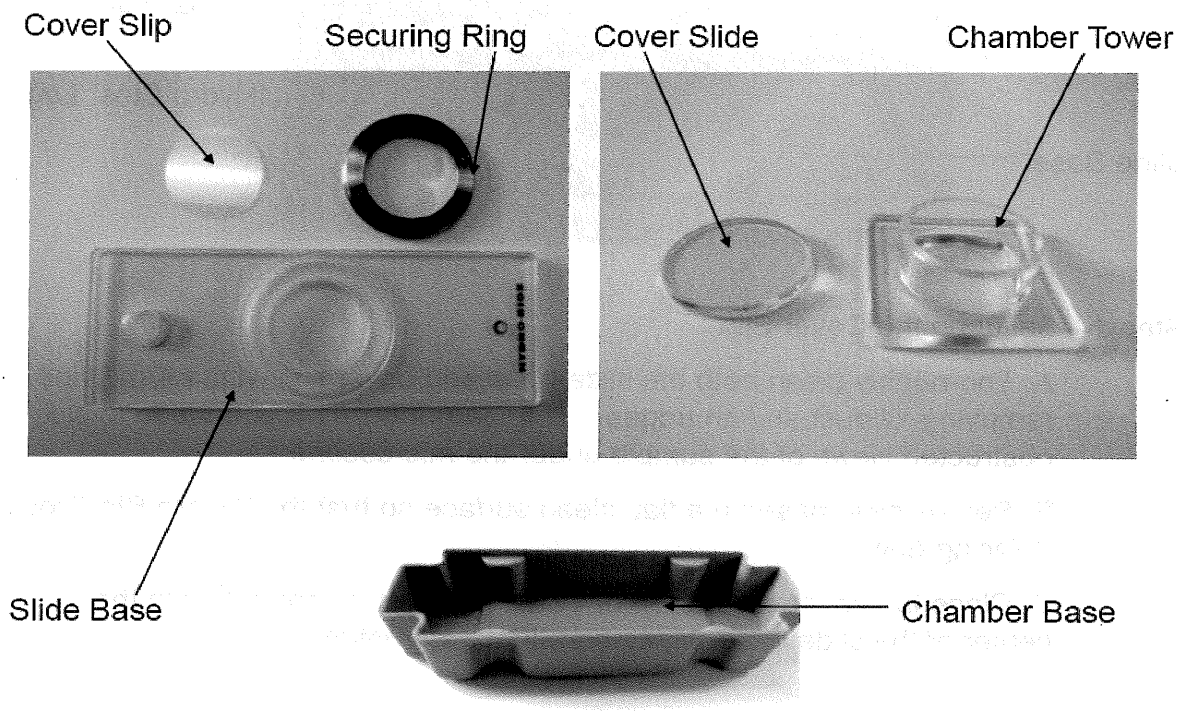
Appendix 1

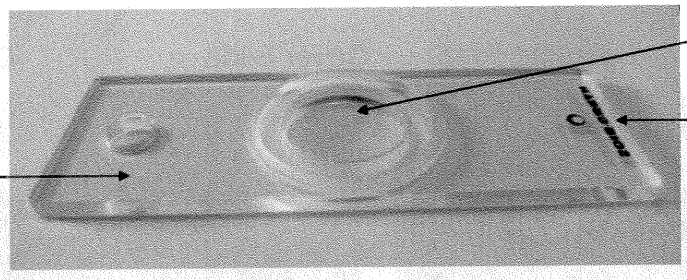
Plankton Enumeration

1. Introduction to settling chamber components
2. Assembling the setting chamber
3. Preparing the sample
4. Completing the settling process
5. Cleaning up
6. Counting phytoplankton cells

Introduction to Settling Chamber Components



Assembling The Settling Chamber



Step 1—Securing the Cover Slip

A. Thoroughly clean both the slide base and cover slip with a Kim Wipe, removing all dust and smudges (i.e. fingerprints). This is done to avoid obstructed views of the sample under the microscope.

B. Set the slide base on a flat, clean surface so that the "Hydro Bios" logo is facing down.

C. Place the cover slip on top of the raised circular projection in the center of the slide base. Make sure that it is centered.

