Point-by-point response to reviewers

Reviewer 1 - Initial submission, March 8

The study demonstrates the mean inner potential of polystyrene spheres measured at different temperatures by off-axis electron holography. The experiments are carefully performed and presented, the results are supported by simulations, and the paper is overall very well written.

We appreciate the referee's positive comments. All points have been revised using red font in the manuscript and Highlights.

The following points can be improved:

1. In Highlights, the last item could be rephrased.

We changed it to "Charge density does not change as temperature, maintaining 9×10^8 e/cm^2."

2. Figures captions should begin with one line describing the figure as a whole.

We have added for Figures 1 to 5, while Figures 6 to 8 had introductory statements.

3. On page 1, more information about how to obtain the C_E value should be provided. Is this value provided as a parameter of a microscope, or is it given in some textbook or tables?

$C_E = \frac{2\pi e}{\lambda}\frac{E+E_0}{E(E+2E_0)}$, where λ is the wavelength of electron beam, E is the energy of electron beam and E_0 is the rest mass energy of electron beam. This form has been added in the text and a review article is cited. The detailed calculation at 300 keV is following.

e = 1.6×10^{-19} C

E = 300 keV

$\lambda = 1.97 \times 10^{-12}$ m

$E_0 = m_e c^2$ = 511 keV

Therefore, $C_E = 6.52 \times 10^6$ rad/(mV)

4. "Close to RT, each hologram was acquired with an exposure time of 6 s at a dose rate of 1 to 2 e/˚A2/s. At lower sample temperature, 30 frames at 0.2 s per frame were recorded." Writing doubled division …/../… should be avoided. What was the dose at the lower temperature?

The unit was changed to e/(Å^{2}s). The dose rate wasn't changed during cooling. We have added "At lower sample temperature, 30 frames at 0.2 s per frame were recorded while maintaining the same dose rate."

5. "magnification of 18.3 kx" I am not familiar with "kx" units, can the magnification factor be expressed in more familiar units or numbers?

The magnification has been rewritten as 18 300×, and the same modification has been applied to all magnification values throughout the manuscript.

6. More details about the reconstruction procedure should be provided.

A few sentences were added when describing Fig. 1a, and a review article of introduction to electron holography was cited on Page 2.

"The intensity of the hologram includes amplitude and phase in a complex form. In order to reconstruct the phase that comprises electrostatic potential, a Fourier transform is applied to the hologram, followed by an inverse Fourier transform of one of the two sidebands [14]. The phase signs of two sidebands are opposite, and all the reconstruction was processed by choosing the sideband which contains positive phase."

The equations of reconstruction were omitted in this manuscript because it is classical knowledge.

7. The story about the twin image and Fig. 2 is not very clear. Is it the same "twin image" as the artefact arising during the reconstruction of a hologram due to the (RO*+R*O) terms? But the twin image term should be filtered out during the reconstruction.

When a particle is charged, the electrostatic potential surrounding the particle decays from the sample surface until far distance. The field of view of biprism is limited, 2 μm in this study. The electrostatic potential spreads beyond the field of view and consequently distributes in the reference wave. When the reference and objective wave gets interfered, the electrostatic potential in the reference wave contribute to the interreference and eventually to the resulted phase. Such a contribution from the reference wave is equal to the electrostatic potential from an oppositely charged particle placed perpendicular to the biprism direction and in the distance of 2 μm. This oppositely charged particle is defined as "twin image".

The twin image is different from the sidebands of Fourier transform. In the term of (RO*+R*O), the two components have different signs of electron phase φ, one positive in the form of $e^{i\varphi}$ and the other negative $e^{-i\varphi}$. In this study, we chose the sideband comprising the positive phase.

To response to the referee's comment and to avoid potential confusion, we have added a few words on perturbed reference wave on Page 5:

"The electrostatic potential of the charged sphere decays from the surface to the edge of the FOV and further spreads in the reference wave. The PRW contributes to the recorded hologram when interfering with the objective wave. This contribution is equivalent to that of a point charge -Q at the position (0,*D*,0), where *D* is the FOV width (2 μm in this study) [34,37], as shown in Fig. 2a."

8. The direction of the biprism should be indicated in Fig. 2a.

The direction of biprism is parallel to the dashed lines in Fig. 2a. It has been added both in figure caption and in the main text.

9. All physical values should be written in italic, all units and subscripts – in roman font (ISO 31 rules).

We thank the referee's careful reading. We have checked and revised all font in the main text, figures and supplementary information.

Reviewer 3 - Initial submission, May 27

The authors investigate the mean inner potential (MIP) of polystyrene spheres with different diameters and different supports at different temperatures using off-axis electron holography. They carefully distinguish between MIP, surface and bulk potentials due to charging because of secondary electron emission, and electrostatic stray fields into the area of the reference wave. It is clearly demonstrated that the measured MIP is increasing with decreasing temperature, which is related to an increase in electron scattering factor through the Debye-Waller factor. This manuscript represents original work on 20 measurements with a detailed analysis of experimental data through extensive simulations. Because of its methodical character, the manuscript fits perfectly into the scope of Ultramicroscopy. Only a few minor things should be tackled before publication, hence I recommend the publication in Ultramicroscopy with minor revision:

We appreciate the referee's careful reading and positive review. All points have been revised using red font in the manuscript.

Page 1, abstract: "Integrating the the model" => "Integrating the model"

The extra "the" has been deleted.

Page 1, introduction: "An additional empty reference hologram (with the sample removed from the field of view) is typically used to remove distortions from the imaging and recording system of the microscope." Well, the distortions are not removed, but only artificial phase modulations due to distortions are compensated by means of the empty reference hologram. Please correct.

We appreciate the referee's correction. It has been revised as "An additional empty reference hologram (with the sample removed from the field of view) is typically used to compensate the distortion-induced phase modulations by subtracting it."

Page 1, introduction: "[…] V_t is the total potential (including the MIP and additional contributions to the potential resulting from charge redistribution in the sample) and C_E a constant […]". The measured phase shift accordingly to eqn. 1 is additionally due to the electrostatic stray field around the sample, hence also above and below the sphere. Please supplement.

We thank the referee's supplement. The sentence is now "V_t is the total potential, including the MIP, contributions to the potential resulting from charge redistribution in the sample and the electrostatic potential above and below the sphere in the vacuum."

Figure 2: What is the distance between the charged sphere in FOV and the twin image induced by the biprism? Please supplement.

The distance between the charged and twin sphere is around 2 um that is equal to the width of FOV. This information has been added to main text describing Fig. 2a on Page 5.

Page 8, Temperature dependence: It is assumed that there is a Schottky barrier on the interface between metal support and the polymer interface. From the experiments you conclude that "the electron-beam-induced charge does not exhibits clear dependence on temperature." This would suggest that the Schottky barrier does neither exhibit a temperature dependence. Have you tried to estimate the influence of temperature on the Schottky barrier?

We appreciate the referee's valuable insight. We have added a few sentences of discussion in main text describing Fig. 6 on Page 9:

"This would suggest the Schottky barrier between the supporting substrate and sphere rarely changes as temperature. The Schottky barrier is the difference between work function of metallic substrate (Au 5.45 eV at RT [39]) and electron affinity of the insulate sphere (polystyrene 0.4 eV at RT [40]), resulting in a barrier of 5.05 eV. The temperature dependence of work function of metals is approximately on the scale of 10^{-5} to 10^{-4} eV/K [41,42,43], and the temperature dependence of band gap of polymer is around on the scale of -10^{-3} eV/K [44]. At low temperatures, the Schottky barrier decreases by at most 0.32 eV, still maintaining a large barrier height."