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1 **Title:**

2 Quasi-static 3D structure of graphene ripple measured using aberration-corrected TEM

3

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11 **KEYWORDS:**

12 graphene, ripple, aberration-corrected TEM, 3D reconstruction

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1 ABSTRACT

2 Free-standing graphene has a three-dimensional (3D) structure, called a ripple, rather than a perfect
3 two-dimensional (2D) crystal. Since theoretical calculations suggest that a ripple strongly
4 influences various fundamental physicochemical properties of graphene, it is important to clarify
5 the ripple structure quantitatively in experiments. This paper proposes a new method of measuring
6 the 3D atomic structure of a ripple by using aberration-corrected transmission electron microscopy
7 (TEM). The method utilizes the fact that the 2D contrast of a TEM image is sensitive to the height
8 of a six-membered ring. The proposed method is experimentally applied to a monolayer graphene,
9 and the 3D atomic arrangements of consecutively acquired TEM images are reconstructed. In that
10 experiment, the specimen is found to be moving upward. Furthermore, the atomic arrangement
11 can be approximated as a composite of two structures consisting of a 3D ripple and a 2D plane.
12 The ripple is represented as a superposition of sinusoidal waves, with their wave vectors coinciding
13 with the specific direction of the six-membered ring. The time dependences of the height and
14 lateral size of the ripple are also measured.

15

1 **Introduction**

2 Graphene is a two-dimensional (2D) crystal in which hexagonal lattice structures
3 composed of sp^2 bonds of carbon atoms are arranged ¹. On the other hand, it is known to have a
4 three-dimensional (3D) structure that deviates from a completely flat 2D structure and that is
5 classified into three types, depending on size: ripple, wrinkle, and crumple, with the ripple being
6 the smallest ². The existence of the ripple structure was first revealed in an experiment ³. That
7 analysis of electron diffraction patterns of free-standing monolayer graphene revealed the
8 existence of a ripple with a height and lateral size of about 0.5 nm and 5 nm, respectively. Monte
9 Carlo simulations supported the results of that experiment ⁴. The ripple's effects on graphene
10 properties have also been reported ⁵⁻⁷.

11 There are two main kinds of reports on the origin of the ripple. One kind focuses on an
12 intrinsic property that eliminates the thermodynamic instability of graphene ^{4,6}. The other focuses
13 on extrinsic properties such as defects and grain boundaries in graphene ⁸⁻¹⁰, the structure of a
14 substrate supporting graphene ^{11,12}, or the tension applied from the substrates ¹³. Moreover, whether
15 the ripple is static or dynamic has been a subject of studies. Transmission electron microscopy
16 (TEM) measurements of electron diffraction patterns revealed that ripples are static ³. The first
17 measurement by scanning tunneling microscopy (STM) concluded that a ripple is static because
18 similar structures were observed repeatedly ¹⁴. On the other hand, in a study that used STM to
19 observe the movement of one carbon atom in the height direction by STM, the atom moved several
20 tens of Å on the order of seconds ¹⁵. A study of atomic force microscopy (AFM) observations
21 found that one carbon atom continues to oscillate about 1 Å on the order of nanoseconds ¹⁶. The
22 TEM diffraction pattern revealed that the lattice vibration increases with increasing temperature ¹⁷.

1 Using ultrafast electron crystallography (UEC), two types of dynamic responses on the order of
2 picosecond were elucidated¹⁸.

3 Two kinds of methods using aberration-corrected electron microscopy have been reported
4 for the 3D imaging of ripples in free-standing monolayer graphene. One kind includes scanning
5 atomic electron tomography¹⁹ and the measurement of the apparent shrinkage of the interstitial
6 distance due to the slope on an atomic-resolution image of TEM/STEM^{10,20}. The other kind uses
7 the fact that the phase contrast of aberration-corrected TEM changes sensitively with respect to
8 height or defocus^{21,22}. In two reports, height was calculated from the intensity of one carbon atom
9 and a monolayer graphene was 3D reconstructed with an accuracy within 1 Å^{23,24}. However, the
10 method can be applied only to an image acquired very close to the exact focus where the intensity
11 is sensitive linearly to height differences; it becomes difficult to measure a ripple if the specimen
12 fluctuates or tilts significantly.

13 In this paper, we propose a new method of measuring the quasi-static 3D structure of a
14 pristine monolayer graphene. This method makes use of the height sensitivity of the phase contrast
15 of the six-membered ring in an aberration-corrected TEM image. The atomic arrangement can be
16 accurately reconstructed over a wide range of height changes without being significantly affected
17 by specimen tilt. We have also devised a method of quantitatively analyzing the quasi-static 3D
18 structure of graphene. This method revealed that a ripple can be represented as a superposition of
19 sinusoidal waves, with their wave vectors coinciding approximately with the specific direction of
20 the six-membered ring.

21

1 **Materials and methods**

2 **Library of simulated images of six-membered rings**

3 Using a flat monolayer graphene model, we constructed a library of 201 simulated images
4 with defocus varying from -100 Å to +100 Å in 1 Å steps. The library was acquired using the
5 multislice method (software: MacTempasX from HULINKS), which is an established wavefield
6 calculation method for electron microscopy. The procedure is described as follows. First, the size
7 of the supercell was set so that the size of one pixel coincided with the TEM image in the
8 experiment. In the center of the supercell, a flat monolayer graphene with a nearest-neighbor
9 atomic distance of 1.421 Å was placed with the rotation in the x-y plane aligned with the TEM
10 image. In the simulation, the same acceleration voltage and aberration (spherical aberration, two-
11 fold astigmatism, three-fold astigmatism, axial coma aberration) as in the observation conditions
12 of the experiment were introduced, and the defocus was changed in steps of 1 Å. After Fourier
13 inverse filtering with the first-order spots of 2.13 Å, each image for the library was made by cutting
14 out a $3.22 \times 3.22 \text{ \AA}^2$ square containing the intensity around the six-membered ring. The fine
15 structure, more so than the first-order spots, was hardly discernible in the experimental TEM image
16 due to noise. Therefore, the experimental TEM images were filtered with the first-order spots to
17 remove noise. Noise-free simulation images in the library were also filtered to intentionally cut
18 higher-order structural information so as to meet each other's conditions.

19

1 **Identification of the x-y-z coordinates of the six-membered ring**

2 The x-y-z coordinates of the six-membered ring were identified by the following pattern-
3 matching procedure. First, using a 2D TEM image, the coordinates were moved pixel by pixel,
4 and $3.22 \times 3.22 \text{ \AA}^2$ images were cut out. Correlation values were calculated for all the cut-out
5 TEM images and all the libraries of simulated images, and the maximum correlation value of each
6 TEM image and its height z were obtained by selecting the corresponding simulated image with
7 the highest correlation value. The zero-mean normalized cross-correlation (ZNCC) shown in the
8 following equation was used as the function for obtaining the correlation value.

9

$$R_{f,g} = \frac{\sum_{x,y} ((f(x,y) - \bar{f})(g(x,y) - \bar{g}))}{\sqrt{\sum_{x,y} (f(x,y) - \bar{f})^2} \sqrt{\sum_{x,y} (g(x,y) - \bar{g})^2}}$$

10 Where $f(x,y)$ and \bar{f} are the experimental intensity at (x,y) and the experimental average
11 intensity, respectively. $g(x,y)$ and \bar{g} correspond to the simulations.

