Imaging of soft material with carbon nanotube tip using near-field scanning microwave microscopy

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In this manuscript, a near-field scanning microwave microscope (NSMM) of our own design is introduced while using a multi-walled carbon nanotube (MWCNT) bundle as the tip (referred to as ‘CNT tip’). Clear images of gold-patterned numbers, photoresist stripes and corneal endothelial cells (cell line B4G12) were obtained by mapping the resonant frequency f r and S 11 amplitude of a given area while the NSMM is operating in tapping mode. The CNT tip helps to improve image quality and reveals more information about the sample as compared to a traditional metallic tip. The CNT tip is flexible and does not scratch the surface of the sample during the scan, which is useful for imaging soft material in biological science. In the imaging of the B4G12 endothelial cells, the nuclei and cytoplasm can be clearly distinguished from the rest of the cell and its surrounding medium.

1. Introduction

The developments of the near-field scanning microwave microscope (NSMM) over the last two decades have enhanced the NSMM’s ability in making local quantitative measurements such as the dielectric constant and to perform surface imaging. The NSMM is used to observe minute changes in the structure and electromagnetic properties of the sample that perturb the electrical interaction between the probe tip and the sample in the microwave near-field region. These perturbations change the amplitude of the reflected signal S 11 and shifts the resonant frequency f r , which can be measured by the NSMM. As a result, the NSMM [1–3] is capable of revealing the distribution of electrical properties of bulk materials and dielectric films in ‘soft contact’ mode by applying a tiny force between the probe and the sample. However, a metallic tip is required to have a non-conductive coating when measuring highly conductive materials when the NSMM is operating in contact mode [4].

The NSMM is also capable of producing scanned images in other modes like in fixed-distance mode, where the tip–sample separation is held constant during the scan, or in constant resonant frequency mode, where the tip–sample distance is regulated while the conductive samples are being scanned [5]. Hybrid scanning techniques were explored with the NSMM, and combinations with the scanning tunneling microscope (STM)/NSMM [6,7] and atomic force microscope (AFM)/NSMM [8,9] were also developed to exploit the tomographic capability of both non-biological [10] and biological materials [11–13]. Specialized tips like the GaN nanowire probe [14] were also developed for the NSMM, with the aim of improving the spatial resolution of imaging.

The performance of NSMM depends heavily on the material and geometry of the tip. The probes in NSMM are usually hard and both the sample surface and the probe may inevitably be damaged after long periods of scanning. In scanning microscopy, carbon nanotubes have been manufactured as a probe due to their extraordinary thermal conductivity, mechanical and electrical properties [15,16]. Experimental results and simulations demonstrate that multi-walled carbon nanotube (MWCNT) bundles which are used as tips (referred to as ‘CNT tips’) are nearly independent on frequency within the microwave frequency range [17]. Although commercial CNT tips are available for use as an atomic force microscopy (AFM) cantilever probe, the use of the CNT tip in the AFM is still not popular. For the NSMM, the use of the CNT is even rarer.
In this article, we describe the use of a MWCNT bundle as a tip in a NSMM of our own design. The distribution of the resonant frequency \( f_r \) and the \( S_{11} \) amplitude of a scanned area were plotted for both the contact mode and tapping mode. The use of the CNT tip on highly-conducting and dielectric materials were also investigated, and clear images of gold numbers, photoresist stripes and corneal endothelial cells (cell line B4G12) were obtained with CNT tip with the NSMM operating in tapping mode. The CNT tip does not only improve the image quality, but as a flexible probe, it also protects the sample surface from being scratched. The CNT tip in NSMM is also much more durable as compared to metallic probes (e.g. Tungsten probes) and based on our initial experience, the CNT tip (in tapping mode) can repeatedly tap and make contact with the sample for at least \( 10^6 \) times without being damaged. Thus, the use of CNT tips in the NSMM has great potential, especially when imaging soft material.

2. Experimental setup and details

Fig. 1(a) shows a near-field scanning microwave microscope (NSMM) system of our own design [18,19]. It consists of a high quality factor \( Q \) \( \lambda/4 \) coaxial resonator [3], a scanning platform attached to \( x-y \) direction motors, a piezoelectric motor, a vector network analyzer (VNA) (Agilent Technologies N5230A), and some lenses mounted to a charge-coupled device (CCD). The resonator is placed in an aluminum holder and is attached to the piezoelectric motor which controls the \( z \)-direction, while the sample is placed on the top of the scanning platform which is controlled by the \( x-y \) direction motors. Side-view images of the tip and sample are captured in real-time by the lens and CCD, which are displayed on a LCD monitor. The VNA measures the amplitude of the reflection coefficient \( S_{11} \) (in dB) as a function of frequency and displays these values on a graph. The maximum value of the \( S_{11} \) amplitude and the resonant frequency \( f_r \) are both determined at the maximum of the resonance peak. A program written in LabVIEW is used to control the system and to collect data from the VNA.

