

# A Compact Automated Liquid Handler for Microbatch Protein Crystallography

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## Abstract:

- Automating the process used in protein crystallization, namely microbatch, is extremely advantageous especially in high throughput laboratories. In general, automation improves throughput, decreases error within and between experiments, and generates a report of the steps performed. The liquid handler employed in this work is an X, Y, Z robot, with 4 independent probes and varying pitch capable of automating protein crystallization; microbatch experiments. The liquid handler is capable of performing a wide range of experiments both through variations of constituents and plate accessibility. The use of disposable tips make the liquid handler very versatile not only for protein crystallization, but also other liquid handling techniques performed in the lab. The software provides a user-friendly interface to the liquid handler. The capabilities and performance specifications for the liquid handler will be presented in regard to protein crystallization for microbatch, and common liquid handling practices used in crystallography laboratories.



## Outline:

- Hardware of the System
- Software
- Experiments
- Results
- Summary and Conclusion

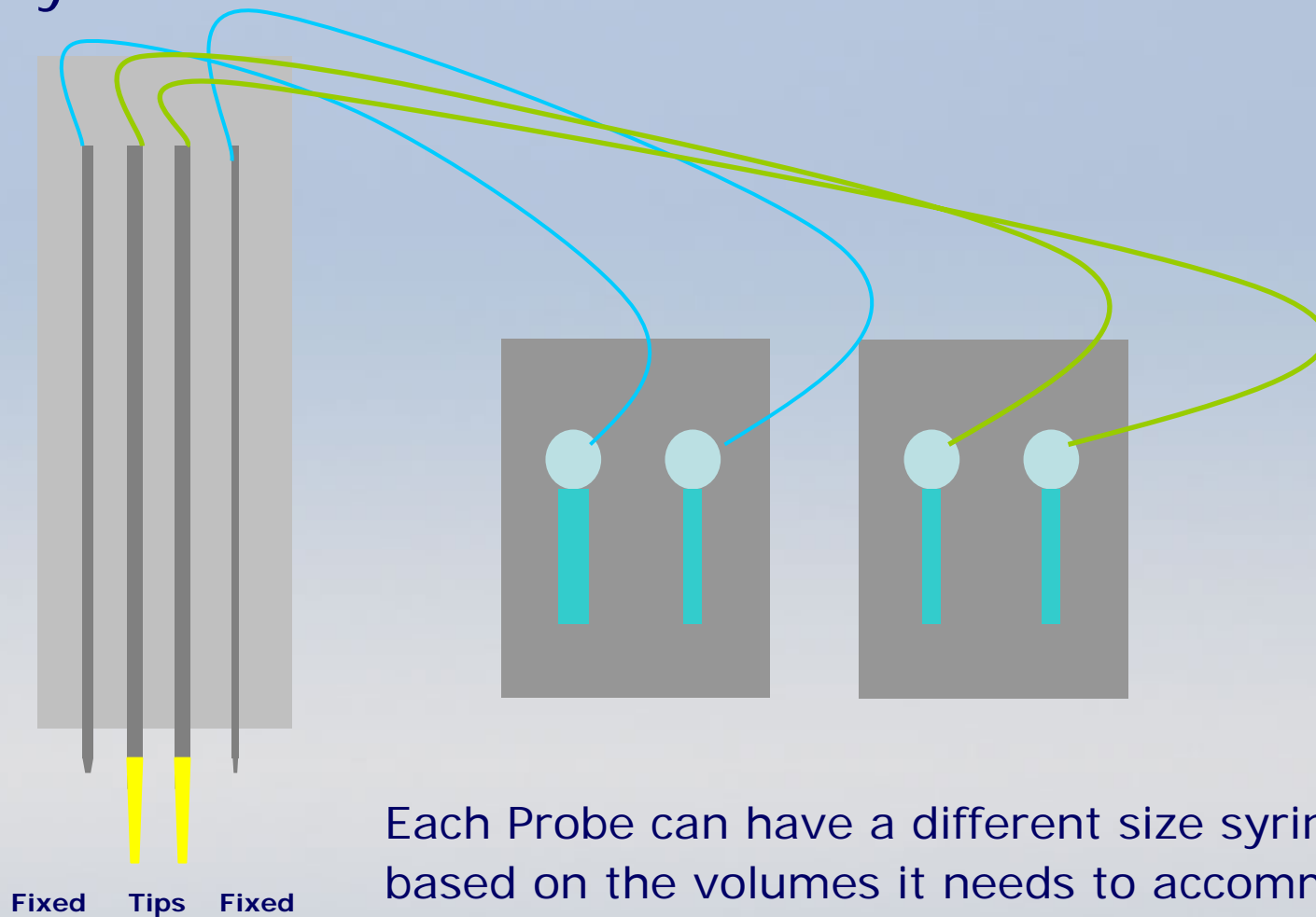


## Hardware:

- QuadZ-215 with 4 independent probes
- Probe 1: Fixed, flat 1.4 mm ID
- Probe 2 & 3: Disposable 10 ul Tips, B version
- Probe 4: Fixed, flat 0.4 mm ID
- 2 402 Dual Dilutors; supplying independent syringe size and control
- Rack, custom racks and Peltier controlled racks



## System Hardware:



Each Probe can have a different size syringe based on the volumes it needs to accommodate

## System Hardware:



- The 4 Probes have independent Z movement
- Each Probe can be unique to accommodate its tasks
  - Oil dispensing 1
  - Protein dispensing 2
  - Screening Solutions 3
- The Probes have variable Pitch accessing 9-18 mm spacing

# Software:

- Bed Layout: Racks, Custom Design, Attributes

Bed Layout Builder - ACA Quad-Z Microbatch\*

Zone: Inside Rinse

