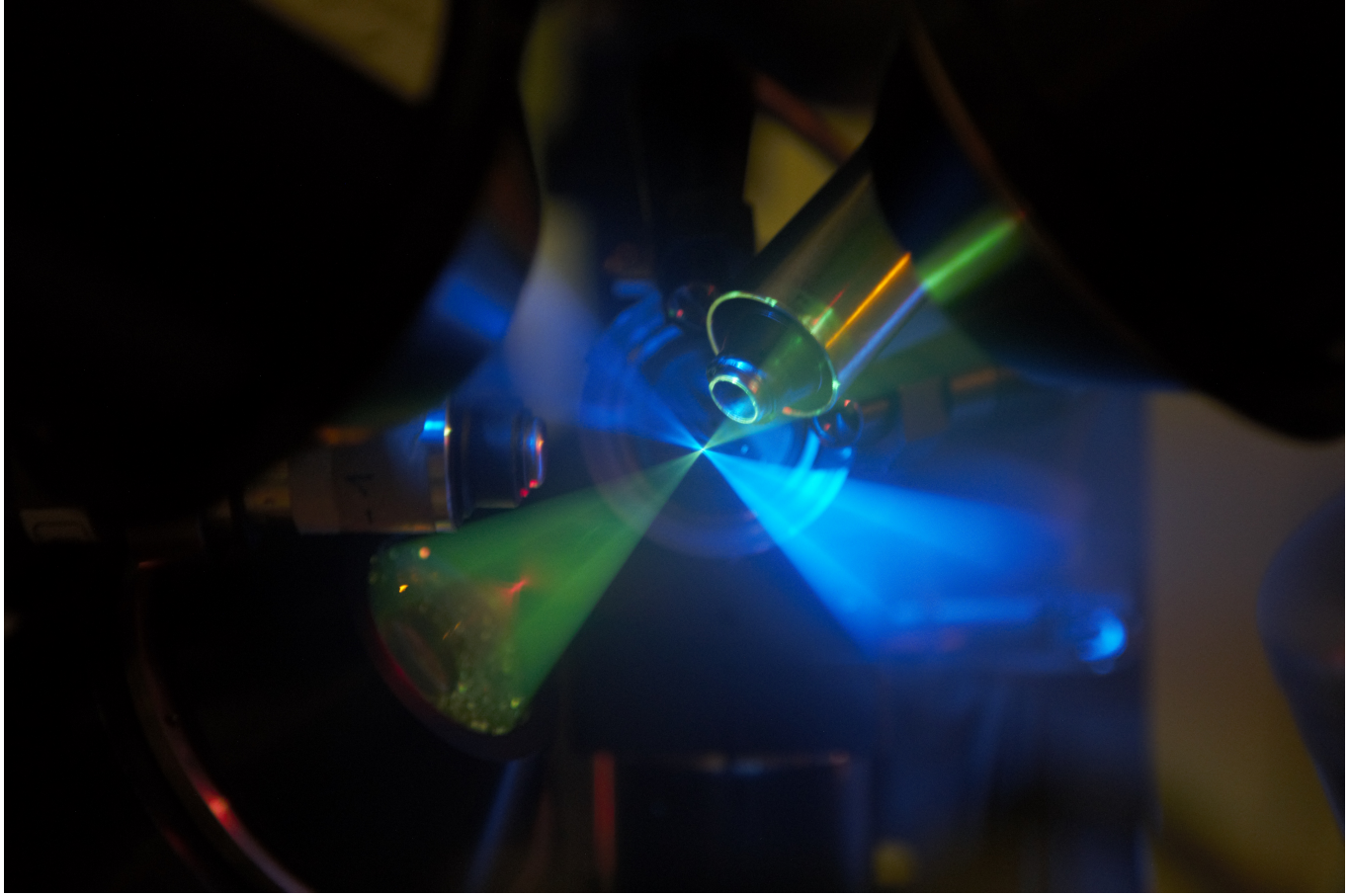
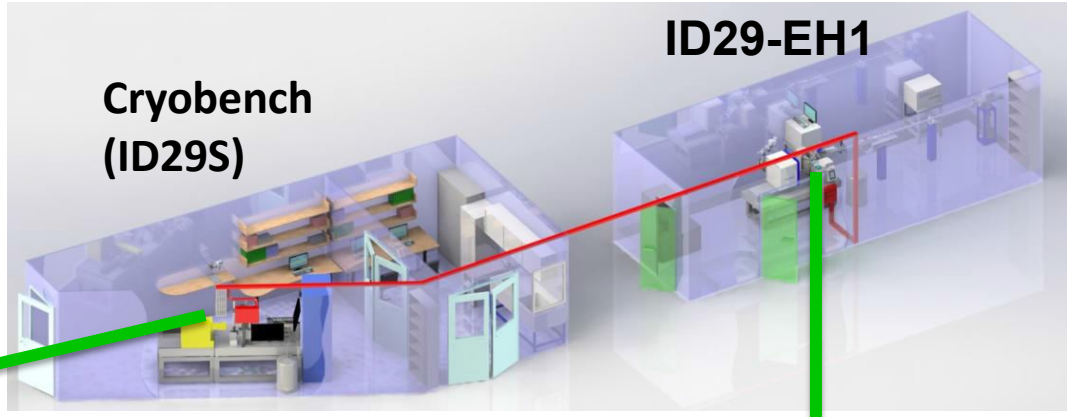


