Screening of magnetic field by self-assembled mammalian and fungal microtubules

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***Abstract*— Microtubules are essential structural elements in living organisms, which form scaffolding of the cells and participate in transport of proteins and separation of chromosomes. They are highly ordered nanotubes built of two types of tubulin proteins and filled with water. It was suggested that additionally to the transport and mechanical functions, microtubules are crucial for the processing of information. Moreover, this processing is considered to be quantum-mechanical and even based on superconductivity. Previously, screening of magnetic field, which supports superconductivity, has been observed by magnetic force microscopy in the microtubules assembled from the mammalian tubulin. Here the study is extended to the fungal self-assembled microtubules. In spite of observed structural differences between the mammalian and fungal microtubules, both display full screening of magnetic field. The temporal scans reveal steady screening in the mammalian microtubules and a fluctuating screening in the fungal microtubules. The formation of links between the microtubules and their implication for the processing and transfer of information is discussed.**

Keywords—self-assembly, microtubules, Magnetic Force Microscopy, screening, superconductivity.

I. INTRODUCTION

Microtubules are widespread in living organisms [1]. They are highly ordered nanotubes built of two types of tubulin proteins and filled with water [2]. Their typical length is 50 μm, outer diameter - from 23 to 27 nm and inner diameter - from 11 to 15 nm. Their functions range from forming scaffolding of the cells and transporting proteins along their length to being responsible for motility of the cells. Another important function of microtubules is pulling apart chromosomes at the cell division. More than that, microtubules are considered to be of high importance in the nervous system, where chemo-electrical signaling takes place [3]. Frequently, it is prescribed to their ability to form extended dendrites and axons [3] of the neurons, which are responsible for the inter-cellular communication. However, there are indications that microtubules are also crucial for quantum-mechanical processing of information [4,5]. This could be another reason why starting with bacteria [6], they are carefully preserved during the evolution of living organisms.

To process information quantum-mechanically, an organism must be in control of a quantum effect. A known effect suitable for the processing is superconductivity [4,5]. It is a macroscopic quantum phenomenon, which operates at large scales, but in this case it needs to work at room temperature. It is clear that this should be unusual type of superconductivity, because in bulk materials it commonly operates at low temperatures. This could be different, however, in systems with reduced dimensionality. High-temperature superconductors [7] are quasi two-dimensional systems, and there are supporting evidences for superconductivity in the mammalian brain coming from microtubules [8,9], which are highly ordered quasi one-dimensional structures, like in [10]. To extend the evidences for quasi two-dimensional superconductivity, and perhaps clarify trends in the evolution of microtubules, a very different from the mammalian organisms' evolutionary branch, namely fungi, has been chosen for further experiments. Microtubules in fungi are not that different from the microtubules in the mammalian cells, as they are built from the tubulin proteins, which retain 90–95% genetic similarity between the species [11,12]. However, the set of proteins facilitating their assembly is different in the species, and even small changes in their structure on the molecular level may lead to their very different behavior.

The study is based on the self-assembly of microtubules from the tubulin extracted together with the assisting proteins from a mammalian brain and a fruit body of a fungi. The Meissner effect, or expulsion of magnetic field from the microtubules [9], is used as a criterion for superconductivity. The paper reports experimental results and concludes that in spite of structural differences, there is evidence of superconductivity in both types of organisms. In fungi, superconductivity may exist in a fluctuating form with a long period of fluctuations on the scale of tens of seconds, which is much longer than time necessary for the processing of information [4].

II. EXPERIMENTAL TECHNIQUES

A. Preparation of samples and imaging

A simple technique used previously [9] was employed for preparing the samples. In case of mammalian microtubules, fixed in formalin slice of porcine brain was dissolved in water solution of graphene nano-flakes by multiple rinsing. After that, a small amount of solution, which contained tubulin and other proteins extracted from the slice, was dropped on a glass slide and set on the stage of magnetic force microscope allowing water evaporating in the presence of magnetic field. At the evaporation of water, the concentration of tubulin protein, which is main component in the solution, reaches critical value and microtubules start to assembly on the surface. The imaging of microtubules begins straight away with a nanometer resolution using a magnetic probe [9]. Consequentially recorded images allow tracing the process of the growth of microtubules, their spread on the surface, brunching, bundling parallel to each other and formation of connections between each other. During the

imaging, several signals has been recorded, including phase shift of probe oscillations, which carriers information about the distribution of magnetic field [13-15].