Zone Management

Numbering Pattern

| Attributes      | Values        |
|-----------------|---------------|
| {Zone} PC Plate | 43            |
| Kind            | Microbatch 48 |
| Absolute X      | 380.5 mm      |
| Absolute Y      | 215.3 mm      |
| Absolute Z      | 104.1 mm      |
| Relative X      | -32.5 mm      |
| Relative Y      | -45.5 mm      |
| Relative Z      | 2.5 mm        |
| Shape           | Circle        |
| Diameter        | 11.5 mm       |

Bed Layout

- ACA Quad-Z Microbatch
- NANOLITER VOLUME TEST
- QUADZ\_VOLUME\_TEST\_TIPS

X: 709.774 Y: 370.496

Scale Factor : 252

Auto Scale

Bed Layout Builder - ACA Quad-Z Mi...\*

New Open... Save Save As... Delete Export... Import...

Current User : Administrator  
 Bed Layout : ACA Quad-Z Microbatch  
 Created Date : 4/26/2005 3:17:13 PM  
 Created By : Gilson  
 Last Modified Date : 5/5/2005 10:10:43 AM  
 Last Modified By : Administrator



# Software:

- VPS: Virtual Pumping System, Smart Pumps

Configuration Builder - ACA Quad-Z DISP \*

Available Instruments ( Liquid Handlers )

Liquid Handlers

Accessories

All Instruments

Pumps

Communication

Scan

215 Liquid Handler

215 Liquid Handler without Pump

215 SW Liquid Handler

221 XL Liquid Handler

222 XL Liquid Handler

223 Sample Changer

ASPEC XL

ASPEC XL4

Micro 215

Multiple Probe 215

Quad-Z 215

SPE 215

Workspace

Instrument: Virtual Pumping System

Fluid System Name: Virtual Pumping System

| Probe(s) | Syringe Names       | VALVEMATE | Position | Use                                 |
|----------|---------------------|-----------|----------|-------------------------------------|
| ▶ B      | A (402 Pump Dual)   |           |          | <input checked="" type="checkbox"/> |
| C        | B (402 Pump Dual)   |           |          | <input checked="" type="checkbox"/> |
|          | A (402 Pump Dual 1) |           |          | <input type="checkbox"/>            |
|          | B (402 Pump Dual 1) |           |          | <input type="checkbox"/>            |

Configuration Builder - ACA Quad-Z DISP \*

New Open... Save Save As...

Delete Export... Import...

