

Supplementary Information to Accompany:

## Removal of Attached Zinc Oxide and Titanium Dioxide Nanoparticles from Spinach Leaves by Rinsing in the Absence and Presence of Preexisting Surface Extracellular Polymeric Substances (EPS)

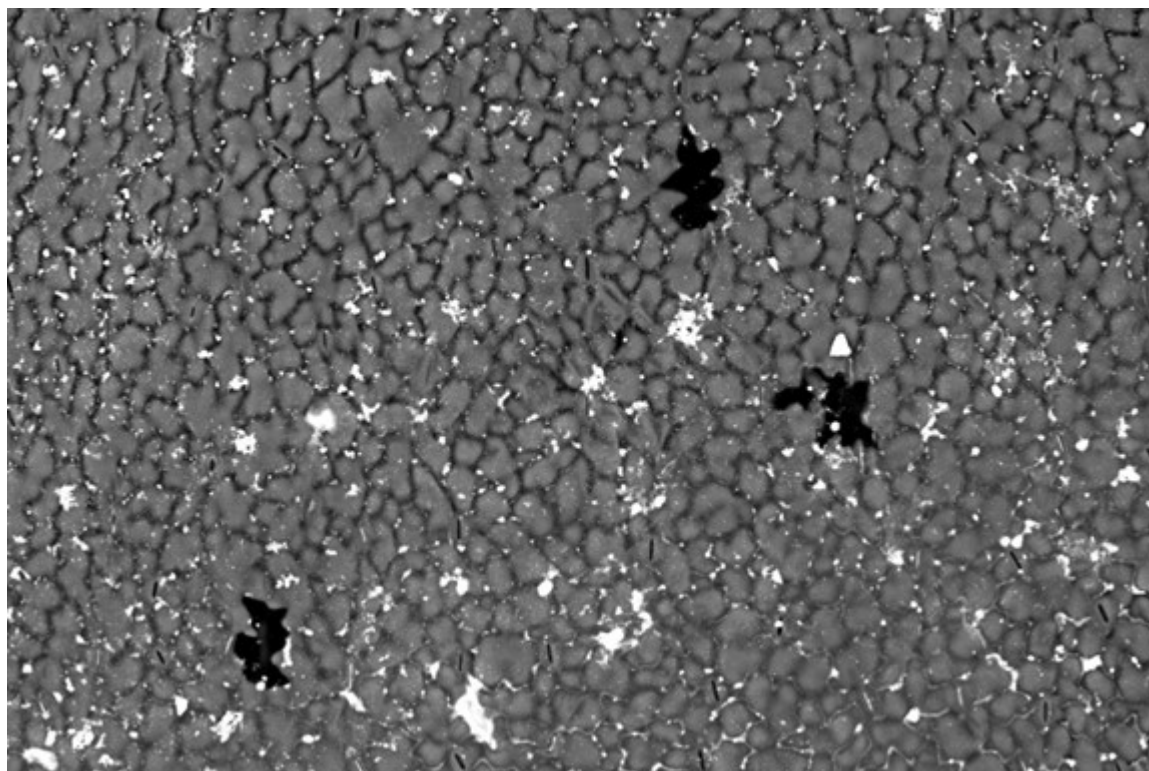
Jack E. Bezdek, Keith A. Strevett and Tohren C. G. Kibbey \*

School of Civil Engineering and Environmental Science, University of Oklahoma, Norman, OK 73019, USA;  
jack.bezdek-1@ou.edu (J.E.B.); strevett@ou.edu (K.A.S.)

