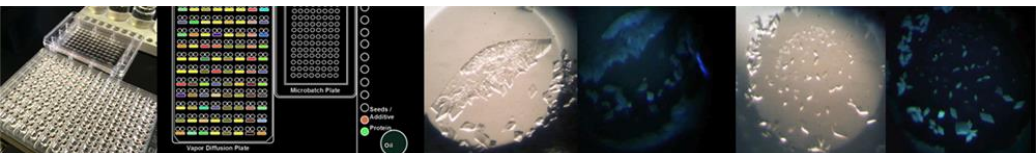


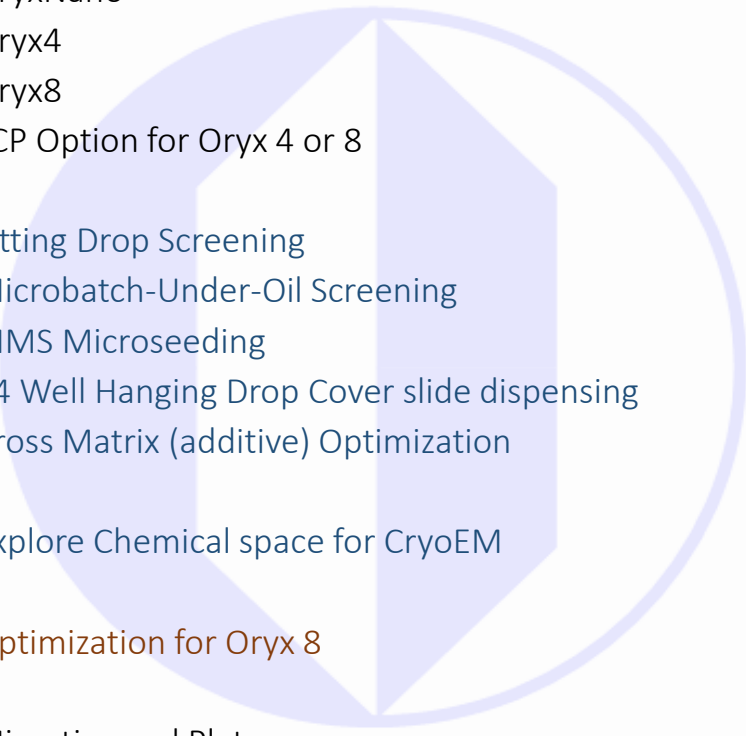


Douglas Instruments

Success in protein crystallization

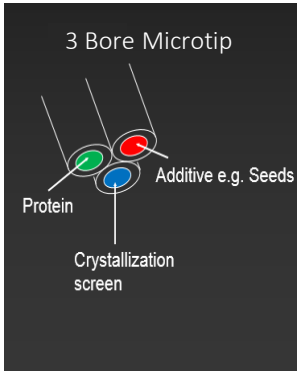


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Introduction to Oryx



The Oryx is a nanolitre dispensing system for protein crystallization. Three models are available with LCP-dispensing available for Oryx4 and 8. All systems have these features in common:

■ Unique dispensing method

All Oryx systems use re-usable multi-bore dispensing tips (microtips), which have several independent channels to dispense small volumes.

At the end of e.g. a 3 bore microtip there are three holes. Each channel dispenses a different solution. The solutions do not mix in the tip - they mix in the drop after they are dispensed. This means that there is no dead-volume.

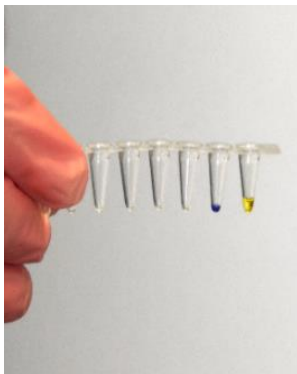
■ Contact dispensing essential for MMS

The microtip always touches the plate when liquids are dispensed.

This gives very reliable dispensing, especially when suspensions of e.g. seed crystals are used, which makes the system ideal for microseeding experiments.

■ Minimal protein wasted

Contact dispensing has another advantage: minimal protein remains in the tip at the end of the experiment.

