VMXi
&
Harwell Campus Structural Biology Partnership Crystallisation Facility

Dr Halina Mikolajek

Crystallisation Facility Manager/VMXi Senior Support Scientist

December 2019

Outline

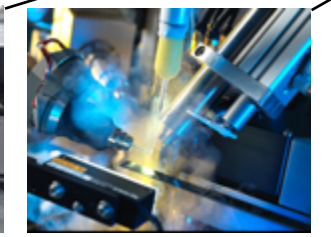
- Detail of crystallisation lab @ Harwell
- Some crystallisation tips

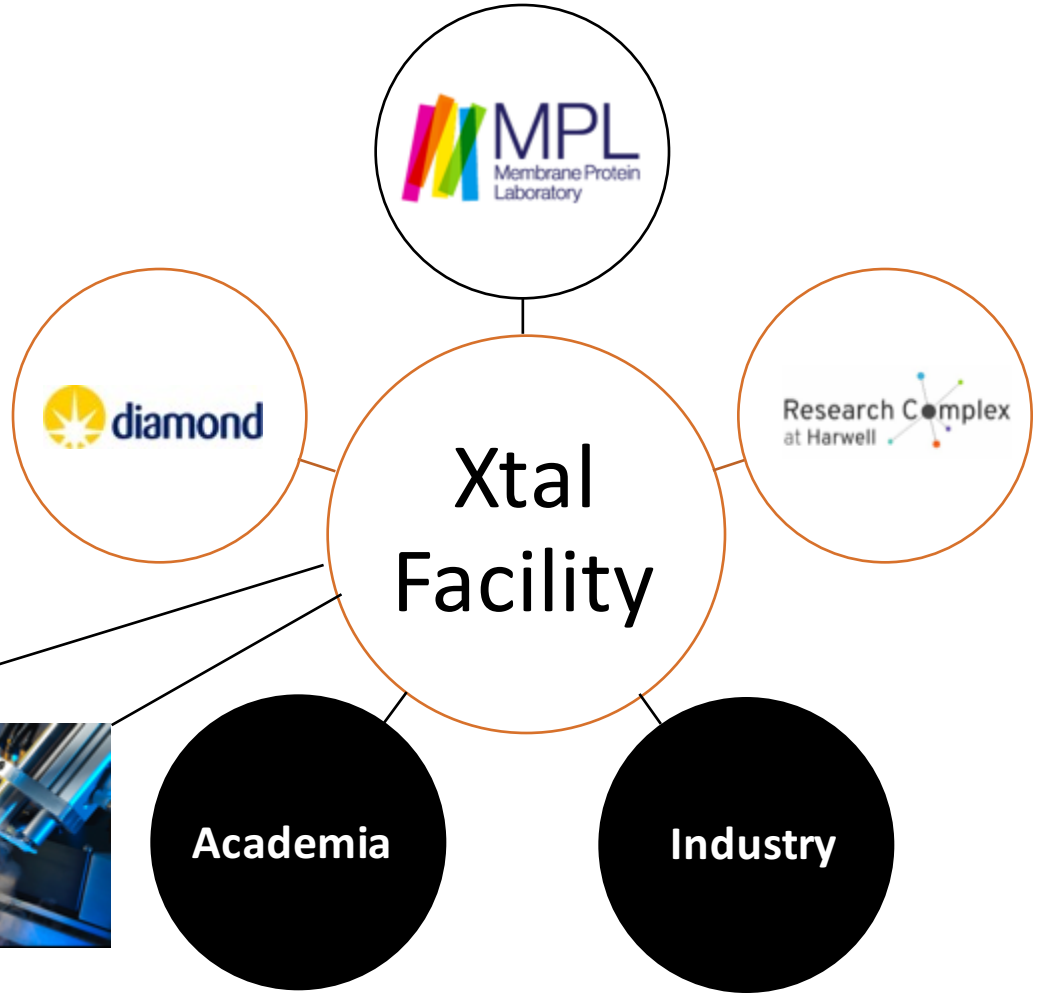
History

Set up under **OPPF** in 2010

Reinvestment & relaunch in 2018

Combining expertise: PPUK, Diamond & MPL



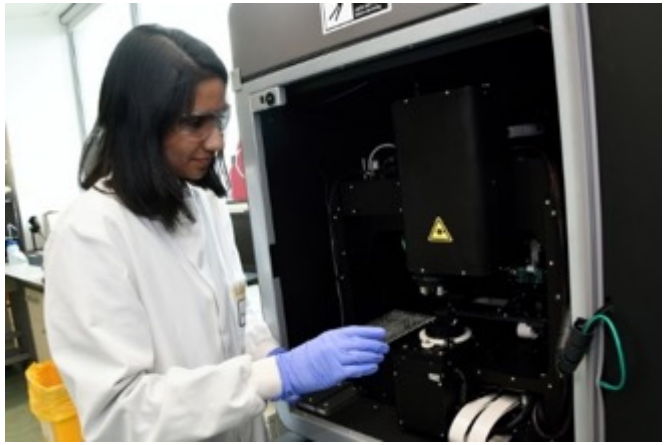


Currently open to users within RCaH, friendly VMXi users

Research Complex at Harwell diamond