For the fungal microtubules, tubulin protein was extracted from the fruit body (mushroom) of *Boletus edulis* in the same way as it was done for the porcine brain. It should be mentioned that it was difficult to dissolve fragmented slice of the mushroom by multiple rinsing with the water solution of graphene nano-flakes. Therefore, one should expect a small concentration of tubulin in the final solution. The rest of the preparation and imaging was as it is described above for the porcine microtubules.

B. Magnetic force microscopy and imaging technique

For magnetic force microscopy (MFM), a scanning probe microscope JPK NanoWizard 4.0 was used. The magnetized probes NANOSENSORS (TM) PPP-MFMR were set in to monitor the magnetic state of the surface. The measurements were performed in tapping mode at the frequency of about 74 KHz. A permanent magnet of the strength of ≈0.6 T was attached to the microscope stage. Routinely, the two-scan technique [14-17] was used, with the first scan taken directly at the surface to measure sample topography and another one taken at a predetermined height of few nanometers above it following the topography revealed by the first scan. This technique allows separating short-range van der Waals interaction from the magnetic one. However, it is not perfect in cancelling effect of topography and, sometimes, electrostatic interaction is admixed to the magnetic one [15] complicating detection of the screening of the magnetic field.

Considering this, main focus here is on the scans very close to the surface allowing probe directly entering the area of screened magnetic field. Due to big difference in magnetic field outside and inside the screened area, this can lead to the instability of the probe oscillations and result in large diamagnetic shift of the phase of the oscillations. Specifically, since in the screened area probe loses attraction to the magnet, it starts drifting out of surface. In a positive feedback, this reduces attractive van der Waals and electrostatic interaction. When the probe stops drifting, all interactions except the magnetic one become weak. Moreover, at the exit from the screened area, gradient of magnetic force becomes positive. This leads to a sudden increase in the phase shift from large negative to a positive value. This technique requires a strongly magnetized probe to initiate the feedback loop before it passes the screened area. The advantage to the techniques is in high contrast of the images and fast monitoring of large areas of the samples. By the convention, the phase shift maps of the areas with negative gradients of forces are dark. Therefore, screened areas are expected to be bright.

III. Results and Discussion

Figs. 1a and 2a give two examples of the phase shift maps in the regime of probe instability, showing initial stage of the growth of mammalian microtubules and the microtubules of the mushroom *Boletus edulis*, respectively. The lower parts (b) of each figure show linear scans through the microtubules along the white lines in the upper parts. According to color scale on the top-right, the range of phase shifts is nearly the same in both figures, namely between 90 and 100 degrees with the noise level of a fraction of degree. The phase shift in the background is similar too, about minus 60 degrees. Above the microtubules (bright contrast on the images), it changes its sign. The width of the microtubules estimated from the linear scans is about 25 nm in both samples, but structurally they look very different. The mammalian microtubules are long, with a tendency of branching (see Fig. 1). They are growing in all directions from a tubulin spot visible in the right part of the figure. In contrast, microtubules of Boletus edulis (Fig. 2) are just short fragments, which, nevertheless, are well connected to each other.