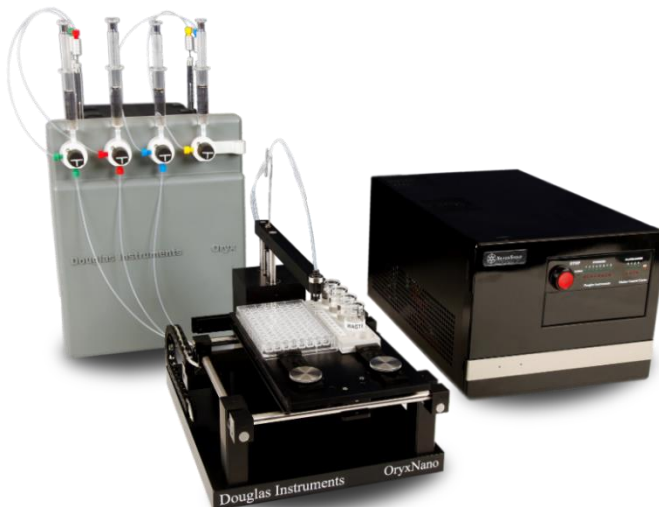


	<i>Protein Vol (nl)</i>	<i>Reservoir (Screen) Vol (nl)</i>	<i>Protein Required (μl)</i>	<i>Protein wasted (μl)</i>
<i>96x1 Drop</i>	100	100	10.0	0.4
<i>96x1 Drop</i>	300	300	29.4	0.6

What's more, since only one (multi-channel) tip is used, all of the protein for an experiment can be placed in a single PCR tube, which also reduces waste. If your pipette is accurate, there is no need to put more than the specified amount into the tube.

The Oryx range of robots

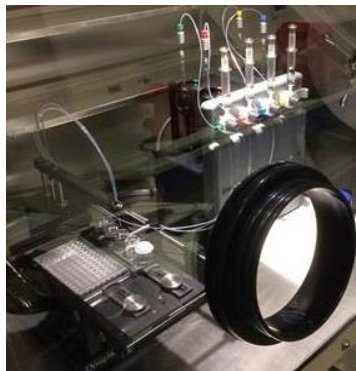
OryxNano



- ❶ Sitting drop down to 100+100nl for screening
- ❶ Up to 8+8µl drops for harvesting and soaking
- ❶ MMS and microseeding for optimization
- ❶ Only 10.0µl of protein for 96 wells (100+100 nl)
- ❶ Simple optimization:
 - 2D grids
 - Cross-Matrix (Combinatorial) experiments
 - Additive Scatter Optimization

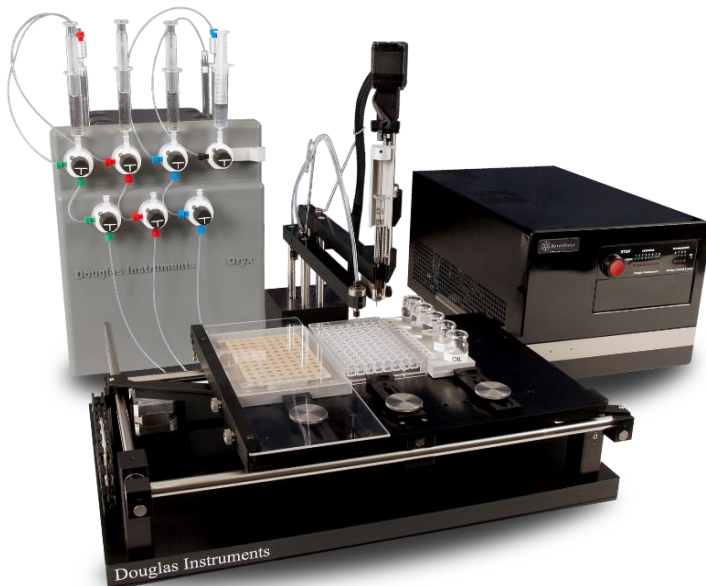
OryxNano is a compact option which has the capability of the Oryx4, except for larger format 24 well sitting drop, hanging drop, microbatch and additive experiments. OryxNano can do quick-and-easy 2-D grid optimizations with up to 4 ingredients.

The OryxNano is well suited to crystallization at 4°C and anaerobic crystallization due to its compact size (photo below).



