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Installation of an IR/Raman measuring station at the ESRF for simultaneous detection of vibrational and nuclear resonant scattering spectra

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Abstract. Research on micro-structured iron-containing samples can be efficiently carried out by means of nuclear resonant scattering techniques at synchrotron facilities, because the high brilliance and low divergence of the beam facilitates the characterization of micrometer-sized samples containing Mössbauer active isotopes. Additional information can be obtained if the nuclear scattering techniques are combined with classical vibrational methods such as Raman and IR microspectroscopy. The sample environment presented within this paper provides the possibility to measure Raman spectra and to obtain optical (visible) on-line information about possible changes/damages of the sample during synchrotron beam exposition.

1. Introduction

Analytical methods like vibrational spectroscopy, and nuclear resonance scattering techniques have become independently evolved and established. Raman and infrared spectrometers are applied as standard instruments for solving analytical problems in academia and industry, furthermore, they are actually applied for routine analytics concerning quality processes as well as for quantitative measurements in large scale industrial process analytics by now. However, the fundamental aspect within the material and biophysical sciences is to enlighten the structure-property relationships. Due to the high brilliance of 3rd generation synchrotron radiation sources even small samples with a low content of ~ 10^{12} atoms of ^{57}Fe can be probed.

Thus, the nuclear inelastic scattering (NIS-) and nuclear forward scattering (NFS-) techniques can be used as an efficient tool for analyzing metabolic steps, which involve iron-containing enzymes, due to obtain information about the iron's surrounding as well as information about the iron's central atom itself. These techniques are meanwhile well established for characterization of iron-containing organic and inorganic samples [1,2].

There is an increasing demand for the investigation of micro-structured iron-containing samples not only from biogenous material but also from synthetic inorganic and organic compounds by application of synchrotron radiation simultaneously with vibrational spectroscopic methods such as infrared spectroscopy and Raman scattering, respectively. Davies et al. already described an experimental set-up at ESRF ID 13, which enables combined microfocus Raman and microfocus XRD to probe deformation of high performance fibers [3].

This contribution describes the set-up of the first combined Raman-microscope and NFS/NIS measuring station at a synchrotron radiation facility enabling the simultaneous measurement of vibrational nuclear scattering techniques while the sample is additionally probed by optical methods with an applied microscope. As an example first results obtained from this sample environment on the reaction of a metmyoglobin single crystal with hydrogen peroxide are presented.

2. Experimental Set-up of the Measuring station

The installed measuring station currently allows the simultaneous probing of iron containing micro-structured samples by Raman spectroscopy - via a SENTERRA® Raman microscope - and NFS/NIS techniques, provided and developed as in-house methods at ESRF / ID-18. It is planned to extend the workplace with the IR microscope HYPERION® in 2010. The key features of the already provided and installed techniques are presented within this subchapter.

2.1. Raman microsope SENTERRA®

The SENTERRA® dispersive Raman microscope (figure 1, Bruker Optik GmbH, Germany) is a full featured confocal system which is capable to accommodate different excitation wavelengths with a high spatial resolution. The microscope features three different lasers with excitation wavelengths of 488 nm, 532 nm and 785 nm with a corresponding power of 20 mW, 20 mW, and 100 mW, respectively. Since the microscope was primarily developed as stand-alone system the integration into an existing NFS/NIS workplace requires small modifications in particular those affecting general safety regulations. Usually the Raman-experiments were carried out with closed safety doors of the measurement compartment, thus the system fulfill the laser safety class I. If the microscope is put into the synchrotron beam it is beneficial with respect to the intensity of nuclear forward scattering signals to endue the safety doors with pin holes enabling the lossless input and output of the beam or the scattered signals, respectively. During operation at the synchrotron the microscope therefore fulfills the laser safety class IIIb.

The optical device of the Raman microscope is based on the Olympus BX51 microscope, which provides the necessary tools concerning sample visualization and characterization. Visualization can be achieved using the standard objectives with a magnification of 20x (NA: 0.4, working distance is 1.3 mm) or 50x (NA: 0.75, working distance is 0.38 mm). Larger working distances can be realized by the additionally provided long distance working objective (20x, NA: 0.4, working distance is 12 mm).

Spectral resolution of the system is smaller than 3 cm^{-1} and the spatial resolution is 1 µm depending on the excitation laser wavelength. Confocal depth resolution is higher than 2 µm and depends on the utilized objective. For achieving a higher flexibility concerning the probing of larger samples or if the examination requires a higher working distance (e.g. investigation of samples in cooling or heating stages), the system is equipped with an optical Raman probe, which is connected to the excitation laser (only 785 nm) and the detector via fiber optic cables. The working distance of the current system is 15 mm and 60 mm, but other lenses with smaller or larger working distances are available.

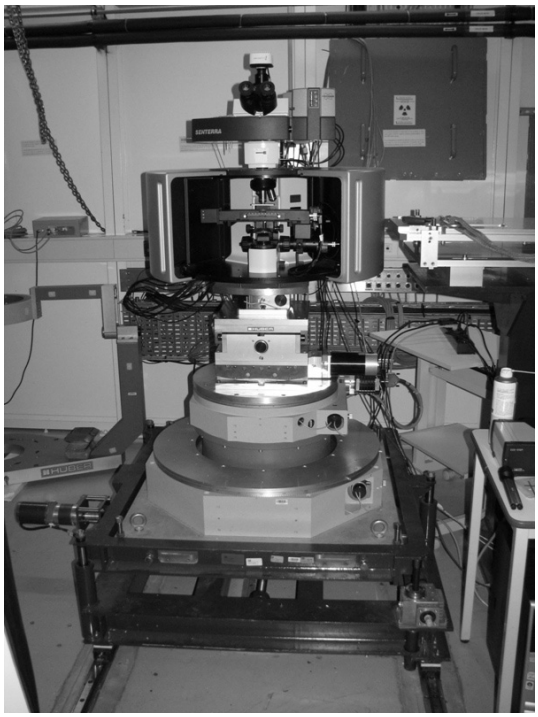

Figure 1. Set-up of the Raman microscope (Bruker Optik GmbH, Germany) inside experimental hutch of beamline ID 18 during experiment CH 2839; the microscope is mounted on a goniometer with an adequate adapter plate. The figure shows the microscope with opened safety doors.

