様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　５ 年　 ５ 月 ２２ 日現在

機関番号：８２６７５
研究種目：基盤研究(C)（一般）
研究期間：2020～2022
課題番号：２０Ｋ０３８８９

研究課題名（和文）Mechanical stability of microtubules investigated under unidirectional strain using combined fluorescence microscopy and high-speed atomic force microscopy

研究課題名（英文）Mechanical stability of microtubules investigated under unidirectional strain using combined fluorescence microscopy and high-speed atomic force microscopy

研究代表者

Ｇａｎｓｅｒ　Ｃｈｒｉｓｔｉａｎ（Ganser, Christian）

大学共同利用機関法人自然科学研究機構（新分野創成センター、アストロバイオロジーセンター、生命創成探究・生命創成探究センター・特任助教

研究者番号：５０８４６０９５

交付決定額（研究期間全体）：（直接経費）　　2,500,000円

研究成果の概要（和文）：HS-AFMと弾性基板伸縮装置を使用し、スパースに配置されたキネシンによって固定された微小管を荷重しました。ポアソン効果による弾性基板の同時的な引張りと圧縮を経験することで、微小管の屈曲を制御的に記録することができました。解像度は数ナノメートルで撮像され、従来の蛍光顕微鏡を超える高解像度で行われました。さらに、微小管の伸張も観察され、これにより微小管が破断し、脱重合することがありました。
また、異なるアプローチを用いて、キネシン基板上でのDNA修飾により微小管をリング状に自己組織化しました。これらのリングは複雑な3D構造を持ち、HS-AFMとTIRFMの組み合わせによって解明されました。

研究成果の学術的意義や社会的意義
A system that allows to intrinsically apply stress to any number of samples was developed and can be used study membrane mechanics or mechanical properties of soft material on a large scale.
Furthermore, ring-shaped microtubule swarms could be utilized as motors for nanomachines.

研究成果の概要（英文）：An elastic substrate stretching device to be used with a tip-scan high-speed atomic force microscope (HS-AFM) was develeloped and used to load microtubules fixed by sparsely distributed kinesin. Due to the elastic substrate experiencing simultaneous tension and compression caused by Poisson's effect, buckling of microtubules could be recorded in a controlled manner. Imaging was perfomed with a resolution of several nanometers, surpassing the resolution of conventional fluorescence microscopy. Further, stretching of microtubules could also be observed, which lead to the microtubules fracturing and depolymerizing.
Using a different approach, microtubules were self-assembled into rings by DNA-modification on kinesin substrates. These rings turned out to have a complex 3D structure that was elucidated by combined HS-AFM and TIRFM.

研究分野：Materials Science

キーワード：Microtubules　HS-AFM　TIRFM　deformation

１．研究開始当初の背景

Microtubules are protein polymer tubes that are part of the cytoskeleton. They act as transport pathways for motor proteins such as kinesin and dynein. During their activity, microtubules can experience stresses that will cause deformation by bending, compressing or stretching. These deformations are influencing the interaction of motor proteins, basically turning the microtubules into force sensors[1]. Investigating the effects of such stresses on the nanometer scale is challenging, because a precisely controllable force must be applied directly to the microtubules. Typically, this is overcome by introducing an elastic substrate covered in kinesin, which enables the transduction of deformations from the substrate to the individual microtubules. Such setups are usually used with optical microscopy methods, limiting the resolution to hundreds of nanometers. Atomic force microscopy (AFM), specifically high-speed AFM (HS-AFM) would allow for a much higher resolution in the order of 1 nm. However, such large-scale deformations make it difficult to keep the position for targeting one and the same sample over the time-span of the stress application.

２．研究の目的

The purpose was to investigate deformed microtubules on the nanometer scale using HS-AFM and apply the deformation reliably and in a controlled manner. For this a device enabling the stretching of elastic substrates needed to be developed. Further, a method to intrinsically deform the microtubules was utilized and eliminated the need for elastic substrates, allowing to use the established glass substrates.

