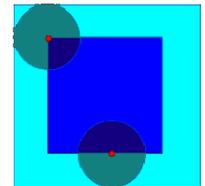
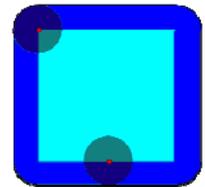




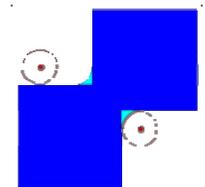
UNIQUE PSA300 IMAGE ANALYSIS SOFTWARE FEATURES

The PSA300 software created by Clemex comes from a group of scientists working in the field of image analysis for many years. The software has now been customized for particle size and shape applications for use with the PSA300 image analyzer. This is a radically different approach than the software designed by those particle sizing companies who recently learned to apply image analysis to this application. Because of this difference in development path the PSA300 provides many powerful software features that, although standard tools in image analysis, are uniquely valuable in the field of particle characterization. This document explains many of these features how users benefit when applying them to particle characterization applications.

Erosion and Dilation are two of the most common image processing functions performed. These functions improve edge definition by performing opposite tasks. The operations take two pieces of data as inputs: the image to be modified, and a set of coordinate points known as the *kernel*. Although these operations are rarely applied individually to particle characterization applications they are explained here because more advanced functions include these steps. **Erosion** is a transformation filter that uses the kernel size and shape selected by the operator to reduce the size of features. The picture to the right shows the erosion of the light blue square by a spherical kernel shape (in gray), creating the smaller dark blue square. **Dilation** is the opposite of erode. This filter adds layers of pixels around the edge of the particle according to the size and shape of the chosen kernel. The picture to the right shows the dilation of the light blue square into the dark blue square with rounded corners.



Closing is the combination of two binary image operations: erode and dilate. The size parameter determines the degree of erosion that will be used to reconstruct certain features. In a Closing instruction, smaller valleys and craters are filled (made darker) based on the value of the size parameter as seen in the picture to the right. This is a fairly common tool used in particle characterization to smooth edges.



Delineation: Whenever there is a transition from a dark to light pixel (or vice versa) at the edge of features, the camera detects intermediate gray levels at the transition point. The delineation filter removes intermediate gray levels to increase contrast wherever there is a transition from dark to light or light to dark. This is done by changing the gray value of pixels in the transition zone by assigning them either the maximum or minimum value in the kernel depending on which is closest to their original value.



Technical Note

Unique Software Features

When the kernel size is increased, it is more likely that the range separating the minimum and maximum kernel values will be great. This accentuates the delineation attained in a single iteration (size=1=3X3 kernel, size=2=5X5 kernel, size=3=7X7 kernel, etc.). *Figure 1* shows the effect of delineation to the ultimate pixel level. The top image shows the gradual transition between the particle and the background. The bottom image shows the abrupt transition achieved with the delineation function. *Figure 2* shows the effect of applying two delineation steps on a particle image. Notice the improved edge transition.

White feature				Transition zone				Black feature			
255	255	255	255	210	170	110	80	0	0	0	0
255	255	255	255	210	170	110	80	0	0	0	0
255	255	255	255	210	170	110	80	0	0	0	0
255	255	255	255	210	170	110	80	0	0	0	0

New Transition zone											
255	255	255	255	255	255	0	0	0	0	0	0
255	255	255	255	255	255	0	0	0	0	0	0
255	255	255	255	255	255	0	0	0	0	0	0
255	255	255	255	255	255	0	0	0	0	0	0

