Image Analysis Protocol Instructions #1: Spatial Calibration of Images
Using Image-Pro Plus, Clemex Vision PE, and ImageJ

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1.   Introduction

In computers, images are represented in the form of picture elements, or pixels, which are small dots making up the image. In order to perform measurements, the image analysis tools need to know how many pixels there are in one unit length of the image. The unit length may be in millimeters, micrometers, or any other unit chosen according to the overall size of the image and its important components. The process of assigning the number of pixels per unit length to an image is known as calibration. In this section we summarize how calibration information can be added using each of the three image analysis tools under study: Image-Pro Plus 5.0, Clemex Vision Professional Edition (PE) 3.5, and ImageJ (Version 1.33). We then offer a brief assessment of how well these image analysis packages perform for the calibration of images.

2.   Obtaining the Calibration Measure

We use the term calibration measure to refer to the number that indicates the number of pixels per unit length of the image. The calibration measure of an image depends upon:
1.   The magnification of the objective lens that is used to view the image under the microscope.
2.   The digital camera/microscope system that is being used to acquire the image.
3.   The resolution under which the image is stored.

Thus we need to obtain the calibration measure only once for each objective lens-camera-resolution combination and the measure obtained will apply to all images taken with that combination. Most digital microscope cameras include the capability of calibrating directly at the time of image capture. If this was not done, the calibration measure can also be obtained with some image analysis software packages.

To obtain the calibration measure that can then be applied to image calibration, refer to the documentation for your digital camera. Obtaining a calibration measure will involve measuring the pixels per millimeter or micron for each objective lens on a specific digital camera/microscope system. Many digital microscope cameras have a calibration feature as part of their software that permits the user to calibrate in turn each objective lens on a given system.

For example, with the Polaroid DMC-ES used here, in the calibration procedure a stage micrometer is used with each lens. The user clicks on a beginning point on the scale bar, then again on an ending point of known length, to determine the number of pixels per given unit for

each objective lens. Since there tends to be variation, it is best to do repeated measures for each distance (we use 10-20 measurements), and take the average. The final pixel information will be needed to provide spatial calibration information for use in any image analysis.

Once the calibration measurements are known, for some digital microscope cameras they can be used to add a scale bar automatically at the time of image capture. For many image analysis applications, however, the scale bar would have to be removed or masked off prior to analysis, as all items in the image may be subject to analysis. In that case, when the image is captured it could also be placed within an image database that contains information regarding the objective lens, microscope, and image capture system used, and that information can be accessed later to add a scale bar using the image analysis software.

In all cases, the scale bar should appear in any final published photographs. Listing of a "magnification" is not an effective substitute for a scale bar, because such calculations in digital photomicroscopy can be complex and prone to error. Factors that have to be considered in determining magnification include not only the value of the objective lens and any other components within the microscope system that can create enlargement and affect magnification, but also changes in image manipulation done with the computer and in the printing process that change image size and final print magnification.

3. Calibrating an Image

3.1 Image-Pro Plus 5.0

1. Open Image-Pro Plus.
2. Open the image that needs to be calibrated. This can be done by selecting **File** on the main menu and then selecting **Open**.
3. You can use Image-Pro Plus to calibrate your microscope objectives and microscope-camera system, if you have not already done so using software accompanying your digital microscope camera as mentioned in Step 2 above. To do so, click on **Measure** in the main menu, then **Calibration**, and then **Spatial Calibration Wizard**. Follow the instructions in the calibration wizard to create a set of reference calibrations. If you already have obtained the information regarding your number of pixels per unit for each objective lens in your system, follow the procedure as given in the steps below.
4. Select **Measure** from the main menu and then select **Calibration**. In the submenu that appears, choose **Spatial**. The following dialog box will appear:
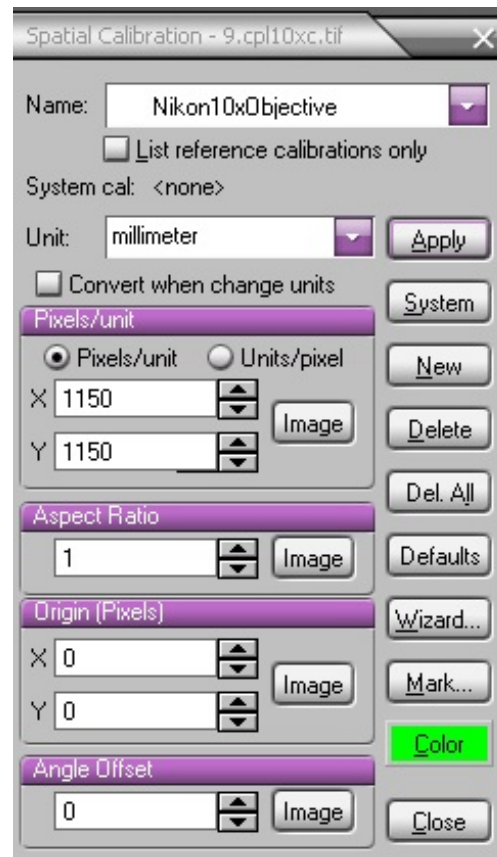
5. Select **New**. A default name will appear in the **Name** text box. Delete it and type in your own identifier (for example, *Nikon10xObjective*). Select from the drop-down menu the unit of measure you prefer (for example, *millimeter*) and type the calibration measure in the X and Y text box (for example, the number of pixels per millimeter that you measured along both the X and Y axes using your digital microscope camera's calibration software with a Nikon system and its 10x objective). Make sure that the appropriate box is marked (pixels/unit or units/pixel, depending upon how you recorded your calibration measures).
6. Select **Apply**. Continue to repeat the process from the step of **New** through **Apply** for as many calibrations as you wish to save; generally, you will have one for each microscope-camera-objective lens combination for which you will be capturing images.
7. When finished, with the spatial calibration dialog box still active, select **Measure** from the main menu bar, then select **Calibration**, and from that drop-down menu select **Save All**. This will save the named calibration measures for future use.
8. To apply any of these saved calibration measures to a particular image you have taken from one of those microscope-camera-objective lens combinations, select **File** on the main menu, select **Open** and choose your image file, select **Measure**, and **Calibrate**. Then, under the drop-down menu select **Open** and select your saved calibration file (which will have a .cal extension).

9. You can now go back to the main menu and select **Measure** and **Calibration** and go to **Select Spatial**, and all of your previously-saved calibration measures will be available to apply to any image you are working with, through the dialog box below. Select **OK** to apply a calibration measure to the active image.
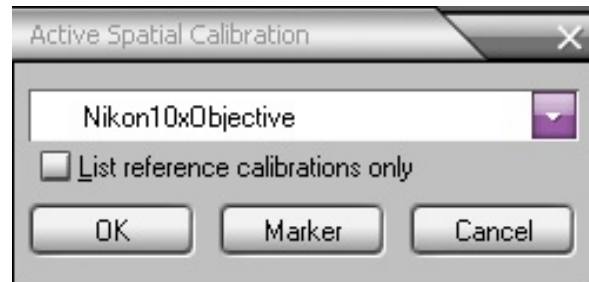

10. Selecting **Marker** allows you to place a scale bar onto the image, with choices as to marker length, color, and location if you have not already done so at the time of image capture; it can also be set non-permanently onto the image (for publication of images, for example), so that the marker does not interfere with future image analyses (although any markers permanently set onto an image now or at the time of image capture can also be masked off if necessary during image analysis).


### 3.2   Clemex Vision Professional Edition (PE) 3.5

1. Open Clemex Vision PE.
2. Open the image that needs to be calibrated. To do this, select **File** on the main menu and then select **Open Image**. Select the image that you wish to open and then click on **Open**.
3. The following dialog box will appear:
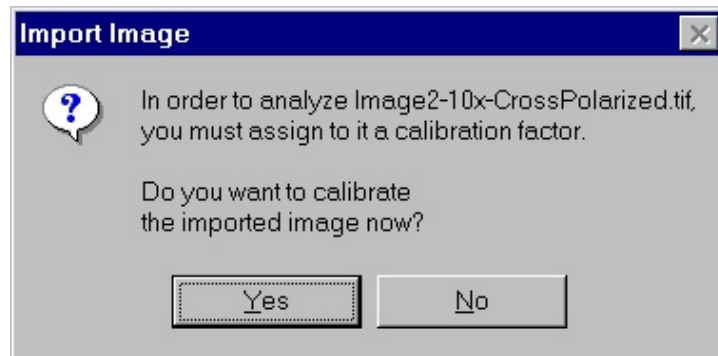
4. Select **Yes**.
5. The **Video Input Calibration** dialog box will appear as shown below. If the system-objective lens calibration measure that you used to capture the image is not already present on the list, click on **Add**.
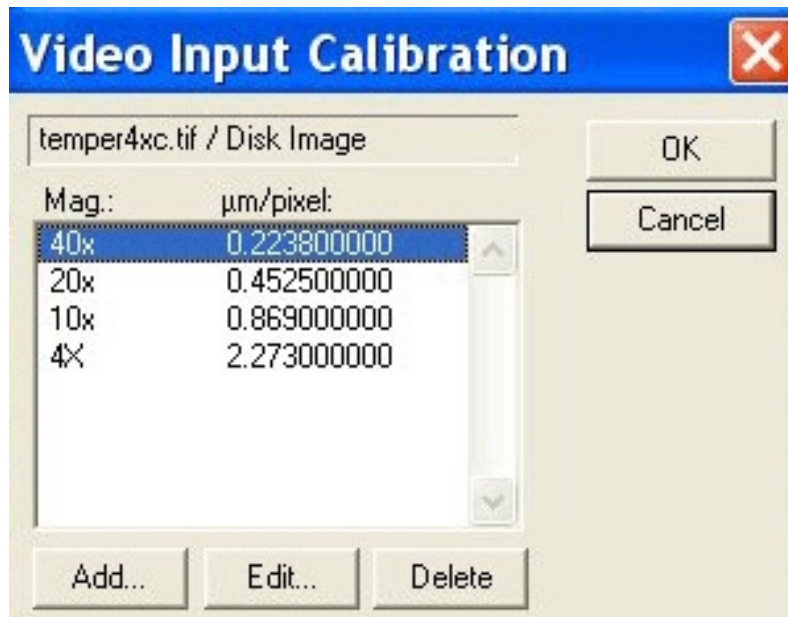

6. The following dialog box will appear:
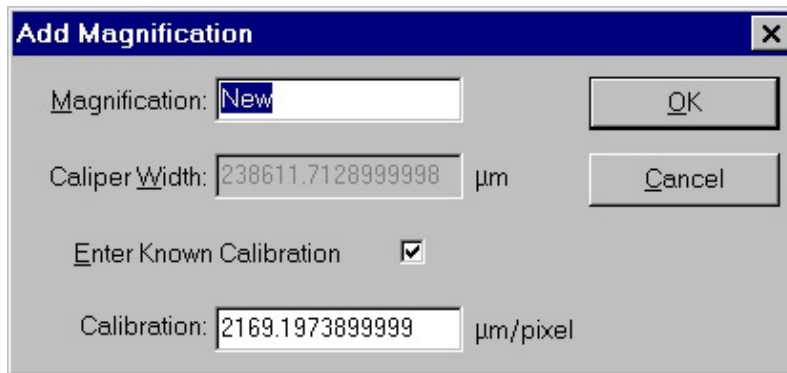
7. Enter the title of the microscope/objective lens combination in the magnification text box. Select **Enter Known Calibration** and then enter the calibration measure in microns per pixel (obtained from the calibration process with your digital microscope camera). Click on **OK**. To change the units (such as from microns to millimeters), choose **Preferences** in the **Options** selection of the main window toolbar.
8. As with Image-Pro Plus, in Clemex Vision PE one can calibrate a video input directly, if this is not done with software accompanying the digital microscope camera. And, as with Image-Pro Plus, a scale bar can be added, once calibration has been completed.

3.3   ImageJ (Version 1.33)

1. Open ImageJ.
2. Open the image that needs to be calibrated. To do this, select **File** on the main menu and then select **Open**. Select the image that you wish to open and then click on **Open**.
3. Select **Image** from the main menu and then select **Properties**. The following dialog box will appear:
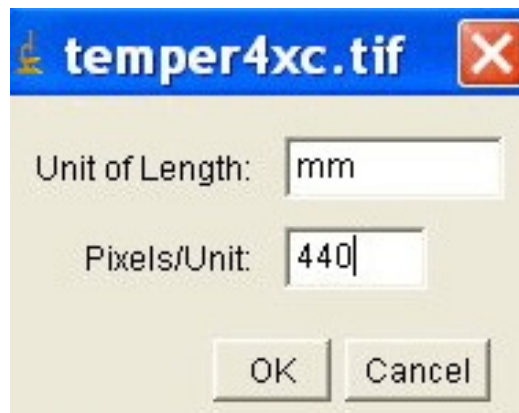
4. Enter millimeter, micron, or another unit of length as appropriate, and enter the calibration measure (obtained from the digital camera calibration process) in, for example, pixels/mm.

5. Click on **OK**.
6. Alternatively, select **Plugins** from the main menu, then select **Spatial Calibration**. From the list that appears, select **Set Scale**. A dialog box will appear as below:
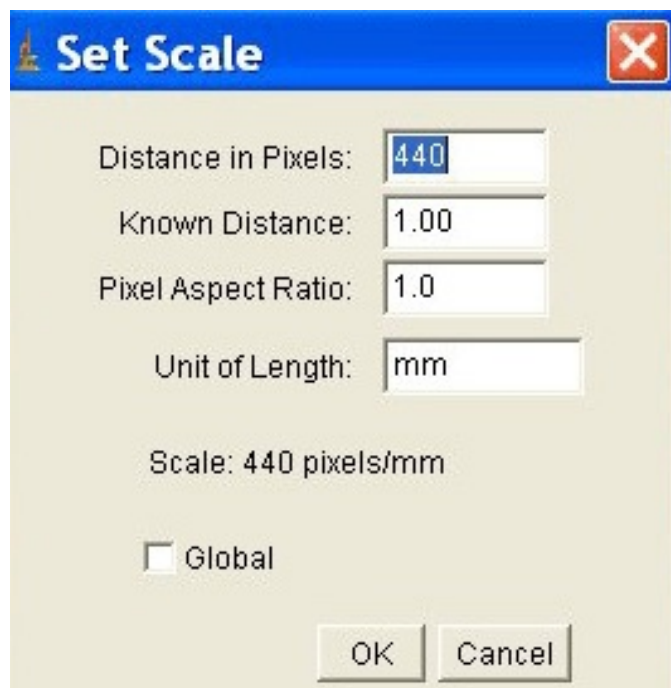
7. From the information on number of pixels per given unit, obtained when calibrating with your digital camera, enter your unit of length and the distance in pixels for the microscope/ objective lens combination that was used to take the image under study. This process also allows you to count the number of pixels for a given length (such as a scale bar) and at that now if you did not calibrate at the time of image capture.
8. The **Set Scale** operation can also be accessed by selecting **Analyze** from the main menu, and then choosing **Set Scale** from the drop-down list.
9. Once the calibration measure is entered, a scale bar can be added, by choosing **Add Scale Bar** from the list that appears under **Plugins – Spatial Calibration**.
10. Unlike Image-Pro Plus and Clemex PE, the ImageJ software cannot save these calibrations, so the pixel/unit information must be re-entered each time an image is calibrated. An exception is that under **Plugins - Spatial Calibration** there is a selection that says **Microscope Scale** where a list of microscopes and objectives is found with their respective calibration measures. This plugin can be customized to apply your own system calibration measures. Once can also open an image that has already been calibrated, then select **Plugins -- Spatial Calibration – Copy Pixel Size**, to copy calibration information from one image to another.

4.   Analysis

Now we will compare the three image analysis tools and give an assessment regarding their performance in spatial calibration tasks. We will evaluate each approach using the following criteria:

1. Ability to perform spatial calibration for a given image under study.
2. Ability to retain information that can easily be accessed for future images using the same microscope/objective lens/camera system.
3. Ease of use for calibration of images.
4. Ability to perform calibration measurements as well as to accept calibration measures obtained directly with digital camera software.
5. Ability to add a scale bar or other measurement markers if these were not added during image capture.

4.1   Ability to Perform Spatial Calibration on an Image Under Study

We found that all three of the image analysis tools contain the ability to calibrate an image. This is important because many of the operations performed on petrographic thin sections of cultural materials require spatial calibration (such as measuring length, perimeters, area, etc.).

4.2   Ability to Retain Information

Both Image-Pro Plus and Clemex Vision PE easily allow a user to name microscope/objective lens/camera combinations, and specify a calibration measure for that combination. At any time that an image using that combination is under study, the named specification can be recalled and used for calibrating the image. ImageJ requires the user either customize the **Microscope Scale** plugin to add their own system measures, copy information from an image previously calibrated, or re-enter the pixels and unit length data each time an image is calibrated.

4.3   Ease of Use for Calibration of Images

We found all three software tools to have easy and intuitive calibration procedures. A strong highlight of Clemex Vision PE is that a statement automatically appears each time you open an image, reminding you that the image must be calibrated before any analyses are performed. Image-Pro Plus and ImageJ do not remind the user; we find that it is easy to forget to calibrate the image, especially when analyzing many images. In Image-Pro Plus, however, there is always a notation present on the lower right screen indicating what the calibration measure is. If it says *(Pixels) System: <none>* then you know you have not yet calibrated the image. Thus, when using Image-Pro Plus it is a good idea to develop the habit of always checking that portion of the screen.

However, we found that the Clemex software was frustrating in that it requires the user to enter calibration information in the form of units/pixels, whereas the calibration procedure with our digital camera collects the information in the form of pixels/unit, so we had to do a conversion. With Image-Pro Plus one can enter the information in any unit of choice, and as pixels/unit or unit/pixel. If one wishes to change formats later, the software can automatically convert the numbers. In ImageJ one can choose any unit of length, and then enter in the number of pixels/unit.

4.4   Ability to Perform Calibration Measurements Directly

We prefer to obtain a calibration measure (number of pixels per unit length) at the time of image capture, using the digital microscope camera software. However, other users may prefer to do this step using image analysis software, or may not have the capability with their microscope camera. Both Image-Pro Plus and Clemex Vision PE provide procedures for obtaining the calibration measures that can then be used to calibrate all images taken with the same microscope/objective lens combination.

4.5   Ability to Add a Scale Bar

We prefer to add the scale bar directly at the time of image capture. However, some cameras may not perform this step, the scale bar may be removed for the purpose of image analyses, or the researcher may wish to change the appearance or location of the scale marker. It is therefore useful to be able to add the scale marker within an image analysis program. All three image analysis packages include the capability of easily adding a scale bar, and to vary the appearance of that scale marker.

4.6   Evaluation Conclusions

We find Image-Pro Plus easiest to use for spatial calibration. ImageJ was cumbersome in contrast. Clemex Vision PE requires one to use microns/pixels, which did not always suit our applications. However, the automatic reminder to calibrate is a very helpful feature of Clemex Vision PE.

Product Sources

Image-Pro Plus: MediaCybernetics, www.mediacy.com
Clemex Vision PE: www.clemex.com
ImageJ: author Wayne Rasband, National Institutes of Health, http://rsb.info.nih.gov/ij/

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Image Analysis Protcol Instructions #2:
Measuring Microcracks in Quartz Grains
Using Image-Pro Plus, Clemex Vision PE, and ImageJ

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1.   Introduction

In this set of protocol instructions, we discuss the measurement of length of microcracks in quartz grains within stone specimens, used as a measure of deterioration or means of monitoring continuing deterioration of stone sculpture or architecture. Most of the cracks seen within quartz grains are curving and wandering lines, so the traditional method of placing a stage micrometer with a straight edge next to such cracks is problematic. For analysis here we used thin section images of microcracks in quartz grains within granite specimens. All of the thin sections were observed using crossed polarized light. We discuss the measurement of these features using the three image analysis tools under study: Image-Pro Plus 5.0, Clemex Vision PE 3.5, and ImageJ (Version 1.33)

2.   Measuring Length of a Microcrack

2.1   Image-Pro Plus 5.0

There are two methods for tracing and highlighting a microcrack, the **Trace** tool and **Trace Object**. However, only the **Trace** tool provides a measurement of the length of that microcrack. **Trace Object** does automatically find and highlight microcracks, but as will be seen, this feature tends to perform poorly with geological or heat-treated specimens that may contain many small cracks in addition to the cracks of interest.

### 2.1.1   Using the Trace Tool

1. Load Image-Pro Plus.
2. Open the image that needs to be measured. This can be done by selecting **File** on the main menu and then selection **Open**..
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image, if required, so that the cracks are distinctly visible (such as with the **Enhance: Contrast Enhancement** tool in the main menu).
5. Select **Measure** from the main menu and click on **Measurements** from the drop-down list.
6. A **Measurement** tool box pops up which contains all the tools needed to proceed with a variety of measurements.

7. Select the **Trace** button ⌇ from the **Measurement** tool box. A **Trace** box appears which has the option to auto trace. Click on the **Auto** check box.

8. Click on the starting point of the crack by left clicking the mouse button. Then, follow along the crack line, highlighting the crack in red. When finished, right click the mouse button, and the traced crack length will become yellow. The length appears in the central area of the measurements window, in the units to which you have calibrated. The right side of the lower line of the computer screen shows which spatial calibration you used. If you forgot to calibrate the image, this line will read *<none> (Pixels) System*, and your measured line lengths will simply be calculated as number of pixels rather than as microns or millimeters. Because there can be some variation each time you outline the crack, it is a good idea to take multiple measurements and use the average for the final length measurement. To delete all measurements and start again, press the red X button in the **Measurements** window.
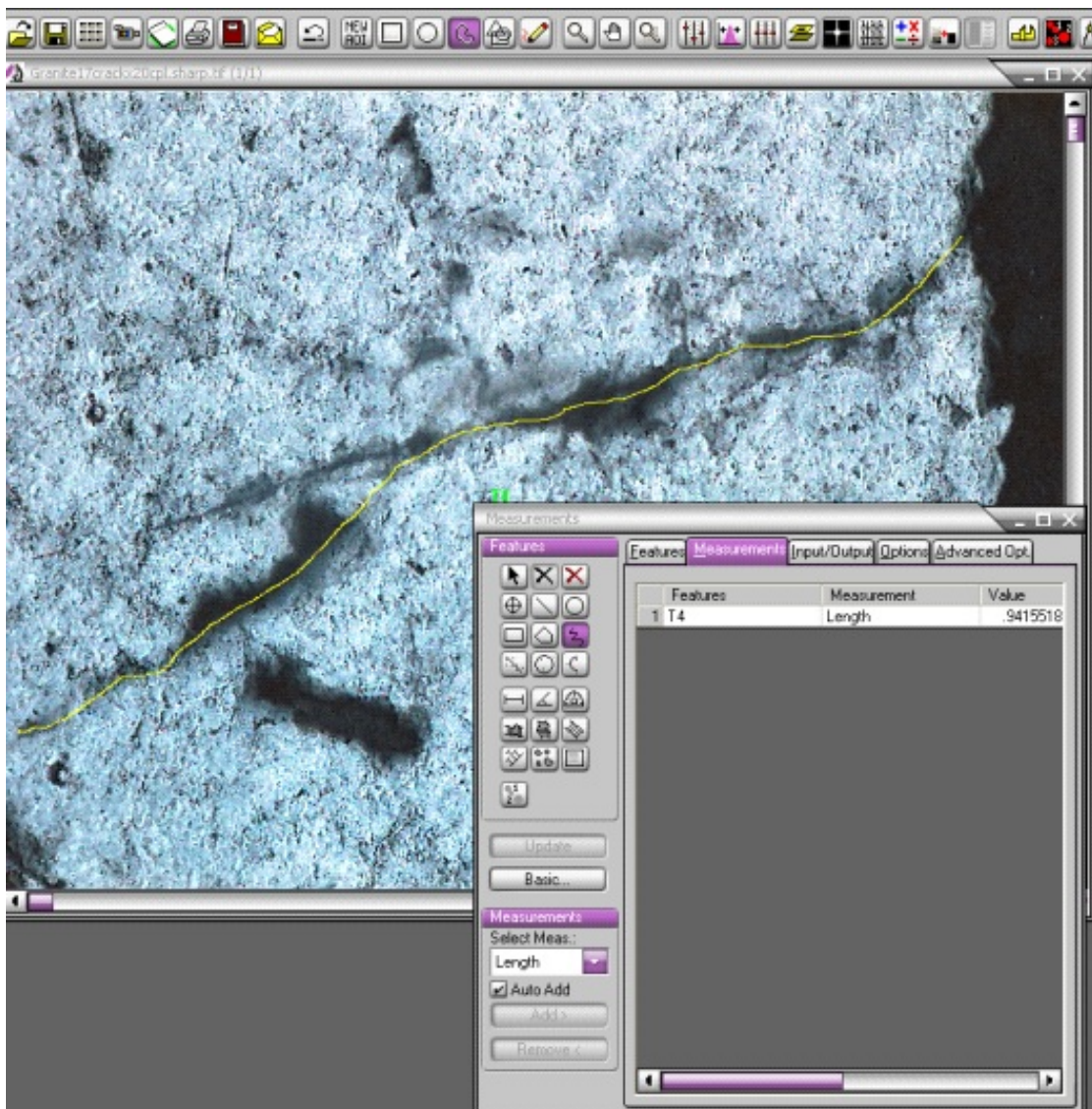
9. Alternatively, one can begin by left clicking twice to set autotrace on. A wand appears that moves along a straight line; simply click at the beginning and ending points of the crack and end with a right click; a trace line will automatically be generated. If there are many subcracks or other surface features that could mistakenly get incorporated into the trace, one can click at multiple points along the crack to keep the autotrace line on track. However, we found that with the complicated cracking geometry of most geological and cultural materials, manually tracing along a crack tends to work better.

10. For completing additional measurements like width of a crack or distance between two cracks, at least two traces are required.

11. Using the **Measurement** tools, vertical thickness ( ), horizontal thickness ( ), and distance ( ) can be measured. See the protocol instructions for **Measuring the Thickness of Layers** for an illustration of the use of these tools.