12 A six-membered ring was placed at the coordinates where the correlation value is the
13 local maximum. The height z of the arranged six-membered ring was determined at the same
14 time. From the above, the x-y coordinates and height z of the six-membered ring were obtained
15 in all the regions of the TEM image.

16

1 **Preparation of free-standing graphene**

2 We made free-standing graphene using a chemical vapor deposition (CVD) device and a
3 recipe that we independently developed ²⁵. The detailed steps are described as follows. First, Cu
4 foil (purity 99.8%) cut into a $1.5 \times 1.5 \text{ cm}^2$ square was placed on a quartz plate and inserted into
5 a quartz tube in the CVD device to grow graphene. The Cu foil had been immersed in acetic acid
6 overnight to remove the natural oxide layer. The inside of the quartz tube was set to 60 kPa and
7 300° C, and Ar was introduced at 50 sccm for 15 minutes. The temperature inside the tube was
8 then raised to 1000° C, and graphene was grown for 60 min in a 97.5 sccm Ar, 2 sccm H₂, 0.5
9 sccm CH₄ mixed gas flow. The copper was then rapidly cooled while the CH₄ flowed. Next, the
10 graphene grown on the copper foil was floated on a mixed solution of sulfuric acid: hydrogen
11 peroxide solution (3:1) in about 2 seconds, and only the backside Cu surface was etched. To
12 dissolve the copper foil, it was floated on an ammonium persulfate aqueous solution for 4 hours.
13 Finally, that solution was replaced with pure water, and the graphene was scooped up directly with
14 a carbon-supported Cu TEM grid to prevent sample contamination. The TEM grid had been
15 subjected to hydrophilization treatment for easy transfer.

16

1 **TEM observation**

2 The aberration-corrected TEM was a TITAN Cubed G2 60-300 manufactured by FEI. The
3 accelerating voltage of the electron beam was set to 80 kV so that the graphene would not be
4 damaged by knock-on²⁶. First, the aberrations of the electron lens were corrected with amorphous
5 carbon on the TEM grid. Based on the diffractogram tableau, spherical aberration was corrected
6 to 0.0048 mm, and two-fold astigmatism, three-fold astigmatism, and axial coma aberration were
7 also corrected to (Angle [°], Magnitude [\AA]) = (-115.0, 40), (-100.0, 200), and (141.2, 72),
8 respectively.

9 Through-focus TEM images were acquired as follows. The monolayer region in the
10 specimen was searched using the electron diffraction pattern³, and the exact focus was estimated
11 by moving the defocus to where the contrast was smallest. Starting at the defocus, which was
12 moved several tens of \AA from the estimated exact focus to the underfocus (decreasing the
13 excitation of the objective lens), a through-focus TEM image was acquired by moving the defocus
14 to the overfocus side in steps of 5.26 \AA (the error is $\pm 0.1 \text{\AA}$), which is the device's minimum step
15 size. The electron beam exposure time for acquiring a TEM image was set to 1 second, and the
16 next TEM image was acquired after 4 seconds by moving the defocus. This procedure makes it
17 possible to obtain through-focus TEM images including an exact focus.

18 In the experiment, it was carefully confirmed that aberrations were stable. The aberration
19 coefficients were measured twice: before and after acquisition of the through-focus images. The
20 dose of each TEM image was $1\sim2 \times 10^4 e^{-1}/\text{\AA}^2$.

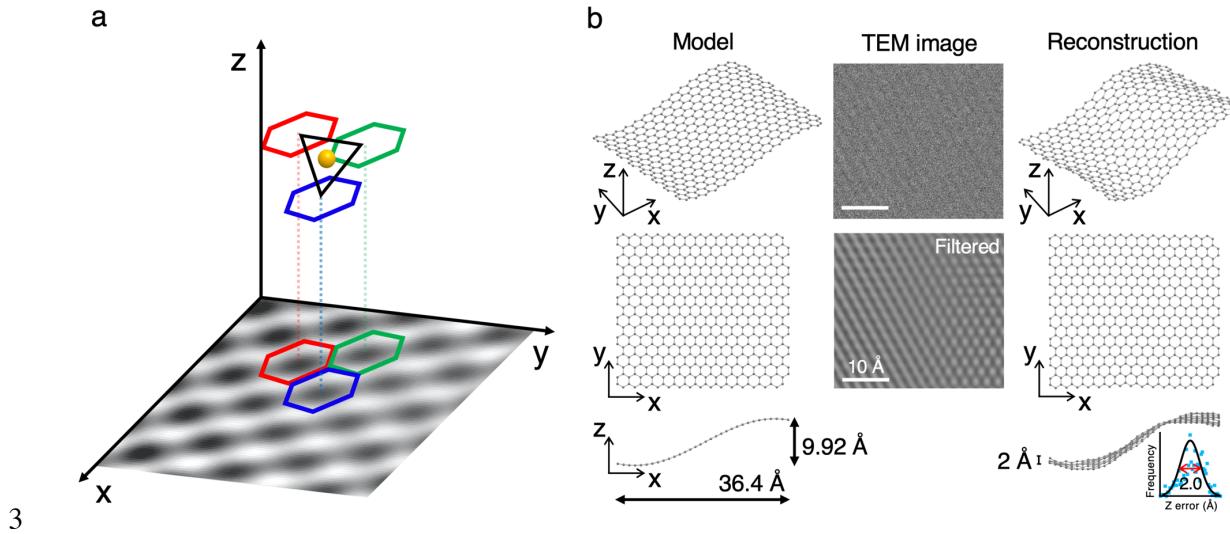
1 **Atomic force microscopy (AFM) observation**

2 The topography of the free-standing graphene was observed by the cyclic contact mode of
3 AFM (SPA-400, Hitachi High-Technologies Corporation) equipped with a closed-loop system.
4 We used a silicon cantilever with a spring constant of 26 N/m (catalog value provided by the
5 manufacturer). The Q value was controlled so that the excitation voltage was 0.3 V and the
6 vibration amplitude was 1.0 V. AFM and TEM observations were made in almost, but not exactly,
7 the same location.

8

1 Results and discussion

2 Proposed method and verification by numerical simulation



4 **Figure 1.** Proposed method and verification by numerical simulation.

5 a. Schematic of the proposed method for extracting the 3D positions of carbon atoms from a 2D
6 TEM image. In the x-y plane, a 2D TEM image of a monolayer graphene and three adjacent six-
7 membered rings (red, blue, green) extracted by pattern matching are shown. The height
8 information for each six-membered ring is indicated by red, blue, and green dotted lines. The center
9 of gravity of the triangle (black triangle) formed by connecting the centers of these three six-
10 membered rings is the 3D position of the carbon atom (yellow sphere).

