ESRF	**Experiment title:** Stroboscopic X-ray diffraction studies of photoexcited transient states of Bacteriorhodopsin	**Experiment number:** LS 481
Beamline: ID09	**Date of experiment:** from: 21 Aug 96 to: 30 Aug 96	**Date of report:** 10 Sept 96
Shifts: 17	**Local contact(s):** Friedrich Schotte	***Received at ESRF:***

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Report:

Summary

Nine time points from 50 μs to 20 ms could be recorded. Observed differences for ms time points seem to be in qualitative agreement with published data. But the difference signal is noisy and sample excitation could be improved.

Details

For this experiment the time resolution of the X-ray diffraction was obtained by the time structure of the beam using a detector without any time resolution. So at the starup of the experiment we gave the priority to optimize the pulsed peak flux in order to achieve tolerable accumulation times. We tried the most performant configuration using the "monoharmonic" undulator and the X-ray chopper.

The monoharmonic undulator, installed in June on ID9, is an X-ray source which produces a quasi-monochromatic beam. Most of the intensity is concentrated in a peak around 1.1 Å, the undulator's fundamental.

The beamline's built-in monochromator does not work very efficiently at this wavelength because it uses a 400 μm silicon crystal in transmission (Laue-Bragg geometry) which absorbs 90% at this wavelength. So we used a silicon channel cut monochromator which works in reflexion (Bragg-Bragg geometry) and has less than 10% absorption. It had to be installed in the experimental hutch, 50 cm from the sample and was protected against the 25 W heat load from the unfiltered beam by the X-ray chopper, so it could be operated without cooling.

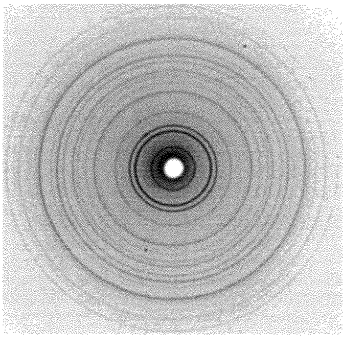
The X-ray chopper had been designed for single pulse Laue exposures and runs at 445 Hz normally. But it could be operated 10 Hz as well, the maximum rate for the laser excitation, where it has a 115 μs opening window. Unfortunately the X-ray chopper's driving electronics burned out due to a startup problem. It took two days to replace the drive by a stepper motor which could be operated by standard electronics used for the beamline control. Fortunately it turned out that the chopper worked very reliable driven by this motor at 2000 steps/s. The mechanical jitter was only ±25 μs per 100 ms revolution. This allowed us to use delays down to 50 μs.

The first stroboscopic images taken using the pulsed monochromator looked quite disappointing: only two rings visible, signal to background 1:50. The drawback of the pulsed monochromator is that the unfiltered beam from the undulator enters the experimental hutch where the chopper selects 1/800 of the intensity and the monochromator reflects a wavelength band of 1.3" 10-4 width. So the only 10^{-7} of the intensity coming in the hutch is used for the experiment and the local shielding is critical. We to spend a whole day looking for radiation leaks in the lead housing surrounding the chopper and monochromator by placing image plates at strategic positions and installing additional shielding around the beam pipe. Finally we succeeded to get the white beam background down by a factor of 1000 and improved the signal to background to 4:1.