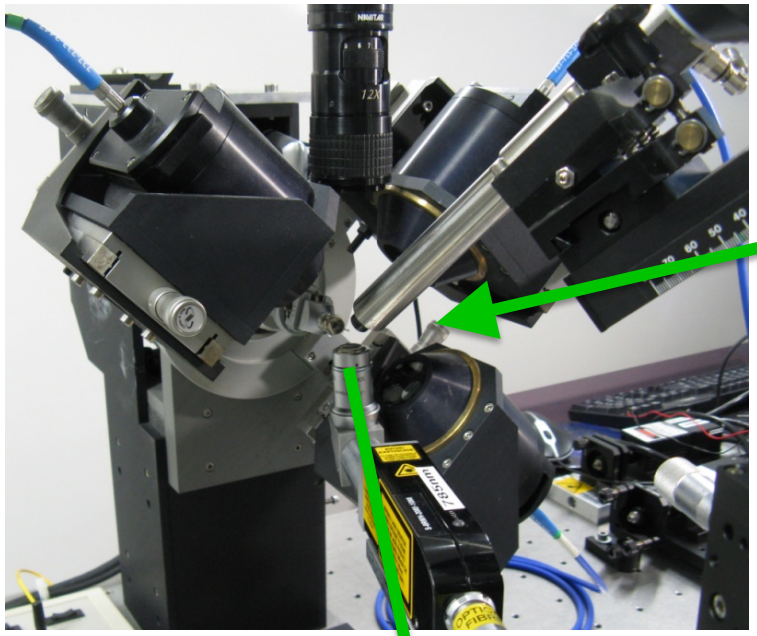
News from ID29S-Cryobench

Antoine ROYANT

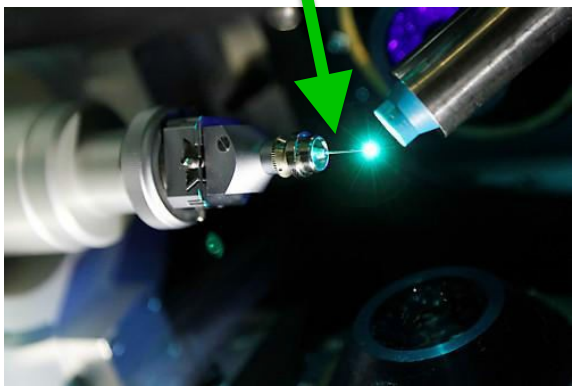
Feb 5th, 2018

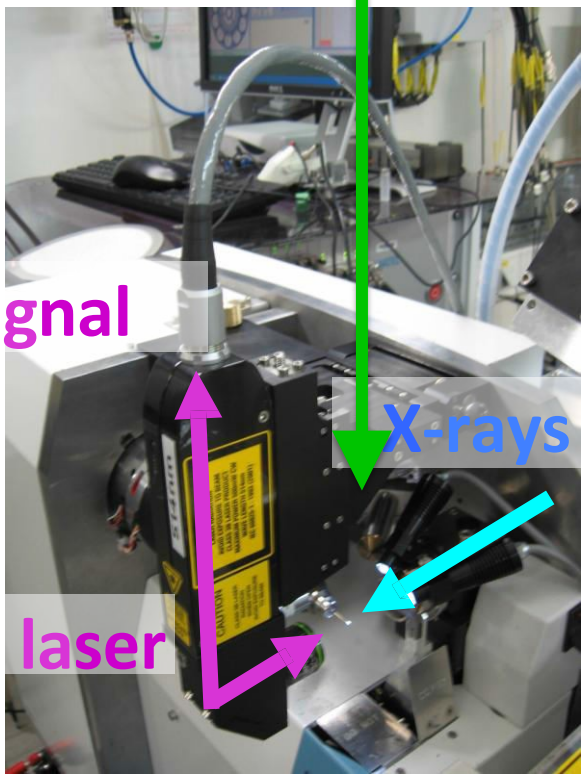
Current setups





+microspec on BM30A



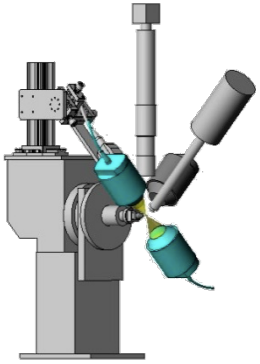


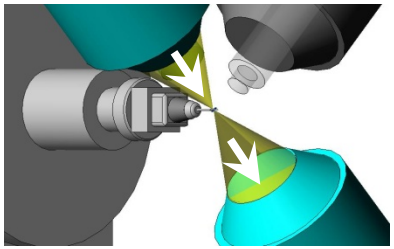
Citation publications:

von Stetten *et al.*, *Acta Crystallographica D* (2015)
von Stetten *et al.*, *Journal of Structural Biology* (2017)

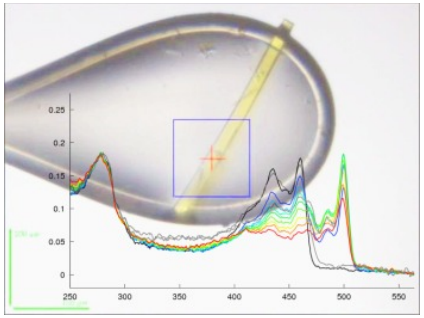
Different modes of operation

Absorption mode

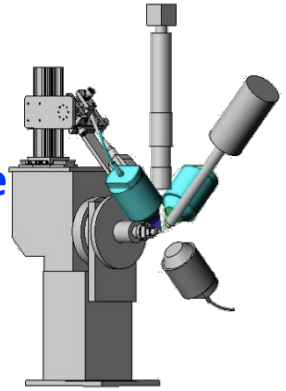


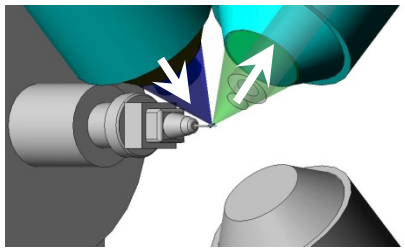


Transmission geometry (0°)

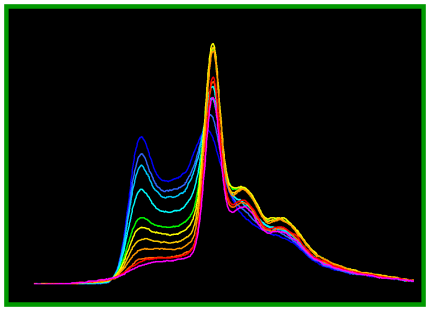


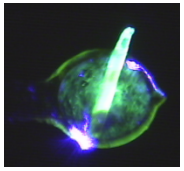
Fluorescence mode



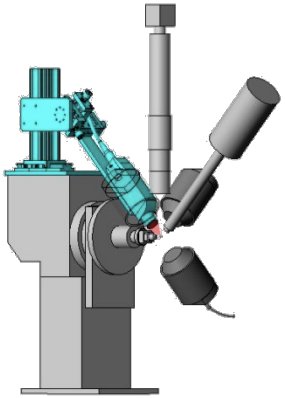


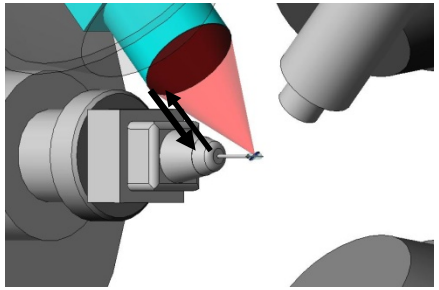
Reflection geometry (90°)



