

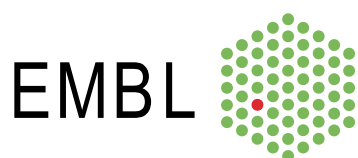


CrystalDirect®

Automatic crystal harvester

Technical Document

May 2017



CrystalDirect[®] at a glance

Overview

CrystalDirect[®] is a fully-automatic crystal harvester which combines high efficiency with strong reliability and multiple selectable methods to harvest a single crystal or a group of crystals.

Harvesting process

CrystalDirect[™] plates are composed of a membrane on which samples are deposited.

The CrystalDirect[®] robot cuts the membrane around selected crystals by means of laser photoablation (Cipriani *et al.*, 2012).

Thereafter the crystal support is fixed on a SPINE sample pin and immediately cryo-cooled.

Thanks to this photoablation laser technology, CrystalDirect[®] also gives the possibility to separate nested crystals by laser cutting without risk to deteriorate them.

Key features

- ~1 harvest/min
- Success rate > 99 %
- Flexible harvesting methods
- Automatic cryo-protection
- Compatible with sample changer robot
- Web based sample management

The CrystalDirect[™] plate is a 96-well vapor diffusion crystallization plate that uses an ultrathin film as crystallization support (25 µm and thinner). CrystalDirect[™] plates are entirely compatible with standard equipment.



CrystalDirect® Key points

High throughput harvesting process

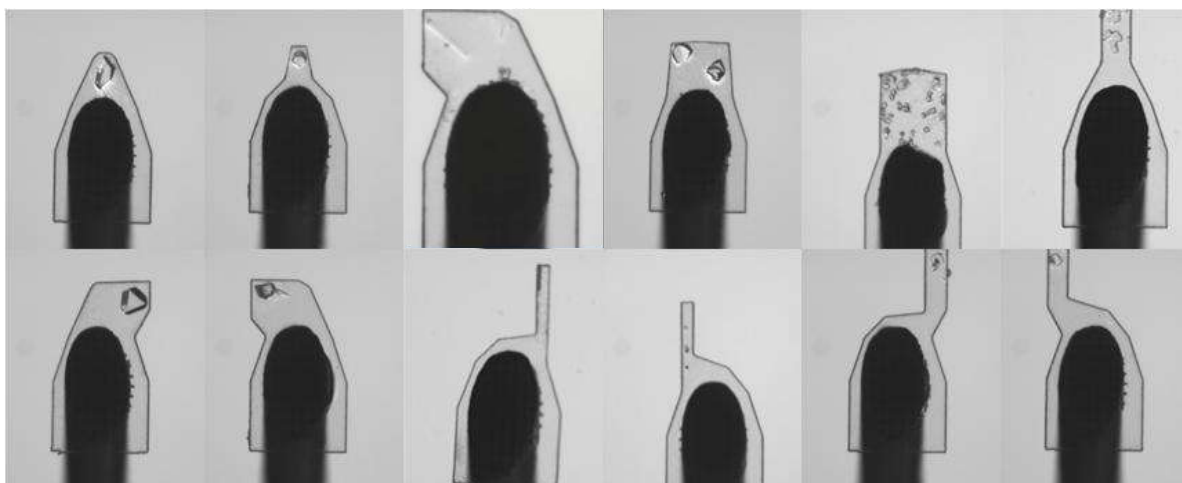
CrystalDirect® is a fully-automated system designed for high throughput crystal harvesting that fits perfectly to modern crystallization platforms: CrystalDirect® needs about 1 minute per harvest and reaches this performance with a success rate superior to 99 %.

Thought for highly automated workflow, CrystalDirect® is fully-compatible with the Flex ED sample changer robot, developed by the EMBL.

Flexible harvesting methods

CrystalDirect® offers a large amount of cut shapes adapted to different distributions and types of crystal: CrystalDirect® can select and harvest single crystals, batches of crystals or micro-crystals and single needles from clusters.