2.1.2   Using the Trace Object Tool

1. Follow steps 1-4 in section **2.1.1**.
2. Select **Process** from the main menu and click on **Trace Objects** from the list.
3. A trace window pops up which has the option to automatically trace long the crack:
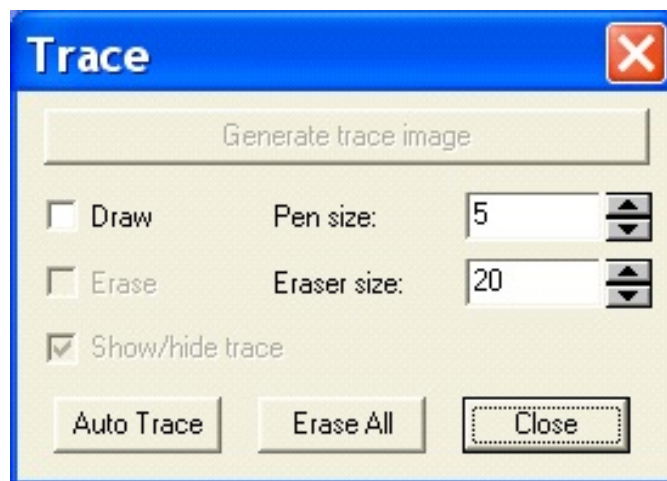
4. Using the spin buttons, select the **Pen size** of the drawing tools in pixels. Enter the pen size as 5.
5. Using the spin buttons, select the **Erase size** of the erasing tools in pixels. Enter the eraser size as 20.
6. Check the **Show/hide trace** box to display or hide trace lines in the image.
7. Click the **Auto-Trace** button to automatically trace all the cracks in an image with a red line. The problem with geological samples will be immediately apparent, in that the surface tends to have numerous small cracks, all of which are traced by the automatic tracing tool. These unwanted lines will then have to be removed using the eraser tool.

8. Check the **Erase** box to remove unwanted traced lines. To erase the unwanted lines move the mouse on the image, hold the right button down, and move the mouse over the areas of unwanted lines. The result will look as below:
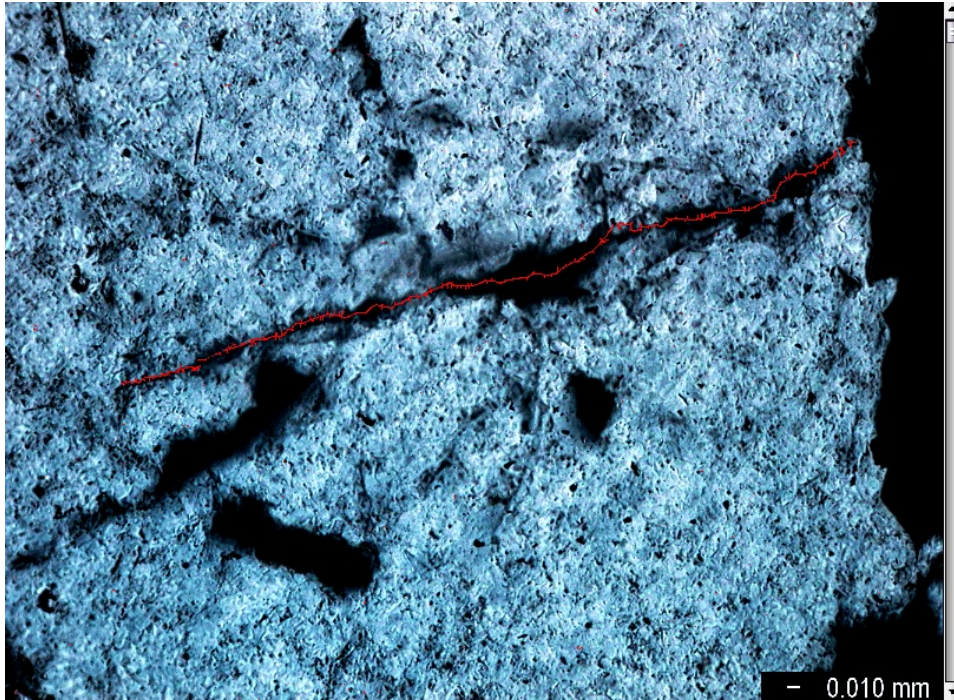
9. The **Trace Object** tool is of very limited usefulness compared to the **Trace** tool described in **2.1.1** because, although it sometimes can help identify and outline a line of interest, it cannot provide information about the length or any other measurement parameters for the cracks. We found that identifying cracks by eye as in **2.1.1** was also more effective in finding and highlighting cracks than the automatic method of **2.1.1**.

2.2   Clemex Vision PE 3.5

1. Load Clemex Vision PE.
2. Open the image that needs to be measured. To do this, select **File** from the main menu and then select **Open Image**. Select the image that you wish to open and then click **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image if required so that the cracks are as distinctly visible as possible.
5. From the **Image Window Edit and Annotation Toolbar** (shown below), click on the **Measure Tools** button (shown encircled in red below). The **Measure Tools** toolbar will open.
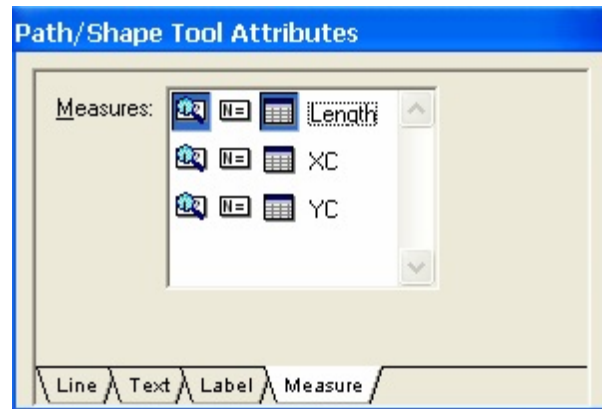
Image Window
Edit and
Annotation
 Toolbar
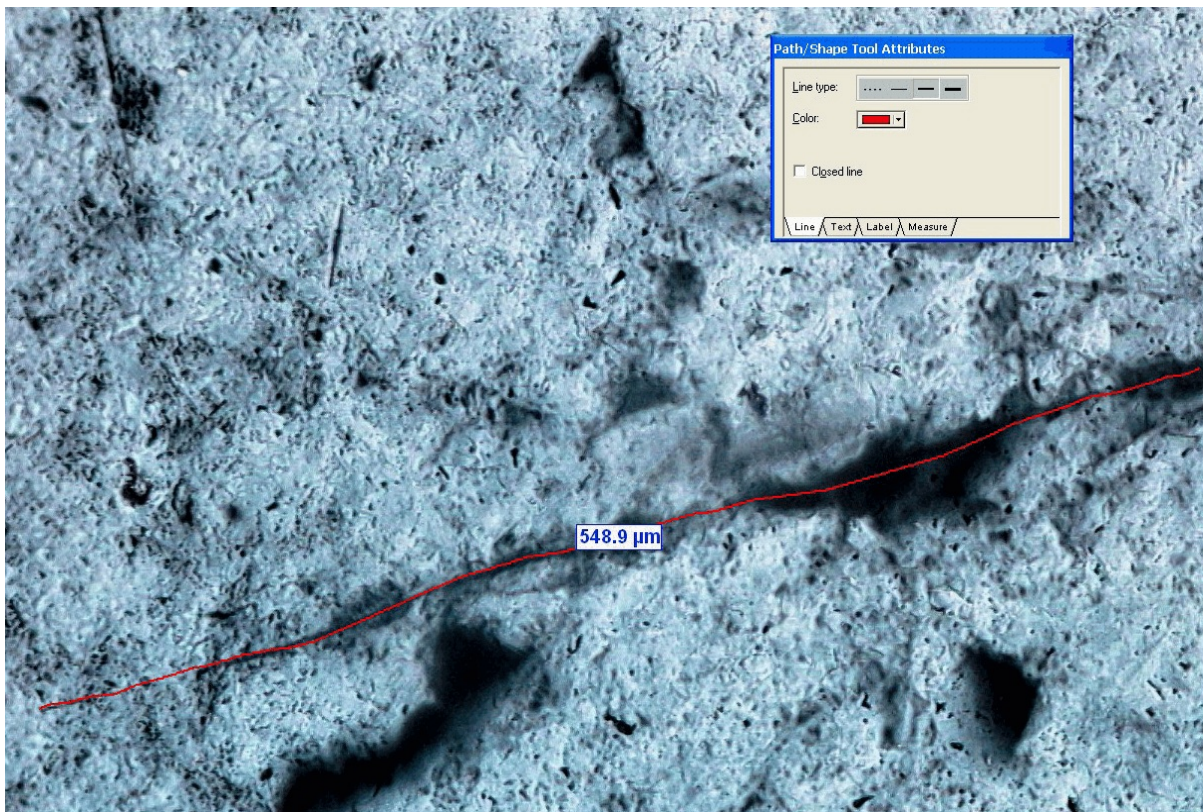
Measure Tools Toolbar
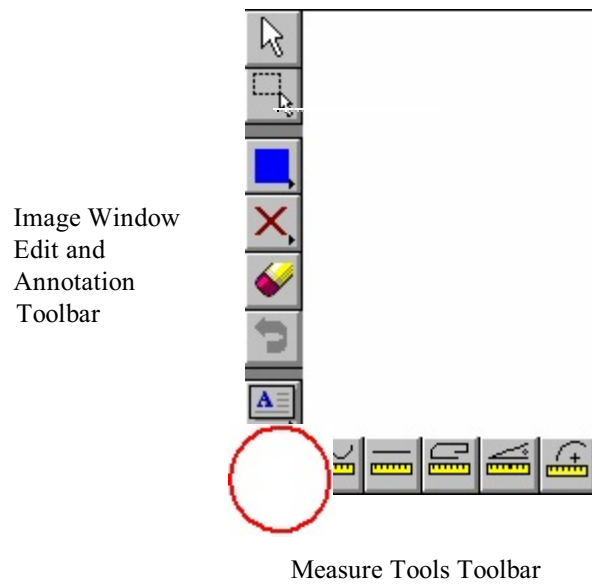
6.   To measure an unconstrained single crack:

A. Choose **Free Draw** in the **Measure Tools** flyout.
B. Choose a line style and color in the **Attribute** property sheet:

Path/Shape Tool Attributes

Line type: .... — **—** **—**

Color: ▭ ▾

☐ Closed line

Line \ Text \ Label \ Measure /
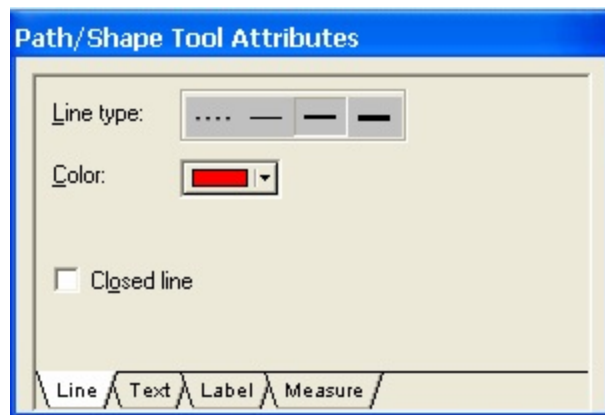
C. Make sure that Length is selected on the **Measures** list.

D. Click the left mouse button and trace the mouse over the crack. The result is displayed in the image window. As soon as you let go of the mouse button, the length measurement will appear on the traced crack, as in the following image:

7. Alternatively, to measure a complex crack with multiple segments::

    A. Choose the **Polyline** tool located in the **Measure Tools** flyout.
    B. Choose a line style and color in the **Attribute** property sheet.
    C. As before, make sure that Length is selected on the **Measures** list.
    D. Define the start point of the polyline by left clicking the mouse.
    E. Define the end point of the first segment by left clicking the mouse.
    F. Double click the end of the last segment to end the polyline. The result is displayed in the document window as shown below:
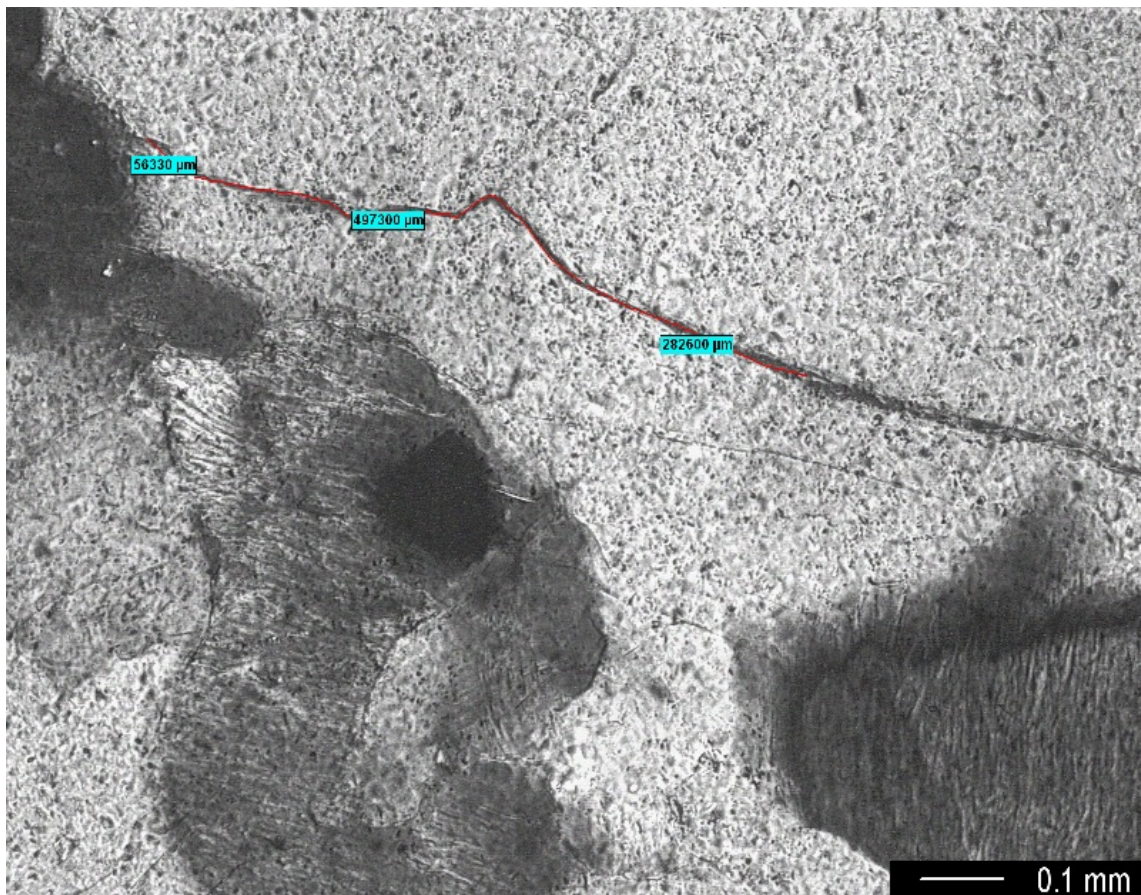

2.3 ImageJ (Version 1.33)

There are two ways of conducting this operation using ImageJ; depending upon the character of the line(s) you are trying to trace and measure, one may work better than another. We found that for microcracks in quartz grains, option **2.3.2 (**the **Segmented Line** tool) tends to work better for tracing the outlines of complex cracks. You may need to zoom in or out on the cracks, using the zoom tool (a small magnifying class) on the main ImageJ toolbar.

2.3.1   Freehand Tool

1. Load ImageJ.
2. Open the image that needs to be measured. To do this, select **File** on the main menu, and then select **Open**. Select the image that you wish to open and then click **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image if required, to ensure that the cracks are as distinctly visible as possible (select **Image - Adjust - Brightness/Contrast**).
5. Select the **Freehand** line tool from the main window toolbar:
6. Define the starting point of the crack by left clicking the mouse and holding the button until the crack ends; then release the button.
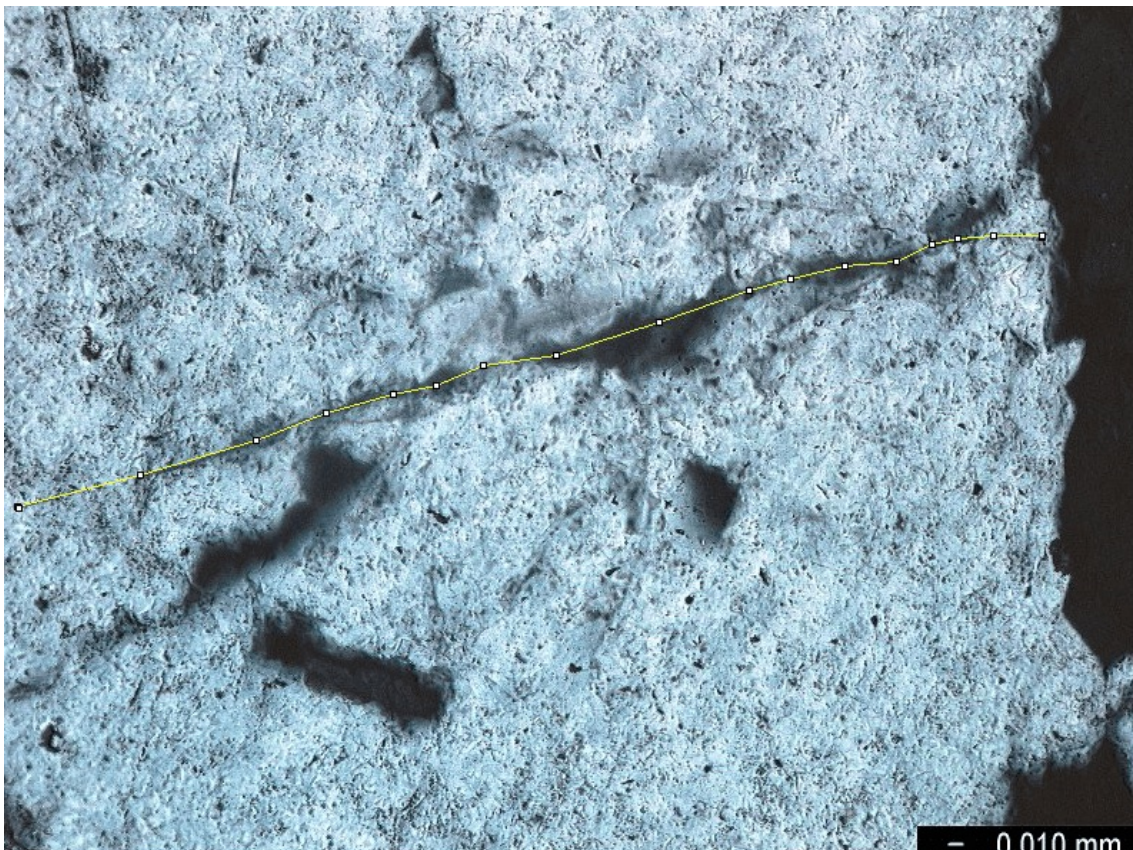

8. Select **Analyze** from the main menu and click on **Measure** from the list.
9. A **Results** window shows the length of the crack.
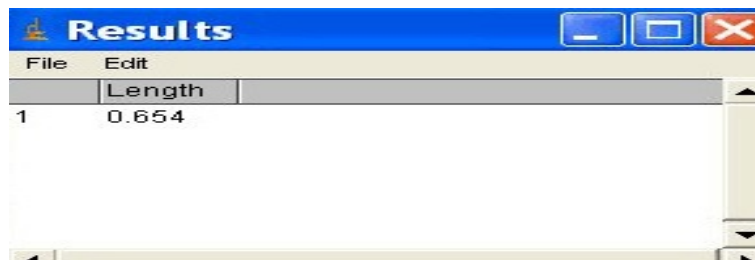
10. The units shown will be those used in the image calibration. If no calibration was performed, the units will simply refer to number of pixels.
11. The results can be transferred to a spreadsheet or statistical program file by selecting **Copy All** from the **Results** popup menu, switching to the spreadsheet or statistical program file, and pasting.

2.3.2   Segmented Line Tool

1. Load ImageJ.
2. Open the image that needs to be measured. To do this, select **File** on the main menu, and then select **Open**. Select the image that you wish to open and then click **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image if required, to ensure that the cracks are as distinctly visible as possible.
5. Select the **Segmented Line** tool from the main window toolbar:
6. Define the starting point of the crack by left clicking the mouse and holding the button until the crack goes along a different angle or segment; release the mouse and move to a new starting point, then click and trace the crack again as far as possible. Repeat as needed through any directional changes or interrupted segments. Double click when done, and small boxes will appear along the line to indicate angle or segment changes, as below.

7. The line selection can be adjusted if needed by clicking and dragging the tiny boxes.
8. Select **Analyze** from the main menu and click on **Measure** from the list.
9. A **Results** window shows the length of the crack.
10. The units shown will be those used in the image calibration. If no calibration was performed, the units will simply refer to number of pixels.
11. The results can be transferred to a spreadsheet or statistical program file by selecting **Copy All** from the **Results** menu, switching to the other application, and pasting.

3.   Analysis

Now we will compare the three image analysis tools and give an assessment regarding their performance in the task of measuring the length of microcracks. We will evaluate each approach using the following criteria:

1. Ability to trace along the contours of an individual or complex cracking using visual assessments to identify the crack parameters.
2. Ability to provide a length measurement for the microcrack.
3. Ability to provide a width measurement for the microcrack.
3. Ability to automatically identify crack parameters.
4. Ease of use.

3.1   Ability to Trace an Individual or Complex Microcrack

We found that all three image analysis packages contain one or more approaches to tracing along the outlines of a crack line.

3.2   Ability to Provide Length Measurement for a Crack Line

We found that all three image analysis tools can provide length information for a traced crack line. The accuracy of such a measurements depends upon one having calibrated the image prior to taking the measurements, as otherwise the numbers are simply in pixels rather than in the measurement unit of interest.

3.3   Ability to Provide a Width Measurement for a Microcrack

A width measurement at individual points along a crack can be obtained using all three image analysis tools. Both Image-Pro Plus and Clemex Vision PE can also provide automatic sampling along the entire length of a defined crack, giving the minimum, maximum, and average width of a crack. However, we found those operations to be much easier to perform with Image Pro-Plus. These operations are discussed in more detail in the protocol instructions on **Measuring the Thickness of Layers**.

Although ImageJ can be used to make width measurements at individual points, it has no tool that will automatically sample the width of an entire crack and calculate a minimum, maximum, and average width for the microcracks.

3.4   Ability to Automatically Identify Crack Patterns

We found that none of the image analysis programs are able to do this; the microcracks must be traced individually by eye. Although Image-Pro Plus does have a tool designed to pick out cracks automatically, due to the surface variation and texture of geological and cultural materials, there is too much "noise" on the surface for this tool to work well, even with careful preprocessing.

3.5   Ease of Use

We found that tracing the lines of microcracks and obtaining a length measurement were easy procedures to perform with all three of the image analysis packages.

3.6   Evaluation Conclusions

For measuring the length of meandering microcracks in quartz grains, all three image analysis tools perform well and are easy to use.

Product Sources

Image-Pro Plus: MediaCybernetics, www.mediacy.com

Clemex Vision PE: www.clemex.com

ImageJ: author Wayne Rasband, National Institutes of Health, http://rsb.info.nih.gov/ij/

Image Analysis Protocol Instructions #3: Measuring the Thickness of Layers
Using Image-Pro Plus, Clemex Vision PE, and ImageJ

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1.   Introduction

The measurement of the thickness of layers is an important element of conservation research studies since the thickness or width of a deterioration layer provides one assessment of the amount of weathering present in the specimen.   Layer thickness in some cultural materials (such as a decorative layer on a ceramic) may also be an important indicator of workshop technology and may be useful in characterizing the technique of an artist, a workshop, or a cultural tradition.

In this section we discuss a set of protocols for measuring the thickness of layers using each of three image analysis tools: Image-Pro Plus 5.0, Clemex Vision PE 3.5, and ImageJ (Version 1.33). In addition, we provide an assessment of the features offered by the three tools with regard to layer thickness measurements.

2.   Images Studied

We experimented with the following petrographic thin sections as examples for measuring the thickness of a layer, observed under a Nikon Labophot polarizing light microscope using both plane polarized and crossed polarized light:
1. A volcanic tuff specimen with a weathered exterior surface.
2. A Gandharan phyllite sculpture specimen with a weathering layer.
3. A glazed ceramic specimen.

Our results and conclusions are partially dependent on applying the measurements to the above images, and hence those working with specimens that differ significantly from these may encounter a different set of experiences and problems.

3.   Performing the Measurements.

3.1   Image-Pro Plus 5.0

We found the taking of thickness measurements to be the simplest and most intuitive in Image-Pro Plus 5.0. Image-Pro Plus allows the user to specify the boundary of the layer by simply drawing it – thus making use of an important resource to detect the layers: the human eye. On the downside, this method might not be the most accurate since image perception varies from human to human. Like Clemex Vision PE (see 3.2 below), Image-Pro Plus allows us to take both individual measurements and automatic samples across the layer, thus allowing us to calculate the minimum, maximum and average thickness of the layer.

