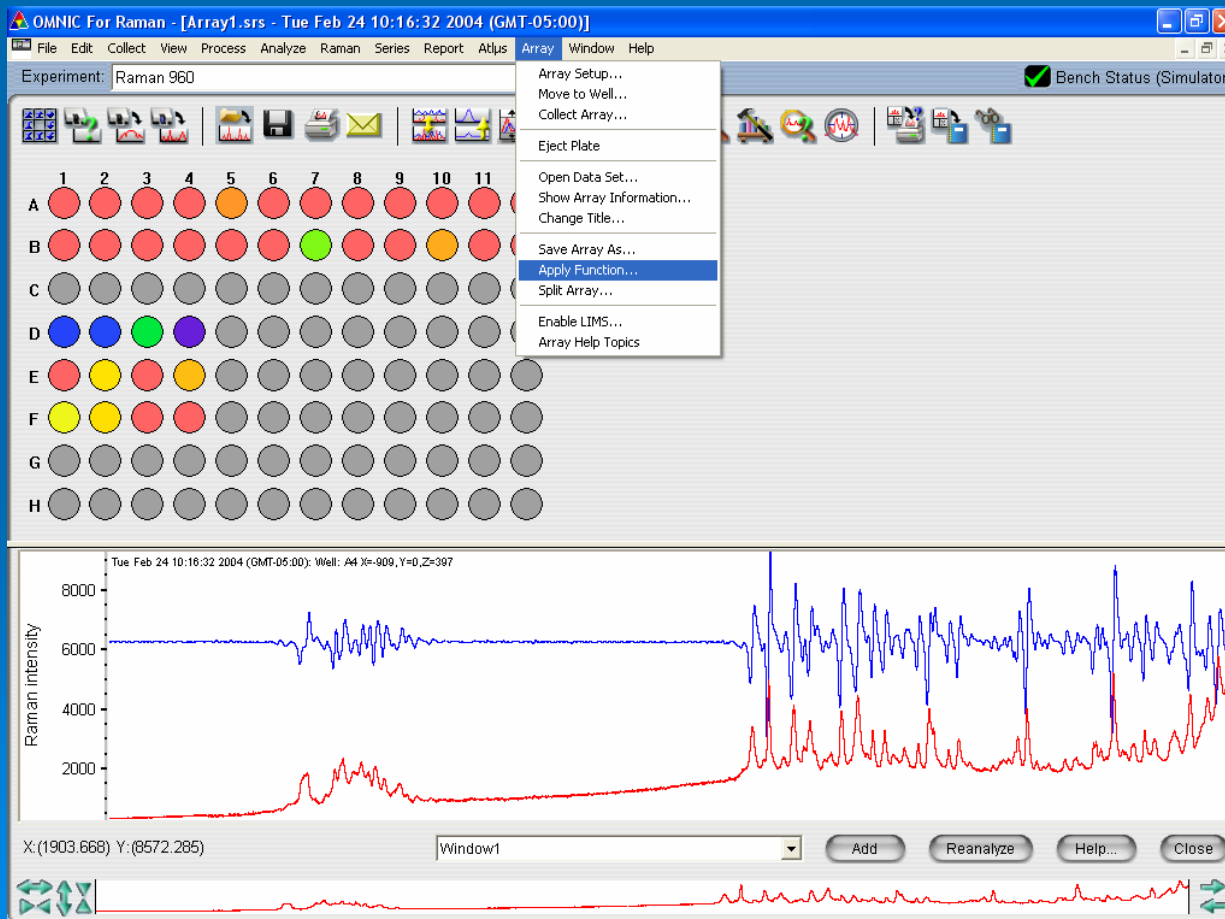
ASL File as **RAMAN INPUT**



Data Analysis

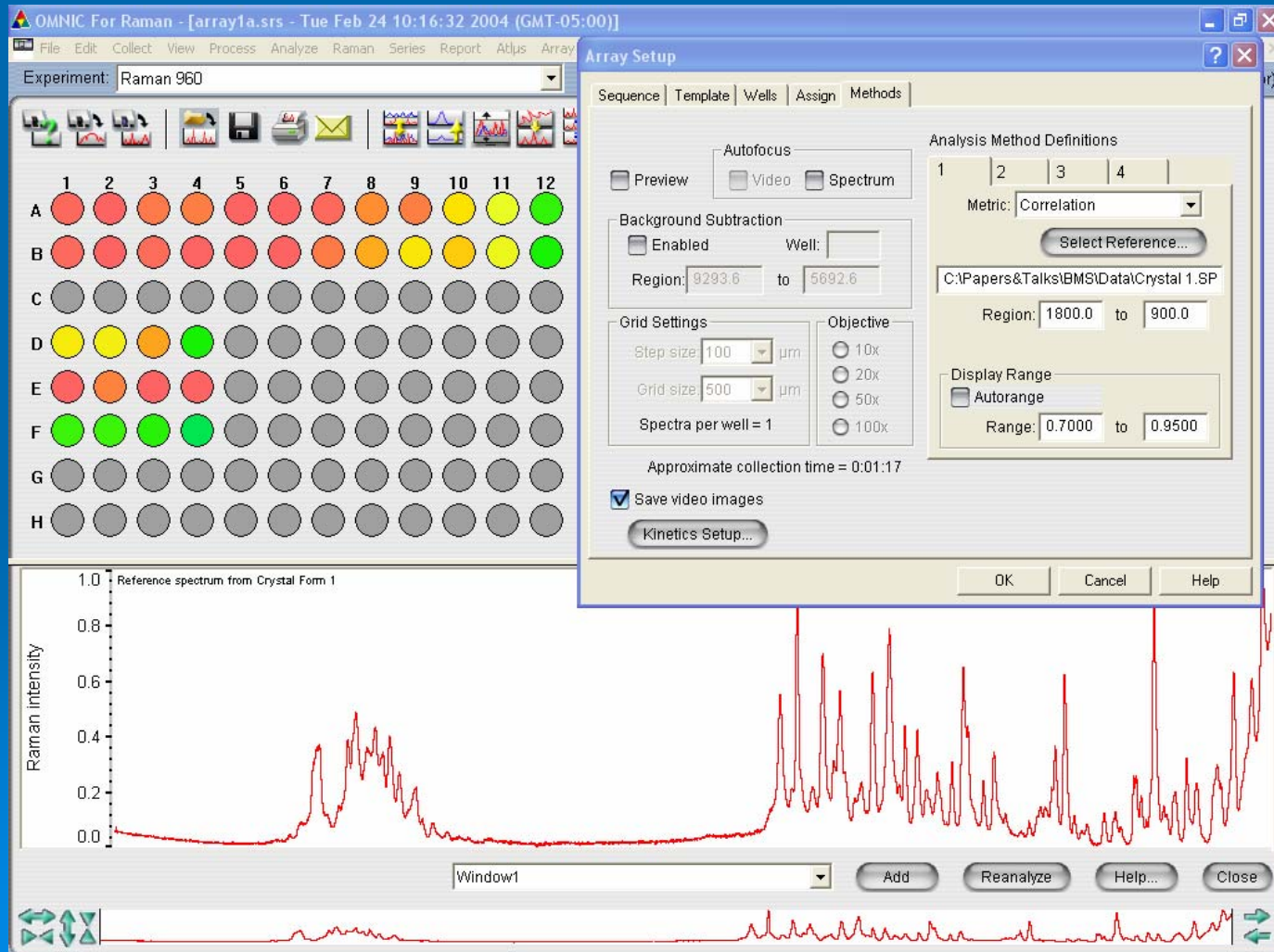
Spectral Preprocessing

