Counting Small Particles in Electron Microscopy Images— Proposal for Rules and Their Application in Practice

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Overview of Particle Counting Rules
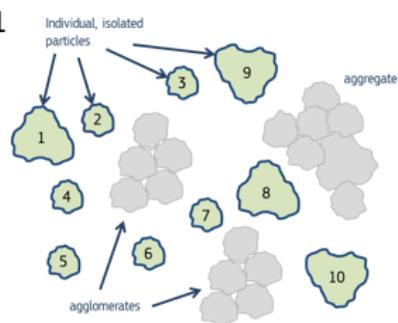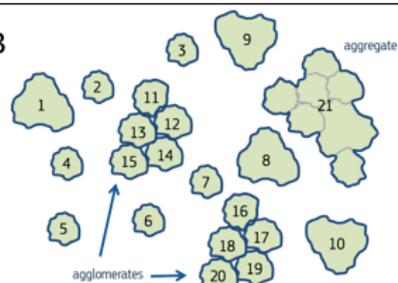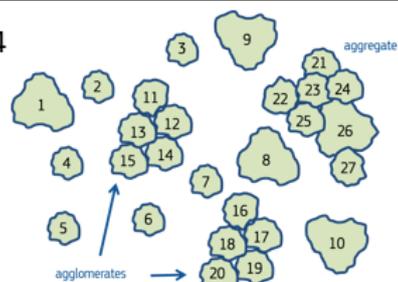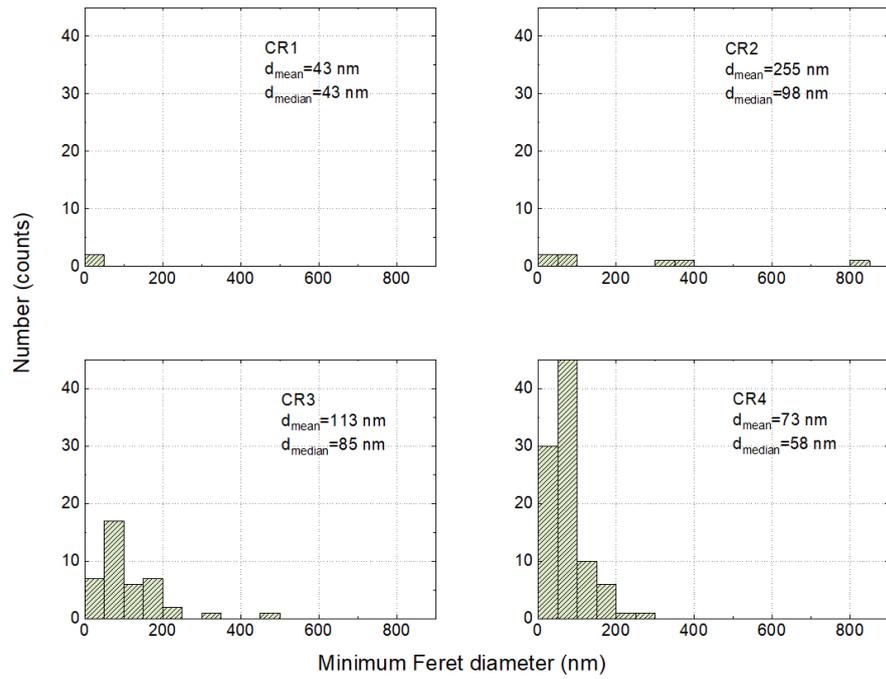
Figure S1. Minimum Feret Diameter of zinc oxide based on the application of the CR1 to CR4 to the TEM image in Fig. 6. As seen also from these graphs the number of particles counted varies significantly with counting rule applied as does the mean and median size of these particles.
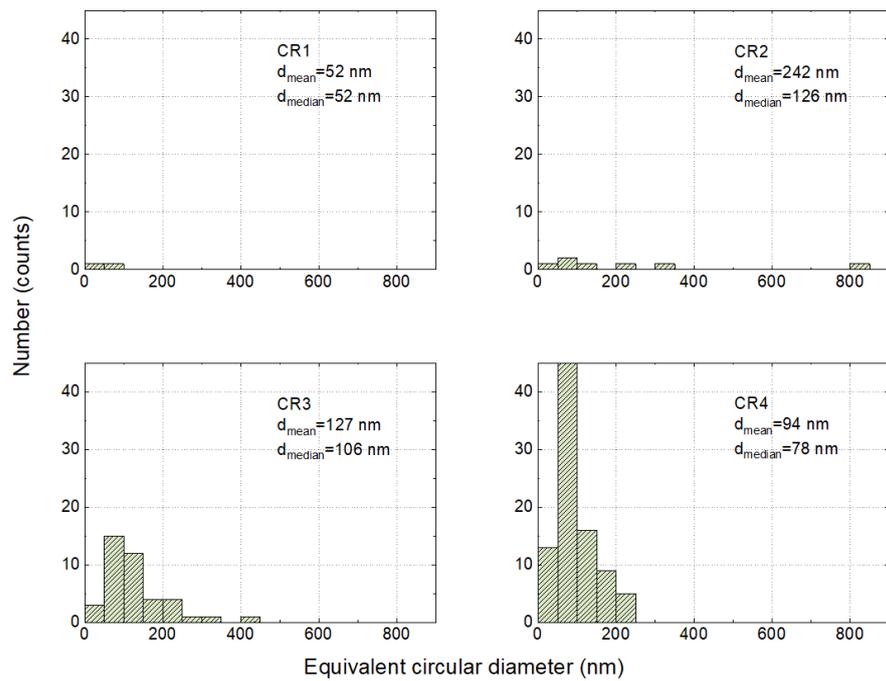