The Oryx range of robots

Oryx4



Oryx4 is the product for those users who do not want to do multi-variate optimization. As with the Oryx8, it performs standard screening, additive, microseeding, sitting drop and hanging drop experiments and microbatch-under-oil screens (also down to 100+100 nL).

Oryx4 can be upgraded to Oryx8 if multi-variate optimization becomes a requirement.

- ❶ Sitting drop down to 100+100nL for screening
- ❶ Up to 8+8µL drops for harvesting and soaking
- ❶ MMS and microseeding for optimization
- ❶ Only 10.0µL of protein for 96 wells (100+100 nL)
- ❶ Simple optimization:
 - 2D grids
 - Cross-Matrix (Combinatorial) experiments
 - Additive Scatter Optimization
- ❶ Hanging drops up to 8+8µL
- ❶ Additive experiments
- ❶ Microbatch-under-oil down to 100+100nL
- ❶ Lipidic Cubic Phase (LCP) dispensing option

The Oryx range of robots

Oryx8



Oryx8 is the flagship Douglas Instruments protein crystallization robot. It can set up sitting drops down to 100 + 100 nl and up to 2 + 2 μ l for 96 wells. For hanging drop experiments up to five drops can be set up per cover slide, with volumes up to 8 + 8 μ l. For screening experiments the reservoirs must be pre-dispensed by some other method such as a 12-channel pipette.

For optimization experiments the reservoirs are filled automatically. Standard screening, additive and microseeding experiments are included as well as microbatch-under-oil screens (also down to 100 + 100 nl).

Optimization experiments can be carried out using up to 7 ingredients with full pH control, including multi-variate designs such as the Central Composite. In addition, quick-and-easy 2-D grid optimizations with up to 4 ingredients can be carried out in a few minutes.

- ① Sitting drop down to 100+100nl for screening
- ① Up to 8+8 μ l drops for harvesting and soaking
- ① MMS and microseeding for optimization
- ① Only 10.0 μ l of protein for 96 wells (100+100 nl)
- ① Simple optimization:
 - 2D grids
 - Cross-Matrix (Combinatorial) experiments
 - Additive Scatter Optimization
- ① Hanging drops up to 8+8 μ l
- ① Additive experiments
- ① Microbatch-under-oil down to 100+100nl
- ① Lipidic Cubic Phase (LCP) dispensing option
- ① Autodesign optimization capability:
 - 7-dimensional optimization
 - Central Composite and Box-Behnken designs
- ① Rapid reservoir filling for optimization
- ① Multi-component pH control
- ① LCP Optimization (Oryx8 LCP)
- ① Reduced need to swap microtips

LCP option for Oryx4 or 8

Lipidic Cubic Phase (LCP) dispensing is an option for Oryx4 and Oryx8. The option is available for new systems and can also be installed to recent compatible systems. The LCP arm can dispense volumes as small as 10 nl and up to 1 µl or larger.

The shape of the volume (dispensing technique) can be selected: taller conical volumes or wider pancake shaped volumes. precipitant(s) and additives are immediately dispensed onto the LCP volume preventing evaporation or phase change of the LCP. Below are examples of LCP screening experiments with the Oryx LCP systems.



LCP Screening:

- Recommended bolus size 30 nl – 200+ nl
- Recommended aqueous drop volume 400 nl – 2000 nl



Aqueous drop
1000 nl Screen e.g. JCSG
LCP bolus
100 nl

LCP 'Additive effect' Screening:

- Screen with additive effect of known crystallization condition.
- Mimics additive effect of typical rMMS experiment
- Find new hits in different conditions



Aqueous drop
300 nl Previous Hit Solution
700 nl Screen e.g. JCSG
LCP bolus
100 nl

LCP Additive Screening:

- Additive screens can be dispensed onto the LCP
- Simultaneous dispensing of additive and precipitant



Aqueous drop e.g.
300 nl Additive Screen
e.g. Silver Bullet
700 nl Precipitant
LCP bolus
100 nl

LCP Microseed Screening:

- Prepare LCP Seed Stock by incubation method
- Dispense LCP Seed Stock to random screen



Aqueous drop
1000 nl Screen e.g. JCSG
LCP bolus
100 nl LCP Seed Stock

Sitting Drop Screening

All Oryx systems can dispense sitting drop screening experiments. The re-usable multi-bore dispensing tip (microtip) can be used to dispense up to 5 drops to each well of a 96 well plate. The ingredients can be controlled independently and different ratios or volumes of up to 4 ingredients can be dispensed to each drop.