A list of pre-defined cutting shapes is available in the software and the user can also create his/her own cutting shapes



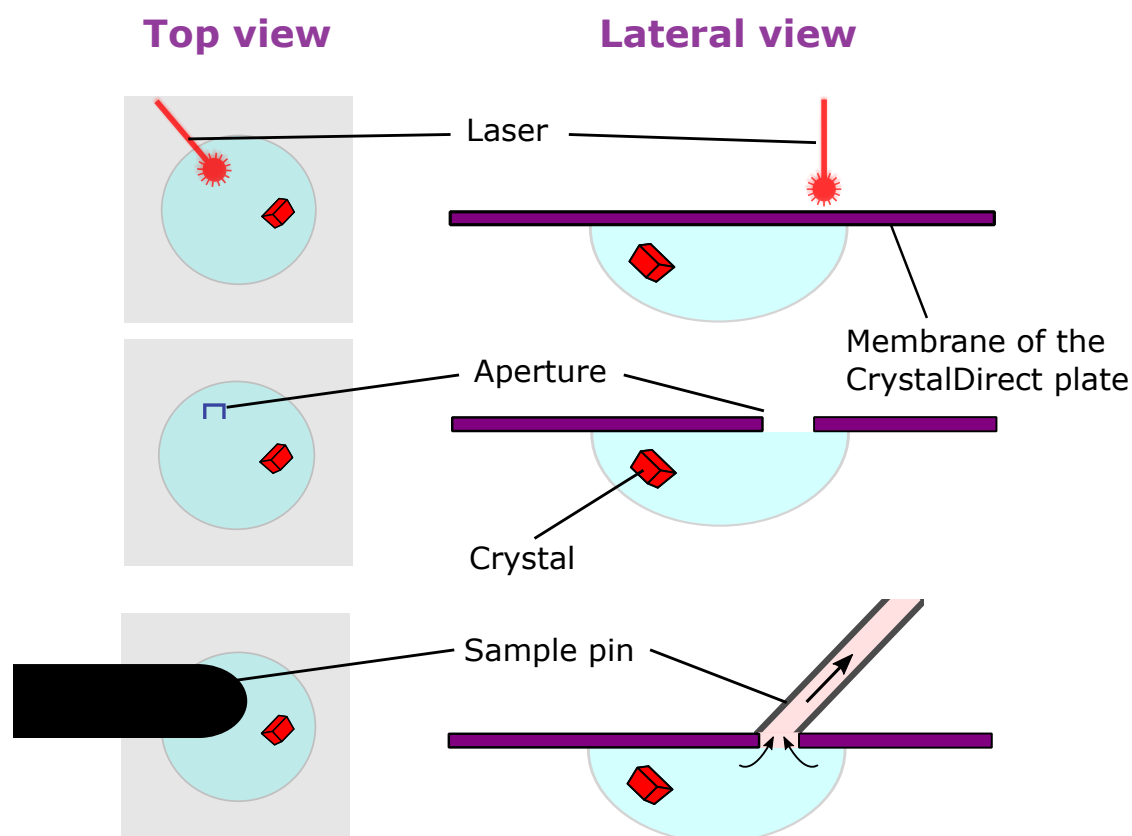
Example of cut shapes available with CrystalDirect® to harvest single crystals or batches of crystals.

Extracted from Zander, U., *et al.*, "Automated harvesting and processing of protein crystals through laser photoablation" *Acta Crystallographica Section D Structural Biology* 04/2016; 72(4)

Harvesting process

Step 01: Preparation of the crystal harvesting

The user selects a cutting shape which is adapted to the chosen crystals. Then, a first laser cut creates an aperture and CrystalDirect® removes the mother liquor surrounding the protein crystals.



This operation improves the laser cutting quality and according to Pellegrini *et al.*, 2012, risks of mechanical stress to the crystal(s) during the cryo-cooling process are strongly reduced. In addition, diffuse X-ray scattering from vitrified mother liquor is minimized.

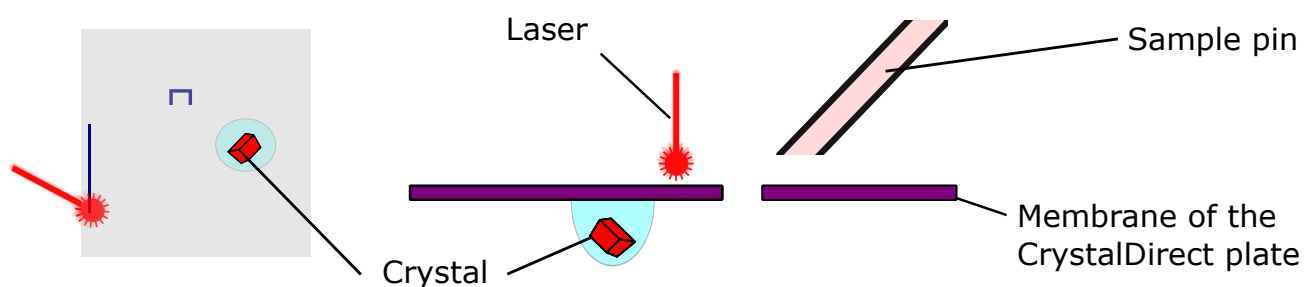
Illustrations extracted from Zander *et al.* Acta Cryst. (2016). D72, 454–466