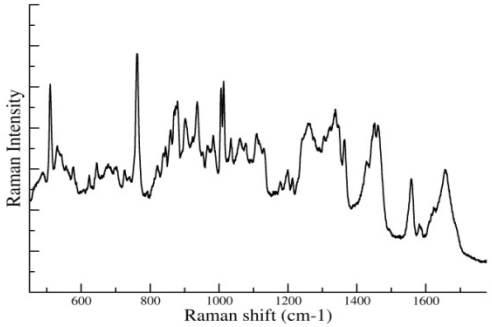


Raman mode



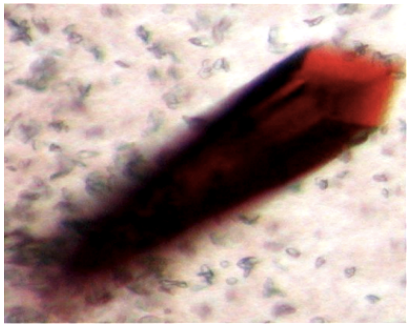


Back-scattering geometry (180°)

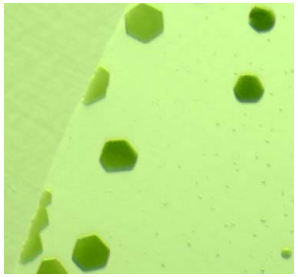


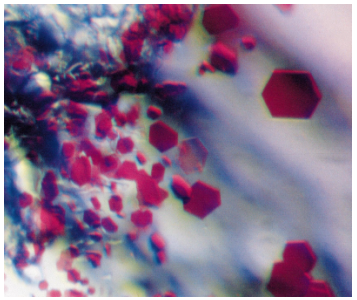
Samples

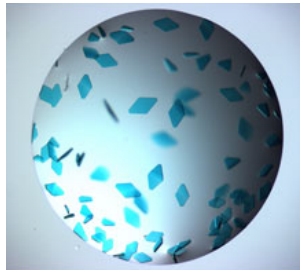
- Metal centers (redox state)



- 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-Cryobench)

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

User output 2017

Arinkin *et al.* "Structure of a LOV protein in apo-state and implications for construction of LOV-based optical tools." (2017) *Sci Rep.* **7**, 42971. **[UV-vis Absorption]**

Genovese *et al.* "Binding of doxorubicin to Sorcin impairs cell death and increases drug resistance in cancer cells" (2017) *Cell Death and Disease* **8***,* e2950 **[UV-vis Absorption, Fluorescence]**

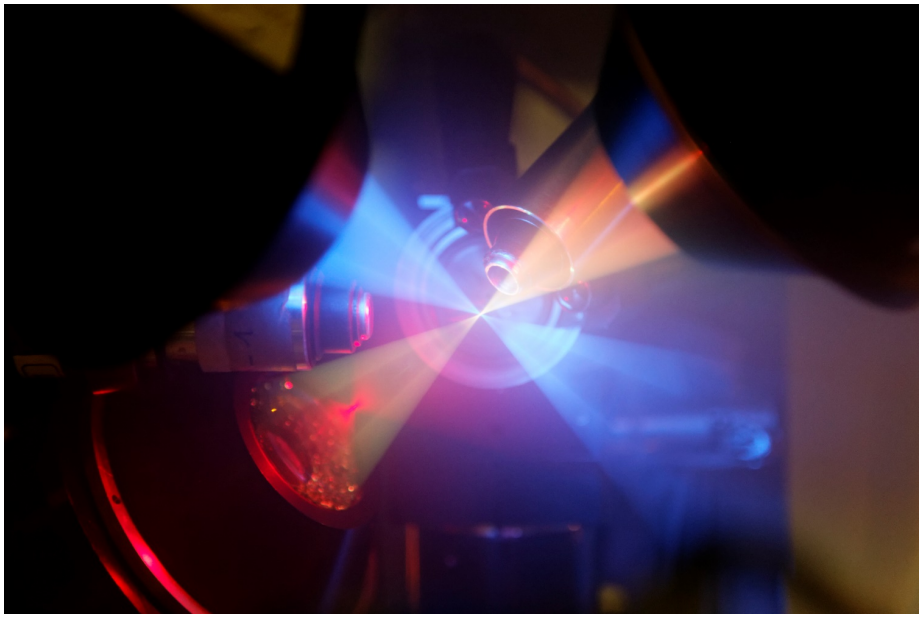
Gotthard *et al.* "Chromophore Isomer Stabilization Is Critical to the Efficient Fluorescence of Cyan Fluorescent Proteins." (2017) *Biochemistry* **56**, 6418-6422 **[UV-vis Absorption, Fluorescence, Actinic]**

Kekilli *et al.* "Photoreduction and validation of haem-ligand intermediate states in protein crystals by in situ single-crystal spectroscopy and diffraction." (2017) *IUCrJ* **4**, 263-270. **[online UV-vis Absorption]**

Rodrigues *et al.* "Lysine relay mechanism coordinates intermediate transfer in vitamin B6 biosynthesis." (2017) *Nat. Chem. Biol.* 13, 290-294. **[(online) UV-vis Absorption]**