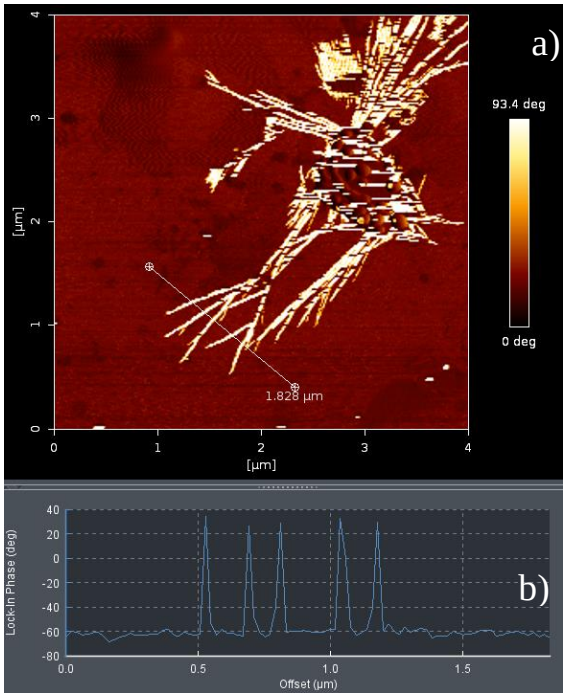

Fig. 1. a) Phase shift map of the surface of a sample with the screened areas above the mammalian microtubules seen as the bright lines. b) Linear scan along the line of the length of 1.828 µm shown by the white color in a).

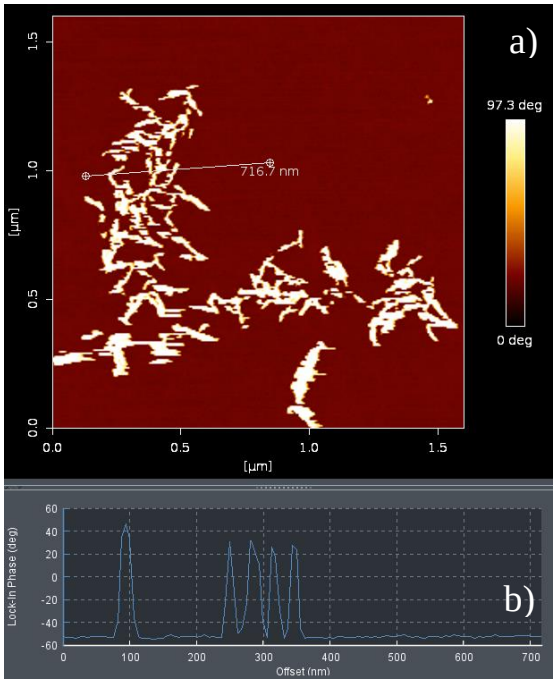

Fig.2. a) Phase shift map of the surface of a sample with the screened areas above the mushroom microtubules seen as the bright fragments. b) Linear scan along the line of the length of 716.7 nm shown by the white color in a).

More details could be found in the images recorded in the regime of stable oscillations. Fig. 3 shows two different stages of the growth of microtubules presented in Fig. 1, but taken two (a) and 21 hours (b) after the image in Fig. 1. The maps show the amplitude of oscillations. In contrast to Fig. 1, fine details are seen in the figure. With time, microtubules are extended in length and align parallel to each other forming bundles like in the neurons of nerve system [3]. This does not happen in the fungi sample. Microtubules in it remain fragmented and disordered as in Fig. 2. Taking into account that the procedure of sample preparation was the same in both cases, this may give an argument in favor of the evolutionary advantage of mammalian microtubules.

