Applications

Below, examples are shown for intrinsic fluorescence imaging in crystallization plates of different types illuminated with white light and UV (blue)

_needle shaped crystals of a ribosome inactivating protein type I as a hanging drop on a siliconized coverslip

Needle shaped crystals of a ribosome inactivating protein Type I. XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

_needles of salt crystals in a NEXTAL QIA1 µplate (QIAGEN, Canada Inc.) Lot No. 2181002077, covered with standard sealing film. XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.
Needles of salt crystals in a NEXTAL QIA1 µplate (QIAGEN, Canada Inc.) Lot No. 2181002077, covered with standard sealing film. XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

Crystals of lysozyme as a hanging drop on a siliconized coverslip in a Linbro Plate (Hampton Research, HR3-110). XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

Needle shaped crystals of a protein-RNA complex among oily phase separation precipitate in a hanging drop. The drop hangs on a siliconized coverslip in a Linbro Plate (Hampton Research, HR3-110). XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

Crystals of the dihydrofolat reductase in a 96 Well, CrystalQuick COC plate (greiner bio-one, 609820). XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

A salt crystal in a NEXTAL QIA1 µplate (QIAGEN, Canada Inc.) Lot No. 2181002077, covered with standard sealing film. XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

A salt crystal in a NEXTAL QIA1 µplate (QIAGEN, Canada Inc.) Lot No. 2181002077, covered with standard sealing film. XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

Crystals of a ribosome inactivating protein type I, together with salt crystals in a hanging drop. The drop hangs on a siliconized coverslip in a Linbro Plate (Hampton Research, HR3-110). XtalLight was combined with an imaging system equipped with a with 1280 x 1024 DP-10 Digital Camera (Olympus).

A Crystal of a unknown protein, in a hanging drop. The drop hangs on a
siliconized coverslip in a Linbro Plate (Hampton Research, HR3-110). XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

Salt crystals among heavy precipitated protein and oily phase separation. The intrinsic fluorescence of the protein crystals is significantly more intense than the fluorescence light of the precipitant. The sample was in a 96 Well, CrystalQuick COC plate (greiner bio-one, 609820).

Crystals of an unknown protein. The sample was in a 96 Well, CrystalQuick COC plate (greiner bio-one, 609820) sealed with a standard sealing film. XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

Crystals of a ribosome inactivating protein type I. The sample was in a 96 Well, CrystalQuick COC plate (greiner bio-one, 609820) sealed with a standard sealing film. XtalLight was combined with an imaging system equipped with a CCD-video camera resolution: 1360 x 1036 pixel.

**Physical background**

![Mercury vapor lamp emission spectrum](image)
XtalLight 100 uses a filtered mercury arc lamp emission spectrum for intrinsic protein fluorescence excitation. Tryptophan fluorescence excitation is most efficient at 280 nm wavelength. The other aromatic aminoacids, tyrosine, phenylalanine and histidine, could only be excited at shorter wavelengths. Therefore they are of minor importance for in situ intrinsic fluorescence crystal detection (Figure 2).

The opacity of glas coverslips and sealing films reduce the light intensity significantly (Figure 3). However, the characteristics of a filtered mercury arc lamp spectrum compensates the weak opacity and
is still sufficient to excite tryptophan fluorescence.

Tryptophan fluorescence is shown in three images of the same glucose isomerase crystal as sitting drop in a 96 Well, CrystalQuick COC plate (greiner bio-one, 609820). All three images were taken using the same exposure time and light sensitivity. On top, the crystal was covered with a quartz cover slip (suprasil). The relative excitation spectrum intensity was calculated for three different proteins (product of the transmission spectrum with the specific molar absorption of the protein) shown on the left side. In the middle the crystal was covered with a standard polymeric film and below with a common glass cover slip. The opacity for wavelengths below 300 nm is significantly reduced when the crystal is covered by glass. However, the appearance of the intrinsic fluorescence seems almost identical, when illuminated with a filtered mercury arc lamp spectrum.
# Technical Data

**UV light source**
- Mercury arc lamp with 120 W
- ✔ Lamp life span > 2000 h
- ✔ Motorised shutter and intensity control

**Filter**
- Motorised filter change up to three positions:
  - ✔ Pos 1: Shortpass 385 nm
  - ☐ Pos 2: (optional) Shortpass 325 nm
  - ☐ Pos 3: (optional) Filter of choice

**Control**
- Control of UV light intensity, filter setting and shutter
  - ✔ Manually
  - ✔ Software control from PC over ethernet XtalLight 100(C) remote software runs on
    - ☐ Linux
    - ☐ Windows
    - ☐ MAC

**Light guide**
- Light guide for UV light 1.5 mm core diameter
  - ✔ Length 1.5 m
  - ☐ Customized length (optional)

**UV light optics**
- Focusing optics for directing UV light onto the sample
  - ✔ Focal length 20 mm built-in blocking filter
<table>
<thead>
<tr>
<th><strong>Hardware</strong></th>
<th>Table-top case</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔ Portable unit</td>
<td></td>
</tr>
<tr>
<td>✔ 400 mm x 300 mm x 200 mm (LxWxH)</td>
<td></td>
</tr>
<tr>
<td>✔ Weight: approx. 12 kg</td>
<td></td>
</tr>
<tr>
<td>✔ Power consumption: 90 to 264 V, 200 W</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Imaging package</strong></th>
<th>Computer</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔ Mini PC attached to Monitor</td>
<td></td>
</tr>
<tr>
<td>✔ Monitor 22 inch for full camera image display</td>
<td></td>
</tr>
<tr>
<td>✔ Operation system: Linux</td>
<td></td>
</tr>
</tbody>
</table>

**Imaging SW**

- ✔ Live display of camera image
- ✔ Control of camera settings for UV and coloured light
- ✔ Easy acquisition of UV images and combinations
- ✔ Storage and retrieval of images in a database
- ✔ Short UV exposure times to protect crystals against damage

| **Positioning and protection** | ✔ Manual Stage for positioning of optics |
Manual Stage for positioning of UV protection shield (optional)

**Adaptable Microscopes**
Adaptable to several microscopes depending on working distance and set-up

**Suitable plates and sealing films**
Crystallization plates with low intrinsic fluorescence (low birefringence) and UV suitable sealing films

---

**References**

**Karsten Dierks, Arne Meyer, Dominik Oberthür, Gert Rapp, Howard Einspahr, and Christian Betzel**

Efficient UV detection of protein crystals enabled by fluorescence excitation at wavelengths longer than 300 nm


**Arne Meyer, Christian Betzel and Marc Pusey**

Latest methods of fluorescence-based protein crystal identification

Download

Flyer_XtalLight100_100C.pdf (1.4 MiB)