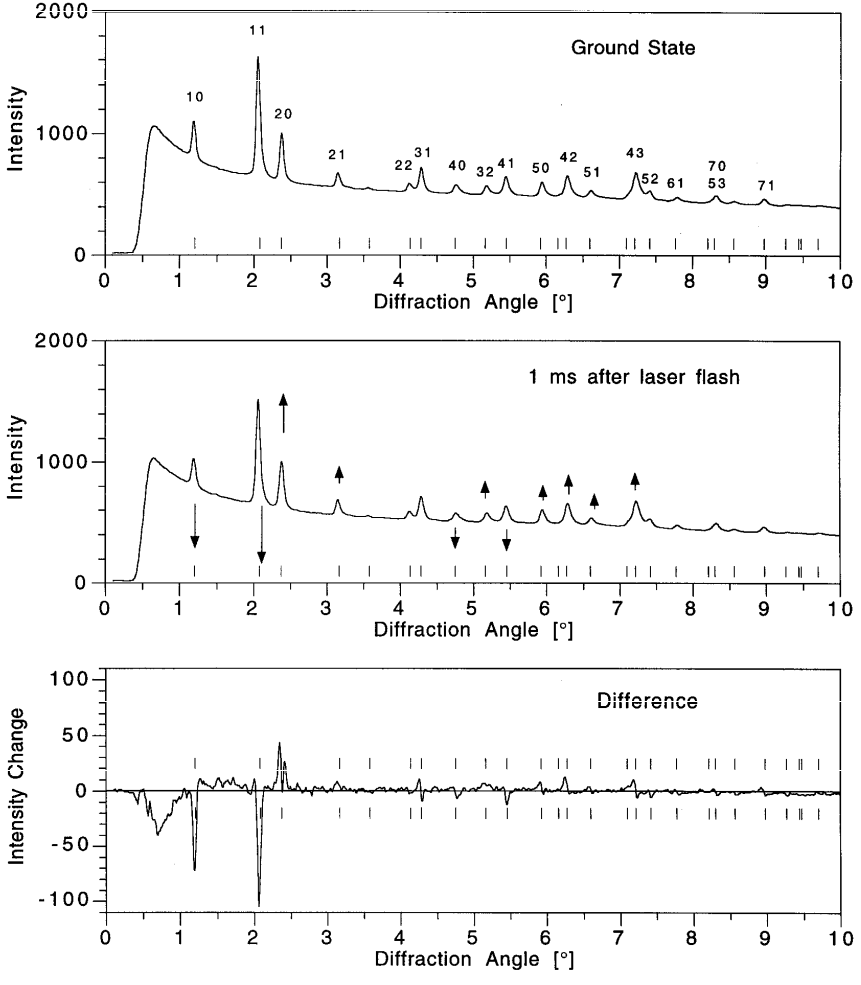
The detector was a Fuji image plate, read offline by a "Storm" scanner by Molecular Dynamics. A helium cone was placed between the sample and the image plate which reduced the air scattering background by more than a factor of 6. A 2 mm beam stop on the exit window of the cone at 130 mm distance allowed diffraction down to 100 Å d-spacing to be recorded.

The sample was excited by 10 ns pulses from a Nd:YAG-Laser at 532 nm with 50 $\mu J/mm^2$. This is probably not optimal but did not give any visible color change during the whole time of the experiment, whereas 200 $\mu J/mm^2$ completely bleached the illuminated spot within one minute.

All time points were collected on on single sample of native purple membrane of optical density 0.8 whithout any treatment at 22 'C. The exposure time was 10 min. per time point. The sample had a surface area of about 1 cm^2. The inner part of 0.5 cm^2 was continuously scannened through the beam in two directions during the exposure with a speed of 0.5 mm/s. Care has been taken that always the same sample area was scanned with the same speed for each time point. To avoid differences from proceeding laser or radiation damage and to eliminate slow intensity drifts, time points and reference points have been collected alternately. For the reference images the laser was still exciting the sample at 10 Hz but 1 ms after the X-ray pulse, so the probe X-ray pulse saw the sample in a maximal relaxed state. This way I tried to minimize possible differences from laser heating and light adaption.



115 µs stroboscopic X-ray diffraction pattern of purple membrane, optical density 0.8 at 570 nm, 6400 repetitions at 10 Hz, 10:40 min accumation time, 0.74 s integrated exposure time, recorded at ID9, 30 Aug 96.



lms after excitation the M-state should be fully developed. The intensity changes of the diffraction rings show the same tendencies as published X-ray data of artificially stabilized M-intermediate [1,2]. However, the changes of the low order rings are much larger with respect to the changes of the high order rings..

[1] Koch, Dencher, Oesterhelt, Plöhn, Rapp & Büldt, ***Time resolved X-ray diffraction study of structural changes associated with the photocycle of Bacteriorhdopsin,*** The EMBO Journal, vol. 10, no. 3, p. 521-26 (1991)

[2] Nakasako, Kataoka, Ameniya & Tokunaga, ***Crystallographic characterization of the M-intermediate from the photocycle of bacteriorhodopsin at room temperature,*** FEBS, vol. 292, no. 1,2,p. 73-75 (1991)