- Smoothing and derivative remove most background
- Norris derivative (smoothed derivative)



Data Analysis

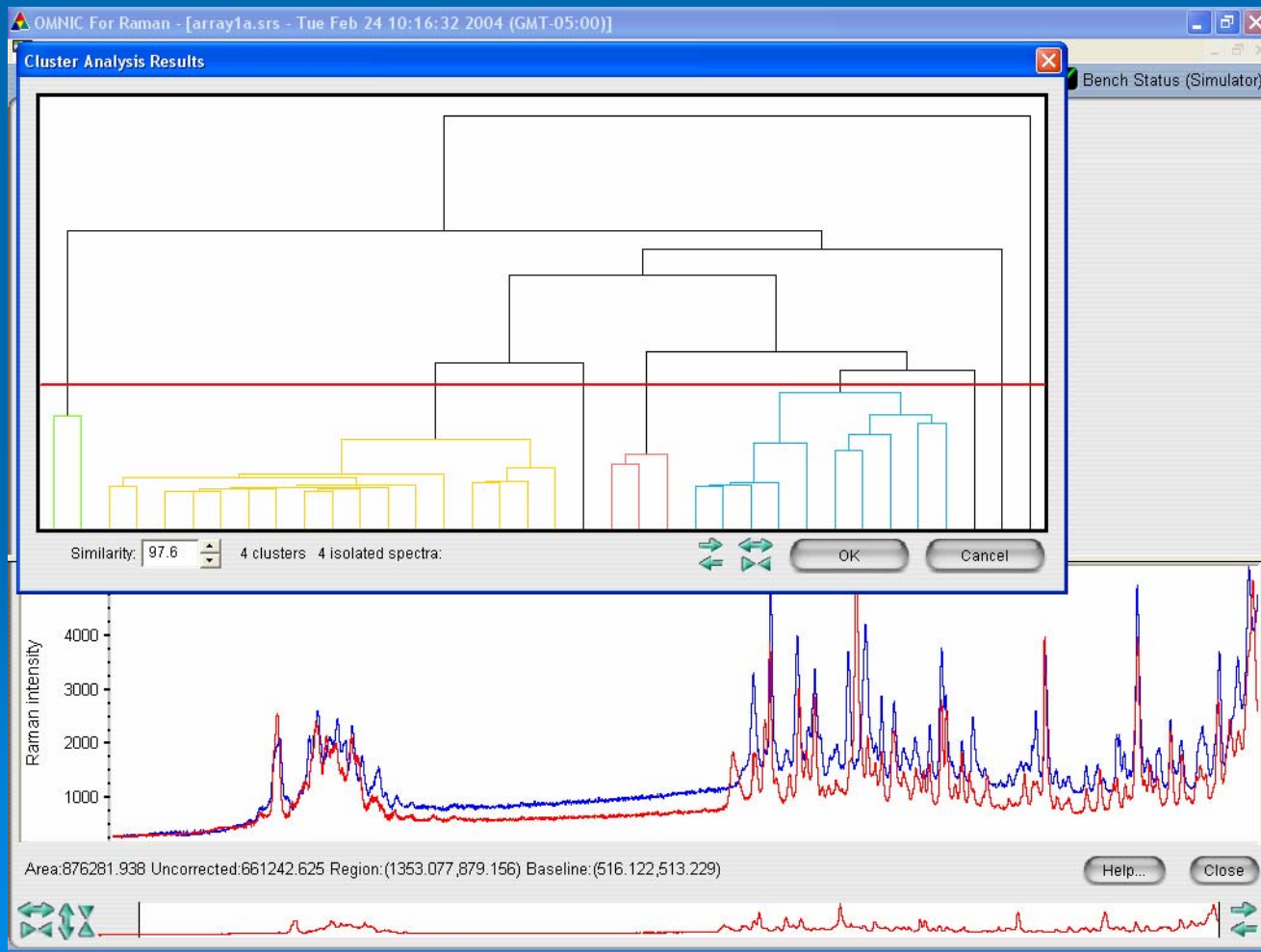
Visual Display of Correlation with Reference



