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Enhanced Environmental Scanning Electron Microscopy Using Phase Reconstruction and Its Application in Condensation

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ABSTRACT

Environmental scanning electron microscopy (ESEM) is a broadly-utilized nanoscale inspection technique capable of imaging wet or insulating samples. It extends the application of conventional scanning electron microscopy (SEM) and has been extensively used to study the behavior of liquid, polymer and biomaterials by allowing for a gaseous environment. However, the presence of gas in the chamber can severely degrade the image resolution and contrast. This typically limits the ESEM operating pressure below 1000 Pa. The dynamic interactions, which require even higher sensitivity and resolution, are particularly challenging to be resolved at high-pressure conditions. Here, we present an enhanced ESEM technique using phase reconstruction to extend the limits of the ESEM operating pressure while improving the image quality, which is useful for sensing weak scattering from transparent or nanoscale samples. We applied this method to investigate the dynamics of condensing droplets, as an example case, which is of fundamental importance and has many industrial applications. We visualized dynamic processes such as single-droplet growth and droplet coalescence where the operating pressure range was extended from 1000 Pa to 2500 Pa. Moreover, we detected the distribution of nucleation sites on the nanostructured surfaces. Such nanoscale sensing has been challenging previously due to the limitation of resolution and
sensitivity. Our work provides a simple approach for high-performance ESEM imaging at high-pressure conditions without changes to the hardware and can be widely applied to investigate a broad range of static and dynamic processes.

**KEYWORDS**: environmental scanning electron microscopy, phase imaging, nanoscale sensing, condensation, dynamic processes, nucleation sites
The ability to image a wide range of wet and insulating materials in the gaseous environment highlights the significant advantage of the environmental scanning electron microscope (ESEM).\textsuperscript{1–4} In contrast to the conventional SEM, the ESEM is capable of detecting the secondary and/or backscattered electrons from ionized gas molecules (typically water vapor molecules) around the samples.\textsuperscript{1–3} The charging artifacts arising from nonconductive samples are effectively suppressed by the positive ions accumulating on the sample surface.\textsuperscript{1,2} Consequently, it is not necessary to maintain a high vacuum environment or coat insulators with a conductive layer when using the ESEM. Accordingly, the ESEM can provide physical insights into many phenomena at the micro-to-nanoscale such as phase changes of matter,\textsuperscript{5–8} colloidal dispersions,\textsuperscript{3,9–11} complex fluids,\textsuperscript{3,12–14} biological specimens,\textsuperscript{15–17} and chemical reactions.\textsuperscript{18–21}

However, the presence of gas in the specimen chamber often leads to degradation of image quality. As depicted in the inset of Figure 1 (a), when the pressure is elevated, incident electrons have a larger probability of being scattered by gas molecules. This scattering of electrons spreads the focused electron beam (e-beam) outside the area of interest (see Figure 1 (a)), thereby lowering the spatial resolution, elevating background noise, and degrading the image contrast. For this reason, the pressure of an ESEM chamber has been kept below 1000 Pa in practice,\textsuperscript{2} which limits the broad exploration at higher pressure conditions. Although the improvement of imaging quality was recently demonstrated, instrument-level modifications on the secondary electron detector and the differential pumping system are required.\textsuperscript{22} Traditional image processing techniques such as noise filters can also be applied, but the improvement in image quality is limited as both the noise and useful information will be filtered.

In this work, we developed a phase reconstruction technique to improve the imaging quality of ESEM without any instrument-level changes on the current ESEM system. Previously, it was
shown that the phase of an optical field is sensitive to weak perturbations in the topology of a sample because the interaction between the incident wavefront and weakly scattering specimen leads to the shift of phase (see Figure 1 (b)), which is detectable even at the deep-subwavelength scale.\textsuperscript{23,24} Accordingly, phase probing using either interferometry or depth scanning were extensively used to extract weak signals from a noisy background in previous biological and nanoscale research efforts.\textsuperscript{23–27} Although phase imaging has been successfully used for optical microscopy and X-ray related applications, its capability in the electron microscopy especially when using an ESEM has not been widely demonstrated. Here, we considered a “phase” associated with the electron wave, where high contrast information is embodied and can be reconstructed. Through the transport of intensity equation, we were able to retrieve the phase information, which is otherwise difficult to measure directly (see Supporting Information Section I for details). The relation between the phase and intensity of the wave can be expressed as,\textsuperscript{28}

