Drexel-SDP GK-12 ACTIVITY

Subject Area(s): Chemistry, Physical Science, Science & Technology

Associated Unit: Nanotechnology

Associated Lesson: "A DNA biosensor"

Activity Title: "Nanocounting with ImageJ"

Grade Level: 11th-12th

Time Required: 2 hours

Group Size: 3 students per group

Summary
In this activity students learn how to use ImageJ to count the number of nanoparticles in a sample and measure their size. Students then determine the mean size and standard deviation of their particles and explore ways to decrease the accuracy of their measurements. This activity works well following the associated modules "A DNA Biosensor" or "PDLC Fabrication." It can also be used to in activities involving the analysis of images from cell cultures.

Engineering Connection
Engineers and scientists commonly encounter situations where they must use image analysis software to interpret the results of an experiment. Biomedical engineers who work with light microscopy must regularly analyze images to track responses to their cells. Materials engineers and those who work in nanotechnology must use tools like ImageJ to determine the size and shape of their nanomaterials.

Keywords
Image analysis, statistics, characterization of nanoparticles, nanotechnology

Educational Standards
- Math: 2.3 Measurement and Estimation
- Math: 2.6 Statistics and Data Analysis
- Science: 3.7.12.D Evaluate the effectiveness of computer software to solve specific problems

Pre-Requisite Knowledge
Knowledge of working with basic formulas in excel

Learning Objectives
After this lesson, students will be able to:
- Use ImageJ software to analyze images
- Use a particle counting technique to estimate the total number of nanoparticles in a sample
- Calculate the average and standard deviation of a the size of particles

Materials List
Each group needs:
- Computer
- ImageJ software
- Image of nanoparticles
- Excel worksheet or a calculator

Introduction / Motivation
Nanoparticles have special optical properties depending on their size, even though we cannot seem them with our naked eye, because they are so small they can change the color of a solution depending on their size. This can be important for biosensors which are designed to analyze biological fluids such as urine or blood by changing the color of the solution depending on the way the molecules interact with the nanoparticles. Their minute size also gives them a huge surface area to volume ratio where many reactions can occur.

To take advantage of their special characteristics it is useful to have a method to check their size and quantity after we make them. Since we cannot seem them with a regular microscope engineers and scientists use a scanning electron microscope which is a type of microscope using electrons instead of light to form a picture of the sample. Using our computer we can take these images and analyze them to determine the characteristics of our nanoparticles.

Vocabulary / Definitions

Word	Definition
Cell viability	Determination of living or dead cells
Standard deviation	The square root of the variance.

Nanoparticles	Usually very small metal spheres with a diameter between 1nm and 100nm.
ROI	Region of interest
Scanning electron microscope	Type of microscope that bombards a sample with electrons instead of light to form an image of it.

Procedure
Background
In biology it if often important to determine cell viability of a culture and this is done by counting the number of cells left on a plate after washing. Usually a cell chamber is used which has a small grid on its surface. Cells found on each square are counted and then an overall cell count is estimated. It is much easier counting cells or particles in a smaller predetermined region of interest (ROI) rather than counting every single cell in the entire sample.

In the case of nanoparticles, small changes in the chemical procedure will yield particles of varying size so it is crucial to characterize the outcome of each batch to know if these particles can be used. Using similar counting methods as are used in cell biology we can estimate the number of particles in our sample and find the variability in diameter.

Before the Activity
This computer requires computers so the computer lab should be reserved ahead of time or the laptop cart. The ImageJ software is public domain and should be downloaded on all computers prior to the start of the activity. It can be downloaded from the following website: http://rsbweb.nih.gov/ij/download.html

Additionally, a couple of images from the previous activity should be chosen that way all students can analyze a similar image. If you are looking at cells or doing the PDLC Fabrication activity then take a few images of the samples through a microscope. If you are looking at the nanoparticles made in the DNA biosensor activity then obtain an SEM image of the particles through a University that collaborates with you. Make sure there is a scale bar included in the picture.