11 b. Verification of the proposed method by numerical simulation. On the left is a 3D atomic
12 arrangement model of monolayer graphene with deformation in the z direction. The top, middle,
13 and bottom are respectively drawn as an x-y-z 3D diagram, an x-y 2D projection diagram, and an
14 x-z 2D projection diagram. For ease of viewing, each atom is connected by a line. The deformation
15 was a sinusoidal wave ($z = \sin(2\pi x/60)$) with an amplitude of 5 Å and a wavelength of 60 Å. The

1 projected region in the x-y plane was $36.4 \times 36.4 \text{ \AA}^2$. The distance between the nearest-neighbor
2 carbon atoms was set to 1.421 \AA . The TEM image in the center is the simulated result (software:
3 MacTempasX, HULINKS) considering the experimental conditions of aberration-corrected TEM.
4 Above is an image in which noise has been introduced with a dose amount of $10^4 \text{ e}^- / \text{\AA}^2$ after
5 multislice calculation. Below is an image that has been filtered to remove noise from the image
6 above. The filtered image was reconstructed three-dimensionally. On the right is the reconstructed
7 result of the 3D carbon atomic arrangement obtained by the proposed method. At the bottom right,
8 the height difference between each atom and the original model (that is, the z error) is shown as a
9 histogram (blue dot), and the approximate curve of the Gaussian function (black solid line) is
10 drawn.

1 We describe the proposed method of identifying the 3D arrangement of carbon atoms by
2 using a 2D TEM image of a free-standing monolayer graphene. The method is divided into two
3 processes. The first is to identify the 3D coordinates of each six-membered ring by pattern
4 matching with the simulated image. The second is to arrange the carbon atoms in 3D.

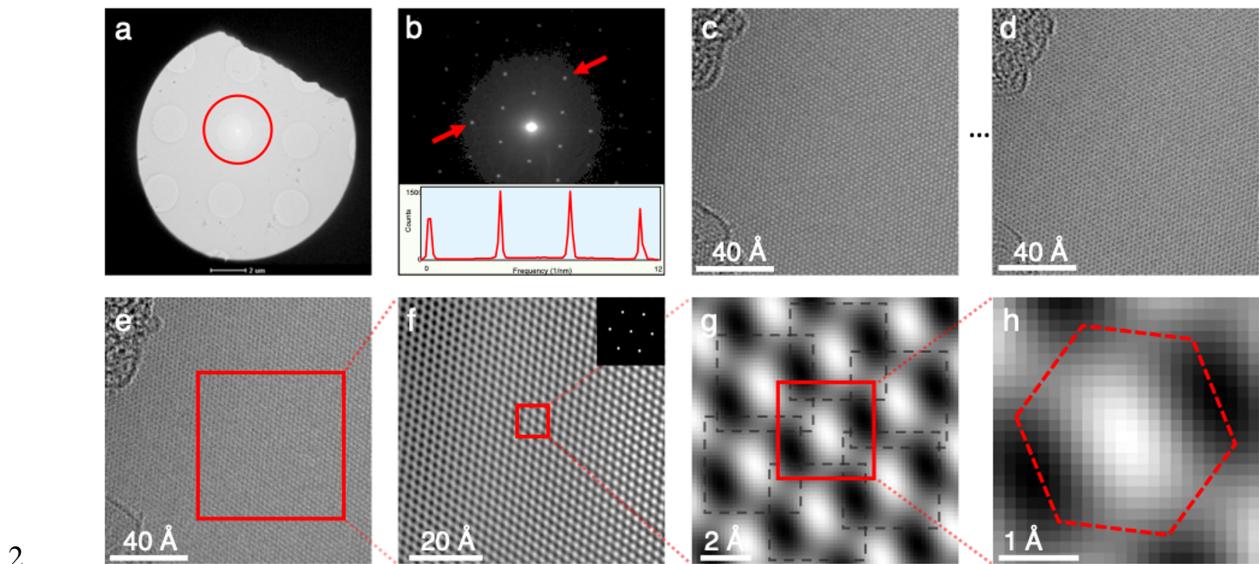
5 Fig. 1a outlines the method. A 2D TEM image is shown in the x-y plane. As an example,
6 three adjacent six-membered rings identified by pattern matching are also shown in red, blue, and
7 green. The heights of these rings are indicated by dotted lines in the respective colors. One atom
8 (yellow sphere) is arranged at the center of gravity of a triangle (black triangle) formed by
9 connecting the centers of the three six-membered rings. This method of arranging carbon atoms
10 was used by Y. Maehara et al. in 2D²⁷, and we applied it to 3D. This allows the 3D position (x,y,z)
11 of one atom to be determined. By repeating the same operation for all three adjacent six-membered
12 rings, the 3D positions of the carbon atoms can be reconstructed from the TEM image of the
13 monolayer graphene obtained in the experiment.

14 Fig. 1b shows an example of the verification of the proposed method's effectiveness by
15 using numerical simulation. The model on the left is a 3D atomic arrangement model of monolayer
16 graphene with deformation in the z direction. Based on a report³ that a ripple of the monolayer
17 graphene is likely to be a wave, part of a sinusoidal wave with an amplitude of 5 Å and a period
18 of 60 Å was used as an atomic model. The TEM image in the center is a simulated 2D TEM image
19 using the experimental conditions of TEM observation at a dose of $1 \times 10^4 e^{-1}/\text{\AA}^2$ ²⁸. The noise
20 was reduced from this image by Fourier inverse filtering with the first-order spots of 2.13 Å using
21 a filter size of 3×3 . The contrast differs between the left and right sides of the image. There are
22 also subtle differences in contrast between adjacent six-membered rings. This indicates that the
23 phase contrast of the TEM image reflects the height of each six-membered ring. The reconstruction

1 on the right shows the result of the 3D atomic arrangement obtained by the proposed method as
2 explained in Fig. 1a. Comparison with the original model on the left shows almost no difference
3 in the x-y projections but a slight difference in the x-y-z 3D image and in the x-z 2D projection.
4 On the bottom right, the height error from the model for each atom is shown as a frequency
5 distribution graph (histogram, blue dot), and the approximate curve (black solid line) of the
6 Gaussian function is drawn. The height error can be approximated by a Gaussian function with a
7 standard deviation of $\pm 1.0 \text{ \AA}$. Since the error in the x-y plane was less than 1 pixel ($= 0.15 \text{ \AA}$), the
8 height error in the z direction was the dominant error in the reconstruction process. 3D structures
9 such as inclined six-membered rings have not been considered. We used the 2D flat monolayer
10 graphene model described in Materials and methods. This is valid from the results of the sine wave
11 simulation shown in Fig. 1b. In this example, the maximum tilt angle is 16 degrees in the central
12 region, but the height error is not the largest in this region. The error is due to noise.

13

1 **TEM observation and image processing.**



2 **Figure 2.** TEM observation and image processing.

3 a. Observed hole (red circle) in TEM grid.

4 b. Electron diffraction pattern at the hole in the red circle. Inset shows the intensity profile along
5 the red arrows.

6 c, d. Through-focus TEM images (raw data) (c. TEM image 1, d. TEM image 15).

7 e. TEM image 6. Red square shows the region used for reconstruction.

8 f. TEM image after inverse Fourier transform using the filter in the inset.

9 g. Examples of identified six-membered rings. Red and black squares represent six-membered
10 rings identified by pattern matching.

11 h. The red dotted line represents a six-membered ring with the nearest-neighbor atomic distance
12 of 1.42 Å. There is a carbon atom at the intersection of each side.