３．研究の方法

For deforming the microtubules with an elastic substrate, a uniaxial, symmetric stretching device was used (Fig. 1). This device enabled symmetric load application to the substrate, limiting the large-scale translocations of the sample to a manageable level [2].

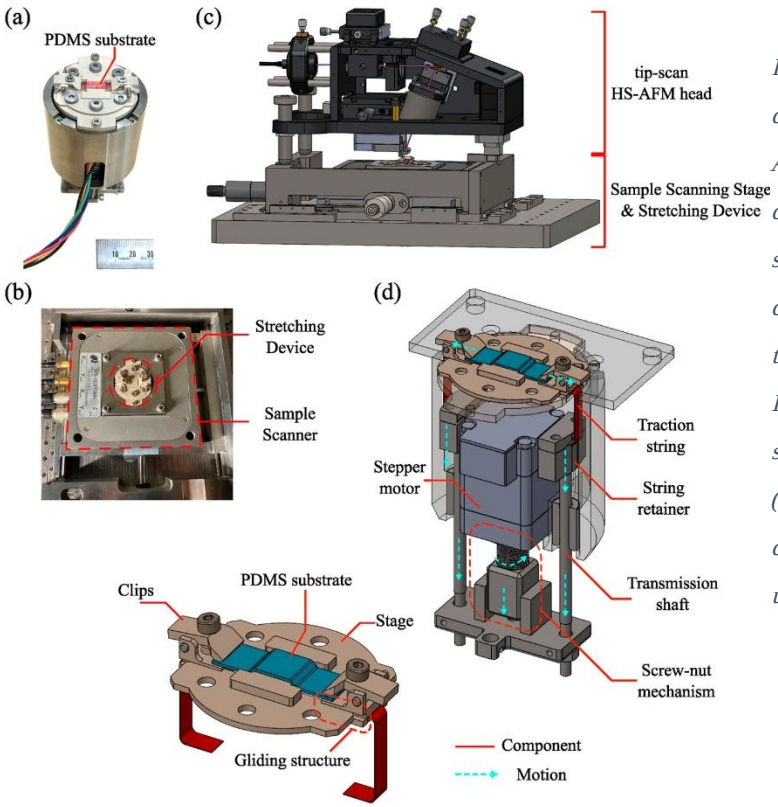

Figure 1: Uniaxial stretching device for use with tip-scan HS-AFM. (a) Photograph of the device loaded with a PDMS substrate. (b) The stretching device placed onto the stage of the HS-AFM. (c) Assembly of HS-AFM scanner head and stage with the stretching device. (d) Scheme of the stretching device, showing the principle of uniaxial stretching. From [2].

In addition to the mechanical deformation through substrate stretching, microtubules were also deformed by self-assembly into rings. In this case, the samples were fluorescently dyed

and investigated by combined HS-AFM and TIRFM on kinesin covered glass substrates. Such investigations might take a long time – typically tens of minutes – photobleaching of the dyes is an issue. Since TIRFM images can be recorded within 100 ms, but AFM images of these complex, 3D structures take around 1 s to 10 s a synchronized imaging system was established that incorporates a shutter that allows to illuminate the sample only for the duration of 100 ms at the beginning of the HS-AFM image, while the rest of the image is dark, allowing for extended investigations.

4．研究成果

An elastic PDMS substrate was sparsely covered with kinesin, which was used to fix microtubules, but leave enough degrees of freedom to allow buckling and deformation of the microtubules (Fig. 2a). Microtubules were randomly oriented on the substrate, some experiencing tension, some compression, but general a mixed load due to Poisson's effect (Fig. 2b). Buckling could be clearly observed (Fig. 2c) and by evaluating the buckling radius of the microtubule a clear decrease from 2000 nm at 3.3% to around 800 nm at 10% stress was observed. Note that a stress over 10% lead to breaking of the microtubule which can be clearly observed with the HS-AFM's superior resolution [2].

