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Imaging Guide Part 1: Supplemental Table 1

0.1 Rank of Auto Threshold Methods in ImageJ (Based on inlens, contrast, nice image)

We tested all auto threshold methods provided by ImageJ on different SEM images of nanoparticles, including nice, uneven contrast and inlens images. The results suggest that **Local Otsu** is the **best** choice. Meanwhile, **Local Bersen** and **Local MidGrey** are alternative options, which are working **good**.

It is also important to avoid using **Local Median**, **Local Phansalker**, **Local Sauvola**, **Global MinError(I)** and **Global Percentile**. They can not give resonable threshold when analyze SEM images of nanopartilces.

By analyzing the thresholded images carefully, we sumimize the effectiveness of these auto methods in the following tables, including 9 local methods and 16 global ones.

Rank of ImageJ Local Auto Threshold Methods ([Open directory](#))

Local Methods	Nice images	Uneven contrast images	Low res Inlens images
Otsu	Best	Best	Bad But close to get clusters
Bersen MidGrey	Best	Good	Bad Fail to draw out clusters
Niblack	Good A little noisy	Good	Worse Poor on the whole image
Mean	Bad Wrong particle size Too large	Bad Poor on the whole image	Worse Poor on the whole image
Contrast	Best	Worse Poor on the brighter part	Worse Poor on the whole image
Median Phamsalker Sauvola	Do not use them.		Worse

Rank of ImageJ Global Auto Threshold Methods ([Open directory](#))

Global Methods	Nice images	Uneven contrast images	Low res inlens images
Intermodes MaxEntropy	Best	Bad	Worse
RenyiEntropy	Best	Bad Poor on the brighter part	Worse
Default Huang Yen	Best	Bad Poor on the brighter part	Worse
Isodata Li Moments Otsu	Best	Worse Poor on the brighter part	Worse
Triangle	Best	Worse Wrong particle size Too small	Worse
Mean	Worse Wrong particle size Too Large	Worse Wrong particle size Too small	Worse
Minimum	Worse Wrong particle size Too small	Worse Wrong particle size Too small	Worse
Shanghag	Worse Wrong particle size Too small	Worse	Worse
MinError(I) Percentile	Do not use them		Worse