3.1.1 Measuring Thickness at a Particular Point:

1. Load Image-Pro Plus.
2. Open the image that needs to be measured. This can be done by selecting **File** on the main menu and then selecting **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image, if required, so that the layers are distinctly visible (such as with the **Enhance: Contrast Enhancement** tool in the main menu).
5. Select **Measure** from the main menu and then click on **Measurements**. The **Measurement** dialog box (shown below) will appear:

6. Select the ⬛ button (**Creating Line**). Move the mouse pointer to the position at which the measurement is to be taken at the start of the layer. Hold down the left mouse button and keep it depressed, drag the mouse pointer to the end of the layer, and then release the mouse button. A line will be drawn across the layer as shown below:
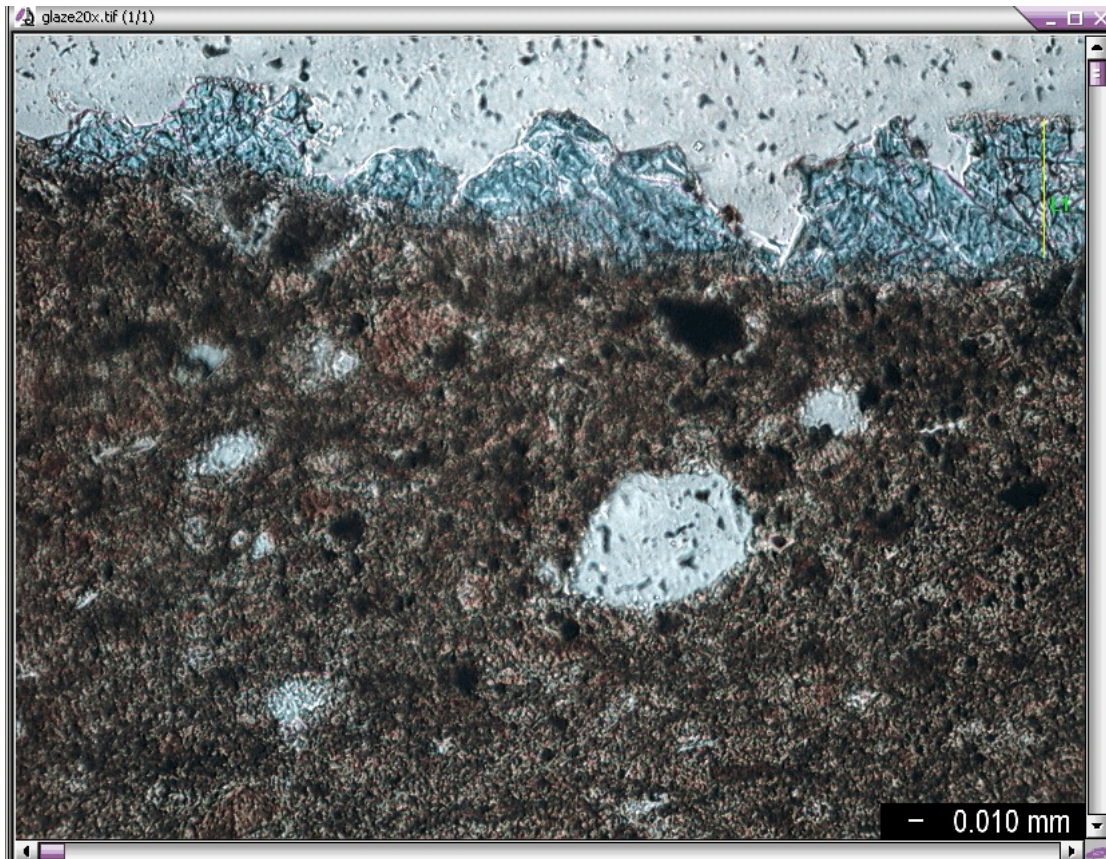

7. Click on the **Measurements** tab. The dialog box should now display the length of the line, as shown below, which will effectively be the width of the layer of interest. The units will be the units to which you have calibrated the image. In this case, the ceramic glaze layer is shown to be .094 millimeters thick at the location measured. Check the lower right screen to ensure that the image calibration step was in fact completed; a notation of *(Pixels) System*: *<none>* indicates that image calibration was not performed and thus the measurements given are all in pixels.
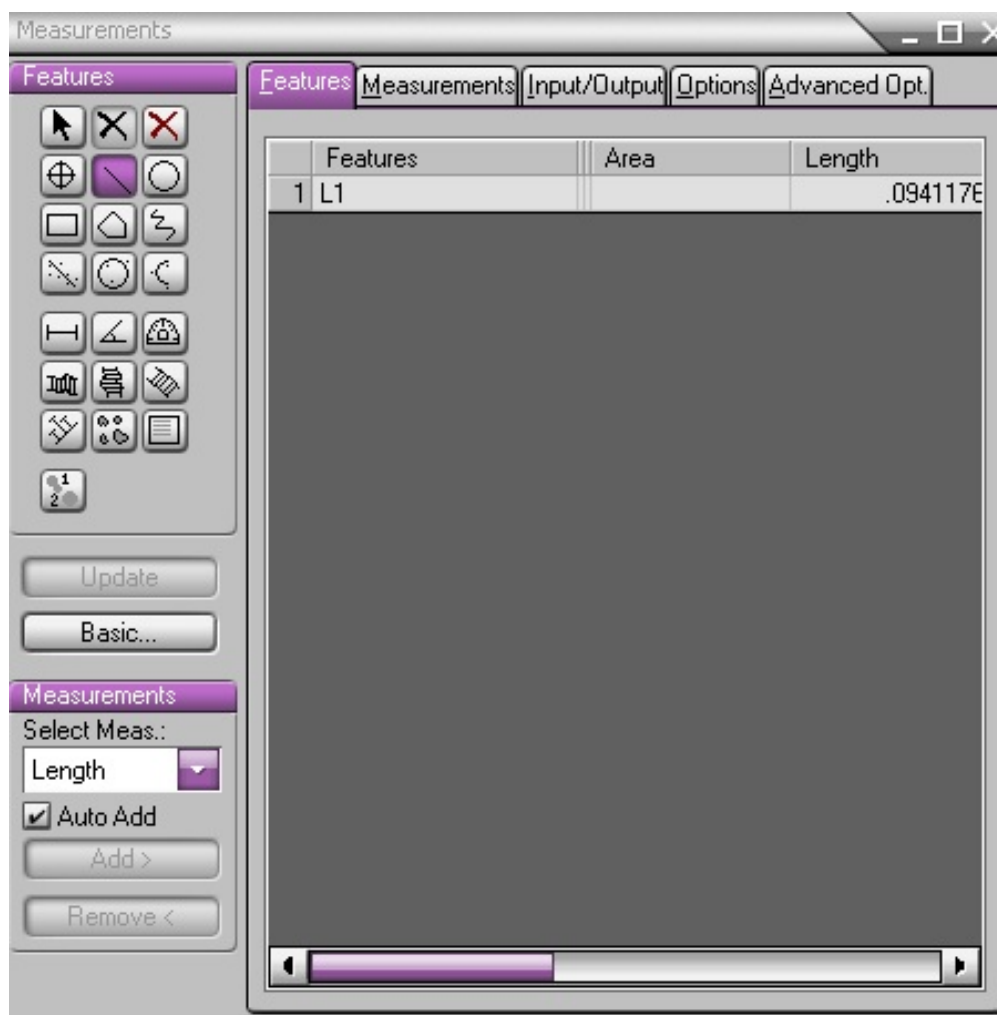
3.1.2 Taking Automatic Samples at Regular Intervals Across the Layer

1. Load Image-Pro Plus.
2. Open the image that needs to be measured. This can be done by selecting **File** on the main menu and then selecting **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images.**
4. Preprocess the image, if required, so that the layers are distinctly visible (such as with the **Enhance: Contrast Enhancement** tool in the main menu).
5. Select **Measure** from the main menu and then click on **Measurements**. The **Measurement** dialog box (shown in the figure above) will appear.
6. Select the [ ] button (**Create Trace Feature**). Now trace the upper boundary of the layer whose thickness is to be measured, by clicking at points at regular intervals along the edge. Image-Pro will automatically trace out the upper edge of the layer. After completing the trace, **right click** to finish. Similarly, trace the lower boundary of the layer. At this point, the image should look something like the one shown below:
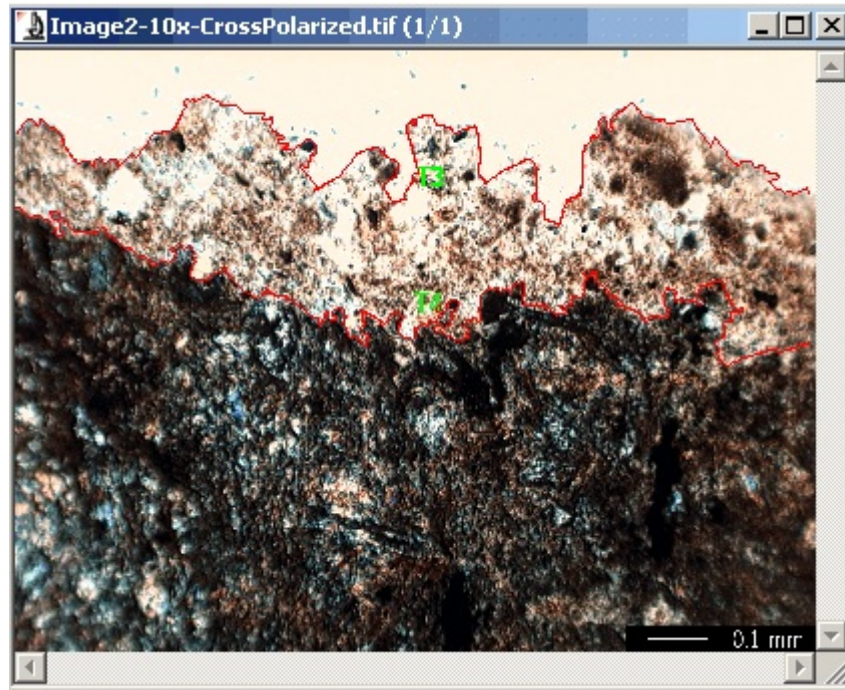
7. Now, in the **Measurement** dialog, click on the ⬚ button, designed to add a thickness measurement between two primarily horizontal lines or traces. Hold down (press and do not release) the *Ctrl* key and click anywhere on one edge of the layer that you traced out in Step 6. Then, while still holding the *Ctrl* key, click anywhere on the second edge of the layer that you traced. Image-Pro Plus will automatically calculate the minimum, maximum, and average distance between the two boundaries of the layer.

8. To view the results, click on the **Features** button in the main **Measurements** window. Then scroll along the page to find the measurements of interest (such as the minimum distance, maximum distance, and average distance between the two edges of the layer), as shown below.

## 3.2   Clemex Vision PE 3.5

As with Image-Pro Plus, we found that the Clemex software allows us to automatically take samples of the thickness at regular intervals across a layer (and calculates the minimum, maximum, and average thickness of the layer) in addition to allowing us to take individual measurements across cross-sections of the layer at a particular position. However, whereas taking a single measurement of the thickness at a particular point is rather simple, taking multiple samples of the thickness can be a very involved process, especially if the layer is broken and/or if the layer cannot be well separated using the thresholding process. We could not find an intuitive way of taking the samples, and the series of steps presented here is just an outline of the actual process that may be required to measure the thickness, depending upon the appearance and parameters of the particular image undergoing measurement.

3.2.1 Measuring Thickness at a Particular Point:

1. Load Clemex Vision PE.
2. Open the image that needs to be measured. This can be done by selecting **File** on the main menu and then selecting **Open Image**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image, if required, so that the layers are as distinctly visible as possible.
5. From the **Image Window Edit and Annotation Toolbar** (shown below), click on the **Measure Tools** button. The **Measure Tools** toolbar will open. From this toolbar select the **Measure Line** button (shown encircled in red).
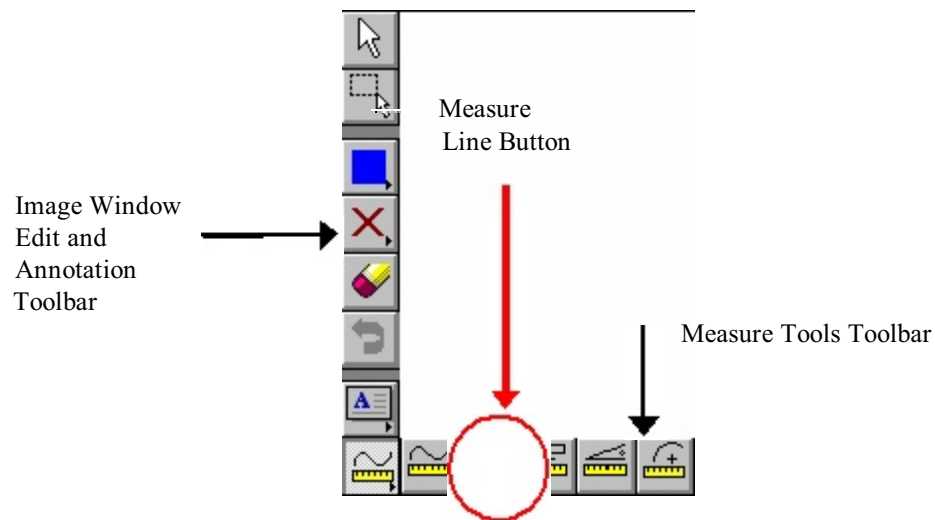
6. Draw a line perpendicular to the layer at the point at which the measurement of the thickness is to be performed. To draw the line, mover the cursor to the inner point at which the layer begins, hold down the left mouse button and drag the mouse to the outer point at which the layer ends and release the mouse button. You will see a line across the cross-section of the layer with the thickness of the layer displayed as shown below.
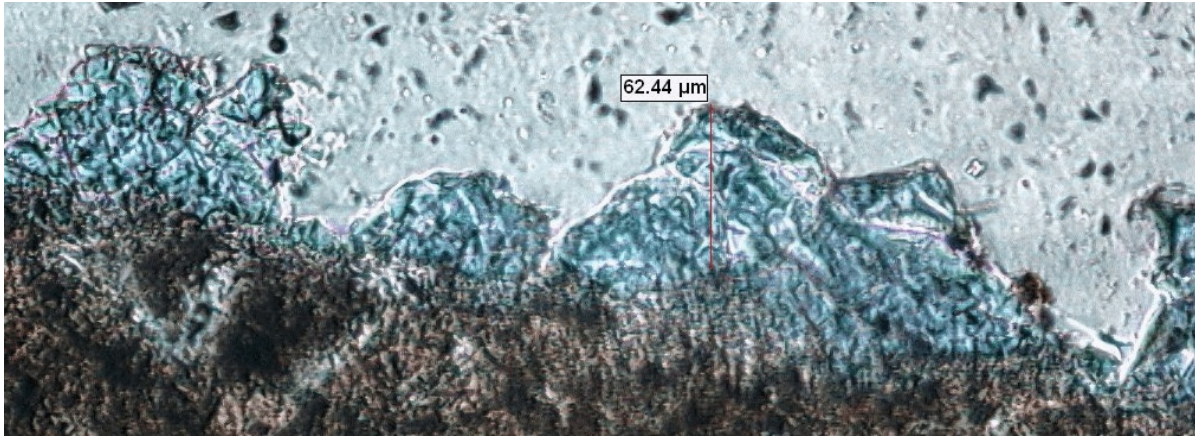
7.   One can take multiple measurements at selected points along the layer to visually identify locations of minimum, maximum, and typical thickness. To change the scale in which distances are shown (for example, to change from microns to millimeters), change the **Preferences** in the **Options** selection of the main window toolbar.

3.2.2   Taking Automatic Samples at Regular Intervals Across the Layer

This section describes how Clemex Vision PE 3.5 can be used to automatically take samples of the image at regular intervals across the cross-section of the layer and then calculate the minimum, maximum, and average of the sampled values. Note that the details in this section are intended to be a general outline and the actual steps that need to be taken may vary depending upon the image being analyzed.

1.   From the main window toolbar, select the **Toolbox** button (shown encircled in red).
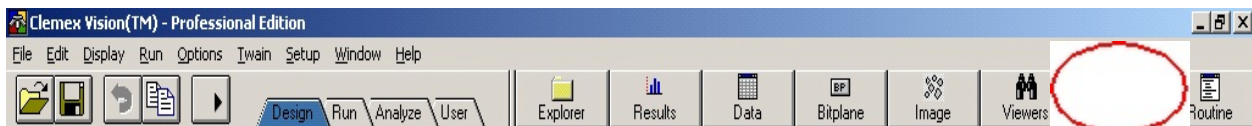
2.   A dialog box will appear as the **Video Input Operations** button:
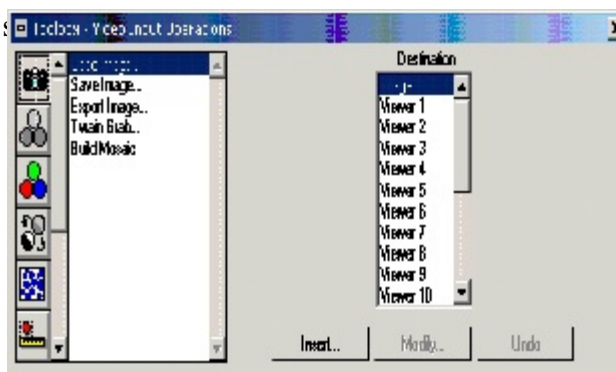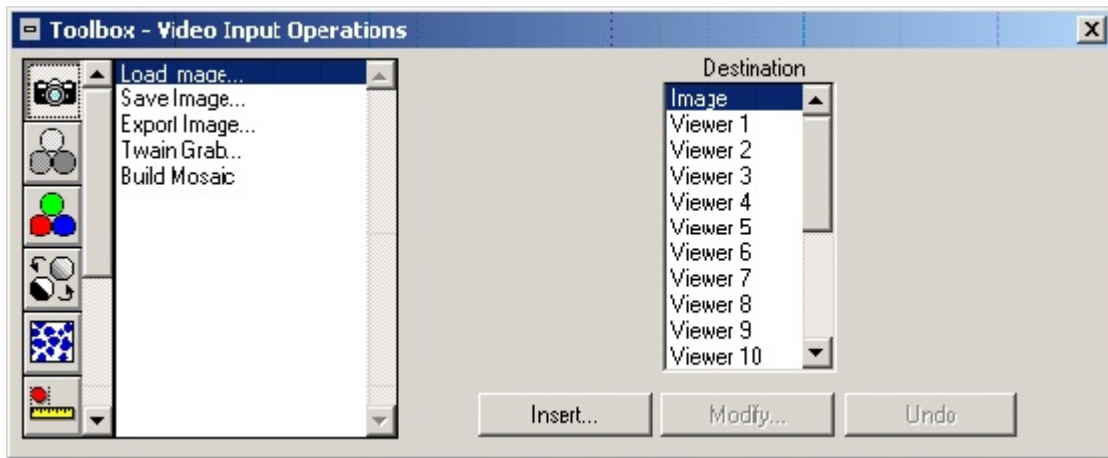
4. Then select **Load Images** and click on **Insert**. The **Insert Load Image** dialog box will appear. Select the image that you wish to work on and click on **Open**. Assign a calibration factor to the image as described in the protocol instructions on **Calibration of Images**.
5. Now perform any steps required for preprocessing the image, so that the layers are as distinctly visible as possible.
6. After the layers are distinctly visible, **thresholding** has to be performed. Thresholding is the process of grouping pixels of a particular color together - all the colors that belong to a particular layer have to be grouped together - whereas the pixels that belong to the background or the underlying material have to be grouped separately. One of the aims of preprocessing the image and separating the layers is to make thresholding possible. Thresholding can be of two types:

    i. Gray thresholding: All the pixels are assumed to be in shades of gray (i.e. the image is assumed to be a grayscale image - if not, it is converted to one.) All the pixels that belong to a particular gray level are grouped together (In Clemex Vision PE terminology, the range of pixels are assigned to a bitplane – a term that is used to denote the color that is used to access the group of pixels as a unit.)
    ii. Color thresholding: Color thresholding needs to be done on color images when the equivalent shades of gray are so close together that they cannot be separated using gray thresholding. Color thresholding is based on the hue, saturation and intensity levels of the pixels.

In this section, we will only discuss gray thresholding since the sequence of steps for performing color thresholding is similar. Please refer to the Clemex Vision PE manual for more information on color thresholding.

In the **Toolbox** dialog, select the **Gray/Binary Transformation** symbol

Click on **Gray Threshold** and then click on **Insert**. The **Insert Gray Thre**
will appear. In the dialog box, start by clicking on **Bitplane 1** (of the eight
that appear on the top left hand corner in the dialog box). Position the left
(by dragging them) in such a way that most of the pixels that belong to a particular layer
are assigned to the same bitplane. (Don't worry if there are gaps in the layers of a different
color – we will fill the gaps later).
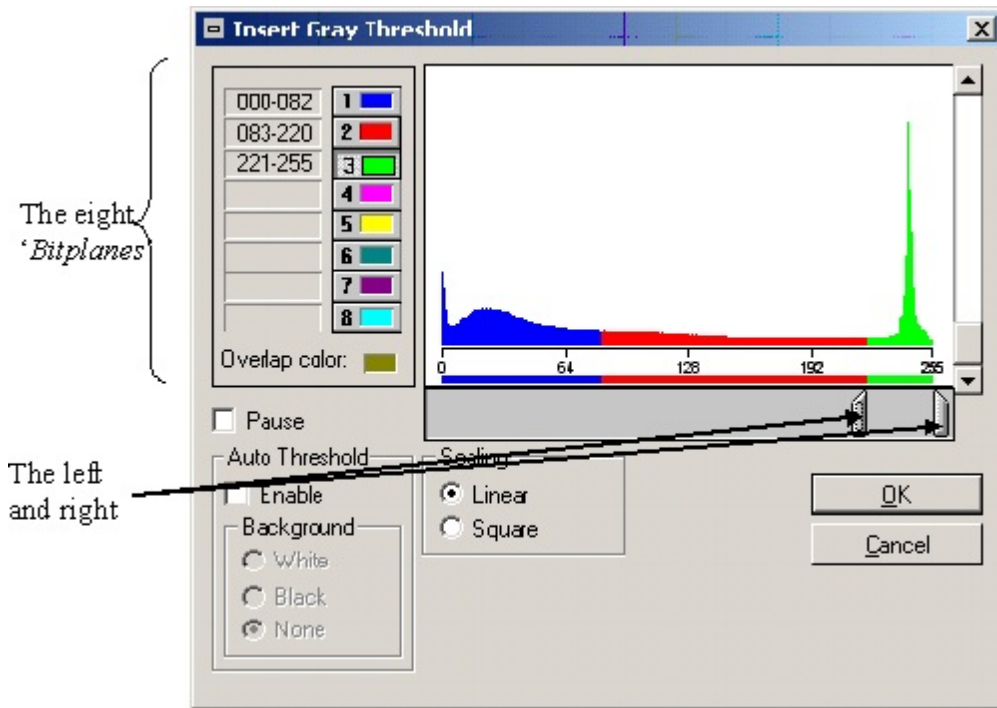
Repeat this step for each layer, assigning a different bitplane each time. After all of the
layers have been assigned to bitplanes, the image will be similar to the one shown below.
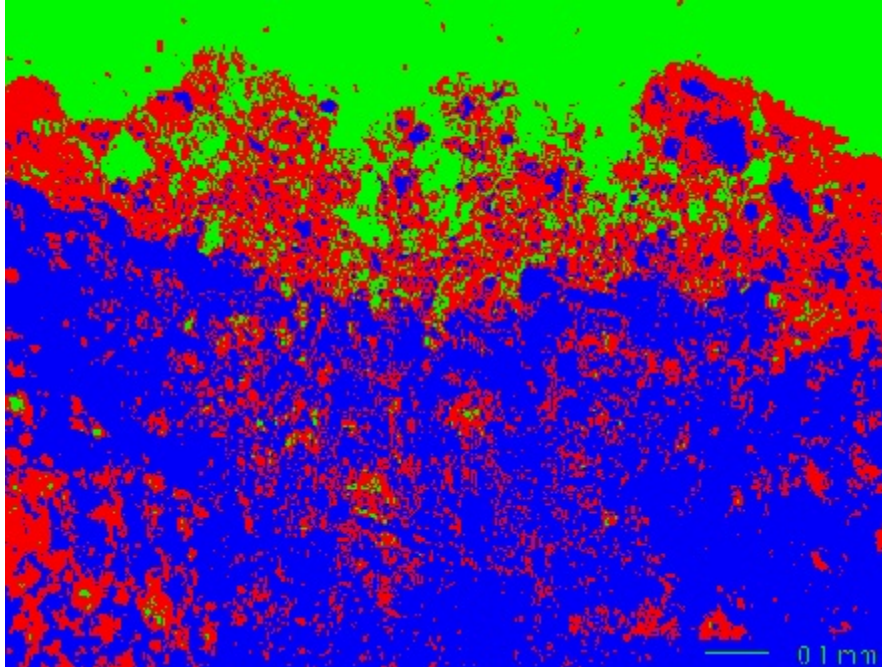
7. As can be seen in the image shown above, after the thresholding has been performed, the distinct layers (belonging to a particular bitplane) contain many "holes" and parts belonging to other bitplanes. We will now need to fill these "holes."

    The first way in which the holes can be filled is by applying a **Chord Size Filter**. A chord size filter filters artifacts (holes, objects, etc.) that are so small that a box or a circle (of a particular size that can be specified) cannot be fitted into the artifact. The selected artifacts can either be removed or converted to another bitplane.

    We will illustrate the application of a chord size filter using our sample image of the Gandharan sculpture. In our sample image, we will apply two chord size filters: one for the weathering layer and the other for the underlying material.

    To apply the chord size filter, select **Binary Operations** (encircled in red) from the **Toolbox** dialog box shown below, and click on **Chord Size**. In the **Source**, select the bitplane from which the artifacts have to be removed. For example, in our sample image, we have to remove the artifacts that are a part of Bitplane 1 (BP1), which are smaller than a circle of diameter 10 pixels (since these artifacts are actually a part of the weathering layer). In the destination choose **To None** since we want to remove them completely. The size of the artifacts can be specified using the diameter spin box. After all of the values have been selected, click on **Insert**.
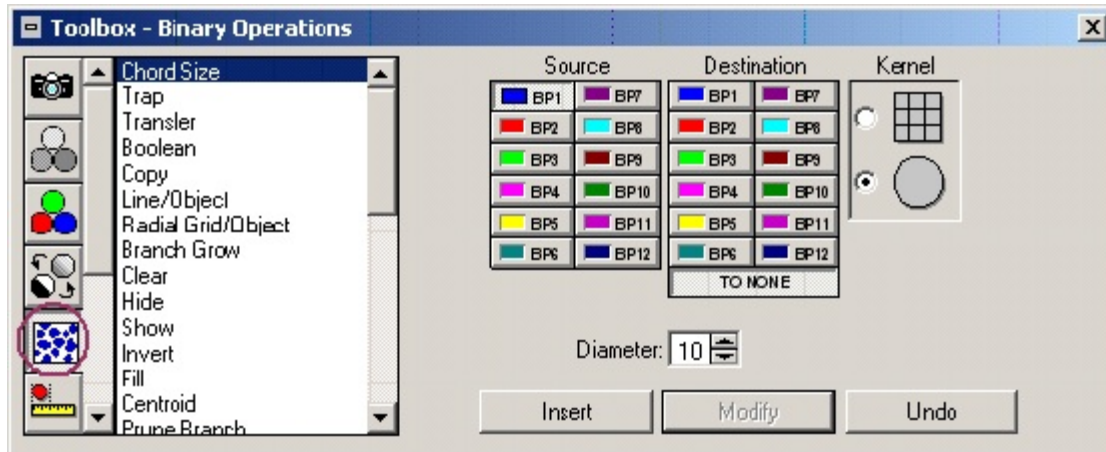
Similarly, we would like to remove the artifacts that are a part of Bitplane 2 (BP2), the weathering layer, since these small artifacts are actually a part of the underlying material below the weathering layer. To do this, insert a chord size filter as explained above, but instead of specifying **BP1** in the source, enter **BP2**.