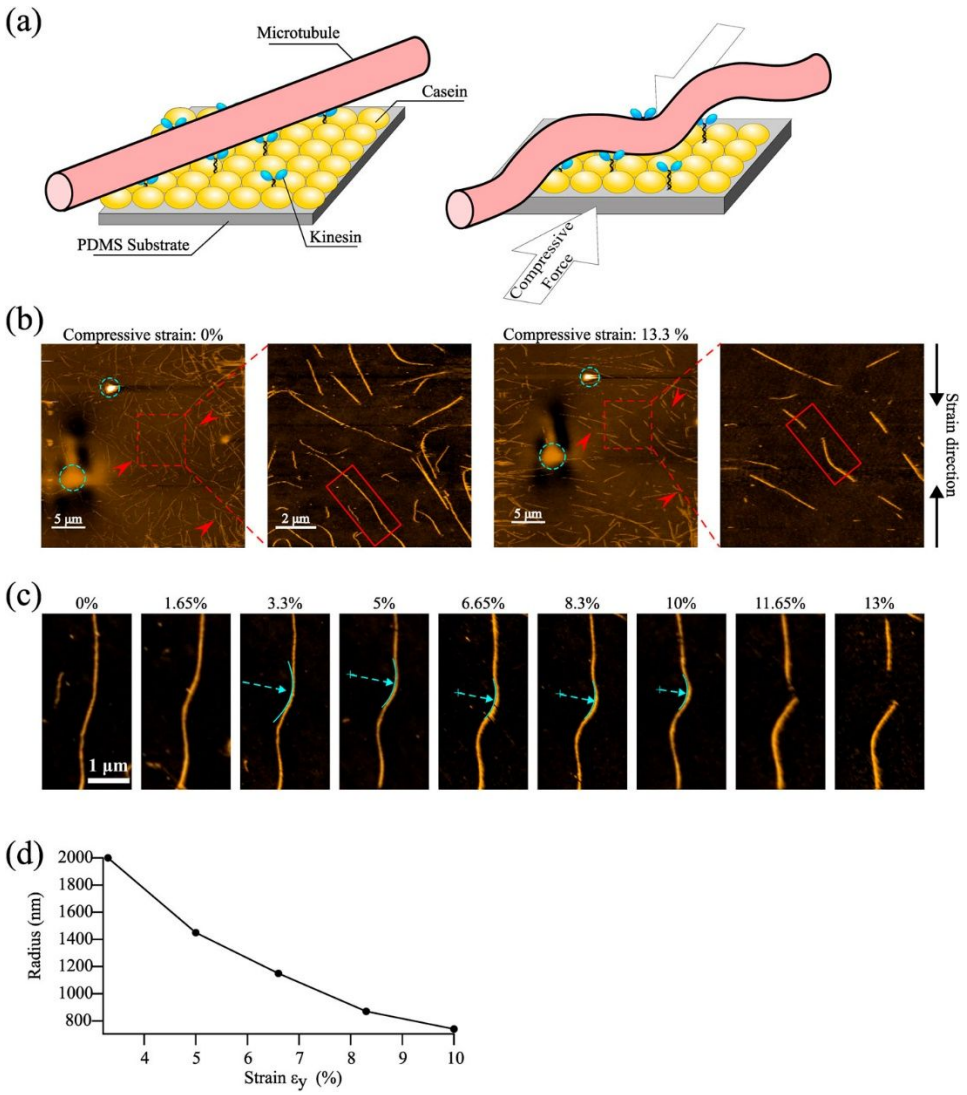

Figure 2: Controlled buckling of microtubules under load. (a) Scheme of microtubule fixation and principle of loading. (b) Large-scale observation of randomly oriented microtubules. (c) Individual buckling microtubule. (d) Relationship of buckling radius with compressive strain. From [2].