\[
\nabla_{xy}^2 \psi(x,y;z_0) = -k \frac{\partial I(x,y,z)}{\partial z} \bigg|_{z_0} \tag{1}
\]

where $\nabla_{xy} \psi = I \nabla_{xy} \phi$, $\nabla_{xy}$ is the two-dimensional Laplacian and $\psi$ is defined as an auxiliary field. $I$ and $\phi$ are the two-dimensional intensity and phase of the scattered field at position $z = z_0$, respectively. Here, the electron wave propagates along the $z$-direction with a wave vector $k$. Note that this “phase” described by Eq. (1) is not necessarily the physical phase defined in the object space, but a pseudo-phase in the imaging space which represents the deformation of the wavefront due to scattering. Eq. (1) is the Poisson equation, because the right-hand side is known from the intensity field captured by ESEM, and the left-hand side contains the information of the phase for which we solve.
Among all of the ESEM applications, imaging of vapor condensation has been of particular interest. Condensation is a common phenomenon in nature and has been widely applied to power plant, energy conversion, water harvesting, anti-fogging, anti-icing, as well as thermal management. Understanding vapor condensation has heavily relied on ESEM as it occurs at the micro-to-nanoscale, but is also very challenging due to the following reasons. (1) Condensation experiments are typically carried out in relatively high-pressure conditions, which often exceed the operating pressure limit of the ESEM. (2) Condensation is a dynamic process. Imaging the droplet morphology, growth, and interaction requires high resolution and contrast. (3) Many nanoscale effects associated with condensation, such as droplet nucleation on nanostructured surfaces, are too weak to be detected and require high imaging sensitivity. Therefore, to demonstrate the advantage of our proposed technique, we applied this concept to enhance the fundamental understanding of condensation, where the two most promising modes, i.e., dropwise and jumping-droplet condensation were studied.

We obtained the phase field by computing the gradient of intensity along the propagation direction. In optical microscopy, the depth scanning technique is typically used to collect the out-of-focus information, which can also be applied to study static phenomena in ESEM imaging. In the case of condensing droplets, however, the out-of-focus information can be conveniently obtained without moving the sample stage along the optical axis. More specifically, the focal plane of the e-beam is fixed (the dashed line of Figure 1 (c)), but the best focal plane of the droplet (typically crossing the center of the droplet) is rising continuously as it grows. Consequently, except for the moment when the best focal plane coincides with the preset focal plane, images captured at any other time intervals are de-focused. As depicted in Figure 1 (c), in the droplet coordinate, to construct the gradient field at \( z_0 \), we need out-of-focus information at planes \( z_0 - \Delta z \) and \( z_0 + \Delta z \).
Note that at the moments \( t_0 - \Delta t \) and \( t_0 + \Delta t \), the preset focal plane coincides with planes \( z_0 - \Delta z \) and \( z_0 + \Delta z \), respectively. Because the scanning rate of the e-beam is much faster than the droplet growth rate, \( \Delta t \) is small enough so that the droplet does not expand too much. The out-of-plane \( (z_0 - \Delta z \) and \( z_0 + \Delta z) \) information at moment \( t_0 \) can be approximated by the images taken at moment \( t_0 - \Delta t \) and \( t_0 + \Delta t \), respectively. Therefore, the relationship between the spatial derivative and the temporal derivative of the intensity field can be formulated as,

\[
\left. \frac{\partial I}{\partial z} \right|_{z_0,t_0} = \left. \frac{\partial I}{\partial t} \right|_{t_0,z_0} \frac{dt}{dz} \bigg|_{t_0,z_0} \tag{2}
\]

where \( \left. \frac{\partial I}{\partial z} \right|_{t_0,z_0} \) can be directly obtained from ESEM images, and \( \frac{dt}{dz} \bigg|_{t_0,z_0} \) is the inverse of the speed of the droplet’s best focal plane which acts only as a scalar factor and will not affect the relative strength of the phase signal in each frame. We solved the discretized form of Eq. (1) numerically using the finite difference method\(^{45} \) (see Supporting Information Section II for the development of the finite difference solver by diagonalizing the Toeplitz matrix and importing Tikhonov-Phillips regularization).