Uroplet One

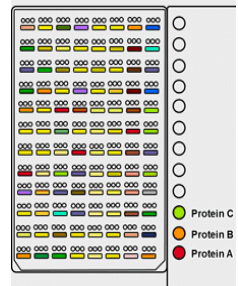
Protein Volume [μl] 0.10
Screen Volume [μl] 0.30
Droplet Position

Uroplet Two

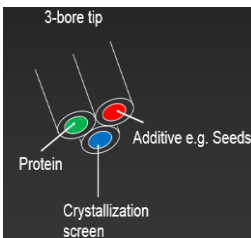
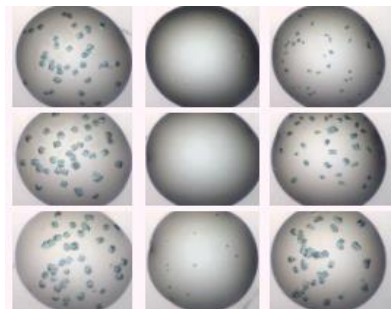
Protein Volume [μl] 0.20
Screen Volume [μl] 0.20
Droplet Position

Uroplet Three

Protein Volume [μl] 0.30
Screen Volume [μl] 0.10
Droplet Position



- Dispense down to **100+100 nl** for screening.
- Dispense up to **8.0+8.0 μl** for crystal harvesting and soaking.
- Dispense down to 5 nl of additive or seed stock.
- Only 10.0 μL required for a 96 well screen (100+100 nl).



2, 3, and 4-bore microtips can be used for screening experiments. The 3 and 4-bore tips allow ingredients such as seed-stock or additives to be dispensed to the drop.

Microtip	Screening experiments available
2 Channel Microtip	Protein + Reservoir
3 Channel Microtip	Protein + Reservoir Protein + Reservoir + Seed-Stock Protein + Precipitant + Additive Screen 2 Different Proteins + Reservoir Protein + Reservoir + Additive
4 Channel Microtip	Protein + Reservoir Protein + Reservoir + Seed-Stock Protein + Reservoir + Seed-Stock A + Seed-Stock B Protein + Precipitant + Additive Screen 2 Different Proteins + Reservoir 2 Different Proteins + Reservoir + Seed-Stock Protein + Reservoir + Additive Protein + Reservoir + Additive + Seed-Stock 3 Different Proteins + Reservoir

Microbatch-Under-Oil for Oryx4 or 8

Microbatch is a very simple approach to crystallization. Small samples of protein (**100 nl to 5.0 μ l**) are mixed with stock solutions to the drop, then immediately covered with oil by the robot to prevent evaporation.

Microbatch-under-oil experiment protocols available:

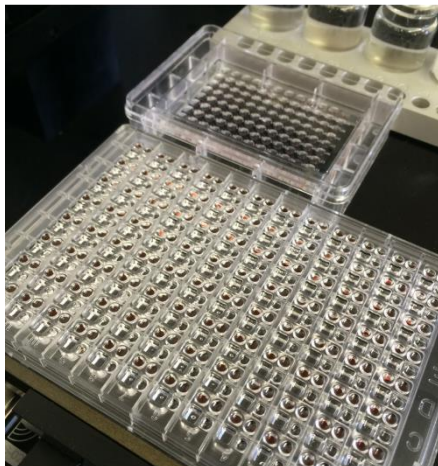
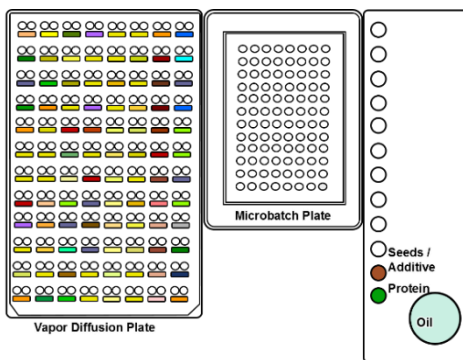
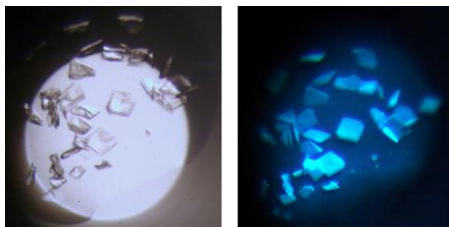
- Down to **100+100 nl** for screening.
- Dispense sitting drop and microbatch plates in one run.
- 2D gradient optimization.
- Microseeding and Additive screening.
- 7D optimization using XStep (Oryx8 only).