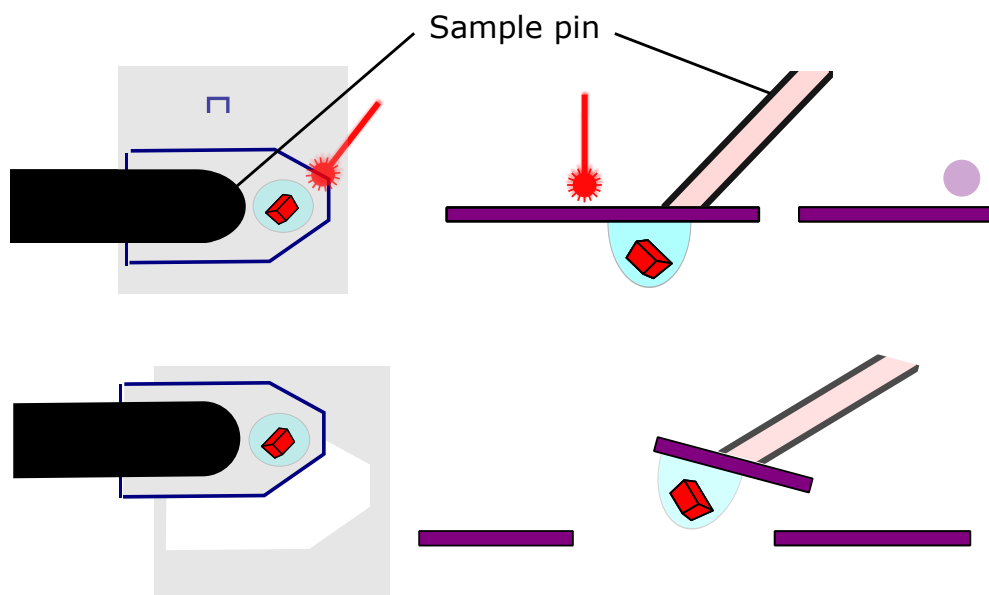
Harvesting process

Step 02: Crystal harvesting

Before gluing the SPINE compatible sample holder pin onto the membrane, a first cut (vertical cut in the image) is operated.



Then, CrystalDirect® cuts the membrane around selected crystals according to the chosen cutting shape.



After the laser cutting, the sample is harvested.

The sample holder pin, together with the harvested crystals on the cut-out piece of membrane, is immediately moved into a gaseous nitrogen stream with a temperature of 100 K.

Illustrations extracted from Zander *et al.* Acta Cryst. (2016). D72, 454–466.



Sample protection

Automatic cryo-protection

To applicate cryo-protectant, an additional step can be added to the harvesting process.

Following the laser cutting of step 01, a drop of cryo-protectant is placed on the aperture. The cryo-protectant will diffuse gradually into the mother liquor. In this way, osmotic shocks are reduced (Zander, *et al.* 2016).

The automatic addition of cryo-protectant during the process can be activated or deactivated.

Automatic cryo-cooling process

Once crystals are harvested from the CrystalDirect™ plate, the CrystalDirect® robot places them automatically into a cryostream (gaseous stream of 100 K cold nitrogen) which is located inside the CrystalDirect® machine.

The cryo-preserved crystals are waiting for transfer to an external storage container. The transfer can be executed by a sample changer robot or it may be done manually.

A video camera gives visual feedback on the quality of the cryo-cooling process.



Sample management

CRIMS - CRystallization Information Management System

CRIMS is a web application designed to take care about data management of the entire crystallization pipeline, giving web access and supporting functionalities for each step: sample preparation, crystallization screening, automated protein crystal harvesting within CrystalDirect® and X-ray data collection.

CrystalDirect® was developed by the EMBL to work with CRIMS to take full-advantage of CRIMS functions.



Sample preparation

Crystallization screening

**Automated crystal harvesting
(within CrystalDirect®)**

X-ray data collection




Sample management

Sample preparation

CRIMS records data related to the samples and the design of crystallization experiments (name, type, concentration and others).

It tracks all experimental parameters (crystallization conditions, temperature, etc.) for each well on every well plate. At any time, users can access to these data and modify them. CRIMS links these data with the next step, the crystallization screening.