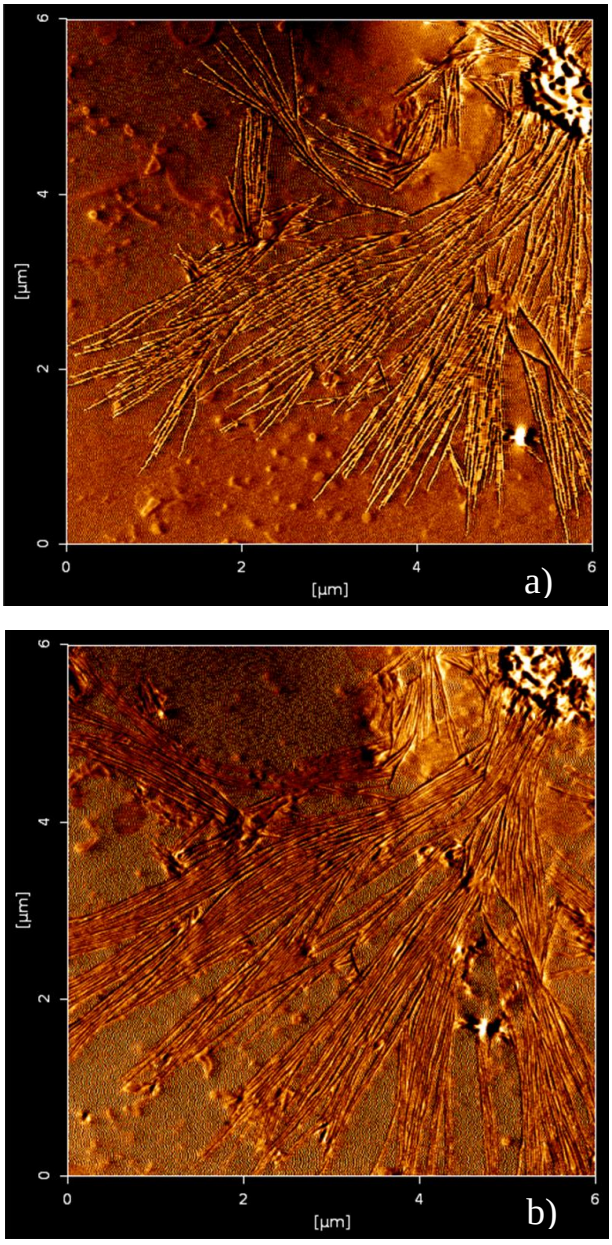

Fig.3. Amplitude of oscillation of the self-assembled mammalian microtubules presented in Fig. 1 and recorded two (a) and 21 hours (b) later than Fig. 1. The images illustrate growth of the microtubules and formation of bundles in which they are aligned parallel to each other.

One can note that in Fig. 3b microtubules are less bright than in Fig. 3a. The same takes place in phase shift maps indicating loss of the screening of magnetic field. This is a result of dehydration of microtubules, which confirms that water is critical to the superconducting state [2,8,9]. The mechanism of superconductivity in the microtubules could be similar to electron-electron interaction suggested in [10]. Here the electrons in the nano-confined water channel could interact with oscillating electrical dipoles of ordered tubulin proteins [12]. The electron-electron interaction can greatly enhance critical temperature of superconductor in comparison with its electron-phonon analog [10].

The microtubules in Fig. 2a look more evenly highlighted than in Fig. 1a. However, the images taken with higher resolution show opposite. The mammalian microtubules demonstrate reasonably homogeneous screening of magnetic field, while microtubules of *Boletus edulis*, as it is shown in Fig. 4a, have a patchy structure, in which the distribution of white spots constantly changes from image to image. This looks like propagation of waves of screening along the microtubules. These waves are slow, on the scale of tens of seconds and if processing of information takes place in the microtubules [4,5], it is unlikely to be affected by these waves.

