# Employing Confidence Intervals in Electron Microscopy Digital Image Analysis to Promote Better Analytical Practices

Penn Master of Chemical Sciences

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## Background

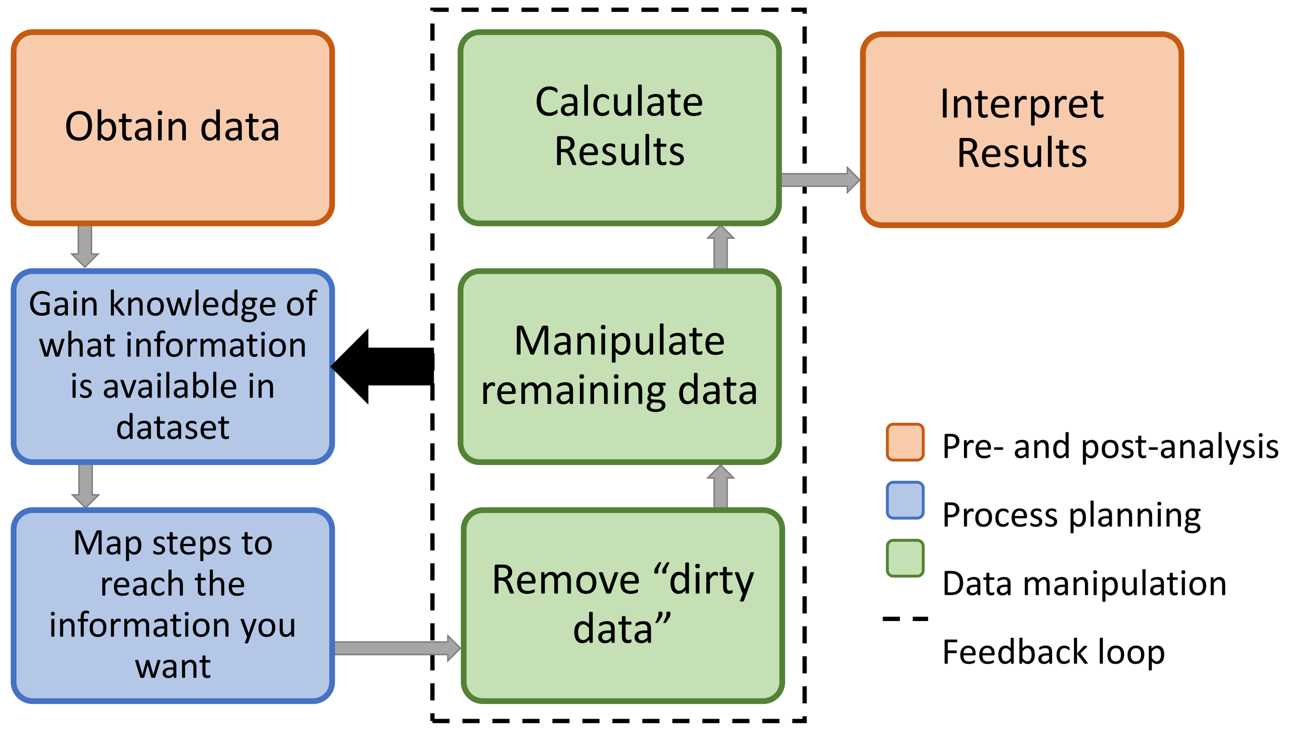



**Figure 1.** Schematic of data processing procedure

Benchmark software used to analyze transmission electron microscopy (TEM) images of nanoparticle systems, such as ImageJ, are limited by their propensity to misidentify particles, requiring post-analysis manual removal, and their failure to divulge data manipulation bias introduced during processing. The data analytics field can help design better TEM image analysis processes by providing techniques such as removing unusable data before analysis and including feedback loops to improve analysis parameters in a non-linear procedure **(Figure 1)**. A MATLAB® Application was developed that includes novel algorithms to measure particles in TEM images and confidence intervals, a quantitative indicator of the results' reliability and feedback loop trigger for the procedure.[1,2]

