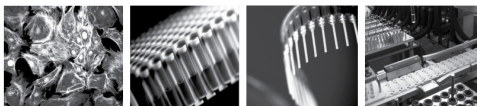


mosquito® Crystal: Fast, reliable automation of Protein Crystallization drop set-up

Joby Jenkins, David Smith, Chloe Carter, Wendy Gaisford



Introduction

Automation of protein crystallography screening has contributed significantly to the rapid progress of crystallography based structural biology.

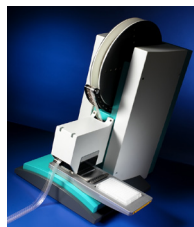
Automation allows samples to be screened using smaller volumes of both protein and screen solutions, reducing costs and saving valuable protein. Additional benefits include increased throughput and accuracy.

One of the challenges to automating this process is the necessity to accurately pipette solutions of varying viscosities. Another challenge is that of drop positioning. The low volume drops have to be placed extremely accurately in order that protein and screen drops coalesce and are not distorted by the edge of the crystallization plates' subwell.

The ability of mosquito® Crystal to address these issues and to automate both vapour diffusion methods of protein crystallography (sitting, drop, hanging drop) as well as the microbatch method without instrument configuration change offers ultimate flexibility for the crystallography laboratory.

1. mosquito® Crystal

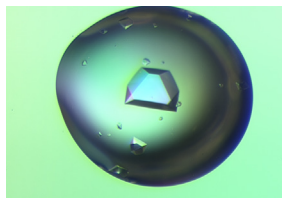
mosquito® Crystal is a compact low volume liquid handling instrument combining a low-cost disposable tip system with a positive displacement pipette to ensure zero cross-contamination. mosquito is capable of pipetting volumes from 1.2 µL down to 25 nL with no washing required.



2. Automated Hanging Drop Set-up

For automated hanging drop set-ups, the mosquito deck is loaded with an inverted, self-adhesive, hanging drop plate seal, a 96-well plate of screen buffers and reservoir(s) of protein sample. Seed stocks or additive screens could also be added.

mosquito pipettes from, and into, plates one column at a time allowing protein solution to be aliquoted from a single source column to all 96 'windows' on the hanging drop plate seal. Droplets of the solutions in the screen plate are placed on top of the protein drops in a mirror image. The plate seal is then inverted using a simple alignment jig – placing droplets over their correct wells.

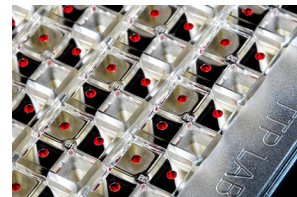


mosquito Crystal's advantages for hanging drop set-up include:

- Drop set-up completed in <2 minutes
- Uses a normal (inexpensive) flat bottomed 96-well plate and 96-well plate seal
- Pipette changes for screen addition avoids slow washing steps and eliminates cross-contamination
- Unrivalled low volume accuracy and repeatability
- Highly accurate drop placement.

3. Automated Sitting Drop Set-up

mosquito's X, Y and Z axes are accurately driven by a stepper motor with a resolution of <0.05mm. This, along with the tightly toleranced and relatively short pipette tips, means that drops can be placed with a high degree of accuracy in the centre of the subwells of any standard crystallization plate.



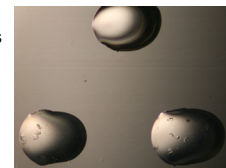
Triple-well sitting drop iQ plate set up with protein, seeds and buffer in 200 nL total drop volume

mosquito Crystal has many advantages for sitting drop set-up:

- Drops perfectly centred in subwells
- Fast set up (<2 mins for 96 wells, <4 mins for 288 wells)
- Pipette changes for screen addition avoids slow washing steps and eliminates cross-contamination
- Unrivalled low volume accuracy and repeatability
- Highly accurate drop placement.

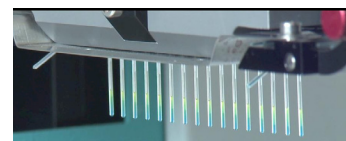
4. Multiple Experiments in One Well

mosquito's accuracy, repeatability and speed allows users to create several multi-component drops per well – even in 96-well hanging drop set-ups. Such drops allow different constructs, volume ratios or protein concentrations to be assessed at the same time. This can yield 288 conditions in a single sitting or hanging drop plate which can be set up in less than 4 minutes.



5. Automated Microbatch Set-up

mosquito crystal's multi-aspirate, dispense and drop mixing capabilities enables both protein and screen solutions to be aspirated consecutively within the same tip and be pipetted directly through oil for microbatch set up ensuring that screen and protein drops always combine.



6. Key Features of mosquito Crystal

- mosquito Crystal can be used for all crystallography techniques without instrument set-up changes
- Nanolitre volume range: 25 nL – 1,200 nL (or lower if multi-aspirate functionality is used)
- Disposable positive displacement pipettes guarantee zero cross-contamination of even the stickiest samples
- Excellent repeatability and accuracy: CVs of < 6% at 25 nL and < 2% at 100 nL
- Intuitive operation and programming are ideal for a multi-user environment
- Negligible dead volumes lead to reduced protein and seed stock wastage
- Extremely reliable hardware makes mosquito Crystal the 'work-horse' of many crystallisation labs.

Conclusion

mosquito Crystal's ability to perform automated vapour phase and microbatch techniques without the need for set up changes can save researchers valuable time as well as offering them significant flexibility.

Disposable micropipettes handle a range of viscosities, even at very low volumes, eliminating carry over and cross-contamination risks to valuable protein or reagent stocks.

Accurate and repeatable X, Y and Z movements allow smaller drops to be positioned very accurately, time and time again.

mosquito Crystal's positive displacement technology enables consistent and accurate nanoliter pipetting for repeatable drop setting irrespective of liquid viscosity and surface tension.

Reliable and robust hardware, together with simple, user friendly software, make mosquito Crystal ideal for any type of crystallography laboratory.