For screening experiments it can help to use a 50:50 mixture of paraffin oil and silicone oil. The silicone allows slow evaporation over about a month, which gives a scanning effect across the phase diagram of the protein.

For optimization, pure paraffin oil can be used, which reduces evaporation to a minimum.

Studies have shown that microbatch finds as many or slightly more hits that vapor diffusion, but the main advantage is that (for reasons that may not be well-understood) certain proteins crystallize more effectively in microbatch than other methods.

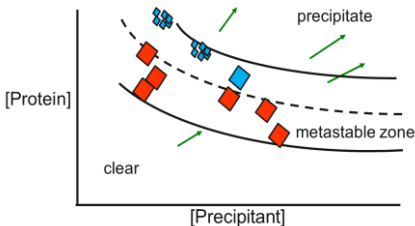
Microbatch can help to protect sensitive proteins such as membrane proteins and anaerobically-produced proteins because it reduces the oxidation and gives thinner skins on the surfaces of drops.



MMS microseeding

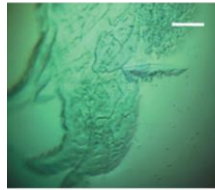
Since all Oryx systems use contact dispensing (the tip always touches the plate) they give very reliable dispensing even when suspensions of solid particles are used. This makes them ideal for microseeding experiments. The technique of adding crystal seed-stock to random screens (rMMS) is a significant breakthrough in protein crystallization that is very effective.

MMS not only produces more hits, it also typically generates better diffracting crystals because crystals are more likely to grow in the metastable zone of the protein's phase diagram (see below).



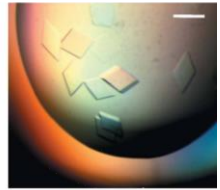
Note also that in cases where only one or a few crystals are obtained in screening experiments, the seed stock that can be made is very valuable – often more valuable than the protein sample. It is therefore a great advantage to be able to use the smallest possible sample of seed stock. Using any robot from the Oryx range, seeding can be performed in a whole 96-well plate using only 1.5 μ l of seed stock. This is particularly helpful for membrane protein crystallization projects because membrane protein crystals are often unstable and it is helpful to make seed stocks without diluting the original mother liquor.

Regular screen



USP7 crystals used for seeds grown in 30% PEG 3350, 100 mM HEPES pH 7.0

MMS screen

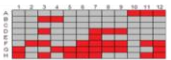
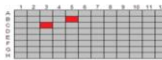


USP7 crystals after seeding in 20% PEG 3350, 200 mM magnesium hexahydrate

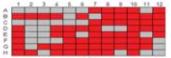
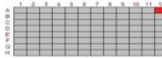
Regular screen

Screen with seeds

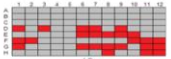
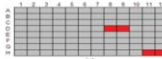
MMP12



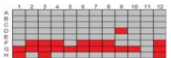
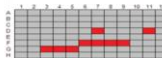
BVP



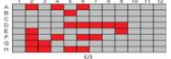
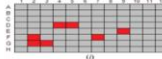
USP7



Trypsin



PPE



D'Arcy et al. Acta Cryst. (2007). D63

MMS with a 4 bore microtip

In the example below a 4-channel microtip is used to dispense protein and two seed stocks. The two seed stocks could be from different sources, for instance a similar protein with some homology, or they could be different dilutions of the same seed stock.

