Chapter 7: Glass Beads

The glass beads sample is a piece of glass covered with a thin layer of very small colloidal silica (i.e., glass) particles. The beads will group in clusters, some of which exhibit crystalline structures. Colloidal particles are often used to make small scale regular structures, such as calibration patterns, hard disk-recording media, and photonic crystals. AFM can be used to characterize defects in such structures, but here the structure is used to characterize the 3D-shape of the AFM tip, as opposed to the 2D characterization done with nanotubes in the previous chapter.

7.1: Measurements

7.1.1: Sample Preparation

The glass bead sample must be prepared. The kit contains an empty glass slide and a vial of diluted bead solution, which will be used to create the sample. In this case the beads have a diameter of 120 nm (may vary in future). Once the sample is prepared, it can be used for measurements as long as it remains clean.To prepare the sample:

1. Take the following materials from the sample kit:
   - Glass slide
   - Diluted bead solution
   - Ethanol
   - Clean tissue
2. Clean the glass slide and remove any accumulated dirt:
   - A. Drip some ethanol on the slide.
   - B. Wipe the ethanol off with the clean tissue.
3. Place the vial with the bead solution in a beaker partly filled with water.
4. Place the beaker in an ultrasonic bath for approximately 20 minutes.
   This process will break up any groupings of beads in the solution. The beads will form aggregates over time due to simple attractive interactions between

them (such as Van der Waals forces), but the goal is to have individual beads come together on the glass slide and eventually form crystalline structures.

5. Use an eyedropper to place a drop of bead solution onto the cleaned glass slide.
6. Try to form the largest drop possible without spilling over the sides of the glass.
7. Dry the sample under ambient conditions.
   This may take several hours.
8. Fix the beads by baking:
   A. Place the sample disc in an oven at 250° for 2–3 hours.
   B. Allow the sample to cool completely before imaging

7.1.2: Image Acquisition

Approaching the Sample

This sample is one of the more difficult to approach, as it is non-metallic, and not very reflective. If you can see the cantilever's shadow or reflection, you can use it to judge the distance. If you find it difficult to recognise the cantilever's reflection, then slightly move the sample holder: the structures on the sample will move, but the reflection will stay in the same place.

If you cannot see the cantilever's reflection, perform a very slow coarse approach while judging the distance on the focal plane of the side view as follows:

- When the tip is on the sample, the focal plane crosses the sample at the tip position.
- When the tip is further away, the focal plane crosses the sample more behind the cantilever.

Scanning

1. Start with a low force set point for best results.

   Applying too much force may move some of the beads around and create wide horizontal stripes across the image.

   If you get stripes in your image:

   A. Lift the tip, and then

   B. Bring it back into contact.

   If the tip is simply dirty, you can remove the dirt by:

   A. Retracting the tip

   B. Re-extending it again.

   If there are still stripes in your image, the problem may be that the region where you are scanning does not have perfectly fixed beads. In a region of more ordered beads, the beads will stay in place. Therefore:

   ➔ Move to another region on the sample.

2. Set the scan range to 1 µm.

   Since the beads are approximately 120 nm in diameter, you should be able to see about 10 of them across the image.

   If your image shows islands of beads surrounded by very flat areas:

   ➔ Move to a region of better ordered beads.

In general, the region with the best ordering is close to the center of the spot on the slide. *Figure 7-1: Well Ordered Beads* shows a well-ordered region near the center of the spot.

Identifying the Bead Structure

In a well ordered region, each bead will be surrounded by 6 others. Identify a single bead, and count the beads around it to see if it is surrounded by 6 others. Then check to see if each of these 6 beads is, in turn, surrounded by 6 others.

In *Figure 7-1: Well Ordered Beads*, for example, the top right corner of the image is far better ordered than the top left. Notice also that there are some beads slightly higher up than others; they are not all perfectly coplanar.

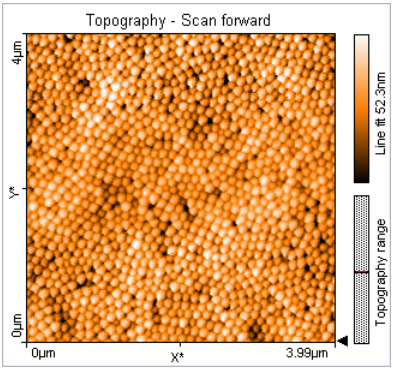

Figure 7-1: Well Ordered Beads. The center part of a spot of bead solution. Some sections have a crystalline structure while others are less ordered.

