



# DiffraX™ User Guide

## Ultra-low background sandwich film for *in situ* diffraction experiments

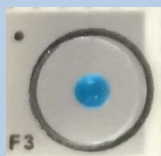
DiffraX can be used for *in situ* screening of crystals grown in the film sandwich. Single crystal or serial crystallography can then be carried out on the whole film sandwich, or by mounting each individual window in the DiffraX sample holder. DiffraX is UV transparent and can be used for crystallization by vapour diffusion, microbatch, counter-diffusion, and *in meso* methods (LCP, Sponge phase etc.).

## Options for experimental set-up

### (a) Batch experiment:

Supersaturation can be achieved directly rather than by diffusion by mixing the precipitant and protein directly in the sandwich. Experiments can be set-up by hand or by robot.

*Recommended drop volume:* up to 2μL total.



### (b) Free interface Diffusion:

Place the sample and precipitant drops next to each other so they touch and an interface forms. This is similar to what you would get in a capillary, where the drops will diffuse gradually into each other. It is recommended a robot is used to set-up drops.

*Recommended drop volume:*  
150 nL per drop.

### (c) Classic vapour diffusion

Set-up the protein + precipitant drop of equal ratio (usually 1:1, but this can be varied). Then place a reservoir drop nearby but not touching. This is now very similar to a vapour diffusion experiment. Experiments can be set-up by hand or by robot.

*Recommended drop volume:*  
75 nL protein +  
75 nL precipitant drop  
500 nL reservoir/precipitant drop.

### (d) Membrane proteins: *in meso* phase

If you are working with membrane proteins you may wish to use a sponge phase or cubic phase (LCP) type of experiment.

Sponge phase: simply place the protein drop on the top of the sponge phase and allow it to diffuse in.

Cubic phase: the protein is premixed in the phase and reagent is allowed to diffuse in by placing over the sample drop.

Pressing lipidic cubic phase drops in a sandwich allows for easier viewing and crystal identification.

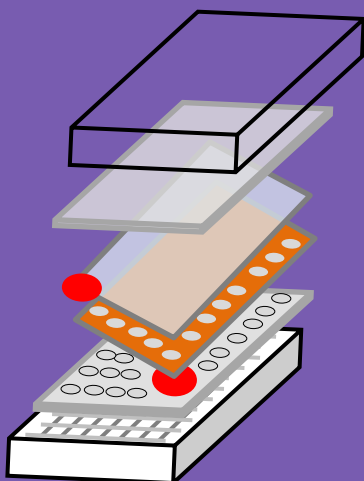
*Recommended drop volume:* up to 2μL total.



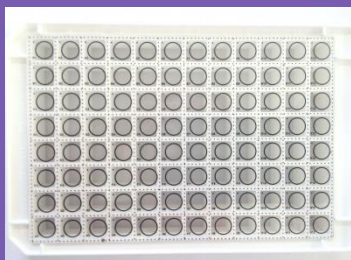
# Step-by-step guide to setting-up a DiffraX experiment

*\*NOTE! The films are protected by a translucent cover which must be removed before use. Ideally carry this out in Step 4 once the sandwich has been fully assembled*

## DiffraX Components:

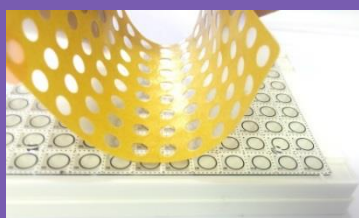


- DiffraX™ Frame Lid
- DiffraX™ Upper Evaporation Cover
- DiffraX™ Cover Film
- DiffraX™ Film Base
- DiffraX™ Printed Lower Evaporation Cover
- DiffraX™ Frame Base



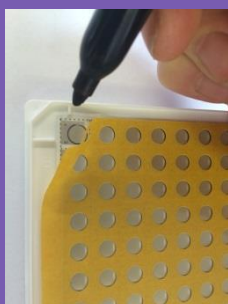
## Step 1

- Place the Printed Lower Evaporation Cover on the white Frame Base.
- Place a few small drops of water onto the Printed Lower Evaporation Cover – this will allow the film to stick to the base in the next step.