DNA-modified microtubules tend to form swarms on kinesin covered surfaces with ATP present. These swarms can self-assemble into rings which will rotate steadily as long as ATP is supplied. Using HS-AFM and TIRFM it was possible to specifically target rings (Fig. 3) and analyze their structure.

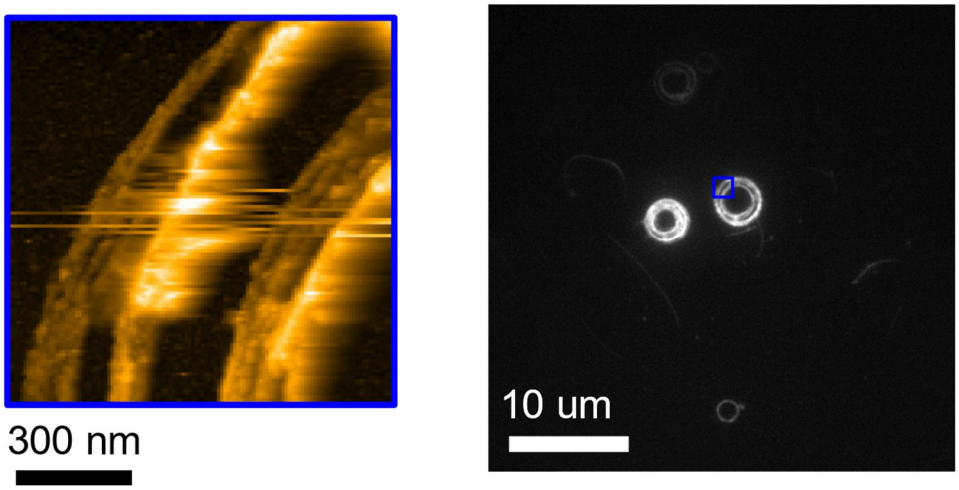

Figure 3: HS-AFM image (left) of the microtubule swarm ring indicated with a blue square in the TIRFM image (right).

This allowed the analysis of the structure of the rings and estimate the number of microtubules contained within a ring (Fig 4). It turns out that there is a clear correlation with the width of the ring and the number of microtubules contained. Furthermore, rings tend to become 3D structures when they are wider (e.g., Fig. 3) while they are single layer structures when less microtubules are contained (Fig. 4) [3].

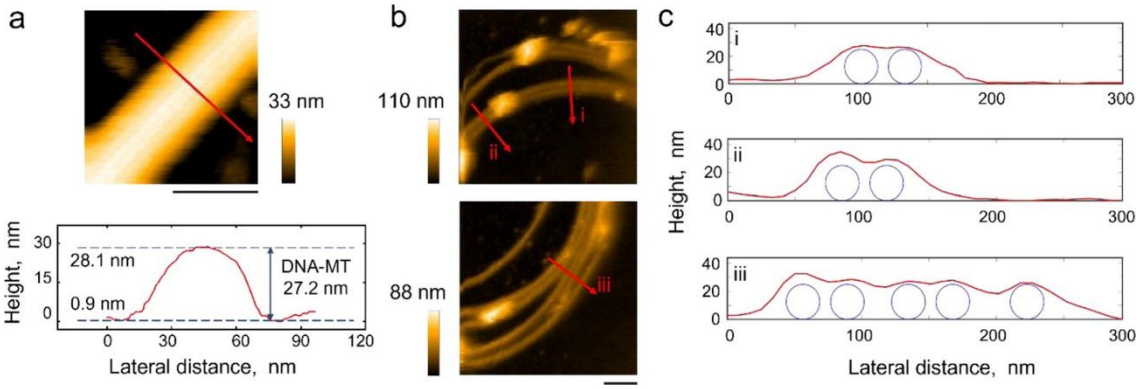

Figure 4: Principle of counting the number of microtubules within a ring. (a) Dimensions of individual microtubules. (b) HS-AFM images of microtubule rings. (c) Line profiles of the rings (red) and automatically fitted microtubules (blue) for counting. From [3].

