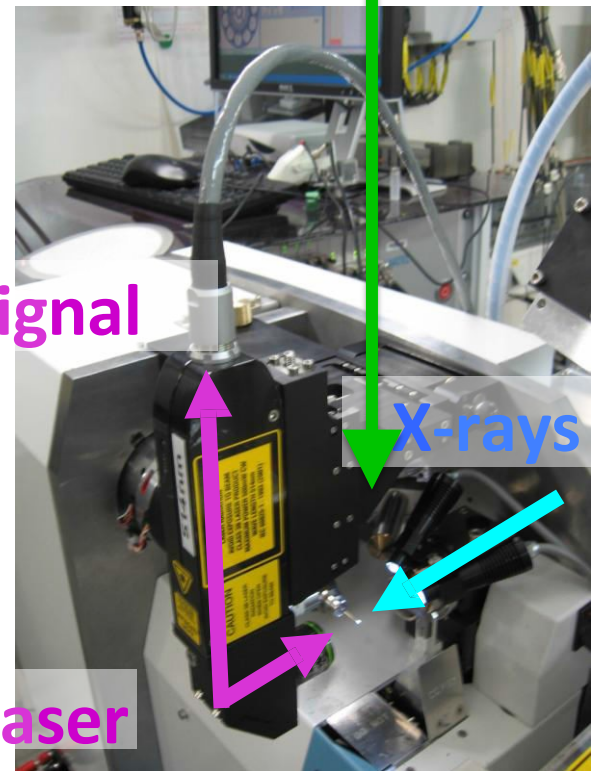
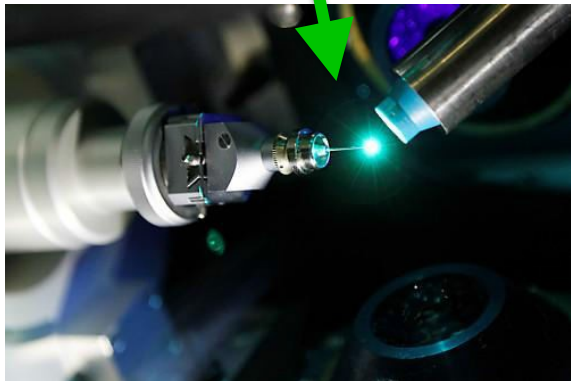
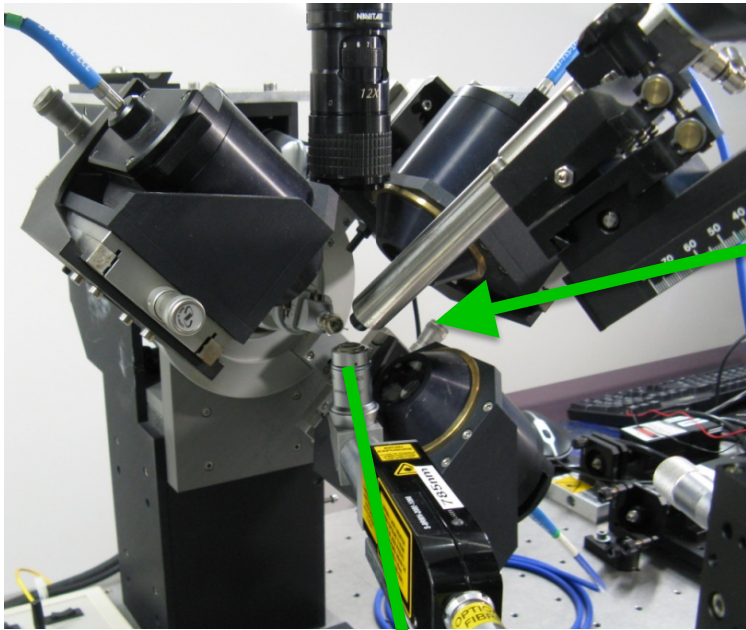
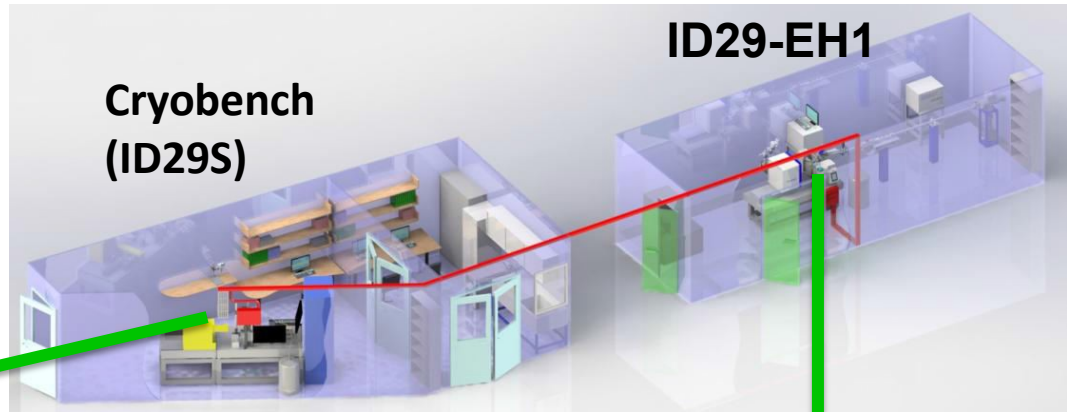


ID29S-Cryobench News

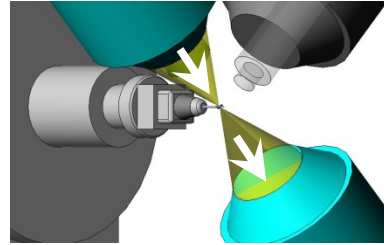
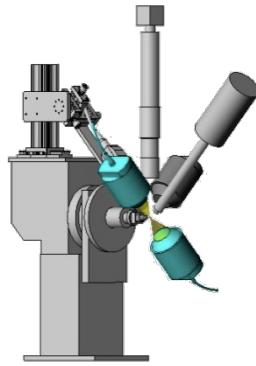
MX BAG Meeting, February 6th 2017