Data Analysis

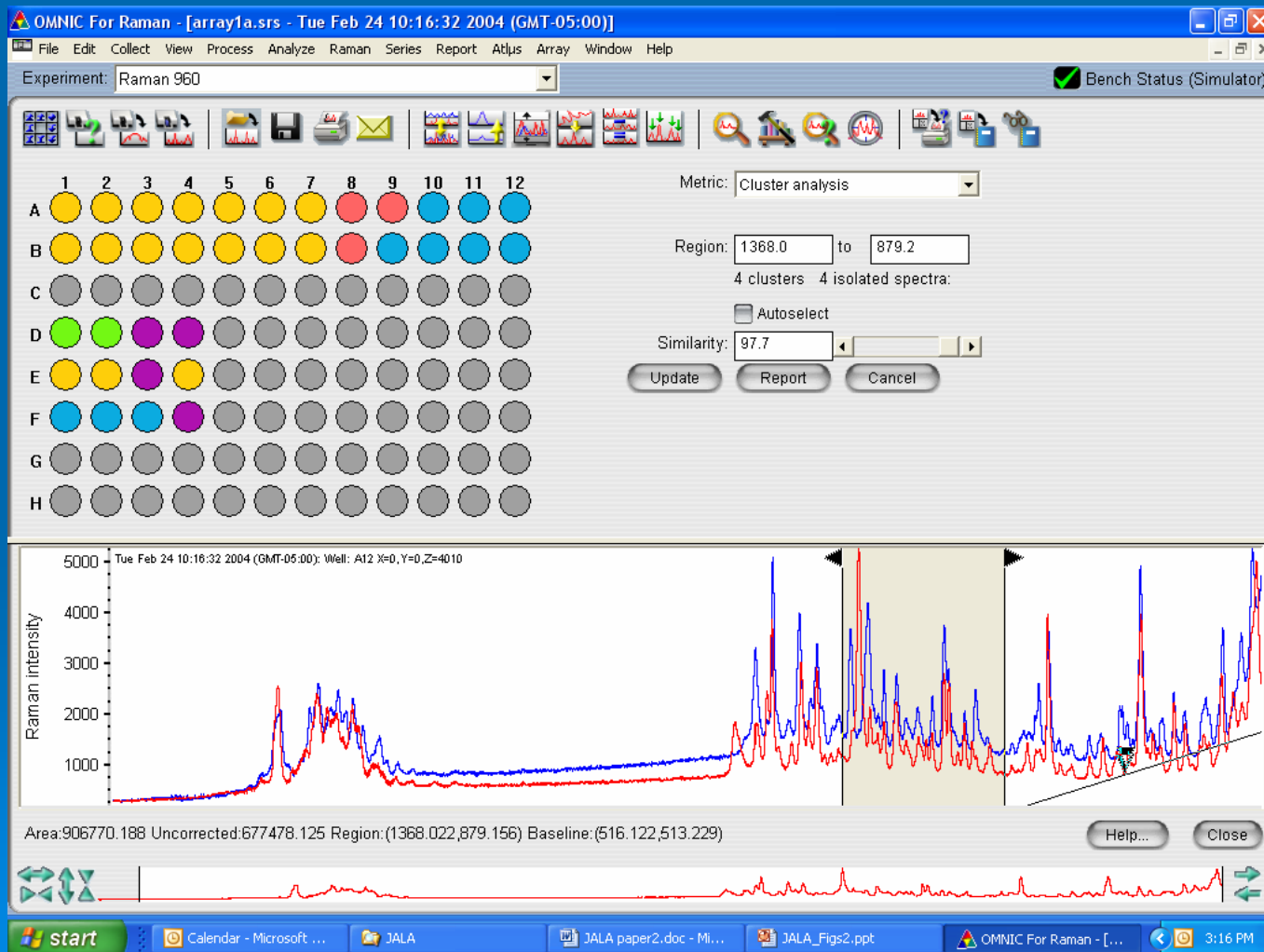
-without reference

Dendrogram from Cluster Analysis



Data Analysis

Results of cluster analysis showing two major groups



Output of Analysis

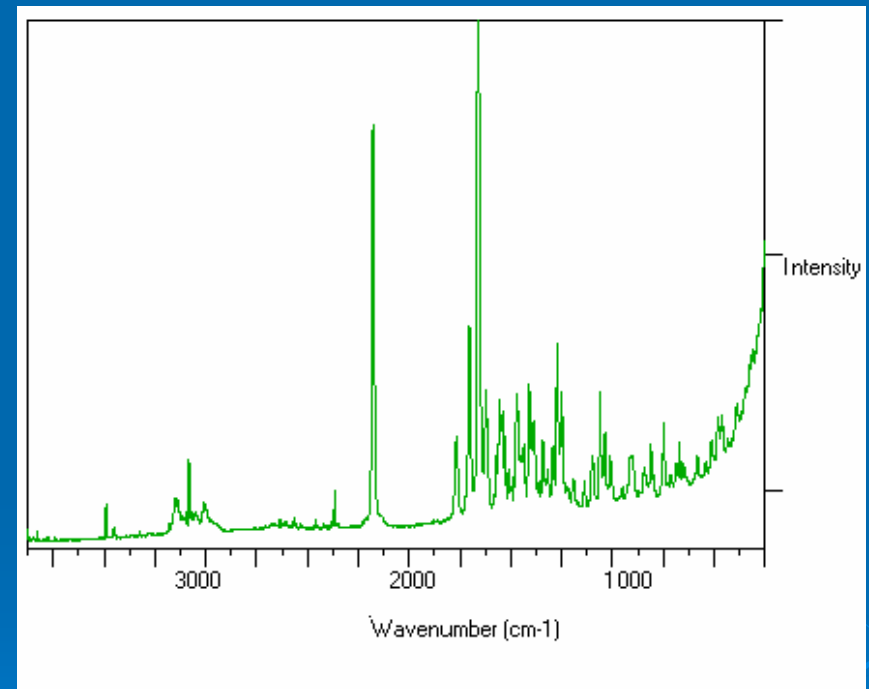
Results of the Analysis in csv File

Sequence file: K:\Raman\VRNEWCOPY.ary						
Comment:						
Method: Group analysis Region: 2600.0 - 300.0						
Similarity: 95						
Well	Mode	X Pos	Y Pos	Z Pos	Group	
A1	Manual	12	-158	702	1	
A2	Manual	-555	-101	702	1	
A3	Manual	-536	-759	1025	1	
A4	Manual	-1070	-806	1090	1	
A4	Manual	-1070	-806	1090	3	
A5	Manual	-146	584	1090	1	
A6	Manual	-1571	588	1129	1	
A7	Manual	-1075	912	1129	1	
A8	Manual	0	-172	1068	2	
A9	Manual	-2640	1817	1298	2	
A9	Manual	-2640	1817	1298	3	
A10	Manual	-1313	1329	771	3	
A11	Manual	-144	0	1084	3	
A12	Manual	-1620	1250	1010	3	