## Radiating Intensity Profiles

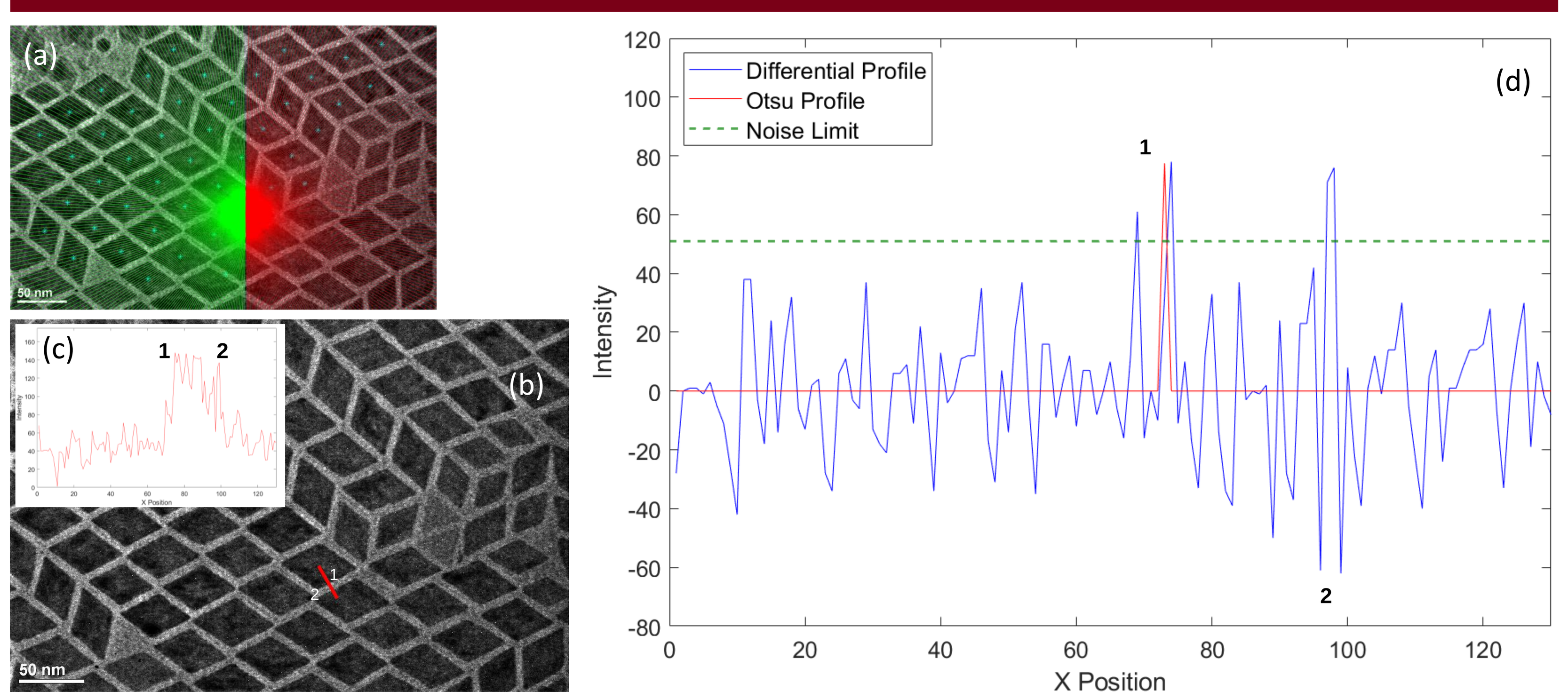



**Figure 2.** Visualization of novel TEM image analysis algorithms including radiating intensity profiles (a), an excerpt indicating particle-background transition (b-c), and differential profile (d). TEM image of a $GdF_3$:Yb/Er (20/2 mol%) courtesy of K. Elbert.