8. As a result of applying the chord size filter, we have created a set of small areas that do not belong to any bitplane. Now we have to fill these areas. This can be done by applying the closing operation. **Binary Closing** fills gaps, holes, and small concavities. To apply the closing filter, select **Binary Operations** in the toolbox dialog (as was done when we applied the chord size filter). Then in the list of binary operations, click on **Closing**. Select the bitplane that needs to be closed. (In our case, we need to close both the biplanes 1 and 2 since gaps in both the weathering layer and the underlying material need to be filled.) Select the various size parameters (such as the kernal type and size) and click on **Insert** (see the figure below). The kernal can be thought of as a polygon or shape. If the polygon does not fit inside a particular artifact, the artifact is selected for whatever operation is to be performed (such as applying a chord size filter or closing gaps). A variety of the kernal shapes and sizes can usually be selected.
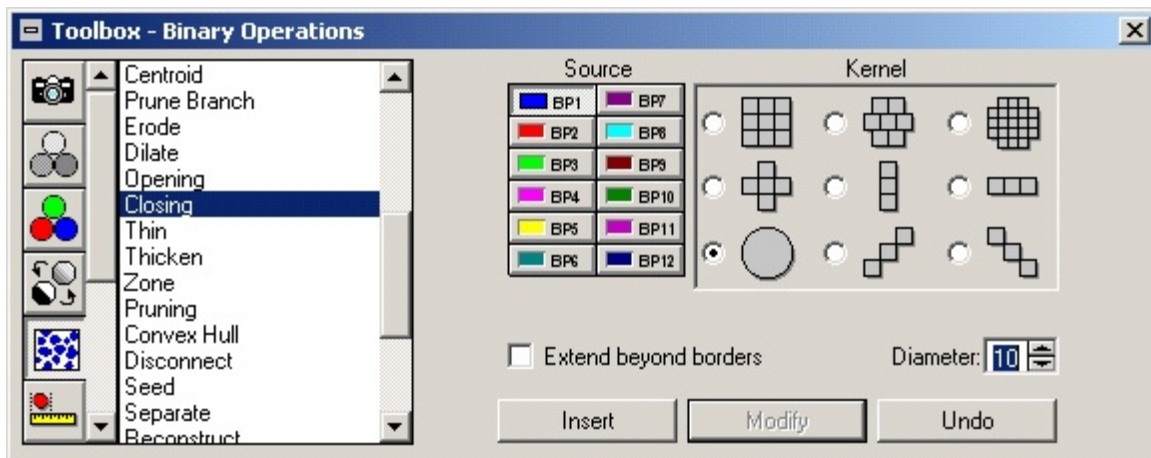
9. The closing operation usually results in overlapping layers since areas which are smaller than the kernal and not a part of the source bitplane are added to the source bitplane. This can be seen in the image shown below.
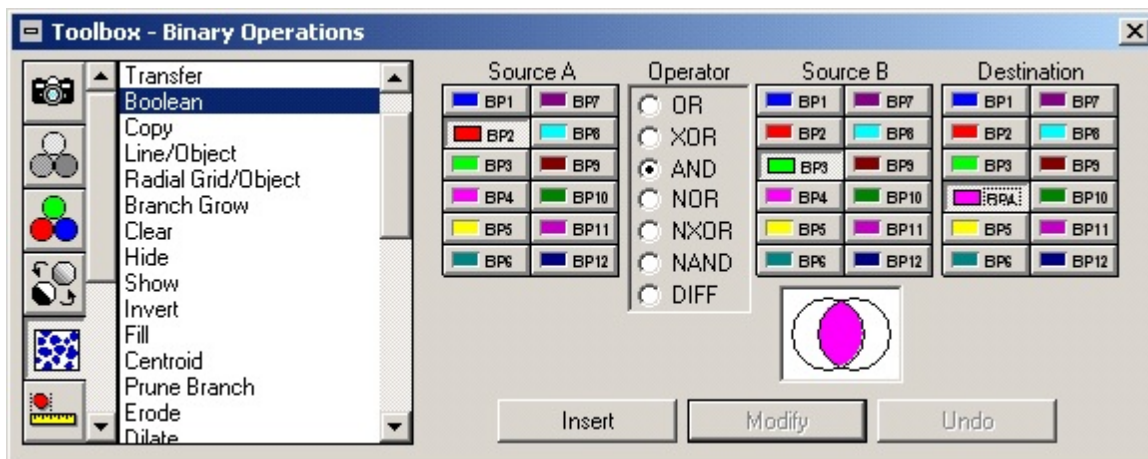

The overlapping layers (in dark
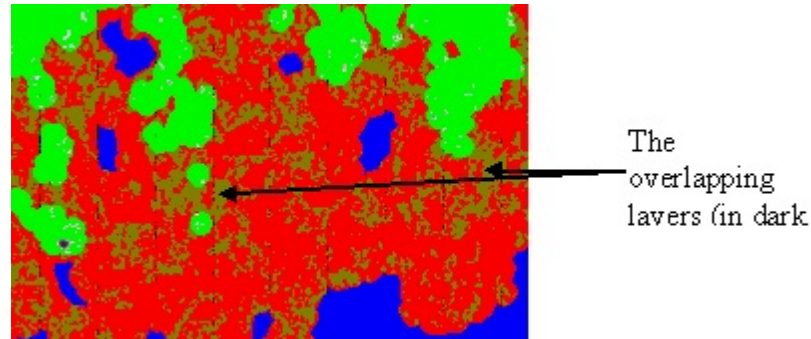
Before the overlapping layers can be removed, we will have to decide to which layer the overlapping layers actually belong. In our sample image, the overlapping between the bitplanes 2 and 3 (the background and the weathering layer) belongs to bitplane 2 (the weathering layer) and the overlapping between the bitplanes 1 and 2 (the weathering layer and the underlying material) belongs to bitplane 1 (the underlying material).

We can correctly remove the overlapping layers by applying Boolean operators. Assuming that the two overlapping layers are the bitplanes b_1 and b_2 and the overlap belongs to bitplane b_2, then the boolean operations required are:

a. The 'AND' (or intersection) of bitplanes that overlap has to be calculated and assigned to another bitplane (say bitplane b_3).

b. The 'DIFF' (or difference) between the bitplanes b_1 and b_3 is assigned to bitplane b_1. This effectively removes the area of bitplane b_1 that overlaps with bitplane b_2.

c. Bitplane b_3 is now cleared since it is not required.

These boolean operations can be performed in Clemex Vision PE by selecting **Binary Operations** and then selecting **Boolean** in the list of binary operations. Select the **Source A** as bitplane b_1 and **Source B** as the bitplane b_2. Also select 'AND' as the **Operator** and bitplane b_3 as the **Destination** bitplane and then click on **Insert**. Similarly, the 'DIFF' operation can be performed by selecting the relevant source and destination bitplanes and 'DIFF' as the operator (see the figures below).

To clear a bitplane, select **Clear** from the Binary Operations list in the **Toolbox** dialog. Select the bitplane to be cleared and click on **Insert**. (See the figure below.)
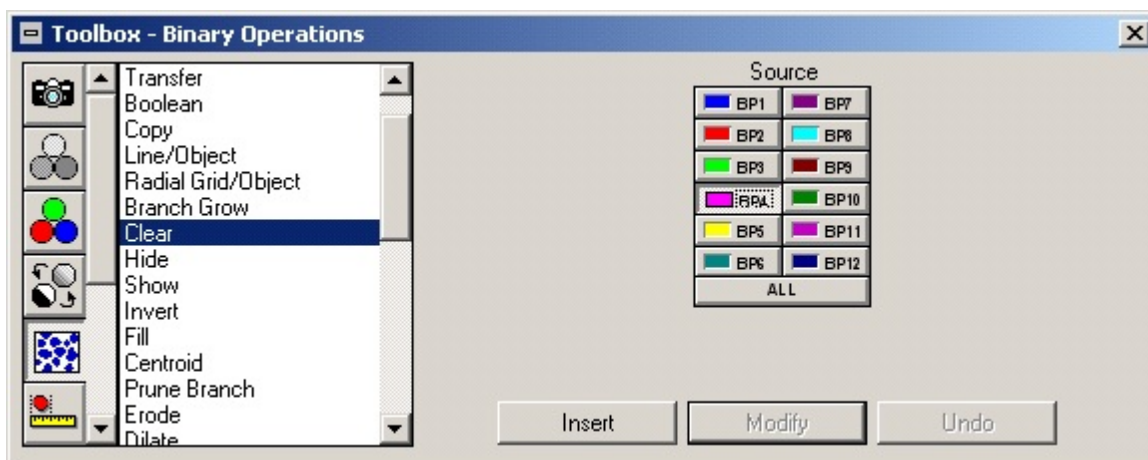

After applying the boolean operators and removing the overlapping layers, our resultant sample image looks as shown in the figure below:
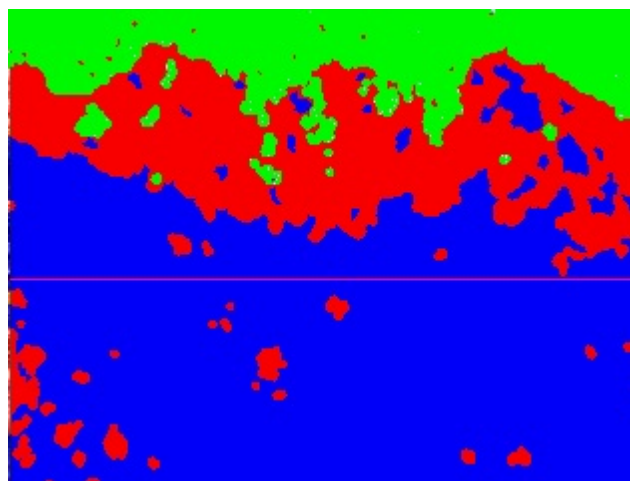
10. By now the layers should be filled and well separated and each layer should be assigned to a bitplane. Now we are ready to perform the actual measurements. To do this, first we need to create a set of vertical lines at regular intervals along the weathering layer and then measure the length of each line. To create the set of vertical lines, select **Square Grid** under the list of Binary Operations in the **Toolbox** dialog. Click on **Insert**. The **Insert Square Grid** dialog shown below appears:
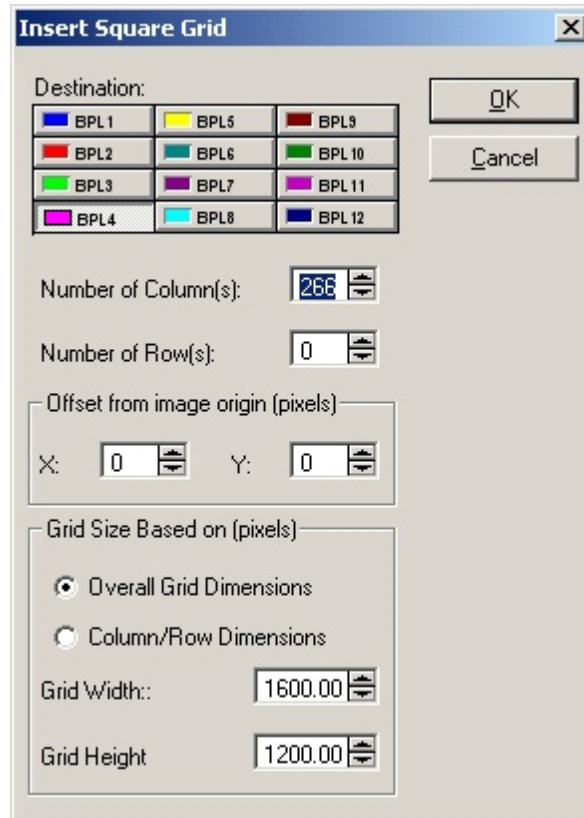
Increase the **Number of Column(s)** to represent the number of samples to be taken. Set the **Number of Row(s)** to 0. You might also want to change the **Grid Width** and **Grid Height** values as desired. Select the **Destination** to be a bitplane that has not already been used. Click on **OK** after you finish. A set of vertical lines as shown below will be inserted:
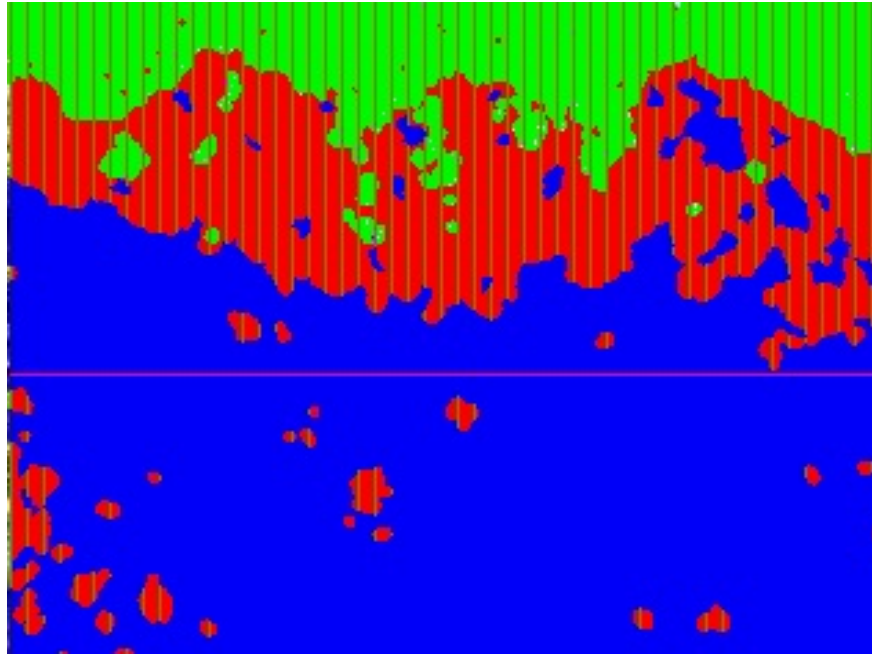
Now since we only need to measure the thickness of the weathering layer, we have to reduce the length of the vertical lines to equal the length of the weathering layer. This can be easily done by taking the 'AND' of the bitplane representing the vertical lines and the bitplane 2 (representing the weathering layer). The procedure for taking the 'AND' is the same as that described in step 9 above.

11. Finally, we have to apply a frame guard, to remove the unwanted areas from the measurement. To do this, click on the **Additional Operations** button ⟩⟩

   in the **Toolbox** dialog and then select **Guard Frame**. Click on **Insert**. The **Insert Guard Frame** dialog (shown below) appears and a red rectangle appears on the image. Change the size of the red rectangle so that the weathering layer just fits into the rectangle.

   To change the size of the rectangle, move the mouse pointer to an edge of the rectangle until the mouse pointer becomes a double headed arrow. Now drag (click the mouse without releasing it) the edge to move it as desired. Click on **OK** after you are finished. (See the screenshot below.)
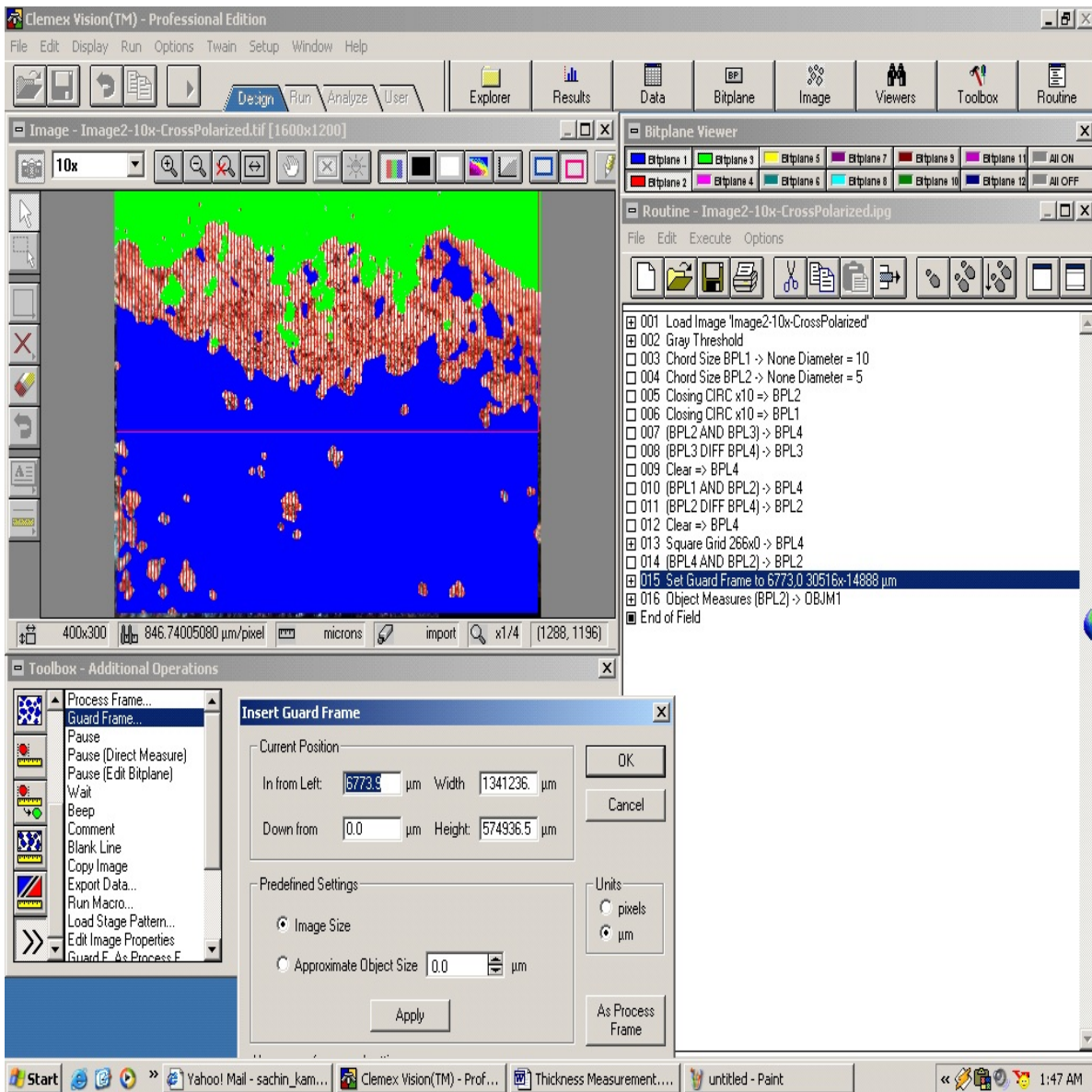
12. Now we can perform the actual measurement by selecting **Object Measure** in the **Toolbox** dialog.

    Select **Length** in the object measures list. Select the source as the bitplane that was assigned to the vertical lines. Then click on **Insert**. (See the figure below.)

13. To see the results, click on the **Results** button in the main toolbar. The results are also shown in the figure below.
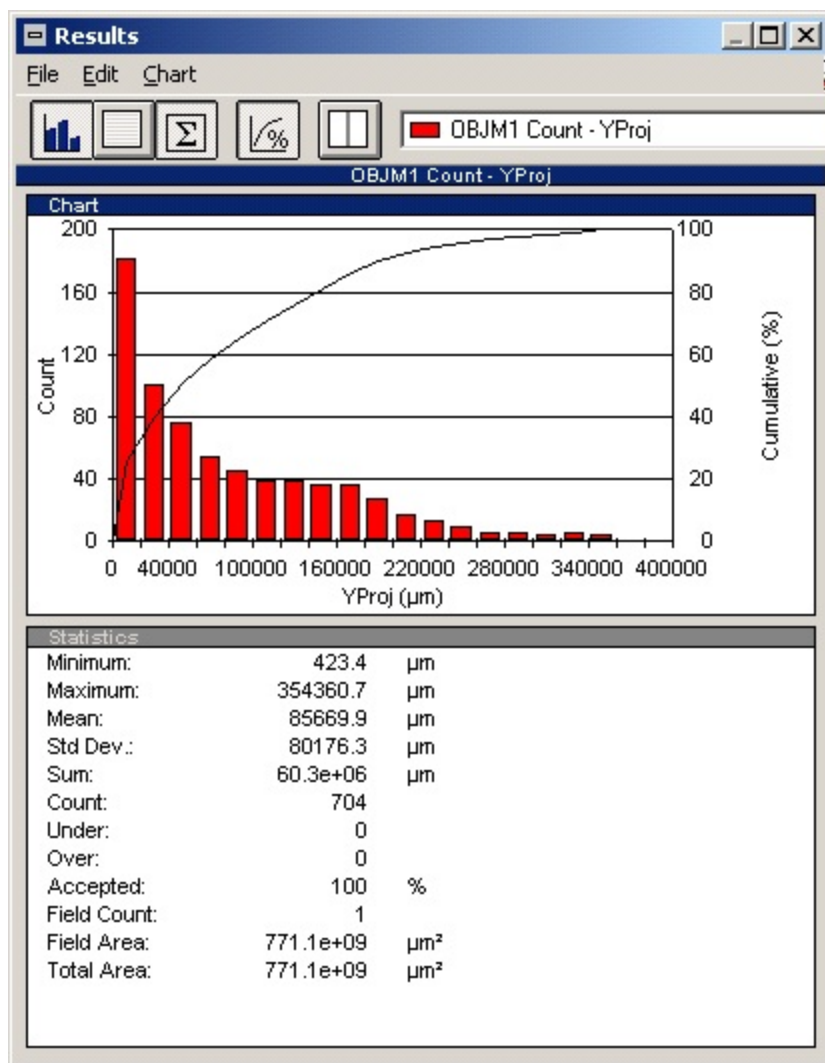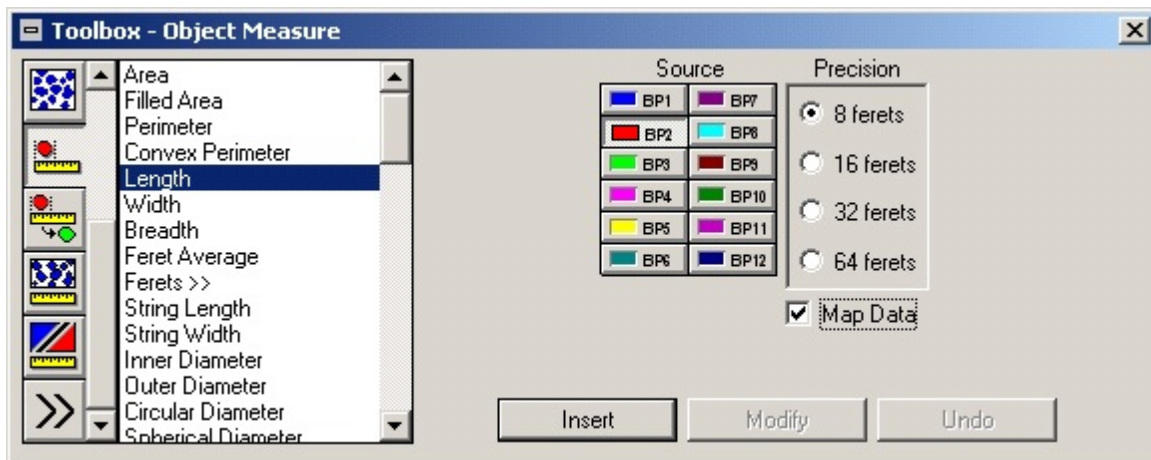
3.3 ImageJ (Version 1.33)

We found that, whereas ImageJ allows us to measure the thickness of the layer at a particular point, it **does not** allow us to automatically take samples of the thickness along cross-sections of the layer and does not automatically calculate the minimum, maximum and average thickness of the layer. This seriously impairs the use of ImageJ in conservation research or technical studies of artifacts. However, one can still take the approach of making multiple single measurements and trying to select a width at the thinnest, thickest, and typical thickness of a layer.

3.3.1 Measuring Thickness at a Particular Point:

1. Load Image J.
2. Open the image that needs to be measured. To do this, select **File** on the main menu and then select **Open**. Select the image that you wish to open and then click on **Open.**
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image if required (if the layers are not distinctly visible). For example, from the **Image** function in the main window, select **Adjust**, and then **Brightness/Contrast**.
5. Select the **Straight Line Selections** tool (shown highlighted, or selected here) from the toolbar shown below.
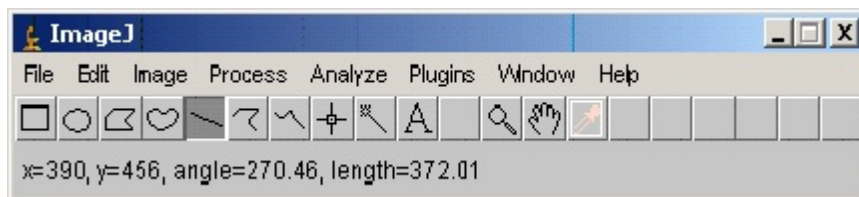

6. Draw a line across the layer at the point at which the measurement of the thickness is to be performed. To draw the line, move the cursor to the point at which the layer begins, hold down the left mouse button, and drag the mouse to the point at which the layer ends; now release the mouse button. You will see a line across the cross-section of the layer:
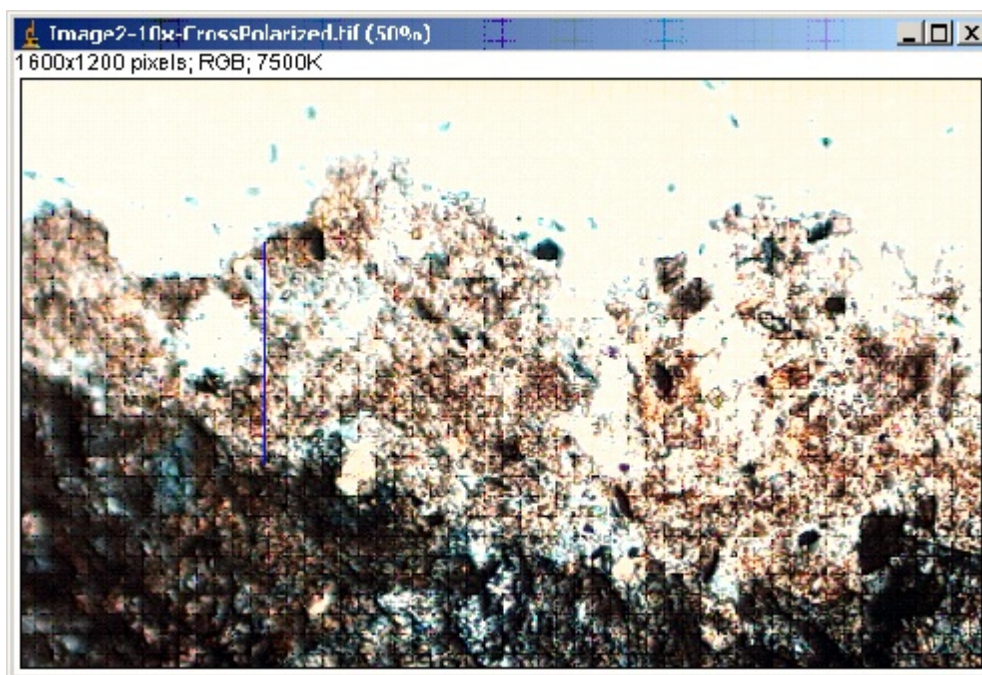
7. Select **Analyze** from the main menu and then click on **Measure**.
8. The **Results** window will display the length of the line (in the units to which you have calibrated the image).
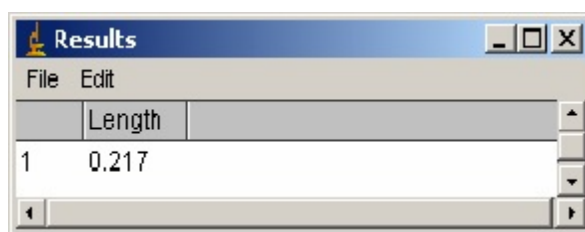
4. Analysis

Now we will compare the three image analysis tools and give an assessment regarding their performance in thickness measurement tasks for thin sections of cultural materials. We will evaluate each approach using the following criteria:

1. Ability to take a single measurement across a cross-section of the layer.
2. Ability to automatically take samples of the thickness at regular intervals across the cross-section of the layer and automatically calculate the minimum, maximum and average thicknesses.