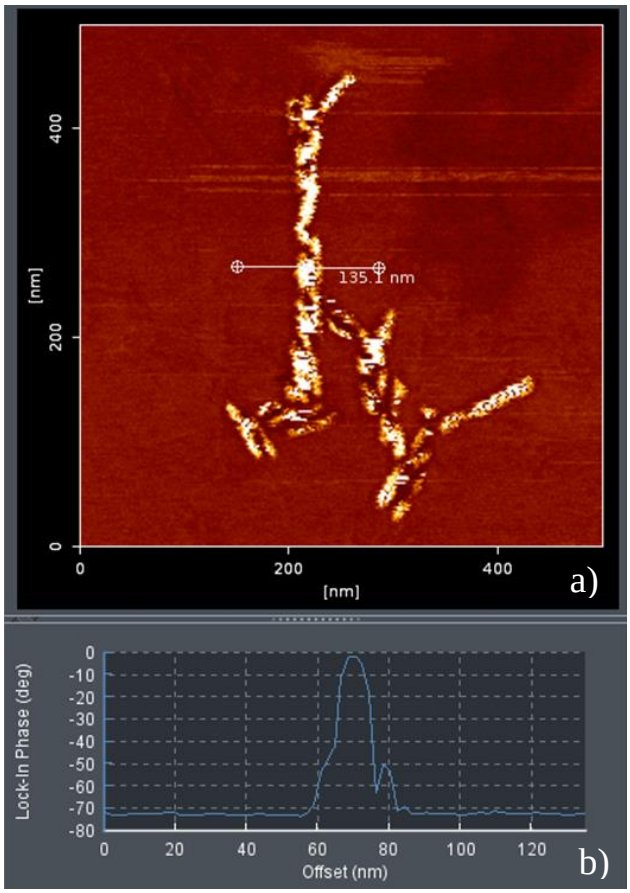

Fig.4. a) High-resolution phase shift map of fungi microtubules demonstrating a patchy structure of the expelled magnetic field. b) Linear scan along the line shown in white color in a).

Fig. 4b shows a line profile across a vertical fragment of microtubule confirming its expected diameter. The instances of branching and development of connections between the microtubules are evident in Fig. 4a. The connections are important for superconducting integrity on the large scale in the organism. It is possible that individual contacts between the microtubules are Josephson junctions, which allow electron pairs passing them without losing energy.

Eventually, after the growth, a fixed network of microtubules is formed. An example of such a network is shown in Fig. 5.

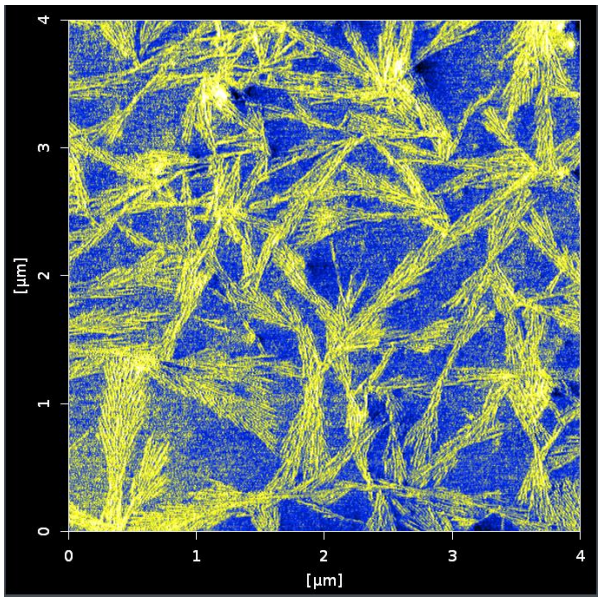

Fig.5. Phase shift map of a developed network of self-assembled mammalian microtubules. Multiple connections between the microtubules are established. The bright contrast of the microtubules reflects expulsion of the magnetic field.

The properties of the network can be controlled by varying density of nucleation centers or by adding certain nanoparticles, specifically magnetic Fe_3O_4 or Pd nanoparticles, which are not difficult to identify in MFM. Preliminary experiments show that such nanoparticles restrict length and reduce branching abilities of the microtubules.

With a high density of connections, and at the application of voltage to such Josephson network, one could expect coherent generation of electromagnetic waves [18]. It can happen naturally in certain parts of the brain. For a voltage of ≈70 mV on individual junctions, which is a membrane potential [19] in nerve cells, this generation will be in the infrared diapason with a wavelength of about 9 µm. Here it is attempted to detect this radiation with infrared camera Flir One Pro, which is sensitive to electromagnetic waves in the wavelength range from 8 to 15 µm. To generate radiation, a voltage of 50 V was applied to the samples. The result of the experiment is shown in Fig. 6 for the slice of porcine brain (a,b) and the *Boletus edulis* mushroom (c).

In (a), no voltage is applied. The red elongated segments are metal electrodes, which reflect ambient radiation present in the room. The slice of brain is seen in the image as blue region between the electrodes. With application of the voltage of 50 V in b), the bright spot of radiation appears between the electrodes. In contrast, in the slice of the mushroom, two bright spots appear close to the electrodes, where the most intensive heating, and, as consequence, strong blackbody radiation is expected. The central peak in b) could be the evidence of partial Josephson radiation, as the voltage of 50 V approximately corresponds to characteristic potential of ≈70 mV on the contacts between the bundles of microtubules in the brain slice. This technique could be a simple way of producing coherent infrared radiation from a natural Josephson network, although it is currently not clear how much blackbody radiation is admixed to it.

