ORIGINAL ARTICLE

Deciphering buried air phases on natural and bioinspired superhydrophobic surfaces using synchrotron radiation-based X-ray phase-contrast imaging

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Superhydrophobicity is an important phenomenon in nature that inspires the design of numerous biomimetic functional materials. A superhydrophobic surface is expected to have a three-phase solid–liquid–vapor interface. To directly image the buried air phase in wetted superhydrophobic surfaces, we employed synchrotron radiation-based X-ray phase-contrast imaging to non-invasively probe the surfaces of natural lotus leaves and artificial carbon nanotube films in three dimensions. Reconstructed images of the three-phase distribution surrounding the superhydrophobic surfaces were presented with high resolution and contrast. Further extraction of the phases enabled direct analysis of the relationship between the surface morphology and its wetting property. We found that the protruding micro/nano-structures trapped a layer of air up to 14 μm thick, which played an important role in the observed superhydrophobicity. Direct evidence of the presence of a buffer layer air cushion deepens our understanding of hydrophobicity and opens new opportunities for designing novel bioinspired materials.

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INTRODUCTION

Superhydrophobic surfaces, such as lotus leaves and insect wings,^{1,2} are often found in nature and have inspired the design of novel materials with numerous applications in anti-water textiles, moisture-resistant electronics, microfluidic systems and biomedical materials.^{2–7} Generally, the superhydrophobicity of a solid surface indicates that its water contact angle (CA) exceeds 150°. The mechanism of this special type of wettability has been explored extensively since the early twentieth century.^{9,10} It is generally accepted that water repellence of a surface is determined by both its material composition and the micro/nano-scale roughness of the surface.¹¹ However, whereas the superhydrophobicity of a surface can be explained by the Cassie or Cassie-to-Wenzel transition classic theoretical models, the role and mechanism of trapped air in the three-phase solid–liquid–vapor (SLV) interface have not been investigated thoroughly.

Various techniques have been developed to probe the SLV contact on the surface in wetted conditions, including environmental scanning electron microscopy (ESEM),^{12,13} optical microscopy,^{14,15} atomic force

microscopy,¹⁶ and cryo-s.e.m.¹⁷ Although these techniques have provided an in-depth understanding of the SLV interfaces, direct and clear-cut evidence of the existence of air cushions is still lacking. For example, optical imaging is inevitably distorted by the droplet lens effect, atomic force microscopy imaging usually perturbs delicate interfaces, and the complicated sample preparation steps often cause artifacts in cryo-s.e.m. Very recently, Yang *et al.*¹⁸ developed an X-ray micro-computed tomography (micro-CT) method to directly image the internal morphology of the SLV interface on textured surfaces. However, absorption-based imaging is intrinsically restricted by the image resolution and contrast, especially with biological samples. Furthermore, the light intensity of desktop X-ray further limits the contrast when the imaging materials consist of light elements.

In our contribution, we employed synchrotron radiation-based X-ray phase-contrast imaging (SR-XPCI) to non-invasively image SLV interfaces on the superhydrophobic surfaces of natural lotus leaves and artificial multi-walled carbon nanotube (MWNT) films (Supplementary Figure S1).¹⁴ Unlike conventional absorption-based X-ray

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Figure 1 ESEM images of (a) the lotus leaf surface and (b) the papilla. Conventional s.e.m. images of (c) the MWNTs/Nafion composite film surface and (d) the MWNT clusters. Insets show water droplets on each surface.

techniques, phase-contrast imaging differentiates matter by the refractive indexes (that is, phase shifts), which provides contrast levels three orders of magnitude greater than absorption techniques.¹⁹ The significant contrast can reveal micro-scale or even nano-scale spatial distribution in materials that can be readily reconstructed.^{20–22} In particular, the use of synchrotron sources with intense and coherent X-rays allows high signal-to-noise ratio imaging and rapid scanning times. Using SR-XPCI, we directly visualized in three-dimensions (3D) the three-phase distribution, the shape of the water contact and the relationship with the superhydrophobic surfaces. The reconstructed images using high resolution and high contrast are distortion free, providing direct evidence for the existence of water cushions in SLV interfaces.

EXPERIMENTAL PROCEDURES

Methods

The water CA was measured by a goniometer (Theta Optical Tensiometer, Attension of Biolin Scientific), and the sliding angle (SA) was measured on a tilted plate. s.e.m. images were captured by JEOL JSM 6360 LV (JEOL Ltd.) with an accelerating voltage of 10 kV. ESEM photos were taken by Quanta 200 (FEI). The SR-XPCI was conducted on the BL13W1 Beamline at Shanghai Synchrotron Radiation Facility (SSRF), a third-generation synchrotron source.