Seed stock A



Reservoir well

Seed stock B

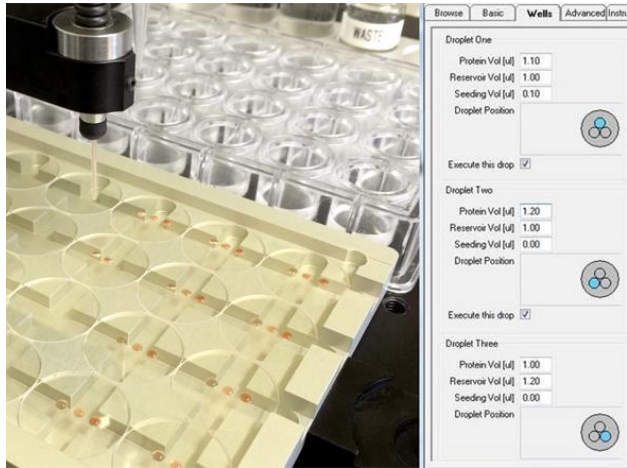


Control (no seed stock)

24 Well Hanging Drop for Oryx4 or 8

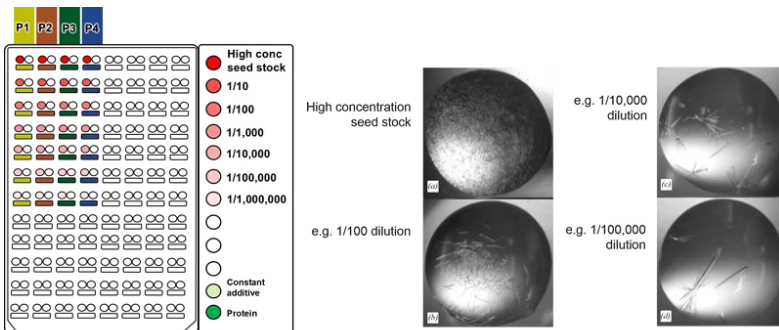
Oryx4 and Oryx8 can set up experiments with 24 well plates and cover slides, with up to 5 drops on each cover slide. Volumes can range from 100+100 nl up to 8+8 μ l (assuming 24 drops).

You can also add one additive to each drop with Oryx4, and up to five additives with Oryx8. This is very useful for leads that are picked up in random microseeding experiments, where it may be necessary to add e.g. diluted seed stock to get crystals.



Cross Matrix (additive) Optimization

The systems' powerful *Combinatorial Optimization* approach allows a different additive or seed-stock to be added to each row.



Each additive or dilution is picked up from the corresponding PCR tube on the right of the table (1/10, 1/100 etc. on the diagram above). By arranging e.g. precipitants in columns (P1, P2 etc.), different combinations of precipitants and additives such as seed-stock can be tested very quickly.

Explore chemical space for cryoEM and crystallization

Oryx8



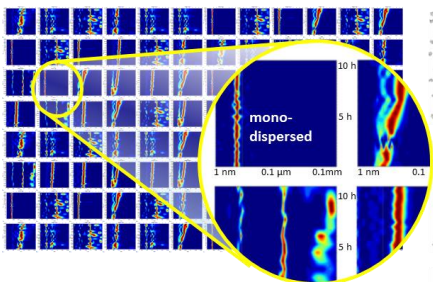
SpectroLight 600



cryoEM

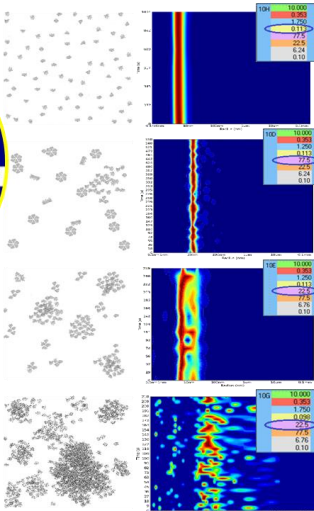


Screening and optimization experiments (with cryoEM and/or crystallography in mind) can be dispensed by the Oryx8, then efficiently analyzed by the SpectroLight 600. Several hundred samples can be analyzed per day, before choosing the best to pass on for analysis by cryoEM.



