A fast and fully automated solution for Lipidic Cubic Phase (LCP) Screening using mosquito LCP

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Abstract

Membrane proteins, such as G-protein-coupled receptors, are known to be much more difficult to purify and crystallise than soluble proteins due to their native environment within the lipid bilayer of the cell membrane. As a result aqueous solutions are unsuitable for their reconstitution as they require lipids or detergents to retain their structural integrity.

The *in meso* (lipidic cubic phase or LCP) crystallisation technique has revolutionised the process of crystallising membrane proteins. This method utilises highly viscous lipid mesophases to contain the membrane proteins for crystallisation. However, there are a number of technical difficulties associated with the LCP method which makes this process difficult to perform and challenging to automate.

One problem is the viscous nature of the lipids which can be almost solid at room temperature. As a result the addition of protein to the lipid and subsequent reconstitution can be hard to achieve. In addition, the accurate dispensing of LCP, required for efficient miniaturisation, and the precise positioning of drops required for efficient imaging of membrane crystals present two other challenges.

TTP LabTech have solved this problem by developing mosquito® LCP, a dedicated instrument that offers a fully automated solution to LCP screening. This instrument offers fast throughput, high precision and unrivalled reproducibility.

Here we describe the benefits of the instrument and how the renowned and reliable mosquito positive displacement tip technology ensures that the LCP screening preparation is performed to the highest standard with the minimum amount of effort.

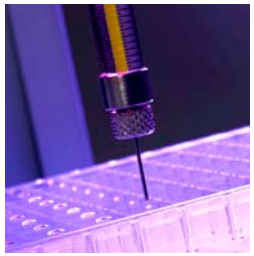
1. mosquito LCP Instrument

mosquito LCP is a low volume liquid handling instrument that has been specifically designed to overcome the challenges presented by the LCP method and provide an automated solution to LCP screening techniques.

LCP is highly viscous, thus difficult to manipulate.

This in turn makes the automation and the miniaturisation of the LCP crystallisation set-up very challenging. With its unique features, mosquito LCP solves these problems.

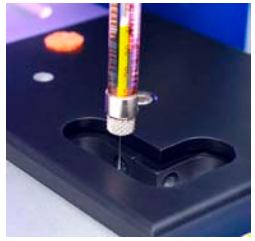
2. mosquito LCP Features



- Accurate and repeatable dispensing of LCP down to 25 nL using a dedicated positive displacement syringe.
- Automatic measurement and calibration of the LCP syringe needle tip position to within +/- 20 µm enables precise drop placement and subsequent automated imaging of membrane protein drops and crystals.
- Choice of either 50 µL or 100 µL capacity syringe
- Loading/changing of syringes is facilitated via user friendly, wizard-based software.
- Software wizards enable simple definition of plate types, number of columns, and volumes of LCP mixture and screen solutions to be pipetted.
- Disposable, positive displacement mosquito tips guarantee zero cross contamination and eliminate time-consuming tip washing.
- Minimized evaporation of the dispensed LCP as a result of screen solutions being dispensed on the columns of LCP within 5 seconds.
- Plate set-up time of less than 5 minutes results in high throughputs of >12 plates an hour.

3. Calibration

- mosquito LCP is able to accurately detect and calculate the position of the central axis and tip of the LCP dispensing needle in the X, Y and Z axes. Calibration is achieved using a crossed pair of photoelectric sensors mounted directly on the mosquito deck.
- Proprietary electronics drive and control the Z height positioning of the LCP syringe needle tip with precision accuracy with repeatable positioning with 5 µm being achievable.
- Positioning of the LCP dispense needle relative to the mosquito deck is accurate to +/- 20 µm in the X axis and +/- 5 µm in the Y and Z axes.

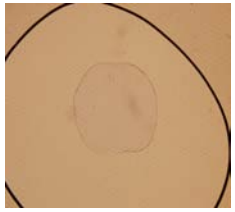


- The electronics also enable rapid and highly accurate loading of a new syringe. Users can directly drive the syringe pump into the correct loading position without having to refer to the user interface on the screen as a result of the addition of the button pad interface for syringe adjustment.

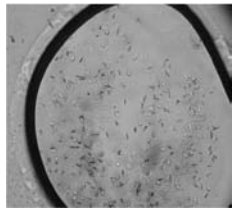
4. LCP dispensing precision and accuracy

- *Repeatability*:
  - at 100 nL, CVs of < 6%
  - at 50 nL, CVs of < 8%
  - at 25 nL, CVs of < 10%
- *Accuracy:*
  - Within 10% accuracy at volumes of 25 nL and higher

Figure 1:



LCP 'sandwich' drop image



Crystal image supplied by Tony Warne, MRC, London

5. Typical protocol for LCP screening set-up

- A 96-well screen plate and LCP 'sandwich plate' are loaded on to the deck of mosquito LCP.
- A syringe containing the pre-mixed LCP/protein solution is loaded on to mosquito LCP using the 'Change Syringe Wizard'.
- Tip position is automatically calibrated to high accuracy to reference its exact position in X, Y and Z dimensions.
- The required protocol is selected or a new protocol is generated using wizard software.
- Step 1: a column (8 wells) of LCP mixture is dispensed, one well at a time. This process takes <20 secs.
- Step 2: a corresponding column of screen solution is aspirated from the source plate and dispensed on top of the LCP drop using the positive displacement tips in the mosquito head. This process takes ~5 secs.
- Steps 1 and 2 are repeated for all 12 columns of the plate.
- The cover sheet of the LCP sandwich plate is applied using an alignment jig, to seal the LCP/screen drops in the wells. Figure 1 shows the LCP screen drops and resulting crystals.
- The overall set-up time for a 96-well plate is <5 mins.

Conclusion

- mosquito LCP offers a fully automated solution to LCP screening set-ups.
- It ensures accurate and repeatable dispensing of LCP down to 25 nL.
- The accurate syringe and pipette positioning permits precise drop-on-drop placement of the LCP solution which facilitates automated imaging.
- mosquito LCP has all the functionality of the standard mosquito Crystal and enables zero loss dispensing of protein solutions or other expensive/highly viscous additives.
- This instrument enables both LCP and traditional crystallisation experiments to be set up in commercially available plates.
- mosquito LCP enables high throughput screening set-ups for membrane proteins such as G-protein-coupled receptors.

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