Visualization of arrangements of carbon atoms in graphene layers by Raman mapping and atomic-resolution TEM

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In-plane and out-of-plane arrangements of carbon atoms in graphene layers play critical roles in the fundamental physics and practical applications of these novel two-dimensional materials. Here, we report initial results on the edge/crystal orientations and stacking orders of bi- and tri-layer graphene (BLG and TLG) from Raman spectroscopy and transmission electron microscopy (TEM) experiments performed on the same sample. We introduce a new method of transferring graphene flakes onto a normal TEM grid. Using this novel method, we probed the BLG and TLG flakes that had been previously investigated by Raman scattering with high-resolution (atomic) TEM.

T
he electronic band structures and physical properties of graphene layers depend on the in-plane and out-of-plane arrangement of their carbon atoms, which are reflected in their edge/crystal orientations and stacking orders, respectively. The influence of the edge orientation on the physical properties of graphene flakes and nanoribbons has been predicted theoretically and measured experimentally1–10. Recently, the stacking sequence of graphene layers, that is, the out-of-plane arrangement of the carbon atoms, has been the focus of research. For example, many interesting and important physical properties have been discovered in ABC-stacked trilayer graphene11–17.

Several techniques, such as atomic force microscopy (AFM), scanning tunneling microscopy (STM), transmission electron microscopy (TEM) and Raman spectroscopy, have been used to study the edge/crystal orientations and the stacking orders of graphene18–30. Among them, Raman spectroscopy with Raman mapping is the most favored due to its high spectral efficiency (tens of milliseconds integration time for a clear spectrum), non-destructiveness and lack of special sample preparation. In addition to identifying the arrangements of carbon atoms in graphene, Raman spectroscopy is also able to determine the number of layers31,32, the electronic structure33–35, electron-phonon interactions36–38, phonon dispersion relations39, doping levels40–42, defects43,44, and strains45–48. Although Raman spectroscopy has been widely adopted in graphene research, its intrinsic spatial resolution of microns limits the direct visualization of the carbon atoms on an atomic scale. Thus, high-resolution AFM, TEM and STM are also useful. It is now possible to resolve every single carbon atom by HRTEM with the development of the aberration-corrected, monochromated TEM microscope20,49,50.

Graphene flakes are usually prepared by mechanical exfoliation of natural graphite crystals or highly oriented pyrolytic graphite (HOPG) on a silicon substrate with a 300 nm silicon dioxide layer (SiO2/Si)51. To perform TEM experiments on graphene flakes on SiO2/Si substrates, it is necessary to transfer pre-selected graphene flakes onto a TEM grid. Thus far, only a few efficient methods to transfer graphene flakes have been proposed. The best one, so far, promises about a 25% success rate using a very expensive and specially fabricated TEM grid52.

In this work, we report on the identification of the in-plane and out-of-plane arrangements of carbon atom layers (bi- and tri-layers) by Raman spectroscopy and high-resolution TEM characterization. With a simple, one-step pre-treatment of the SiO2/Si substrate by O2 plasma, we improved the existing method for transferring graphene flakes and gain a success rate of 70%. Furthermore, we used a normal rather than a specially fabricated TEM grid. Thus, for the first time, we were able to study bi- and tri-layer graphene (BLG and TLG) flakes by Raman spectroscopy and then transfer them onto a normal TEM Cu grid for TEM study. As in our earlier observations of single-layer graphene (SLG), the dominant edge orientations and the crystal orientations of BLG
and TLG could be determined by monitoring the D band intensity and G band position. In addition, we were able to use TEM to visualize the arrangements of the carbon atoms at the atomic level to substantiate our Raman results.

**Results**

Figure 1 shows our modified transfer process. The key step that guarantees a high success rate and relaxes the requirement of a C-flat TEM grid is treatment of the SiO$_2$/Si substrate with O$_2$ plasma. As can be seen in Figures 1(a1) and 1(b1), the hydrophilicity of the surface of the SiO$_2$/Si substrate increased with O$_2$ plasma treatment, as indicated by the change in the contact angle from 49° to 17°. This is mainly due to the modification of the surface structures of the siloxane groups (Si-O-Si) in the as-purchased SiO$_2$/Si substrate to silanol groups (Si-OH) in the O$_2$ plasma-treated SiO$_2$/Si substrate. Immediately after the O$_2$ plasma process, the graphene flakes were mechanically exfoliated onto the pre-treated SiO$_2$/Si substrates.