Figure 1  (a) Spread of the e-beam width due to the electron scattered by gas molecules and ions under high-pressure conditions. Inset: Schematic of electron scattering mechanism. The beam electron (1) can be either elastically scattered to (2) transmit the gas molecule or (3) travel backwards, or (4) inelastically scattered to excite a secondary electron. (b) Schematic of the wavefront distorted by the weakly scattering nanoscale feature. (c) Schematic of the changing best focal plane position, \( z_0 \), due to the growth of droplets. The actual focal plane of the e-beam was fixed during scanning, but the best focal plane elevates with the growth of the droplet. The space-
dependent gradient of intensity at a certain time is approximated by the time-dependent gradient of intensity at the preset focal plane.

RESULTS AND DISCUSSION

We first carried out dropwise condensation experiments in an ESEM chamber (EVO 50, Zeiss). The saturation vapor pressure was maintained at 1000 Pa, 1300 Pa, 1500 Pa, 2000 Pa, and 2500 Pa, and the sub-cool of the temperature controlling stage was set to 1 K (see Supporting Information Section III for experimental setup and technical specifications of ESEM settings). Figures 2 (a) and 2 (b) show the temporal evolution of growing droplets at 1000 Pa and 2500 Pa (see Supporting Information Section IV for results from 1300 Pa to 2000 Pa). Specifically, the time-lapse images in the first row of Figures 2 (a) and 2 (b) were directly captured from ESEM, whereas the second row of Figures 2 (a) and 2 (b) shows the corresponding phase images retrieved from Eq. (1). Examining the experiment at 1000 Pa is useful to validate the accuracy of the proposed method because although the contrast of the raw ESEM images was very low, the position and size of droplets could still be observed and compared using the corresponding phase images. As depicted in Figure 2 (a), phase imaging precisely recovered the size and morphology of each droplet, where the image contrast was significantly improved, the background noise was successfully suppressed, and the phase shift arising from wave propagation in the liquid phase can be well retrieved. Additionally, it is worthwhile to mention that some of the dynamics were revealed from phase imaging. Growth of an individual droplet with time (the white-dashed circles of Figure 2 (a)) was measured from the retrieved phase. Coalescence of two droplets (the yellow-dashed circles of Figure 2 (a)) was also captured. As depicted in the last image of Figure 2 (a), the
size and position distribution of small droplets (<10 µm radius), which was difficult to resolve from raw ESEM images, were extracted from the background noise (see the red-dashed boxes).

When the chamber pressure was increased to 2500 Pa, the large-angle scattering of electrons became so severe that the signal from the droplets was completely masked by the background noise (the raw ESEM images in the first row of Figure 2 (b)). However, the growth and coalescence of multi-droplets can be seen from the retrieved phase images. For example, in the red-dashed boxes of Figure 2 (b), there were two droplets growing initially (from t = 10 s to t = 12 s), and then three more droplets close to those two droplets started to grow at t = 12 s. These five droplets began to interact with adjacent droplets at t = 23 s. At t = 35 s, the interfaces of three droplets (two were close to the upper boundary and the other one was at the center of the red-dash box) approached each other and merged into one larger droplet at t = 52 s.

