ESRF	**Experiment title: X-ray fiber diffraction of micro-tubule: Structural dynamics of native microtubules with anti-cancer tubulin-binding compounds**	**Experiment number**: LS 2732
Beamline: BM26B	**Date of experiment**: from: 06/11/2017 to: 10/11/2107	**Date of report**: 01/08/2018
Shifts: 9	**Local contact(s)**: Daniel HERMIDA-MERINO	*Received at ESRF:*

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Microtubules are key components of the cytoskeleton in eukaryotic cells. Dynamic conversion between tubulin dimers (free unit protein before assembly in cytoplasm, MW=110,000) and assembled microtubules (polymerized state) occurs in a controlled manner, which modifies intracellular microtubule networks along with the whole cell activities such as cell-migration, shape changes, mitosis, differentiation and so on. Since microtubules are one of the most crucial targets of anti-cancer chemicals (*e.g.*, paclitaxel) that knockout cancer cells, our question is how such tubulin-binding drugs and related derivatives affects the structure of microtubules depending on the states of tubulin dimers during chemical reaction of GTP-hydrolysis. For such purposes, we applied our original technique for the rapid shear-flow alignment of biological filaments (Sugiyama et al., 2009; Kamimura et al 2016) to X-ray fiber-diffraction analysis. First, we tried to find out suitable buffer solution conditions as well the concentration of tubulin. We also tried to observe WAXS signals with a short camera length of 0.37 m (higher angle comparing with our last report for #26-02 852) to investigate the signals in a Q-range up to 2.5 Å.

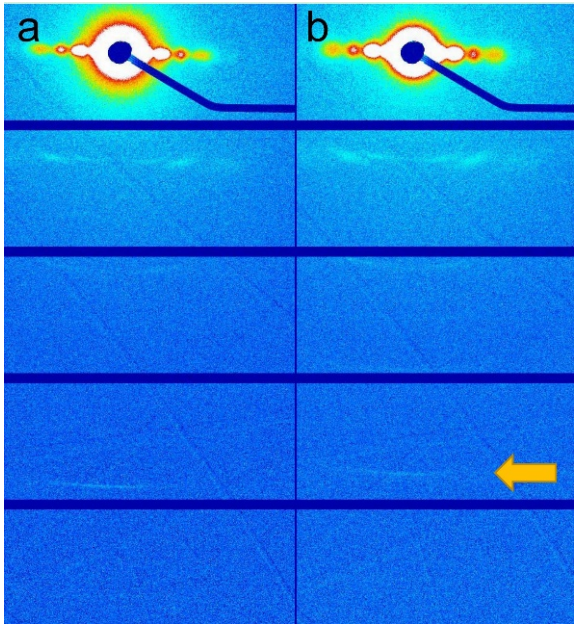

Fig. 1 An example to show the comparison of diffraction patterns of X-ray fiber diffraction of microtubule before (**a**) and after (**b**) adding paclitaxel. Clear shift of approximately 1-nm layer line (4th-order diffraction due to axial tubulin repeat of 4-nm in microtubules) was observed (arrow).

RESULTS & DISCUSSION

We first tested experimental setup to know whether the original setting for shearing machine (Kamimura et al, 2016) can be applied to SAXS/WAXS analysis at BM26B at the camera length of 0.67 m. Fig. 1 show an example to show the difference of diffraction pattern with or without paclitaxel, a potent microtubule stabilizer. As has been reported by one of the authors

(Kamimura et al., 2016), it was clearly show that paclitaxel elongates the axial repeat of tubulin within microtubules, suggesting conformational changes occurring inside tubulin dimer molecules. We also tested 10 different new chemical compounds and nucleotide conditions (details will be published). Fig. 2 shows an example to show the structural variations of microtubules. Using the present technique, the quick shear-flow alignment of microtubule in solution, we can accumulate signals that can readily indicate the wide variations of microtubule structure in solution. The other conventional techniques of structural biology, SAXS, cryo-EM or NMR techniques, cannot repeat similar analysis easily. At the same time, we found some of chemical compounds designed as microtubule stabilizers showed quite different effects on microtubule structures.