Spectral Raw Data to Image File

```
##TITLE= Tue Feb 24 10:16:32 2004 (GMT-05:00): Well: A1 X=346,Y=0,Z=3884
##JCAMP-DX=5.01 $$ Nicoletv. 521
##DATA TYPE=INFRARED SPECTRUM
##ORIGIN=Public Domain
##OWNER=Nicolet
##LONGDATE=2036.02/06
##TIME=01:28:16
##DATA PROCESSING=
##XUNITS=
##YUNITS=ARBITRARY UNITS
##FIRSTX=99.573181
##LASTX=3700.020752
##FIRSTY=13634.541992
##MAXX=3700.020752
##MINX=99.573181
##MAXY=16567.503906
##MINY=689.182007
##XFACTOR=1.000000
##YFACTOR=1.000000E-004
##NPOINTS=7469
##DELTA X=0.482117
##XYDATA=(X+(Y..Y))
99.573 13634.5424 140374576 143333824 145138912 146208144 146148336 145635008
102948 145204544 144883856 144571536 144330704 143976096 143213792
105841 141945808 140499456 139102032 137743744 136674416 136249664
108733 135900960 135336032 134566576 133747752 133020624 132378200
111626 131753056 131011024 130405264 130070504 129914032 129740568
114519 129423440 129061096 129125960 129890808 130627120 131319704
117411 131953936 132478904 132879200 133255040 133762128 134371792
120304 135049216 135685520 136455472 137610288 139204672 140946064
123197 142511712 144096880 146029616 148526592 151169088 153413024
126090 155216112 156852976 158302400 159680864 161151664 162677328
128982 164025424 164988784 165582288 165675040 165231616 164345600
```

Gif file
converter
➔



Text File (JCAMP-DX format)

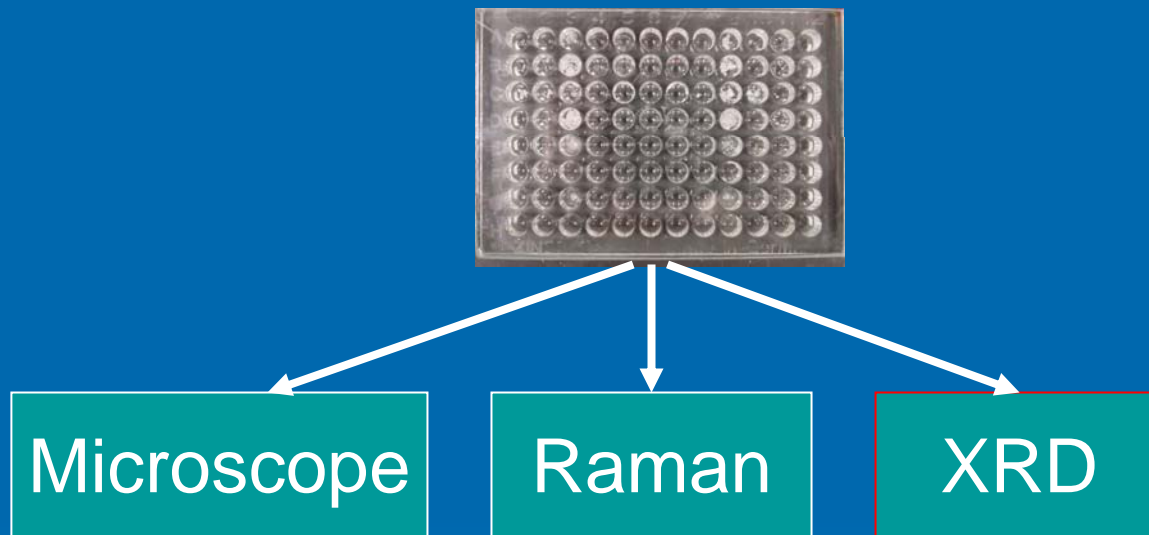
Image File for Data Visualization Tool

Bruker XRD Evaluation



Workflow Enhancement

Single plate for three Analyses with no extra sample preparation or scale-up to obtain P-XRD



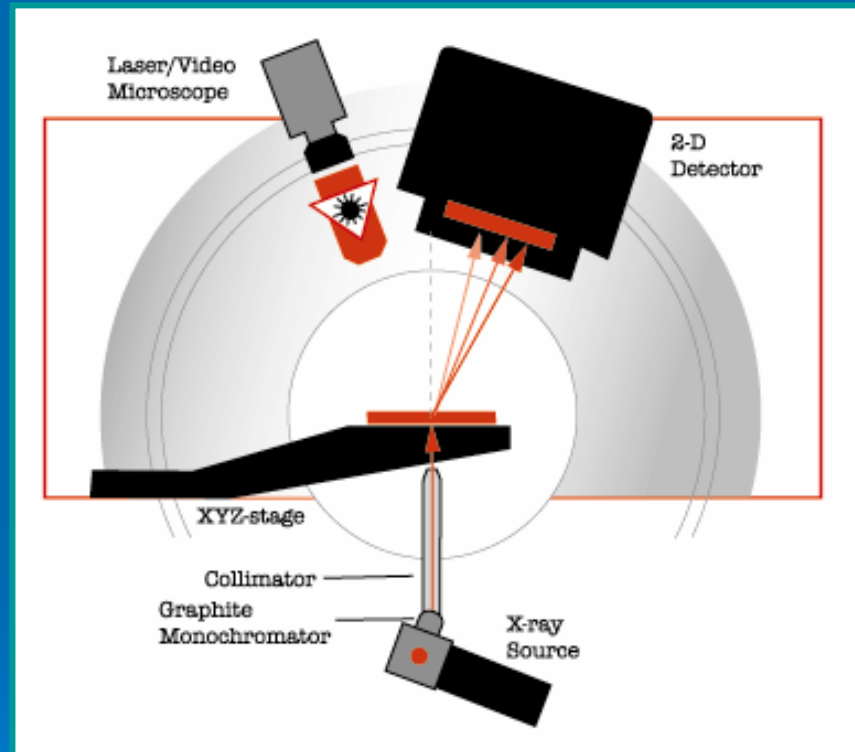
- Borosilicate glass has properties which interfere with Raman and XRD signals
- Several 96 well **quartz** plates with thin bottoms were designed and fabricated for evaluation

Objectives of P-XRD Evaluation

- Optimum Instrument Configuration
- Proper Bottom Thickness of Plate
- Minimum API quantity
- Distinguish mixture of Forms
- One plate for Crystallization and three analyses
Microscope, Raman, and XRD

D8 Discover with GADDS

(general area detector diffraction system)
for Combinatorial Screening (transmission)