Figure 1: Effect on white/black pixel boundary after Delineation

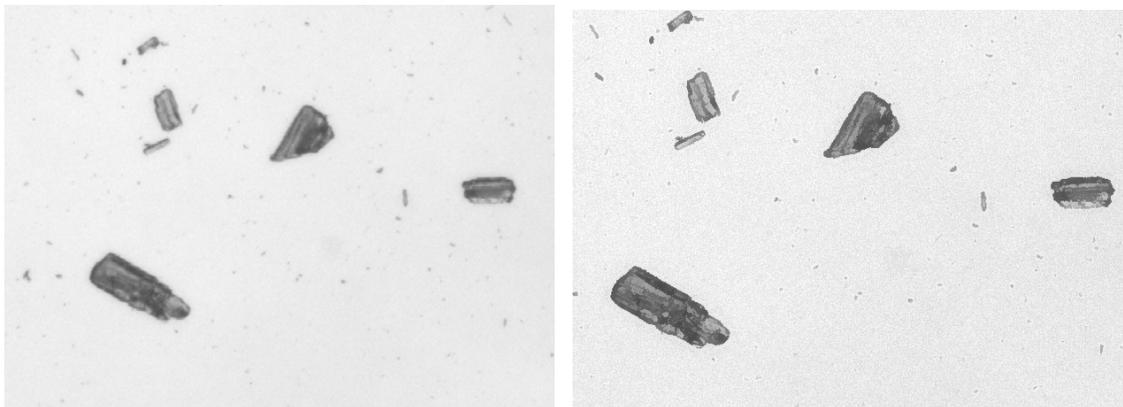


Figure 2: Image on the left without Delineation and the resultant image (right) after the tool is applied

Multi-Layer Grab is a unique PSA300 function useful for samples with a wide range of particle sizes. Limitations in depth-of-field for optical microscopes make it difficult to have both small and large particles in focus at the same time. By adjusting the height (z-axis), focusing on different portions of the image, and combining these views, a sharp image can be generated despite the fact that various portions of the image are within different focal planes. The Layers edit box lets the user determine how many layers, or focal planes, will be considered to generate the final composite image. The alternate approach of measuring the sample using different objectives takes much longer than the multi-layer grab method. *Figure 3* shows the same image with the small particle in focus, large particle in focus, and the combined view with all particles in focus using the multilayer grab.

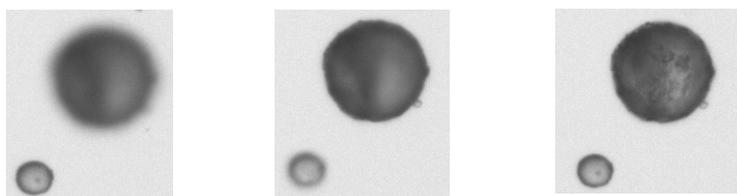


Figure 3: Multi-Layer Grab combines data from the leftmost image (small particle in focus) and center image (large particle in focus) to create the final, accurate composite image on the right



Particle Separation: Many image analysis packages offer ways to separate touching particles. This is important in order to assign individual size and shape parameters to particles in contact with each other. While other software programs only allow the user to turn this feature on and off, the PSA300 allows the user to adjust and set how aggressive the separation algorithms should be. Particle separation functions within the PSA300 software include Reconstruct, Separate, and Bridge Removal.

Reconstruct is actually a combination of several low level operations based on kernels. It separates objects gradually over many iterations as specified in the Cycles edit box (cycling to infinity is similar to using Separate). This operation attempts to maintain the original size of features by dilating features once they have been separated. Note that iterations are not additive but cumulative. Applying 2 cycles followed by a second application of 4 cycles will yield a different effect than applying 6 cycles at once. In many cases it may be useful to apply a Reconstruct in two steps, starting with several cycles followed by another with fewer cycles. The first step separates larger features, step two restores inappropriate separations that affected smaller objects. If used on elongated features, the cycles value should be less than the feature's thickness. *Figure 4* shows an image before and after applying the Reconstruct function.

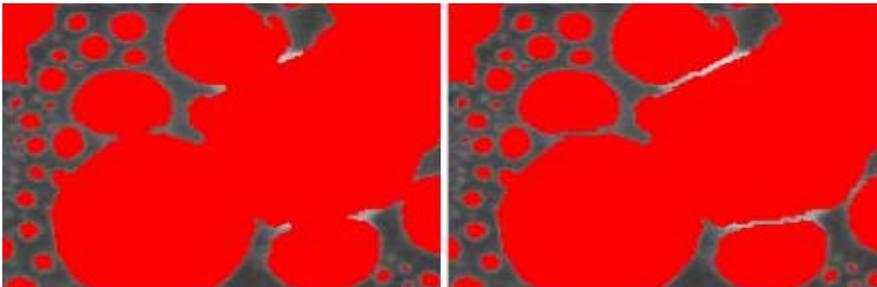


Figure 4: Before (image on the left) and after (right) Reconstruct

Separate: This function is similar to Reconstruct without the ability to control the number of cycles. It is a morphological filter that separates features by distance analysis. It is less sensitive to the size of features than Reconstruct, but can occasionally separate objects arbitrarily. Separate is most effective when the features of interest form a homogeneous ensemble (and are not elongated). Often, applying Convex Hull before Separate will reduce the occurrence of inappropriate separations.