3. Ease of use and simplicity of the measurement process.
4. Consistency of the measurement process across multiple images.
5. Accuracy of the measurement results.
6. Format of the resultant output.

4.1 Ability to Take a Single Measurement

We found that all three tools were able to perform this operation. Furthermore, we found that all three tools were equally easy to use for this measurement task. Taking a single measurement across a cross-section of the layer, in all three tools, involved selecting the desired operation from a toolbar and then drawing a line across the layer at the desired point. Although the measurement toolbar was "hidden" in the Image-Pro Plus software (we had to open the **Measurement** dialog box from the main menu), we did not find this to create any difficulties.

4.2 Ability to Take Samples at Regular Intervals

We found that ImageJ was unable to offer an automatic way of performing this task. Thus we had to rule out ImageJ for thickness measurement tasks for which many multiple measurements are important; our research frequently requires the ability to automatically sample across a layer rather than confining ourselves to single measurements or to multiple measurements made individually. Of the remaining two software packages, we found that both of them did a good job of taking multiple measurements and calculating the minimum, maximum, and average distances.

4.3 Ease of Use and Simplicity of the Measurement Process

This is a very important criterion, since we feel that the interface and measurement procedure offered by the software should be as simple and intuitive as possible, especially for the inexperienced and novice user. Clearly, a software tool that requires a computer science graduate degree to operate is a problem since typical conservation users cannot be expected to have that level of expertise.

Image-Pro Plus easily meets this criterion. It offers a very intuitive way of measuring the thickness by allowing the user to draw the outline of the two layers and then automatically measuring the various parameters such as minimum, maximum, and average thicknesses.

The measurement procedure in Clemex Vision PE, on the other hand, relies on a procedure which is akin to programming in many ways. The measurement procedure requires many steps, which depend to a large extent on the image under consideration and on the accuracy of the preprocessing work.

4.4 Consistency of the Measurement Process Across Multiple Images

The measurement procedure should **not** change just because a different image is under consideration. We found the measurement procedure in Clemex Vision PE to be largely dependent on the image under consideration to the extent that each image might require a different sequence of steps to measure. Thus Image-Pro Plus is more consistent across different types of specimens since it offers the same intuitive way of measuring the thickness irrespective of the image under consideration.

4.5 Accuracy of the Measurement Results

This is a rather difficult criterion to evaluate, since the accuracy depends to a large part on the measurement process, the type of image and layers being assessed, and the preprocessing steps. Image-Pro Plus might be less accurate as compared to the Clemex Vision PE software since it depends on the use of the human eye to detect the boundaries of the layers. It may be difficult for the user to accurately determine the boundary of a layer by using no tool other than the eye. Although the human eye can be a powerful resource for detecting the outlines of layers, there are also times when the human eye can be deceived (such as when encountering optical illusions). Clemex Vision PE, on the other hand, makes use of thresholding and shades of gray to determine the boundary of a layer. Although the accuracy of the Clemex Vision PE software might depend on the accuracy of the thresholding process, it is possible that Clemex Vision PE may be more accurate than Image-Pro Plus. However, some steps in the Clemex Vision PE thickness protocol also require visual estimates (such as identifying artifacts that should be reassigned from layer to background and vice-versa), so the problem of visual judgment is not that of Image-Pro Plus alone.

4.6 Format of the Resultant Output

In this criterion we consider whether the software offers alternative forms of output representation such as graphs and tables. Clemex Vision PE automatically displays the results in the form of a bar chart which may be more desirable than a table of numbers. Image-Pro Plus, on the other hand, does not offer us this ability. However, it, as with Clemex Vision PE, does allow us to export the data to a spreadsheet (such as Excel) or to a statistical package (such as SPSS), where the data can be used for further analysis and for the drawing of more sophisticated graphs and charts. ImageJ data is also easily cut and paste into another application program for analysis or viewing.

4.7   Evaluation Conclusions

Based on the evaluation criteria discussed above, we would recommend Image-Pro Plus for the thickness measurement needs for research using petrographic thin-sections of cultural materials. Image-Pro Plus is easy to use and apply to a variety of image types on the basis of its simple and intuitive way of measuring thicknesses. However, where the user is willing to spend considerable time and effort in learning the tools, Clemex Vision PE may be equally good if not better for the thickness measurement tasks. ImageJ is suitable only if the user is interested in taking a few individual measurements at specific points across a layer.

Product Sources

Image-Pro Plus: MediaCybernetics, www.mediacy.com

Clemex Vision PE: www.clemex.com

ImageJ: author Wayne Rasband, National Institutes of Health, http://rsb.info.nih.gov/ij/

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Image Analysis Protocol Instructions #4: Measuring the Area Percent, Size, and Shape of Phases in a Ceramic Thin Section
Using Image-Pro Plus, Clemex Vision PE, and ImageJ

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1.   Introduction

In this set of protocol instructions we discuss a set of procedures for measuring the area percent of phases (such as non-plastic temper additives or natural clay accessories, pores, etc.) present in ceramic thin sections. We also examine ways of measuring the minium, maximum, and average size and degree of roundness (shape characteristics) of those components, using each of the three image analysis tools under study: Image-Pro Plus 5.0, Clemex Vision PE 3.5, and ImageJ (Version 1.33). In addition, we provide an assessment of the features offered by the three tools with regard to calculation of area percent, size, and shape of specific constituents of a thin section.

We used the following thin section images as samples for experimentation. All of the images were observed in both plane polarized and crossed polarized light:
1. Hard-paste porcelain, German, mid-18th century
2. English-Irish tin-glazed delft, 1760
3. Salt-glazed stoneware, European, 1800
4. Earthenware, historic American (Virginia), 1720-1745
5. Hard-paste dinnerware, European, 19th century
6. Earthenware, native American, Southern Delaware (Townsendware), 1000-1600 A.D.

Due to variation from one location to another on a thin section, an accurate measurement would include averaging the results of several repeated measures from random locations within the thin section, or from a systematic set of measurements taken across the entire thin section. Better yet would be the incorporation of multiple thin sections, including both perpendicular and parallel sections from a sherd or vessel. The goal is to capture the full degree of variation present.

## 2.   Calculating the Area Percent of Ceramic Thin Section Components and Measuring Their Size and Shape

2.1   Image-Pro Plus 5.0

Two procedures are given below, one involving a conversion to grey-scale (Procedure A), and another involving use of manual highlighting of phases to be counted and measured, using a dropper tool (Procedure B). For a given type of thin section, it may be necessary to experiment to see which procedure is most satisfactory. Procedure A allows for fast and automatic counting of bright (or dark) grains. For many types of samples, this procedure works very well.

However, for other types of samples, this procedure can be problematic. For example, when counting light sand grains within a dark clay matrix, in some cases bright areas within the clay background may be counted as sand grains. Sometimes preprocessing can ameliorate the problem, but not always. Procedure B, although slightly more time-consuming, may be more accurate where there are more gradations in color or brightness and so an automatic counting method fails to correctly distinguish between the areas of interest.

### 2.1.1   Compute the Area Percent of Phases: Procedure A

1. Load Image-Pro Plus.
2. Open the image that needs to be measured. This can be done by selecting **File** on the main menu and then selecting **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image, if required, so that the phases of interest are distinctly visible (such as with the **Enhance: Contrast Enhancement** tool in the main menu).
5. Convert the image into a grey scale image (if the original image is a color image) by selecting the **Convert to Grey Scale 8** command in the **Edit** menu.
6. Select the **Count/Size** command from the **Measure** menu. The **Count/Size** dialog box appears as below. Click **Automatic Bright Objects** to analyze the image and automatically set the intensity range when it performs the count, if the bright objects are of interest (or select **Automatic Dark Objects**, as appropriate).
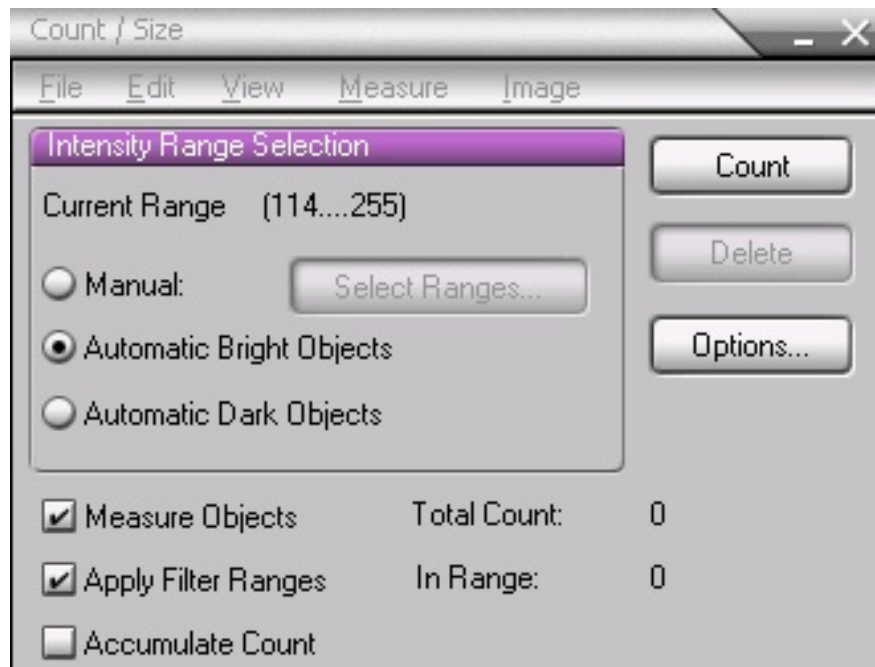
7. Select **Measure** in the **Count/Size** dialog box; then click on **Select Measurements**. The **Select Measurements** dialog box appears as below. Select the **Per-Area** measurement parameter from the **Measurements** scroll box.
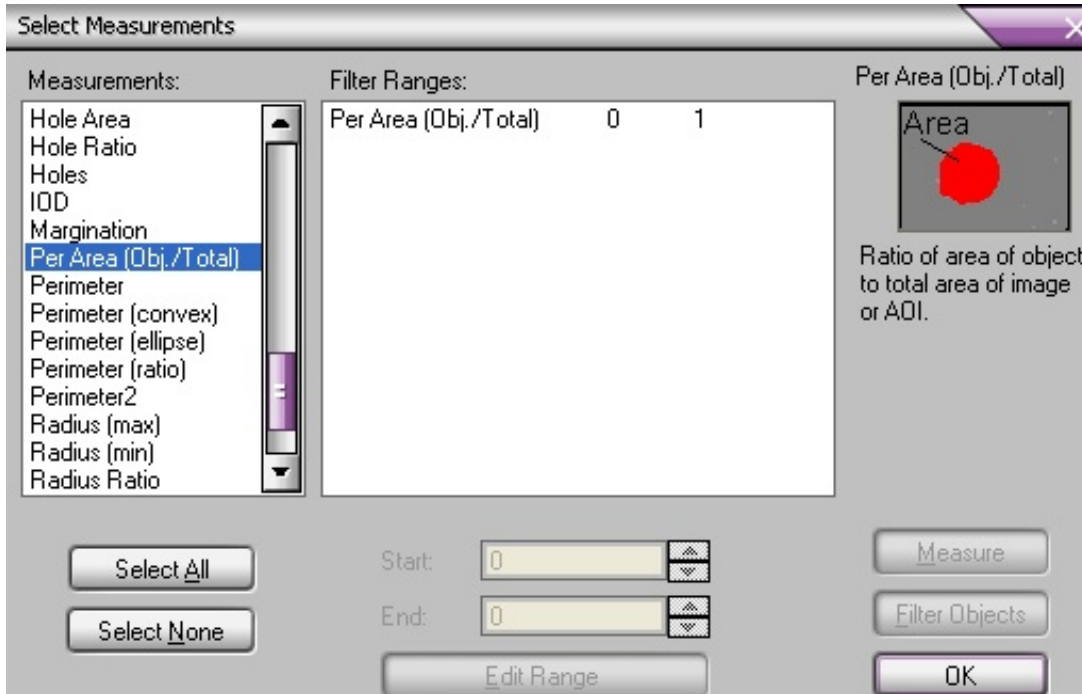
8. Click **OK**. The **Select Measurements** dialog box closes.
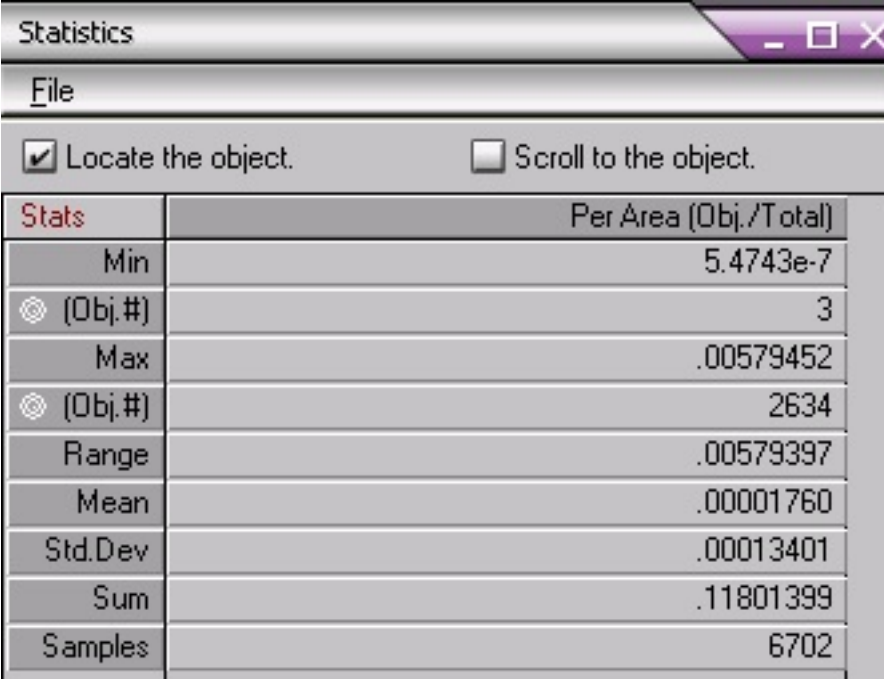9. Click the **Count** button in the **Count/Size** command window.
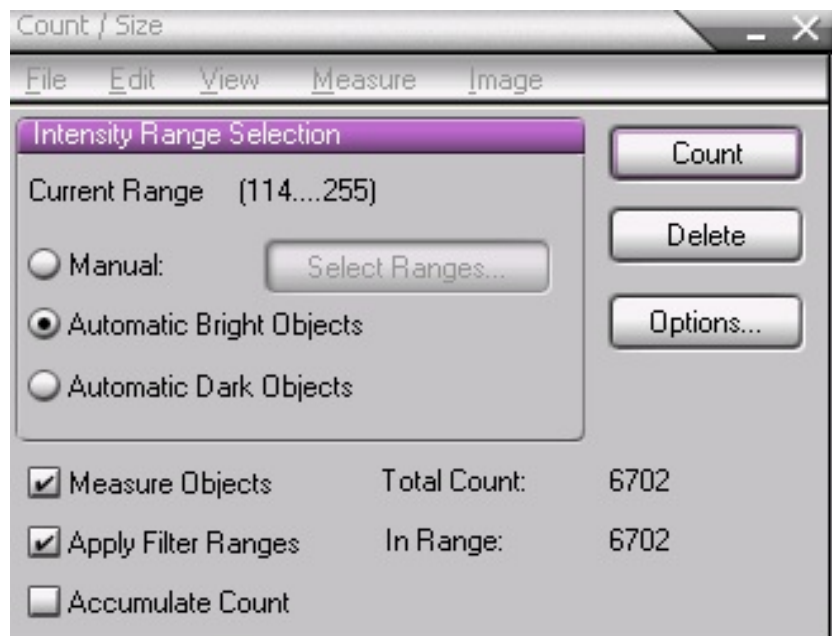
10. The image is analyzed; the phases of interest are highlighted.
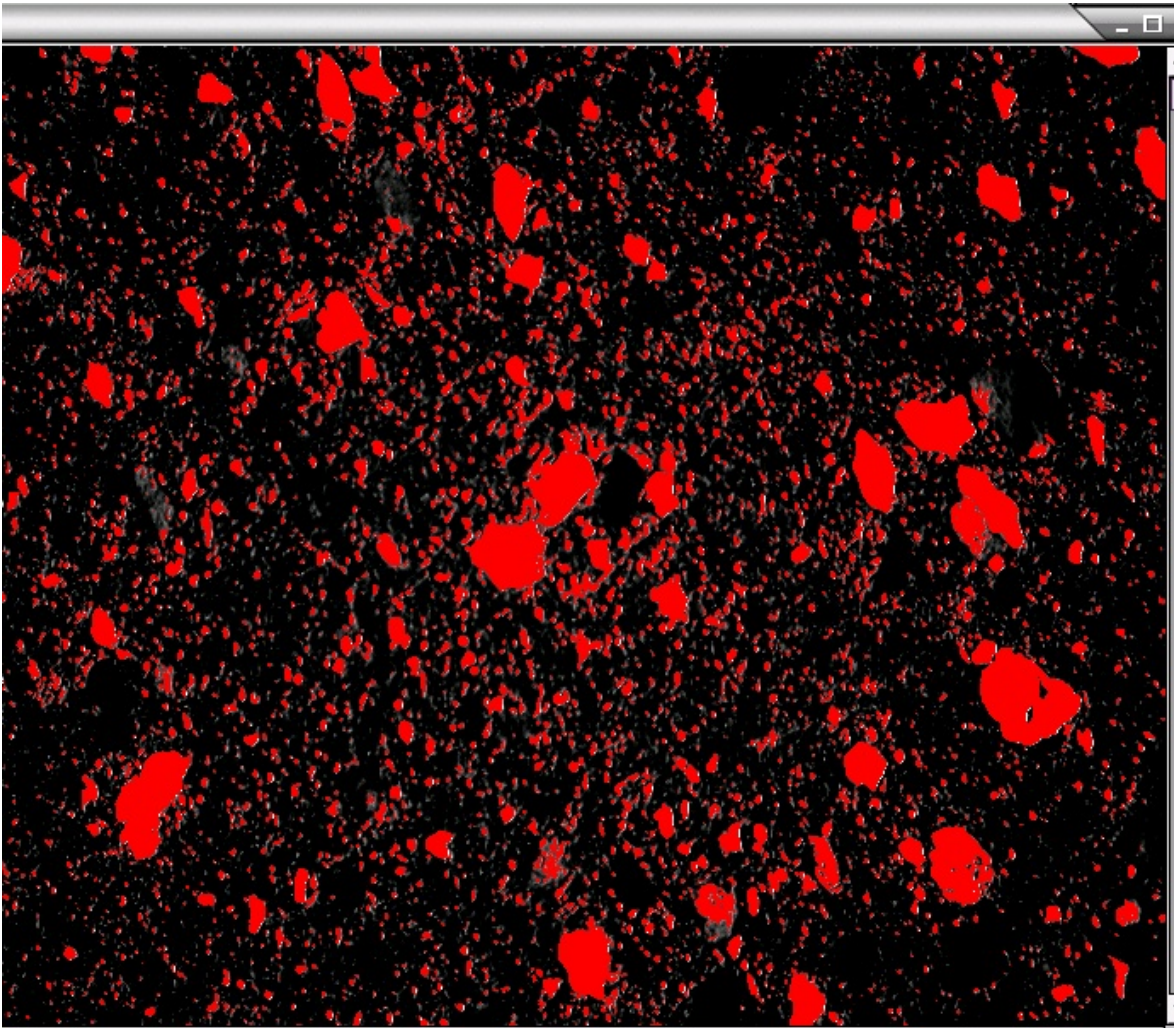
11. Open the statistics table by selecting **Statistics** from the **View** menu in the **Count/Size** command window and find the row titled **Sum** in the table, as shown below. The decimal number found in the **Sum** row of the **Per Area** column gives the percent area of the highlighted objects in the image. This example shows 12% (11.8%) for the bright objects, in this case the sand component of the ceramic thin section.

Stats	Per Area (Obj./Total)
Min	5.4743e-7
◎ (Obj.#)	3
Max	.00579452
◎ (Obj.#)	2634
Range	.00579397
Mean	.00001760
Std.Dev	.00013401
Sum	.11801399
Samples	6702

12. If you wish to cross-check, you can then delete this count (or save the data), and change the **Intensity Range Selection** in the **Count/Size** menu from **Automatic Bright Objects** to **Automatic Dark Objects**. Then, keeping the same settings for **Per Area** measurement, click the **Count** button in the **Count/Size** command window. The result will appear as below.
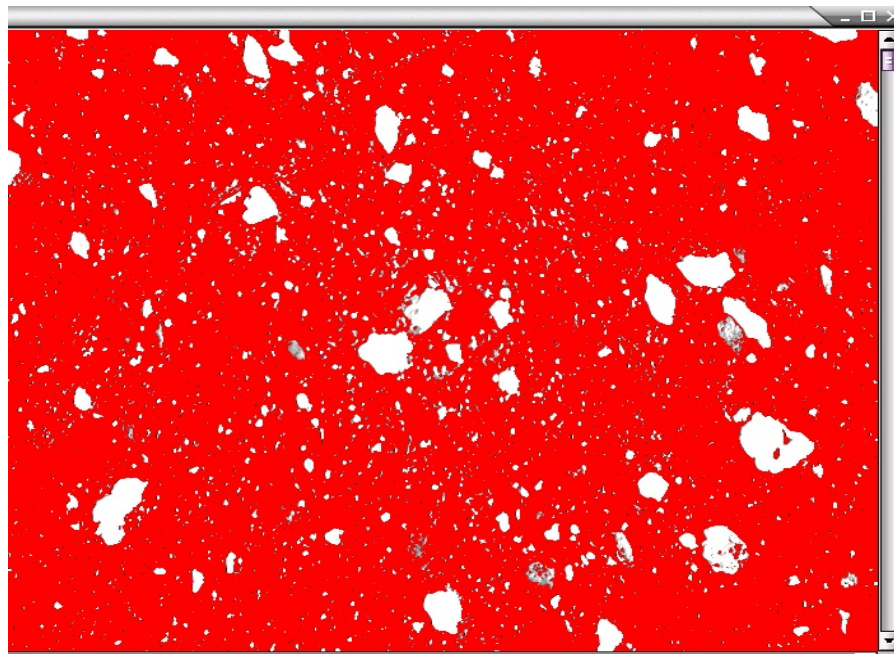
13. Again open the statistics table by selecting **Statistics** from the **View** menu and find the row titled **Sum** in the table, as shown below. Again, the decimal number found in the **Sum** row of the **Per Area** column gives the percent area of those objects in the image. This example shows 88 % for the dark background area, in this case the fired clay component of the ceramic thin section.

Stats	Per Area (Obj./Total)
Min	5.4743e-7
(Obj.#)	2
Max	.88130611
(Obj.#)	1
Range	.88130558
Mean	.00456987
Std.Dev	.06327299
Sum	.88198602
Samples	193

14. You may wish to select the option to fill holes within an object, or to count only those objects of a certain size class. Calculating the percentage of non-plastics or other temper material can be done as a separate operation or it can be combined with collection of other measurement data as discussed below.

2.1.2   Count the Grains in an Image and Measure Their Size and Shape: Procedure A

1. Load Image-Pro Plus.
2. Open the image that needs to be measured. This can be done by selecting **File** on the main menu and then selecting **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image, if required, so that the objects of interest are distinctly visible (such as with the **Enhance: Contrast Enhancement** tool in the main menu).
5. Convert the image into a grey scale image (if the original image is a color image) by selecting the **Convert to Grey Scale 8** command in the **Edit** menu.

6. Select the **Count/Size** command from the **Measure** menu. The **Count/Size** dialog box appears. Click **Automatic Bright Objects** to analyze the image and automatically set the intensity range when it performs the count, if the bright objects are of interest (or select **Automatic Dark Objects**, as appropriate).