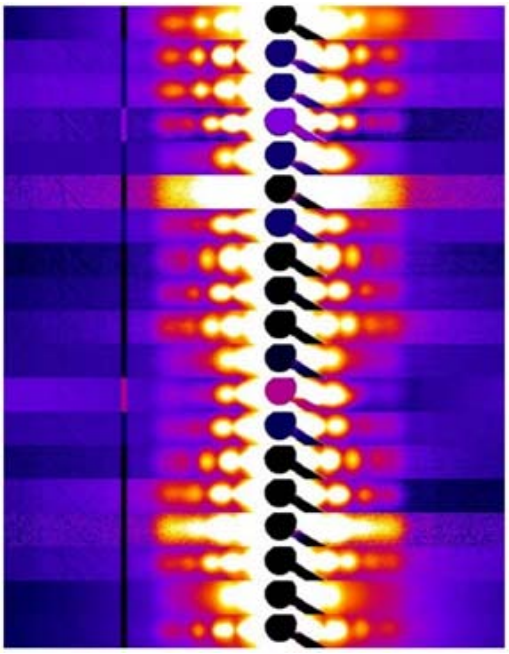

Fig. 2 Equatorial signals extracted from a series of experiments. We observed X-ray fiber-diffraction of microtubules under various chemical and GTP analogue conditions with or without different types of microtubule stabilizers. Equatorial parts were then extracted and arranged here to show the variation of equatorial signals, from which we can estimate mean diameter of microtubules by fitting to 0th-Bessel function.

We also tried to observe WAXS signals with a short camera length of 0.37 m (higher angle comparing with those in our last report for #26-02 852) to investigate the signals in a high Q-range to find out there are some additional WAXS-signals in the range of 0.3 to 0.4 nm in a reciprocal space. An example is shown in Fig. 3. We found here again the problem of scattering from our apparatus or optics, which was observed rather in an enhanced manner comparing with our previous conditions (0.67 m, Q-range<2.0 Å). Some of them is clearly corresponding to the noise or scattering presumably coming from the window materials in the optics, kapton film or CDV diamond. Some improvements of the present apparatus would be required soon to reduce the noise level in this high Q-range.

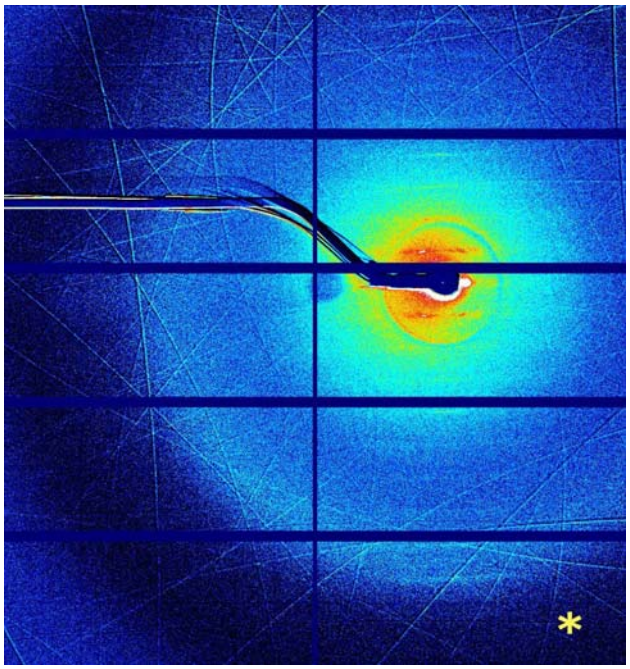

Fig. 3 An example of X-ray fiber-diffraction with a camera length of 0.37 m. In this range of diffraction, scattering coming from window or specimen holder was quite high to detect faint signals from aligned microtubules. * indicate the area of Q=2.5 $Å^{-1}$ (0.4 nm in reciprocal space).

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