Crystallisation capability for soluble and membrane proteins

- Crystallisation robotics (4°C & 20°C, humidity & light control, LCP)
- Scorpion for making screens
- Automated imaging systems (4°C & 20°C, LCP) with remote viewing
- LCP-FRAP
- Assisted HTP crystal harvester (Shifter)



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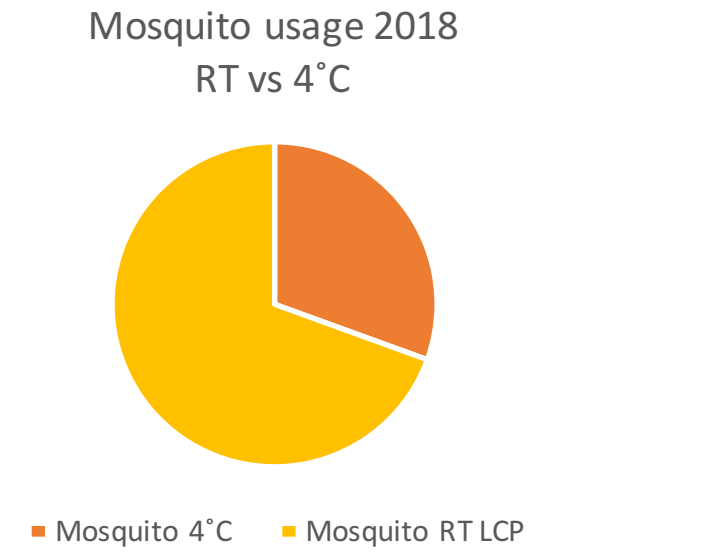
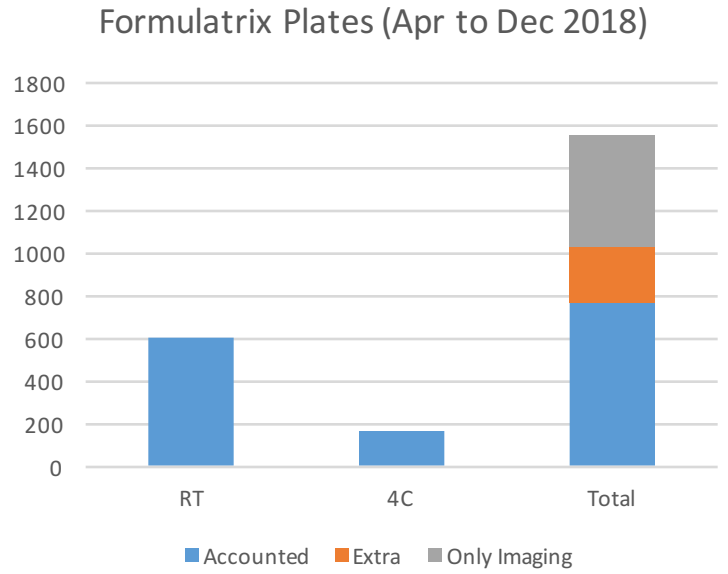
Crystallisation Facility Statistics

+100 registered users



Mosquito at 4°C is useful and being used for ~35 % of total plates set up.