References:

[1] Nasrin, S. R., Ganser, C., Nishikawa, S., Kabir, A. M. R., Sada, K., Yamashita, T., ... & Kakugo, A. (2021). Deformation of microtubules regulates translocation dynamics of kinesin. *Science advances*, *7*(42), eabf2211.

[2] Chan, F. Y., Kurosaki, R., Ganser, C., Takeda, T., & Uchihashi, T. (2022). Tip-scan high-speed atomic force microscopy with a uniaxial substrate stretching device for studying dynamics of biomolecules under mechanical stress. Review of Scientific Instruments, 93(11), 113703.

[3] Rashid, M. R., Ganser, C., Akter, M., Nasrin, S. R., Kabir, A. M. R., Sada, K., ... & Kakugo, A. (2023). 3D structure of ring-shaped microtubule swarms revealed by high-speed atomic force microscopy. Chemistry Letters, 52(X).

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Rashid Mst. Rubaya、Ganser Christian、Akter Mousumi、Nasrin Syeda Rubaiya、Kabir Arif Md. Rashedul、Sada Kazuki、Uchihashi Takayuki、Kakugo Akira	４．巻 52
２．論文標題 3D Structure of Ring-shaped Microtubule Swarms Revealed by High-speed Atomic Force Microscopy	５．発行年 2023年
３．雑誌名 Chemistry Letters	６．最初と最後の頁 100～104
掲載論文のDOI（デジタルオブジェクト識別子） 10.1246/cl.220491	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Chan Feng-Yueh、Kurosaki Ryo、Ganser Christian、Takeda Tetsuya、Uchihashi Takayuki	４．巻 93
２．論文標題 Tip-scan high-speed atomic force microscopy with a uniaxial substrate stretching device for studying dynamics of biomolecules under mechanical stress	５．発行年 2022年
３．雑誌名 Review of Scientific Instruments	６．最初と最後の頁 113703～113703
掲載論文のDOI（デジタルオブジェクト識別子） 10.1063/5.0111017	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計6件（うち招待講演　2件／うち国際学会　1件）

１．発表者名 Christian Ganser
２．発表標題 Studying the motor protein kinesin with high-speed atomic force microscopy
３．学会等名 International Symposium on Nanoscale Research（招待講演）
４．発表年 2021年

１．発表者名 Christian Ganser
２．発表標題 Kinesin motility inhibition studied by HS-AFM
３．学会等名 4th ExCELLS Young Researchers Retreat
４．発表年 2022年

１．発表者名
Christian Ganser

２．発表標題
The influence of microtubule deformation on kinesin motility studied with HS-AFM

３．学会等名
4th ExCELLS Symposium

４．発表年
2021年

１．発表者名
Christian Ganser

２．発表標題
High-speed atomic force microscopy based force mapping and multiscale observation in combination with fluorescence microscopy

３．学会等名
3rd ExCELLS retreat for young researchers

４．発表年
2020年

１．発表者名
Christian Ganser, Kimitoshi Takeda, Ryota Iino, Koichi Kato, Takayuki Uchihashi

２．発表標題
High-speed atomic force microscopy as a versatile tool to study dynamical and mechanical properties of proteins

３．学会等名
The 58th Annual Meeting of the Biophysical Society of Japan（招待講演）（国際学会）

４．発表年
2020年

１．発表者名
Shigetaka Nishiguchi, Christian Ganser

２．発表標題
Multimodal high-speed atomic force microscopy to investigate nano-scalemechanical properties and dynamics on the protein and cell level

３．学会等名
3rd ExCELLS Symposium

４．発表年
2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
研究協力者	角五　彰 (Kakugo Akira)		
研究協力者	内橋　貴之 (Uchihashi Takayuki)		

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関