Figure 2  ESEM and corresponding phase images of condensing droplets at (a) 1000 Pa and (b) 2500 Pa. Droplets condensed on a smooth silicon wafer coated with 137 nm of Teflon AF with an advancing contact angle of 115.5°. The first row of (a) and (b) depicts the raw time-lapse ESEM images during droplet condensation. The droplet dynamics, including the growth of a single droplet (the white-dashed circles) and the coalescence of droplets (the yellow-dashed circles), were clearly resolved from the phase images shown in the second row of (a). The distribution of 11 tiny droplets (the red-dashed boxes in the second row of (a)) and droplet interaction at high-pressures (the red-dashed boxes in the second row of (b)) could also be retrieved through phase imaging. (c) Normalized intensity distribution (from raw ESEM images) along (1) in (a). (d) Phase distribution along (2) in (a). The white-dashed lines (1) and (2) shown in (a) passed through the center of a droplet (≈ 10 µm in radius). The radius of this droplet could not be determined from the intensity
distribution in (c) due to the high noise level but could be measured from the phase reconstruction (the blue band with strong phase signal in (d)).

To demonstrate the advantage of phase imaging, we plot the intensity and retrieved phase distribution along a line (see lines (1) and (2) in Figure 2 (a)) crossing the center of a droplet in Figures 2 (c) and 2 (d), respectively. The intensity signal in Figure 2 (c) is comparable to the amplitude of the background noise, which made it difficult to distinguish the droplet using the intensity distribution. On the contrary, a strong phase signal was found in Figure 2 (d), where the position of the droplet could be estimated by the center of the peak and the size was determined from the width of the peak (see the blue band in Figure 2 (d) which indicates the radius of this droplet was \( \approx 10 \mu m \)). Therefore, counting a large number of droplets as well as measuring their spatial and size distribution can be rapidly achieved by fitting the peaks using a computer program. This information could not be obtained using the ESEM raw images. Our result demonstrates the practical significance in computer-aided analysis of droplets with the proposed method.

To quantitatively analyze the enhancement in image quality, we compared the contrast of ESEM raw images and corresponding phase images at different chamber pressures. The image contrast in this study was defined as the ratio of the strength of the peak signal from a droplet to the amplitude of background noise, which can be expressed as,

\[
\gamma = \frac{I_p}{\max(I_b) - \min(I_b)}
\]  

(3)

where \( I_p \) is the peak signal from a droplet, and \( I_b \) is the distribution of the background signal along a reference line. Figure 3 shows the image contrast \( \gamma \) of the raw ESEM and reconstructed phase as a function chamber pressures (see Supporting Information Section IV for the calculation of image contrast). \( \gamma \) of ESEM always stayed at around 1 (note the minimum value of the image contrast is
1 according to Eq. (3)), indicating the peak intensity signal was comparable to the background noise. Note that although $\gamma$ of the raw ESEM image is always close to 1 when the pressure is higher than 1000 Pa, the raw ESEM image at 1000 Pa can still look visually better than that at 2500 Pa (Figure 2) because more high intensity pixels can be seen on the droplet at lower-pressure conditions. Through phase reconstruction, $\gamma$ improved by a factor of approximately six at 1000 Pa. $\gamma$ of the phase image decayed with pressure rapidly due to electron scattering and decreased to about 2 at 2500 Pa. Above 2500 Pa, $\gamma$ converged to 1, indicating no further enhancement could be achieved. Additionally, we demonstrated this improvement in image contrast through phase reconstruction cannot be achieved by conventional image processing techniques such as noise filters due to the loss of information within a certain frequency range after filtering (see Supporting Information Section IV for detailed analysis).

Figure 3 A comparison of the ESEM and corresponding phase image contrast at different pressure conditions. The contrast of the ESEM raw image remained at around 1, indicating the peak signal from the droplets was comparable to the amplitude of noise. The image contrast was significantly improved through phase retrieval. The improvement decayed as the pressure increased. The error bars represent the standard deviations of multiple measurements on different droplets and images at the same pressure.