2.2. NFS/NIS technique at ESRF/ID 18

For the protein single crystal NIS measurements presented below the incident beam was monochromatized by a Si(111) double-crystal premonochromator to a bandwidth of 2 eV. A further decrease of bandwidth down to 1 meV (8 cm^{-1}) was obtained with a refractive beryllium collimator and a high-resolution monochromator. This beam was focused by a Kirkpatrick Baez mirror optic to a spot size of 8*15 µm. The NIS data were collected during several energy scans with 0.25 meV step size. The metmyoglobin single crystal was oxidized by treatment with hydrogen peroxide according to a procedure described in [4], frozen in a loop, and placed into the focused beam.

Cooling during the NIS measurements was performed by a cryostream from Oxford Cryostream Ltd. (figure 2). The examined sperm whale metmyoglobin was enriched in the Mössbauer isotope ^{57}Fe according to [5].

3. Initial experiments using the installed technical equipments

Figure 3 shows the NIS spectra obtained at 110 K of a ^{57}Fe-Myoglobin single crystal (700*150*150 μm^3) after addition of $H_2{}^{18}O_2$ and subsequent freezing. The single crystal data identify a localized iron mode of 18 cm^{-1}. Modes in this region have also been observed near 25 cm^{-1} in MbCO and deoxyMb and have been assigned to translational modes in response to torsional oscillations of the polypeptide backbone and side chains [6]. This assignment was confirmed by a NIS study of millimeter-sized metmyoglobin single crystals which reports haem sliding motions in the region of 32 - 40 cm^{-1} [7]. The reason why in the case of $H_2{}^{18}O_2$ treated myoglobin a localized mode is seen at lower energy is unclear and subject to further investigations by combined quantum mechanical and molecular mechanics calculations. Unfortunately the obtained Raman spectra do only show a significant band at 975 cm^{-1} using an excitation wavelength of 785 nm which corresponds to the ammonium sulfate (usually found at 983 cm^{-1}) within the crystal. Clearly more measurements also at 488 and 532 nm excitation wavelength as well as directly in the Soret band e.g. at 407 or 413 nm have to be performed to increase the up to now none significant scattering signals which are related to the

protein vibrations. However, these data might be correlated with recently published synchrotron-derived results concerning investigations on different protonation states of oxidized myoglobin [8].

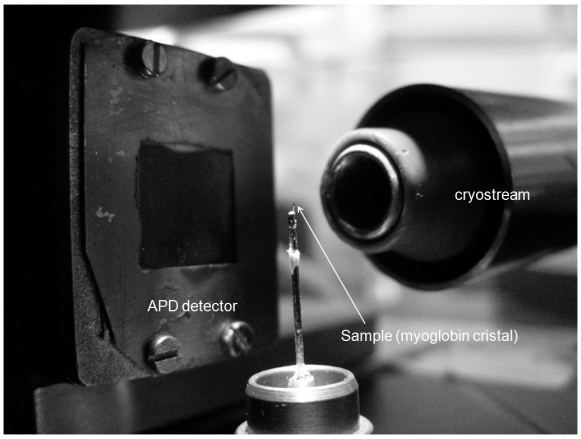

Figure 2. Experimental set-up of the cryostream and APD detector for probing the myoglobin crystal. The pin of the crystal mount has a diameter of 0.65 mm.

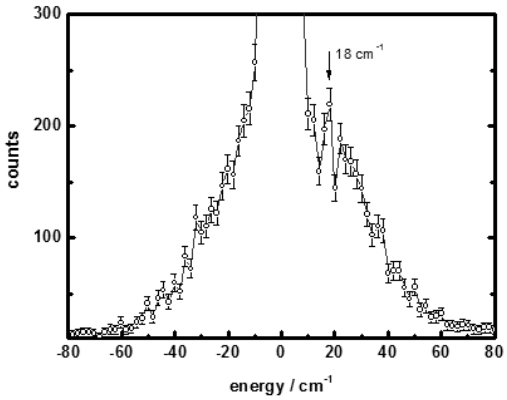

Figure 3. Nuclear Inelastic Scattering spectra obtained at 110 K of a ^{57}Fe-Myoglobin single crystal (dimension of crystal: 700*150*150 μm^3) after addition of $H_2{}^{18}O_2$ and subsequent freezing.

4. Conclusion and Outlook

The installed unique measuring station at ID 18 (ESRF, Grenoble) provides analytical information by complementary vibrational techniques and nuclear resonant methods. Thus, proteins, organic films, and micro-structures can be investigated spatially resolved, whereby the resolution depends on the selected vibrational technique, which is in the μm range. Since the molecular vibrational techniques are probed via corresponding microscopes, possible structural changes and potential damages of the sample during synchrotron beam exposition can be detected online with this equipment. New users can use the systems with their full potential within short adaptational training phases. This world-wide unique workplace will be available for the whole Mössbauer community.

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