ID29S/ID29

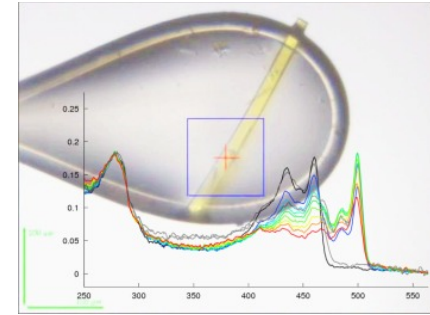


Different modes of operation

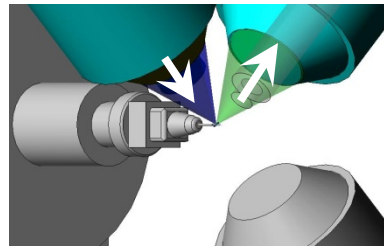
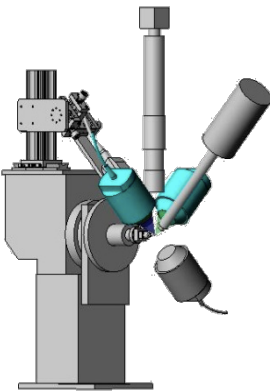
Absorption mode



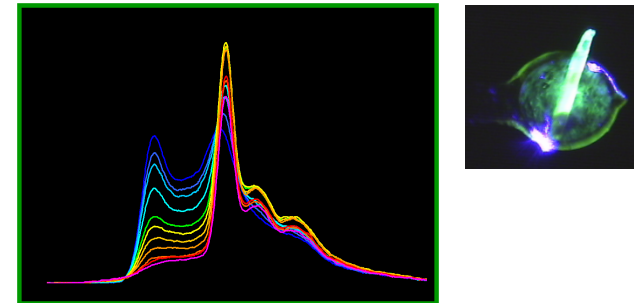
Transmission geometry (0°)



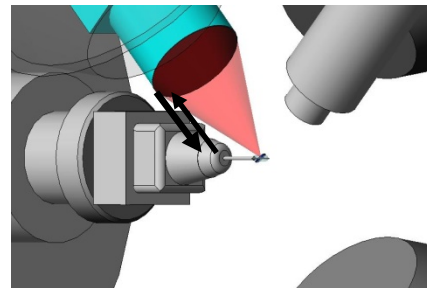
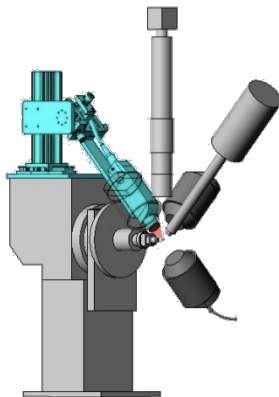
Fluorescence mode



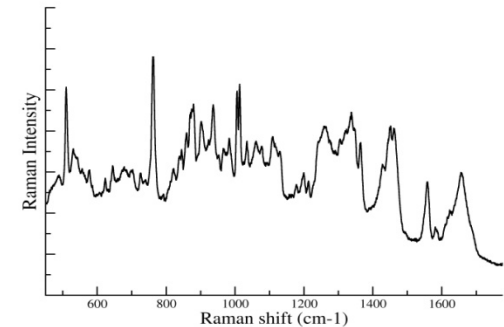
Reflection geometry (90°)



Raman mode

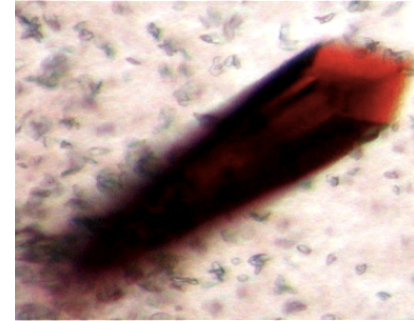


Back-scattering geometry (180°)

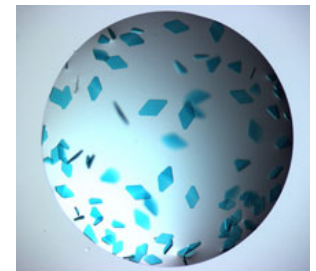
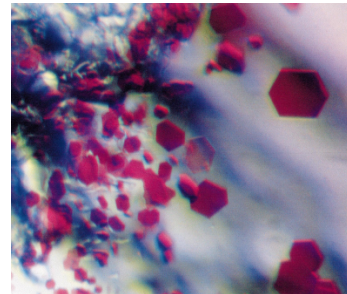
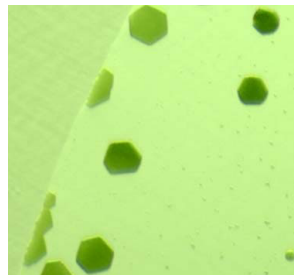


Samples

- Metal centers (redox state)



- Light-absorbing cofactors (chromophores)



- Bonds involving heavy atoms: disulfide, C-Br, Fe-O (potentially non-coloured)

Applications

Why performing optical spectroscopy experiments on crystals?