Particle center points are identified using Otsu's thresholding.[3] Then, 358 radiating intensity profiles are used to determine a particle's perimeter **(Figure 2a)**. For each profile, the transition from particle to background is in a high-slope area, indicating a point along the edge of the particle **(Figure 2b-c)**. A differential profile (blue) is calculated, where the peak indicating the position of the edge point is a positive peak most closely matching a reference binary peak (red) from Otsu's thresholding **(Figure 2d)**. The 358 edge points combine to form a single particle perimeter.

## Confidence Intervals

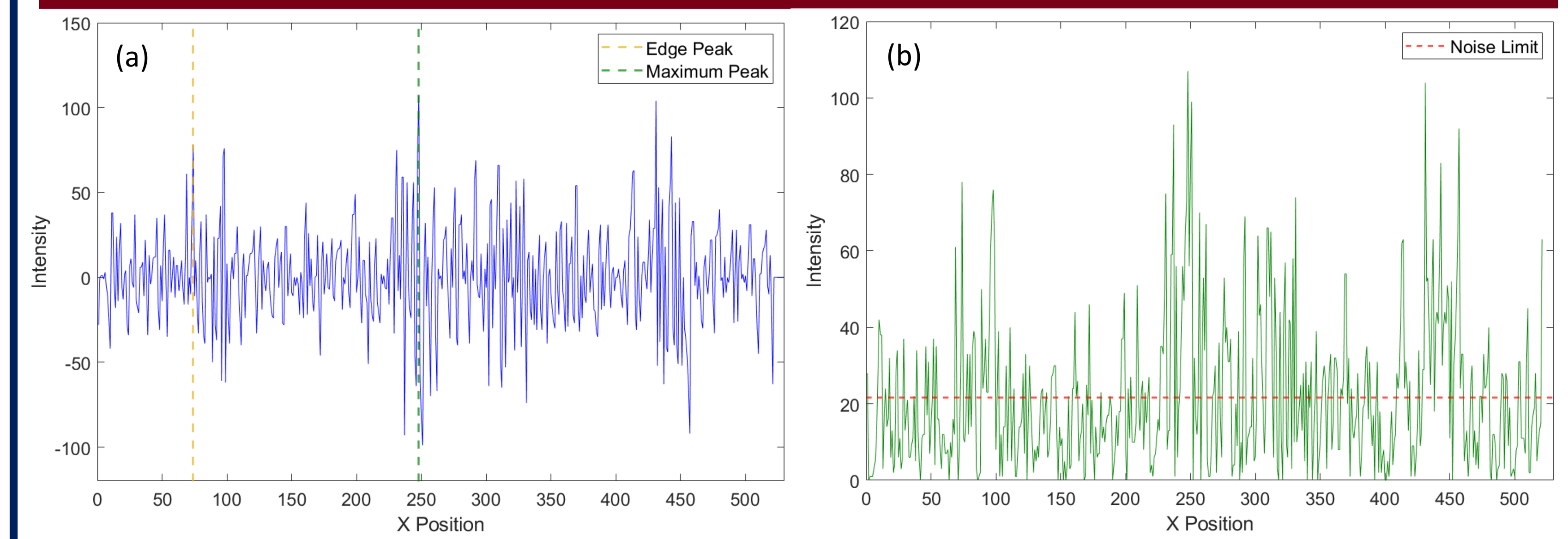



**Figure 3.** Visualization of confidence interval algorithms, (a) edge point attribute, (b) noise level attribute. The confidence interval was calculated as 76.52%

Each edge point's confidence interval indicates its position's reliability reported as a percentage average of two attributes. The first attribute is the proportion of the edge peak to the maximum amplitude of the differential profile **(Figure 3a).** The second is the inverse proportion of the average noise level of the absolute value differential profile to the maximum amplitude. Edge point confidence intervals are averaged to find the reliability of particle perimeters or overall image results **(Figure 3b).**