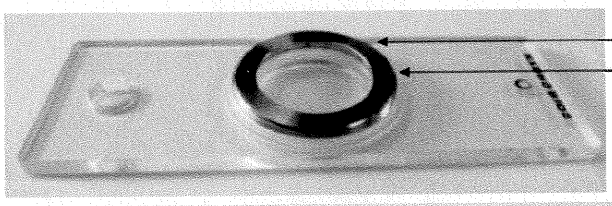
Assembling The Settling Chamber

Step 1—Securing the Cover Slip (Cont.)

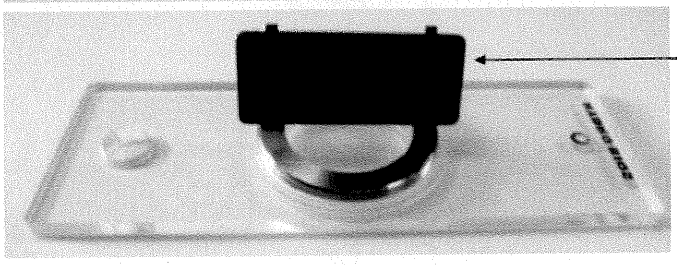
D. Place the securing ring with the two circular indentions facing upward on top of the groove surrounding the circular projection on the slide base.

E. Use the tightening tool to screw the securing ring into place. Affix the securing ring with care; a tight seal is desired so that sample loss can be prevented, however, screwing the securing ring on too tightly can cause the cover slip to crack.

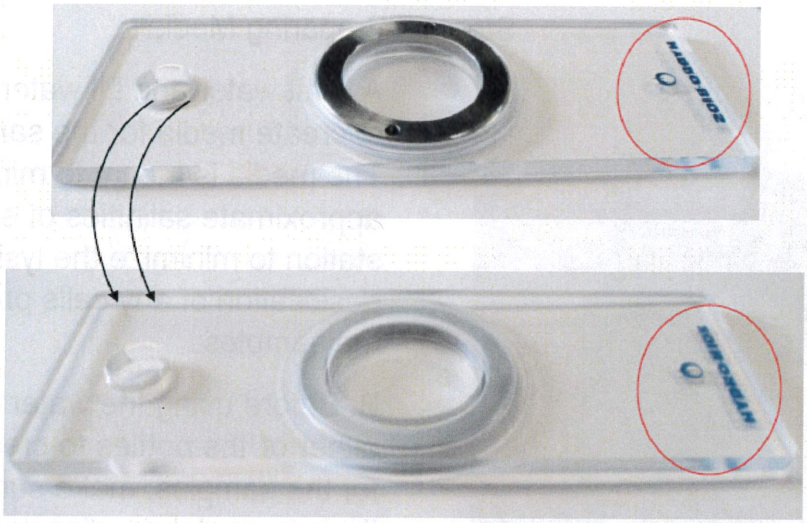
D.



E.