7. Select **Measure** in the **Count/Size** dialog box, then choose the **Select Measurements** command. The **Select Measurements** dialog box appears as below. Add the **Area (Polygon)**, **Per-Area**, **Roundness**, **Size (length)**, and **Size (Width)** or any other parameters you are interested in that are listed in the **Measurements** scroll box. When a range for a measurement is selected, only objects that fall within the range in the count are recorded in the measurement data sheet. When a measurement type is selected, a description of how that parameter is calculated appears in the upper right corner of the **Select Measurements** dialog box, as shown below.
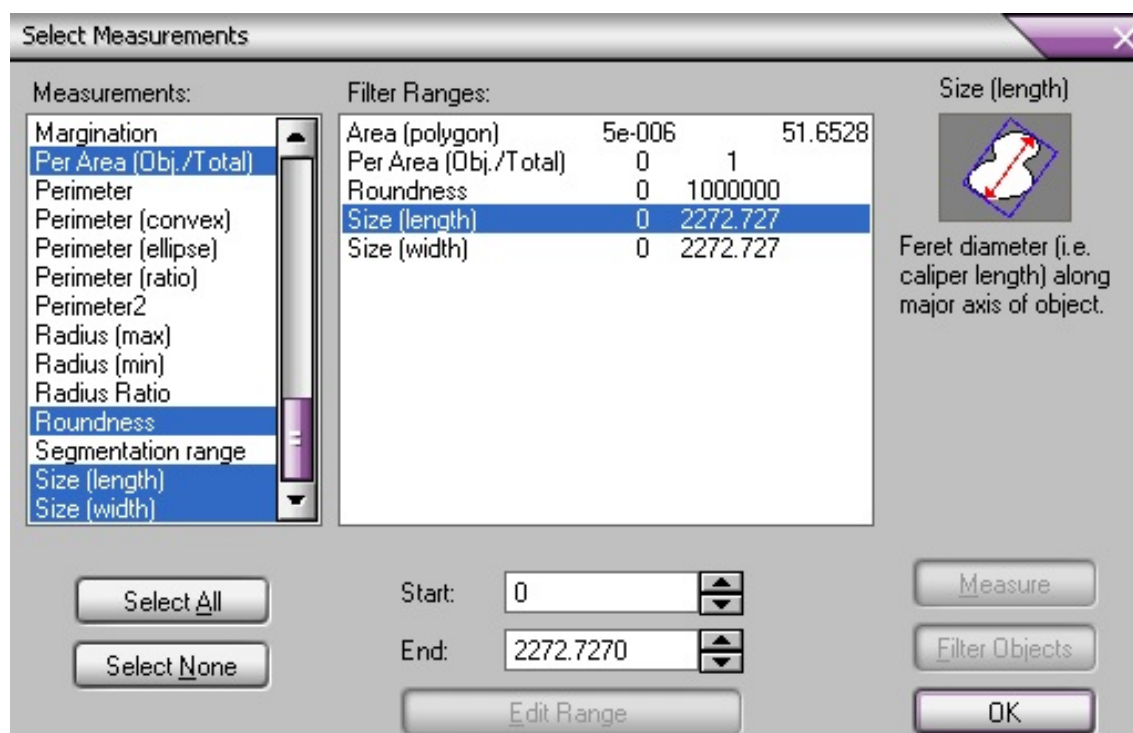
8. Click **OK**. The **Select Measurements** dialog box closes.
9. Click the **Count** button in the **Count/Size** command window.
10. The image is analyzed; grains are identified with a reference number, and counted.
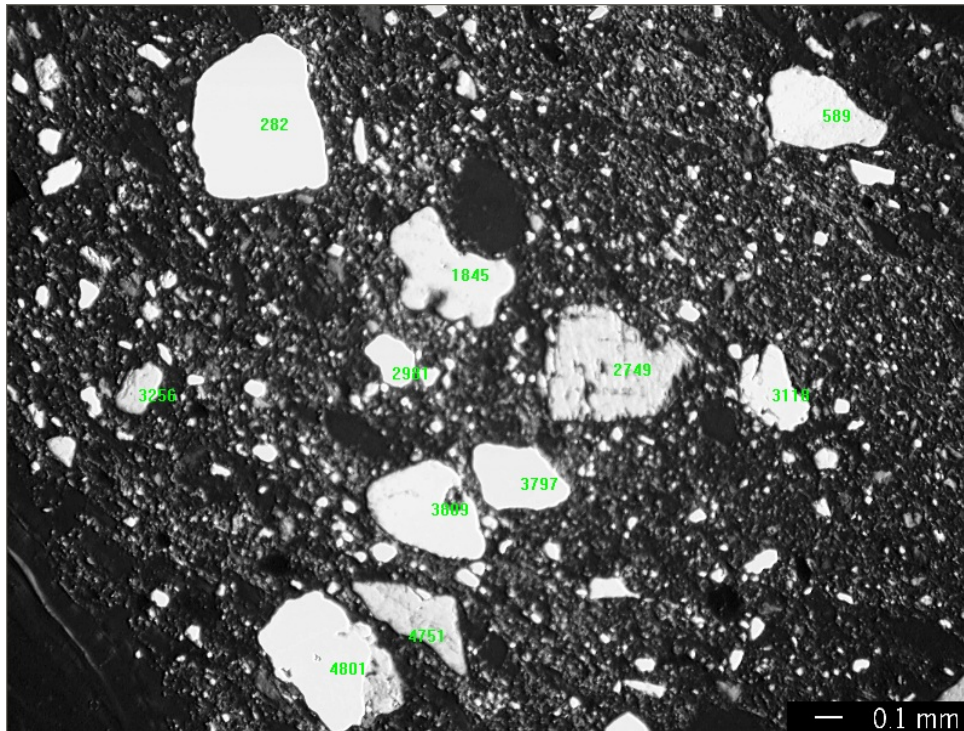
If labeling each grain with a reference number clutters the image, the numbers can be left off by selecting **Options** in the **Count/Size** dialog box, then selecting *Label Style: None*.

11. Under the **View: Measurement Data** window, the data for each counted grain is given, as shown below. Clicking on any given grain number will highlight it and show where it is within the image.

| Obj.#	Roundness	r Area (Obj./Total)	Size (length)
1797	2.4667299	.00334971	.31640625
314	2.4058840	.00224883	.28282928
1007	2.1893942	.00183881	.26902771
1521	1.6389354	.00464383	.26611328
916	6.1696760	.00271250	.25109863
911	1.8916808	.00178461	.22811890
1118	2.8158452	.00299552	.22224426
2000	3.5534171	.00195760	.22027588
822	2.0337905	.00244262	.21866226

12. Select the **Statistics** command on the **Count/Size** window's **View** menu to get the statistics of each measurement parameter selected.

13. Consult the detailed information in the Image-Pro Plus manual to determine which measurements may be useful to you and to understand how they are calculated.
14. Measurement data or computed statistics can be saved using the **File–Data to File** command in the **Measurement** window. The settings you have selected can also be saved so that they can be recalled whenever you are working with a similar image.

### 2.1.3   Compute the Are Percent of the Phases: Procedure B

1. Load Image-Pro Plus.
2. Open the image that needs to be measured. This can be done by selecting **File** on the main menu and then selecting **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image, if required, so that the objects of interest are distinctly visible (such as with the **Enhance: Contrast Enhancement** tool in the main menu).
5. Select the **Count/Size** command from the **Measure** menu. The **Count/Size** dialog box. In this case, if we try **Automatic Bright Objects**, light areas in the clay matrix background are erroneously highlighted along with the sand grains, as below.
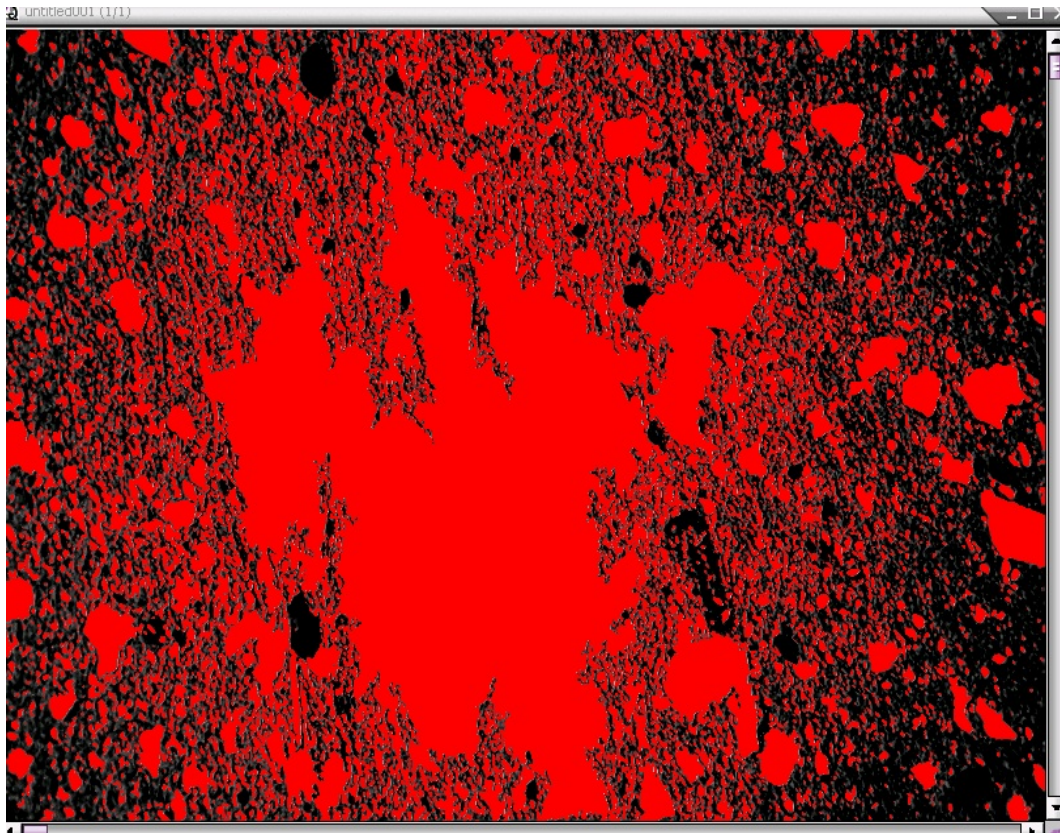
The highlighting of areas not actually part of the sand temper fraction gives statistics that are also incorrect. Here, for example, the sand temper fraction (bright object fraction) is calculated as being 47% of the total area.

6. To better mark the sand temper fraction for this type of sample, we would choose **Manual** in the **Count/Size** dialog box, as below. Make sure that the three boxes in the lower left are all checked (**Measure Objects**, **Apply Filter Ranges**, and **Accumulate Count**).
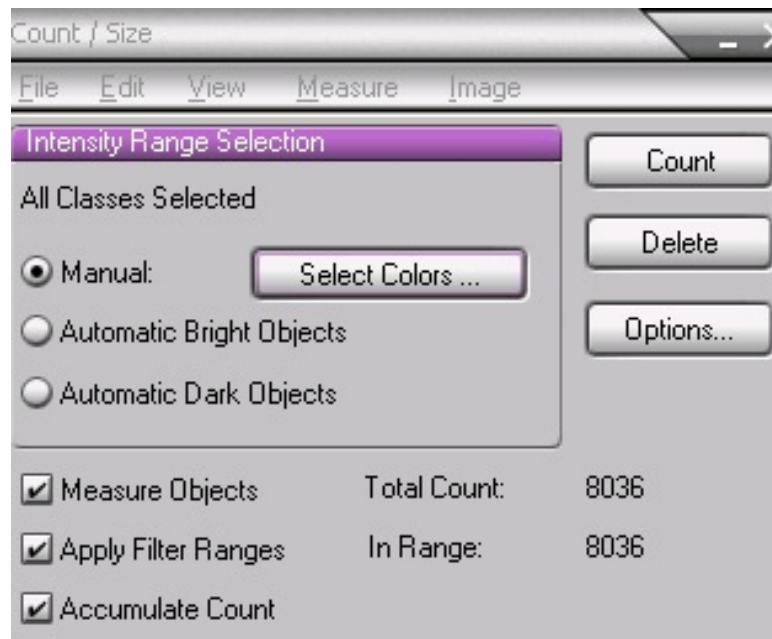
7. Next, click on **Select Colors**.
8. The **Segmentation** dialog box then appears as below. Click on **Create Preview Image**, and make sure that **All Classes** appears in the window next to that box, which will keep all of the image areas highlighted in color as you mark them so that you can decide when you have finished (when all parts of the image have been assigned to a color class). Now, click on the eyedropper symbol, then on the image, to a representative color of the first component you wish to mark (such as the sand). Everything with that color will be highlighted with the first color (in the example below, with red). Then click on **New**, to move to the next color class (such as yellow). Click on another representative color of the sand, and those portions will be highlighted with that color. Continue with clicking on **New** and moving through various color classes until you have highlighted all components of the image.

.
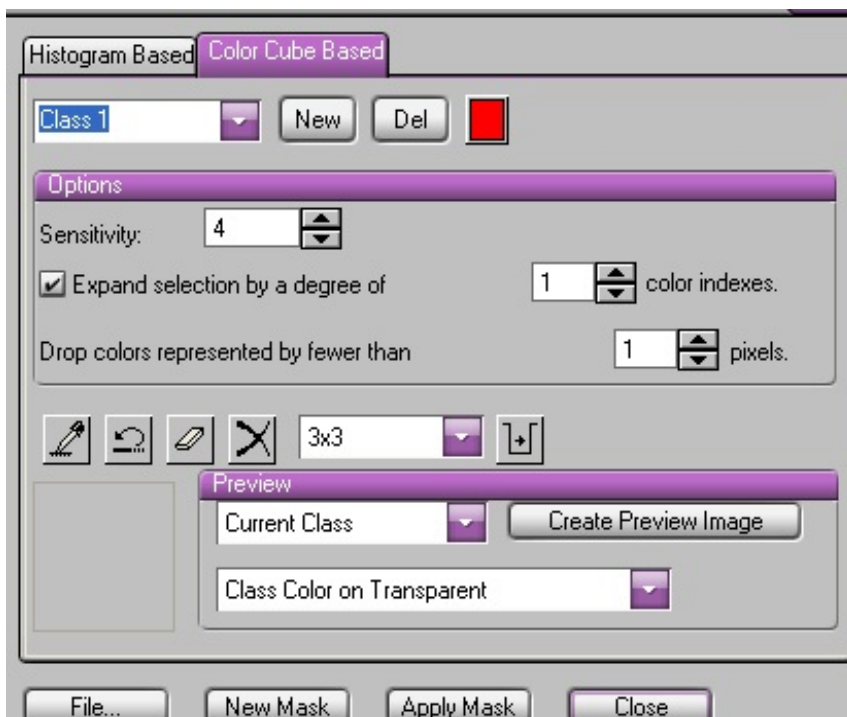
The highlighted image will then look similar to the one below:
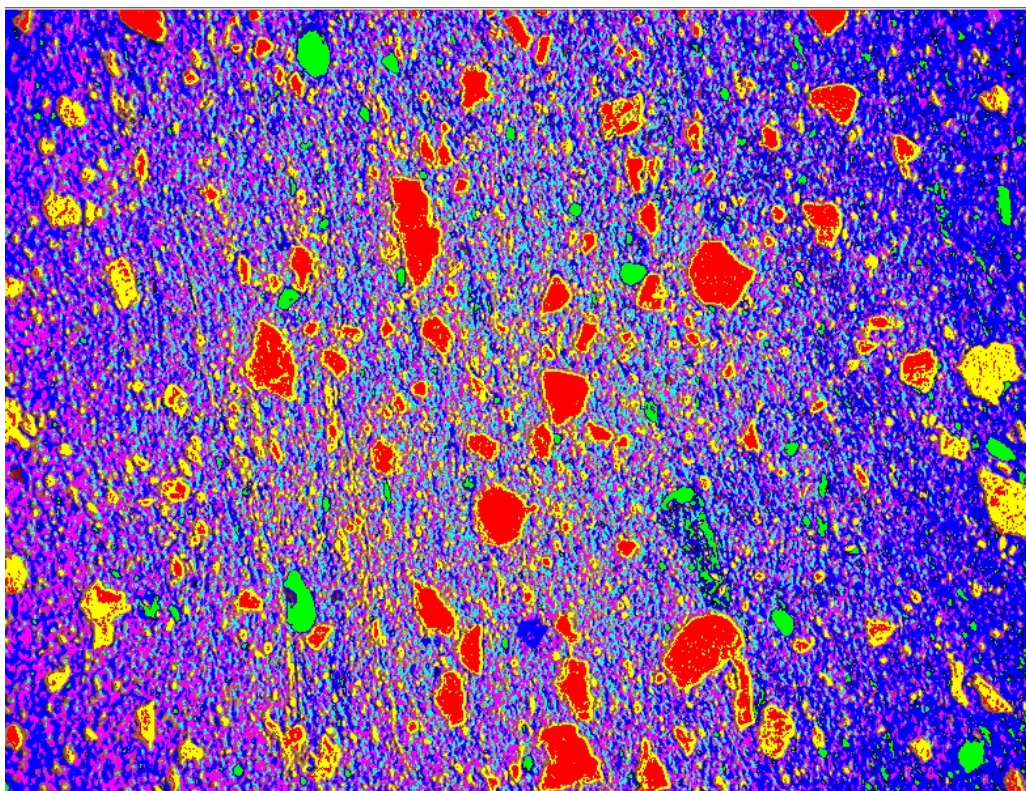
Here the red and yellow are the quartz and feldspars within the sand temper (color classes 1 and 2); the green is a dark red hematite found within the clay (color class 3); and the remaining colors (color classes 4, 5, and 6 which are light blue, dark blue, and violet) are the clay matrix. Now click on **Close**. The **Count/Size** dialog box reappears.

8. In the **Count/Size** dialog box, click on **Select Measurements**. As before, we will select **Per Area**, and click **OK**. Now, back in the **Count/Size** dialog box, click on **Count.** All of the color classes will be counted.

9. Now when we open the statistics table for all color classes by selecting **Range Statistics** from the **View** menu and find the row titled **Sum** in the table shown below, we see the number of the **Per Area** measurement for each color class in the image. We simply add the color areas that are of interest. In this case we find that the sand temper (red = 6.7% and yellow = 13.9 %) comprises 20.6%; hematite (green) is found to be 2.1%, and clay (the remaining 3 color classes) is 77.3 % (only three color classes are displayed at once in the **Range Statistics** dialog box, and you will have to scroll down to view the next three).

Range	Objects	% Objects	% er Area (Obj./Total)
1	488	1.5546353	6.6774063
2	2309	7.3558459	13.914869
3	502	1.5992354	2.1423202

10. Procedure B is actually rather fast and simple to perform, yet can be very useful whenever the automatic bright or automatic dark do not perform well with the thin section images under study. Some experimentation with the types of images under study will identify when this method works better than automatic bright or dark object counts. Measuring several thin sections and/or areas within a thin section, under both plane polarized and crossed polarized light, will help assess how well the automatic measurements are performing.

For example, there may be some pores that look like quartz or feldspar in plane polarized light, and dark quartz grains that look like the background clay matrix in crossed polarized light. Sometimes small grains and edges of large grains may appear somewhat differently in color and brightness than the centers of large grains, so might not get counted in an automatic bright count. In the manual option, such areas can be marked with a second

color class that can be included in any counts or statistics along with the other appropriate color class. However, for other specimens there will be very little if any difference in percentages using the manual versus an automatic setting. For your particular thin sections, you can choose how best to count the grains of interest.

### 2.1.4 Count the Grains in an Image and Measure Their Size and Shape: Procedure B

1. Perform steps 1-7 in section **2.1.3** above so that all components of the image have been highlighted with an appropriate color class. After clicking on **Close** in the **Segmentation** dialog box, the **Count/Size** dialog box will reappear.
2. Select **Measure** in the **Count/Size** dialog box, then choose the **Select Measurements** command. The **Select Measurements** dialog box appears. Add the **Area (Polygon)**, **Per-Area**, **Roundness**, **Size (length)**, and **Size (Width)** or any other parameters you are interested in that are listed in the **Measurements** scroll box. When a range for a measurement is selected, only objects that fall within the range in the count are recorded in the measurement data sheet.
3. Click **OK**. The **Select Measurements** dialog box closes.
4. Click the **Count** button in the **Count/Size** command window.
5. The image is analyzed; grains are identified with a reference number, and counted.
6. If labeling each grain with a reference number clutters the image, the numbers can be left off. Under the **View: Measurement Data** window, the data for each counted grain in each color class is given. Clicking on any given grain number will highlight it and show where it is within the image.
7. Select the **Statistics** command on the **Count/Size** window's **View** menu to get the statistics of each measurement parameter selected for an individual color class, or use **Range Statistics** to view all color classes.
8. Measurement data or computed statistics can be saved using the **File–Data to File** command in the **Measurement** window. The settings you have selected can also be saved so that they can be recalled whenever you are working with a similar image.

2.1.5 Using Graphics to View Measurement Results

1. A useful feature for examining ceramic thin section data in Image-Pro Plus is to view histograms to study the distribution of counts for objects measured. For example, we can focus on the non-plastic sand fraction, and look at the size distribution (for example, using length measurements).
2. Open up the image of interest, calibrate the image, preprocess to enhance the objects of interest, as in sections **2.1.1-2.1.4** above. In this case in the **Count/Size** dialog box, under **Select Measurements**, we will include **Size(length)**. In the example image shown below, the **Automatic Bright Objects** procedure works well for separating the sand fraction from the clay matrix.
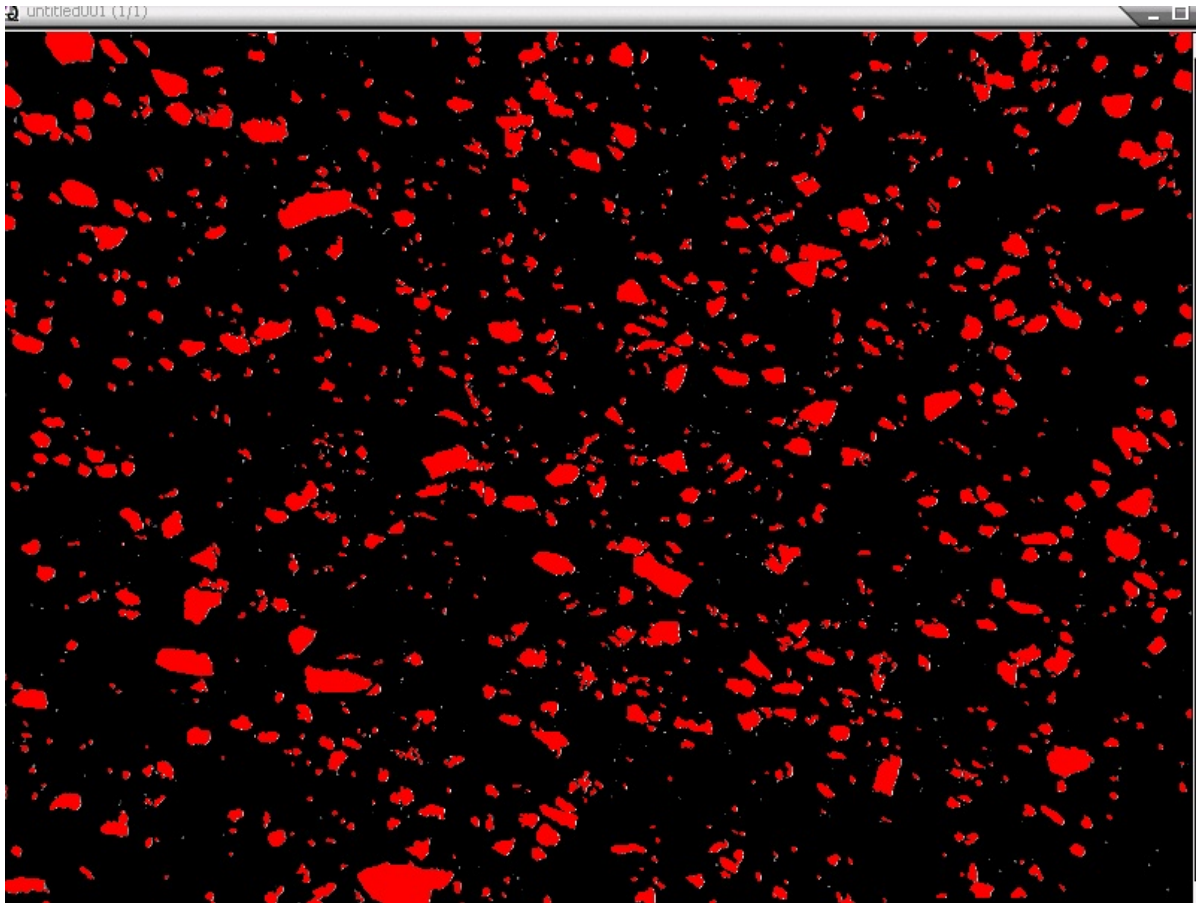
3. After clicking **Count**, we can go to the **View** menu and click on **Statistics**, as was done in earlier examples, to view the minimum, maximum, and mean length of the highlighted bright objects (the sand grains). However, from the same **View** menu we can also click on **Histograms**, to get to the view shown below. If multiple measurements were performed during the count operation, we need to select the appropriate measurement parameter, in this case **Size(length)**. Depending upon the spread of the data and the number of objects counted, we may need to adjust the number of bins for a finer view of the data. Here **Bins** was set at 25.