## MATLAB® Application Dashboard

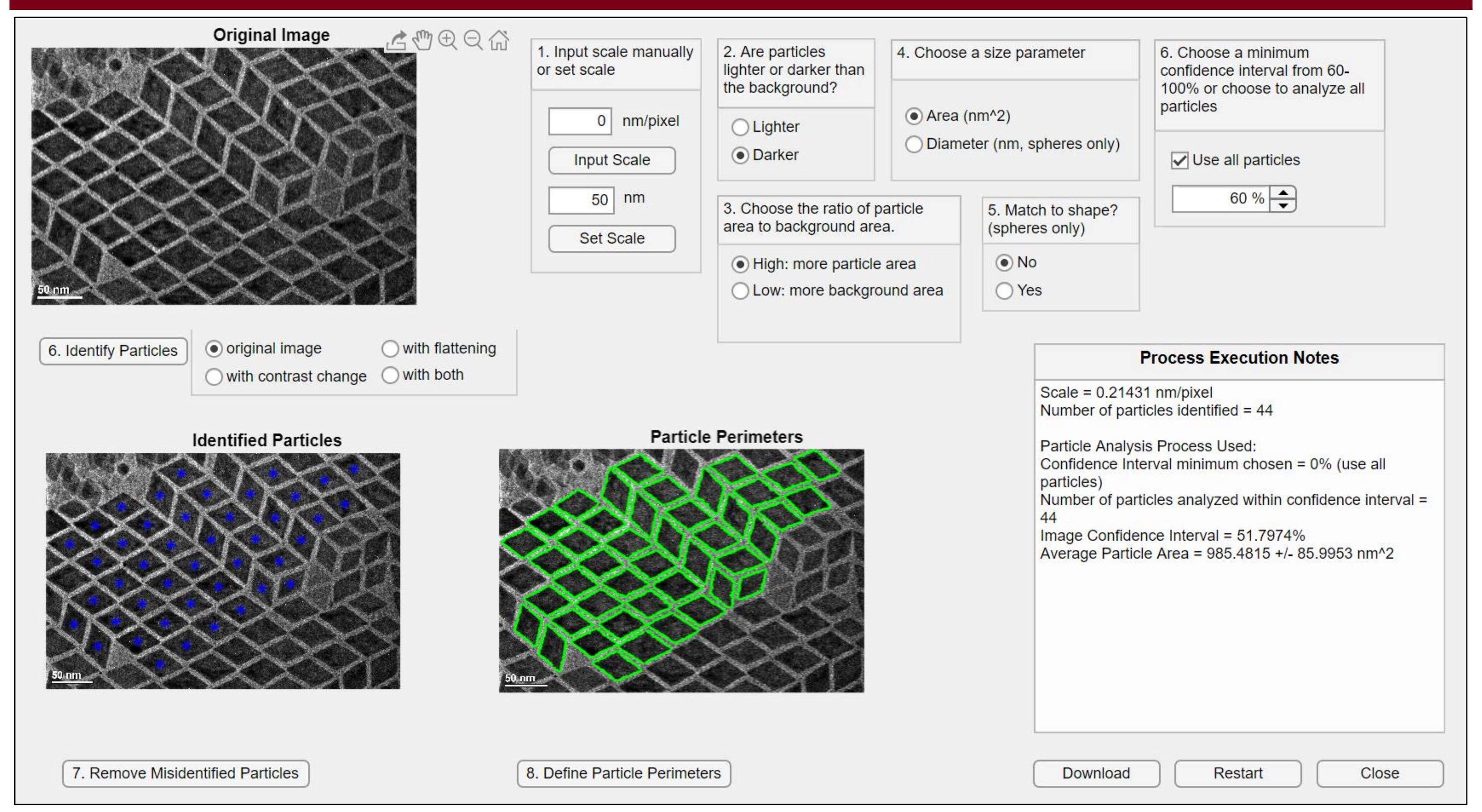
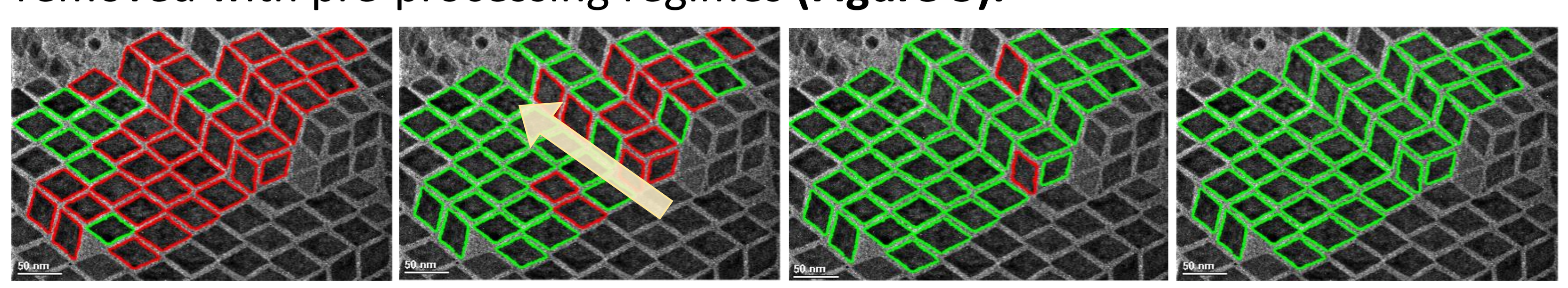