Most users image & store at RT, again ~35 % image at 4°C. ~35 % only image (Xchem)

Facility

Plate types available:

- Greiner Crystal Quick X (VMXi)
- Mitegen *in situ (VMXi)*
- *Swiss SCI 3 well (Xchem)*
- *Intelli (for Gryphon)*
- *LCP glass plates*

Screens:

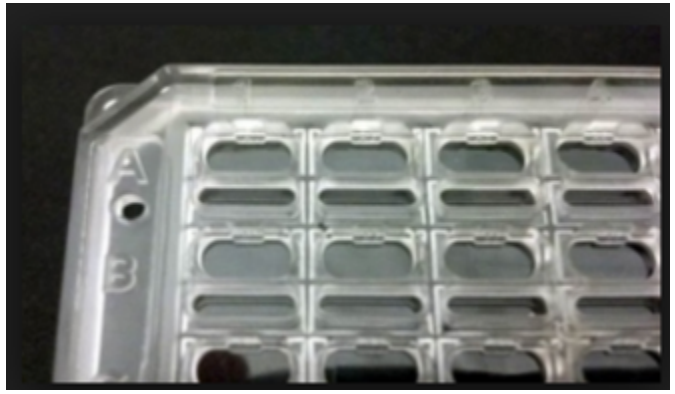
Molecular Dimensions

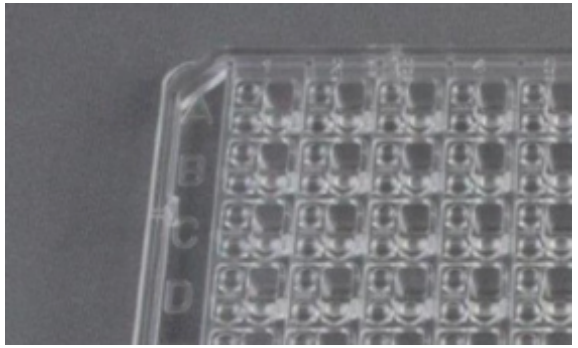
Hampton research

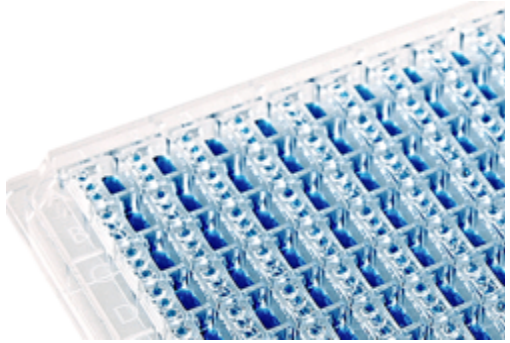
Quiagen

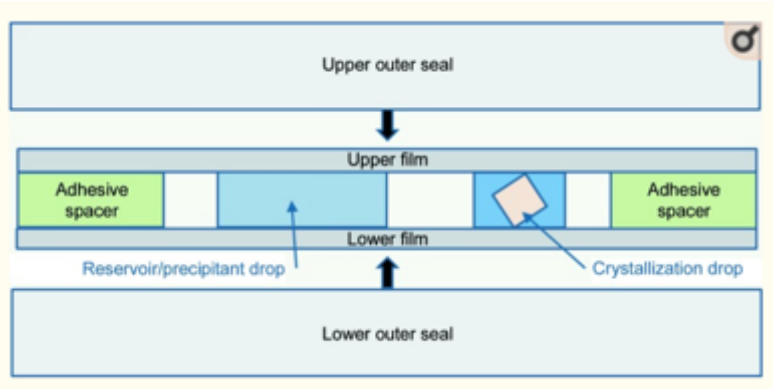
Films:

