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Fluorescence Visualization of Carbon Nanotubes Using Quenching Effect for Nanomanipulation

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Abstract—We demonstrate visualization method of carbon nanotubes (CNTs) in water with fluorescent microscopy through quenching effect that cause a decrease of fluorescent intensity around CNTs. Reversal contrast in fluorescence imaging of the CNTs was observed in fluorescent dye solution. We could observe CNTs under a constant excitation light for more than ten minutes. Dielectrophoretic force was used for CNTs deposition onto electrodes that consist of transparent conductive film. Scanning electron microscope revealed that CNTs of 80 nm in diameter were observed with fluorescent microscopy. The positions of the CNTs tips were observed under optical microscope. It can be applied for biological sensing devices with nanomanipulation.

Keywords- optical microscopy; carbon nanotubes; dielectrophoresis; fluorescence; quenching effect

I. INTRODUCTION

Carbon nanotubes (CNTs) are molecular wires that exhibit interesting structural, mechanical, electrical, and thermal properties [1]. CNTs are used as building blocks such as scanning probes [2], field emission sources [3], chemical sensors [4], and transistors [5].

Observation of CNTs in water could make a device for biology and *in situ* assembly using dielectrophoresis. In order to apply CNTs in water, nanometer-scale resolution microscopes, e.g. electron microscopes or atomic force microscopes are needed, however, it's practically difficult to observe CNTs with these microscopes in water.

Fluorescent labeling of CNTs was attempted to observe CNTs in water using both covalent and noncovalent modifications. Fluorescent imaging has been demonstrated using organic dyes [6], fluorescent polymers [7], quantum dots [8], and fluorescent DNA [9]. The imaging of CNTs in optical microscopes has caught some attention recently, but results have been limited. Mostly, approaches related to immobilization of fluorescent dyes suffer from less quantum yield and short fluorescence lifetime. This was attributed to fluorescence-quenching through pi-stacking of CNTs. It was reported that a decrease of fluorescence intensity in the porphyrin CNTs solution compared with that of the porphyrin solely solution [10]. To reduce quenching effect, many fluorescent dyes should be attached onto CNTs surface. But this excessive attachment may deteriorate the useful properties of CNTs, e.g. high thermal conductivity.

For achieving visualization of CNTs, we focused on a quenching effect. It was expected that an intensity of fluorescent dyes around metallic CNTs can be decreased due to energy transfer. CNTs proximity to fluorescent dyes quenched fluorescence and dark clouds were observed under optical microscope [11]. Photoluminescence intensity of carbon nanotubes is dramatically reduced by aggregation of the isolated nanotubes [12]. It was presumed that the presence of a metallic nanotube within a bundle quench electronic excitation on an adjacent semiconducting tube preventing its luminescence.

Dielectrophoretic force was used to deposit CNTs onto a large number of electrodes [13], fabricate short CNT tips [14], and retrieve DNA from an aqueous solution [15]. In our case, transparent conductive oxide film was used to retrieve CNTs from an aqueous solution for the same sample comparison under both an optical and electron microscope. The transparent film is suitable for observation under optical microscope.

To capture individual CNTs from water, CNTs should be dispersed by chemical treatment. Conventional dissolution methods of CNTs in water by physical adsorption are as follows. CNTs are dispersed in aqueous micellar solution of surfactants, sodium dodecylsulfate [16]. DNA molecules dissolve CNTs in aqueous solutions [17]. Cyclic aromatic compounds can be used to obtain soluble nanotubes [18]. Our approach is use of N-(1-pyrene) iodoacetamide. This substance is reactive to a thiol group and able to immobilize proteins onto sidewall of CNTs.

II. EXPERIMENTS

CNTs were prepared by a conventional direct current arc discharge method. Nanotubes thus prepared had multiple walls, were 20-90nm in diameter with an average of 60 nm. Solubilization procedure was as follows: For noncovalent functionalization of suspended CNTs, a sample was incubated in N-(1-pyrene) iodoacetamide solution in dimethyl sulfoxide for 24 hours at room temperature, after which the sample was rinsed three times in distilled water to wash away excess reagent. Then we made a mixture of dispersed CNTs and fluorescent dye. Water soluble Rhodamine B was used in this study for observation of quenching effect.