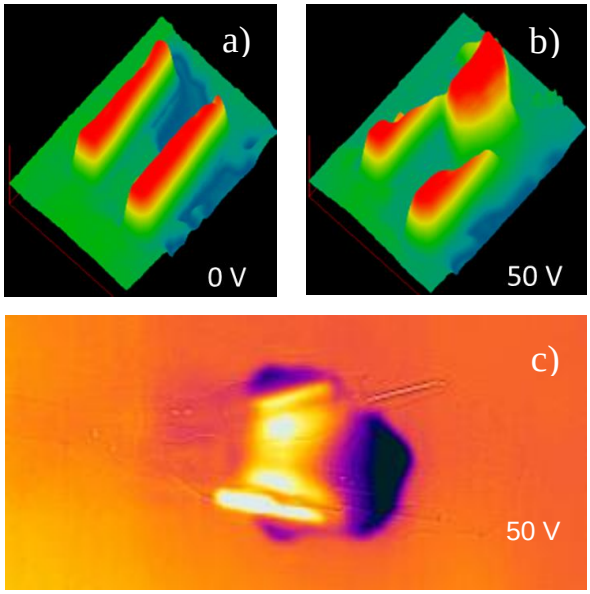

Fig.6. Infrared radiation from the slice of porcine brain (b) and mushroom (c) at application of voltage of 50 V. In (a), no voltage to the slice of brain is applied. The red segments in a) are metal electrodes that reflect ambient radiation. The slice of brain is seen as blue region between the electrodes in a).

Proving existence of coherent Josephson radiation in living organisms would be additional argument in favor of superconductivity and an evidence of mechanism for the fast, with the speed of light, transfer of information inside and between living organisms. Thus, superconductivity could offers not only quantum processing in the organisms [5], or effects based on entanglement of united electrons [20], but also fast delivery of quantum information.

IV. Conclusions

Magnetic force microscopy of self-assembled microtubules coming from different evolutional branches of living organisms, namely mammals and fungi, reveals differences and similarities in their growth and formation of contacts between them. The microtubules assembled from the tubulin of mammalian brain are long, spread in all directions from a nucleation cite, branch intensively and form parallel bundles. In contrast, microtubules formed from the tubulin of the fruit body of a fungus *Boletus edulis* are short, fragmented, but still well connected with each other. In spite of these differences, both types of microtubules show screening of magnetic field, which is considered to be consequence of superconductivity. Due to the entanglement of paired electrons in the superconducting state or other effects, nanoscale superconductivity might be a key to quantum processing of information in living organisms. Additionally, superconductivity can provide fast, with the speed of light, delivery of quantum information via coherent Josephson radiation. The detection of infrared waves from a slice of brain at application of voltage could be the evidence of such a radiation. To be coherent, a high density of Josephson contacts is required. The control over the growth

of microtubules by the addition of nanoparticles might be a solution for achieving required density of the contacts.

REFERENCES

[1] D. A. Fletcher and R. D. Mullins, “Cell mechanics and the cytoskeleton,” Nature, vol. 463, pp. 485–492, January 2010.

[2] S. Sahu, S. Ghosh, B. Ghosh, K. Aswani, K. Hirata, D. Fujita, and A. Bandyopadhyay, “Atomic water channel controlling remarkable properties of a single brain microtubule: Correlating single protein to its supramolecular assembly,” Biosens. Bioelectron., vol. 47, pp.141–148, September 2013.

[3] W. Yu and P. W. Baas, “Changes in microtubule number and length during axon differentiation,” Journal of Neuroscience, vol. 14, pp. 2818-2829, May 1994.

[4] S. Hameroff, “Quantum computation in brain microtubules? The Penrose–Hameroff ‘Orch OR’ model of consciousness,” Phil. Trans. R. Soc. A, vol. 356, pp. 1869-1896, August 1998.