A tungsten tip with a CNT bundle is mounted on the central conductor of the \( \lambda/4 \) coaxial resonator. The schematic diagram of the CNT tip in the NSMM during scan is shown in Fig. 1(b), with the CNT tip bending as it makes contact with the sample surface. The tungsten tip is fabricated by anode corrosion with 1 M NaOH and the diameter of the tip is approximately 50 \( \mu \)m. A MWCNT bundle (average diameter is 5 \( \mu \)m) is adhered to the tungsten tip using silver paste, and the CNT tip is covered with cyanoacrylate (Loctite 460, Henkel Technologies, \( \varepsilon_f = 2.75 \)), which helps to bundle the individual CNTs together and to reduce mechanical damage to the CNTs when the scanning is being performed by the NSMM. The cyanoacrylate layer also helps to stabilize the microwave signal when the CNT tip is swept across a dielectric and conductive boundary of the sample during contact mode. The tip can be adjusted vertically in the \( z \)-direction by using the piezoelectric motor, while the sample can move horizontally in the \( x-y \) direction by controlling the \( x-y \) motors.

Fig. 1(c) is the equivalent lumped series resonant circuit that has different electrical components representing different parts of the NSMM. The interaction between the CNT tip and the sample is represented by an effective resistance \( R_{\text{tip}} \) and effective capacitance \( C_{\text{tip}} \), which may vary as the tip-sample distance is changed. \( R_{\text{sample}} \) is the effective resistance of the sample, while the substrate has an effective resistance \( R_{\text{sub}} \) and effective capacitance \( C_{\text{sub}} \). Any change within the NSMM such as moving to a different measuring point or varying the tip sample distance would cause a response to be generated by the system, which can be seen as a change in the effective resistance or capacitance of a component within the electrical circuit.

3. Results and discussions

The vertical position of the CNT tip can be controlled by changing the input voltage of the piezoelectric motor as seen in Fig. 1(a), Fig. 2(a) and (b) shows the measured resonant frequency \( f_r \) and the amplitude of the reflection coefficient \( S_{11} \) respectively as a function of the tip-sample distance for a glass plate, photoresist film (Allresist AR-P 5350) on a glass plate, a silicon wafer and an aluminum plate. The zero point of the tip position is defined to be at the inflection of the resonant frequency and \( S_{11} \) amplitude curves and these points are marked with a vertical dashed line as shown in Fig. 2. At the zero point, the CNT tip makes contact with the sample beneath it. The resonant frequency and \( S_{11} \) amplitude changes as the tip-sample distance increases.

For a positive tip position as shown in Fig. 2, the tip is not in contact with the sample. Thus, the tip position is related to the distance between the tip and the sample (i.e. tip-sample distance, \( d \)). The resonant frequency \( f_r \) increases while the \( S_{11} \) amplitude decreases with increasing tip-sample distances for all the samples shown in Fig. 2. As the tip-sample distance increases, the tip-sample capacitance \( C_{\text{tip}} \) (in Fig. 1(c)) decreases (because \( C_{\text{tip}} \propto 1/d \)), which decreases the total capacitance of the system. Since \( f \propto 1/\sqrt{C} \), the resonant frequency \( f_r \) will therefore increase with an increasing tip-sample distance. Meanwhile, the tip-sample distance directly affects the amount of power reflected back into the tip and hence the \( S_{11} \) amplitude. The \( S_{11} \) amplitude is at maximum at 0 dB and indicates total reflection of the incoming signal towards the tip. As the tip leaves the sample (i.e. increasing \( d \)), more power will be radiated into
from both the resonant frequency and frequency Fig. 2 would be useful in identifying the material composition of a sample distance) when the tip does not make contact with the sample, while a on the tip-sample distance respectively for a glass plate, a photoresist the tip position is de...


tip position makes contact with the sample at a fixed tip position, which is controlled by the piezoelectric motor. The CNT tip would then be swept across the sample like a broom during the scan. In tapping mode, the CNT tip is driven up and down by the piezo-electric motor mounted in the holder which contains the resonator with the CNT tip. By fine tuning the voltage of the piezoelectric motor, it can ensure that the CNT tip makes contact with the sample to obtain better images. This also helps to prevent the sample surface from being scratched by the CNT tip.

For a fixed tip position, a comparison of the resonant frequency and $S_{11}$ amplitude curves of the different materials presented in Fig. 2 would be useful in identifying the material composition of a given scanned image. For example, glass has a higher resonant frequency $f_r$ and lower $S_{11}$ amplitude as compared to photoresist. Hence, this result allows the glass and photoresist to be identified from both the resonant frequency and $S_{11}$ plots which are generated by the NSMM.