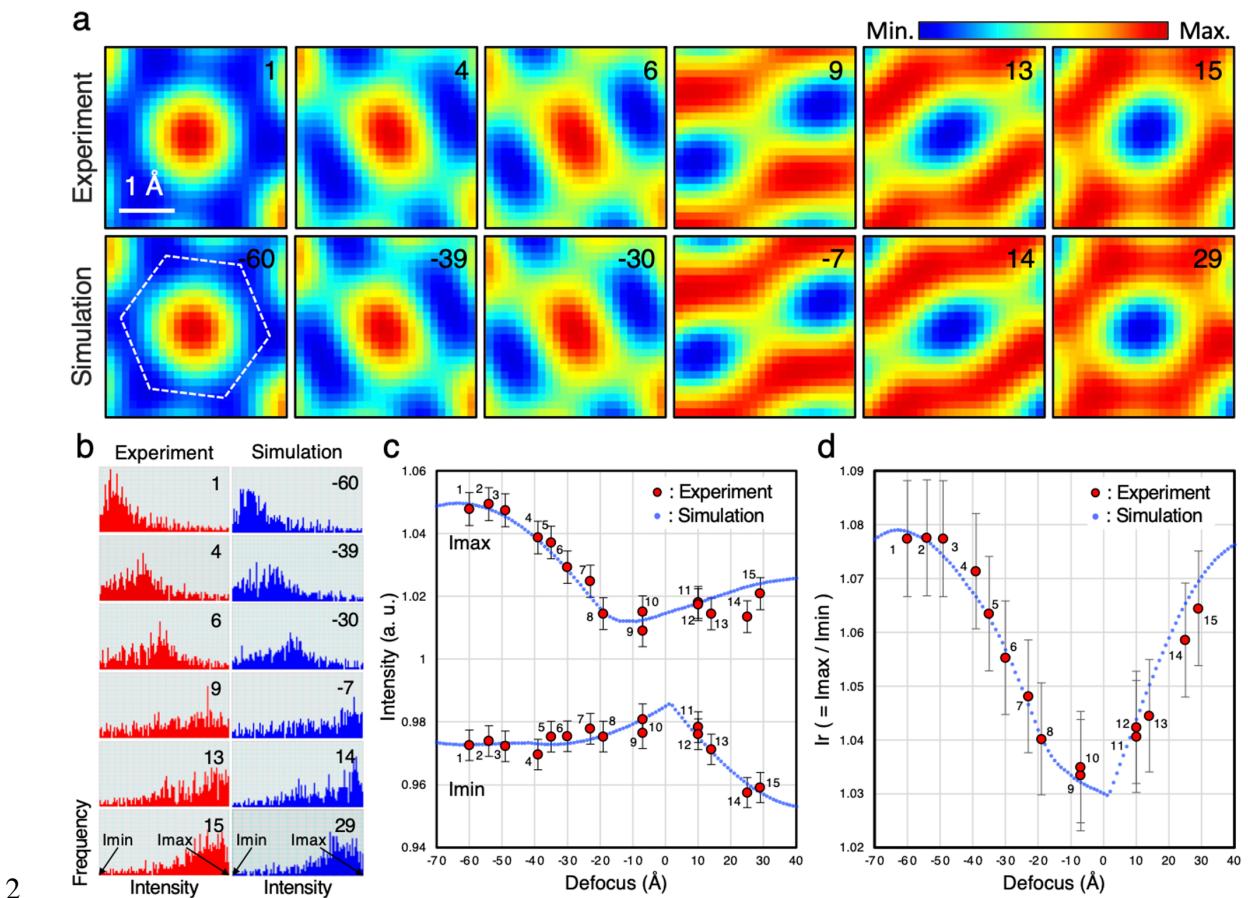
1 An experiment using an aberration-corrected TEM was performed to obtain the 3D
2 reconstruction of monolayer graphene by the proposed method. To compare the experimental and
3 simulated images over a wide defocus range, a series of through-focus TEM images were acquired.
4 Specimen preparation and TEM observation are described in the Materials and methods section.

5 Fig. 2a shows an observed hole (red circle) in the TEM grid. Fig. 2b shows the selected-
6 area electron diffraction pattern with a diameter of about 126 nm and its intensity profile. The two
7 inner (first-order) diffraction spots are larger than the outer (second-order) spots. In the case of
8 monolayer graphene, the first-order diffraction spot of the electron diffraction pattern is larger than
9 the second-order spot²⁹. Fig. 2c and 2d are examples of the acquired through-focus 15 TEM images.
10 Next, drift correction was performed on each acquired through-focus image, and a pristine region
11 in Fig. 2e was cut into an area of $72.76 \times 72.76 \text{ \AA}^2$. Fig. 2f shows the TEM image after inverse
12 Fourier transform using the filter in the inset. The S/N ratio was improved by extracting only the
13 first-order spots by filtering. Fig. 2g shows examples of an identified six-membered ring by pattern
14 matching as presented in the Materials and methods section. Fig. 2h represents a six-membered
15 ring. There is a carbon atom at the intersection of each side.

16 Subsequently, 3D reconstruction of the atomic arrangement was performed on the 15 TEM
17 images. Hereinafter, they are referred to as TEM image 1, TEM image 2,... TEM image 15 in the
18 order of acquisition.

19

1 **Comparison of experiment and simulation for a six-membered ring**



2 **Figure 3.** Comparison of experiment and simulation for a six-membered ring.

3 a. The upper images show experimental through-focus TEM images cut out with the six-membered ring (size of $3.22 \times 3.22 \text{ \AA}^2$, 24×24 pixels, 6 of 15 images, pseudo-color display). The number in the upper right of each TEM image indicates the order in which the image was acquired. For example, the image of the number 6 corresponds to the TEM image 6. Each image shows the center of the whole TEM image from which it was derived at the position indicated by the red square in Fig. 2g. Lower images are simulated images that show the highest correlations with the upper images. The number in the upper right is the defocus value (\AA). As a guide, a six-membered ring

1 with a nearest-neighbor atomic distance of 1.42 Å is drawn in the simulated image at the left end
2 with a dotted white line. There is a carbon atom at the intersection of each side.

3 b. Intensity distribution of the images in Fig. 3a. The results of the experiment (red) and simulation
4 (blue) are drawn. The horizontal and vertical axes represent the pixel intensity and frequency,
5 respectively. The both sides of the horizontal axes are the maximum intensity (I_{max}) and the
6 minimum intensity (I_{min}), respectively.

7 c. Defocus dependence of I_{max} and I_{min} . These intensities are the minimum and maximum
8 intensities of each six-membered ring. The blue circles in 1 Å steps represent the simulated result
9 and the other 15 red circles represent the experimental result. Error bars were estimated from
10 statistical error at a dose of $1.0 \times 10^4 e^{-1}/\text{\AA}^2$.

11 d. Defocus dependence of the intensity ratio (Ir) defined as $Ir = I_{max}/I_{min}$. The Ir was calculated
12 using Fig. 3c. The blue and red circles are drawn in the same way as in Fig. 3c.