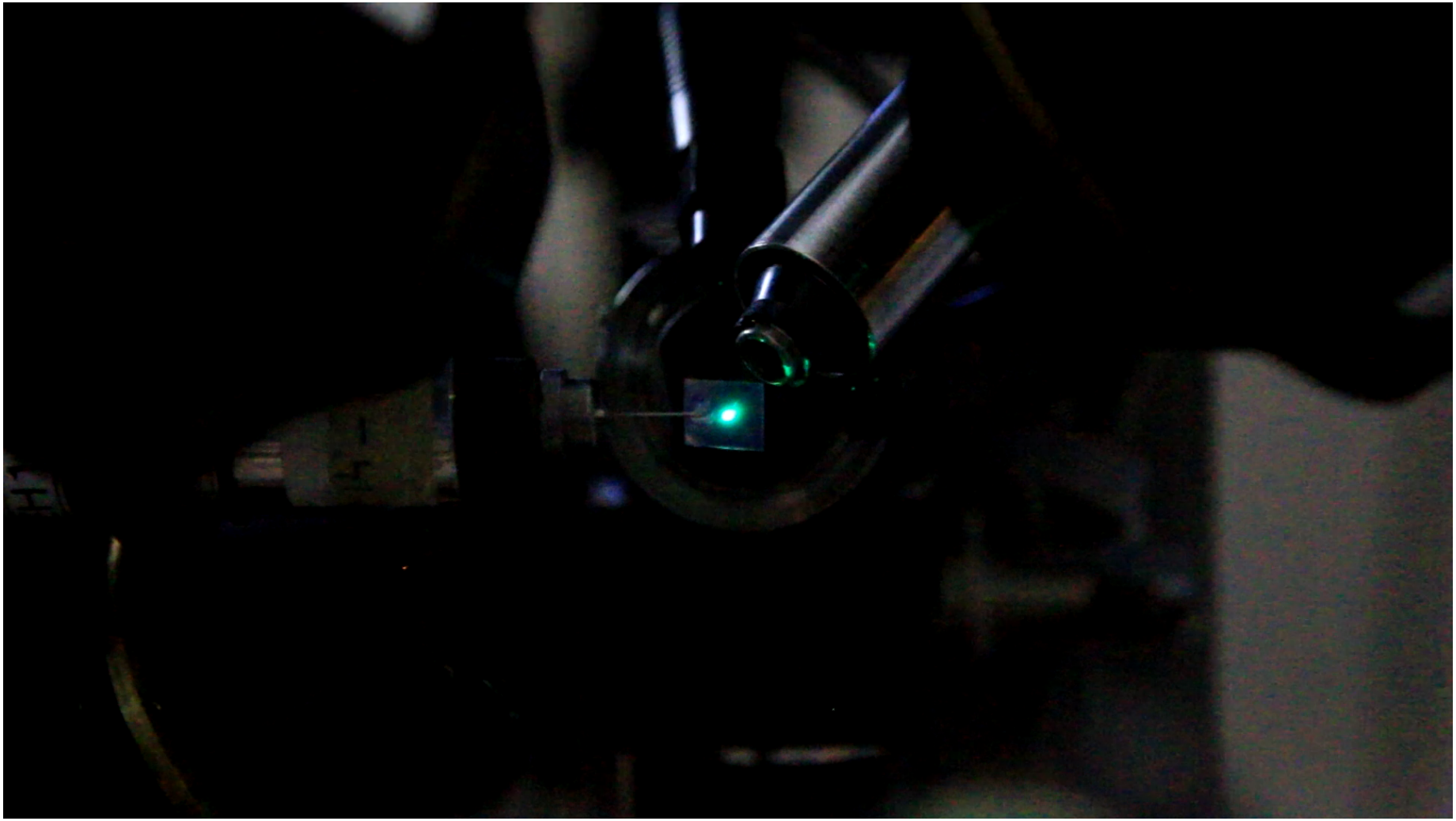
Romero *et al.* "Characterization and Crystal Structure of a Robust Cyclohexanone Monooxygenase." (2017) *Angew. Chem. Int. Ed.* 55, 15852-15855. **[(online) UV-vis Absorption]**

New setup at ID29S-Cryobench

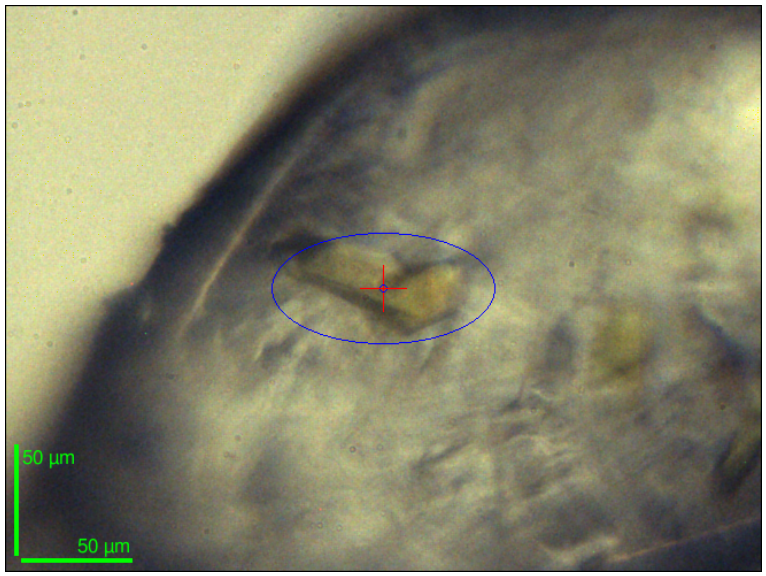


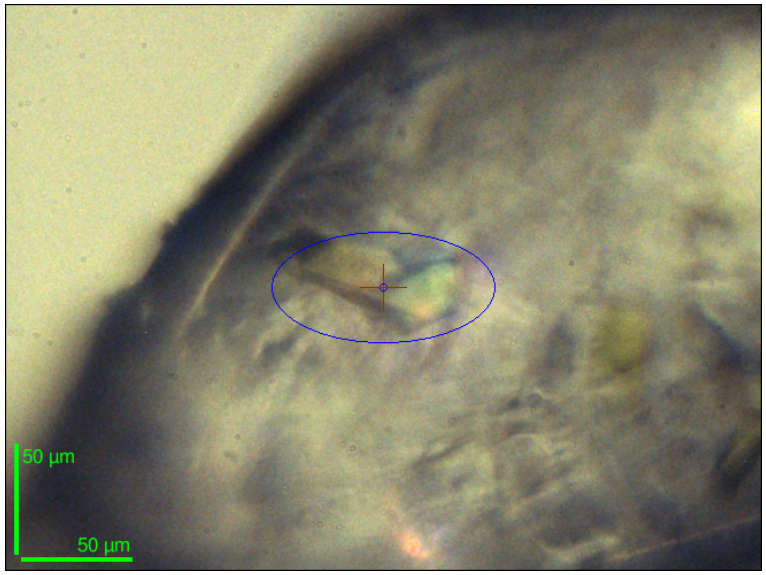
- 4 x 3 motorized translation stages
- 3-click centering
- Modified version of MxCuBE