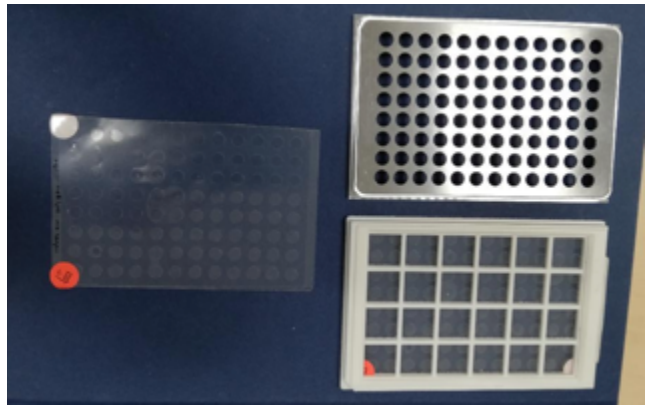
- In development phase



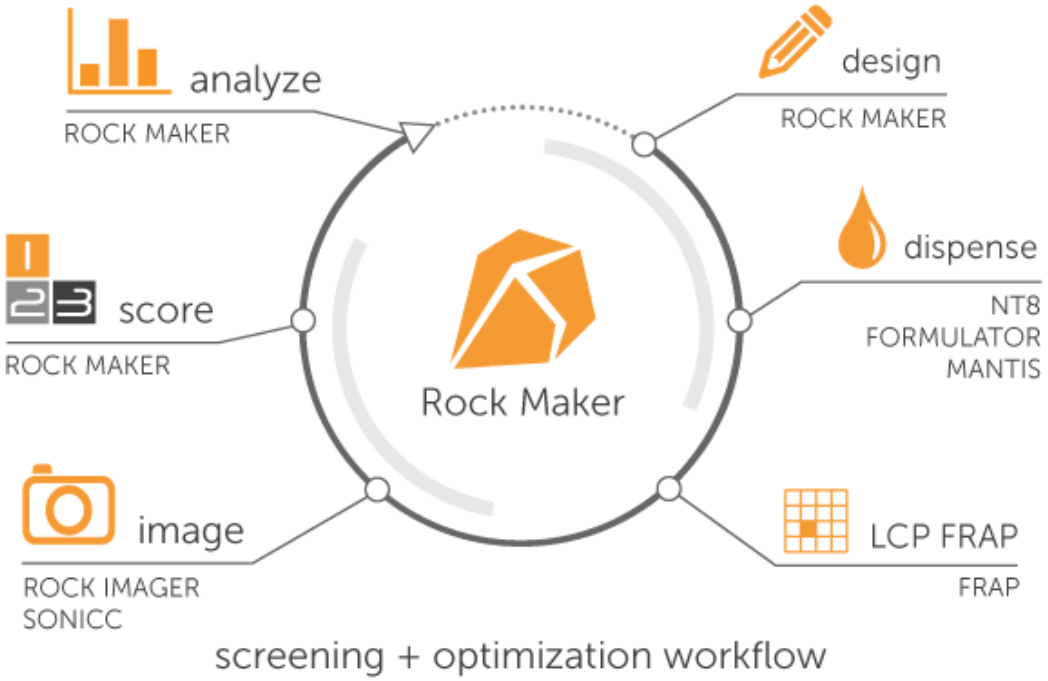


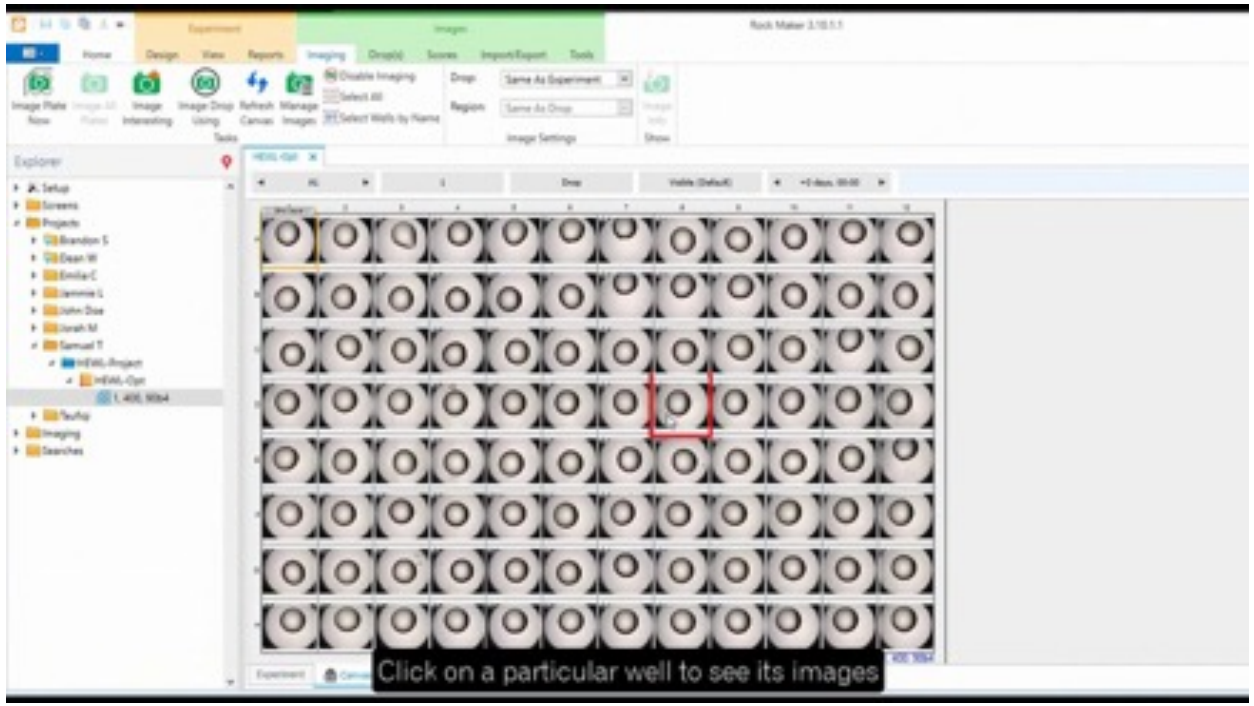






Rock Maker Software Web : Remote access using Fed ID from anywhere in the world





Crystallisation is still the bottle neck

Success rates for steps in crystallographic structure determination.

Percentages are given in relation to the previous step.

Total	Cloned	Expressed	Purified	Crystallized	Structures
54744	35893	24306	16833	2390	1711
100%	65.6%	67.7%	69.3%	14.2%	71.6%

http://sbkb.org/metrics/milestonestables.html)