## Step 2

- **! Tip** - Leave the protective layer (indicated with a round red sticker) on until the sandwich has been assembled.
- Position the Film Base (brown paper side facing up) onto the Printed Lower Evaporation Cover - the water drops spread to stick the Film Base to the Printed Lower Evaporation Cover.
- **! Tip** - Bend the film in the middle and lower it onto the water from the centre outward
- Peel back a small corner of the brown paper and place a mark on the Film Base to indicate position A1.

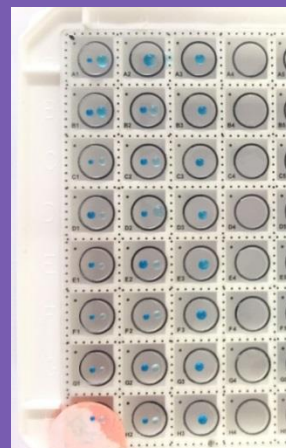




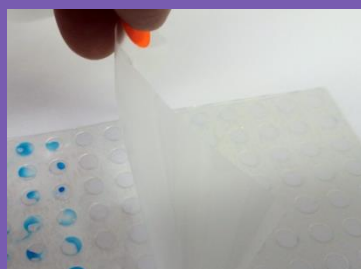
### Step 3



- Remove the brown paper release liner from the Film Base and dispense sample and reagent drops onto the windows of the Film Base.
- **! Tip** - If using a contact dispensing robot it may be beneficial to place the Film Base and Lower Evaporation Cover assembly directly onto the Robot (without white Frame Base).



### Step 4



- Cover the Film Base with the Cover Film and press down firmly with a roller or a Sealing Sheet Applicator. This is now the DiffraX Film Sandwich.
- Remove protective film from both the Film Base and the Cover Film (indicated by round red stickers).
- Cover the Film Sandwich with the Evaporation Cover (Plain - not printed).

### Step 5



- Place the Lid on the whole assembly and set aside to incubate crystal growth.
- During crystal growth the Film sandwich can be examined without the lid under a microscope or on an imaging robot.

**Note!** The evaporation Covers and Films are UV Transparent.

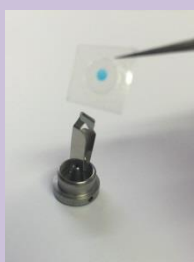


## Data collection with DiffraX

**Screening:** The whole film sandwich can be mounted in a suitable frame and typically 50 degrees of data can be collected from crystals in every window.

**Complete data sets:** Cut out a single window from the DiffraX film sandwich with a scalpel (MD9-30, included in Starter Pack) or pair of fine scissors and mount using tweezers or fingertips in the Spring Clip of the DiffraX Sample Holder (just gently squeeze the clip and it will open). Mount the CryoCap with clip on the goniometer in the usual manner.

**Note!** *If you need to store your sample in a cryovial- make sure you cut the window narrow enough to fit in the vial.*



**Cryo protection:** DiffraX windows can be flash cooled in place by opening the cryo-shutter.

**Serial crystallography:** On a micro-focus beam line serial data can be collected from different crystals in a single window.

## Re-ordering Details

Code	Description	Pack size
MD11-56	DiffraX™ Frame (Reusable)	10
MD11-83	DiffraX™ Printed Lower Evaporation Cover (Reusable)	10
MD11-84	DiffraX™ Evaporation Cover (Reusable)	10
MD11-85	DiffraX™ Lid (Reusable)	10
MD9-30	Scalpel (Reusable)	5
MD11-87	DiffraX™ Sample Holder (Reusable) Includes Spring clip mounted in a Magnetic CryoCap and CryoVial	10
MD11-88	DiffraX™ Starter pack – enough to set up 5 <i>in situ</i> film sandwich experiments.	5 components plus 1 scalpel
MD11-82	DiffraX™ Film Sandwich Includes 10 each of DiffraX™ Film Base and DiffraX™ Film Cover	10