Two optical microscopes were used for this work: a BX51 microscope and an IX70 inverted microscope with charge

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coupled device (CCD) camera. Samples were observed using dark-field and fluorescence imaging with BX51. 100x oil immersion objective lens was used to observe samples under an optical microscope. In order to avoid soiling the samples from oil, 40x dry objective lens was used to compare images captured with optical and scanning electron microscope.

For the comparison of an optical microscopy and electron microscopy image, a transparent electrode was fabricated. Indium tin oxide (ITO) was chosen as the transparent electrode material, because of its high transparency and conductivity. ITO was etched with Focused ion beam and deep ultra violet laser in Fig.1 (a), (b). The electrode gap of 1 μm was designed according to the length of carbon nanotubes in this study.

Figure 1 shows an illustration of the experimental setup of MWNTs retrieval. In Fig.1(c) the electrode was put up side down to keep the electrode surface clean by precipitation of CNTs. Double-coated conductive tape was used as a flow chamber component and a wiring to an alternating power supply. After the flow chamber was filled with CNTs dispersion, an AC field of 300 mV at 1 MHz was applied between the ITO electrodes, which contained the CNTs/water suspension. The motion of CNTs was recorded as a dark-field microscope image during this operation. Samples were imaged using dark field, fluorescence imaging. After the BX51, optical microscope observation, CNTs were dried and put into the scanning electron microscope chamber to measure the diameter of CNTs.

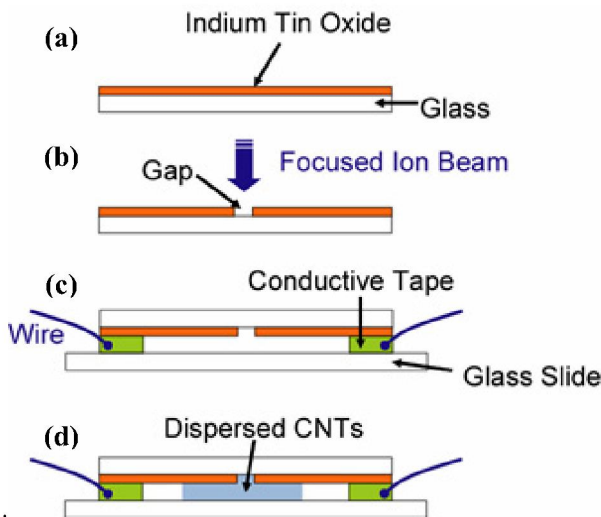


Figure 1. Schematic illustrations of an ITO flow chamber fabrication process (a) Indium tin oxide coated glass substrate. (b) Etched gap with focused ion beam. (c) Flow chamber assembled with conductive tape. (d) After filling the flow chamber with dispersed CNTs.

To attach CNTs onto tungsten needle, the needle was coated with an adhesive by dipping it into hydrophobic photo curable resin. An AC field of 1V at 1MHz was applied between the tungsten needle and the metal holder, which contained the nanotube/water suspension. CNTs were attracted to the tungsten needle tip and the needle was pulled out from CNTs suspension under the supply of the AC current. CNT were fixed with photo cured resin by irradiating near ultraviolet light with

a mercury lamp. Optical microscope examination revealed that the position of CNTs in water.

III. RESULTS AND DISCUSSION

CNTs solely were insoluble in water as shown in Fig. 2(a). Figure 2(b) shows that N-(1-pyrene) iodoacetamide can disperse CNTs. Although precipitation were noticed after one day at room temperature, CNTs in water dispersed again by pipetting several times.

