

Facilitating Low Volume Protein Crystallography Set-ups Using the mosquito® Liquid Handler

Jas Sanghera, Joby Jenkins, Rob Lewis, Chloë Milburn

TTP LabTech Ltd, Melbourn Science Park, Melbourn, Royston, UK

Abstract

A prerequisite for efficient high throughput protein crystallisation screening is the accurate pipetting and positioning of the low volume drops used in hanging and sitting drop setups. Solutions exist for low volume pipetting, however, the variable viscosities of protein and reservoir/screen solutions present significant challenges for many liquid handling systems. The mosquito® (TTP LabTech) offers fast positive displacement pipetting for accurate and reproducible aspiration and dispensing throughout the 50 nL - 1.2 µL range, producing CVs of <8% at 50 nL irrespective of viscosity. Mosquito's micropipettes are also disposable, thus guaranteeing zero cross-contamination where required.

Introduction

Crystallising proteins, required for subsequent structure determination by X-ray diffraction, is a difficult and labour-intensive task. It is essential to automate parts of this process in order to increase the rate at which new protein structures can be determined. Screening the many different conditions under which a protein crystal may form lends itself to automation, since it requires hundreds of similar experiments to be set up to find the few 'hits'. Automation significantly increases throughput, improves repeatability, and reduces cost by allowing much lower volumes of expensive protein and screen solutions to be used.

One of the challenges to automating this process is the necessity to 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 subwell.

Conclusion

Mosquito can save researchers valuable time and protein. Disposable pipettes handle a range of viscosities with no cross-contamination. Accurate and repeatable X, Y and Z movements allow smaller drops to be positioned very accurately, thus demonstrating mosquito's capability for automating high throughput protein crystallisation production and screening using a variety of microplate formats

1 mosquito® Instrument

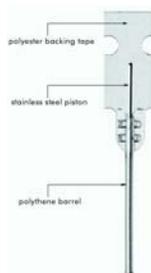


mosquito is a low volume liquid handling instrument combining a low-cost disposable tip system with a positive displacement pipette to ensure zero cross-contamination.

mosquito is capable of pipetting volumes from 1.2 µL down to 50 nL with no washing required. CVs are <8% at 50 nL irrespective of viscosity.

2 Pipetting Precision

Mosquito's pipettes are made of inert materials and manufactured to tight engineering tolerances to provide an accurate, low-cost disposable pipette. Their relatively wide bore (0.4mm) means blockages are unlikely. The pipette size has been reduced to little more than the working volume, thus enabling extremely high density storage on reels of 26,000 or 36,000 tips.

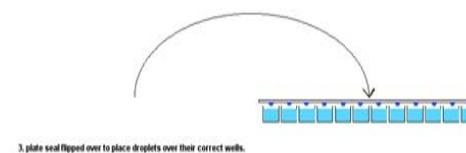
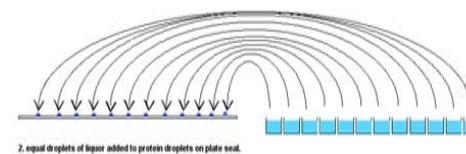
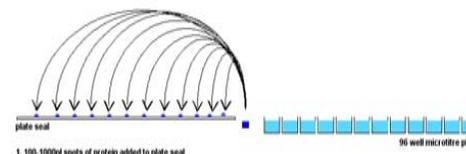


Mosquito's pipettes offer the following advantages:

- 50nL to 1,200nL aspirate and dispense range.
- positive displacement pipetting handles liquids of varying viscosities accurately without recalibration.
- disposable pipettes guarantee zero cross-contamination
- excellent repeatability and accuracy. Mosquito offers CVs of <8% at 50nL and <4% at 100nL across a 384 well plate; accuracy is within +/-5% throughout the volume range.
- negligible dead volumes reduce protein wastage.

3 Automating Hanging Drop Setup

mosquito is the only instrument currently capable of automating 96-well hanging drop plate setup. mosquito pipettes from, and into, plates one column at a time. This allows protein solution to be aliquoted from a single source column to all 96 'windows' on a hanging drop plate seal. It also means droplets of the solutions in the screen plate can be placed on top of the protein drops in a mirror image. The seal can then be inverted – placing droplets over their correct wells.

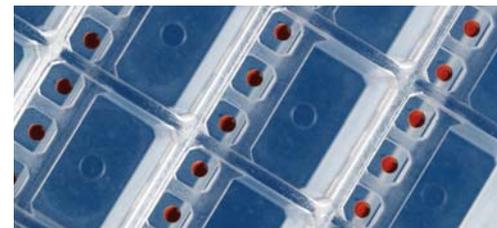


Mosquito's advantages for hanging drop set-up are:

- Drop set-up is complete in <2 minutes so there is no need for humidity control.
- Only a normal (inexpensive) flat bottomed 96-well plate and 96-well plate seal are required
- One set of pipettes used for sample
- Pipette changes for screen addition avoid washing steps
- Set-up is automated for increased accuracy and repeatability.

4 Automating Sitting Drop Setup

mosquito uses precise, stepper motor driven, linear drives in conjunction with optical sensors to achieve positional accuracy better than 0.05 mm in the X, Y and Z axes. This, along with the tightly tolerated and relatively short pipette tips, means that drops can be placed with a high degree of accuracy in the centre of the sub-wells of any standard crystallisation plate.



Smaller drops can be used without the worry of protein and screen drops not coinciding. Set-up is extremely fast (under 4 minutes for a triple sub-well Greiner crystal ledge plate). A further advantage is found when imaging drops automatically since the area of interest can be much smaller, hence making crystals easier to identify.

5 Multiple Experiments in One Well



Mosquito's accuracy and repeatability allows users to create several multi-component drops per well – even in high density 96-well hanging drop set-ups (see image left).

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.

