High-throughput Crystallisation Experiments

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The Oxford Protein Production Facility (OPPF) is a new structural genomics facility funded by the Medical Research Council (U.K.). It seeks to achieve high-throughput production of proteins and protein crystals by automating and miniaturising all stages of the process. Instruments used to carry out high-throughput crystallisation experiments include a Robbins Hydra dispensing system, a Cartesian Microsys dispenser, a robotic storage vault and an automated imaging system.

Crystallisation experiments are carried out in 96-well crystallisation plates using the sitting drop vapour diffusion method. The 96 reservoir solutions are transferred from a deep well Masterkit to the crystallisation plate in a single pipetting step using a Robbins Hydra instrument. Small droplets of protein and reservoir solution of typically 100 nl are pipetted with an eight-head Cartesian Microsys dispenser. The instrument was modified to reduce drop evaporation during the pipetting process.

Crystallisation plates are placed in a temperature controlled, robotic storage vault, which is connected to an automated imaging system. Images of the crystallisation droplets are taken at regular intervals and the whole process is fully automated.

Preparing Crystallisation Plates

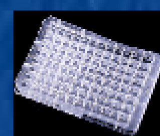
Greiner crystallisation plates are used for our experiments. Features include 3 shelf positions for sitting-drop vapour-diffusion experiments which can be used for different protein concentratrions, ligands, cryoprotectant solutions or even different proteins.

Currently we use crystal screen solutions from Hampton Research and Emerald

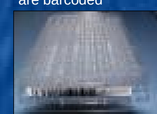
Deep-well blocks contain the screen solutions in 96-well format

The Robbins Hydra dispenses the screens into the crystallisation plates

The crystal screening solutions are placed into 2 ml deep-well blocks. These are then used to produce crystallisation plates complete with the screens whilst retaining the format. Each aspiration by the Robbins Hydra is enough to fill the reservoirs of 3 crystallisation plates with 95ul of the screening solutions.

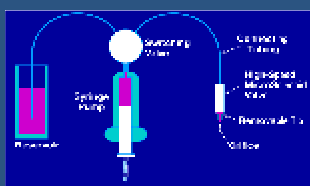


The Greiner 96-well crystallisation plates are barcoded



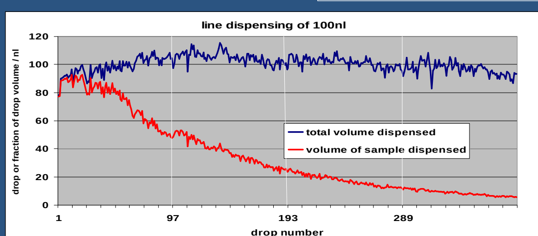
Cartesian Dispensing Robot

The crystallisation drops are set up on the centre of the shelf positions in the Greiner plates. Normally 100nl protein + 100nl screen is used. Either protein or screen solution can be pipetted first. The screen solutions are dispensed as single drops with washing between dispense actions. The protein is dispensed in line mode, "on-the-fly", using only one tip.

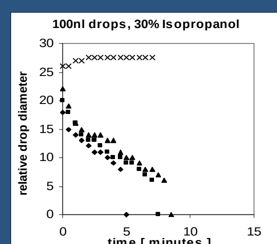


The Cartesian robot functions by pressurising 8 dispensing lines and operating a fast solenoid valve to release the drops.

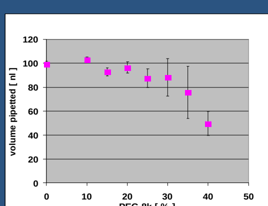
A cover has been fitted to prevent evaporation of the screens and the drops The evaporation rates were monitored for both water and 30% isopropanol and show that the cover is efficient (below).

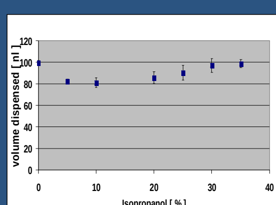


The line-dispensing mode is used for distributing protein to the individual crystallisation experiments and appears to be consistent in volume. The concentration of the protein drops by 50% due to mixing with the system fluid in the lines (water)

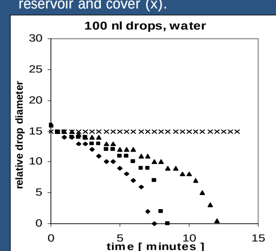


Evaporation of 100nl drops of water and 30% isopropanol from a flat surface (?) and Greiner crystallisation plates without (?) and with reservoir (?), and with reservoir and cover (x).





High and low viscosity single-dispensing (as for screen solutions) was investigated using fluorescein in PEG 8000 and isopropanol. The Cartesian robot is able to dispense solutions up to 30% PEG 8000 consistently.



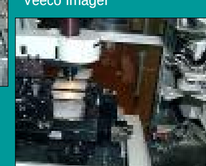
Storage & Monitoring of Crystal Growth

The crystallisation experiments are placed into a temperature-controlled storage vault (TAP "Homebase") of capacity 10000 crystallisation plates. An automated imaging system (Veeco) is attached to the storage vault. Images of the crystallisation droplets can be taken at regular intervals and the whole process is fully automated

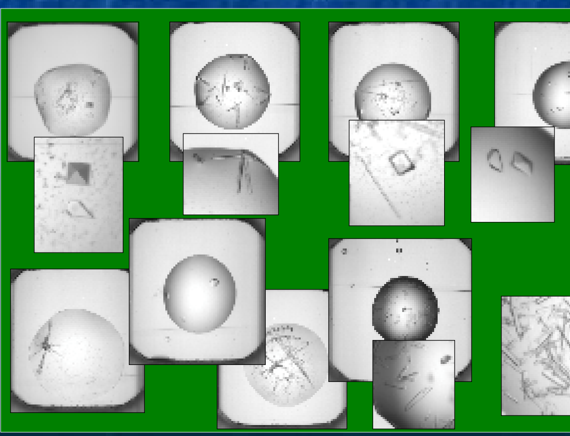
The outside of the storage vault showing the Veeco enclosure

The grippers for transferring crystallisation plates between the Homebase vault and the Veeco imager

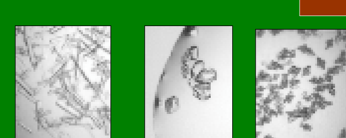
The interior of the storage vault. Visible are the racking units, the tray transfer robot (bottom centre) and the temperature control unit.



Crystallisation plates being imaged by the Veeco



Work is currently progressing on automated image analysis software for integration with an information management system.



This protocol for crystallisation has been used for projects of the Division of Structural Biology and has yielded many crystals. Crystals grown in 100nl drops were often tested for diffraction and on several occasions could be used for data collection.

The OPPF is a UK Medical Research Council (MRC) –funded pilot project to develop methods and protocols for high-throughput structural genomics. It is also a member of the Structural Proteomics IN Europe (SPINE) initiative.