(1) To determine the **functional state** of the crystalline protein

(2) To evaluate the extent of **radiation damage** effects

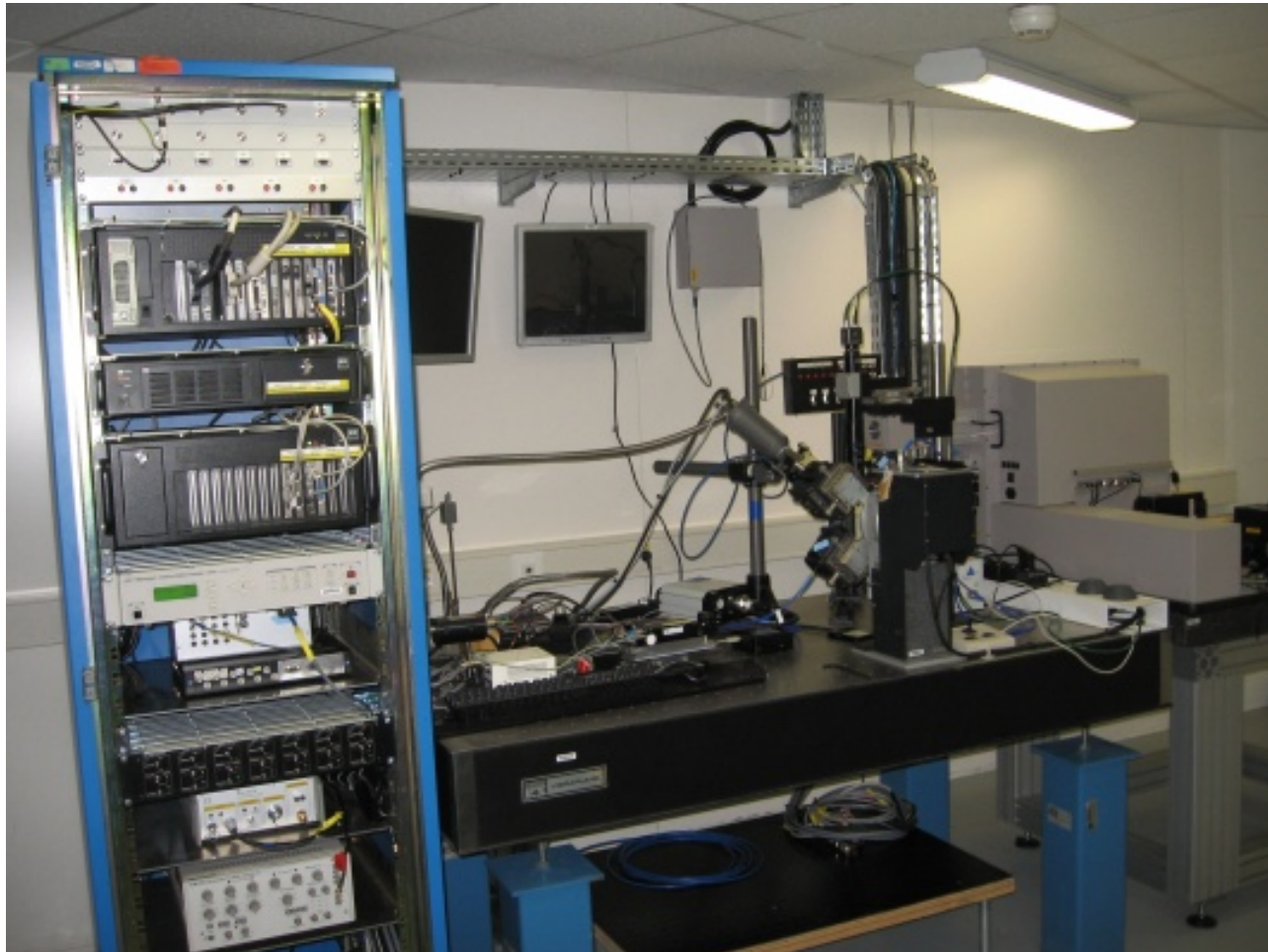
(3) To perform **kinetic crystallography** experiments (Structure determination of unstable species in time or dose)

When and where?

Before or after the diffraction experiment: **Offline setup** (ID29S)

During the diffraction experiment: **Online setup** (ID29, ID30A-3, BM30A)

