Instructions for ParticlePicker plugin

1. Install ImageJ
   run ij128.exe and extract all file to a directory (ImageJ home directory).

   To run ImageJ program, just double click the ^(Ij-icon.ico) in the ImageJ home directory. (You can also download the newest ImageJ from http://rsb.info.nih.gov/ij/)

2. Install the ParticlePicker plugin
   extract all file in particle_picker.zip into the plugins directory in the ImageJ home directory. You will see particlepicker in the Plugins menu of ImageJ after you restart the ImageJ.

3. Before using the plugin, make sure to select "center of mass" in Set Measurements in Analyze menu of ImageJ.

4. Open an image in ImageJ

5. Threshold the image
   Press Ctrl-Shift-T (or menu "Image/Adjust/Threshold") to open the thresholding window (see below). Use this tool to interactively set lower and upper threshold values. Pixels with brightness values greater than or equal to the lower threshold and less than or equal to the upper threshold are displayed in red. Try to select gold particles in the image as many as possible. Leave the window open and do not click any button in the window.
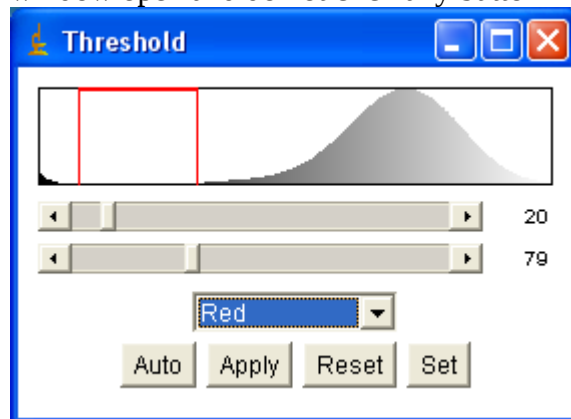
6. Open the ParticlePicker Plugin
   Click menu "Plugin/ParticlePicker", the toolbar of ImageJ will change to the following

7. Choose the particle sizes you are looking for
   Click the forth button in the toolbar, the following window will show up.
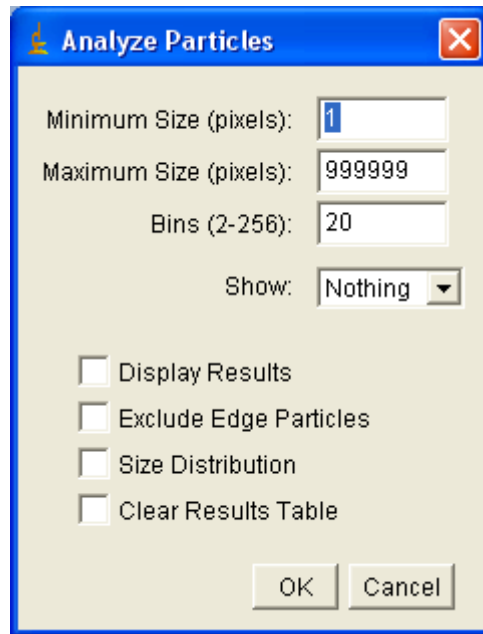
Choose the min and max sizes in pixels that correspond to the particles. Size of particles can be estimated by area of circle. Pick a typical particle and zoom in and find the radius of the particle in pixels and calculate the area of the particle. Min and max are usually set to ½ and 2 of the area of the typical particle, respectively.

8. Click Ok and the plugin will find the particles and give a summary. Click the reset button in the thresholding window, you will see the particles are circled in the image.

9. Use the first button in the toolbar to add/remove particles and correct any errors. First click the button, then click left/right mouse button within the image to add/remove a particle.

10. Click the third button to export, import or show the list of particles. By clicking on the tool with a file icon, you access a menu that allows you to either save the list of points as a text file [Save as], to display the same list in the ImageJ main window [Show], or to retrieve a previously saved file [Open]. In the latter case, the actual list of points (if any) will be overwritten by the new one.

11. Click the sixth button to exit the plugin and return to ImageJ

12. Click the fifth button to run the clustering analysis. You first need to specify the cutoff distance and then choose "open" (importing a list of particles from a file) or "Calculate" (using the list of particles you just obtained using the plugin). It will find the clusters of particle and give results and an image with the clusters identified by rectangles.