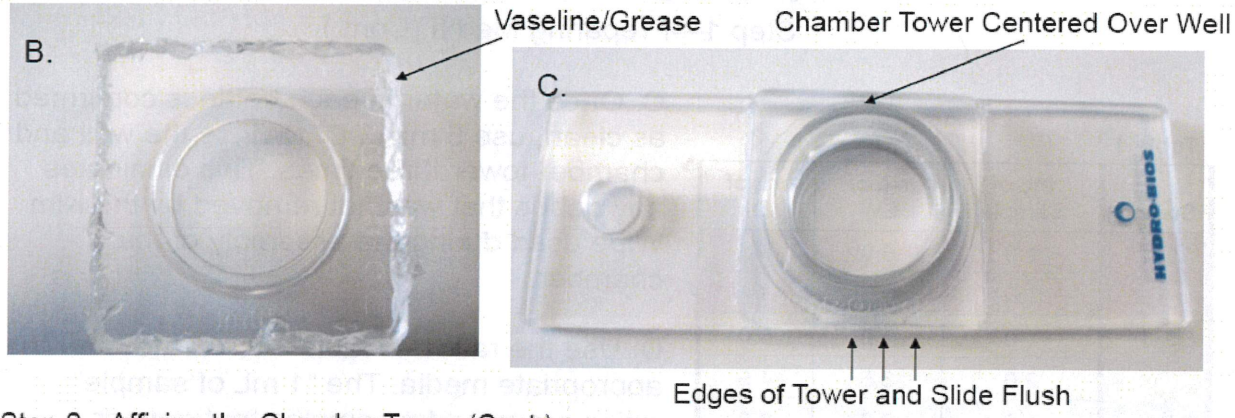
Assembling The Settling Chamber



Step 2—Affixing the Chamber Tower

A. Flip the slide base so that the "Hydro Bios" logo is now facing upward.

Assembling The Settling Chamber



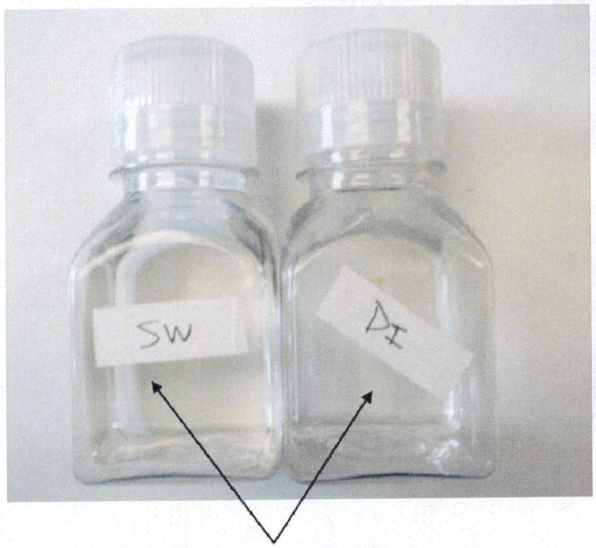
Step 2—Affixing the Chamber Tower (Cont.)

B. Apply a moderate layer of Vaseline or silicone grease only to the perimeter of the bottom of the base of the chamber tower with a cotton swab. This method is used to avoid getting Vaseline/grease inside the chamber tower and potentially the sample.

C. Center the chamber tower over the well formed by the sides of the circular projection on the slide base and the cover slip. Make sure that the edges of the chamber tower are flush with the edges of the slide base. Gently press the chamber tower down on the surface of the slide base so that the Vaseline/grease forms a tight seal between the two objects. A tight seal is necessary to retain sample/media in later steps.

Preparing The Sample

Step 1—Preparing Media



Clear Water, No Contamination

A. Salt water and DI water are used to create media for the sample. The media is meant to mimic the approximate salinities of each station to minimize the lysing or desiccation of any cells present in the samples.

B. Before using the water from either of the bottles to create media for the samples, make sure that there is no debris, discoloration or cloudiness in the water to avoid contaminating the sample.

Preparing The Sample

Step 1—Preparing Media (Cont.)

Table 1

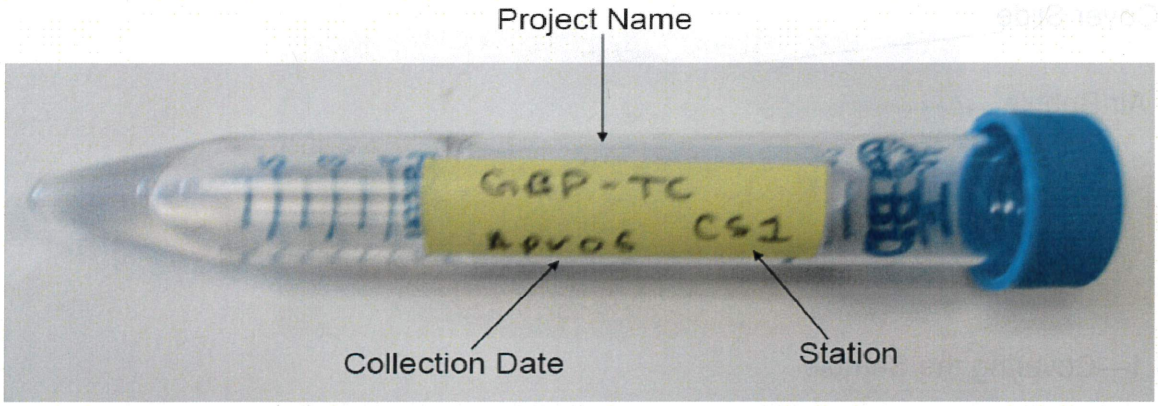
Station	mL of Sample	mL of SW	mL of DI
CS-1	1.0	5.0	4.0
	2.0	4.5	3.5
CS-4	1.0	4.0	5.0
	2.0	3.5	4.5
CS-6	1.0	3.0	6.0
	2.0	2.5	5.5

C. Once the water in each bottle is confirmed as clean, use 5 mL of DI to rinse the well and chamber tower three times. This eliminates any debris that was not removed by the Kim Wipe used during the assembly of the chamber.