These flakes, identified by optical microscopy and Raman spectroscopy, were then carefully covered by a normal TEM grid. We added a small drop of isopropanol alcohol (IPA) on top of the grid, which instantly spread out across the entire surface including the areas underneath graphene flakes because the silanol groups populate the surface of the O$_2$ plasma-treated SiO$_2$/Si substrate and attract the IPA molecules through hydrogen bonding. Thus, the superhydrophobic graphene flakes were lifted up and came into contact with the carbon film of the grid. The contact between the graphene flakes and carbon film of the grid was strengthened by the surface tension during the full evaporation of the IPA through illumination with a small incandescent lamp. To lift up the grid with the graphene flakes, we dropped a second droplet of IPA next to the grid. After the grid was then picked up and dried carefully in air, the suspended graphene flakes were ready for further investigation with TEM and Raman spectroscopy after some necessary cleaning processes.

The transfer of a TLG flake mechanically exfoliated from natural graphite crystals is shown in Figure 2. The graphene flake on the O$_2$ plasma-treated SiO$_2$/Si substrate was located by an optical microscope and further probed by Raman spectroscopy and mapping. After the first drop of IPA, the flake had the same contrast as the as-exfoliated flake (Figure 2(b)). As shown in Figure 2(c), the color of the flake changed from blue to gray after the second drop of IPA. This color change is a sign of success: the graphene flake had fully detached from the substrate and had become strongly anchored to the carbon film of the grid. Figure 2(d) shows a low-magnification TEM image and Raman images of the graphene flake after transfer onto the TEM grid.

Optical images recording the transfer of a BLG flake exfoliated from natural graphite crystals are presented in the Supporting Information (SI; Figure S1). Optical images of recording the transfer of graphene sheets with different numbers of layers exfoliated from HOPG are shown in Figure S2.

As previously described, we are interested in graphene flakes that have an angle of $(2n + 1) \times 30$ degrees (where $n$ is an integer between 0 and 5) between two adjacent edges. In such flakes, it is highly likely that one edge is dominated by zigzag orientation while...
the other is dominated by armchair orientation or vice versa. A BLG flake with a right angle corner is shown in Figure 3(a). The lineshape and width of the G' (2D) peak indicate that this flake is a Bernal-stacked BLG. The Raman spectra of the two edges indicate a remarkable difference in the defect (D) and G modes, particularly the integrated intensity of the D mode and the position of the G mode in relation to one edge and the other. We used Raman mapping to clarify details about the entire region, especially the two edges, with the polarization of the incident laser 45 degrees to each edge, which ensured that the polarization effect influenced the two edges equally. Figures 3(b) and 3(c) show the Raman images extracted from the D mode integrated intensity and the G mode position, respectively. As observed in SLG\textsuperscript{21}, one edge is bright, indicating an armchair orientation, while the other is relatively dark, suggesting its zigzag orientation. The obvious contrast of the D mode intensities from zigzag and armchair orientations could be understood by inter-valley double resonant scattering\textsuperscript{53}. Compared to the SLG\textsuperscript{21}, the contrast of BLG is not remarkable, at least in this flake, which might be due to the lower chance of having a neat edge in thick layers prepared by mechanical exfoliation. The Raman modes selection rule and the Kohn anomaly result in a difference in the G mode positions in zigzag- and armchair-dominated edges\textsuperscript{23,24}, which agrees with the identification by the D mode intensity.