Bridge Removal: This instruction is used to separate juxtaposed or slightly superimposed particles. It is generally used after the binarization of close objects connected by a bridge, as illustrated in *Figure 5*. The Bridge Removal instruction is more effective when marked concavities (cusps) are present at the junctions of the objects to separate. It is usually good practice to fill inner holes to avoid spurious openings after the execution of the instruction.

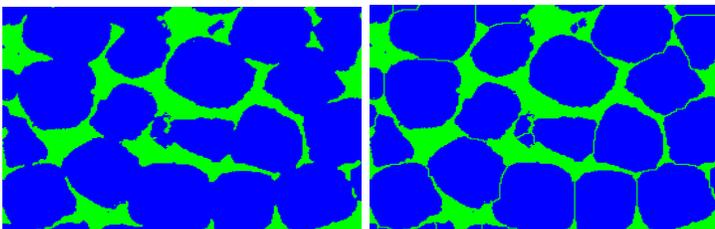


Figure 5: Before and after Bridge Removal. Notice that the particles have been binarized and any inclusions filled



Separate Long Objects is the unique PSA300 feature used to separate fibers or acicular crystals for accurate chord length distribution measurement. The other separation algorithms discussed in this document would break crossed fibers into four individual segments, none of them equal to the actual fiber length. Other solutions for the separation of two crossed particles would inaccurately report four pieces. Using **Separate Long Objects**, the PSA300 separates crossing fibers as individual particles and assigns each one accurate size and shape parameters. *Figure 6* shows fibers before and after applying the **Separate Long Objects** function.

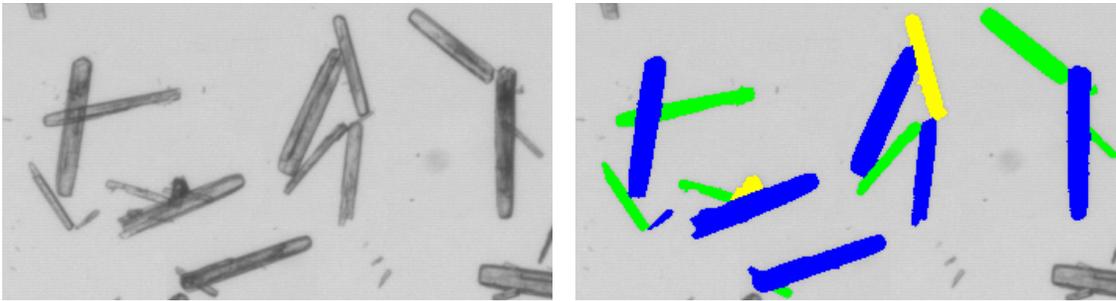


Figure 6: Fibers before and after applying the Separate Long Objects function

Thresholding: All image analysis programs offer some kind of threshold function to separate the particles from the background. The PSA300 software offers multiple paths for achieving this goal. **Gray Threshold** allows the user to perform a manual threshold based on selecting an intensity value while watching an image. Unique features within the PSA300 software include Auto Gray Threshold and Contrast Thresholding.

Auto Gray Threshold automatically detects phases in a grayscale image. With Auto-thresholding, you no longer need to manually set intensity intervals, but instead allow the software to choose optimum conditions.

The **Contrast Threshold** function is extremely useful when measuring particles when the particle/background contrast is weak. *Figure 7* shows an example where Contrast Thresholding is more effective than conventional thresholding. Particles that are more or less transparent fall into this category.