Figure S2. Equivalent circular diameter of zinc oxide as resulted after the application of the CR1 to CR4 to the TEM image in Fig. 6. As seen also from these graphs the number of particles counted varies significantly with counting rule applied as does the mean and median size of these particles.
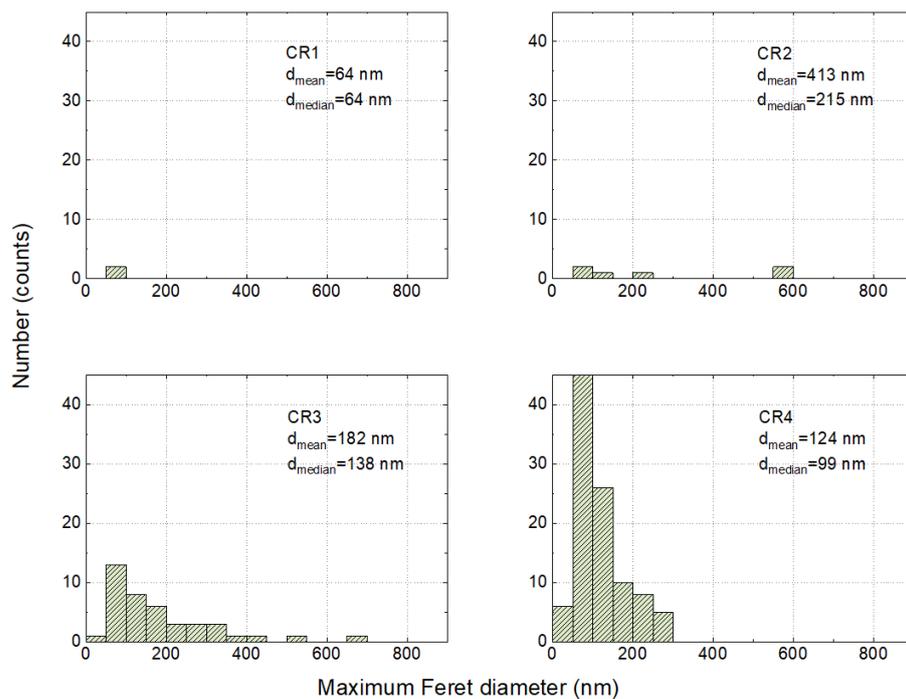
Figure S3. Maximum Feret Diameter of zinc oxide based on the application of the CR1 to CR4 to the TEM image in Fig. 6. As seen also from these graphs the number of particles counted varies significantly with counting rule applied as does the mean and median size of these particles.
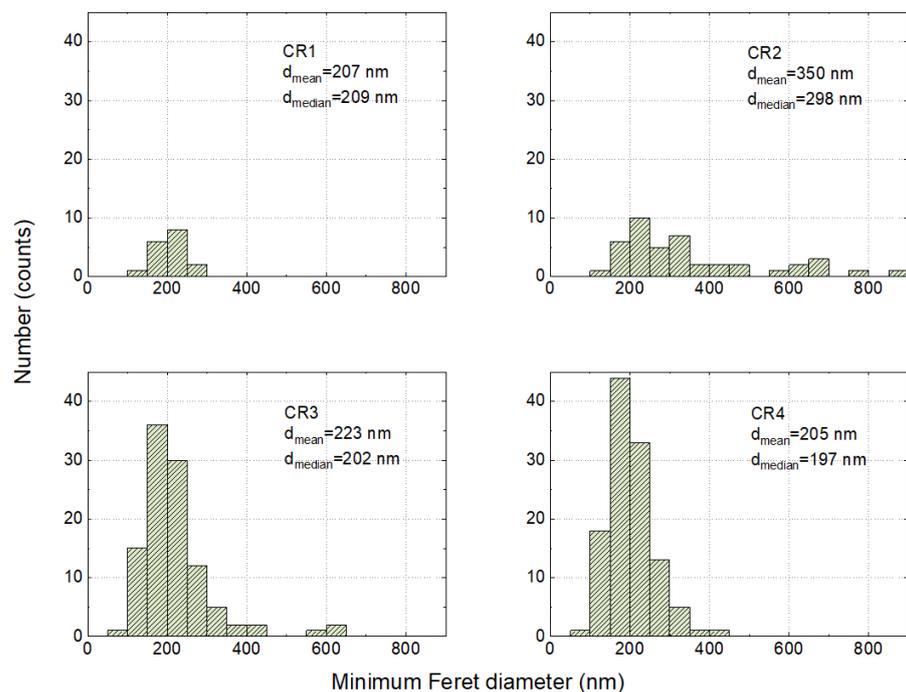