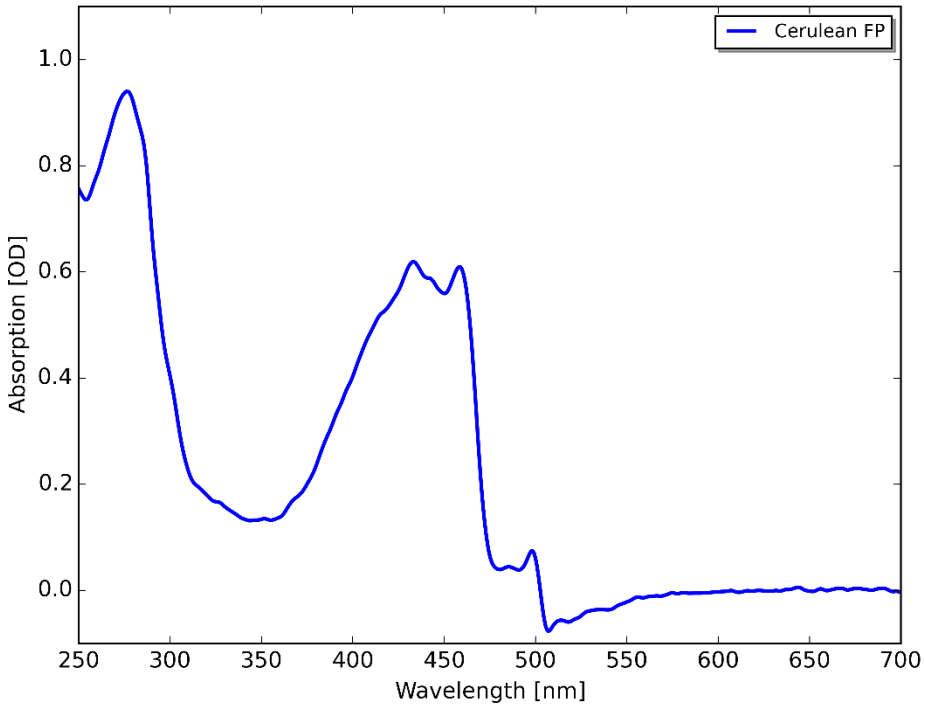
Rough alignment tool



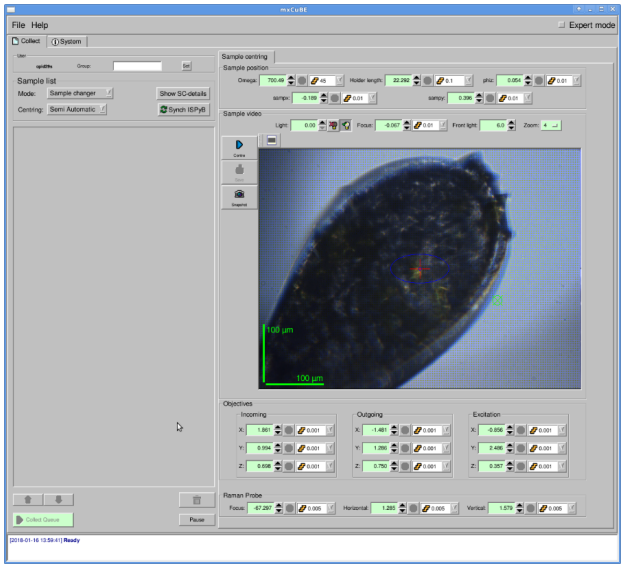
1st UV-Vis absorption spectrum

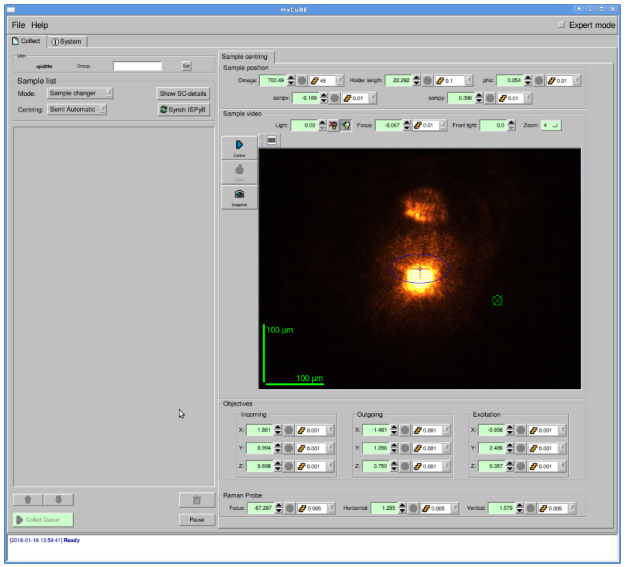


