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.

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.

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

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.

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.

Sample protection

**Automatic cryo-protection**

To apply 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

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

<table>
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<th>Performances</th>
<th>Harvesting time</th>
<th>~1 harvest/min</th>
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<tr>
<td></td>
<td>Success rate</td>
<td>&gt;99 %</td>
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<tr>
<td></td>
<td>Laser cutting</td>
<td>No heat transfer</td>
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<td>Micrometric precision</td>
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<td>Multiple cut profiles</td>
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<td></td>
<td>Crystal soaking process</td>
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<td>film piercing: photo ablation</td>
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<td>Crystals Support</td>
<td>CrystalDirect plates</td>
<td>96-well vapor diffusion plate</td>
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<tr>
<td>Software</td>
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<td></td>
<td>Web based crystal selection</td>
<td>CRIMS, customizable</td>
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<tr>
<td></td>
<td>Multi-device servers</td>
<td>TINE, TANGO, EPICS</td>
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<tr>
<td></td>
<td>Sample changer robot</td>
<td>Robot hardware &amp; software integration</td>
</tr>
<tr>
<td>Power</td>
<td>230 VAC; 50 Hz</td>
<td>&lt;3680 W</td>
</tr>
<tr>
<td></td>
<td>110 VAC; 60 Hz</td>
<td>&lt;3680 W</td>
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<tr>
<td>Dimensions (W,D,H)</td>
<td>1.0 x 1.0 x 2.3 m³</td>
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**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
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:


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
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