ESRF	Experiment title: **X-ray fiber diffraction of microtubules: Analysis of the secondary structure dynamics of tubulin molecules within native microtubules**	**Experiment number**: LS 2882
Beamline: BM26B	**Date of experiment**: from: 22/08 to: 24/08	**Date of report**: 11/09/2018
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Microtubules are key components of the cytoskeleton in eukaryotic cells. Dynamic conversion between unit molecules (tubulin dimers) and assembled state (microtubules) 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 affects the structure of microtubules, and how such binding depends on the chemical states of tubulin dimers during the chemical reaction of GTP-hydrolysis. For these purposes, we applied our original technique for the rapid shear-flow alignment of biological filaments (Sugiyama et al., 2009; Kamimura et al 2016) to observe X-ray fiber-diffraction signals from microtubules at higher resolutions (up to 0.4 nm), the Q-range of which is expected to provide us with crucial signals from the internal structures of tubulin molecules.

RESULTS & DISCUSSION

Before starting present experiments, we executed the following two improvements of machine setting. First, according to the test results obtained with our previous beam time (LS2805), which showed WAXS signals from aligned microtubules at a high Q-range (1.25 $Å^{-1}$, ~0.5 nm in a reciprocal space), we modified the camera positions to cover 0.4 nm (~1.6 $Å^{-1}$). Second, we modified the position and tilting angle of shear-flow chamber to cover higher angles (>10 degrees) of specimen tilting, β, angles between the axis of X-ray beam and the orientation of aligned microtubule in solution (Fig. 1a).

After these modifications, we could observe from -4^{th}- (1 nm) to $+10^{th}$- (0.4 nm) order layer lines derived from the axial repeat of tubulin molecules within assembled microtubules. This is the first demonstration to observe such high-order signals from microtubules in solution. Although we need to determine the exact peak position of each layer line for further details discussions, it is obvious that the signal intensity and position were varied depending on β. This property of layer line signals was as we had expected from simple theory of fiber diffraction (Fig. 1a, *i.e.*, $n \cdot \lambda/\delta = \sin\beta + \sin(\beta+\theta)$). However, we found there was difference of angle-dependency between $+n^{th}$ and $-n^{th}$-order signals, *e.g.*, the highest peak of -4^{th}-order

signal was observed at around +7 deg of β, but +4th-order one was peaked at around –3 deg. This difference would be corresponding to the helix angle of tubulin arrangement within microtubules, which is estimated to be 9~12 degrees (3-start left-handed helix with 12-nm pitch). Precise simulations of fiber diffraction from aligned microtubules should be required, however, our observation is the first evidence to obtain the helix angle of tubulin molecules directly from fiber-diffraction pattern. We are going to carefully analyze the position of layer lines around 0.5 nm, that are expected to be reflecting secondary (internal) structure of tubulin molecules.

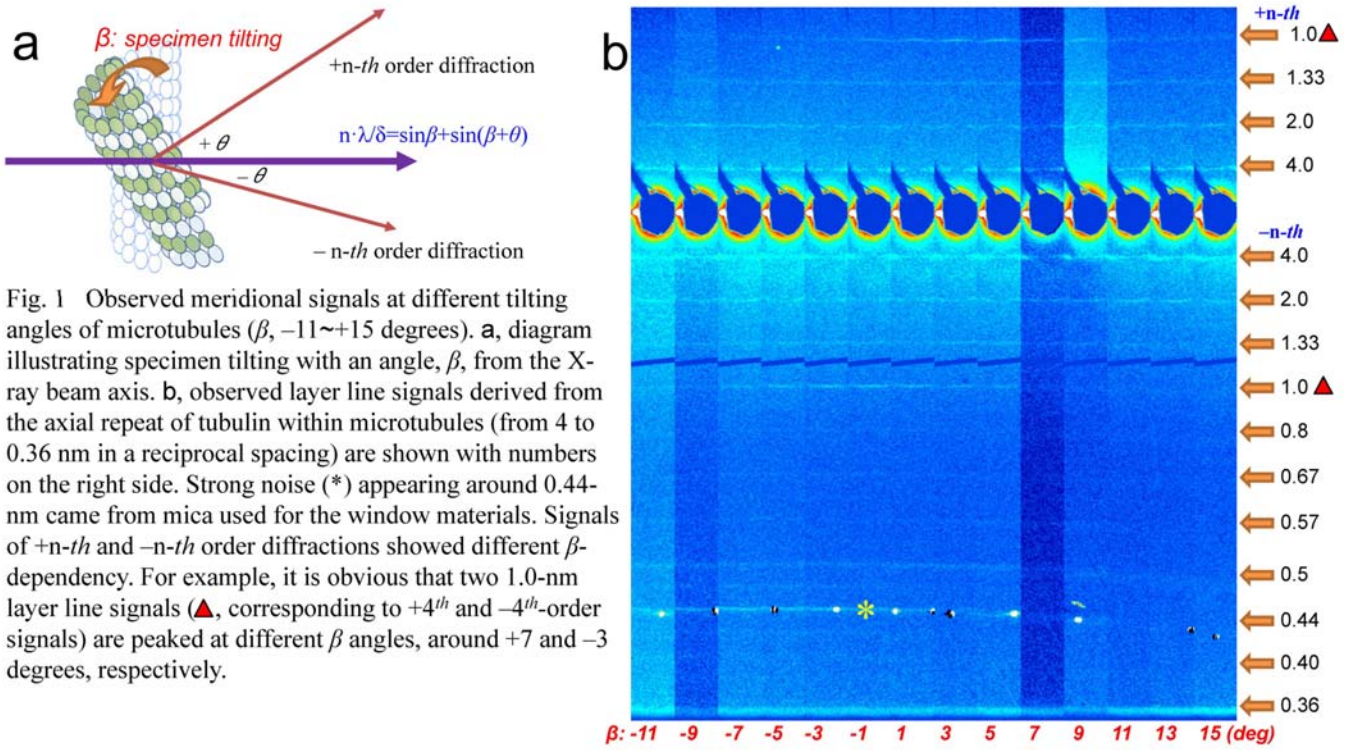

Fig. 1 Observed meridional signals at different tilting angles of microtubules (β, –11~+15 degrees). a, diagram illustrating specimen tilting with an angle, β, from the X-ray beam axis. b, observed layer line signals derived from the axial repeat of tubulin within microtubules (from 4 to 0.36 nm in a reciprocal spacing) are shown with numbers on the right side. Strong noise (*) appearing around 0.44-nm came from mica used for the window materials. Signals of +n-*th* and –n-*th* order diffractions showed different β-dependency. For example, it is obvious that two 1.0-nm layer line signals (▲, corresponding to +4th and –4th-order signals) are peaked at different β angles, around +7 and –3 degrees, respectively.

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