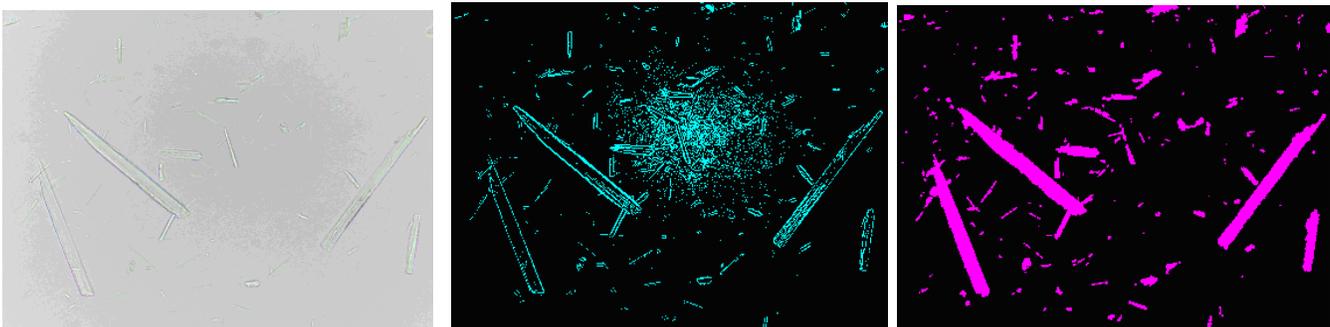


Figure 7: The leftmost image shows the as-is image without thresholding, the center image shows the effect of conventional thresholding, and the final image shows the PSA300 Contrast Threshold



The **Convex Hull** feature is used to fill the perimeter concavities of a particle. The result is similar to what would happen if a rubber band were placed around an object. This filter selectively dilates concave portions of a particle's contours until they become convex. This operation is most effective with small concavities. A few cycles of Convex Hull are useful when particle perimeters need to be measured at high magnification. As the magnification increases, the edges of particles can become too rough (a fractal effect) and lead to erratic perimeter measurements. Since Convex Hull reduces concavities, applying a few cycles can improve the accuracy of perimeter measurements as shown in *Figure 8*.

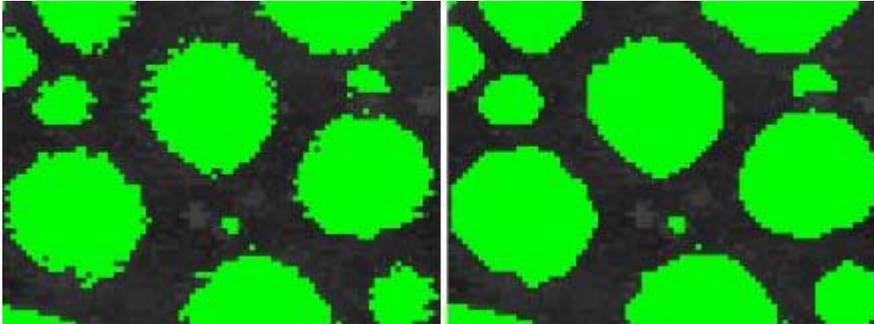


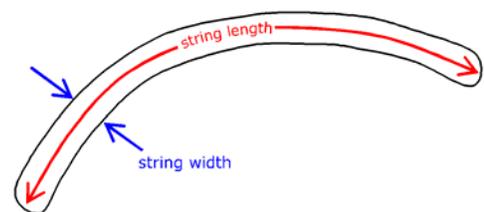
Figure 8: Before (left) and after (right) applying the Convex Hull operation

Accurate Volume Distributions: Image analysis results are collected as a number distribution, which is often later converted to a volume distribution. Transforming the two dimensional projected area into a volume distribution requires an assumption on the shape of the particles. Other software packages always assume spherical shaped particles. The PSA300 software can build the volume distribution based on spherical, ellipsoidal, cylindrical, and trapezoidal shapes. Combining the length and width measurements of the 2-D projection of each particle with an accurate shape basis generates a more accurate volume distribution.

Some applications may include smaller particles encapsulated within larger particles. The **Child Area** function calculates the percent of the parent particle occupied by the child particle. The **Child Count** function totals the number of child particles within the parents.

The **String Length** calculation provided in the PSA300 software measures the true length of thin, curved, and elongated particles such as curved fibers. A regular length measurement based on the longest Feret diameter would underestimate the actual length of certain particles. The equation used to measure string length is shown below.

$$\text{String Length} = \frac{\text{Perimeter} + \sqrt{\text{perimeter}^2 - 16(\text{area})}}{4}$$



**Conclusions**

The PSA300 software includes the functions described here and many more not covered that aid the user to acquire the highest quality raw data and generate the most accurate results possible for particle characterization. While other software packages make decisions without explanations or options, the PSA300 software allows the user to optimize the algorithms for specific samples and applications. But don't believe you need to be an expert in image analysis to make best use of these powerful functions. Pre-designed measurement routines and expert technical support assures that all customers generate the best possible results.

**The PSA300 Image Analysis System**

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