D. Use the ratios in Table 1 to create appropriate media. The "1 mL of sample" ratios are used for samples that appear cloudy, the "2 mL of sample" ratios are used for samples that appear clear.

E. Regardless of the station for which you are creating media, pipette DI water into the well and chamber tower before SW. This way, the same pipette tip can be used to draw up SW without affecting its purity.

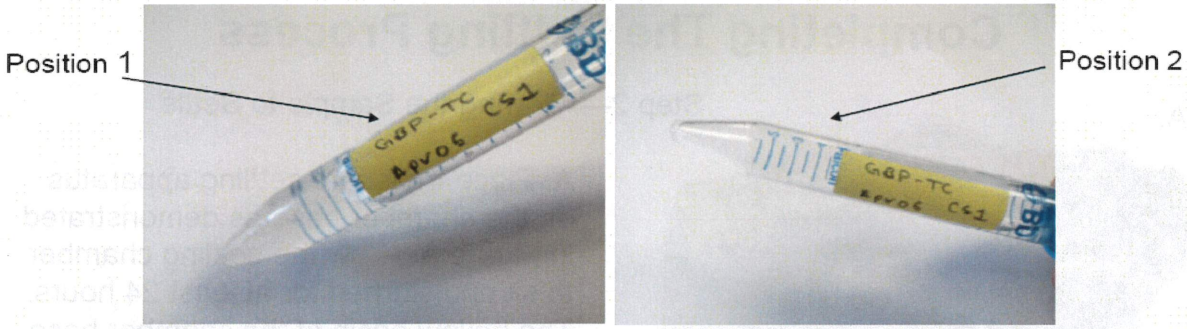
Preparing The Sample



Step 2—Adding Sample

A. Samples are contained in 15 mL falcon tubes and are organized in a test tube rack by month and station. All samples are preserved with toxic formalin! Wear gloves when handling the sample and dispose of the sample and all things that touched it (i.e. pipette tips) in toxic waste containers!

Preparing The Sample

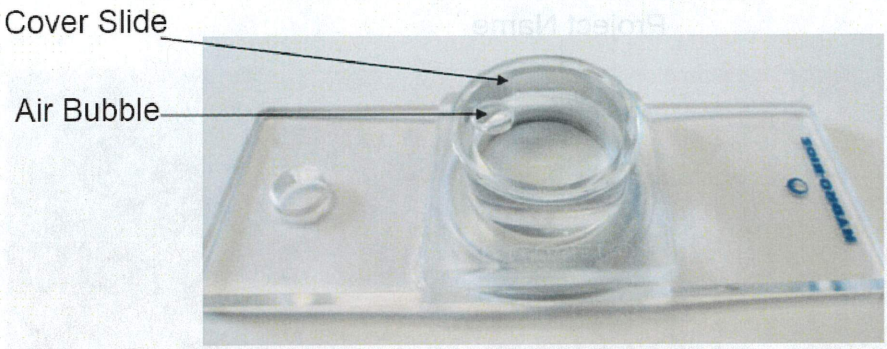


Step 2—Adding Sample (Cont.)

B. Before adding sample to the media, the falcon tube containing the sample must be *gently* inverted 50 times to maintain homogeneity. One inversion consists of first pointing the tube down (Position 1) and then rocking it back (Position 2).Exercise care when inverting, handling a sample too roughly can result in rupturing the cells within it.

C. When extracting sample from the tube, first draw up the sample then eject it and re-draw it. This is another method of maintaining homogeneity. Use a similar technique when ejecting sample into the media; eject the sample below the surface of the media, draw it back into the pipet tip, then eject it a final time. As stated before, use 1 mL of sample for samples that appear cloudy (high concentration of objects) and 2 mL of sample for samples that appear clear (low concentration of objects).

Completing The Settling Process



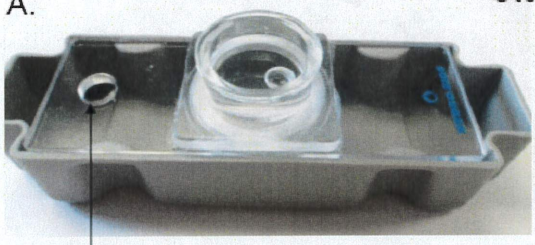
Step 1—Covering the Sample

A. Once the media and sample are placed in the well and chamber tower, cover the opening of the chamber tower with the cover slide. The presence of a small air bubble is perfectly normal. However, if the bubble gets larger, it indicates that the integrity of the seal has been compromised (a leak has occurred). In the event of a leak at *any time* during the settling process, it is imperative to *start over*. The procedure is dependent on constant liquid levels, salinities and sample contents in order to maintain the consistency of calculations used for the project.