Crystallization screening



The screenshot displays the CRIMS software interface. On the left, a grid represents a well plate with columns labeled A through L and rows numbered 1 through 12. A red dot is visible in well A1. The central area shows a large, dark, circular image of a crystal drop. Below this image is a 'Set Name' field with a dropdown menu and a button labeled '(You can use these buttons to score your drops)'. On the right, a 'Viewing Settings' panel is visible, containing options for 'Select imaging sequence' (with a dropdown menu showing 'Position 1', 'Position 2', and 'Position 3'), 'Show samples Side-to-Side', and checkboxes for 'Position 1', 'Position 2', 'Position 3', and 'Depth'. A 'Scoring Schema' legend is overlaid on the bottom left of the image, listing 10 categories with corresponding colored squares: 0 Clear drop (light blue), 1 denatured (dark blue), 2 Precipitate (medium blue), 3 Interesting (yellow), 4 Microcrystals (orange), 5 Clusters, needles (brown), Single crystal (red), * Interesting, put in Summary (pink), and X crystal pointing in Image (purple).

Scoring Schema

- 0 Clear drop
- 1 denatured
- 2 Precipitate
- 3 Interesting
- 4 Microcrystals
- 5 Clusters, needles
- Single crystal
- * Interesting, put in Summary
- X crystal pointing in Image

CRIMS can collect pictures generated by Crystal Farms (e.g. Rock imagers).

The lab' manager and expert users can inspect and score all of these pictures for each crystallization well. This "image viewing & scoring" function allows the expert user to analyze and annotate the results for each well.



Sample management

Automated crystal harvesting - within CrystalDirect®

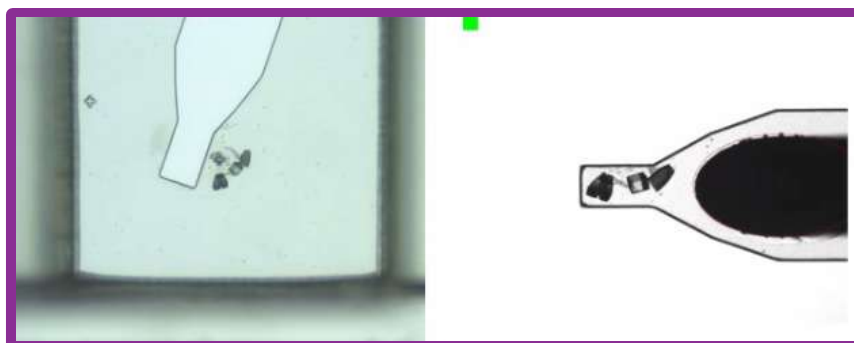
The EMBL has developed the automated protein crystal harvester CrystalDirect® to take full advantage of the features offered by CRIMS.

Based on the crystallization screening results and for each harvest, the expert user can:

- Select the crystals to be harvested
- Define the cutting shape and its orientation

Then, CRIMS transfers the information to CrystalDirect® which will perform the harvest as set. It cryo-preserves the harvested crystals, takes pictures of them and transfers these images to CRIMS.

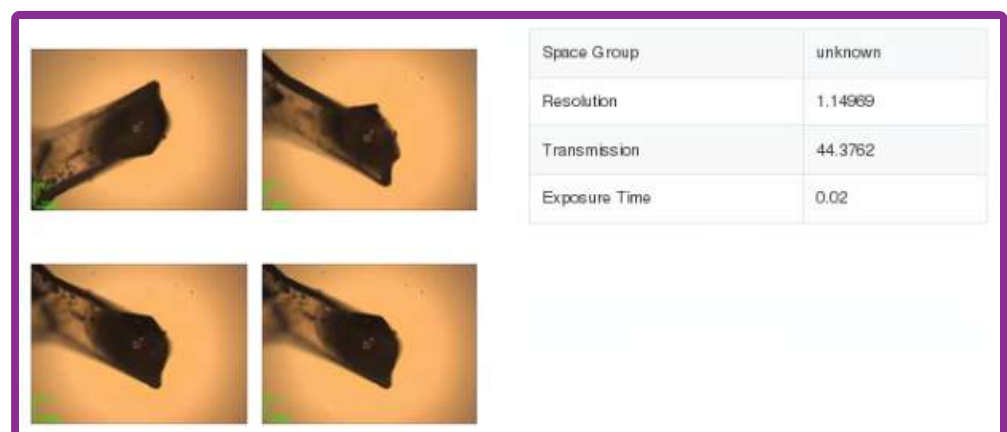
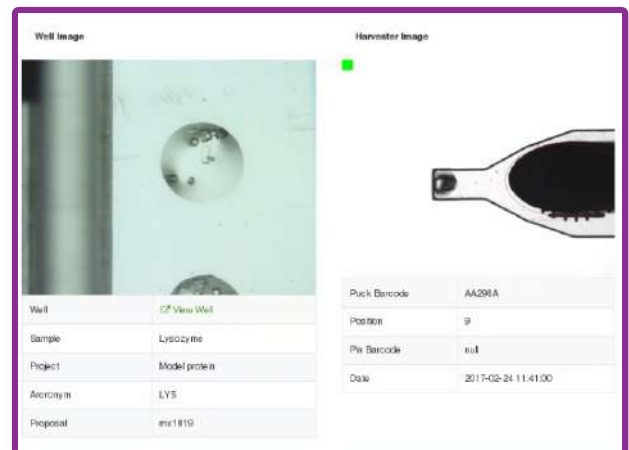
The expert user can check these images by means of CRIMS and hence, gets visual feedback on the success of the harvesting process and the final sample quality.