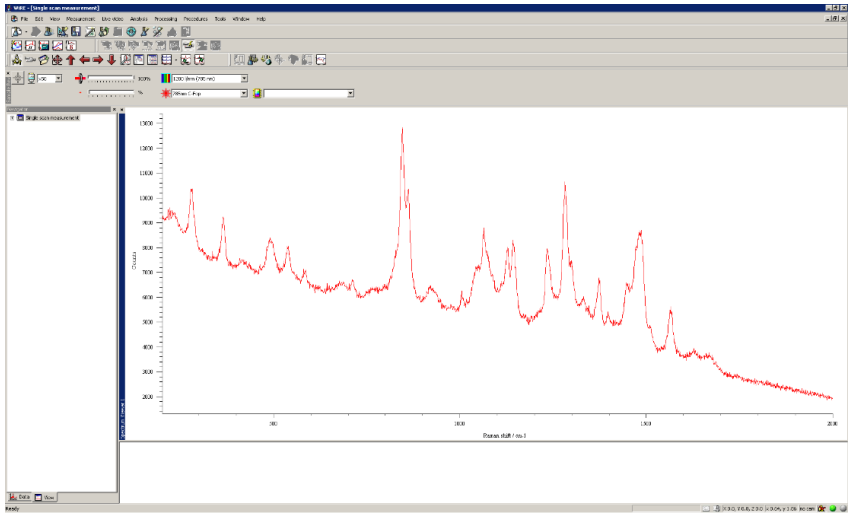




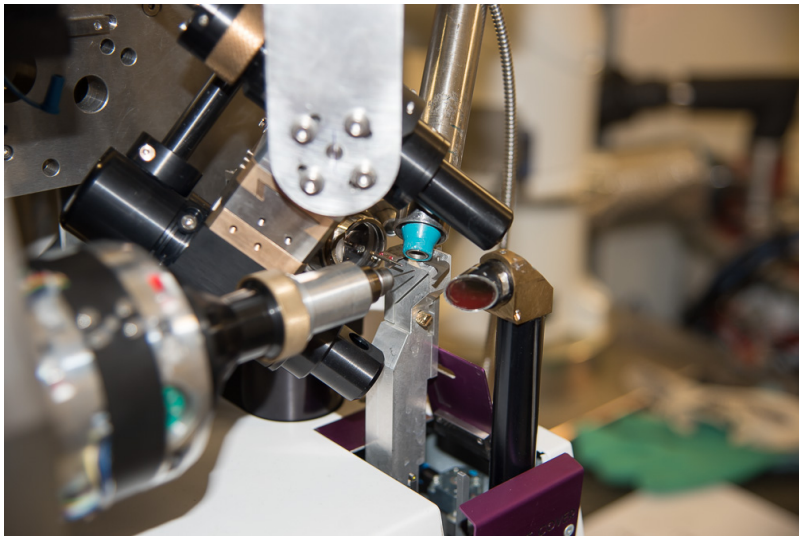
1st Raman spectrum

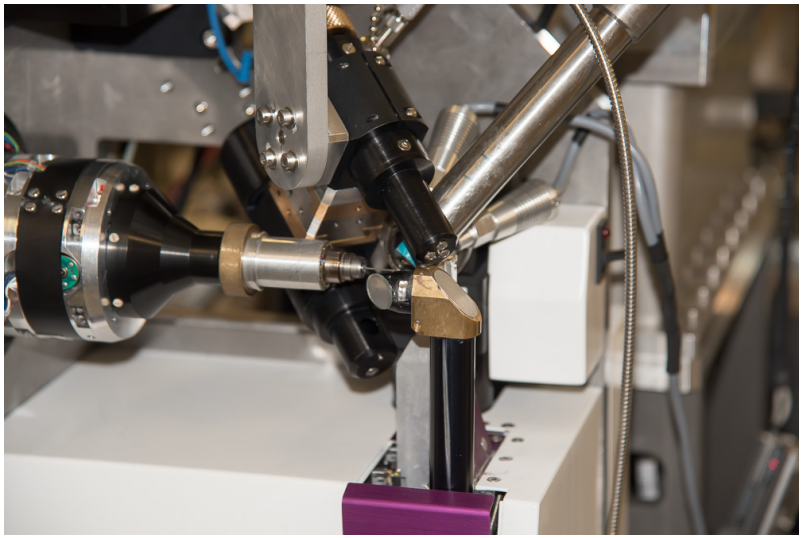






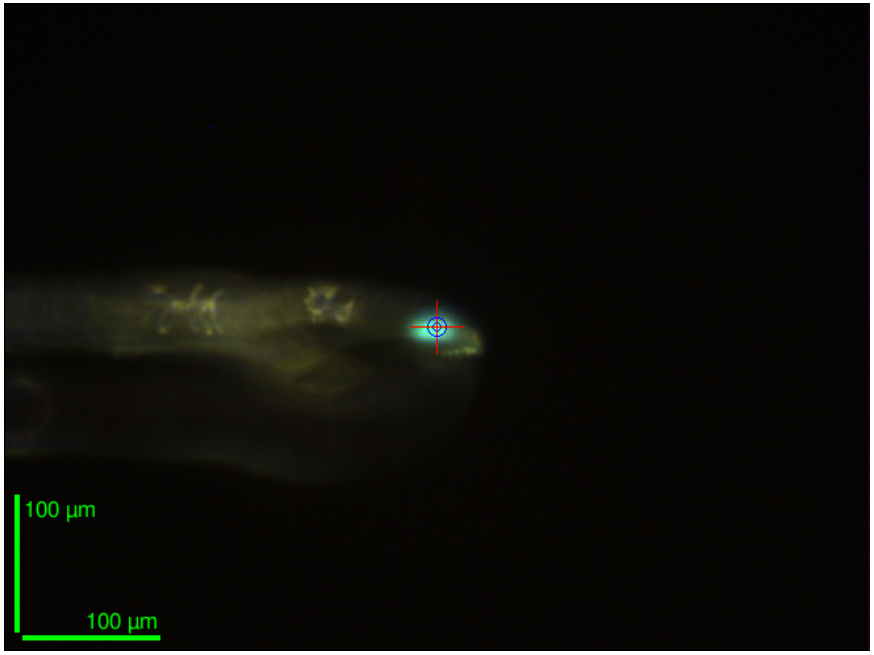