The CNT tip as shown in Fig. 1(b) is flexible and durable, which allows our NSMM system to operate in two different scanning modes: contact mode and tapping mode. In contact mode, the CNT tip position makes contact with the sample at a fixed position, which is controlled by the piezoelectric motor. The CNT tip would then be swept across the sample like a broom during the scan. In tapping mode, the CNT tip is driven up and down by the piezo-electric motor mounted in the holder which contains the resonator with the CNT tip. By fine tuning the voltage of the piezoelectric motor, it can ensure that the CNT tip makes contact with the sample to obtain better images. This also helps to prevent the sample surface from being scratched by the CNT tip.

Fig. 3 shows images of a gold-patterned number ‘02’ collected by the NSMM while operating in both contact mode and tapping mode. The images obtained in contact mode are shown in Fig. 3(a) and (d) with plots of the $f_r$ and $S_{11}$ values respectively. Although the number ‘02’ can be clearly seen in both images, scan lines and ghosting of the images are also observed. The scan lines may be due to the CNT tip picking up dirt particles as it sweeps across the image, while the ghosting and distortion of the image may be caused by the deformation of the CNT tip. Thus, it may not be preferable to have the CNT tip make contact and be dragged across the sample surface during the scanning process. In contrast, Fig. 3(b) and (e) presents the same image of the gold-patterned number ‘02’ obtained by the NSMM in tapping mode for the $f_r$ and $S_{11}$ values respectively. These images are relatively clearer and have no dragged lines which make the tapping mode the preferred method of scanning. Also, in the optical image of Fig. 3(e), a defect is observed in the number ‘0’. This detail is also clearly observed in the NSMM tapping mode images of Fig. 3(b) and (e). Both the lower $f_r$ and higher $S_{11}$ values of the defect in Fig. 3(b) and (e) respectively indicate that the defect is protruding outwards from the gold-patterned number surface (i.e. the defect is higher than all other parts of the sample). This was deduced from the data in Fig. 2 as a lower $f_r$ and a higher $S_{11}$ value indicate a smaller tip-sample distance.

To demonstrate the non-invasive operation of the NSMM with a CNT tip, the NSMM was made to scan stripes of photoresist in tapping mode. A poor image quality may result if the CNT tip is able to scratch the surface of the photoresist and damage it. However, Fig. 4 show clear images of photoresist stripes on a glass plate when the NSMM is operating in tapping mode with a CNT tip. The stripes of photoresist were prepared by photolithography with a width of 10 μm (periodicity of 20 μm) and a thickness of around 1.2 μm. The height difference of the photoresist stripes affects the resonant frequency $f_r$ and $S_{11}$ amplitude during the NSMM measurement. As seen in the Fig. 2 data, the photoresist on glass has a lower resonant frequency $f_r$ and a higher $S_{11}$ amplitude as compared to a bare glass plate. Thus, in Fig. 4(a), the lower resonant frequency corresponds to a photoresist surface, while a higher resonant frequency corresponds to the exposed glass plate which is located between the gaps of the photoresist stripes. Similarly, in Fig. 4(b), a higher $S_{11}$ amplitude corresponds to a photoresist surface, while a lower $S_{11}$ amplitude corresponds to the glass plate. Fig. 4(c) is a line scan of the resonant frequency $f_r$ along the black line shown in Fig. 4(a), while Fig. 4(d) is the line scan of the $S_{11}$ amplitude along the black line in Fig. 4(b). The step size of the line scan is 0.24 μm for both Fig. 4(c) and (d). It can be observed from the respective resonant frequency $f_r$ and $S_{11}$ amplitude curves of Fig. 4(c) and (d) that the NSMM can resolve stripes that are 10 μm wide and are spaced 20 μm apart. A clear difference of 0.3 MHz in the resonant frequency $f_r$ and about 0.1 dB in the $S_{11}$ amplitude is observed for the stripes of photoresist and the glass plate in Fig. 4(c) and (d) respectively. From the measurement of the fuzzy fringes in Fig. 4(c) and (d), the lateral resolution of the NSMM with a CNT tip and operating in tapping mode is found to be about 2.5 μm.

The NSMM is also capable of imaging biological soft tissue, which we have done for corneal endothelial cells (cell line B4G12) [20]. To prepare the cells for imaging, the glass cover slips that were used to hold the cells were treated with plasma for three minutes, sterilized and left overnight with a coat of FNC Coating Mix™ to improve cell adhesion. The B4G12 corneal endothelial cells were then seeded with a seeding density of 6000 cells/cm² and cultured for three days in Dulbecco’s Modified

Eagle’s Medium (Sigma Aldrich) supplemented with 10% fetal bovine serum (Gibco, Invitrogen) and 1% Penicillin Streptomycin (Life Technologies). After three days, the cells were fixed with 4% paraformaldehyde. The cover slips with the fixed cells were then taken out of the paraformaldehyde solution and dehydrated naturally in ambient atmosphere for one day.