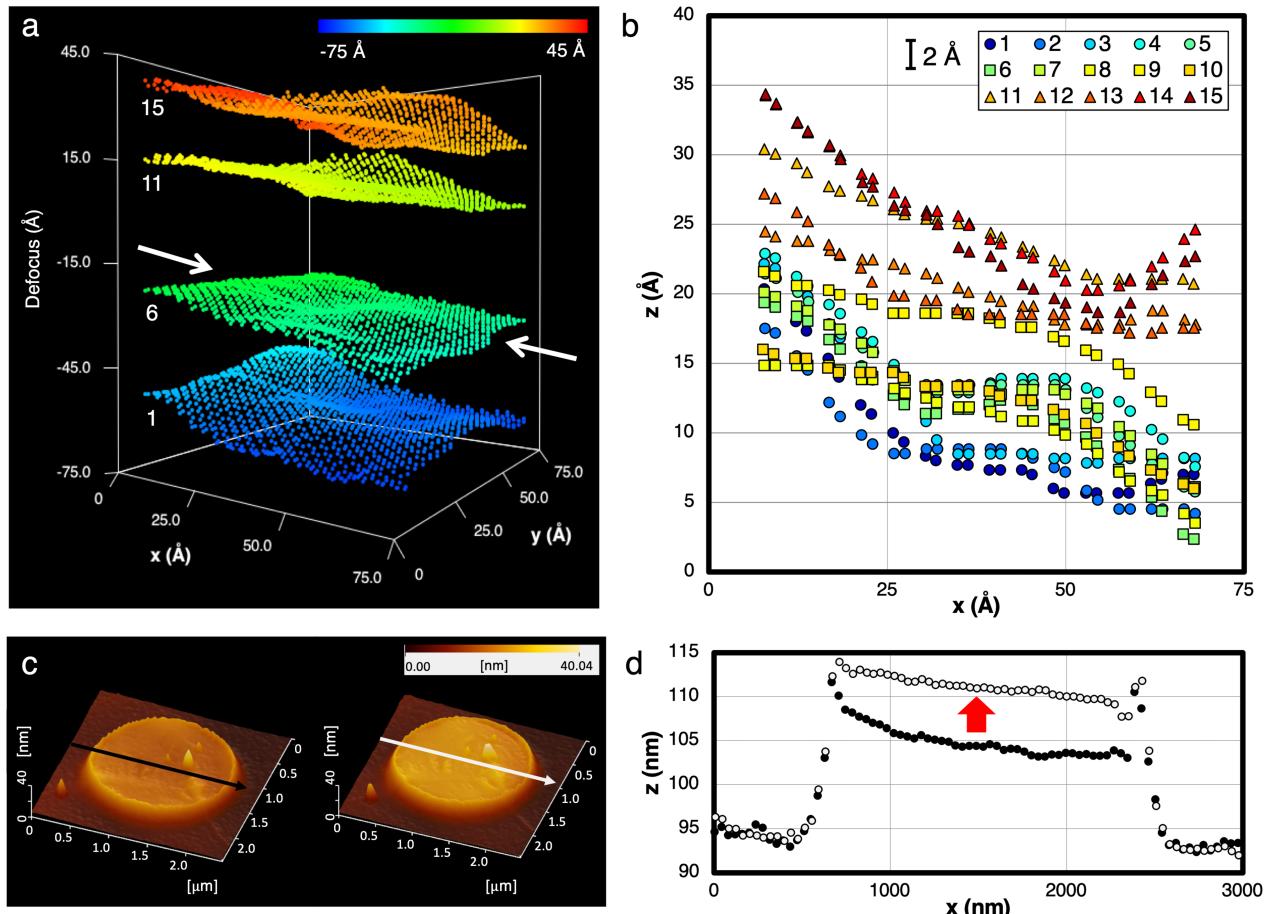
13

1 Fig. 3a shows examples of through-focus TEM images of the six-membered ring (upper
2 images) and the corresponding simulated images (lower images). It can be seen that the 2D contrast
3 patterns of TEM images were in good agreement with the simulated images. Details on how to
4 determine the defocus value of the six-membered ring are shown in Fig. S1. The results of all 15
5 TEM images of the six-membered ring are shown in Fig. S2. The correlation values defined in the
6 Materials and methods section were above 0.95 over a wide defocus range of 89 Å. In Fig. 3a,
7 from left to right, as the focus shifted to overfocus, the intensity of the carbon atoms increased and
8 the intensity of the center of the six-membered ring decreased. It should be noted here that the six-
9 fold symmetry was broken for any defocused TEM image of the six-membered ring. This is due
10 to the residual aberrations of two-fold and three-fold astigmatisms. Through-focus TEM images
11 were acquired with each defocus in steps of 5.26 Å, but the step size of the highly correlated
12 simulated images does not match 5.26 Å. This is because the specimen was moving. We describe
13 this in detail in Fig. 4.

14 Fig. 3b shows the intensity distribution of the images in Fig. 3a. The results of all 15
15 intensity distributions of the TEM images are shown in Fig. S3. Fig. 3c shows the defocus
16 dependence of the maximum intensity (I_{max}) and the minimum intensity (I_{min}). The experiment
17 and simulation were confirmed to be in good agreement within the error. Fig. 3d shows the defocus
18 dependence of the intensity ratio (I_r). In the simulation (blue dots), it can be seen that I_r is the
19 minimum at defocus = 1 Å. In the ideal case where the aberration is zero, the minimum I_r is at the
20 exact focus, i.e. defocus = 0 Å, but in this experiment, the minimum I_r deviates from zero defocus
21 due to the slight residual aberration. The I_r of the experimental TEM images 1 ~ 10 were plotted
22 on the underfocus side, and those of the TEM images 11 ~ 15 were plotted on the overfocus side
23 with the exact focus in between.

1 **3D Reconstruction of atomic arrangement and AFM measurement**

2



3

4 **Figure 4.** 3D Reconstruction of atomic arrangement and AFM measurement.

5 a. From the bottom, the 3D reconstruction results of TEM images 1, 6, 11, and 15 are drawn. The
6 plot represents carbon atoms. The vertical axis is the defocus (\AA), and the carbon atom is colored
7 by the color bar in the upper right according to the defocus.

8 b. For all 15 TEM images, the x-z positions of carbon atoms are plotted in the cross section
9 indicated by the white arrows in Fig. 4a. In the figure, the defocus when the through-focus image

1 was acquired is subtracted, so that the vertical axis shows height z itself. The lowest carbon atom
2 of TEM image 1 was set to 0. The colors of plots in the figure indicate acquisition times. The error
3 bar was 2 Å from the result shown in Fig. 1b.

4 c. AFM images of holes with graphene transferred (left and right: before and after TEM
5 observation, respectively).

6 d. Cross-sectional profile along the straight line indicated by the black and white arrows in the
7 AFM image of Fig. 4c (black and white plots: before and after TEM observation, respectively).
8 The horizontal axis is x and the vertical axis is z. As shown by the red arrow, the entire cross
9 section rose by about 6 nm.

10

1 The 15 through-focus TEM images were reconstructed in 3D. Fig. 4a shows the 3D
2 reconstruction results obtained from TEM images 1, 6, 11, and 15. The vertical axis is the defocus.
3 Atoms are arranged smoothly in each image. Although they are from the same specimen, they have
4 different 3D structures. All 3D reconstruction images are listed in Fig. S4. It is possible for the
5 structure to change even during the acquisition of a single image, but an average structure or a
6 structure with a long stay time for 1 s appears as the reconstruction image. This indicates that the
7 graphene obtained in this experiment has a quasi-static 3D structure.