With the Students
There are multiple ways students can find the length of their nanoparticles using ImageJ. Below is just one possible way with few steps to follow.

Getting started and calibrating to the scale bar
1. Open the ImageJ program and open the desired jpg file.
2. Click on the line icon and draw a line the length of the scale bar and take note of the pixels that it measures on the ImageJ box and correlate this to the true length of the scale bar.

3. Go to Analyze → Set Scale and enter the information in the given boxes and click on the global box.

Measuring the length of nanoparticles
1. Go to Analyze → Set Measurements and uncheck all of the boxes that are checked and exit menu.
2. Click on the box tool and draw a box around the area that you want to analyze. Make sure the box is about ¼ the size of the complete image. Take note of the area of the box. Also try to minimize the number of particles that cross the edges of the box. See Figure 1 for an example
3. Hit Control-D that way the box stays in place.
4. Click on the line icon and use it to trace the diameter of one particle within the box.
5. Hit Control-M and a "results" box will appear showing you the angle and length of the line you just drew. This is the diameter of your particle.
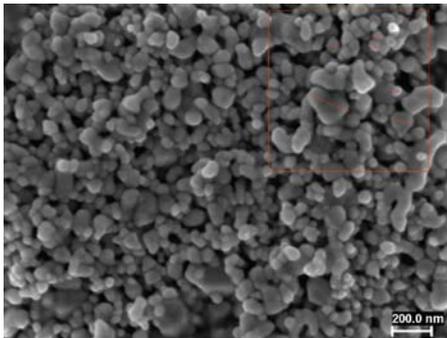
Figure 1: SEM image silver nanoparticles (Left). Red box shows ROI with lines drawn across the diameter of the nanoparticles. Results box shows the angle and length of each nanoparticle (Right).

6. Go back to the image and with the text tool write a #1 on the particle you just measured.
7. Go back to line icon and use it to measure your next particle.
8. Repeat steps 4-7 until you have measured all of your particles in the box.
9. Copy the measurements from the "results" window and paste them on an excel sheet.
10. Add all the values together and find the average and standard deviation of all of the particles. You can do this on excel by using the AVERAGE and STDEV formulas.
11. Then make a bar graph of the diameter of your particles.

Calculating the total number of particles
1. The number of particles in your region of interest is the same as the number of measurements that you made.
2. To calculate the total number of particles in the entire sample you must make a ratio of the number of particles in your ROI area to the number of particles in the entire sample area.

3. The ROI area will just be the area of the box you created in the image while the area of the entire sample will most likely be the area that is covered by your sample.

Particles in ROI / area of ROI = Total # of particles / area of sample

Troubleshooting Tips

It is important that students set the scale on their image and check the global box. If not then ImageJ will give lengths in pixels instead of nanometers.

Investigating Questions

1. What happens to your mean and standard deviation when you analyze a larger region of interest?
2. What about a smaller region?
3. Can you think of a better way of getting a true representation of the size of your nanoparticles using image analysis?
4. Using the bar graph that you created, what does the standard deviation tell you about your sample?

Assessment

Pre-Activity Assessment

Title: Group brainstorm

Students are asked to brainstorm a way to count all of the nanoparticles and check the size of each one using a scanning electron microscope.

Activity Embedded Assessment

Title: Graphing particle diameter

After conducting the ImageJ analysis of the diameter of each particle in the ROI, students must find the average and standard deviation of their particle diameter. Additionally they must graph their results and interpret what the standard deviation tells about their sample. Finally, they must calculate their total particle count.

Post-Activity Assessment

Title: Class Discussion

After the activity, students are asked to design a protocol to accurately determine the size and count of their particles. Students should consider taking multiple images of their sample, establishing the size of their ROI, which way to measure their diameter, how to deal with particles that cross the boundaries of the ROI box, etc.

Reference

1. Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2009.

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