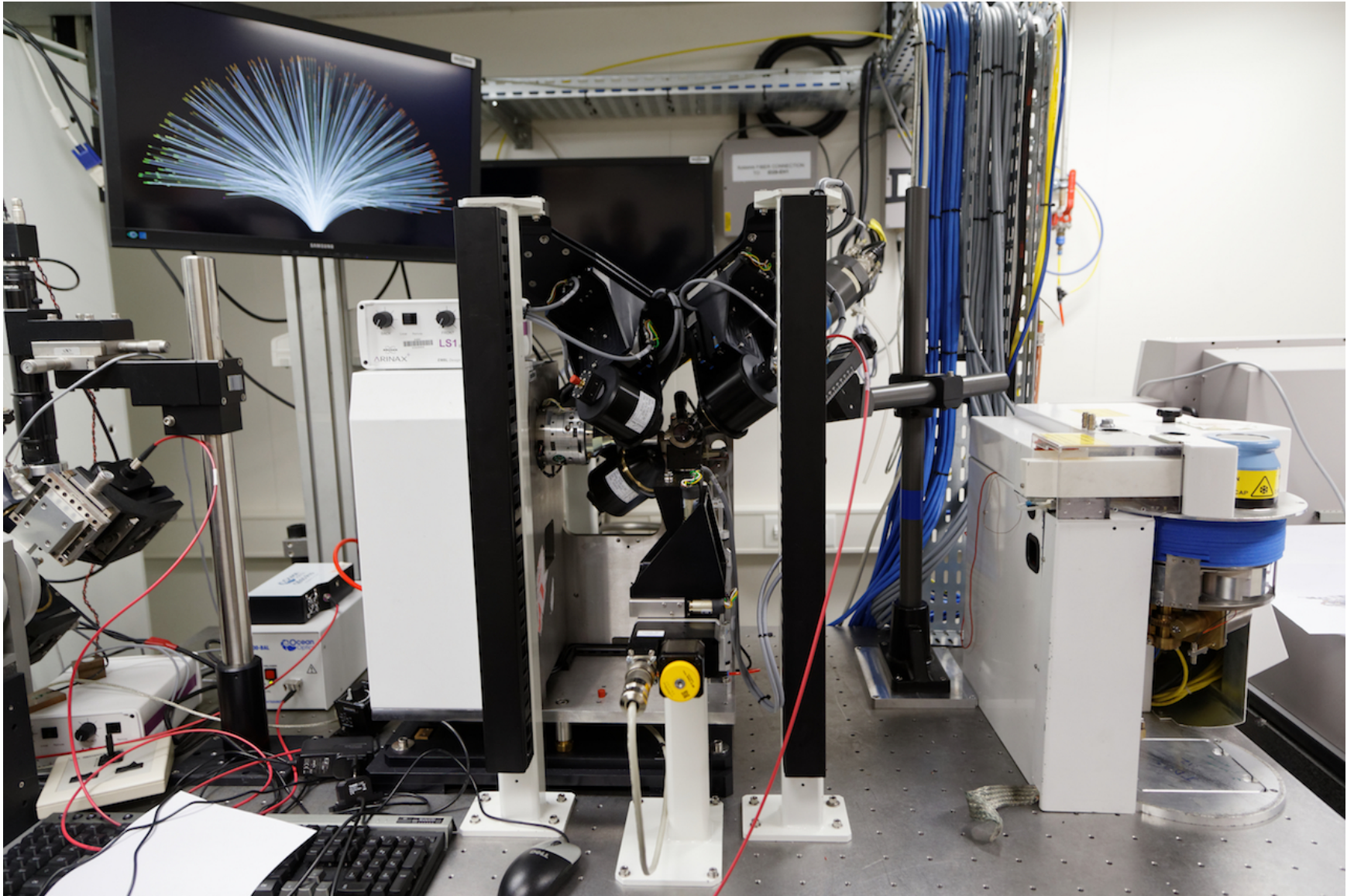
ID29S: old Cryobench



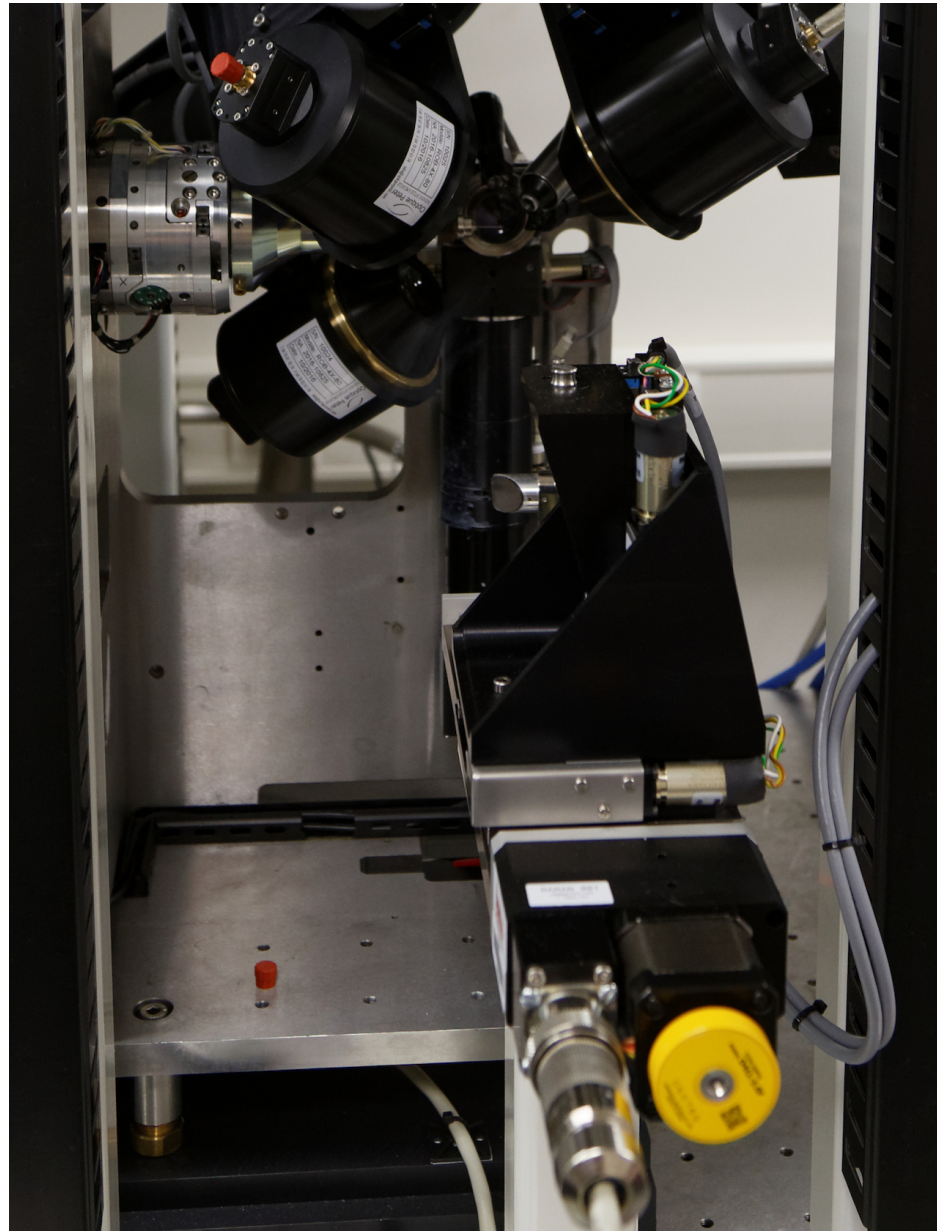
ID29S: new Cryobench



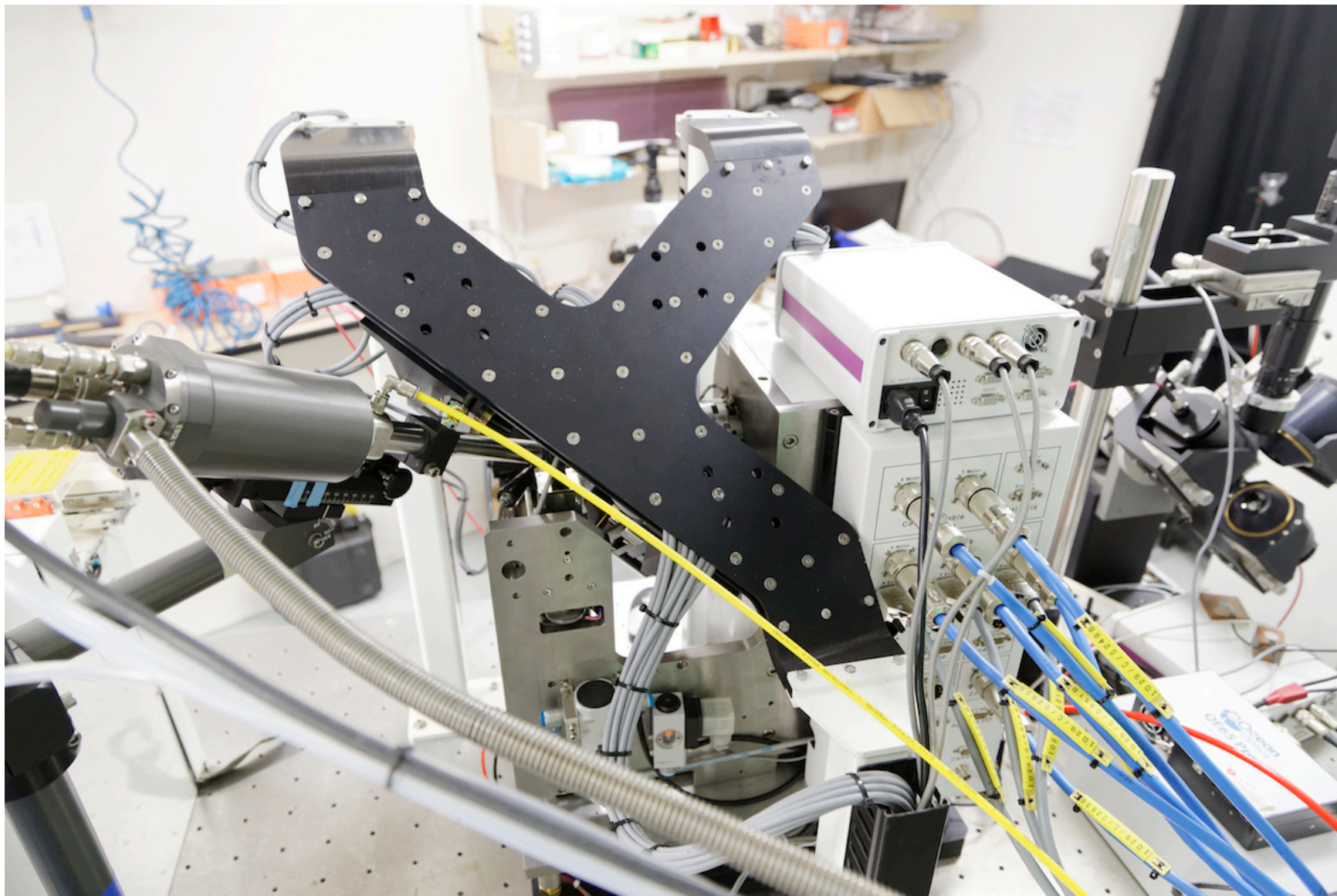
ID29S: new Cryobench



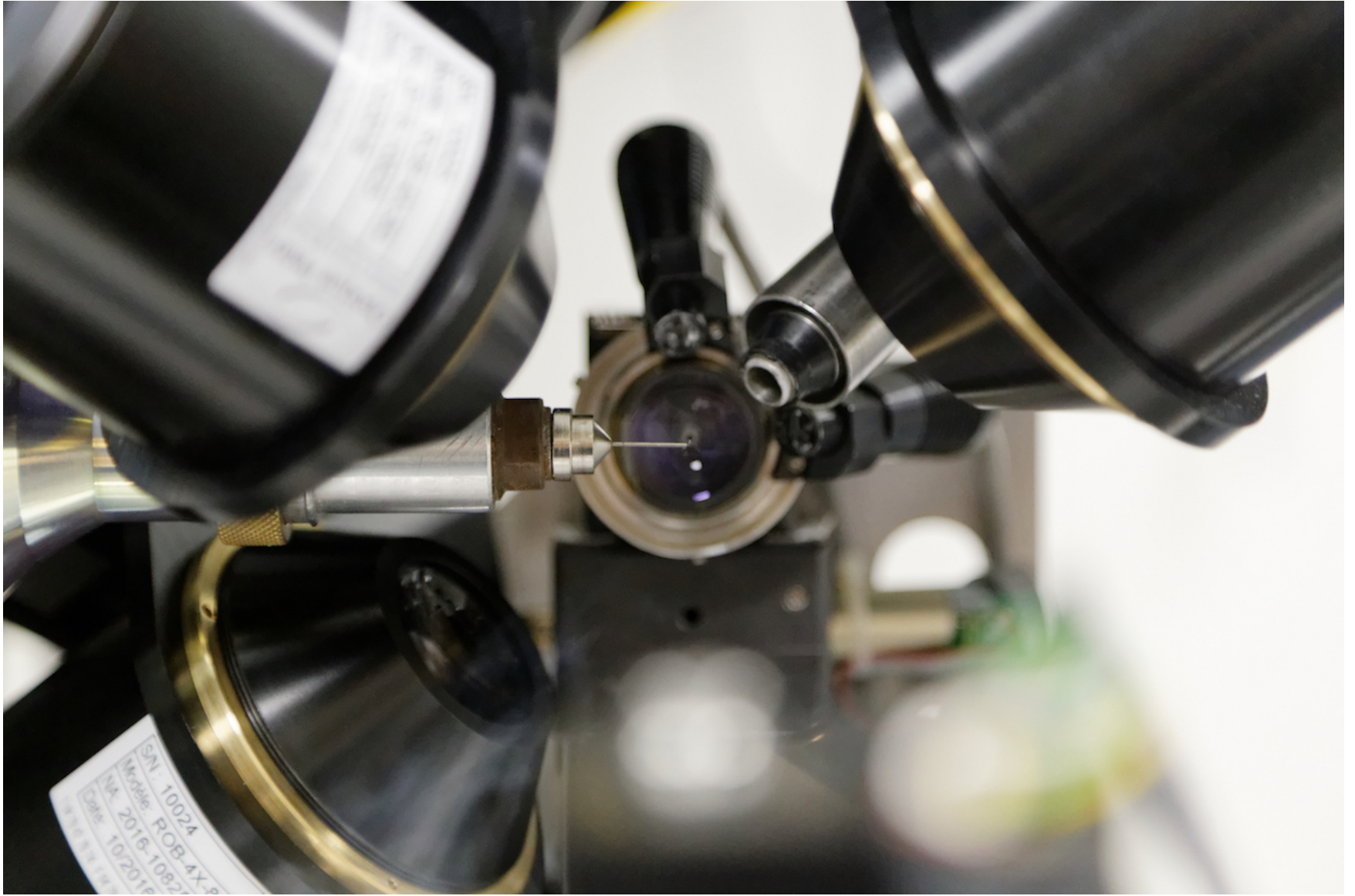
On-axis Raman



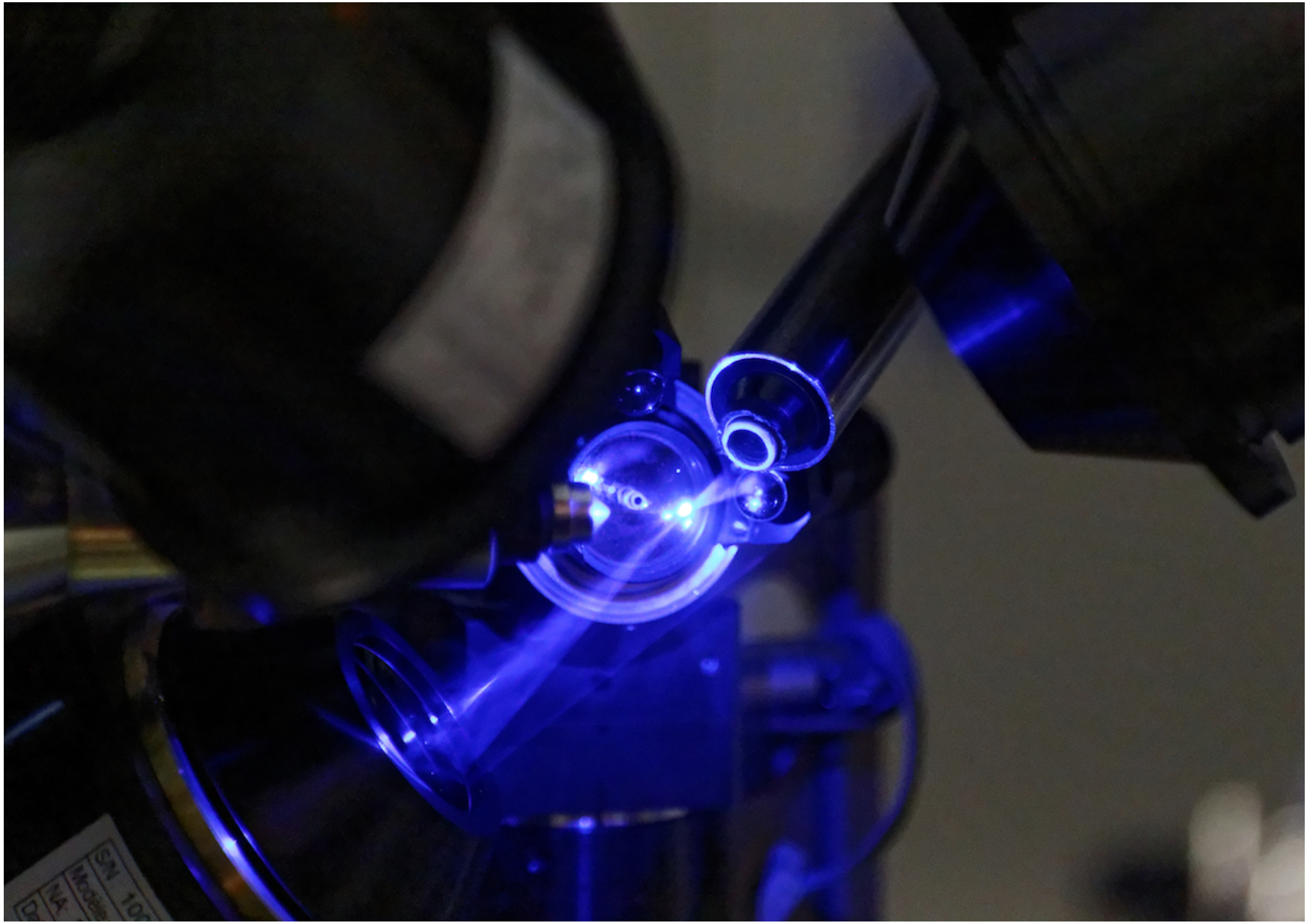
Holding the three objectives



Sample environment



Light path



Cryobench-related equipment

- **UV-vis abs / fluo microspec**

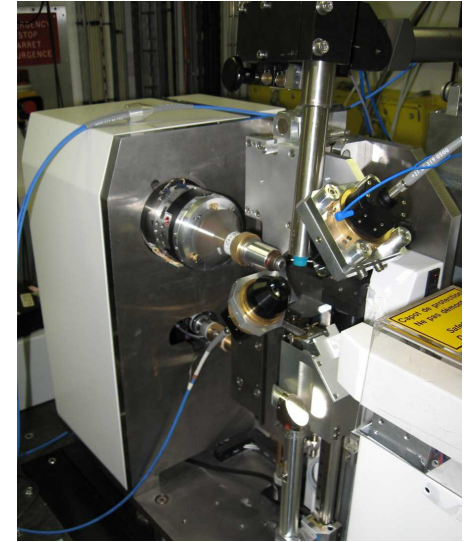
- Permanently mounted on MASSIF3 (1E+13 ph/s - 15 um diameter beam)

- Can be mounted on FIP (1E+11 ph/s – 300 um square top-hat beam)

**New microspec in preparation
(David von Stetten)**

- **Online Raman on ID29**

- Identify suitable radiation damage project



Experiment scheduling/declaration

- Contact by email: antoine.royant@esrf.fr
- Experiment scheduling (BAG / IHR)
- A-form declaration

We will use the following equipment and consumables:

<input type="checkbox"/> Automatic Sample Changer		
<input type="checkbox"/> Mini-kappa goniometer		
<input type="checkbox"/> Laser	If checked, specify Class <input type="text"/>	and its wavelength <input type="text"/>
<input type="checkbox"/> Dehydration Device		
<input type="checkbox"/> HPLC set-up (BioSAXS only)		
<input type="checkbox"/> Pressurised cell	<input type="radio"/> Krypton <input type="radio"/> Xenon <input type="radio"/> Other (specify) <input type="text"/>	
<input type="checkbox"/> Propane		
<input type="checkbox"/> Dry Ice	If checked, specify when <input type="text"/>	and weight <input type="text"/>

We need support from:

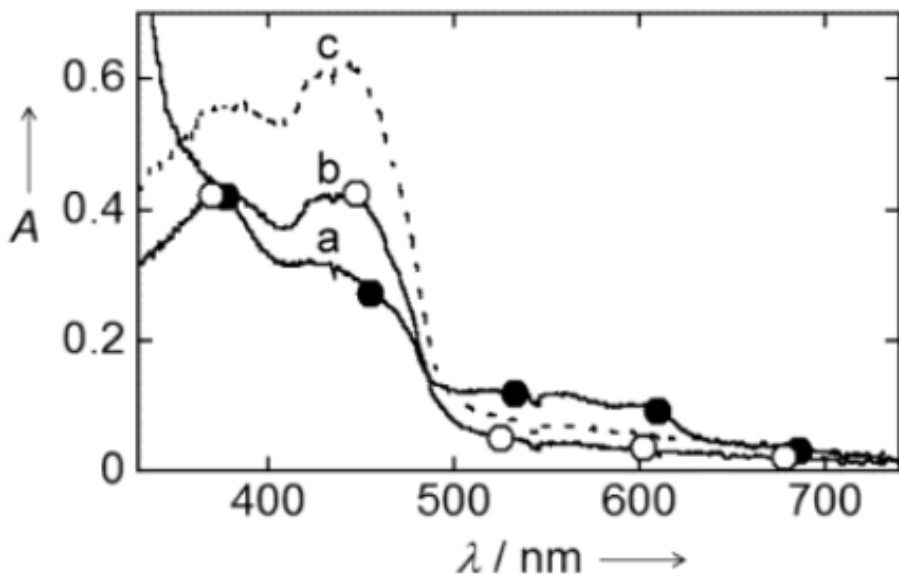
<input type="checkbox"/> AFM Platform (PSCM Labs in Science Building)	The AFM Platform IS NOW PART of the PSCM Labs: if you wish to use the AFM services please complete and send the <i>PSCM REQUEST FORM</i> to: pscm-support@esrf.fr
<input type="checkbox"/> EMBL Lab	If you wish to use EMBL facility you must send <i>THIS FORM</i> back one month before your needs. Please return the form by email to lab_support@embl.fr and to expsaf@esrf.fr .
<input type="checkbox"/> PSCM Labs (Science Building)	If you wish to use the PSCM support facilities and have not yet contacted the lab managers you should contact them at your earliest convenience by emailing the <i>PSCM REQUEST FORM</i> to pscm-support@esrf.fr . Further information about the Instruments offered can be found HERE . The PSCM facilities you need may already be assigned to other users at this late stage.

2016 scientific results (1)

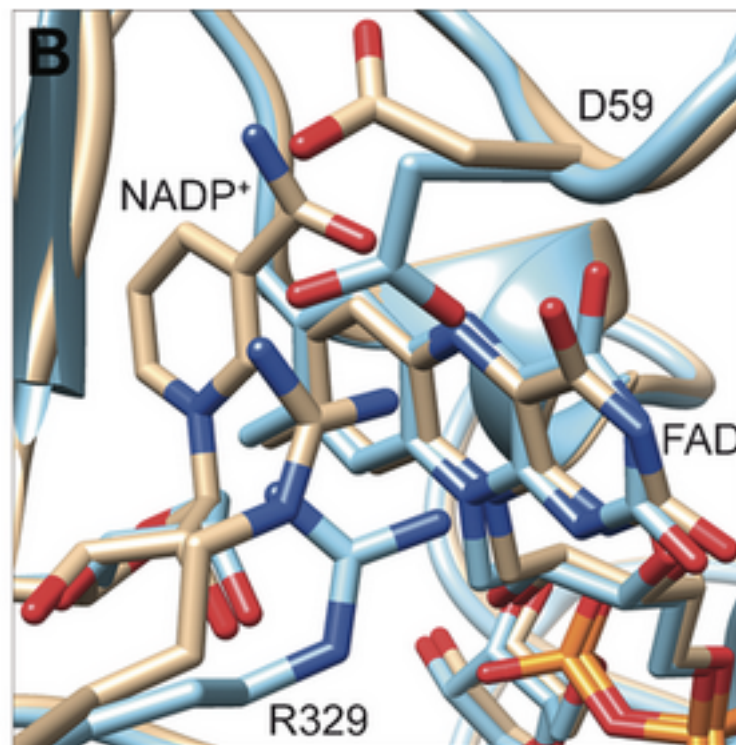
- Romero *et al.* *Angewandte Chemie* (2016)

Characterization and Crystal Structure of a Robust Cyclohexanone Monooxygenase

Offline UV-Vis Abs



Functional study: Red/Ox structural comparison



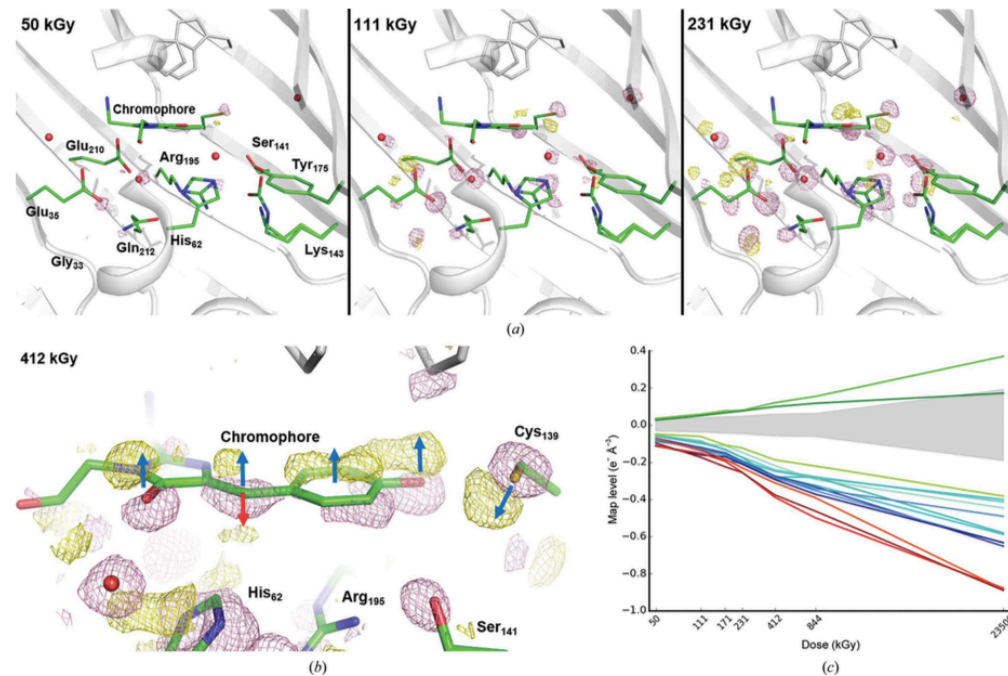
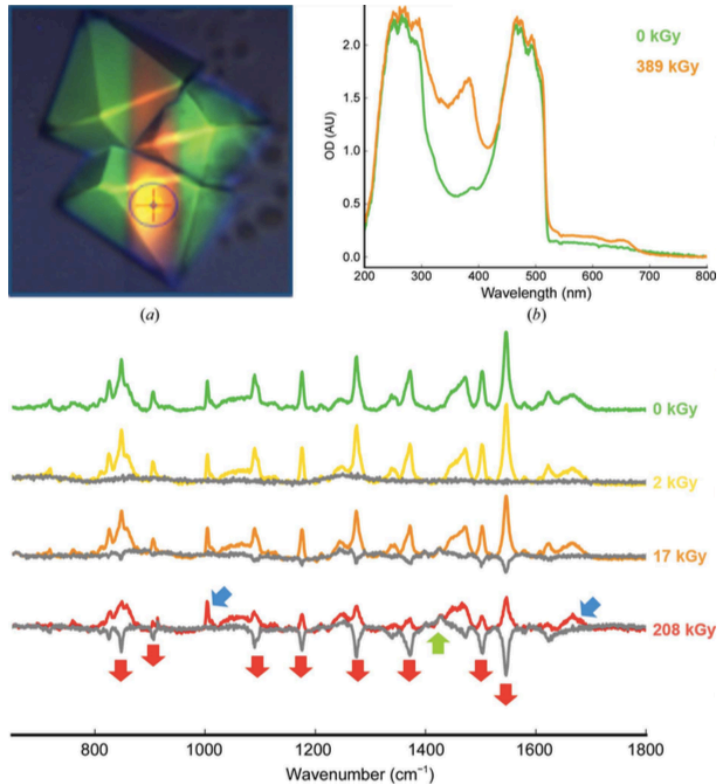
2016 scientific results (2)