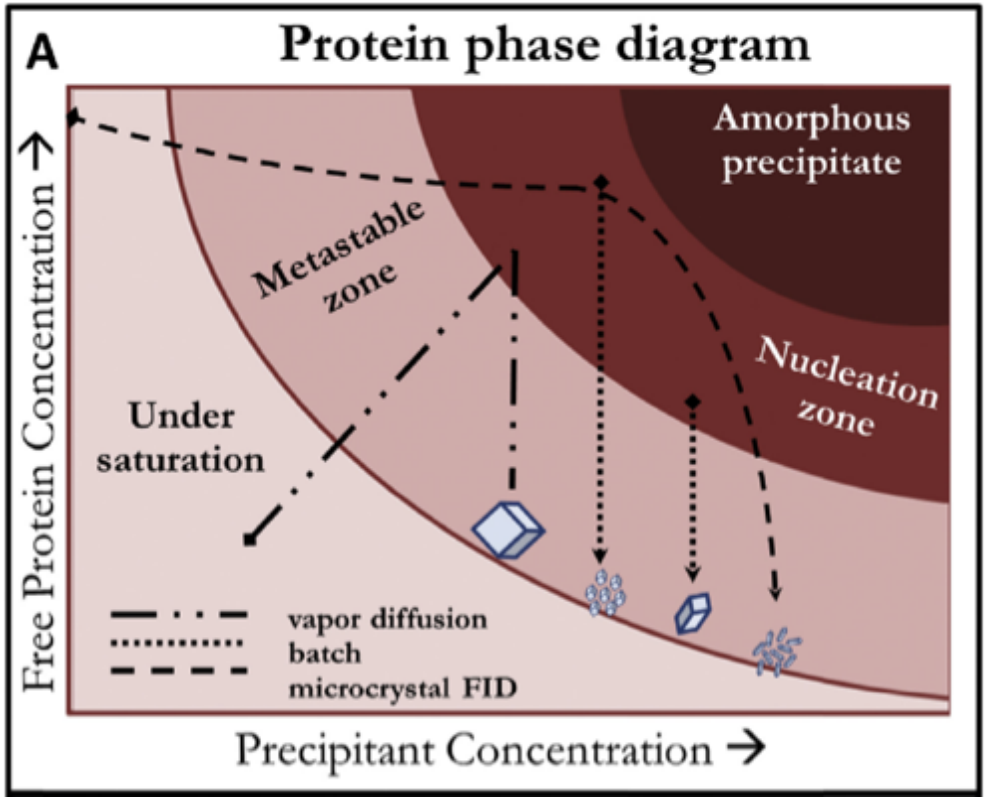


Figure from Garcia et al. 2016

Controlling your experiments: Nucleation

- Nucleation: key to crystallisation
- First step and pre-requisite for the process that allows crystal growth

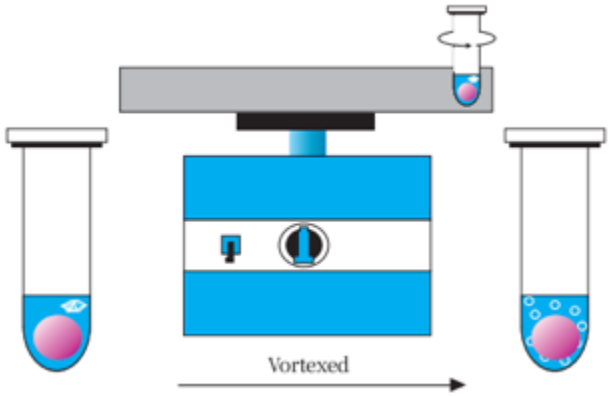
- A parameter difficult to control

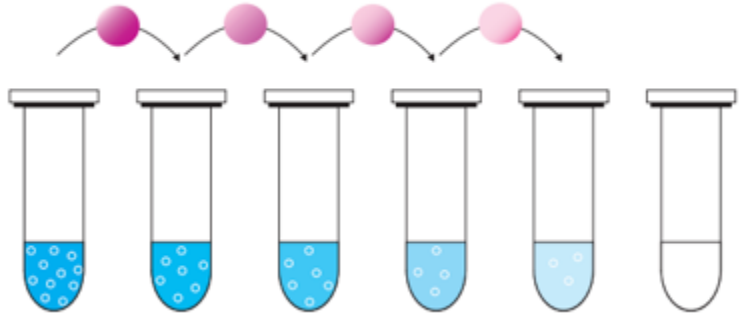
- It depends on:
- Sample concentration
- Sample preparation (Filtering, Storage)
- Method (Vapour diffusion, Microbatch
- Mixing
- Surface

Controlling Your Experiment: Seeding

- Macro: transfer of washing crystal to pre-equilibrated solution
- Micro dilution: Seed beads
- Streak: with needle or cat whisker

- Allows reproduction of crystals, favouring crystal forms





- May Marsh has some interesting publications

Crystallisation Problems

- No crystals
- Tiny low quality crystals, amorphous precipitate, phase separation
- Beautiful crystals, but poorly diffracting
- --> new constructs, different species, co-crystallisaton
- If all fails, try other structural biology methods.