Histogram

File

Size (length) Bins: 25
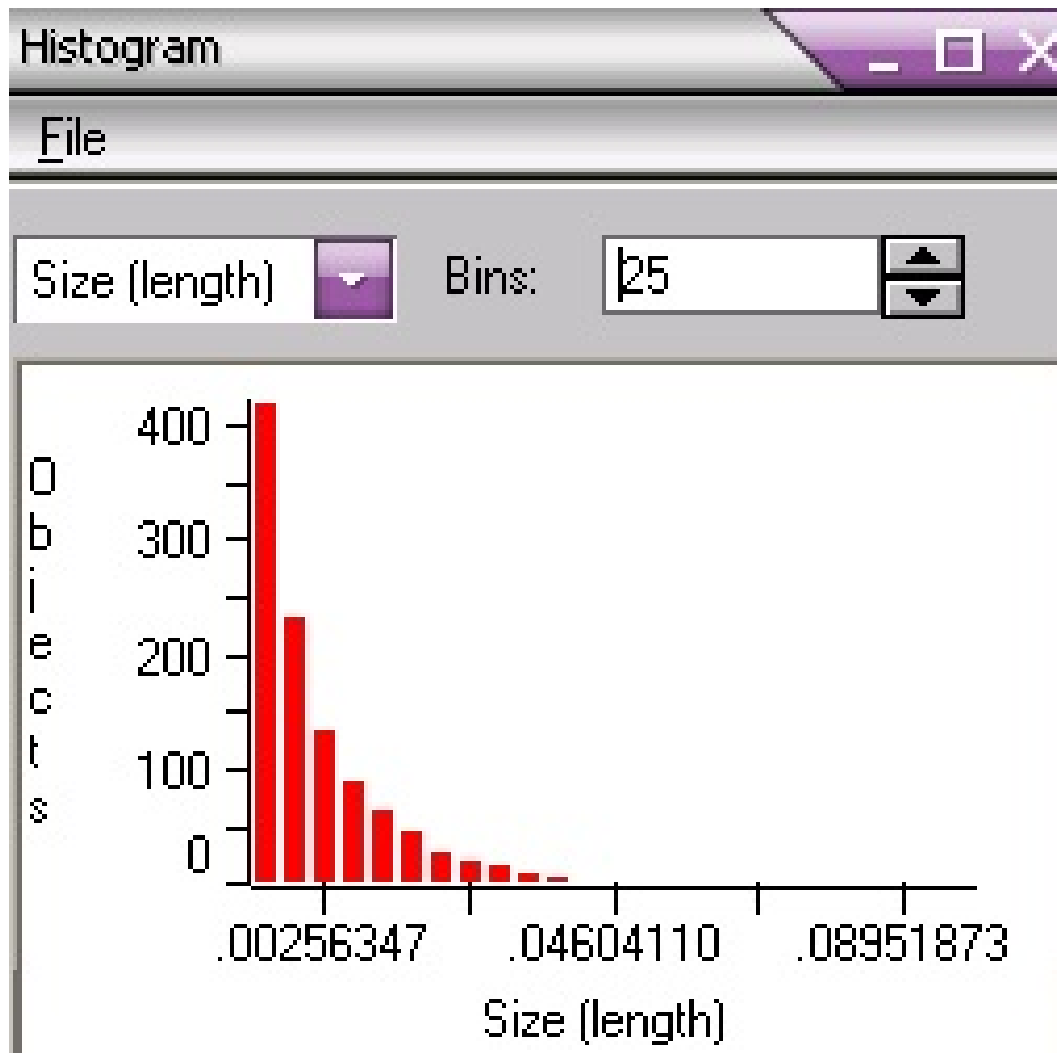
The histogram shows a continuous distribution of sand grain sizes, rather than two clear populations, which may indicate that the sand is a natural component of the clay rather than being a separately-added temper. We can compare this histogram with a different sherd specimen below:
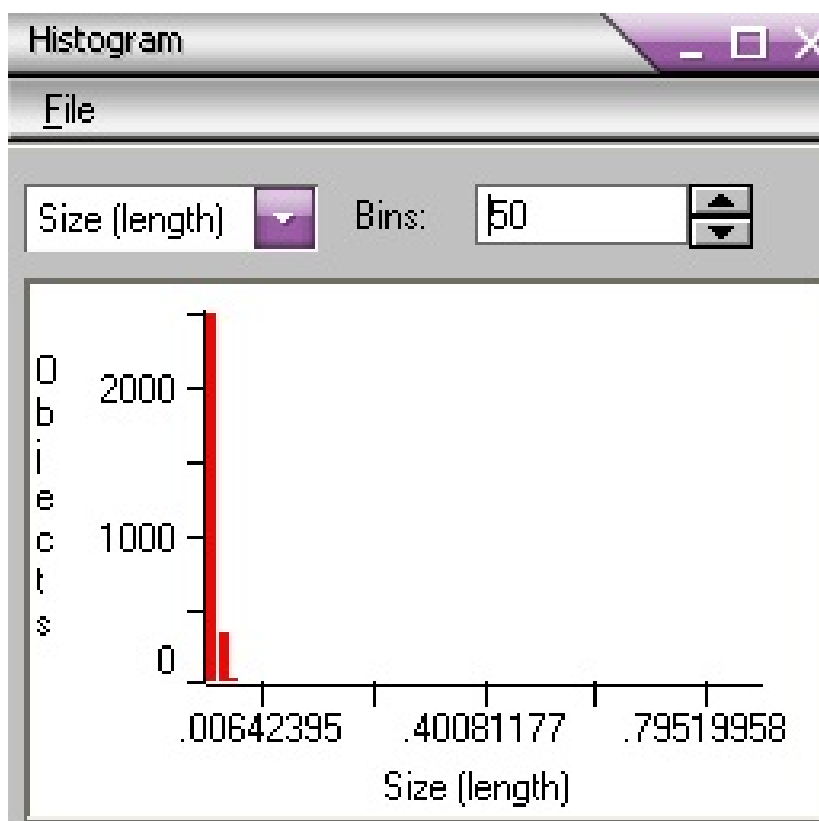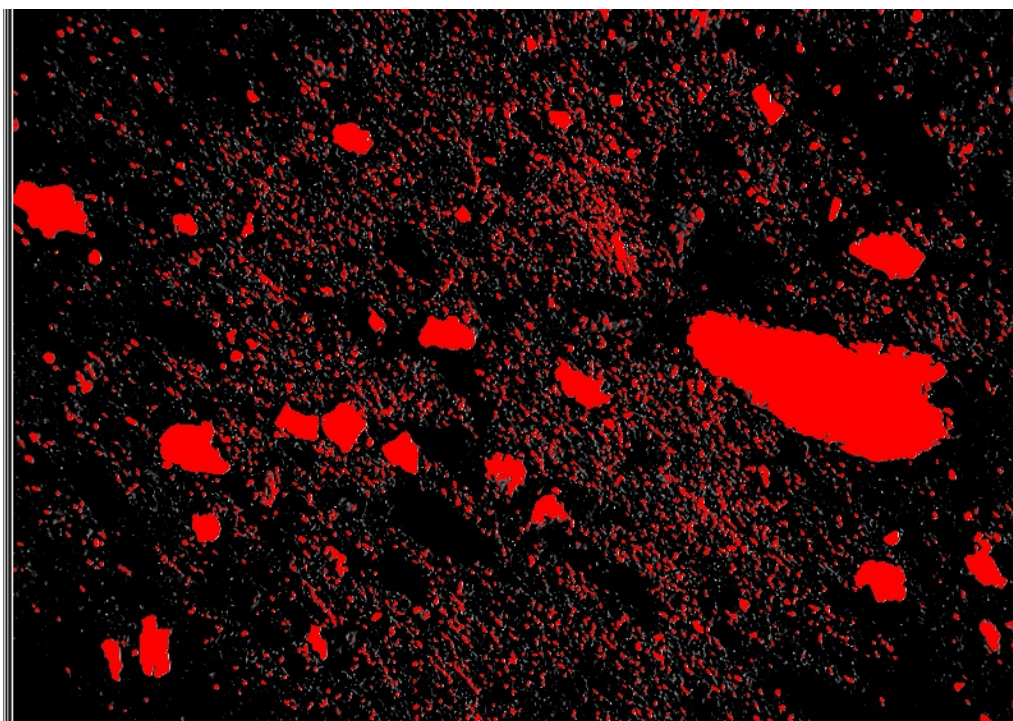
This second example shows, rather than a continuous distribution of sand sizes, two distinct populations. Most of the grains are very small, a few are larger. These results may indicate that a larger-sized sand temper was added to the clay, which itself may have contained small-sized grains of quartz and feldspar contaminants.

From viewing the statistics we can also see that the first example has 8.6 % sand component; with minimum size (length) as .0025 mm, maximum .11 mm, and mean .013 mm; and roundness (perimeter/area) as minimum 1.05, maximum 5.6, and mean 1.7.

The second example has 11 % sand, with size(length) at minimum .006 mm, maximum .99 mm, and mean .019 mm; and roundness at minimum 1.2, maximum 18.3, and mean 2.8.

All of these data can be exported to a statistical package such as SPSS. A multivariate statistical analysis using multiple thin sections, each with repeated readings, can be used to see if such variables are helping in distinguishing between different workshops, sites, or regions, taking into account internal variation.


2.2   Clemex Vision PE 3.5

Two procedures are given below, one involving grey thresholding and the other involving color thresholding. For a given type of thin section, it may be necessary to experiment to see which procedure is most satistfactory. We found that Procedure A is fast and relatively easy to perform; but this procedure is designed for specimens where the phases of interest are well separated on a grey scale (in terms of light and dark phases). We found Procedure B to be more involved and it requires a longer learning curve to master. However, it does allow for careful separation of more subtle differences that represent different phases of interest.


2.2.1   Compute the Area Percent of Phases: Procedure A

1. Load Clemex Vision PE 3.5.
2. Open the image that needs to be measured. To do this, select **File** from the main menu and then select **Open Images**. Select the image that you wish to open and then click on **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**.
4. Preprocess the image if required, in order to make the objects of interest as distinctive as possible.
5. Select the **Tool Box** from the main menu. Insert the **Delineate** function from the **Gray Operations** in the **Tool Box**.

    Use **Size 1**. This instruction sharpens object boundaries. Usually **Size 1** delineation is sufficient, but more might be required for very large grains, to compensate for the shadowed effect around the edges of grains.
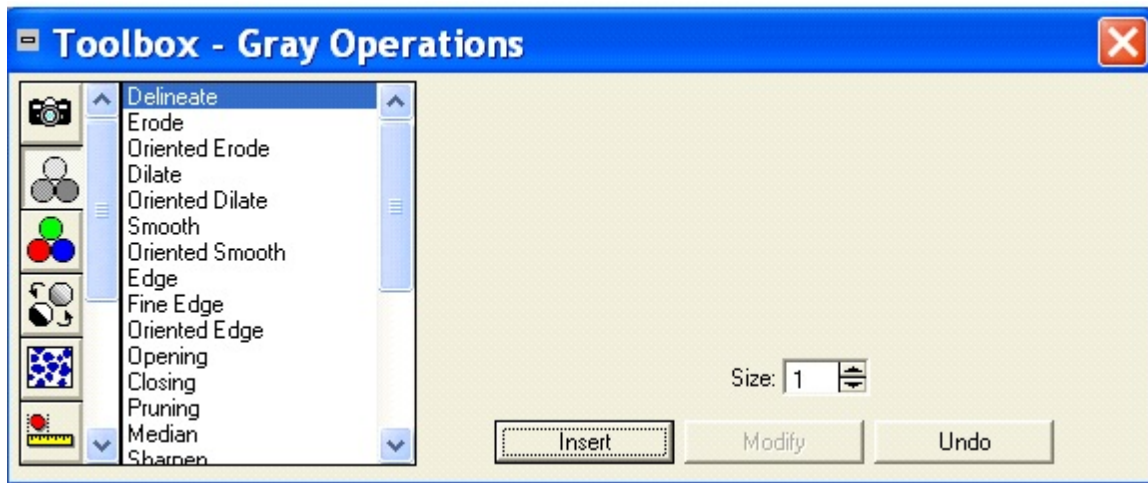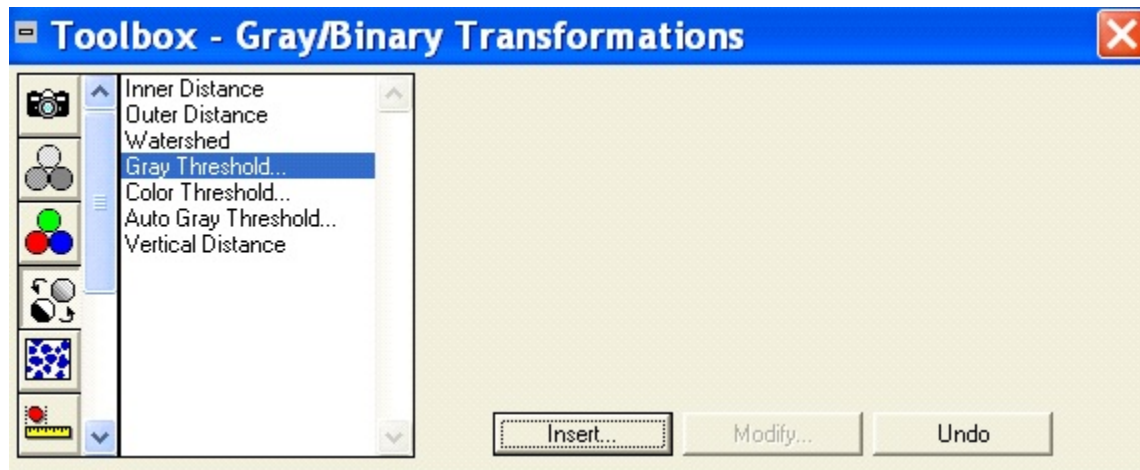
6. Choose **Insert** to execute the operation. A **Routine** window pops up which gives the instruction added to be executed.
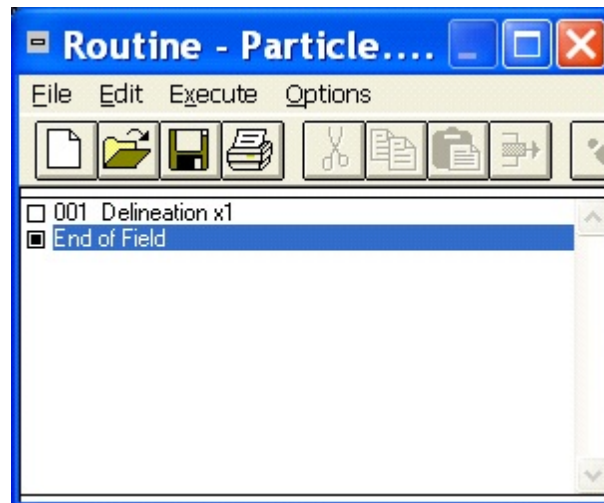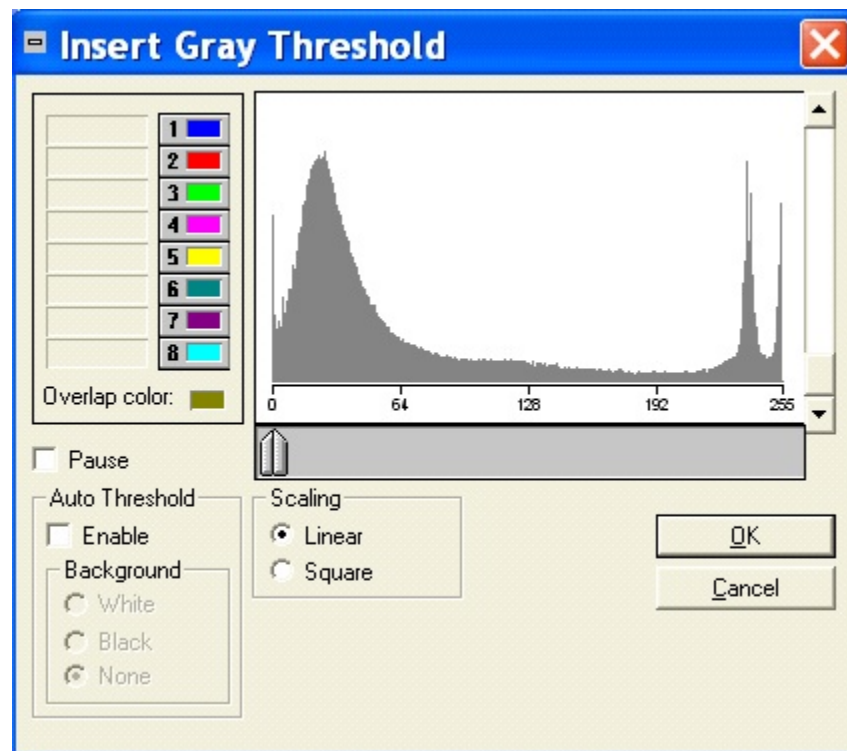

7. Select the **Gray/Binary Transformation** icon in the **Tool Box:**

    Then select **Gray Threshold** in the list box, and **Insert**.

8. The gray level histogram of the **Gray Threshold** pops up, which shows clearly defined peaks indicating distinct phases on gray levels. The histogram indicates the distribution of pixels within each gray level.

9. Each phase will be assigned to a bitplane and represented by a different color. The color selection for the bitplanes is arbitrary and any color can be selected to represent the phases. It is helpful to rename the bitplanes according to the phases in your thin section. This can be done by clicking on **Options** in the main window menu, then clicking on **Bitplane Names**. New names can be assigned to each bitplane using the popup window, as below. Here, for example, we will rename **Bitplane 1** (blue) to **Clay Matrix**, and **Bitplane 2** (red), to **Sand**.

A. Choose the blue square button corresponding to **Bitplane 1** by clicking on it, and place the cursor over the right threshold slider that is below the gray level histogram.
B. The cursor will change to a double-ended arrow. Make sure that the cursor is placed on the right slider rather than on both sliders simultaneously.
C. Drag the mouse (keep the left mouse button pressed) along the gray level axis until the background phase appears blue in the image window. Use the gray threshold histogram peaks to help guide you in best highlighting the phase represented by **Bitplane 1**. Release the mouse button to set the threshold.
D. Click the red button corresponding to **Bitplane 2** representing the bright phase.
E. Place the cursor on both threshold sliders and drag the mouse until the lower limit is set. Release the mouse button to set the threshold limit.
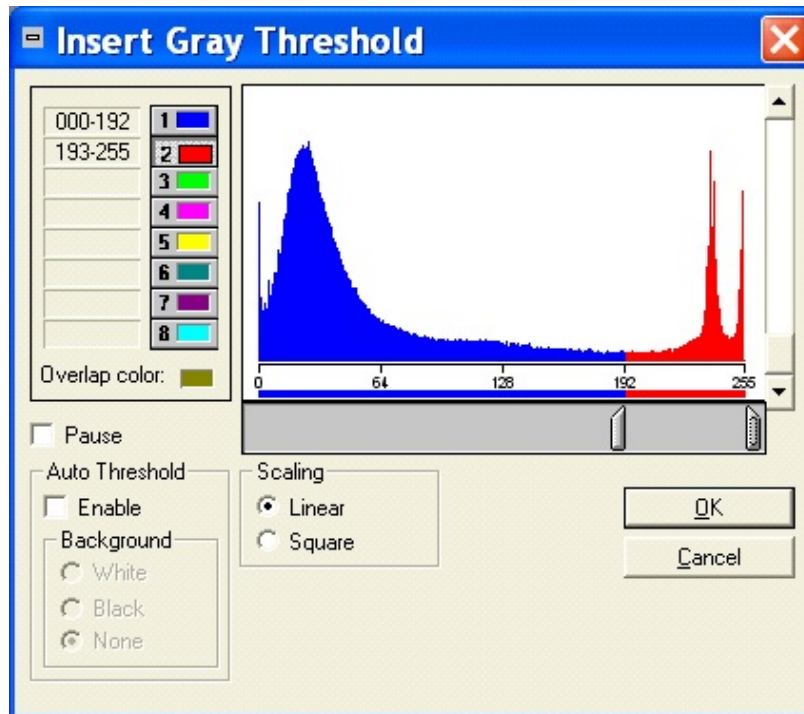
10. Press **OK** to close the grey threshold histogram and the instruction is added to the **Routine**. The image now consists of a blue (clay) background and red sand grains.
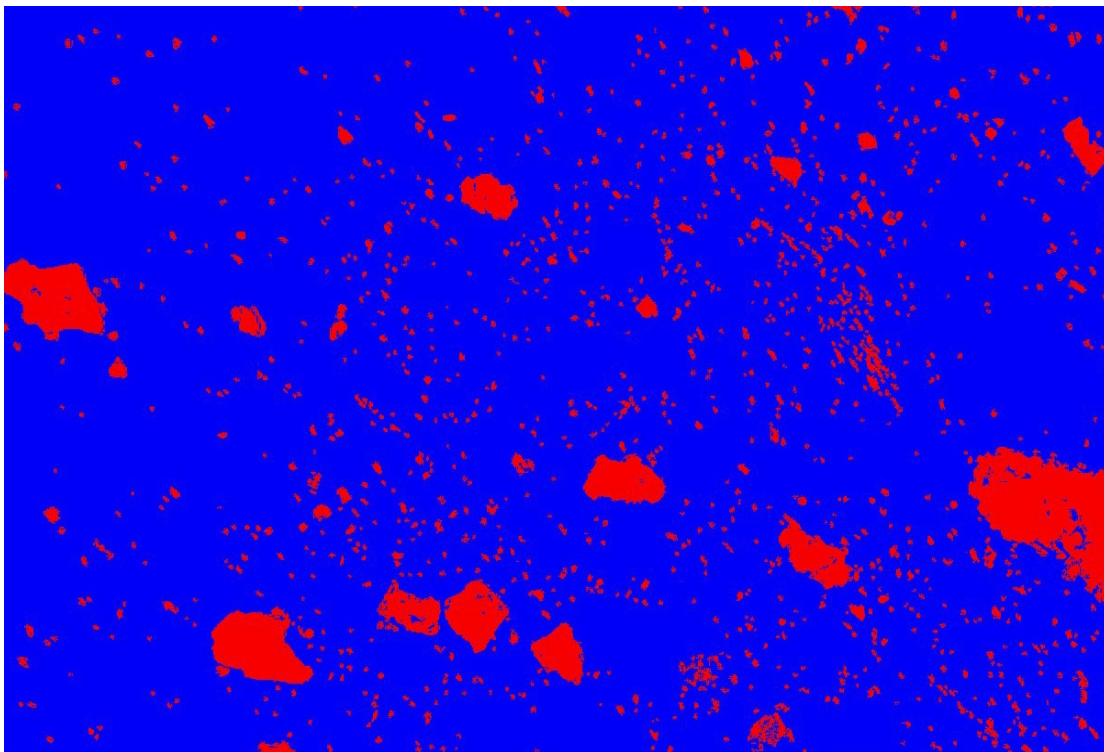
11. Now select the **Relative Measure** icon from the **Tool Box**:
    Using the **Area Percent** measure in the **Relative Measure** group, we can obtain the area of sand grains to the total field area. Select **Area Percent, with** the **Source** as **Bitplane 1** (blue square) and **Bitplane 2** (red square) and click on **Field** under **Compare Plane**.
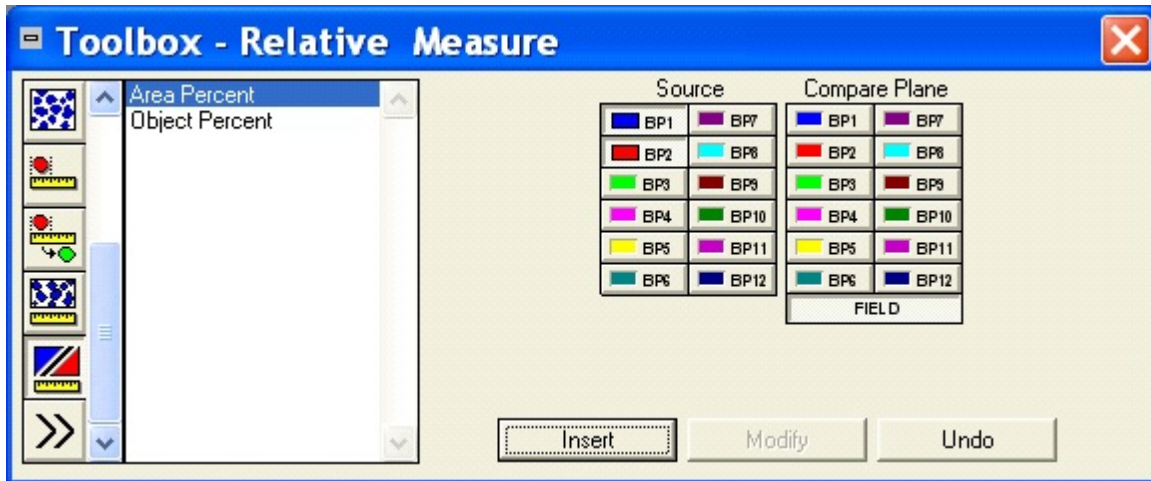
12. Press **Insert** to add the instructions to the **Routine** box.
13. Select the **Results** window from the main menu to obtain the statistical chart. We find that sand comprises about 9% of the area, with the clay matrix at about 91%.
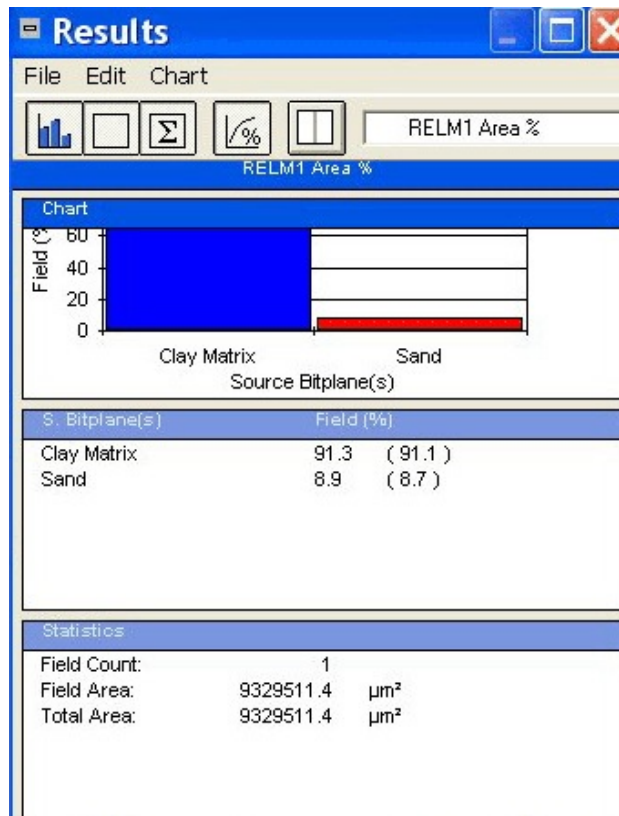
2.2.2 Count the Grains in an Image and Measure Their Size and Shape: Procedure A

Begin by completing Steps 1-10 in **2.2.1** above.