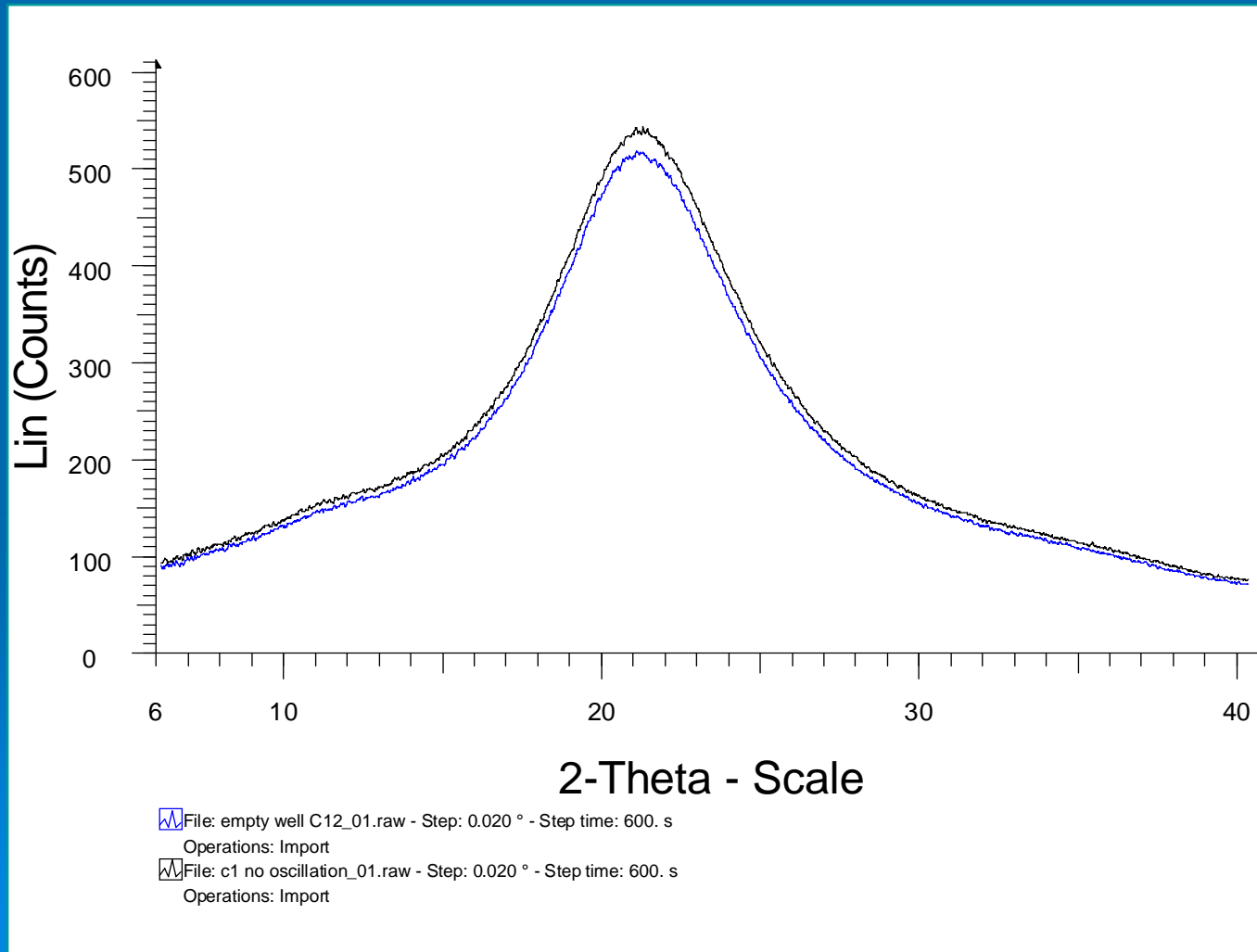


Instrument configuration used:

Inversion of radiation source to avoid shading by the 96 well

Two empty wells (C1 and C12)

