Fluid Imaging Exam

1) Given that AFM imaging in fluid is so much more difficult, time consuming, and risky in terms of potential damage to the AFM than air imaging, why do you want (or need) to image in fluid? Your explanation should include both what you hope to gain by imaging in fluid and the physical phenomena responsible for this.

2) Is the E scanner for the MultiMode 8 a) waterproof or b) water resistant? What are the practical implications of this when conducting fluid imaging on the MM8?

3) What is the approximate (order of magnitude) cost to replace/repair an AFM scanner head if the piezos are damaged by fluid? Do you have that kind of cash handy? If not, where would the money to replace the damaged piezos have to come from?
    a. $500-$2,500
    b. $2,500-$5,000
    c. $5,000-$25,000
    d. >$25,000

4) Fill in the blanks to correctly complete the following sentences, which come directly from the fluid imaging experiment guides found in the electronic user manuals for the AFMs.

a. Users occasionally experience problems with

_____.

b. Users should avoid

_____.

c. Any moisture present on the end of the scanner must be

_____.

d. Do not turn the scanner

_____.

e. When imaging fluid samples, use

_____.

f. Fluids must not be spilled on or around the

_____.

_____.

g. Avoid spilling

_____;

otherwise,

_____.

h. In the case of a spill,

_____.

5) The piezoelectric crystals (piezos) are the most delicate component found in each AFM. They are relatively easy to break, and are extremely expensive to replace. Which piezo(s) are most susceptible to water damage and where are they located in each of the following AFMs?
    a. MultiMode 8

    b. FastScan

    c. Dimension Series (3100 or Icon)

6) What special accessories (e.g., fluid cells/probe holders, piezo tube skirts, etc.) are necessary to carry out fluid imaging on each of the following AFMs?
    a. MultiMode 8

    b. FastScan

    c. Dimension Series (3100 or Icon)

7) For each AFM listed, name the best probe(s) for i) tapping and ii) PeakForce tapping (ScanAsyst) in fluid.
    a. MultiMode 8 or Dimension Icon
        i.

        ii.

    b. FastScan
        i.

        ii.

8) Approximately how much fluid should be micropipetted into each of the following locations prior to carrying out fluid imaging?
    a. Between the probe and the laser window

    b. Onto the sample surface

9) Why is it necessary to merge the sample droplet and the droplet surrounding the AFM probe prior to optimizing the laser alignment, tuning the cantilever (if necessary), and engaging? Are you likely to need to move the probe further down towards the sample surface, or away from it before starting the engage process?

10) Describe how one obtains a tuning curve in fluid and chooses the best/correct peak frequency and drive amplitude. Include sketches of typical tuning curves to illustrate your answer.

11) When operating in fluid, the FastScan has a checkbox in the Navigate menu that should be selected to compensate for the index of refraction of the fluid.
    a. Why is this? What process(es) are affected by this change in index relative to air?

    b. Why is this not necessary on the MM8?

    c. What is the index of refraction of water? (Note that the refractive index is wavelength dependent, so you should specify what wavelength and why you have chosen that wavelength.)

    d. What is the default refractive index used by the FastScan when imaging in fluid? If you are using a fluid other than water, where can you go to modify this value?

12) You just started a fluid scan that will take a while to complete. You're tempted to leave and come back later to check on the results of the scan, but know that you then might not return for several hours to clean up. Is this a good idea? Why or why not?

13) Why is it important to clean the fluid cell/probe holder both immediately before and after use (i.e., what are you trying to prevent and/or remove)?

14) Describe the proper cleaning procedure for each of the following.
    a. MultiMode fluid cell/probe holder

    b. FastScan Z scanner

15) Describe some symptoms that would suggest an air bubble is present. What should you do?

16) What are some ways that you can prevent fluid from overflowing the edges of your sample and leaking onto the stage and/or piezo tube?

17) It's possible that the DNA in your sample may be more attracted to the surface of the AFM cantilever than to the negatively charged mica surface. Why might this be a problem, and what can be done in terms of sample prep and imaging technique to minimize this?

18) To obtain high quality images in fluid, great care must be exercised in every aspect of sample prep. Please answer the following questions.
    a. What type of mica should be used?

    b. What type of adhesive should be used to adhere the mica to the steel puck?

    c. What should be done to all buffer solutions immediately prior to use? Should you use buffer solutions that are old or whose history you don't know?

19) The following are commonly used buffers for DNA solutions. Define each acronym by giving the full name and chemical formula for each buffer component. List the molarity of each component in a "1x" solution of each buffer and indicate the resultant pH.
    a. TAE

    b. TBE

20) Both nickel and magnesium are commonly used as divalent cation sources for fluid imaging of DNA on mica. Why is each one used/how are they different? Does the counterion (anion) make a difference? If so, is simply labeling a solution as "Ni^{2+}" or "Mg^{2+}" sufficient, or instead potentially misleading?

21) Correct concentrations/amounts of DNA, divalent cations, and buffer are often key to obtaining good AFM fluid images.
    a. What are typical concentration ranges for DNA, Mg^{2+} and/or Ni^{2+}, and buffer?

    b. What effect might the "E" in TAE and TBE buffer have on your divalent cation concentration? To minimize this effect, would you want a high or low ionic strength buffer? Would changing the ionic strength affect the pH? Why or why not?

    c. Is the concentration of "E" in the TAE/TBE buffers you typically use high enough to significantly affect the divalent cation concentration? Explain.

    d. Suppose you prepare a sample for fluid imaging by adding 5 µL of 10 mM $NiCl_2$ solution to 20 µL of 1 nM DNA solution, both prepared in 1x TAE. What are the final divalent cation, "E", and DNA concentrations?

22) Below is a list of common chemicals/solvents. Circle the ones that are NOT compatible with the FastScan wash station and/or Z scanner.

- Acetone or other ketones (e.g., methyl ethyl ketone or 2-butanone)
- Acids (dilute)
- Alcohols (e.g., methanol, ethanol, or isopropanol)
- Alkalies (bases, dilute)
- Ammonia (NH_3 gas or aqueous ammonium hydroxide, NH_4OH)
- Benzene or toluene
- Bleach (sodium hypochlorite solution, dilute)
- Bleach (liquid, full strength)
- Butane (C_4H_{10})
- Chlorine (dilute liquid solution)
- Chromic acid
- Dow Corning greases/lubricants or silicone-based oils and greases
- Hot water and steam (up to 350°F)
- Hydrochloric acid (HCl)
- Hydrogen peroxide (H_2O_2)
- Lye (sodium or potassium hydroxide)
- Petroleum-based greases and lubricants
- Polar solvents
- Saline (salt) solutions
- Sodium bicarbonate (baking soda)
- Vinegar (acetic acid, dilute)
- Water (tap, deionized, or ultrapure)