SR-XPCI set-up

An in-line configuration of the experimental set-up is illustrated in Supplementary Figure S1. The white beam emitted from a wiggler unit is first modulated by a double-crystal monochromator to obtain a beam with an energy of 15 keV. The attenuator is used to adjust the intensity while the

ionization chamber monitors the flux. A sample is cut into small pieces and inserted into a pipette tip. The tip with the sample inside is filled with deionized water and mounted on a polytetrafluoroethylene substrate that is fixed to the sample stage. During the X-ray scan, the stage rotates around its vertical axis in 1° steps. The scattered rays that transmit the sample interfere with the uninterrupted rays and form Fresnel fringes behind the sample. After being converted into visible light through a scintillator, the interference patterns are recorded by the Optique Peter CCD detector (effective pixel size is 0.41 μm with a ×20 lens). One complete scan of the sample takes 1–2 h. Special care is taken to avoid heat damage to the sample during the scan. Two-dimensional projection images (slices) of the sample from all angles are acquired and reconstructed into 3D images using computers.

RESULTS AND DISCUSSION

The water-repelling effect of lotus leaves is probably the best-known superhydrophobic phenomenon in nature. The self-cleaning property of lotus leaves has been extensively investigated for years.^{1,2} The water CA on the surface of a *Nelumbo nucifera* leaf is ~152.4° with a SA of 3.0° (Supplementary Figure S2a, Supplementary Table S1) and represents a typical water-repellent surface. The ESEM images revealed distinct features of protruding papillae on the surface with lateral spacings of several tens of μm (Figure 1a and b). The magnified images also clearly showed soft hydrophobic wax and fine structures on the papillae. Previous studies suggested that when wetted, micro/nano-textures readily trap an abundance air under water droplets, which is responsible for the large CA and small SA observed on lotus leaves.^{23,24}

To provide direct evidence of the air cushion, we employed SR-XPCI to image the SLV interface on the lotus leaf. Phase-contrast

Figure 2 (a) Schematic illustration of SR-XPCI to observe the SLV interface on a lotus leaf and to reconstruct the 3D image from the X-ray scanned two-dimensional projection slices around the leaf. A sub-region (marked in the red box in d) bottom) near the leaf surface was selected for analysis. (b) The air (purple) and water (blue) phases were extracted and highlighted. (c) A special isolation of the air phase. (d) Schematics of the papilla structures on the bare leaf and when immersed in water. Also highlighted is the wavy triple-line characteristic of the Cassie-to-Wenzel transit state in both (c and d). All grids have a scale of 100 μm .

imaging detects the phase shift of the transmitted X-ray, which is suitable for probing structures of soft samples with little light absorption. The scan set-up and SR-XPCI imaging procedure are shown in the Supplementary Information (Supplementary Figure S1). In brief, a small piece of the lotus leaf was first immersed in water and then mounted on a rotating stage (Figure 2a). Two-dimensional slice images were then generated by scanning the sample from all angles in 1° steps, and these images were used to create 3D reconstructions of the sample using a filtered back projection algorithm. Because the 3D image revealed the electron density distribution information in the sample,¹⁹ the solid, water and air phases could be reliably identified in high resolution by their contrast. A sub-region near the leaf surface was then selected for phase extraction. Figure 2b shows the phases of air in purple and water in blue; the solid phase is opaque. Note that the external water, in which the leaf was immersed, was intentionally excluded from the analyzed region to simplify analysis of the internal phase distribution. As a result, all the water shown in the images corresponded to the tissue fluid inside the leaf.

In the colored 3D image (Figure 2b), round-shaped voids representing the solid phase could be seen at the surface corresponding to the protruding papillae on the leaf. Most of them are discretely distributed, whereas others are so close that they fuse to each other. This is consistent with the distribution of papillae observed in ESEM images (Figure 1a). Significantly, we found that a large amount of air was buried in the grooves on the surface underneath the water. By specifically isolating the air phase from the image of Figure 2b, we discovered the presence of a network-like air cushion that serves as a buffer layer between the water and the leaf surface (Figure 2c). Previous studies showed that water droplets on lotus leaves remain at a transitional state between the Cassie and Wenzel state.²⁵ We observed a consistent water–air boundary shape (Figure 2d) that arises from the static pressure induced by the water surface tension.²⁴ Furthermore, the air portions were found to occupy most of the space on top of the surface, and only a limited amount of external water reached the solid phase, which clearly provides evidence for the observed superhydrophobic property of lotus leaves.

The sizes of the features revealed in the 3D images showed excellent consistency with those derived from s.e.m. Figure 3 shows histograms of the extracted profile parameters of the papillae. We found that the

papilla diameters (d) were in a range of 5–10 μm , with lateral spacings (s) in the range of 5–20 μm and heights (h) of 2–14 μm . The height of the air cushions reached as high as 14 μm , which is almost as high as the tallest papillae, indicating the efficient water repellence on the lotus leaf.