SpectroLight 600: in-Plate DLS

- Fully automated DLS
- Use 96 well plates
- Characterization of biomolecules for further sample evaluation like NMR, CryoEM, SAXS or Crystallization
- Assess homogeneity, aggregation and assembly state
- Optimize buffer conditions, e.g. by selection of a specific detergent or concentration for membrane protein solubility
- Investigate protein interactions
- No cleaning required



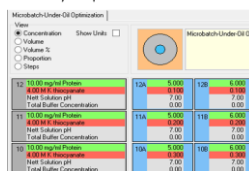
Oryx8 screening under-oil



In situ under-oil DLS measurement



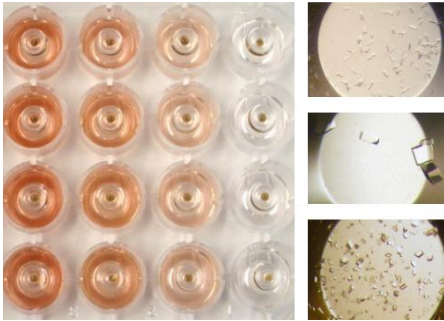
Oryx8 optimization software



Oryx8: multi-channel dispensing system

- Microbatch-under-oil suitable for crystallization and cryoEM
- Only **7.0 μl** of protein required for a 96-well experiment
- Auto design and gradient optimization with up to 7 ingredients

XStep Optimization for Oryx8



Automatic Optimization

XStep for Oryx8 provides a **spreadsheet environment** for designing optimization experiments.

Xstep optimization experiment types:

- **Microbatch-under-oil**
- **LCP**
- **Vapor diffusion**
- **Stock plate preparation** (up to 500 μL)

Experiment Design:

- **Gradients**
- **pH**
- **Auto design (statistical based experiment design)**
- **Up to 7 ingredients per experiment**

Rapid reservoir filling

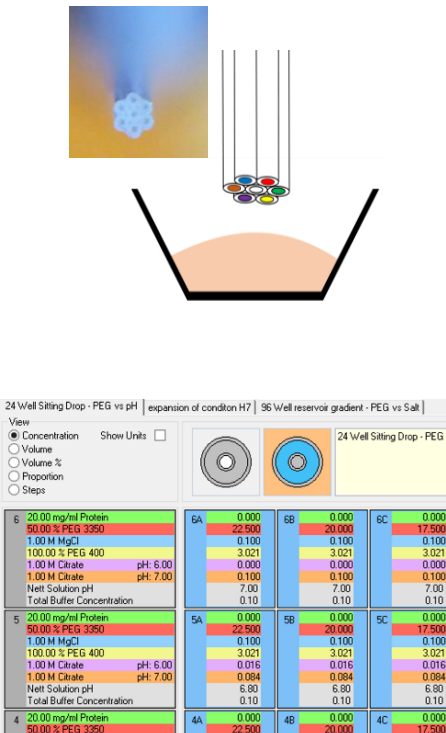
Oryx8 can quickly fill the reservoirs of sitting drop plates using the air-driven 1 ml channel. The microtip can be used to increase dispensing accuracy.

Multi-buffer pH control

Our optimization software includes a sophisticated algorithm that can calculate the pH of a mixture containing any number of buffers, using the pHs, pKas and the concentrations of the buffers.

7-Bore Microtip

The 7-bore tip is used for Microbatch optimization, Vapor diffusion optimization drop dispensing, reservoir filling (where high accuracy is required) and dispensing aqueous solution onto LCP for LCP optimization.



Microtips and Plates

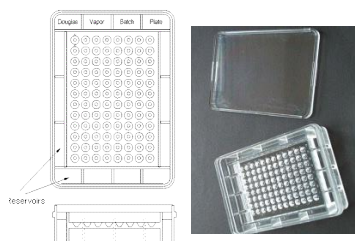
Microtip	7-bore (<i>Oryx8 only</i>)	MTIP7
Microtip	4-bore Fine (<i>OryxNano and Oryx8</i>)	MTIP4-F
Microtip	3-bore Fine	MTIP3-F
Microtip	2-bore Fine (recommended for drops < 0.2 µl)	MTIP2-F
Microtip	2-bore Medium	MTIP2-M