To study the crystal structure of this BLG flake at the atomic level, we transferred the same sample to a normal TEM Cu grid. Figure 4(a) shows a typical low-magnification TEM image of the BLG flake together with that of a TLG flake. The schematic diagram of the edge/crystal orientation of the BLG, identified by our Raman study, is presented in the optical image in Figure 4(b), where the two flakes are oriented along the same direction in the TEM image. Although the most interesting corner (as indicated by Raman mapping in Figure 3) was scrolled during transfer process, the entire BLG flake should share the same crystal orientation because it was prepared by peeling it off from natural single crystalline graphite. In fact, our HRTEM study indicates that the TLG has the same crystal orientation (not shown here). Figure 4(c) presents an HRTEM image of the BLG without a filter. Although we controlled the voltage during our TEM measurements to as low as 60 kV, obvious damage, such as “kicking off” the top layer by the e-beam, can be clearly seen. The damage rate is reduced in this image as compared with images made at higher kV. Inset of Figure 4(c) is the fast Fourier transform (FFT) of the HRTEM image of Figure 4(c). The peak positions of the FFT at different spatial frequencies show the armchair and zigzag orientations of the graphene layers. As shown in the inset of Figure 4(c), the red line connecting the symmetrical pair of dots on the inner circle (corresponding to 0.213 nm spacing) is perpendicular to the zigzag direction of the graphene layers, while the green line connecting the symmetrical pairs of dots on the outer circle (corresponding to 0.123 nm spacing) is parallel to the armchair direction of the graphene layers. (d) An atomic-level HRTEM image of the BLG. The inset of (d) shows the line intensity profile of the light blue dotted line. The obtained readings perfectly match the lattice spacing of graphene.

**Figure 3** | (a) An optical image of BLG and TLG. The right-angle corner of BLG is highlighted. (b) A Raman D band integrated intensity image and (c) the Raman G band position of the highlighted area. The green arrows indicate the polarization of the incident laser. (d) Typical Raman spectra of the zigzag and armchair edges of BLG.

**Figure 4** | (a) A TEM image of the BLG and TLG flakes after being transferred onto a normal TEM Cu grid. (b) An optical image of the BLG and TLG with the schematic diagram of the crystal orientation determined by Raman spectroscopy. The green and red lines indicate the armchair and zigzag directions, respectively. (c) An HRTEM image of the BLG. The inset of (c) is FFT of (c). The red line connecting the symmetrical pair of dots on the inner circle (corresponding to 0.213 nm spacing) is perpendicular to the zigzag direction of the graphene layers, while the green line connecting the symmetrical pairs of dots on the outer circle (corresponding to 0.123 nm spacing) is parallel to the armchair direction of the graphene layers. (d) An atomic-level HRTEM image of the BLG. The inset of (d) shows the line intensity profile of the light blue dotted line. The obtained readings perfectly match the lattice spacing of graphene.
Figure 5 shows an optical image and Raman investigations of TLG. In addition to the in-plane arrangement, TLG has interesting out-of-plane arrangements, its so-called stacking order. It can be seen from the optical image of the graphene sample shown in Figure 5(a) that there are angles of 120 and 150 (or 210) degrees between two adjacent edges. The homogeneous contrast of the Raman images of the G band intensity shown in Figures 5(c) and 5(f) clearly indicates that the sample thickness or the number of graphene layers is the same over the entire sheet except for the folded part at the bottom. The white light contrast spectrum (not shown here) and the Raman spectra (Figures 5(i) and 5(j)) indicate that this is a TLG flake except for the folded part. The detailed Raman mapping of the D mode (Figures 5(d) and 5(g)) reveals that the two edges of the 120 degree angle are dominated by the armchair orientation while the other edge of the 150 degree is dominated by the zigzag orientation. To determine the stacking order, we plotted the Raman images of the G' mode width in Figures 5(e) and 5(h). The broader linewidth of the G' mode in the ABC-stacked TLG is the consequence of its unique electronic band structures, especially at the low energy level, and of the strong electron-phonon coupling in graphene as discussed previously. The other spectral differences between ABA- and ABC-stacked TLG, such as the G mode position and width and the relative shift of the combinational modes, are also observed here (Figure 5(i)).

Figure 6 shows the TEM measurements of the same TLG studied by Raman spectroscopy are presented in Figure 6. The low-magnification TEM image (Figure 6(a)) indicates that the TLG flake was transferred from the SiO$_2$/Si substrate to the normal TEM Cu grid without any noticeable change. The edge/crystal orientation of the TLG, identified by the Raman study, is shown in the optical image in Figure 6(b), where the TLG flake is rotated along the same direction in the TEM image for comparison. Figure 6(c) shows the HRTEM image of the ABA-stacked domain without a filter. The enlarged image (Figure 6(d))
allows for zigzag and armchair orientation identification, which can also be identified by the FFT of the image, as in inset of Figure 4(c). The zigzag and armchair directions in the image and the line intensity profile also match our Raman measurements.