Crystallisation facility and VMXi team

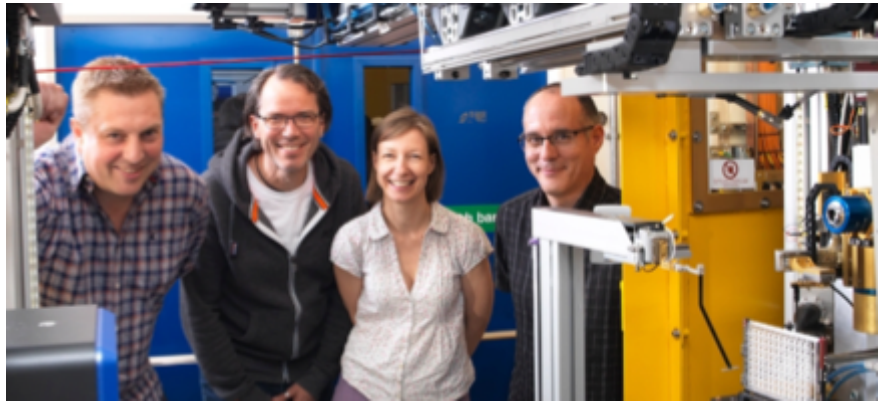
VMXi Team

Prof Juan Weatherby-Sanchez
Dr James Sandy
Dr Halina Mikolajek
Dr Dave Hall

*Dr Thomas Sorensen







MPL Team

Dr Andrew Quigley
James Birch
Dr. Harish Cheruvara
Nadisha Gamage




PPUK & MX Team

Claire Strain-Damerell
Prof Ray Owens

Research Complex at Harwell

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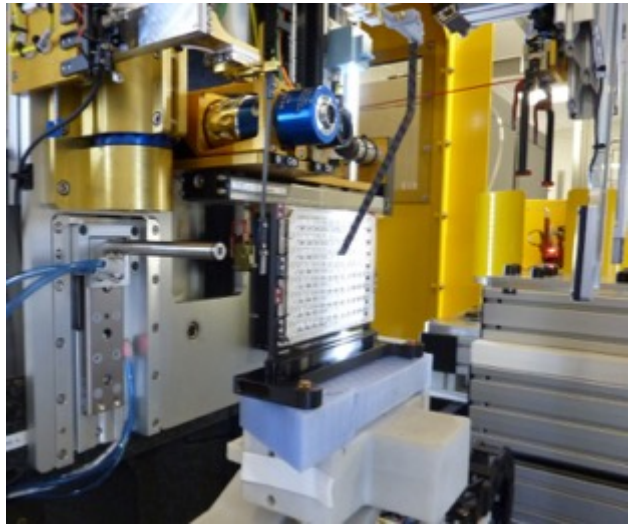
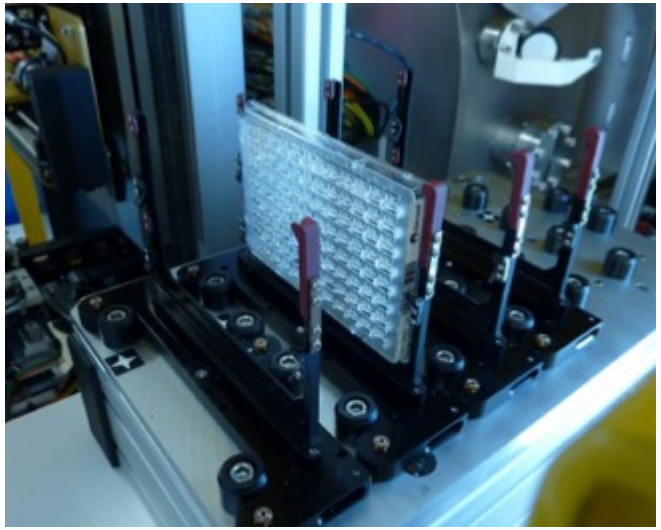
Linking the crystallisation facility to VMXi - the new fully automated *in situ* and serial crystallography beamline

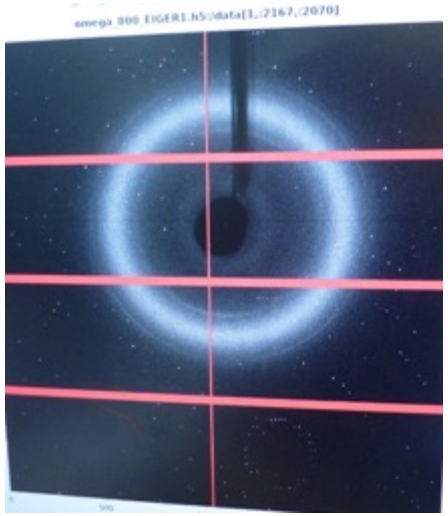
The crystallisation facility is high-throughput and provides users with an easy route into the VMXi beamline, the new fully automated *in situ* and serial crystallography beamline at Diamond.

High flux and fast data collection.

We only accept Greiner Crystal QuickX and Mitegen In-situ plates. We can store and collect data at 20 °C.







We look forward to seeing you here

Questions ?

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