We next applied this enhanced ESEM to understand the growth dynamics of condensing droplets. Due to the high noise level of the raw ESEM image (Figure 2 (c)), it is challenging to perform any quantitative analysis even though the droplets can be seen at the relatively low-pressure condition (e.g., 1000 Pa). However, with the significant enhancement of image contrast through phase reconstruction, quantitative analysis of the evolution of droplet growth at relatively high-pressure
conditions becomes possible. Figure 4 shows the growth of an individual droplet with different sub-cooling temperatures at 1000 Pa. The sub-cooling temperature is defined as the temperature difference between the saturated vapor and solid-liquid interface. The radius of the droplets was measured from the retrieved phase images. The droplet grew almost linearly with time when the radius ranged from 10 µm to 50 µm and had larger growth rates at higher sub-cooling temperatures. The trend of the growth curve appears linear mainly because we only show the droplet growth in a small range before coalescence. In addition, when the droplet grows to tens of microns in radius, the internal convection starts to develop due to the liquid-vapor interfacial mass flow, which improves the heat transfer and results in the linear trend of the droplet growth (see Supporting Information Section V for more discussions). We compared our measurements of the droplet radius to a droplet growth model and showed good agreement in Figure 4 (see Supporting Information Section V of for details about the dynamic growth model).

Figure 4  Temporal evolution of a single-droplet radius at different sub-cooling temperatures ranging from a 0.45 K to 3 K before droplet coalescence. The sub-cooling temperature is defined as the temperature difference between the saturated vapor and solid-liquid interface. The pressure was maintained at 1000 Pa. Single-droplet growth study for ~5 droplets for each sub-cooling temperature was performed. The single-droplet radius was averaged for this group of ~5 droplets. The error bar accounts for both the resolution of ESEM as well as the standard deviation of multiple measurements on different droplets. The error bar increased with the increase of droplet radii, because the shadowed area surrounding the droplet became larger, making it harder to determine the size of the droplets.
Finally, to demonstrate the high sensitivity of the proposed method in nanoscale detection, we characterized the nucleation sites on copper oxide (CuO) nanostructured surfaces with a hydrophobic coating (Figure 5 (a)) using the conventional ESEM and compared the results with the corresponding phase image (see Supporting Information Section VI for the nanostructured surface fabrication). The experiment was carried out at 1300 Pa with about 0.5 K sub-cool. Figures 5 (b) and (c) show that more droplets were observed through phase reconstruction even when the droplet radii were only a few microns. The line-shape patterns shown in the background of Figure 5 (b) due to the electron scattering at the nanostructured surface were also effectively eliminated. We analyzed the number and location of the nucleation sites (Figure 5 (d)) in a 160 µm × 160 µm area. The average number of nucleation sites per unit area was $1.05 \times 10^9$ m$^{-2}$ from the phase image while this number was only $2.73 \times 10^8$ m$^{-2}$ according to the raw ESEM image, indicating the present approach can provide a more accurate estimation in determining the number of nucleation sites.

Figure 5 Spatial distribution of nucleation sites on CuO nanostructured surfaces. (a) SEM image of the CuO nanoblade structures. (b) ESEM raw image and (c) corresponding retrieved phase image of the condensing droplets on the CuO nanostructured surfaces. Few microns radius droplets were masked by the noise in the ESEM raw image, but more droplets were visualized through the phase retrieval process. (d) Coordinate map depicting the position of the nucleation sites where (□) represents the nucleation sites found from the ESEM raw image and (○) represents the nucleation sites found from the corresponding phase image. The error bars of the nucleation sites in the raw ESEM image were defined by the radius of droplets through manual inspection by
eye, whereas the error bars shown in the phase image were the full width half maximum of the signal peaks (referring Figure 2 (d)).

CONCLUSIONS

In summary, we presented an approach to enhance the quality of ESEM by extending the concept of phase to the field of e-beam imaging. We retrieved the phase of the electron wave by solving the transport of intensity equation numerically and carried out a proof-of-concept study with droplet condensation in high-pressure environments. Under these conditions, the noise of conventional ESEM became comparable to the signal of droplets. Experimental results showed that this enhanced ESEM extended the operating pressure range to 250%, without any instrumental changes in the experimental setup. Because of this improvement in image contrast, dynamics including droplet growth and coalescence were clearly extracted from low contrast intensity images, and computer-aided analysis on large numbers of droplets became possible. We applied this enhanced ESEM to analyze the effect of sub-cooling temperature on individual droplet growth and showed good agreement with theory. Finally, we analyzed the nucleation site distribution on nanostructured surfaces, and demonstrated more nucleation sites were discovered through phase imaging. Our study suggests a direction to interpret ESEM images through phase reconstruction and demonstrated its capability in analyzing vapor condensation. This phase imaging technique is generally applicable to any electron-beam microscopy analysis and has great potential for high sensitivity electron-beam probing.

