XtalLight 100C
XtalLight 100C is a combined UV-light and a green light source (100 nm and 20 nm spectral width respectively). The UV-light source is designed for intrinsic protein fluorescence imaging. The green light is predominantly used for trace fluorescence imaging when the protein was covalently labeled with the fluorophore carboxyrhodamine.

**Applications**

A single 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.

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Lysozyme crystals covalently stained with Atto 550 Protein Labelling Kit prior to crystallisation. The crystals were illuminated with 525 nm for fluorescence imaging.

Thaumatin crystal soaked in an Atto 550 Protein Labelling solution and transferred to mother liquor. The crystals were illuminated with 525 nm for fluorescence imaging.

Thaumatin crystals unstained illuminated with a UV-spectrum from 280 to 380 nm and with 525 nm. Intrinsic fluorescence of tryptophan indicated a protein crystal.

Physical background
Intrinsic Fluorescence Imaging

XtalLight 100C 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 glass coverslips and sealing films reduce the light intensity significantly (Figure 3). However, the characteristics of a filtered mercury arc lamp spectrum compensate 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.

**Trace Fluorescence Imaging**

Covalently fluorescence labeled proteins with the fluorophore carboxyrhodamine: Absorption and emission maxima: $\lambda_{\text{ex}}$ 522 nm, $\lambda_{\text{em}}$ 550 nm. The colored light source of XtalLight100C provides a green light spectrum (green curve) and the emission maximum and the absorption peak of carboxyrhodamine match exactly.

However part of the green light spectrum overlays with the
fluorescence spectrum of carboxyrhodamine, therefore a bandpass filter 525 nm with 20 nm spectral width is used for excitation and a 550 nm longpass filter is used to separate the fluorescence light.

Summarized spectra of carboxyrhodamine emission and excitation and the applied optical filters.

Carboxyrhodamine stained crystals with a 0.5 to 2 % labeling efficiency excite with 515 – 535 nm spectral width, exposure time: 1.2 s.
Technical Data

**UV light source**

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

**Green light source**

- **Green LED 525 nm with 150 LM at 700 mA**
  - ✔ LED life span 50,000 h
  - ✔ Motorised intensity control
  - ✔ Optional other wavelengths are available

**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/green LED intensity, filter setting and shutter**
  - ✔ Manually
  - ✔ Software control from PC over ethernet (Windows, Linux, Mac)
  - ☐ Open interfacing to third party software

**Light guides**

- **Light guide for UV light 1.5 mm**
Light guide for **green** light 1.5 mm diameter

✅ Length 1.5 m

☐ Customized length.................

**Dimensions**

Table-top case

✅ Portable unit

✅ 400 mm x 300 mm x 200 mm (LxWxH)

✅ Weight: approx. 12 kg

✅ Power consumption: 90 to 264 V, 200 W

**Software**

XtalLight 100C remote software runs on Linux, Windows and Mac

✅ No installation required, runs from removable USB-Source

✅ Control of light source parameters

**UV optics**

Focusing optics for directing excitation light onto the sample

✅ Focal length 20 mm with built-in blocking filter

☐ Focal length ______mm

**Stage**

☐ Manual stage
**Imaging package**

✓ CCD Camera for adaptation to a microscope or to a stage

☐ 1024x768 pixels colour

☐ 1280x1024 pixels colour

**Computer**

✓ Combined Mini PC and 22 inch Monitor for full camera image display

✓ Linux

**Imaging SW**

✓ Live display of camera image

✓ Control of camera settings for UV and white light

✓ Easy acquisition of fluorescence light images and combinations

✓ Storage and retrieval of images in a data base

✓ Short UV exposure times to protect crystals against damage

**Optional Plexiglas UV-Shielding**

☐ Plexiglas® UV 100, 200 x 300 x 3 mm (w, h, t), UV – transmission 0.3% (DIN EN 410)

**Adaptable Microscopes**

Adaptable to several microscopes depending on working distance and set-up
Efficient UV detection of protein crystals enabled by fluorescence excitation at wavelengths longer than 300 nm

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


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