**Figure 4.** MATLAB® Application Dashboard

The MATLAB® Application Dashboard allows the user to set analysis parameters, including a confidence interval minimum, and remove misidentified particles before analysis, eliminating the necessity for post-analysis statistics **(Figure 4).** Varying the confidence minimum helps determine the ideal minimum to maximize image confidence and number of particles identified and reveals a background gradient that could be removed with pre-processing regimes **(Figure 5).**



55% confidence minimum | 50% confidence minimum | 45% confidence minimum | 40% confidence minimum

**Figure 5.** Confidence interval minimum used to indicate background gradient

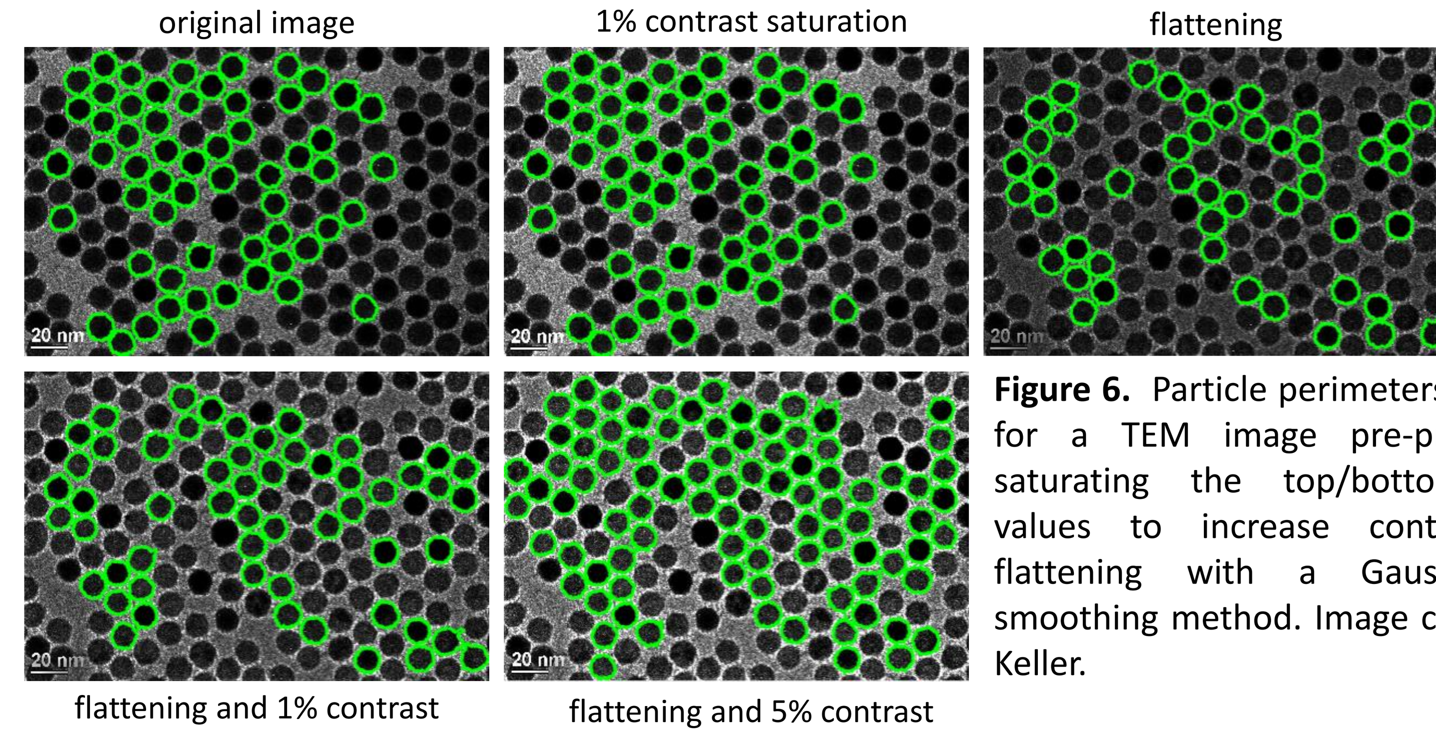



**Figure 6.** Particle perimeters determined for a TEM image pre-processed by saturating the top/bottom intensity values to increase contrast and/or flattening with a Gaussian kernel smoothing method. Image courtesy of A. Keller.

**Table 1.** Results from analyzing eight TEM images of a $Fe_3O_4$-OA system with pre-processing regimes

| Pre-Processing Regime | Number Particles Identified | Average Diameter (nm) | Image Confidence |
|---|---|---|---|
| Original Image | 472 | 13.7 ± 0.6 | 51.05% |
| 1% Contrast Saturation | 483 | 13.7 ± 0.6 | 56.80% |
| Flattening | 466 | 13.7 ± 0.5 | 49.77% |
| 1% Contrast and Flattening | 537 | 13.6 ± 0.5 | 58.21% |
| 5% Contrast and Flattening | 746 | 13.5 ± 0.5 | 61.33% |

Trials pre-processing eight TEM images of a $Fe_3O_4$-OA system revealed the data manipulation bias introduced by these regimes **(Figure 6).** Increasing the contrast was found to increase the number of particles identified, increase the image confidence, and decrease the average diameter. Flattening could be used in conjunction with contrast increase without decreasing the number of particles identified or reducing the image confidence. The standard deviation, or size distribution, decreased with more robust pre-processing **(Table 1).**

## Conclusion and Future Work

The presented MATLAB® Application can determine particle perimeters with greater precision than global thresholding. Confidence intervals give size parameters' reliability and provide a feedback loop trigger for better process planning. The application could be applied to analyze SEM/TEM darkfield images, calculate inter-particle distances, or analyze images of reference standard systems because the reference particles can be removed before analysis.

## References

1. MATLAB R2019a; The MathWorks, Inc.: Natick, Massachusetts, 2019.
2. *App Designer*, MATLAB R2019a; The MathWorks, Inc.: Natick, Massachusetts, 2019.
3. Otsu, N. A Threshold Selection Method from Gray-Level Histograms. *IEEE T Syst Man Cyb*. **1979**, *9*(1), 62-66.

## Acknowledgements

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**provided TEM image datasets*