In Fig. 3, the CNTs are clearly visible in both the dark-field image and fluorescent image. The fluorescence image shows a reversal of contrast. We interpret this image as indicating energy transfer from fluorescent dyes to the CNTs. We could observe CNTs under a constant excitation light for more ten minutes. It's more difficult to observe samples above cover slip than on the surface of cover slip, because of background

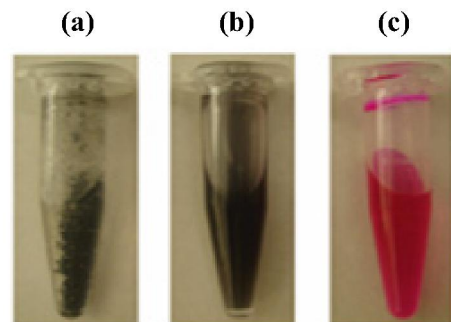


Figure 2. (a) a water dispersion of CNT solely, (b) a water solution/dispersion of CNTs and N-(1-pyrene) iodoacetamide and (c) a water solution of Rhodamine B.

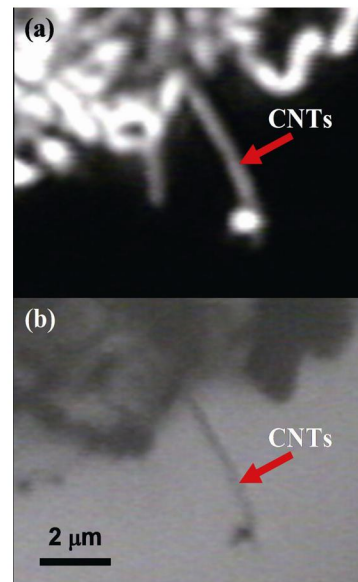


Figure 3. (a) Dark field and (b) fluorescence images of CNTs in a water solution of Rhodamine B.

light which came from fluorescent dyes. And a contrast of images between CNTs and background became worse. Furthermore, if density of dyes was dense, fluorescent intensity was too strong to observe samples with a CCD camera. When density of dyes became little, fluorescent intensity was too weak to observe samples with a CCD camera. In this study, concentration of fluorescent dyes $2 \mu\text{M}$ made a good result. Some CNTs were not observed with fluorescent microscopy. Difference between observable CNTs and not observable one might be based on CNTs properties, metallic and semiconducting. Another reason was difference of CNTs diameter. For observation of CNTs by quenching effect, fluorescent molecules around CNTs are needed. Therefore compared to dark-field and bright-field microscope, observation conditions by quenching effect are limited in fluorescent molecules solution.

We observed that CNTs stacked on the edges of the electrodes gradually under dark-field microscope. The ITO electrode make observation under SEM easier to find the same sample attached onto the electrodes under optical microscope. Compared to using gold electrode, the ITO electrodes reduce reflection and scattering from electrode material. When the voltage was increased, it was observed that not only CNTs but also amorphous carbon and bundles of carbon nanotubes attracted to the electrode. To observe CNTs in SEM, gently removing CNTs from water was required. CNTs were hard to remained, because CNTs were attached weakly via van der Waals force. Letting the samples dry naturally made a success. On the other hand, blowing or sucking water caused strong flow, and CNTs were not remained. When not using dispersion treatment, although shortly after CNTs were sonicated, dielectrophoretic force at 300 mV couldn't retrieve CNTs from water, because of the insolubility of the nanotubes in water. Many of samples attracted to electrode were thought as metallic CNTs, because metallic CNTs have high dielectric constants [19]. For applying this method to another material, dielectric constant of the sample should be higher than that of solvent to attract the sample onto electrode.

We could observe CNTs attached onto both sides of ITO electrodes as shown in Fig.4 (a), (c). SEM observation revealed that the diameter of CNTs was 80nm in Fig.4 (d). Because of high light absorption of CNTs, bright-field microscopy image can be seen without using phase contrast and differential interference contrast microscopy. If a high numerical aperture of an objective lens, for example, oil immersion lens is used, thinner CNTs are assumed to be observed under optical microscope.