500 μm monocal, small well plate

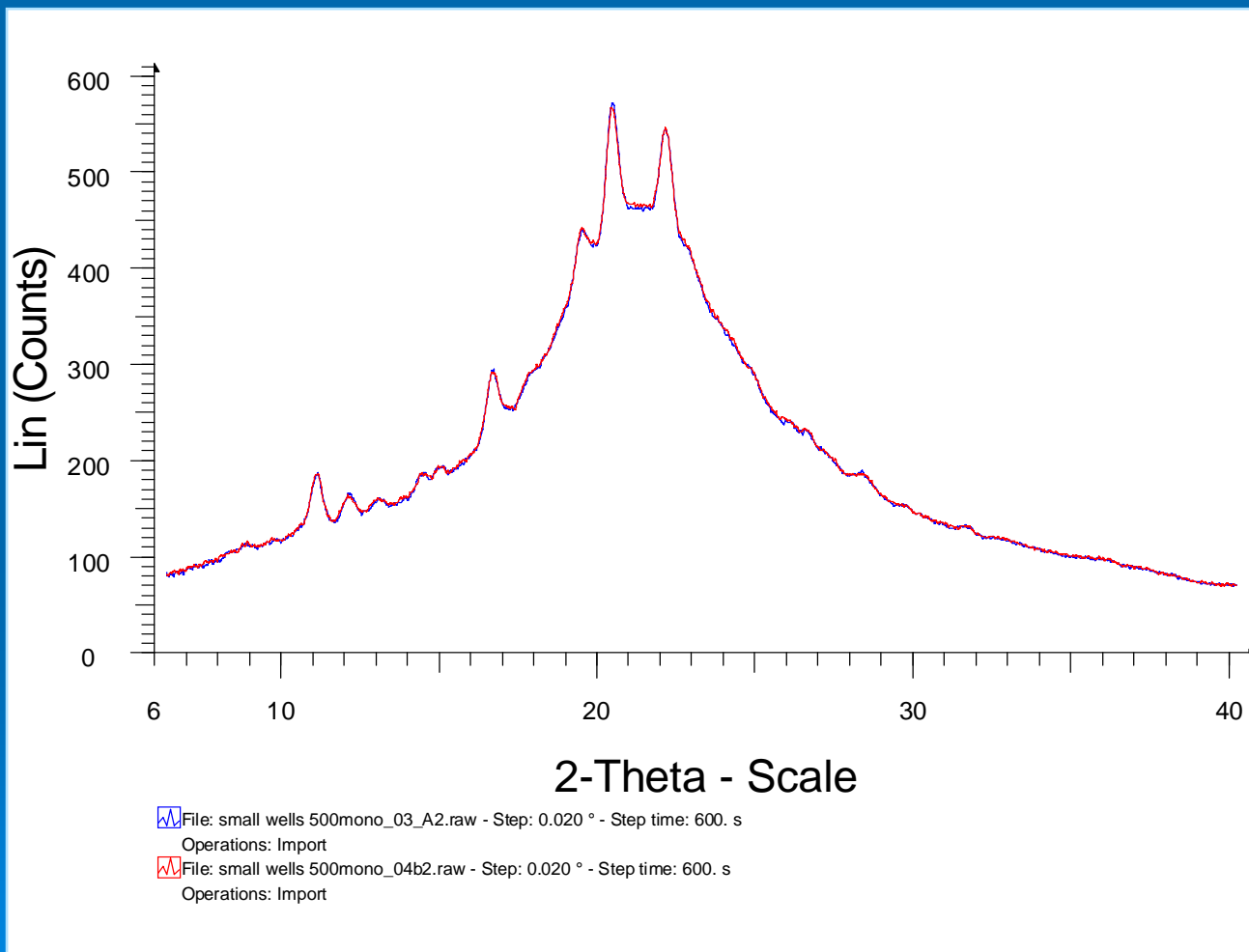


The difference in intensity might be attribute to thickness tolerances

The scaling factor of 1.05 between the two scans would correspond to 5% or **9 μm** difference in bottom thickness

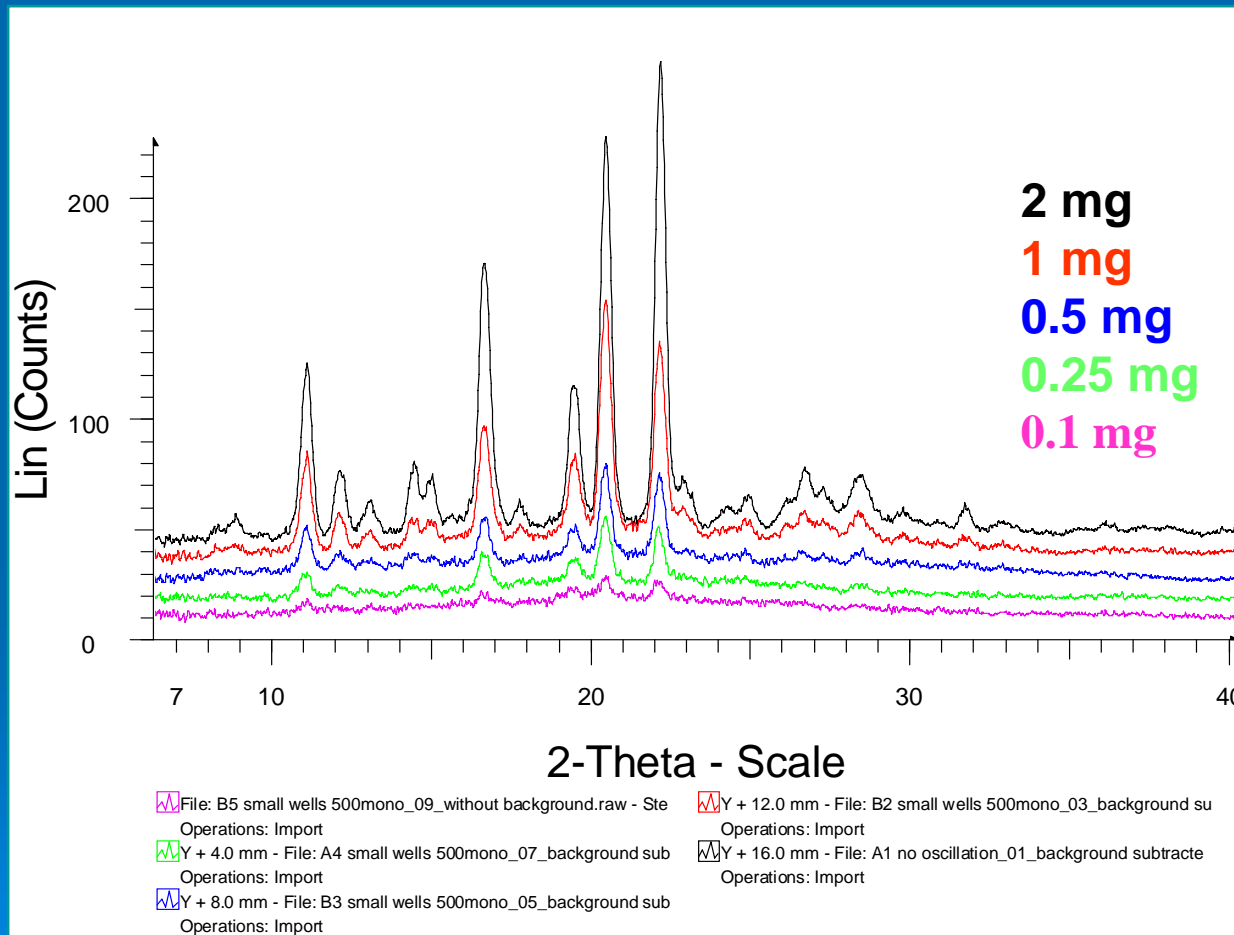
Reproducibility Between Two Wells

(with identical sample amounts)