Workspace

Instrument: 402 Pump Dual

Instrument Name: 402 Pump Dual 1

GSIOC ID: 1

Syringes to Use:  A  B

| Syringe ID | Syringe Name        | Syringe Size (uL) | Reservoir Name |
|------------|---------------------|-------------------|----------------|
| A          | A (402 Pump Dual 1) | 1000              |                |
| B          | B (402 Pump Dual 1) | 500               |                |

Set



## Software:

- Drag and Drop Tasks into the Method Builder

The screenshot displays the 'Method Builder - Mother Solution' software interface. On the left, there are three panels: 'Tasks' (listing operations like '24V Off', 'Add (1 Solution)', etc.), 'Operators' (with 'Variable' and 'Expression' options), and 'Methods' (listing various method templates). The main workspace contains a sequence of buttons: 'Start', 'Load Tips', 'Dispense Random to R...', 'Eject Tips', and 'End'. A 'Task Properties...' dialog box is open, showing a table of properties for the 'Dispense Random to Random with Tips' task.

| Property                  | Value    |
|---------------------------|----------|
| Source Z Option           | Bottom   |
| Source Z Offset (mm)      | 23       |
| Wet Tip                   | FALSE    |
| Result Zone               | PC Plate |
| Result Well Variable      |          |
| Result Flow Rate (mL/min) | 3.0      |
| Result Z Option           | Bottom   |
| Result Z Offset (mm)      | 1.7      |
| Touch Off Droplet Less    | FALSE    |
| Touch Off Auto            | FALSE    |

At the bottom of the interface, there is a toolbar with buttons for 'New', 'Open...', 'Save', 'Save As...', 'Reference', 'Delete', 'Export...', 'Import...', and 'Reload'. A status bar on the right provides system information:

- Current User : Administrator
- Method : Mother Solution
- Created Date : 4/18/2005 5:45:20 PM
- Created By : Administrator
- Last Modified Date : 5/6/2005 10:33:01 AM



# Software:

- Stacking of Methods in the Application Builder

Application Builder - ACA PC Under OIL

Filter criteria

Method - Configuration

| Methods          | Configuration |
|------------------|---------------|
| ACA_PRIME_D...   | ACA-PRIME     |
| Home Quad-Z      | ACA Quad-Z    |
| HOME_MICRO...    | ACA Quad-Z    |
| HOME_PRIME       | NANOLITER T.  |
| Mother Solution  | ACA Quad-Z .  |
| NANOLITER V...   | NANOLITER T.  |
| Oil Aspirate     | ACA Quad-Z .  |
| Oil Dispense     | ACA Quad-Z .  |
| Oil Rinse        | ACA Quad-Z .  |
| Protein Aspirate | ACA Quad-Z .  |
| Protein Dispense | ACA Quad-Z .  |

Applications

- ACA MOTHER SOLUTION
- ACA PC NO OIL
- ACA PC Under OIL
- ACA PRIME DILUTORS
- HOME\_MICROBATCH
- HOME\_PRIME
- NANOLITER VOLUME TEST QUAD
- UL VOLUME TEST QUAD Z NOTIPS
- UL VOLUME TEST QUAD Z TIPS
- VOLUME TEST QUAD Z notips

| Method             | Configuration        | Author        |
|--------------------|----------------------|---------------|
| Home Quad-Z        | ACA Quad-Z           | Administrator |
| Oil Aspirate       | ACA Quad-Z oil probe | Administrator |
| Oil Dispense       | ACA Quad-Z oil probe | Administrator |
| Protein Aspirate   | ACA Quad-Z Protein   | Administrator |
| Protein Dispense   | ACA Quad-Z Protein   | Administrator |
| Mother Solution    | ACA Quad-Z DISP      | Administrator |
| Rinse Probes       | ACA Quad-Z           | Administrator |
| Oil Rinse          | ACA Quad-Z oil probe | Administrator |
| ACA_PRIME_DILUTORS | ACA-PRIME            | Administrator |

Application Builder - ACA PC Under OIL

Current User : Administrator  
 Application : ACA PC Under OIL  
 Created Date : 4/18/2005 5:45:21 PM  
 Created By : Administrator  
 Last Modified Date : 5/3/2005 2:09:13 PM

New Open... Save Save As... Simulate  
 Delete Export... Import... Reload



# Software:

- Simulation

**Simulate**

Application Name: ACA PC Under OIL      Simulation Speed: High

Sample List Name: ACA PC Under OIL-4/26/200512:41:24 PM       Auto Scale

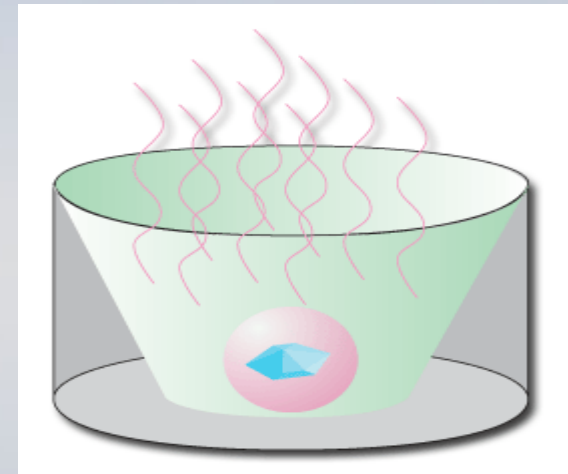
Quad-Z 215 with Tips

Running Move Z command for Probes:A Distance:0mm Speed:200mm/sec Option:Top  
End of Loop  
End of Task : Dispense  
End of Method : Oil Dispense  
Start of Method : Oil Dispense  
Start of Task : Dispense