Attachment of CNTs to a tungsten needle was observed under the optical microscope in real time. When applied voltage was increased, the amount of CNTs to be attached at the needle tip was increased. By observing the CNTs, we can fabricate CNT tips easily, since we can control the amount of CNTs. When a CNT tip was moved in and out of water, the CNT bent and adhered to the surface of the tungsten needle. Therefore CNT tip couldn't hold the surface tension. CNT high Young's modulus is suitable for a probe application. Although CNT has high mechanical property, long carbon nanotube was broken down easily. To increase a spring constant of CNT tip, the length of CNT should be shorten. For shortening process,

cutting method of CNT in SEM by electron beam with oxygen gas [20] or mechanical manipulation can be applied. Pyrene succinimidyl esters, amine reactive probes can be used to disperse CNTs. In this case, however, dispersibility is worse than using N-(1-pyrene) iodoacetamide. CNT probe can be fabricated by using these amine reactive probes. This amine reactive pyrene can be used as a linker to protein adhesive [21]. This adhesive property is able to attach streptavidin onto a CNT probe for an investigation of myosin and actin filament interaction [22].

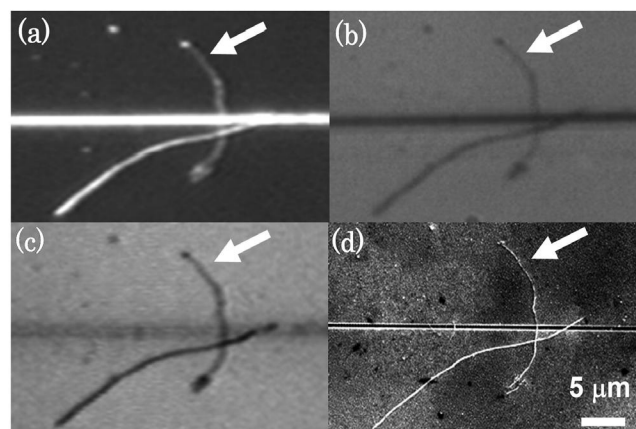


Figure 4. (a) Dark-field, (b) bright field, (c) fluorescent, and (d) scanning electron microscopy image of CNTs attached onto ITO electrode. The CNTs indicated by the red arrows are 80 nm in diameter.

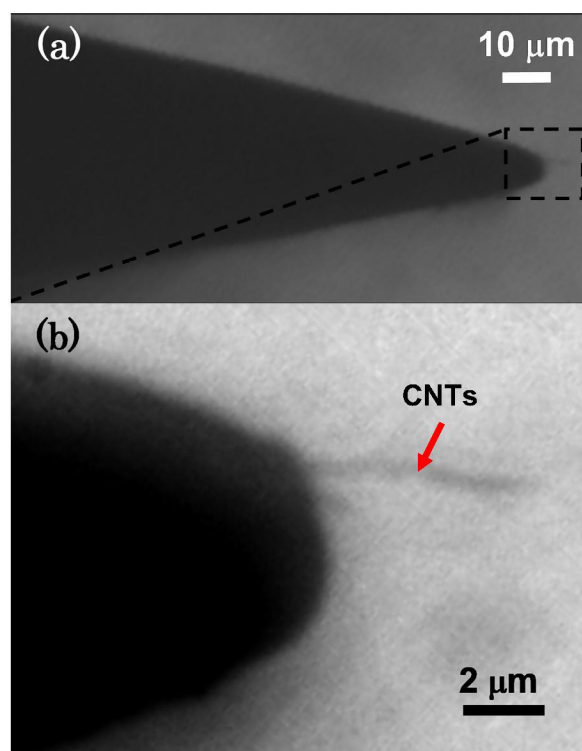


Figure 5. Bright-field microscopy image showing the CNTs attached onto the tungsten needle (a) and the high magnification image of the CNT with 5 μm long on the needle tip (b).

IV. CONCLUSION

We have demonstrated real-time observation of CNTs with optical microscope with fluorescent dyes. For easy manipulation of CNTs in water, firstly, CNTs were dispersed by chemical treatment. Then, dielectrophoretic force was used to assemble CNT on the ITO transparent electrodes. Finally, CNTs were visualized under optical microscopes. We have confined that our optical visualization method worked properly by the comparison of images of optical and electron microscope. Using the proposed real-time observation method, CNT tips were fabricated and the amount of CNTs was controlled. Observation of CNTs will lead us to make several devices for manipulation under the optical microscope for cytological investigation in an aqueous environment.

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