A Solution for Low Volume Pipetting Applications Requiring High Accuracy of Sample Placement

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Abstract

The main engineering challenge for low volume pipetting is the aspiration of liquid from a source and its accurate and precise dispensation in nanolitre volumes. In addition, some applications require highly accurate drop placement in a small area (e.g. for assay miniaturisation in 1536 plates) and/or excellent repeatability (e.g. for drop on drop placement used in assay-ready dilutions or protein crystallography) for both the aspirate and dispense operations.

The mosquito® nanolitre pipettor (TTP LabTech) 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, in conjunction with positive displacement, disposable pipettes which guarantee zero cross-contamination, gives the mosquito® unrivalled accuracy and repeatability for these more complex transfer operations.

Introduction

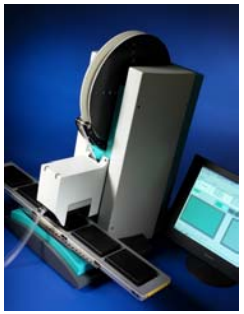
mosquito is capable of multiple aspirations before a dispense. This allows a combination of solutions to be dispensed simultaneously – with additional mixing if required – resulting in perfect drop formation.

mosquito is also able to perform multiple dispenses from a single aspiration. This allows the rapid preparation of multiple daughter plates or set-ups requiring multiple drops within a plate or even single well.

Conclusion

mosquito's unique positive displacement disposable tips and precise X, Y and Z movements allow smaller drops with accurate and repeatable volumes to be positioned very precisely. This ability is essential for successful assay miniaturisation and the set-up of more effective serial dilutions or protein crystallography screens.

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 1.2µL down to 50nL with no washing required.

2 Accuracy of drop placement

mosquito's X, Y and Z axes are accurately driven by stepper motors 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 wells of any SBS plate, up to and including the high density 1536 format.

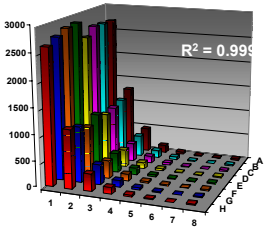
Such accuracy also means that smaller drops can be placed directly on one another, without the worry of drops not coinciding or being distorted by the well walls.

Mosquito's accurate movement and disposable 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 sample wastage.

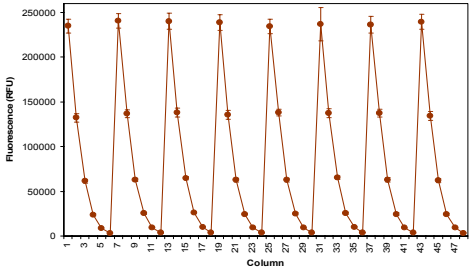
3 Automating assay-ready serial dilutions in high density plates

mosquito's micropipettes are arranged in a column of 8 or 16 tips. The pipettes use positive displacement and direct contact, allowing them to aspirate, dispense and even mix. This enables mosquito to automate serial dilutions directly into the final assay plate, transferring accurate nanolitre quantities even into high density formats such as 384 and 1536.



Serial dilutions in a 384-well plate. Dilution volumes - 315nL fluorescein: 685nL PBS in a ShallowWell Nunc plate.

To assess mosquito's suitability for performing serial dilutions in 1536 plates using nanolitre volumes, hits selected for confirmation were serial diluted (half-log) in a 1536-well plate using a standard 16-tip mosquito and the resultant fluorescence quantified using an Acumen Explorer microplate cytometer. The maximum CV for any series of terminal dilutions was 8% (n = 32) indicating the high precision and accuracy of pipetting, as well as good consistency of mixing.

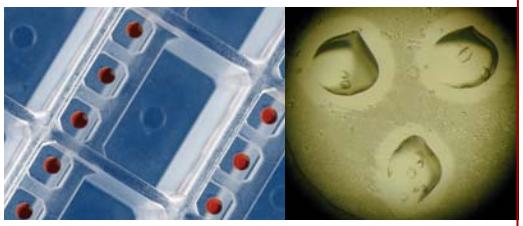


For Kalypsys' assessment of using mosquito for pipetting in 1536 plates, please refer to poster No MP12.

4 Applications for accurate protein crystallography set-ups

mosquito's accuracy has key advantages for sitting drop protein crystallisation set-ups:

- Drops are centred in sub-wells, avoiding problems with drops not coinciding or being distorted by the walls (see image below left)
- Fast set-up so no need for humidity control: Greiner triple-well crystal ledge plate complete in <4 mins
- Placement precision facilitates automated crystal identification, as the area of interest can be smaller.



mosquito's accuracy and repeatability allows users to create several multi-component drops per well - even in high density 96-well hanging drop setups (see image above right). 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.

5 Flexibility to address other formats

mosquito allows the user to precisely specify non-standard destinations for drops. mosquito can thus address formats such as MALDI target plates or Meso Scale Discovery's multi-spot electrode plates.

For complete flexibility, mosquito X1 is a single tip version of the mosquito with a full range of movement so it can address unique (i.e. non SBS format) plate types with multiple targets within a well, or even pipette onto microscope slides or cover slides.

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