7.1.3: Image Analysis

Section analysis can be used to determine the distance between desired markers. *Figure 7-2: Bead Section Analysis* (page 62) illustrates how to find the vertical distance from the point from where two beads touch to the top of one of the two beads. Since the beads are approximately spherical, and have a 120 nm diameter, the vertical distance between one peak and the adjacent valley should be half the diameter (60 nm). However, in this section analysis, the vertical distance is only about 15 nm. The discrepancy is due to the fact that scanning is limited by the shape of the tip, which cannot fully extend down between beads.

Figure 7-3: Bead-tip geometry (page 63) illustrates how this occurs with a tip that is approximately spherical at the end. The tip may track some of the height drop between two adjacent beads, but it will not track the full extent of the drop. While this effect is negligible for larger beads, it becomes more significant the smaller the beads are.

The radius of the tip can be calculated from three pieces of information:

1. The radius R of the beads.
2. The radius r of the tip.
3. the height h between the tops of the beads and the lowest point that the tip reaches between them.

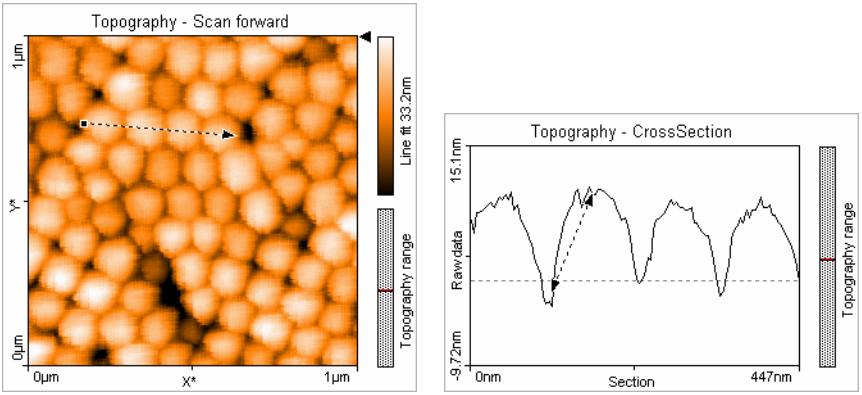

Figure 7-2: Bead Section Analysis. A section analysis of a zoomed in area of the bead sample.

Simple geometry predicts:

$$R^2 + (R + r - h)^2 = (R + r)^2$$

Rearranging that formula provides the following equation to calculate the tip radius:

$$r = \frac{(R - h)^2}{2h}$$

Using h = 15 nm and R = 60 nm, we find that the tip has an approximate radius of 68 nm. The manufacturer specifications for the tip radius is 10 nm, so this is rather large. It is possible that one or more beads are clinging to the end of the tip, causing the aparent increasy in tip radius.

The method outlined above is an easy way to estimate the actual tip radius. Also, we learned that the tip radius influences the image feature size, especially when the two have comparable dimensions.

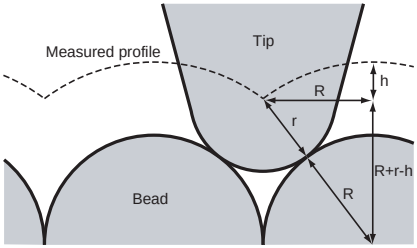

Figure 7-3: Bead-tip geometry. The tip may not fit all the way down between two beads. The geometry of the beads and the tip allow the determination of the tip radius.

7.2: Relevance of the Glass Beads

The glass beads sample demonstrates the small scale interactions of sub-micron bodies. This alone is an interesting use for the beads, but many other uses exist.

Different sized beads have been shown to disrupt different cells. Cell disruption is the process wherein the cell wall growth is disturbed for the purpose of extracting products out of the cells in which they are produced. This disruption can be used, for example, to obtain DNA from within a cell nucleus. Extensive research has been performed, and now sold in different sizes according to their use in specific cells.

Beads have also been very useful in the study of chromatography, the separation of a mixture of substances in a phase separated medium. There is a stationary phase, held in some sort of container, and a moving phase flowing through it. The different substances in a given mixture are drawn to either the stationary or mobile phases based on some property (size, charge, etc.) that they exhibit.

Silica beads coated with a substance similar to that of a cell membrane have been used to simulate cellular interactions. This provides a simple way to reproduce crucial biological phenomena that are not fully understood. In other experiments, researchers have suggested that silica beads coated with gold may kill cancerous cells when exposed to near-infrared light.