The function of the large-area air cushion seen in the images can be interpreted well by Cassie's Law.¹⁰ The relationship between the CA of a water droplet on a pure solid surface (θ) and the CA on a composite surface (θ_c) consisting of air pockets and solid surfaces is described as:

$$\cos \theta_c = f_1 \cos \theta - f_2 \quad (1)$$

where f_1 and f_2 represent the areal fractions of the solid surface and air pockets touched with liquid, respectively ($f_1 + f_2 = 1$). From the equation, it is clear that the larger the area the air occupies, the more hydrophobic the composition surface becomes. In the case of the lotus leaf surface, the air pockets trapped in the micro-scale papilla spacings are small enough to prevent water penetration into the grooves. In the meantime, the connecting air pockets form a large network of air cushions, which dramatically reduce the area of the solid–water contact, leading to the surface superhydrophobic property.

Superhydrophobicity in nature has inspired researchers to develop artificial surfaces with desirable wetting properties for various applications.^{3,26} We previously developed superhydrophobic composite films made of MWNT/Nafion¹⁴ and examined them with SR-XPCI (Supplementary Figure S2b). Pure MWNT film exhibits excellent electrical conductivity that can be exploited for various applications. However, the weak intermolecular attractions (van der Waals forces) between these submillimeter-long nanotubes may prevent them from longtime operation in practical applications where bending and twisting more or less take place. By adding a small fraction of Nafion as a polymer filler, the densely packed nanotube network structure can be loosened to form a hierarchical 3D interpenetrating network. The trapped air pockets make the film surface superhydrophobic while retaining its electric conductivity. No rupture or reduction in the surface's conductivity or superhydrophobicity was observed even after 1000 bending cycles, which shows its excellent mechanical stability. In our current study, the MWNT/Nafion film was fabricated by vacuum filtration from a solution of MWNT and Nafion dispersed in ethanol. The water CA and SA on the

Figure 3 Histograms showing the dimensions of (a) the papilla diameter, spacing and height on the lotus leaf and (b) the cluster size and spacing on the MWNTs/Nafion film. The data compared are extracted from the s.e.m. images and the reconstructed 3D SR-XPCI images.

Figure 4 (a) Schematic illustration of SR-XPCI to observe the SLV interface on a MWNTs/Nafion film and to reconstructed the 3D image from the X-ray scanned two-dimensional projection slices around the sample. A sub-region (marked in the red box in c) near the surface is selected for analysis. The grid has a scale of 100 μm . (b) The air (purple) and water (blue) phases are extracted and highlighted, viewing from different angles. The grid has a scale of 25 μm . (c) Schematic of the SLV interface on the film.

films were measured to be an average of 167.9° and 3.3° , respectively, and a uniform superhydrophobicity across the surface was observed (Supplementary Table S2).

s.e.m. images of the surface of MWNTs/Nafion films are shown in Figure 1c and d. We observed networks of entangled nanotubes. Lumps formed in regions with dense clusters, which probably originated from the non-uniform dispersion of MWNTs in the solution or from fluid turbulence during the vacuum-filtering process. The size of the clusters varied from several to 20 μm . In other regions,

the MWNT networks were loosely packed and formed gaps of several hundreds of nanometers. In general, the micro/nano-structures on the surface of the MWNTs/Nafion films resembled those on lotus leaves, which efficiently repel water.

The SR-XPCI imaging procedure of the MWNT/Nafion film is illustrated in Figure 4a. Similar to the 3D image reconstruction of the lotus leaf, the extraction of air and water phases (purple for air, blue for water) was implemented within a selected region near the surface (Figure 4b and c). In the colored 3D image, we observed that a

network of air portions effectively buffered the water above the solid surface in most areas. The fragmented region of the air phase revealed irregularly shaped pores formed by the intertwined nanotubes and their efficient imprisonment of air. The protruding clusters (uncolored, corresponds to the void volumes) provided the main contact sites for water on the surface, mimicking the role of protruding papillae on lotus leaves. The similarity in the cluster size and the lateral spacing between lotus leaf and MWNT/Nafion films further confirms that the presence of air cushions plays an important role in the observed superhydrophobicity.

CONCLUSION

In summary, for the first time, SR-XPCI was employed for *in situ* observation of two types of superhydrophobic surfaces. By taking advantage of SR-XPCI that provides an *in situ* and non-invasive approach to image the underlying interfaces with high resolution and high contrast in 3D, we observed the buried SLV three-phase interfaces from 3D-reconstructed images. We found that the water on the superhydrophobic surface existed in the Cassie-to-Wenzel transit state. We also substantiated that micro/nano-structures on the superhydrophobic surface trap a large amount of air to form an air cushion to repel water. The protruding structures mainly function as supporting sites for water droplets. We expect that this study will deepen the understanding of the physical mechanisms of superhydrophobicity and open up new possibilities for the design of functional surfaces and bioinspired materials.^{27–31}

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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