A reliable way to identify the ABA-stacked (Bernal) and ABC-stacked (rhombohedral) TLG by HRTEM is to compare their defocus-dependent patterns\(^2\). As shown in Figures 7(c) and 7(d), in addition to the inversion of the contrast (black to white, marked with red circles in Figures 7(c) and 7(d)), the contrast of the individual dots in ABA-stacked TLG is significantly different when the defocus value is changed. For example, it can be seen in Figure 7(d) that three dots appear to have the most intense contrast (either darkest (marked with red solid dots) or brightest (marked with green solid dots)). This is due to the strongest diffraction of the e-beam by the three overlapping carbon atoms in the three layers, in contrast to the relatively weak contrast of the dots caused by the two overlapping carbon atoms or by non-overlapping carbon atoms. In contrast to ABA-stacked TLG, there are always two overlapping carbon atoms along the c-axis in ABC-stacked TLG. Therefore, the contrast of the diffracted dots is uniform regardless of the focus level\(^2\). This is demonstrated in the HRTEM images shown in Figures 8(c) and 8(d). All the dots (marked with red and green solid dots in Figures 8(c) and 8(d), respectively) have the same intensity even though their contrast changes from white to black (marked with red circles in Figures 8(c) and 8(d)) when the defocus is varied.

**Discussion**

Meyer et al.\(^52\) achieved a ~25% success rate by transferring graphene flakes from SiO\(_2\)/Si substrates onto a specially fabricated TEM grid. We found that treatment of the SiO\(_2\)/Si substrate by O\(_2\) plasma increased the success rate to more than 70% even when the graphene flakes were transferred from SiO\(_2\)/Si substrates onto a normal TEM grid. This was mainly because graphene samples on the O\(_2\) plasma-treated SiO\(_2\)/Si substrates were easier to detach from the substrate because of the enhanced hydrophilicity of the SiO\(_2\) surfaces from O\(_2\) plasma treatment.

Our simple yet efficient method for transferring graphene flakes onto a normal TEM grid allowed us to study the in-plane and out-of-plane arrangements of carbon atoms in BLG and TLG by both Raman spectroscopy and HRTEM. By monitoring the Raman spectral features such as the integrated intensity of the D mode, the position of the G mode and the width of the G’ mode, we could precisely identify the edge/crystal orientations and the stacking orders of BLG and TLG. HRTEM measurements of exactly the same BLG and TLG flakes allowed us to visualize the structures of individual carbon atoms and reiterated the Raman measurements. Our work has successfully demonstrated that Raman spectroscopy and HRTEM imaging can be used together to determine the edge/crystal orientations and stacking orders of graphene layers, which is useful for our further understanding of the fundamental physics of graphene layers and for developing applications based on the unique properties of graphene layers influenced by their edge/crystal orientations and stacking orders.

**Methods**

In this work, graphene samples were prepared by micromechanical cleavage of natural graphite crystals or HOPG\(^5\) and transferred on O\(_2\) plasma-treated 300 nm SiO\(_2\)/Si substrates. The treatment of SiO\(_2\)/Si substrates by O\(_2\) plasma was performed using March PX-250 plasma system with 100 W power and 70 mTorr base pressure. Pure oxygen gas was used as the plasma source. The targeted graphene flakes were transferred onto a normal TEM Cu grid based on the method developed by Meyer et al.\(^52\). Raman spectra and images were obtained by a WITec CRM200 confocal microscopy Raman system with a piezocrystal controlled scanning stage. The excitation laser wavelength was 532 nm and its spot size was around 500 nm. To avoid damage and heating, the laser power was controlled below 2 mW. TEM measurements were carried out on an aberration-corrected and monochromated FEI Titan 60–300 microscope. The microscope was operated at 60 kV to minimize the knock-on damage to the graphene. The spherical aberration of the microscope was tuned to a negative value of about 6 μm, and the energy spread of the electron beam was reduced to below 0.2 eV from about 0.8 eV so that the information limit was better than 1.4 angstroms. The images were recorded on a charge-coupled device (CCD) camera (2 k × 2 k, Gatan UltraScan\(^3\))

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