**Procedure for Counting Particles Using ToupView and Mathematica**

1. **Scope**

The following procedures are for the comparison of particulate counts on the surface of a compression pack by using the ToupView microscope program and a Mathematica script.

1. **Preliminary Steps**

In order to capture pictures to be examined, the AmScope microscope was used. A calibration slide was placed on the surface to give an accurate count of the particles. The calibration slide used for this procedure was 1 DIV = 0.01mm, and 0.5mm for the mini compression packs since it was easier to work with. A green light shined at an angle was used instead of the usual white light from the telescope since the particles showed up more with the green light. The microscope was zoomed all the way in to capture as many particles as possible.

1. **ToupView Procedure**

Conveniently, there is an extension installed in ToupView that counts particles. First, a photo of the sample was opened in ToupView. A problem arose when the calibration measurement ruler interfered with the count due to its dark lines, so several steps were taken to counteract that.

First, the photo was cropped such that only one quadrant was shown. Since a calibration slide was used, it was easy to maintain a constant size to work with. This way, the cropped section could be placed such that the lines do not interfere.

Due to the lighting, the particles were a lighter color than the background. Since the particle counter counts darker spots, the colors were inverted. Then, the segmentation feature was used to distinguish the darker particles from the background. The colors were binarized, and the particle count feature under the Plugin option was used. Only black spots were counted, so the GrayMin parameter was set to 0 and GrayMax was set to 1. Also, only particles of significant size were counted (an arbitrary area greater than 10 micrometers was chosen) since the smaller ones could be from the background. The particles were then counted and checked. The resulting counts included the relative size of the particles.

1. **Mathematica Procedure**

A script was made to count the particles using the same principles of the ToupView version. A photo was opened and one quadrant was cropped to work with, setting it so the lines from the calibration slide do not interfere. The photo size was inputted in order to find the size of each relative particle later on. The photo color was then negated so that the lighter particles would become dark, then the background was removed to more accurately count the particles. With the background removed and the particles darkened, the photo was then binarized so the particles were easy to see.

The size of one pixel was then calculated by dividing the size of the area by the pixel size of the photo. Finally, the particles were counted by size and recorded.

1. **Comparison**

The ToupView procedure seems more accurate in that it can define the background versus the particles clearer. The segmentation feature in the program helps a lot in that the user can choose the degree at which it separates the particles and the background. With some of the lighting from the AmScope pictures, it is difficult to discern the spots, so ToupView has a better grasp of that aspect.

However, it does take more user input and time to go through all the features one by one in ToupView. Mathematica has all the features already inputted so it is easier to go through them and is less time consuming. Only the picture and size has to be inputted and the script goes through the commands easily.

The number of particles these two procedures did not differ greatly. ToupView was more precise and counted more particles since it was able to differentiate them better from the background, but the count from Mathematica was not too far off, as shown in the excel spreadsheet.

Some photos were not able to be used since the color of the background and the particles were too similar and did not give an accurate count. However, as many photos and samples were used as possible to give a fairly accurate count.