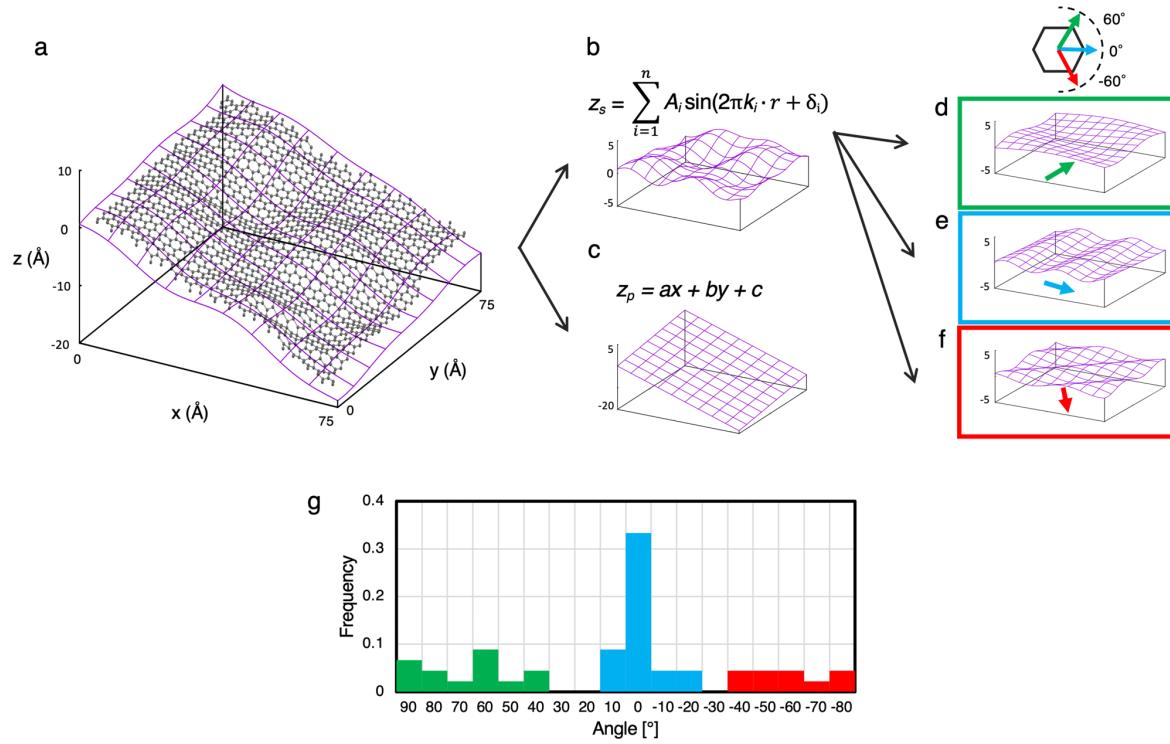
8 Fig. 4b shows cross sections of the 15 3D structures, in which are plotted the carbon atoms
9 in the cross section indicated by the white arrows in Fig. 4a. The same carbon atoms are drawn in
10 all the cross sections of all 15 through-focus TEM images. The vertical axis of each cross section
11 was converted to height z by removing 5.26 Å, which was moved step by step during the
12 acquisition of the through-focus TEM images, from the defocus value of each carbon atom. Cross
13 section 1 was located at $z = 5 \sim 20$ Å, while cross section 15 was located at $z = 20 \sim 35$ Å. This
14 indicates that the specimen rose $15 \sim 20$ Å during TEM observation (about 70 seconds). This is
15 why the step width of defocus in Fig. 3a is not equal to 5.26 Å. In addition, all cross sections had
16 a negative slope with respect to the x-axis. For example, in TEM image 6, the tilt angle is about
17 16 degrees.

18 Figs. 4a and 4b revealed not only that the 3D structure varied but also that the specimen
19 moved in one direction. The latter was observed by AFM before and after the TEM observation.
20 Fig. 4c shows an example of AFM measurements, and Fig. 4d is a cross-sectional view. The AFM
21 experimental method is described in the Materials and methods section, and Fig. S5 shows
22 additional measurements to confirm that the AFM itself is not causing morphological changes in
23 the graphene. As shown by the red arrow in Fig. 4d, the entire graphene rose by about 6 nm (from

1 the black plot to the white plot). The results obtained from AFM are consistent with those in Fig.
2 4b obtained by TEM and strongly support that the whole graphene rose during TEM observation.
3 However, note that under the measurement conditions this time, the resolution in the x-y plane of
4 AFM was ± 10 nm, which is significantly different from that of TEM. It is considered that the
5 specimen drift in the z direction is attributable to factors in the environment inside the TEM facility,
6 such as the influence of electron beam irradiation and the direction of vacuuming. However, much
7 more careful examination is needed to arrive at a physical explanation of the specimen drift in the
8 z direction.

9

1 Approximation of 3D atomic arrangement



2
3 **Figure 5.** Approximation of 3D atomic arrangement (TEM image 6 as an example) and directions
4 of the sinusoidal waves.

- 5 a. The reconstructed atomic arrangement (black) and the approximated curved surface (purple).
- 6 b, c. Sinusoidal and plane components.
- 7 d, e, f. Three constituent sinusoidal waves of the sinusoidal component. The green, blue, and red
8 arrows indicate the directions of the respective sinusoidal waves.
- 9 g. A histogram of all directions of the 45 (3×15) sinusoidal waves. The horizontal axis of the graph
10 shows the angle (°), and the vertical axis shows the frequency.

1 We considered approximating the reconstructed atomic arrangement in Fig. 4a as a
2 composite of two functions with multiple sinusoids (z_s) and one plane (z_p) as follows.

3
$$z = z_s + z_p$$

4
$$= \sum_{i=1}^n \{A_i \sin(2\pi k_i \cdot r + \delta_i)\} + ax + by + c$$

5
$$k_i = (k_{xi}, k_{yi}, k_{zi}), r = (x, y, z).$$

6 Where A_i , k_i , r , and δ_i are the amplitude, wave vector, position vector, and phase, respectively; a , b ,
7 c are the linear coefficients of z_p . The function had parameters $(A_i, k_i, \delta_i, a, b, c)$ that minimize the
8 squared error per atom.

9 Fig. 5 shows an example of the approximation. Fig. 5a shows the approximated curved
10 surface and the reconstruction of TEM image 6 in Fig. 4a. It can be seen that they agree very well.
11 The average height error per carbon atom of the approximation was ± 0.62 Å. Figs. 5b and 5c show
12 structures of the sinusoidal term z_s and of the plane term z_p . In this paper, the structure of the
13 sinusoidal term z_s is defined as a ripple. From Fig. 5b, a min-max height difference of 3.6 Å was
14 found in the 72.76×72.76 Å² region, and from Fig. 5c we see that it was tilted by 14.0° in the x
15 direction and 4.4° in the y direction. The results in Fig. 5c are in quantitative agreement with the
16 tilt angles in Fig. 4b. This shows that the approximation can clearly divide a structure with a ripple
17 and a slope. Figs. 5d ~ 5f show the three sinusoidal waves that compose the ripple shown in Fig.
18 5b. These waves correspond to the directions of the green, blue, and red arrows, and their
19 amplitudes and wavelengths were (0.68 Å, 65.8 Å), (0.68 Å, 35.7 Å), and (0.54 Å, 32.5 Å),
20 respectively. It was found that the three directions corresponded to those of the six-membered ring
21 (upper diagram in Fig. 5d).