## Experiments:

- Microbatch (under oil) Protein Crystallization
  - Dispensing of the oil throughout the plate(s), total volume aspirated with individual well dispenses
  - Aspiration of the total volume of protein, dispensing into individual wells
  - Disposable tips aspirate the mother solutions and dispense into the wells two at a time
  - Plates are centrifuged for 5 minutes at 500 RPM



Picture via [www.hamptonresearch.com](http://www.hamptonresearch.com)



## Experiment:

- Microbatch Plates
  - ImPact, 96 well, 30 ul, flat bottom
  - VDX, 48 well, pre-greased 500 ul, flat bottom
- Crystallization Oils
  - Paraffin, Silicone, Al's
- Xylanase 3 mg/ml, standard from Hampton Research
- Crystal Screen, HTS, 96 well format
- EZ\_Pierce, pierceable adhesive free zone film, EXCEL Scientific, Inc., covers the Crystal Screen plate, disposable tip pierceable



## Experiment:

ImPact Plate: 96 wells, 30 ul capacity, flat bottom

| Ratio<br>uls | Protein<br>3 mg/ml | Mother Solutions  | Oils, 8 uls              |
|--------------|--------------------|-------------------|--------------------------|
| 1:1          | Xylanase           | Crystal Screen HT | Paraffin, Silicone, Al's |
| 1:1.5        | Xylanase           | Crystal Screen HT | Paraffin, Silicone, Al's |
| 1:2          | Xylanase           | Crystal Screen HT | Paraffin, Silicone, Al's |

VDX Plate: 48 wells, 500 ul capacity, flat bottom

| Ratio<br>uls | Protein<br>3 mg/ml | Mother Solutions  | Oils, 250 uls            |
|--------------|--------------------|-------------------|--------------------------|
| 1:1          | Xylanase           | Crystal Screen HT | Paraffin, Silicone, Al's |
| 1:2          | Xylanase           | Crystal Screen HT | Paraffin, Silicone, Al's |

## Results:

- With the microbatch technique it is not necessary to make sure that the reagents and sample mix, centrifugation will coalesce the drops into a single drop



- The picture to the left represents this technique
  - A microliter of blue dye (protein) was dispensed and then a microliter of yellow dye (reagent), the resulting mixture after centrifugation is green, ImPact plate

## Results, cont:

**Tips: 1.0 ml Syringe Low Volume**

**10 ul Tips with B-style (crystal reagents)**

| Dispense Volume<br>uls |  | Amount | % Accuracy | % CV | % STD |
|------------------------|--|--------|------------|------|-------|
| 1.0                    |  | 0.96   | 96         | 3.5  | 0.6   |
| 3.0                    |  | 3.02   | 101        | 2.9  | 1.2   |
| 5.0                    |  | 4.83   | 97         | 1.1  | 0.7   |

**Volumetric Testing for 500 ul syringe, entire volume aspirated  
prior to dispensing (protein)**

| Dispense Volume<br>uls |  | Amount | % Accuracy | % CV | % STD |
|------------------------|--|--------|------------|------|-------|
| 1.0                    |  | 0.98   | 98         | 2.7  | 0.5   |