New microspec on MASSIF3

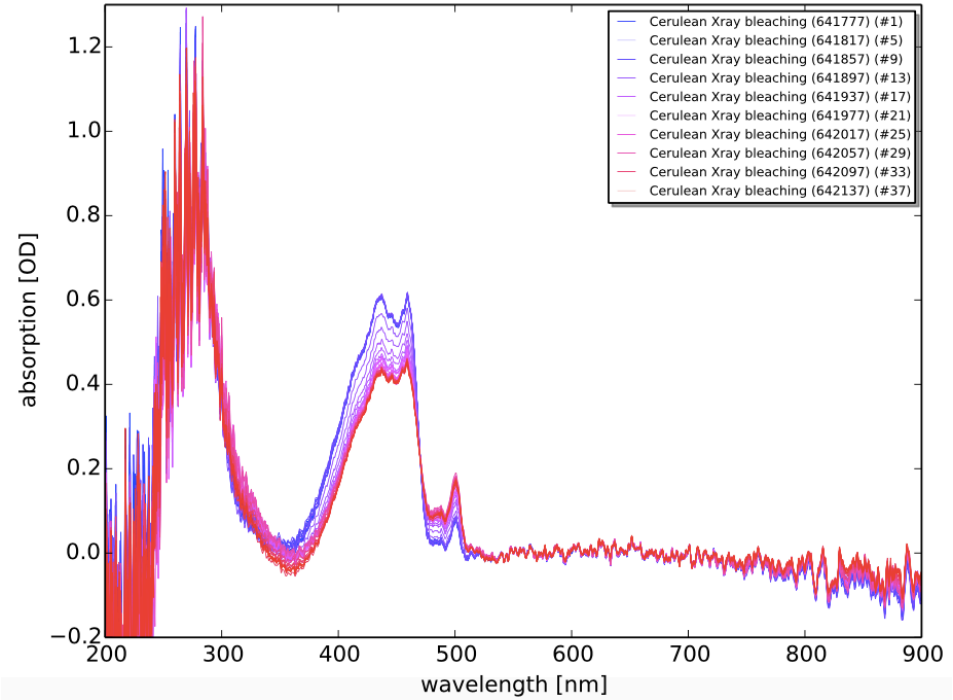




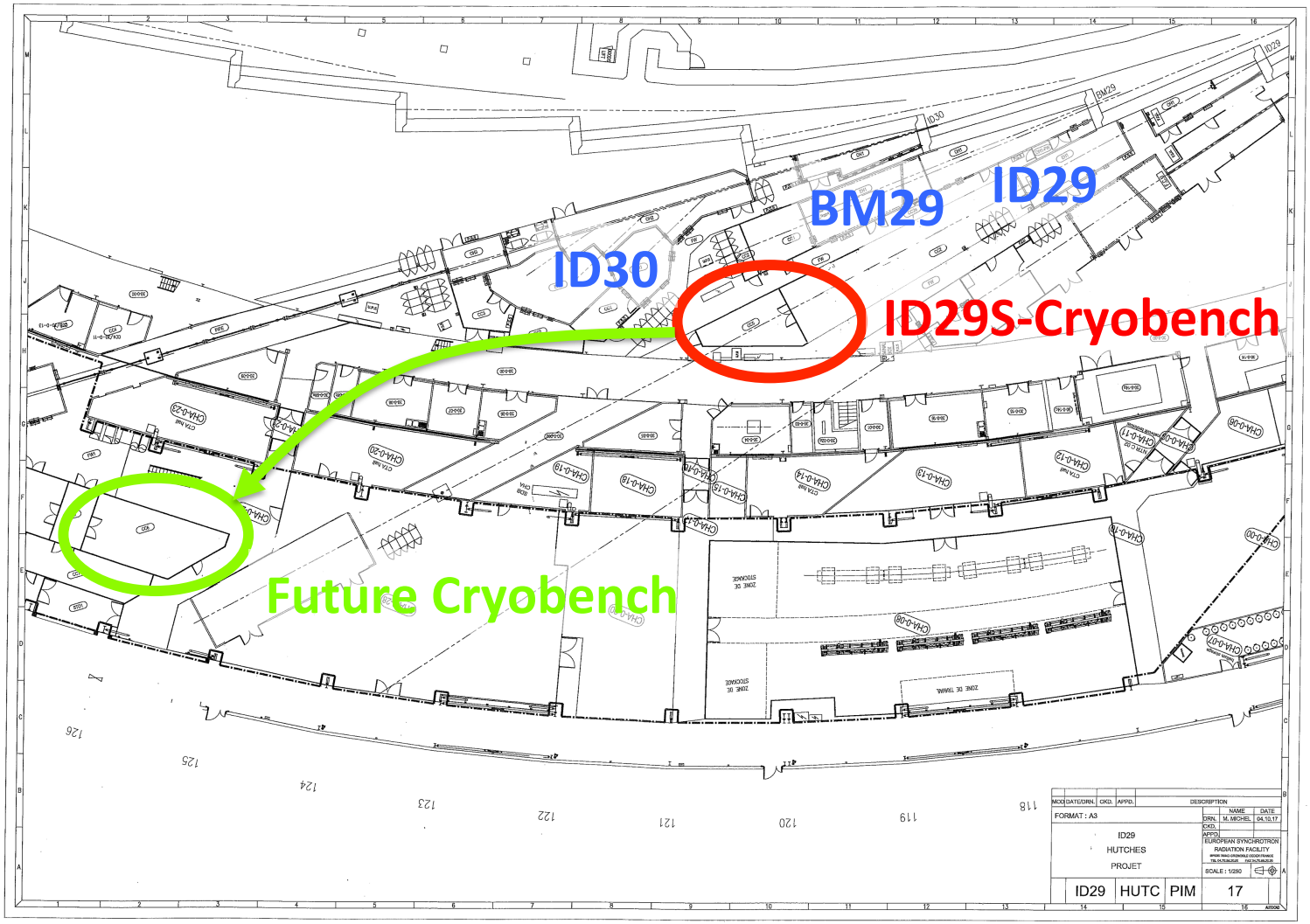
- Mounting on gel jet support
- 25 µm focal spot