**Figure S4.** Minimum Feret Diameter of titanium oxide obtained after the application of the CR1 to CR4 to the TEM image in Fig. 7.
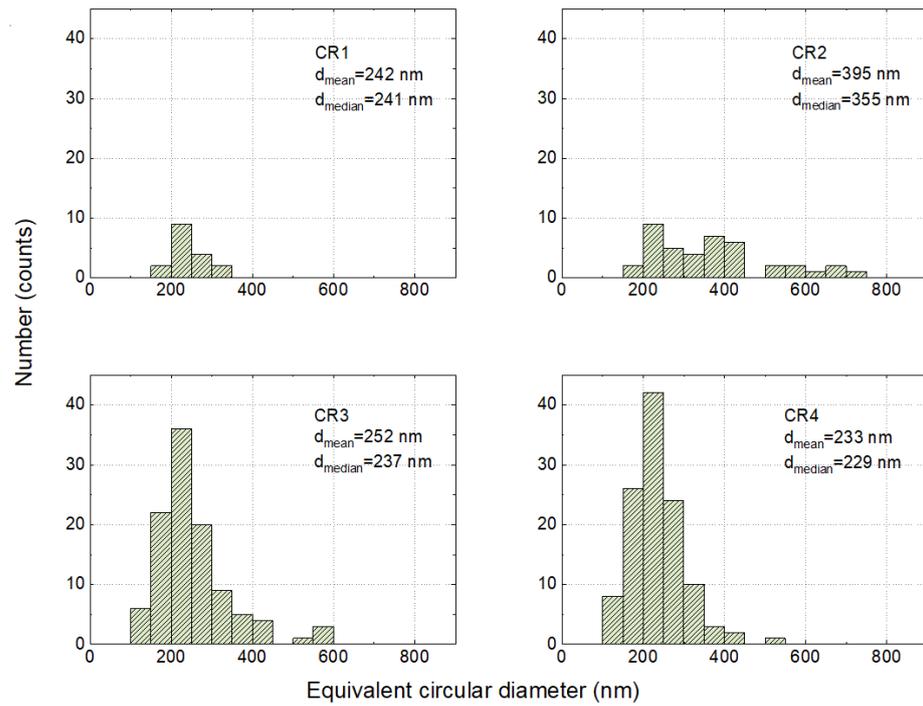
Figure S5. Equivalent spherical diameter of titanium oxide obtained after the application of the CR1 to CR4 to the TEM image in Fig. 7.
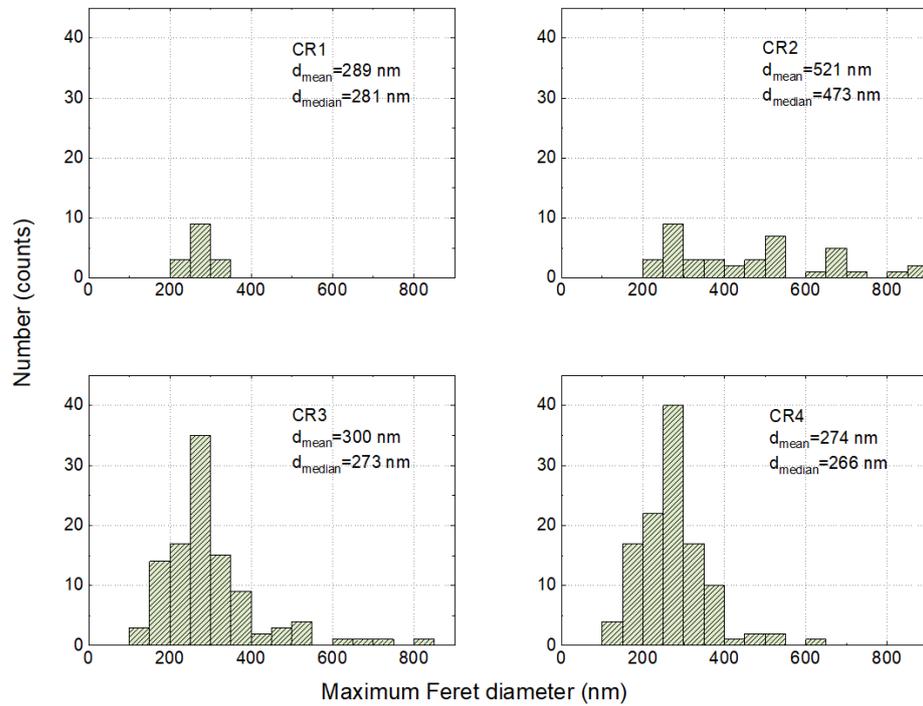