[5] P. Mikheenko, “Nano Superconductivity and Quantum Processing of Information in Living Organisms,” IEEE Xplore Digital Library 9309703, January 2021.

[6] M. Pilhofer, M. S. Ladinsky, A. W. McDowall, G. Petroni, and G. J. Jensen, “Microtubules in Bacteria: Ancient tubulins build a five-protofilament homolog of the eukaryotic cytoskeleton,” PLoS Biol., vol. 9, e1001213, December 2011.

[7] K. A. Müller, and J. G. Bednorz, “The discovery of a class of high-temperature superconductors,” Science, vol. 237, pp. 1133-1139, Sep 1987.

[8] P. Mikheenko ”Possible superconductivity in the brain,” J. Supercond. Novel Magn., vol. 32, pp. 1121–1134, Dec. 2018.

[9] P. Mikheenko, “Ideal Diamagnetism in Brain Microtubules,” IEEE Xplore Digital Library, 9934729, November 2022.M.

[10] W. A. Little, Possibility of synthesizing an organic superconductor, Phys. Rev. 134, A1416–A1424 (1964). DOI: 10.1103/PhysRev.134.A1416.

[11] S. Sahu, S. Ghosh, D. Fujita, and A. Bandyopadhyay, “Live visualizations of single isolated tubulin protein self-assembly via tunneling current: effect of electromagnetic pumping during spontaneous growth of microtubule,” Scientific Reports, vol. 4, 7303, December 2014.

[12] J. A.Tuszynsky, E. J. Carpenter, J. T. Huzil, W. Malinski, T. Luchko, and R. F. Luduena, “The evolution of the structure of tubulin and its potential consequences for the role and function of microtubules in cells and embryos,” Int. J. Dev. Biol., vil. 50, pp. 341–358, March 2006.

[13] D. Sarid. Scanning Force Microscopy, Oxford University Press, New York, U.S.A. (1994).

[14] D. Passeri, C. Dong, M. Reggente, L. Angeloni, M.O Barteri, F. A. Scaramuzzo, F. De Angelis, F. Marinelli, F. Antonelli, F. Rinaldi, C. Marianecci, M. Carafa, A. Sorbo, D. Sordi, I. WCE Arends, and M. Rossi, “Magnetic force microscopy. Quantitative issues in biomaterials,” Biomatter, vol. 4, e29507, July 2014.

[15] L. Angeloni, D. Passeri, M. Reggente, D. Mantovani, and M. Rossi, “Removal of electrostatic artifacts in magnetic force microscopy by controlled magnetization of the tip: application to superparamagnetic nanoparticles,” Scientific Reports, vol. 6, 26293, May 2016.

[16] S. Schreiber, M. Savla, D. V. Pelekhov, D. F. Iscru, C. Selcu, P. C. Hammel, and G. Agarwal, Magnetic Force Microscopy of Superparamagnetic Nanoparticles, Small, 4, No. 2, 270 – 278 (2008). DOI: 10.1002/smll.200700116.

[17] G. Cordova, B. Y. Lee, and Z. Leonenko, “Magnetic Force Microscopy for Nanoparticle Characterization,” NanoWorld J., vol. 2, pp. 10-14, April 2016.

[18] P. Barbara, A. B. Cawthorne, S. V. Shitov, and C. J. Lobb, “Stimulated Emission and Amplification in Josephson Junction Arrays,” Phys. Rev. Lett., vol. 82, 1963, March 1999.

[19] L. A. Kadir, M. Stacey, and R. Barrett-Jolley, “Emerging Roles of the Membrane Potential: Action Beyond the Action Potential,” Front. Physiol. Sec. Membrane Physiology and Membrane Biophysics, vol. 9, 1661, November 2018.

[20] Z. B. Tan, A. Laitinen, N. S. Kirsanov, A. Galda, V. M. Vinokur, M. Haque, A. Savin, D. S. Golubev, G. B. Lesovik, and P. J. Hakonen, “Thermoelectric current in a graphene Cooper pair splitter,” Nature Communications, vol, 12, 138, January 2021.