1st series of spectra

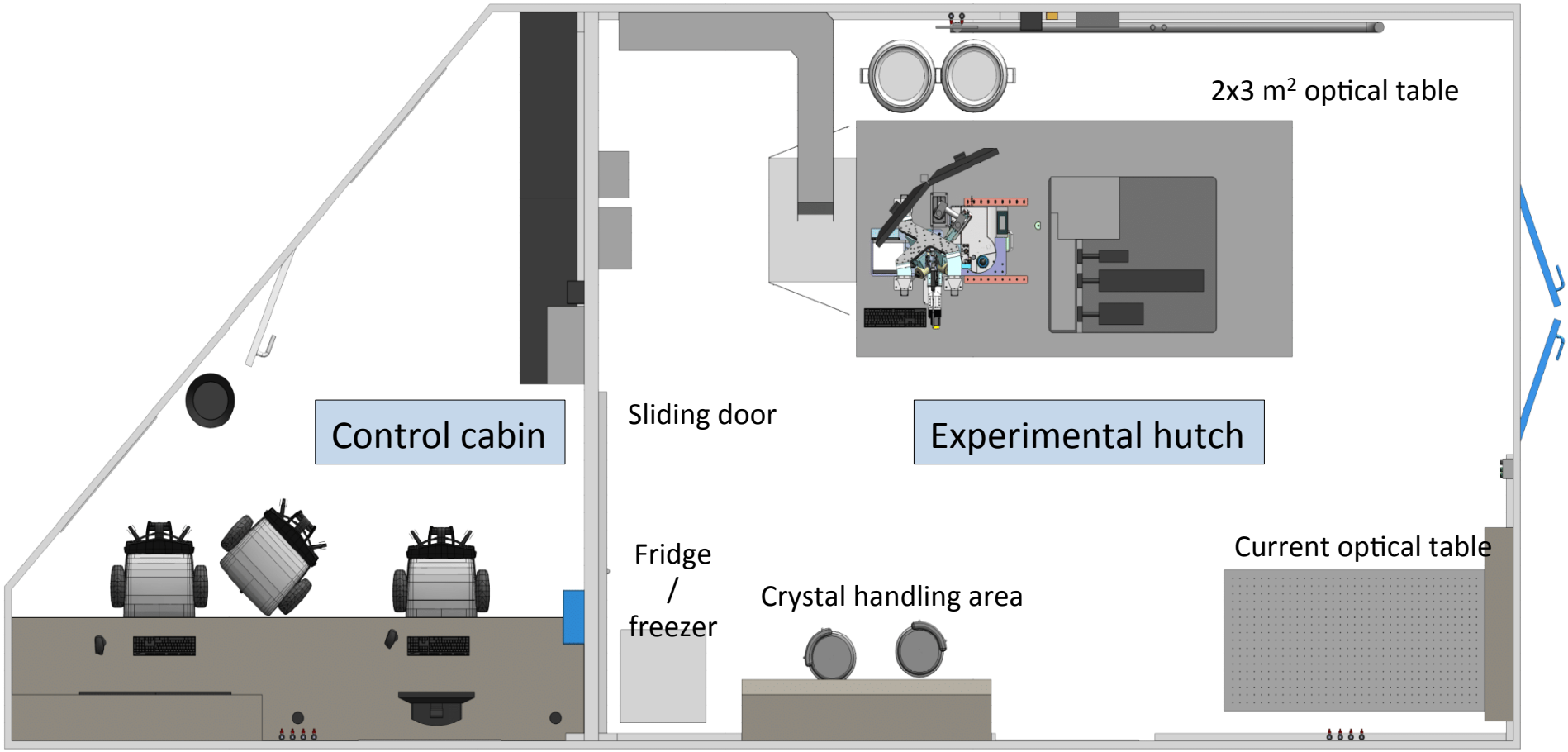




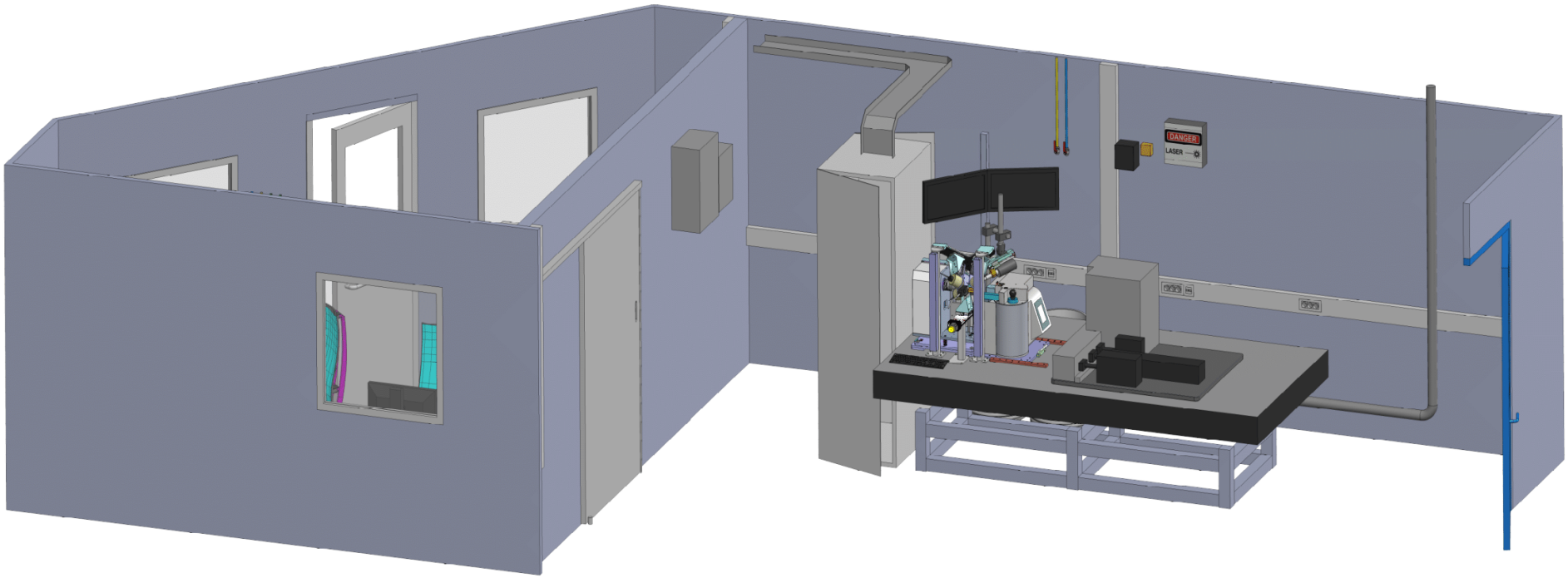
ID29S-Cryobench moving



First draft of Cryobench v4



Side view of the main setup



Control cabin

Operation in 2018 and beyond

Online instruments -> Dec 11th, 2018

- Microspec on BM30A
- Microspec on MASSIF3 (some time during 2018)
- Online Raman on ID29

Offline instruments - available during EBS shutdown

- Downtime for moving: 1-3 months
- Availability of time-resolved setup 2019-2020