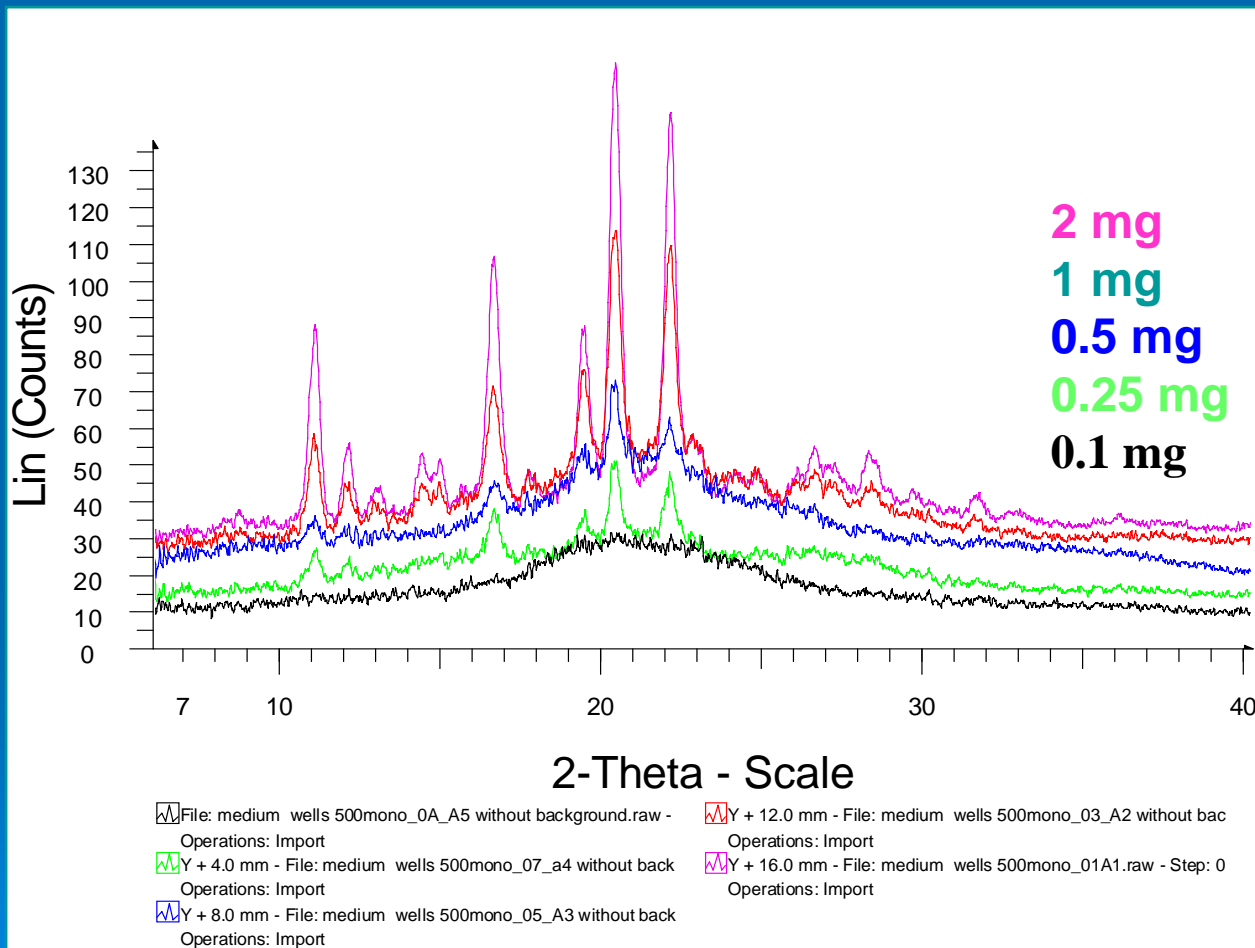
1 mg sample size
(A2 and B2 of
small well plate)

Comparison of Different Sample Amounts (small well plate –background subtracted)

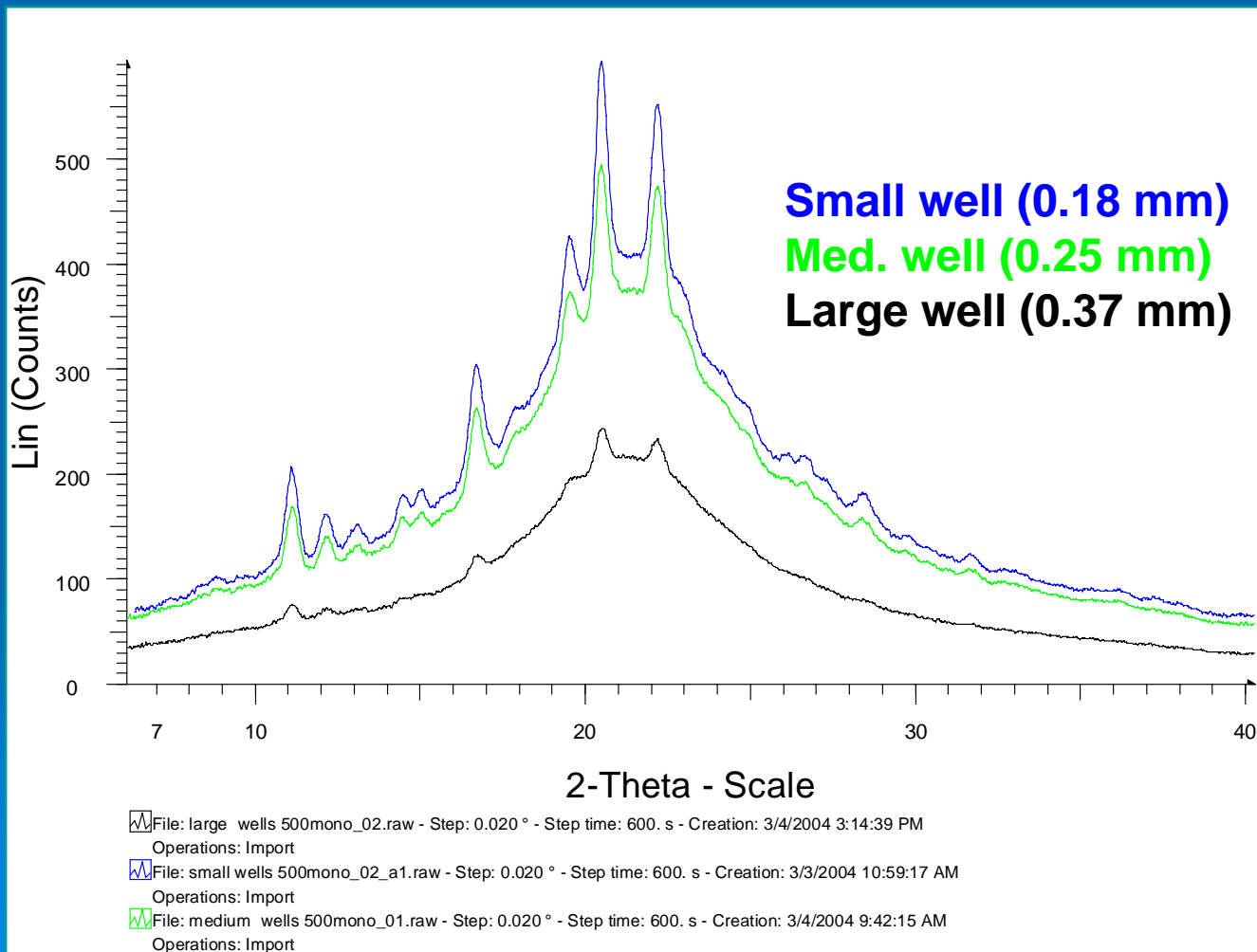


Comparison of Different Sample Amounts

(medium well plate – background subtracted)