Figure S6. Maximum Feret Diameter of titanium oxide obtained after the application of the CR1 to CR4 to the TEM image in Fig. 7.

Table S1. compares descriptor values and results when applying the Counting Rules to the TEM micrograph (titanium dioxide) shown in Figure 7. The numbers are obtained assuming a lognormal distribution. See Figures S4–S6 for details of the corresponding particle size distributions.

d_{mean}; d_{median}; SD (nm)				
Counting rule Descriptor	CR1	CR2	CR3	CR4
Minimum Feret diameter	207; 209; ± 11	350; 298; ± 29	233; 202; ± 9	205; 197; ± 5
Equivalent circular diameter	242; 241; ± 10	395; 355; ± 30	252; 237; ± 9	233; 229; ± 6
Maximum Feret diameter	289; 281; ± 12	521; 473; ± 46	300; 273; ± 12	274; 266; ± 8

Information on image acquisition and used software

Image acquistion:
Titanium dioxide (Figure 7)
      Instrument: ZEISS Gemini Supra 40
      Electron beam voltage: 10 kV
      Pixel size: 4.9 nm
      Type of detector: SE InLens

Zinc oxide
      Figure 6:
      Instrument: JEOL JEM-2200FS
      Electron beam voltage: 200 kV
      Pixel size: 0.853 nm
      Type of detector: Gatan CCD camera

      Figure 8:
      Instrument: ZEISS Gemini Supra 40
      Electron beam voltage: 20 kV
      Pixel size: 1.5 nm
      Detector type: i) SE InLens and ii) conventional SE detector applied to the transmitted electrons via a dedicated sample holder (STEM-in-SEM)

      Figure 9:
      Instrument: ZEISS Gemini Supra 40
      Electron beam voltage: 10 kV
      Pixel size: 4.9 nm
      Type of detector: SE InLens

Software:
      ImageJ 2.1.0/1.53c
      OriginPro2022