Sample management

X-ray data collection

Once the collection of diffraction data is finished, CRIMS retrieves a summary of the data quality from the synchrotron's diffraction database, e.g. ISPyB (ESRF Synchrotron database, France).



Screenshot from CRIMS showing summary of data quality from the synchrotron's diffraction database.

Technical Specifications

Performances

Harvesting time
Success rate

~1 harvest/min
>99 %

Laser cutting

No heat transfer
Micrometric precision
Multiple cut profiles
(pre- & user-defined)

Crystal soaking process

500 µL reservoirs
film piercing: photo ablation

Crystals Support

CrystalDirect plates

96-well vapor diffusion plate
Compatible with standard
equipment

Software

User interface
Web based crystal selection
Multi-device servers
Sample changer robot

Windows® / JAVA®
CRIMS, customizable
TINE, TANGO, EPICS
Robot hardware & software
integration

Power

230 VAC; 50 Hz
110 VAC; 60 Hz

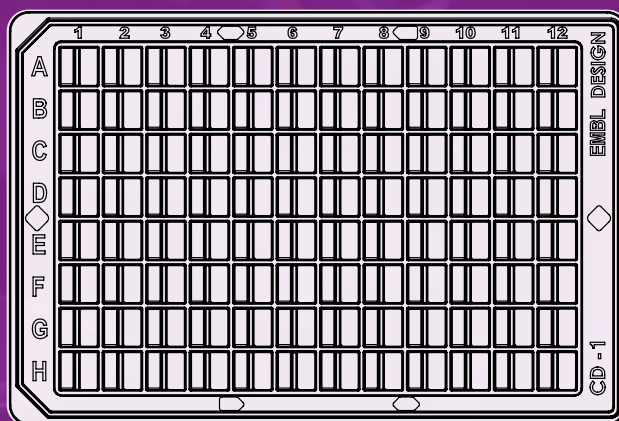
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Dimensions (W,D,H)

1.0 x 1.0 x 2.3 m³

CrystalDirect™ crystallization plates:

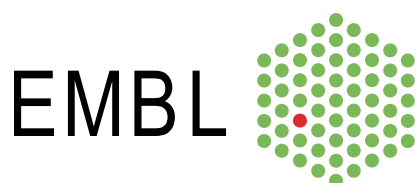
- 96-well standard SBS format
- Up to 3 crystallization drop per well
- 45µl of well solution per reservoir
- Ultrathin growth support (25 µm and thinner)
- Minimal UV background
- Minimal X-ray background



References



CrystalDirect® is a development of the European Molecular Biology Laboratory (EMBL).



The patented CrystalDirect™ plates are also an EMBL development. They are distributed by MiTeGen.

Scientific Publications:

Zander *et al.*, Acta Cryst. (2016). D72, 454–466, "Automated harvesting and processing of protein crystals through laser photoablation."

Cipriani *et al.*, Acta Cryst. (2012). D68, 1393-1399 "CrystalDirect: a new method for automated crystal harvesting based on laser-induced photoablation of thin films"

Pellegrini *et al.*, Acta Cryst. (2012). D67, 902-906 "Direct cryocooling of naked crystals: are cryoprotection agents always necessary?"

Video presentation of CrystalDirect® working with Flex ED sample changer robot in a high throughput environment

See CrystalDirect®
Video





Advanced Research Instrumentation for Neutrons & X-rays

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