- Clavel *et al.* *Acta Crystallographica D* (2016)

Structural analysis of the bright monomeric yellow-green fluorescent protein mNeonGreen obtained by directed evolution

Online Raman + Offline UV-Vis Abs

Radiation damage study

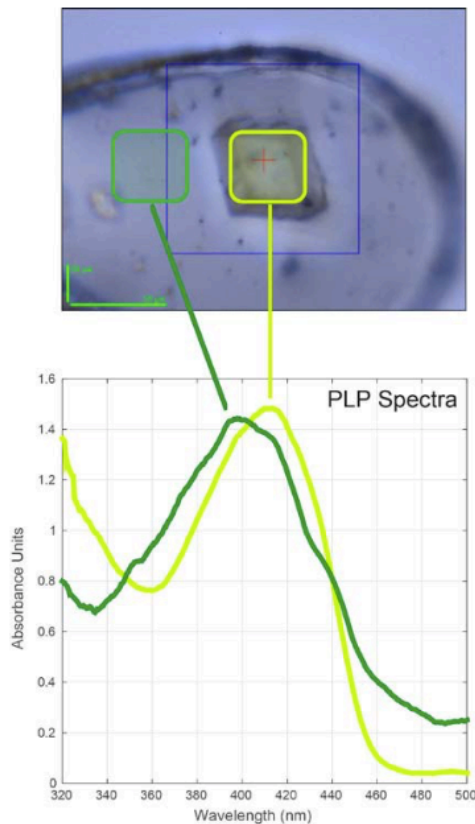


2016 scientific results (3)

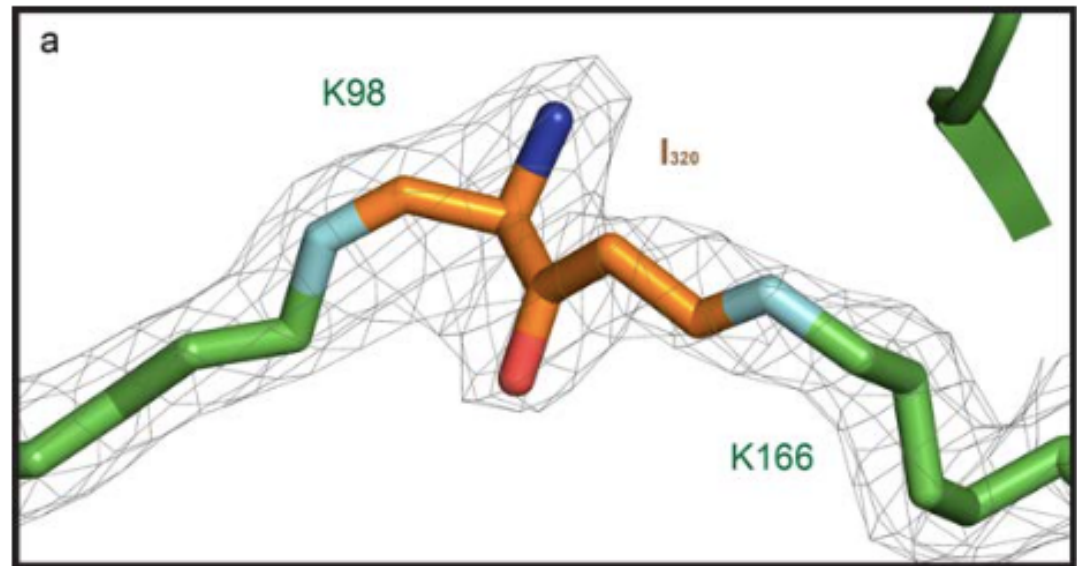
- Rodrigues *et al. Nature Chemical Biology* (2017)

Lysine relay mechanism coordinates intermediate transfer in vitamin B6 biosynthesis

Online + Offline UV-Vis Abs



Functional and radiation damage study:
Presence of the X-ray sensitive intermediate species



Future developments

- Sample changer – end of 2017
- Microsec / millisec UV-vis abs spectroscopy

Acknowledgements

All users

The whole Structural Biology Group, in particular:

- For instrumental support
 - Thierry Giraud
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 - Alexis van der Kleij
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 - David von Stetten
 - Daniele de Sanctis