Please see www.douglas.co.uk for full product list

Vapor batch Plate (Under-Oil) Product Code

96 Well Hydrophilic (GOLD) **VB-GOLD**

96 well Hydrophobic (SILVER) **VB-SILVER**

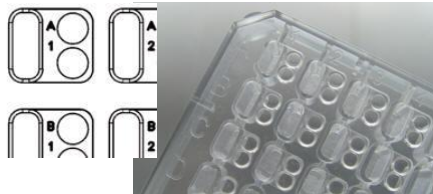


SwissCI 'MRC' 2drop Product Code

Polystyrene **SW2T - PS**

UV (COC, Hydrophobic) **SW2T - UVP**

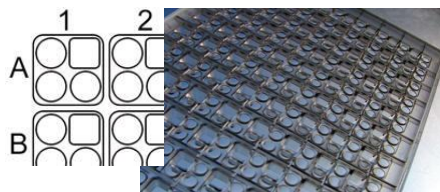
UVXPO (Hydrophilic) **SW2T - UVX**



SwissCI 3drop Product Code

Polystyrene **SW3T - PS**

UV (COC, Hydrophobic) **SW3T - UVP**



SwissCI Maxi Product Code

Polystyrene **SWMX - PS**

UV (COC, Hydrophobic) **SWMX - UVP**



Oryx Comparison Table

Feature	OryxNano	Oryx4	Oryx8
Sitting Drop	✓	✓	✓
Vapor diffusion multiple drops per reservoir, single protein	✓	✓	✓
Vapor diffusion multiple drops per reservoir, 2 proteins	✓	✓	✓
Vapor diffusion multiple drops per reservoir, 3 proteins	✓	✓	✓
Vapor diffusion, Microseed Matrix Screening	✓	✓	✓
Vapor Diffusion, additive experiments		✓	✓
Hanging Drop		✓	✓
Vapor Diffusion with up to 5 drops per cover slide		✓	✓
Microbatch with automatic oiling		✓	✓
Additive experiments		✓	✓
Microseed Matrix Screening		✓	✓
Quick-and-easy 2D grid, 3 ingredients		✓	✓
Quick-and-easy 2D grid, 4 ingredients			✓
Optimization			
Quick-and-easy 2D grid, 3 ingredients	✓	✓	✓
Quick-and-easy 2D grid, 4 ingredients	✓	✓	✓
7D optimization grids			✓
Central Composite, multi-variate experimental designs (7-channel)			✓
Rapid Reservoir filling for Stock Plates, Sitting Drop and Hanging Drop Optimization			✓
Tools			
Microlytic Crystal Former filling		✓	✓
Fluidigm filling		✓	✓
LCP Upgrade			
Compatible with LCP dispensing arm add-on		✓	✓



Douglas Instruments

Success in protein crystallization

Top 14 organic and inorganic main precipitants reported in REMARK 280 of the PDB

(from the 3939 entries that could be parsed.)

www.douglas.co.uk/top14.htm

Organic precipitant (2503 samples)	No. of entries	Average conc. used
PEG 4K	710	21.1%
PEG 8K	488	18.1%
PEG 3.35K	296	20.5%
PEG 6K	212	16.8%
MPD	193	38.6%
PEG 400	142	25.7%
PEG-MME 2000	65	22.7%
PEG-MME 5000	63	20.0%
PEG 1000	57	19.8%
Iso-propanol	48	18.0%
PEG 2000	45	22.3%
Ethylene glycol	43	20.5%
Ethanol	43	28.8%
PEG 10K	32	22.0%
Total, average	2437	22.5%

Salt precipitant (1436 samples)	No. of entries	Average conc. used
Ammonium sulfate	900	1.9 M
Sodium chloride	124	1.7 M
Sodium citrate	76	1.1 M
Sodium, potassium phos.	66	1.8 M
Lithium sulfate	63	1.4 M
Sodium formate	59	3.4 M
Magnesium sulfate	29	1.7 M
Ammonium phosphate	29	1.5 M
Potassium phosphate	25	2.0 M
Sodium acetate	21	1.2 M
Sodium, potassium tart.	13	1.0 M
Cesium chloride	11	2.7 M
Potassium chloride	10	1.4 M
Sodium phosphate	10	1.4 M
Total, average	1436	1.7 M

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