Comparison of small, medium, and large well plates (A1-2mg)

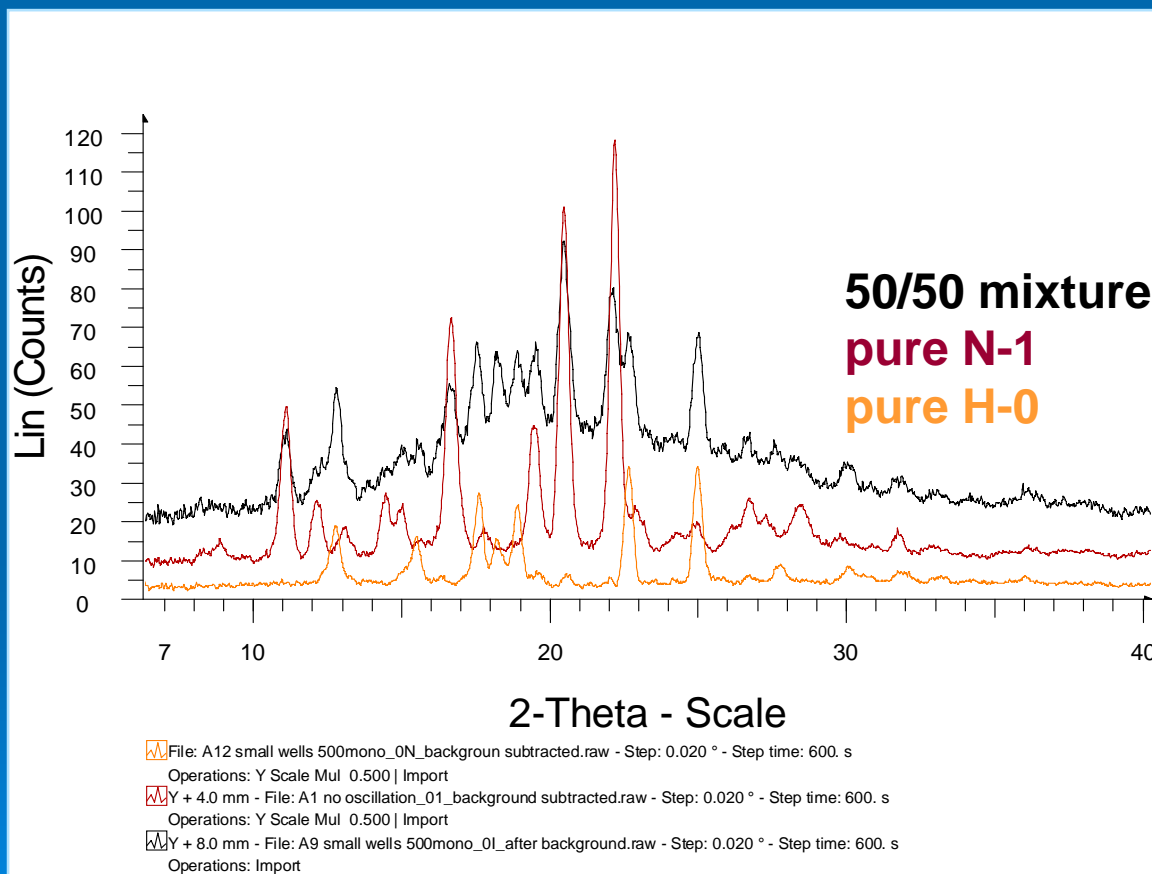


19 % intensity loss
going from small
well to medium well

Mixture 50%-50% of N-1 and H-0

(Comparison with pure samples N-1 and H-0
- backgroup subtracted)

Sample size: 2 mg



Results of XRD Evaluation

- Determined Bottom Thickness of Plate
 - Quality data obtained with Small well (0.18 mm) and Medium well (0.25 mm) plates
- Minimum amount of API
 - 0.25 mg/well
- Distinguish a Mixture of Forms
 - Mixture of two forms detected

Remaining Activities

- Validate plates in crystallization process
- Integration into the HTC system

Lessons Learned

- The system has been successfully used on most of compounds meeting our needs (screened over 150 compounds within 2.5 yrs)
- We gained enough knowledge and expertise to move forward
- With a “difficult to crystallize API” it may be necessary to use multiple techniques
 - To maximize the chances of discovering the optimal form
 - Coverage of greater experimental space
 - Polymer seeding (heteronucleation)
 - Controlled cooling
 - Solid/Solid grinding (co-crystal screening)
- Decisions around internal vs external development should be made in consideration of the best fit for the particular organization

Conclusion

The In-House developed HTC system

- minimized the amount of API required
(1 mg vs. 50 mg / exp)
- maximized the scope of screening and the amount of information collected
(96 parallel crystals vs. 1 crystal/time).
- reduced data handling time and facilitated data sharing within the organization
(minutes vs. hours).
- is easy to use since the software was developed in-house accommodating the existing workflow

ACKNOWLEDGEMENT

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Mauricio Futran

Thermo Electronic

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David Dalrymple

Bruker

Holger Cordes
Uwe Preckwinkel
Dave Bush

Internal Development

➤ Advantages

- Complete control of requirements
- Exact fit to the existing workflow (do you have the optimum workflow?)
- Ease of use (existing workflow based)
- Expandable to fit needs
- Drive Standards within workflow

➤ Disadvantages

- Sole responsibility for support and maintenance
- Potential risk for lacking innovation
- Taking on all of the development risk
- Longer development time and high FTE (Full Time Equivalent) requirements
- Can afford data system islands

Commercial System

➤ Advantages

- Potential for expansion to encompass similar workflow
- Shared risk/solution
- Large array of expertise
- Maintenance of system
- Drive Standards within the industry

➤ Disadvantages

- Purchase of functionality not needed or wanted
- Significant capital investment
- Service or expert based
- Cost for service contract