MATERIALS AND METHODS
Transport of Intensity Equation Solver

The transport of intensity equation was discretized into a matrix form. The matrix was then diagonalized to a Toeplitz matrix. To avoid singularity in numerical computations, Tikhonov-Phillips regularizing filter was applied, which is characterized by a regularizing factor $\epsilon$. The detailed algorithm was discussed in Supporting Information Section II. This solver is also available online: https://figshare.com/articles/Robust_TIE_Solver_m/5504086.

Condensation Experiments

The water condensation experiments were carried out in an ESEM chamber (Zeiss EVO 50 scanning electron microscope) at various pressures. Dropwise condensation was on a smooth silicon wafer coated with 137 nm Teflon AF, and the jumping-droplet condensation was on the copper dioxide nanostructured surface. The sample was held by a sample holder on a cold stage. The temperature of the cold stage and the saturation vapor temperature were changes for different sub-cool conditions. Detailed experimental procedures were discussed in Supporting Information Section III.

Contact Angle Measurements

A custom-built contact angle measurement setup was used. A syringe pump (Micro4, World Precision Instruments) was used to add water droplets on the surface. A DSLR camera (EOS Rebel T3, Cannon) and macro lens were used to collect images of the advancing and receding droplets. Contact angle was obtained from the images using ImageJ. Detailed measurements can be found in Supporting Information Section III.

Copper Dioxide Nanostructured Surface Preparation
Nanostructured copper oxide films were fabricated by immersing a clean copper substrate into a hot (≈ 95 ± 2 °C) alkaline solution composed of NaClO₂, NaOH, Na₃PO₄·12H₂O, and DI water (3.75:5:10:100 wt. %). The surface reaction led to the formation of the CuO nanostructures. The surfaces were then coated with a conformal hydrophobic coating less than 100 nm thick by the company P2i. Details can be seen in Supporting Information Section VI.

**Dynamic Growth Model Simulations**

Numerical simulation on the droplet growth from a few nanometers’ radius to more than 60 μm radius was carried out using COMSOL. In this simulation, the pressure was set at 1000 Pa and the advancing contact angle was 115.5° according to the contact angle measurement. Descriptions about the dynamic growth model was shown in Supporting Information Section V.

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**Author Contributions**

L. Zhang and J. Zhu contributed equally to this work. L. Zhang and J. Zhu conceived the concept, built the theoretical framework, designed the experiments and processed experimental data. K. L. Wilke carried out the ESEM experiments. Z. Xu performed numerical simulation and L. Zhao and Z. Lu analyzed the experimental data. L. L. Goddard and E. N. Wang supervised and guided the project. All authors contributed to the final version of the manuscript.
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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

See Supporting Information for details on phase retrieval formulation using transport of intensity equation; matrix-based finite difference solver for transport of intensity equation; environmental scanning electron microscopy (ESEM) experiments; ESEM and corresponding phase images of condensing droplets for various pressure conditions; dynamic growth model for condensing droplets simulation; copper dioxide nanostructured surface fabrication.

REFERENCES


TOC graphic
Figure 1

448x272mm (300 x 300 DPI)
Figure 2

519x577mm (200 x 200 DPI)
Figure 3

Image contrast \( \gamma \) vs. Chamber pressure (Pa)

220x165mm (300 x 300 DPI)
Figure 4

Droplet radius R (µm) vs. Time (s)

Experiment, ΔT = 0.45 K
Experiment, ΔT = 1.6 K
Experiment, ΔT = 3.0 K
Simulation, ΔT = 0.45 K
Simulation, ΔT = 1.6 K
Simulation, ΔT = 3.0 K

220x165mm (300 x 300 DPI)
Figure 5

280x215mm (300 x 300 DPI)