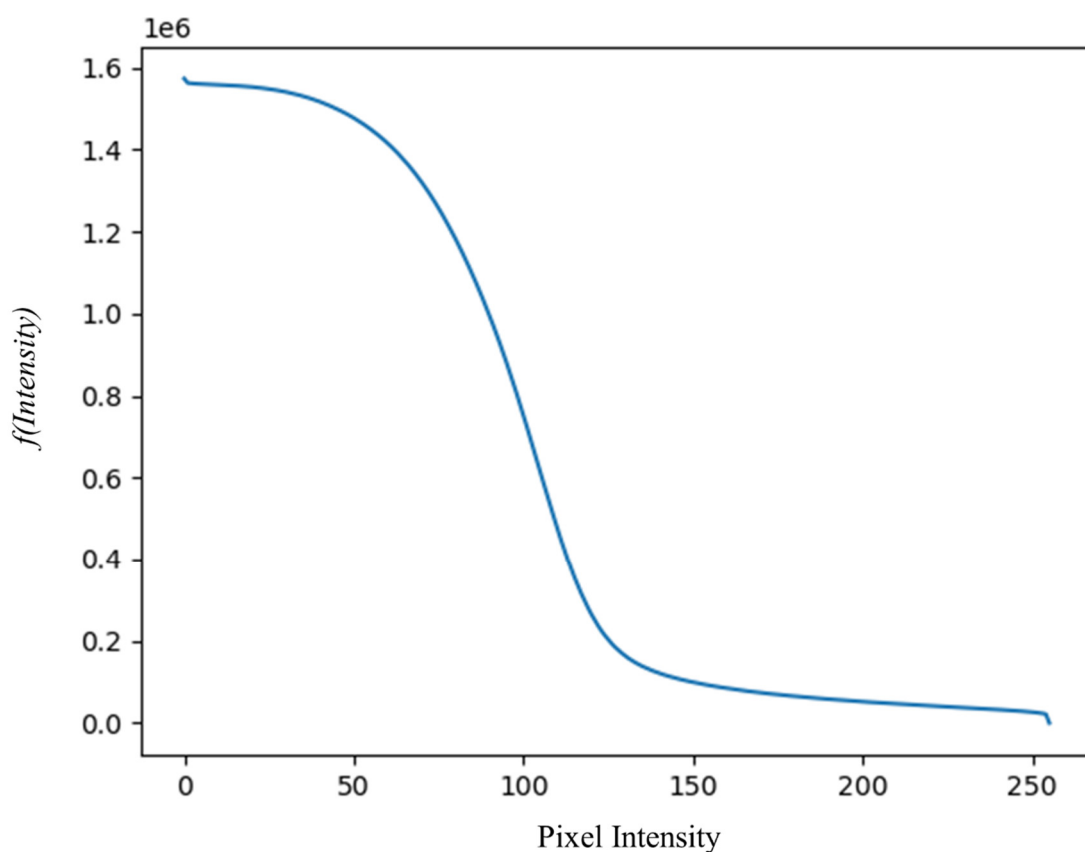
\* Correspondence: [kibbey@ou.edu](mailto:kibbey@ou.edu) (T.C.G.K.)

### Image Analysis of Backscattered Electron Images

This section describes the algorithm used to determine appropriate threshold pixel intensity values for quantification of nanoparticle coverage in backscattered SEM images. The algorithm was coded in Python, and applied for batch processing of all images. The code initially converts the images to 8-bit grayscale images, and then determines a function  $f(intensity)$  which describes the total number of pixels with brightness greater than a given intensity. **Figure S1** shows an image of ZnO on fresh spinach, and **Figure S2** shows  $f(intensity)$  vs pixel intensity.