11. Now select the **Object Measure** icon in the **Tool Box**:
The **Tool Box** changes to the **Object Measure** mode.
12. Select the measurements of interest. Consult the Clemex Vision PE manual for a full description of how these measures are calculated. The software provides the option to define additional custom measurements if the ones of interest are not found on the list. Here we have selected **Length**, **Width**, and **Roundness**.
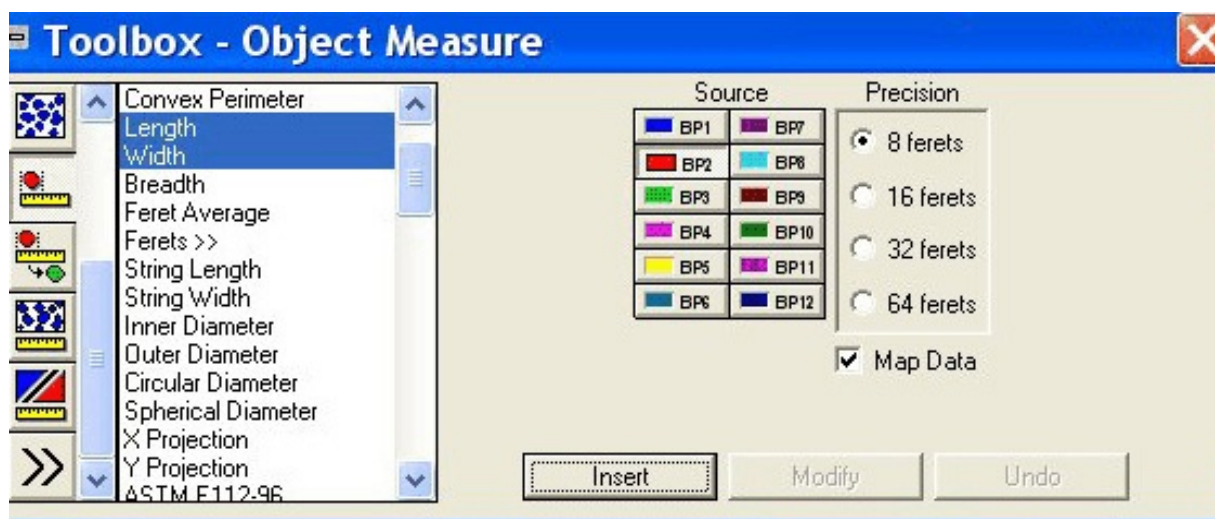
13. Once all of the measurements of interest have been highlighted, select the **Source** as **Bitplane 2** (red, the sand component here), and a **Precision** of **8 ferets**. Press **Insert** and this instruction is added in the **Routine** box.
14. Activate the **Results** window located in the main window by clicking on it. The **Results** window contains data for the minimum, maximum, and standard deviation, as well as a histogram display, for each measurement selected. Use the box in the upper right corner of the **Results** window to move from one measurement parameter to another. Data for the **Roundness parameter** are shown below:
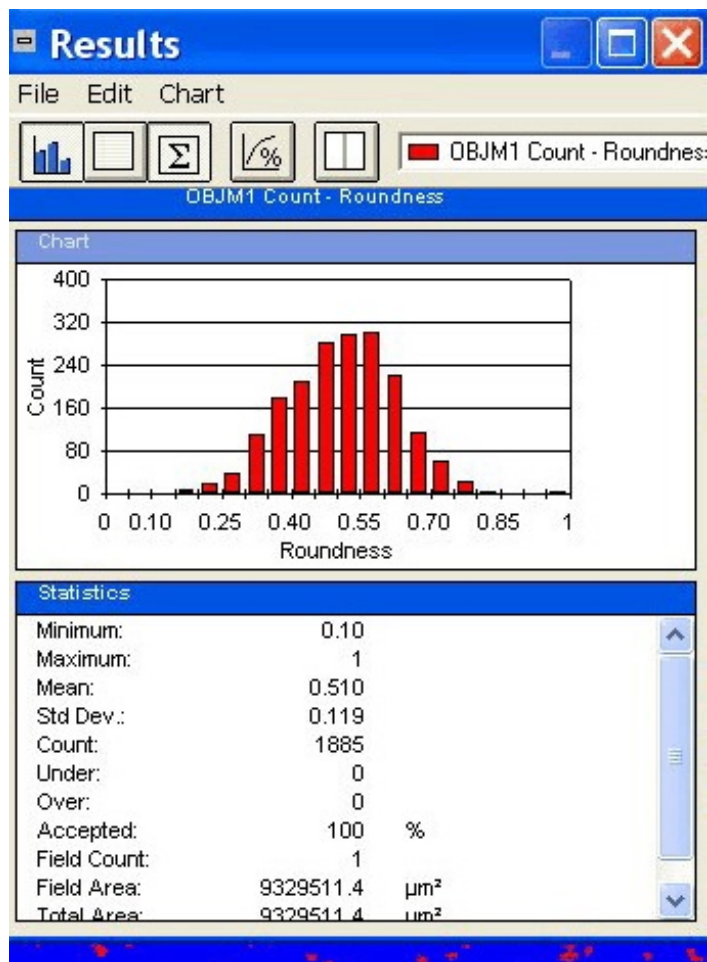
15. There are options available for excluding grains of certain size (for example, we may wish to focus on the larger sand grains that may have been added as temper, rather than on the smaller ones that may be natural constituents of the clay). Mechanisms for excluding objects of certain size include the **Trap** feature and the **Chord Size** tool in the **Binary Operations** mode of the **Tool Box**:

    The data can also be saved to a spreadsheet or ascii text file by clicking on **Data** in the main menu, then selecting **File** and **Export Data**. You will be prompted for a location and format for saving the data file.

2.2.3 Computing the Area Percent of Phases, Counting Grains, and Measuring Size and Shape: Procedure B

In some cases the gray thresholding procedures described in sections **2.2.1** and **2.2.2** may not work satisfactorily due to too many subtle gradations between phases of interest. Sometimes the problem can be solved through improving the quality of the image to enhance the phases of interest, preprocessing measures, or attention to the **Shading Calibration** option in the Clemex software. At times use of color thresholding rather than gray thresholding. To choose this feature, follow steps 1-6 in **2.2.1**. Then proceed as follows:

7. Select the **Gray/Binary Transformations** icon in the **Tool Box** and select **Color Threshold** in the list.
8. A **Color Threshold** window pops up, with a histogram. Starting with **Bitplane 1**, adjust the area of the histogram as needed, using the sliders; the hue and saturation selections can also be adjusted by using the sliders on the color wheel.
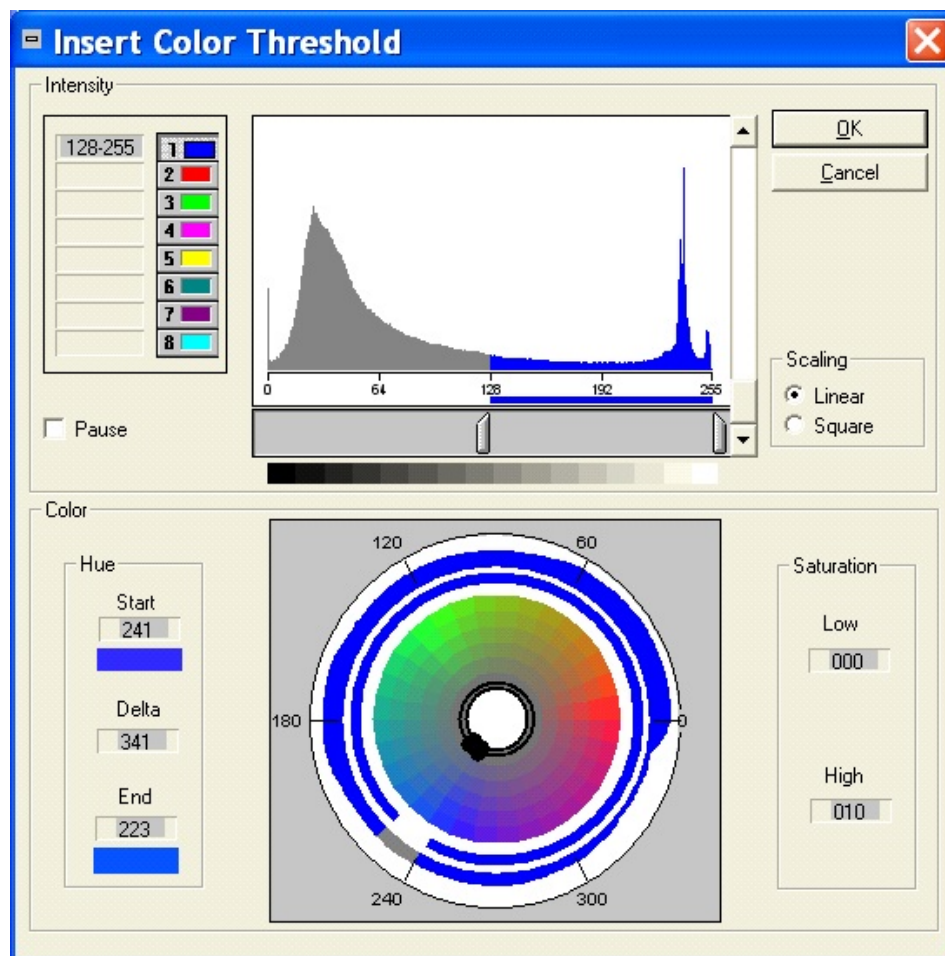
9. However, the easiest way to highlight the pixels of interest for a phase is to move the mouse over to the image window; the pointer changes to a cross. You can now click on the phase of interest, clicking on multiple regions to highlight all of the pixels to be included. The histogram and color wheel will adjust automatically. This procedure is similar to the eyedropper tool in Image-Pro Plus. We found that the **Color Threshold** window disappeared as soon as we clicked on the image with the cross tool. If that happens, click **Window** in the main menu, and a list will drop down of various windows available. Use this tool to go back and forth between the image and the color threshold histogram and color wheel as needed. Click **OK** when the phase(s) of interest are appropriately highlighted. In the image below, we highlighted sand grains in a ceramic sherd viewed in plane polarized light.
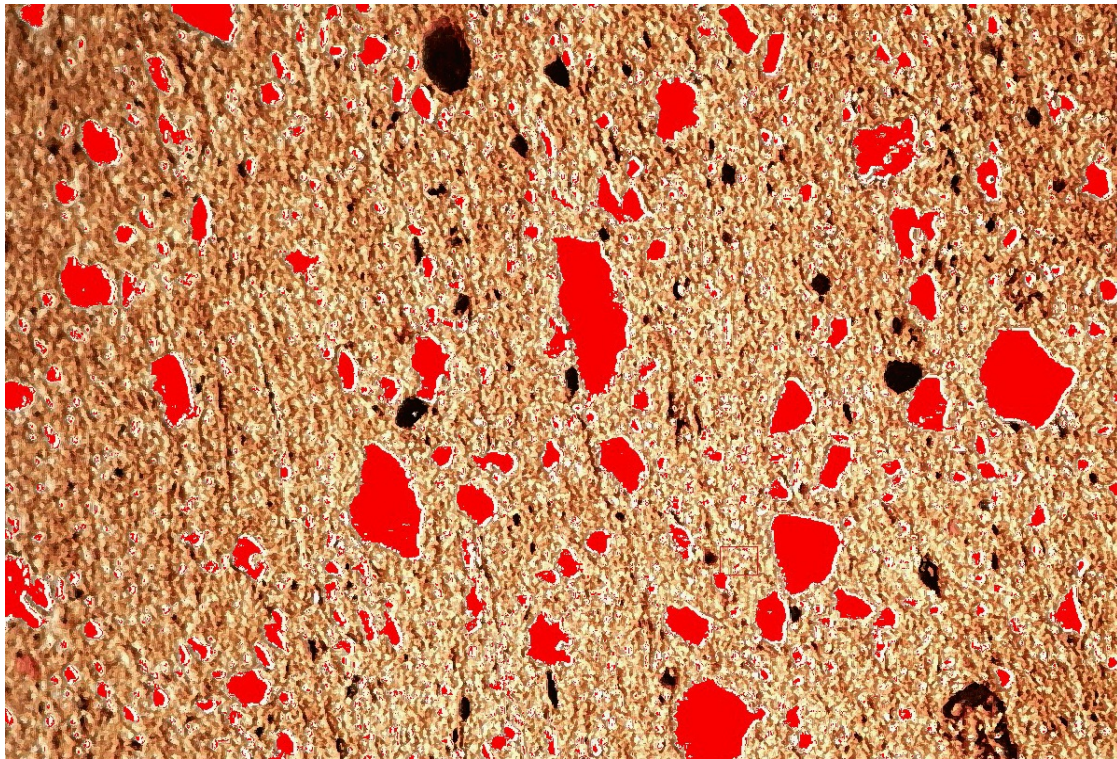

10. To obtain area percent data for the phases of interest, refer to section **2.2.1** and follow steps 11-13.
11. To obtain data on measures such as length, width, and roundness, refer to section **2.2.2** and follow steps 11-15.

2.3   ImageJ (Version 1.33)

2.3.1   Compute the Percentage of the Grains of Interest

1. Load ImageJ.
2. Open the image that needs to be measured. To do this, select **File** on the main menu and then select **Open**. Select the image that you wish to open and then click on **Open**.
3. Calibrate the image as described in the protocol instructions on **Calibration of Images**. Make sure that the **Global** box is checked in the **Set Scale** window.
4. Preprocess the image if required in order to have the objects of interest distinctly visible. For example, from the **Image** function on the main window, select **Adjust**, and then **Brightness/Contrast**.
5. Convert the color image into grey scale by select **Image** from the main menu and then selecting **Type** as **8-bit grey**.
6. Select **Process** from the main menu and select **Binary** function from the **Process** menu. Using the **Threshold** function, the image becomes a binary threshold image. The upper image below is the sand-tempered ceramic thin section chosen for analysis, and the lower image is the binary threshold image obtained using ImageJ.
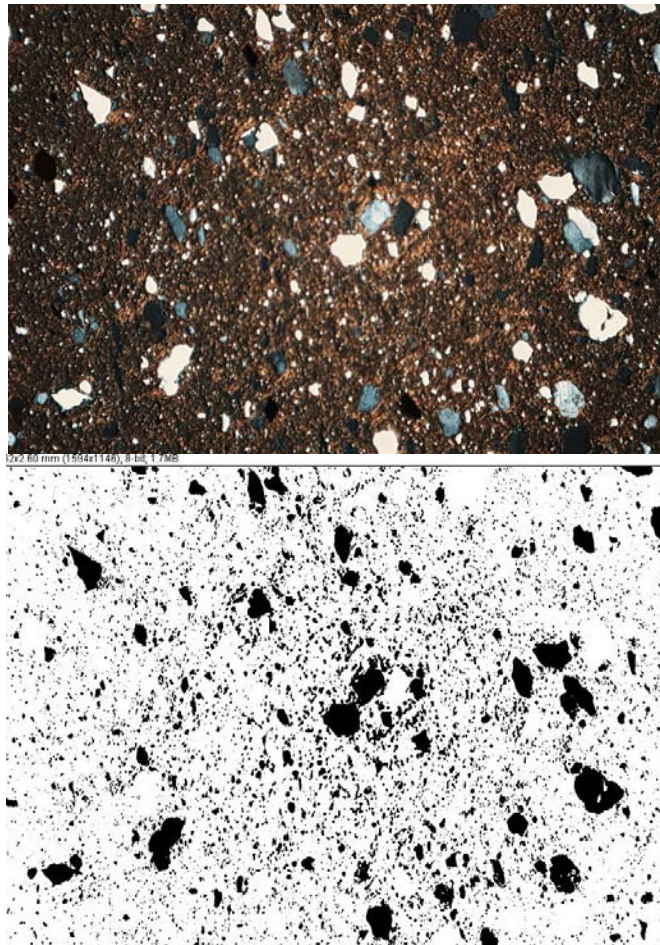
7. Adjustments can be made if necessary using various tools such as **Image** - **Adjust** - **Threshold**.

8. Select **Analyze** from the main menu and click on **Analyze Particles**. The **Analyze Particles** window appears as follows:
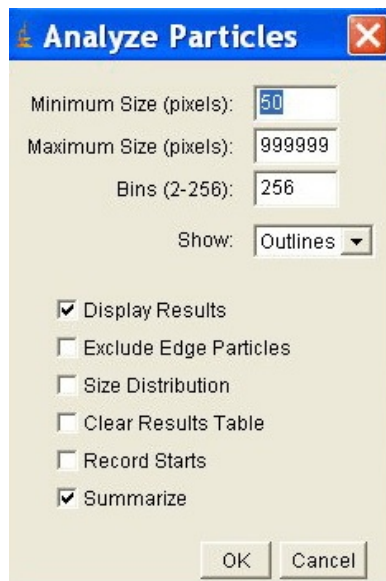
9. In the **Analyze Particles** window, enter a minimum and maximum size of the object for measurement. Here we have selected to have the objects of interest outlined, and have checked the boxes for **Display Results** and **Summarize**.

10. Click **OK**. The objects included in the size parameters have been outlined and assigned a particle number.
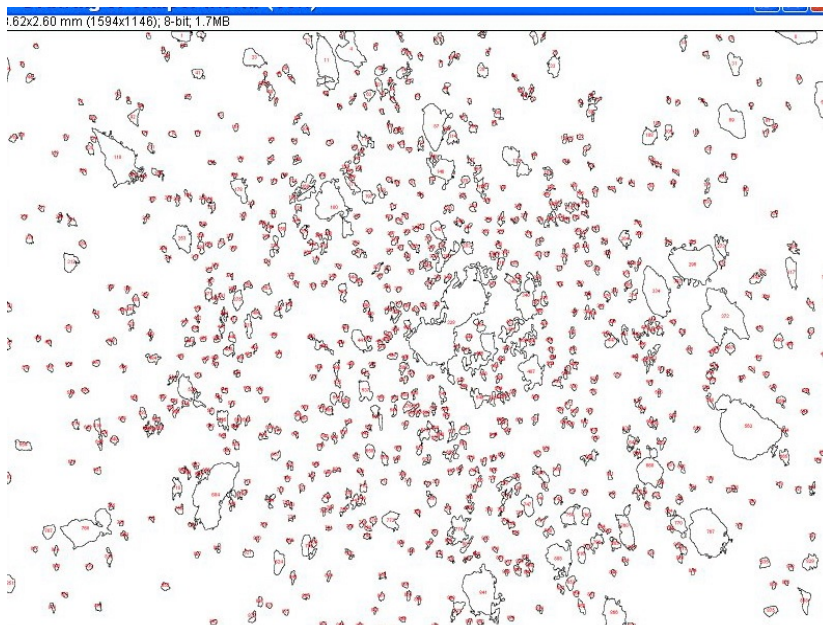
11. A **Summary** window will then pop up, as below. The area percent for the sand grains is shown to be 13.9%.
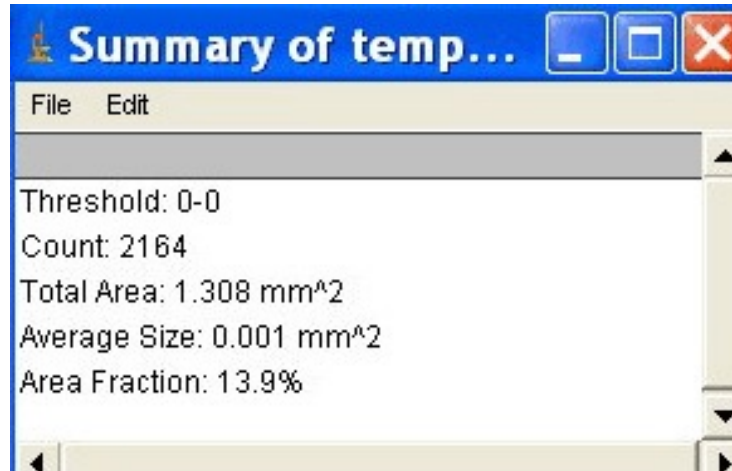
2.3.2 Count the Grains in an Image and Measure Their Size and Shape

Perform steps 1-9 as in section **2.3.1** above.

10. To perform a size measurement, check the box for **Size Distribution.** Then check **OK**.
11. The objects are counted, numbered, and outlined as before.
12. An analyses of particle size distribution is shown in a new window, as shown below.
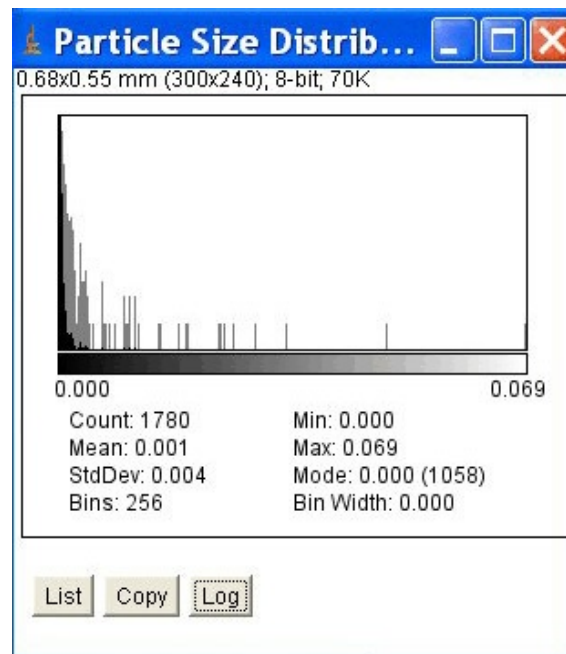
13. In the **Particle Size Distribution** window, clicking on **List** will show size data for each individual particle. Clicking on **Log** will transform the histogram into log distribution. The minimum, maximum, mean, and standard deviation of particle sizes are shown in the window itself; units are those that were set in the calibration procedure (in this case, millimeters).
14. The data can be transferred to a spreadsheet or statistical package by copying and pasting.
15. We did not find measurement parameters at the ImageJ web site for automatic width or roundness of all particles highlighted, although a plugin is available for measuring circularity of particles. Another plugin (at http://home.online.no/~gary.c/IJ/Shape.htm) developed by Gary Chinga provides parameters for shape descriptions, including roundness. And, with ImageJ the user does have the option of programming and adding their own customized plugins using ImageJ's built-in command recorder, editor, and Java compiler.

3.   Analysis

Now we will compare the three image analysis tools and give an assessment regarding their performance in the measurement tasks of greatest interest for the study of ceramic thin sections. We will evaluate each approach using the following criteria:

1. Ability to identify or select the objects of interest (such as temper grains, pores, or non-plastics).
2. Ability to calculate the relative percentage of area of the thin section that the objects of interest comprise.
3. Ability to count grains, and to measure parameters of interest such as size and degree of roundness.
4. Ease of use and simplicity of the measurement process.
5. Consistency of the measurement results.
6. Format of the resultant output.

3.1   Ability to Identify or Select the Objects of Interest

All three image analysis packages have the ability to mark, or highlight, grains of interest for measurement. All three use a somewhat different approach, and multiple approaches within an image analysis tool are also available. Thus one can choose an approach that seems to work best for the type of material under study.

3.2   Ability to Calculate the Relative Area Percentage of Objects of Interest

All three image analysis tools can provide information on relative area percentage of highlighted grains or features of interest within the thin section.

3.3   Ability to Measure Parameters of Interest

Both Image-Pro Plus and Clemex Vision PE provide a full range of parameters of interest to those studying thin sections of ceramic materials. Clemex Vision PE also provides for a means of adding customized parameters. ImageJ and its currently-available plugins do not easily provide all parameters that will be of interest to ceramic petrographers. However, users can extend the parameters available by developing new plugins using ImageJ's built-in command recorder, editor, and Java compiler.

3.4   Ease of Use and Simplicity of the Measurement Process

We found that all three image analysis tools were relatively easy and intuitive to learn and use for area percent measurements, as well as for calculations of length, roundness, and other parameters, with some procedural exceptions. In ImagePro Plus, when the automatic bright objects did not work well for calculating area percentage of a component, the manual procedure was easy to use and worked quite well. However, in marking phases with colors, if one component ended up with more than one color class, the measurements for length, roundness, etc. were difficult to interpret. For Clemex Vision PE, the gray threshold tool was fast and easy to use and worked quite well for most thin sections we examined. However, we found the color threshold tool difficult and non-intuitive, and the instructions in the manual did not match what we saw on the screen. However, the ability to easily record and save a routine in Clemx Vision PE makes it easy to consistently apply the same routine from one specimen to another. In ImageJ the procedure for converting an image to 8-bit grey and doing a binary threshold transformation worked quite well for highlighting the grains of interest, as long as they were bright particles against a dark background. However, to analyze the particles one then had to select an appropriate minimum pixel size. Obtaining area percent for the phase of interest was simple and easy to do. Obtaining additional measurement parameters automatically with mininum, maximum, and mean for the field of view was difficult or not possible for all parameters of interest. Although customized plugins can be added, that requires someone with the appropriate level of expertise.

3.5   Consistency of the Measurement Results

Consistency in measurement of area percent and for the mean of various measurement parameters such as length and roundness depends primarily upon the quality of the image being analyzed. All of the particles of interest need to be clearly visible and distinct from the background matrix, with no extraneous areas in the background appearing too similar to the particles of interest. Keeping the measurement needs in mind when acquiring the image at the microscope, and carefully preprocessing the image, are extremely important, and can affect the results. We found that with good quality images and a standard and careful preprocessing procedure, variation between operators and/or between different sessions with the same operator, were slight. However, variation did exist, thus final results should not depend upon a single analysis.

When we used the same image for all three image analysis packages, some variation was found, but no more than was found within an individual analysis package when the operations were repeated. For example, one thin section analyzed for area percent of a sand temper gave the following results: for ImagePro Plus, 7%; for Clemex Vision PE, 6.7%; and for ImageJ, 7.9%.

If trying to compare some of the other measurement parameters from one image analysis package to another, it is important to check the definitions for how each parameter is calculated or defined in a package. Some parameters with the same or similar names are computed slightly differently from package to package and may not be directly comparable, although all appear to be internally consistent and accurate when checked against known standards (for example, checking length measurements against objects of known length).

3.6   Format of the Resultant Output

In this criterion we consider whether the software offers alternative forms of output representation such as graphs and tables. We found that all three packages provide a listing of measurement data for each particle, which can be exported or cut and paste to bring into another application such as a spreadsheet or statistical package. All three also provide numerical data on area percent, with Image-Pro Plus and Clemex Vision PE also giving histograms to illustrate the relative percentage of each phase of interest. Image-Pro Plus and Clemex Vision PE also give the individual data, statistics, and histograms for all other measurement parameters selected. The ability to change bin sizes for parameters such as particle size in Image-Pro Plus make it easy to identify the presence of two population sizes, as we often see with temper additives mixed in with impure clay. ImageJ provides the data, statistics, and a histogram for size distribution, with the nice feature of being able to switch to a log scale for the histogram view. We were not able to locate statistics and histograms for other parameters such as circularity, although data for each particle was given.

3.7   Evaluation Conclusions

For measuring area percent of a given phase, all three image analysis tools work well. If additional measurement parameters are needed, ImageJ is limited in usefulness for ceramic petrographers. It was originally designed for, and is primarily used by, researchers in the life sciences. Thus the existing plugins are designed to meet their needs. The approach to analysis in Image-Pro Plus and Clemex Vision PE are somewhat different, and different operators may have different preferences. Trying out both packages with the typical thin sections that one will be analyzing, and deciding what one's individual preference is, would be the best way to select a package. Given the nuances of improving preprocessing and analysis, a laboratory will probably want to use a single package and gain a high level of expertise, as well as have a consistent approach for all specimens analyzed within a single research project.

Product Sources

Image-Pro Plus: MediaCybernetics, www.mediacy.com

Clemex Vision PE: www.clemex.com

ImageJ: author Wayne Rasband, National Institutes of Health, http://rsb.info.nih.gov/ij/

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