22 For the approximation of 15 reconstructions, Fig. S6 shows how the average error per atom
23 decreased as the number of sinusoidal waves used for interpolation increased. The results,

combined with the finding that the standard deviation of the height error was 1 Å in the numerical simulation shown in Fig. 1b, allowed us to estimated that the approximation with three or four sinusoidal waves is appropriate. Fig. S7 shows the ripples, i.e., the structures of the sinusoidal term z_s , of all 15 reconstructed structures with three sinusoidal waves. Some of the 15 ripples show similar 3D structures in two adjacent ones (1 and 2, 4 and 5, 8-10, etc., with peaks and valleys in the close positions). In addition, Fig. 5g shows a histogram of all directions of the 45 (= 3×15) sinusoidal waves. It was revealed that three directions were dominant in all 15 approximations and that each ripple was composed of waves in two or three specific directions of the six-membered ring. Because there are areas where no color exists between colors, it turns out that there is a clear boundary. The preference for the zero angle direction may be attributable to contamination, distortion outside the reconstructed field of view, or the tension from boundaries that support graphene. However, further experiments are needed to clarify these reasons.

13

1 Height and lateral size of the ripple structure

2

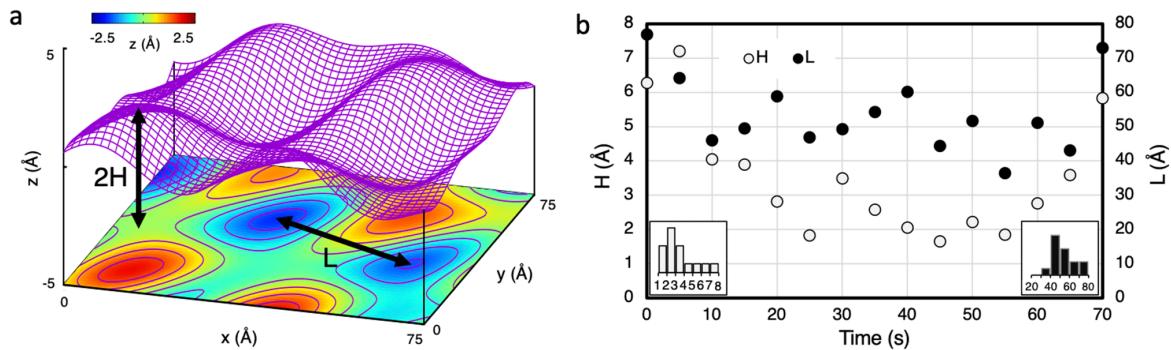


Figure 6. Height and lateral size of the ripple structure.

5 a. Definitions of height (H) and lateral size (L). With the same curved surface as shown in Fig.

6 4b, contour lines are drawn in the x-y plane. The definitions of $2H$ and L are shown by black

7 arrows.

8 b. Time dependence of H and L. H and L were obtained from all 15 approximation results and
9 plotted on a graph whose horizontal axis represented time. The white plot is H and the black plot
10 is L. The insets in the lower left and lower right show the frequency distributions of H and L,
11 respectively (the unit of the horizontal axis is Å).

12

Finally, we investigated the time variations of height (H) and lateral size (L) for the ripple structure. Fig. 6a shows the definitions of H and L of the ripple. $2H$ is the difference between the maximum and minimum values of z, and height H is defined as half of that. In the case of the ripple structure in Fig. 6a, the maximum value was $z = 1.84 \text{ \AA}$ and the minimum value was $z = -1.80 \text{ \AA}$, so that H was $(1.84 - (-1.80))/2 = 1.82 \text{ \AA}$. L is defined as the average for all combinations of the distances between the maximum points (peaks) or the minimum points (valleys) in the whole region. The ripple in Fig. 6a shows three local maxima indicated by red peaks and three local minima indicated by blue peaks of the contour line. Therefore, calculation of the average for 6 (3 combinations to select 2 from 3 convex, 3 combinations to select 2 from 3 concave, total 6 average) different distances revealed that L of the ripple structure was 46.9 \AA .

From theses above definitions, H and L were calculated for all 15 reconstruction results. Fig. 6b shows the time dependence of H and L. H and L changed randomly at 5-second intervals. Furthermore, the frequency distributions of H and L are shown in Fig. 6b. H had a distribution in the range of 1.7 to 7.2 \AA . L had a distribution in the range of 36 to 77 \AA . The ratio H/L varied by $0.03 \sim 0.11$. The ratio was quantitatively consistent with those in some reports but not with those in others^{3,4,23,24,30}. This may be due to differences in conditions, such as sample size, from study to study³.

18

1 Conclusion and discussion

2 We presented a new method for the 3D reconstruction of a ripple from a single 2D TEM
3 image. First, numerical simulation verified that the method is capable of 3D reconstruction with a
4 z error within $\pm 1 \text{ \AA}$ per carbon atom. Next, using an aberration-corrected TEM, 15 through-focus
5 images were acquired during 70 seconds for a wide defocus range of about 100 \AA . There are two
6 reasons to use through-focus images. One is that it is necessary to clearly show that the contrast
7 matches between the experimental and simulated six-membered rings. The other reason is to
8 experimentally show the range of defocus to which this method can be applied. The 2D contrast
9 of the TEM images of the six-membered ring was in good agreement with that of the simulated
10 images. The 3D atomic arrangements of consecutively acquired TEM images were reconstructed.
11 Furthermore, by approximating the obtained 3D reconstructed graphene with multiple sinusoidal
12 waves and a plane function, we were able to cleanly separate the slope component and the ripple
13 component. A true ripple structure was obtained by removing tilt and drift in the z direction, which
14 were problems in high-resolution imaging using aberration-corrected electron microscopy.
15 Moreover, the ripple in graphene was found to consist of the superposition of three or four
16 sinusoidal waves approximately coinciding with the specific direction of the six-membered ring.

17 Since this method uses the six-membered ring as the basic unit, local defects such as
18 dislocations and voids make it difficult to calculate the height without modeling defects where the
19 six-membered ring is broken. In that case, it would be effective to complementarily use the method
20 of locally calculating the height accurately from the TEM intensity of a single atom^{23,24}. An
21 experimentally important parameter that limits accuracy is the signal-to-noise ratio (S/N). The
22 electron dose in the experiment was $1\sim 2 \times 10^4 e^{-1}/\text{\AA}^2$ during an exposure time of 1 second. S/N
23 can be improved by using multiple six-membered rings for pattern matching. This reduces the

1 resolution in the x-y plane but improves height accuracy. In fact, it has been reported that the large
2 size accurately determines the ripple of few-layer graphene³¹, although not that of monolayer
3 graphene.