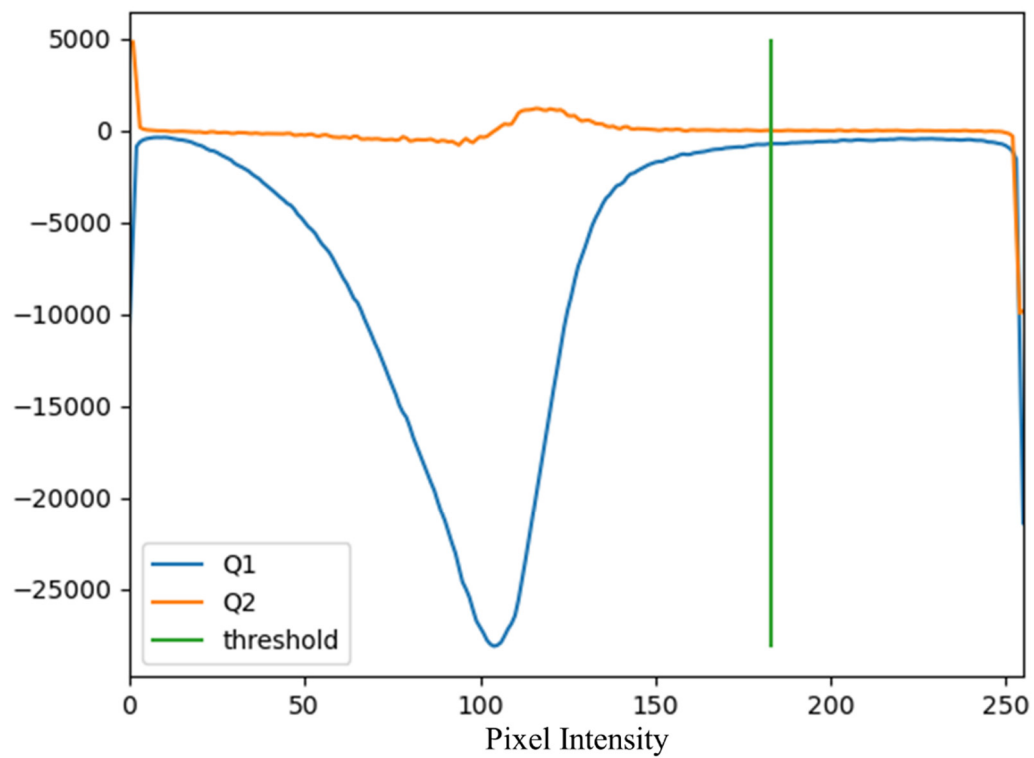


**Figure S1.** Backscattered SEM image of ZnO on fresh spinach.

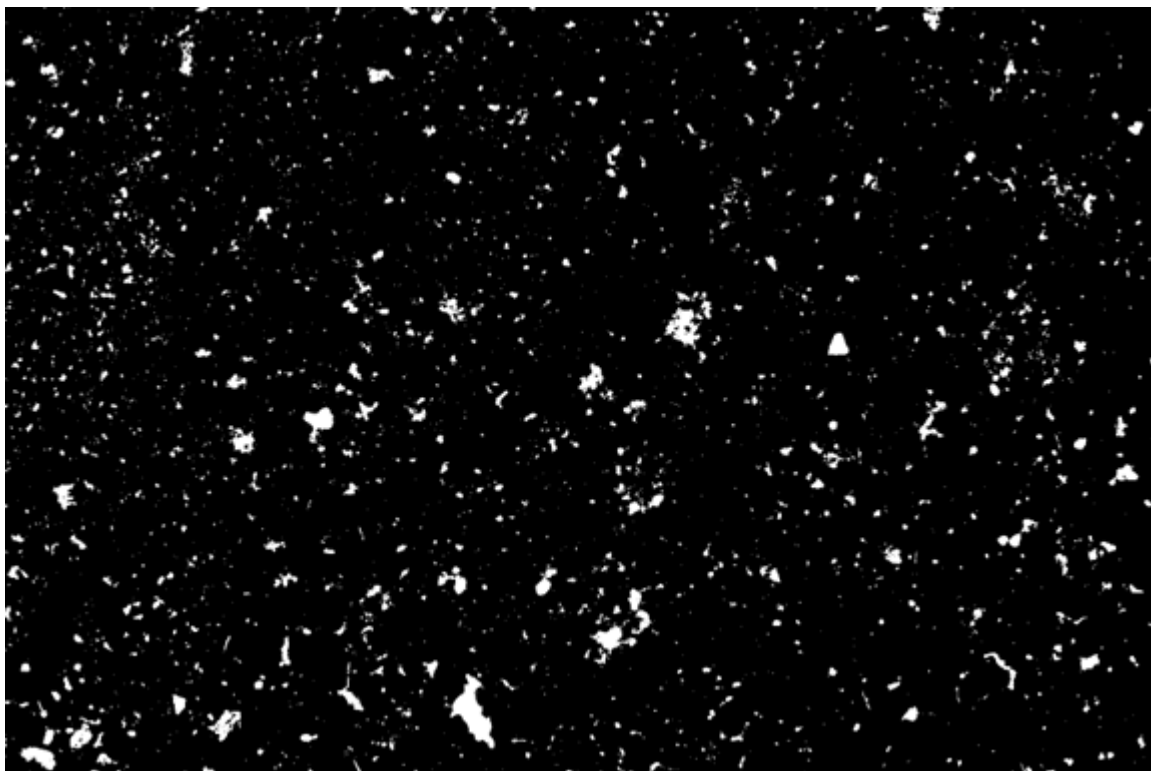


**Figure S2.** Plot showing  $f(intensity)$  for the image in Figure S1, the total number of pixels in the figure greater than each intensity value.

The threshold is then determined from the first derivative of  $f(intensity)$ ,  $Q_1$ . **Figure S3** shows the first ( $Q_1$ ) and second ( $Q_2$ ) derivatives of  $f(intensity)$  as a function of pixel intensity. The threshold intensity (green line) is determined as the intensity where  $Q_1$  is 1% of the way between its maximum and minimum values, for intensities greater than the global minimum. This intensity threshold provides a good empirical measure of a point where increasing or decreasing threshold intensity has minimal impact on the number of nanoparticle pixels identified. **Figure S4** shows the final identified pixels associated with nanoparticles.



**Figure S3.**  $Q_1$ ,  $Q_2$  and the identified threshold for  $f(intensity)$  shown in Fig. S2.



**Figure S4.** Nanoparticle clusters identified based on the threshold in Figure S3.