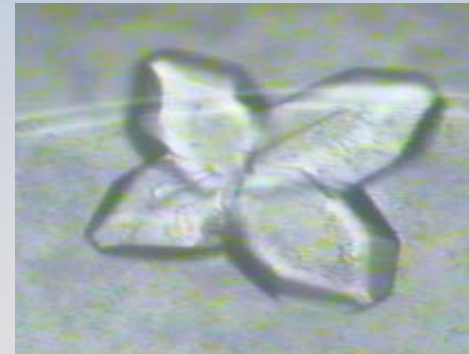
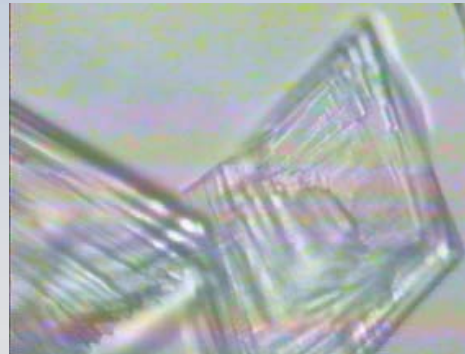
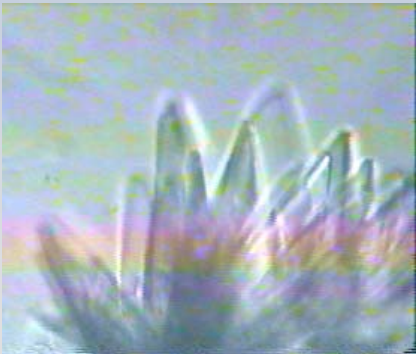
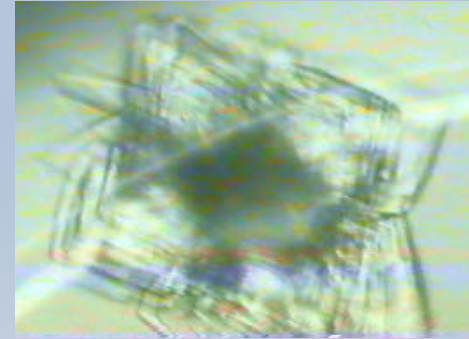
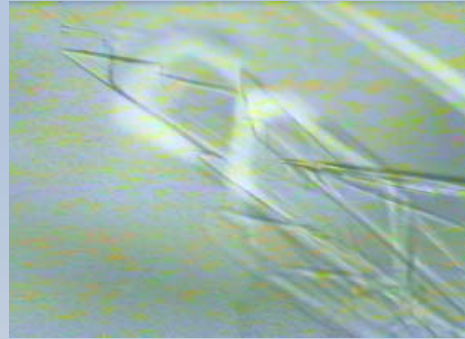
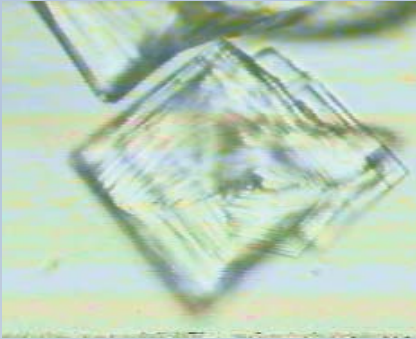
## Results, cont:

- Crystals were found in many of the wells for both the ImPact and VDX plates
- The ratio of 1:1 produced the most abundant crystals
- The ratio of 1:2 protein:reagent produced a high number of wells containing precipitate
- All the Crystallization Oils produced crystals, however Paraffin Oil was the easiest to work with and gave minimal spreading outside of the ImPact wells



## Results, cont:

- Following are pictures of crystals from the microbatch experiments, ImPact and VDX plates



Nikon Microscope 100x magnification, courtesy of MATC, Madison, WI.



## Summary:

- The Quad Z with (2) 402s offers a nice alternative to higher priced crystallization workstations
- The system allows the use of various types of probes/disposable tips without any hardware or tool changes
- Variable pitch allows the system to be as efficient as possible by using the best combination of probes at once
- The system can accommodate all types of microbatch plates without hardware changes
- Volume testing proves that the system is capable of volumes accurately dispensing 1 ul volumes both with and without tips



## Summary, cont:

- Trilution LH software allows a user friendly interface to the Quad-Z while still maintaining a high degree of flexibility
- The task driven software allows the system to be used for a wide range of Liquid Handling application, not just microbatch crystallization e.g. reformatting, filtration, PCR cleanup, SPE, dilutions
- Custom tasks, racks offer an additional degree of flexibility to accommodate the ever changing requirements of research



## Conclusion:

- It's been shown that the QUADZ-215 with (2) 402 dilutors is a nice alternative for automated microbatch experiments
- The system can fit in most hoods or Plexiglas boxes minimizing the plates exposure to airborne particulates
- Using the fixed probe to aspirate all the protein at once is very advantageous with very minimal protein loss less than 2 uls for a 96 well ImPact plate
- Advancements are now underway to accommodate sitting drop crystallization