4 The 3D reconstruction method proposed in this paper can be applied to other 2D materials.
5 In addition, we expect it to be used widely to elucidate the basic properties of 2D materials and to
6 control those materials.

7

1 **Associated content**

2 **Supporting Information.** Detail of TEM image acquisition, all data on through-focus TEM
3 images and simulated images, all data on 3D reconstructions and approximations by sinusoidal
4 waves, detailed results of the analysis (PDF).

5 **Conflict of Interest:** The authors declare no competing financial interest.

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11

12 **Author Contributions**

13 Y.S. and K.G. conceived the idea and designed the study. Y.S. and K.Y. prepared the samples
14 for the experiments and performed the TEM and AFM observations. Y.S. performed the
15 numerical simulations. Y.S. and K.G. analyzed the data. Y.S. and K.G. wrote the manuscript,
16 and all authors commented on it.

1 References

- 2 1 K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V.
3 Grigorieva and A. A. Firsov, *Science*, 2004, **306**, 666–669.
- 4 2 S. Deng and V. Berry, *Mater. Today*, 2016, **19**, 197–212.
- 5 3 J. C. Meyer, A. K. Geim, M. I. Katsnelson, K. S. Novoselov, T. J. Booth and S. Roth,
6 *Nature*, 2007, **446**, 60–63.
- 7 4 A. Fasolino, J. H. Los and M. I. Katsnelson, *Nat. Mater.*, 2007, **6**, 858–861.
- 8 5 F. De Juan, A. Cortijo and M. A. H. Vozmediano, *Phys. Rev. B - Condens. Matter Mater.*
9 *Phys.*, 2007, **76**, 165409.
- 10 6 R. J. T. Nicholl, H. J. Conley, N. V. Lavrik, I. Vlassiouk, Y. S. Puzyrev, V. P. Sreenivas,
11 S. T. Pantelides and K. I. Bolotin, *Nat. Commun.*, 2015, **6**, 8789.
- 12 7 P. Z. Sun, Q. Yang, W. J. Kuang, Y. V. Stebunov, W. Q. Xiong, J. Yu, R. R. Nair, M. I.
13 Katsnelson, S. J. Yuan, I. V. Grigorieva, M. Lozada-Hidalgo, F. C. Wang and A. K. Geim,
14 *Nature*, 2020, **579**, 229–232.
- 15 8 Y. Liu and B. I. Yakobson, *Nano Lett.*, 2010, **10**, 2178–2183.
- 16 9 J. H. Warner, E. R. Margine, M. Mukai, A. W. Robertson, F. Giustino and A. I. Kirkland,
17 *Science*, 2012, **337**, 209–212.
- 18 10 J. H. Warner, Y. Fan, A. W. Robertson, K. He, E. Yoon and G. Do Lee, *Nano Lett.*, 2013,
19 **13**, 4937–4944.

- 1 11 E. Stolyarova, T. R. Kwang, S. Ryu, J. Maultzsch, P. Kim, L. E. Brus, T. F. Heinz, M. S.
2 Hybertsen and G. W. Flynn, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 9209–9212.
- 3 12 M. Ishigami, J. H. Chen, W. G. Cullen, M. S. Fuhrer and E. D. Williams, *Nano Lett.*,
4 2007, **7**, 1643–1648.
- 5 13 U. Ludacka, M. R. A. Monazam, C. Rentenberger, M. Friedrich, U. Stefanelli, J. C. Meyer
6 and J. Kotakoski, *npj 2D Mater. Appl.*, 2018, **2**, 25,
- 7 14 R. Zan, C. Muryn, U. Bangert, P. Mattocks, P. Wincott, D. Vaughan, X. Li, L. Colombo,
8 R. S. Ruoff, B. Hamilton and K. S. Novoselov, *Nanoscale*, 2012, **4**, 3065–3068.
- 9 15 P. Xu, M. Neek-Amal, S. D. Barber, J. K. Schoelz, M. L. Ackerman, P. M. Thibado, A.
10 Sadeghi and F. M. Peeters, *Nat. Commun.*, 2014, **5**, 1–7.
- 11 16 M. L. Ackerman, P. Kumar, M. Neek-Amal, P. M. Thibado, F. M. Peeters and S. Singh,
12 *Phys. Rev. Lett.*, 2016, **117**, 1–5.
- 13 17 C. S. Allen, E. Liberti, J. S. Kim, Q. Xu, Y. Fan, K. He, A. W. Robertson, H. W.
14 Zandbergen, J. H. Warner and A. I. Kirkland, *J. Appl. Phys.*, 2015, **118**, 74302.
- 15 18 J. Hu, G. M. Vanacore, A. Cepellotti, N. Marzari and A. H. Zewail, *Proc. Natl. Acad. Sci.*
16 *U. S. A.*, 2016, **113**, E6555–E6561.
- 17 19 X. Tian, D. S. Kim, S. Yang, C. J. Ciccarino, Y. Gong, Y. Yang, Y. Yang, B. Duschatko,
18 Y. Yuan, P. M. Ajayan, J. C. Idrobo, P. Narang and J. Miao, *Nat. Mater.*, 2020, **19**, 867–
19 873.

- 1 20 U. Bangert, M. H. Gass, A. L. Bleloch, R. R. Nair and A. K. Geim, *Phys. Status Solidi Appl. Mater. Sci.*, 2009, **206**, 1117–1122.

2

3 21 K. Hirahara, K. Saitoh, J. Yamasaki and N. Tanaka, *Nano Lett.*, 2006, **6**, 1778–1783.

4 22 D. Van Dyck, J. R. Jinschek and F. R. Chen, *Nature*, 2012, **486**, 243–246.

5 23 L. G. Chen, J. Warner, A. I. Kirkland, F. R. Chen and D. Van Dyck, *Sci. Rep.*, 2017, **7**, 10839

6

7 24 S. Morishita, R. Senga, Y. C. Lin, R. Kato, H. Sawada and K. Suenaga, *Appl. Phys. Lett.*, 2018, **113**, 233101.

8

9 25 K. Yamazaki, Y. Maehara and K. Gohara, *J. Phys. Soc. Japan*, 2018, **87**, 1–6.

10 26 J. C. Meyer, C. Kisielowski, R. Erni, M. D. Rossell, M. F. Crommie and A. Zettl, *Nano Lett.*, 2008, **8**, 3582–3586.

11

12 27 Y. Maehara, K. Yamazaki and K. Gohara, *Carbon N. Y.*, 2019, **143**, 669–677.

13 28 J. C. Meyer, J. Kotakoski and C. Mangler, *Ultramicroscopy*, 2014, **145**, 13–21.

14 29 J. C. Meyer, A. K. Geim, M. I. Katsnelson, K. S. Novoselov, D. Obergfell, S. Roth, C.

15 Girit and A. Zettl, *Solid State Commun.*, 2007, **143**, 101–109.

16 30 V. Geringer, M. Liebmann, T. Echtermeyer, S. Runte, M. Schmidt, R. Rückamp, M. C.

17 Lemme and M. Morgenstern, *Phys. Rev. Lett.*, 2009, **102**, 076102.

18 31 W. L. Wang, S. Bhandari, W. Yi, D. C. Bell, R. Westervelt and E. Kaxiras, *Nano Lett.*, 2012, **12**, 2278–2282.

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