**Fig. 3.** NSMM images of a gold-patterned number ‘02’ on a glass plate with the CNT tip in different scanning modes. The image of the resonant frequency $f_r$ when the NSMM is operating in (a) contact mode and (b) tapping mode. (c) A binarized optical image of the gold-patterned number ‘02’. The image of the $S_{11}$ amplitude when the NSMM is operating in (d) contact mode and (e) tapping mode. All the arrows point to the position of the defect in the gold-patterned number ‘02’ as shown in (c).

**Fig. 4.** NSMM image of photoresist stripes on a glass plate. The stripe width is 10 $\mu$m and has a periodicity of 20 $\mu$m. The images shown are for (a) the resonant frequency $f_r$ and (b) the $S_{11}$ amplitude when the NSMM is in tapping mode. (c) The line scan image for the resonant frequency $f_r$ along the black line shown in (a). (d) The line scan image for the $S_{11}$ amplitude along the black line shown in (b). The step size for the line scans in (c) and (d) is 0.24 $\mu$m and the lateral resolution is around 2.5 $\mu$m.
Fig. 5 shows the images of endothelial cells (B4G12) that were prepared using the method as described above. Fig. 5(a) shows an optical image of fixed B4G12 cells with an average area of about $600 \mu m^2$ [21], while Fig. 5(b) shows a phase contrast image of a pair of adjacent B4G12 cells which appears flat and tightly bound to the cover slip. It can be observed that each B4G12 cell has a few protrusions along the cell border and while these B4G12 cells are connected. This pair of adjacent cells will be imaged using the NSMM. Fig. 5(c) and (d) shows the three-dimensional plots of resonant frequency $f_r$ and $S_{11}$ amplitude while Fig. 5(e) and (f) show the contour plots of resonant frequency $f_r$ and $S_{11}$ amplitude for the pair of cells shown in Fig. 5(b). Fig. 5(c)–(f) were obtained by operating the NSMM with a CNT tip in tapping mode. In Fig. 5(c) and (e) [Fig. 5(d) and (f)] of the NSMM images, the red [blue] elliptic

![Fig. 5](image)

Fig. 5. (a) The optical image of corneal endothelial cells (B4G12) on a glass cover slip. (b) The phase contrast image of a pair of adjacent B4G12 cells. The same pair of cells in are imaged with the NSMM operating in tapping mode with a CNT tip, and are shown in the following plots: (c) resonant frequency $f_r$ 3D plot, (d) $S_{11}$ amplitude 3D plot, (e) resonant frequency $f_r$ contour plot, and (f) $S_{11}$ amplitude contour plot. The red areas in the NSMM image corresponds to the nuclei, the yellow and green areas are the contour of cells, and the dark blue areas correspond to a thin layer of cytoplasm of the B4G12 cell. Red arrows in (e) and (f) point to the nuclei of cells, while black arrows point to one of the long lobes in the cell that is visible and distinguishable. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
areas correspond to two nuclei of the cells, and they are clearly indicated by red arrows in Fig. 5(e) [Fig. 5(f)]. The yellow and green [light blue] area correspond to the cytoplasm of endothelial cells of B4G12, which are similar to the contours that appear in the optical image of Fig. 5(b). The lobes of the pair of B4G12 cells can also be identified from the NSMM images and the longest lobe is marked with black arrows in Fig. 5(e) and (f). Finally, the dark blue [dark red] area in the NSMM images of Fig. 5(c) and (e) [Fig. 5(d) and (f)] correspond to a thin layer of cytoplasm within the B4G12 cells. It may be difficult to distinguish between the cytoplasm and the medium surrounding the cell in the optical image of Fig. 5(b), but it can clearly be distinguished in the NSMM images of Fig. 5(c)–(f).

4. Conclusions

MWCNTs were used as tips in a near-field scanning microwave microscope (NSMM) of our own design, which were capable of imaging conductive and non-conductive samples in both contact mode or tapping mode. The CNT tip helps to improve the image quality as well as to protect the sample surface from being scratched. Clear images of gold-patterned numbers, photoresist stripes and corneal endothelial cells (B4G12) were obtained by mapping the resonant frequency $f_0$ and $S_{21}$ amplitude of a given scan area by operating the NSMM with the CNT tip in tapping mode. In the imaging of the B4G12 cells, the nuclei and cytoplasm can be clearly distinguished from the rest of the cell and its surrounding medium, which shows the potential of the NSMM in cellular imaging. This work may shed light for further developments and applications of the NSMM technique in imaging soft material in the biological sciences.

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