Completing The Settling Process

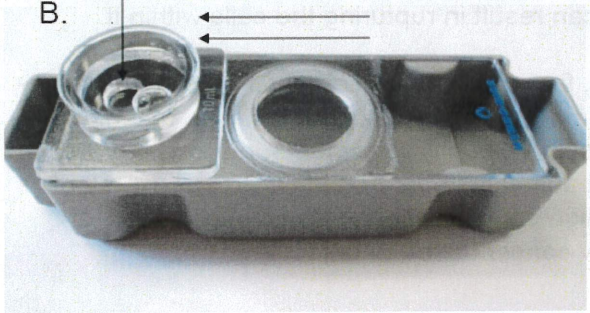
Step 2—Allowing the Sample to Settle

A.



Waste Hole

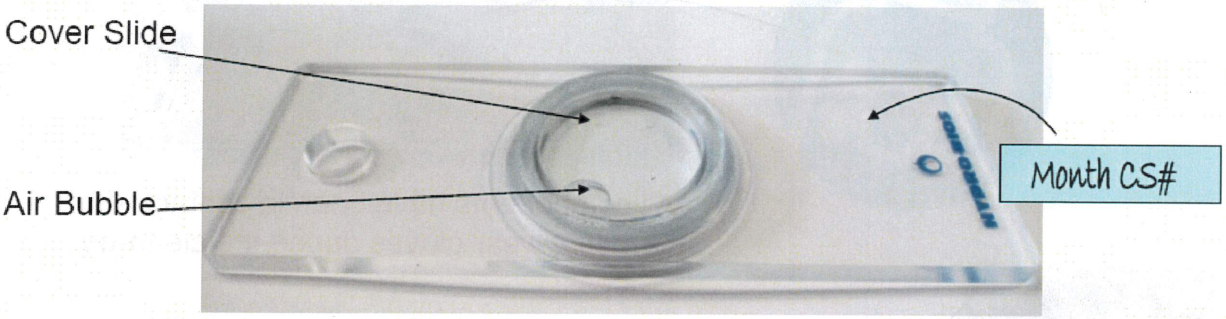
B.



A. Place the entire settling apparatus on the chamber base as demonstrated in Figure A. Allow the settling chamber to sit undisturbed for at least 24 hours. The hollow basin of the chamber base is another instrument for monitoring the success of the seals in the apparatus. If any liquid is found in the chamber base basin, a leak has occurred.

B. After 24 hours, slide the chamber tower complete with cover slide over the waste hole in the slide base. If done successfully, all the liquid above the level of the well will stay within the tower. Remove the cover slide to evacuate the excess liquid.

Completing The Settling Process



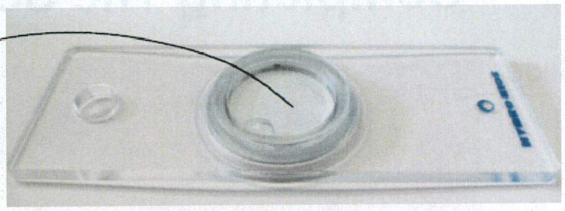
Step 3—Preparing a Slide

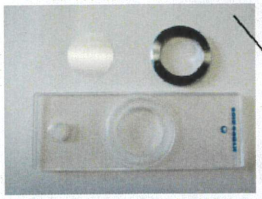
A. Use the cover slide that covered the opening of the chamber tower in previous steps to cover the opening of the well on the slide base. It should adhere to the excess Vaseline/grease left behind after the chamber tower was removed. Again, the presence of an air bubble is normal.

B. Clean excess Vaseline/grease, dust, etc. off the slide using a Kim Wipe.

C. Label the slide with the collection date and station specific to the sample. The label can be placed directly on the slide with labeling tape in the area between the well and the "Hydro Bios" logo.

Cleaning Up





A. All components have been in contact with formalin, wear gloves during the clean-up process!

B. Dispose of sample in toxic waste container!

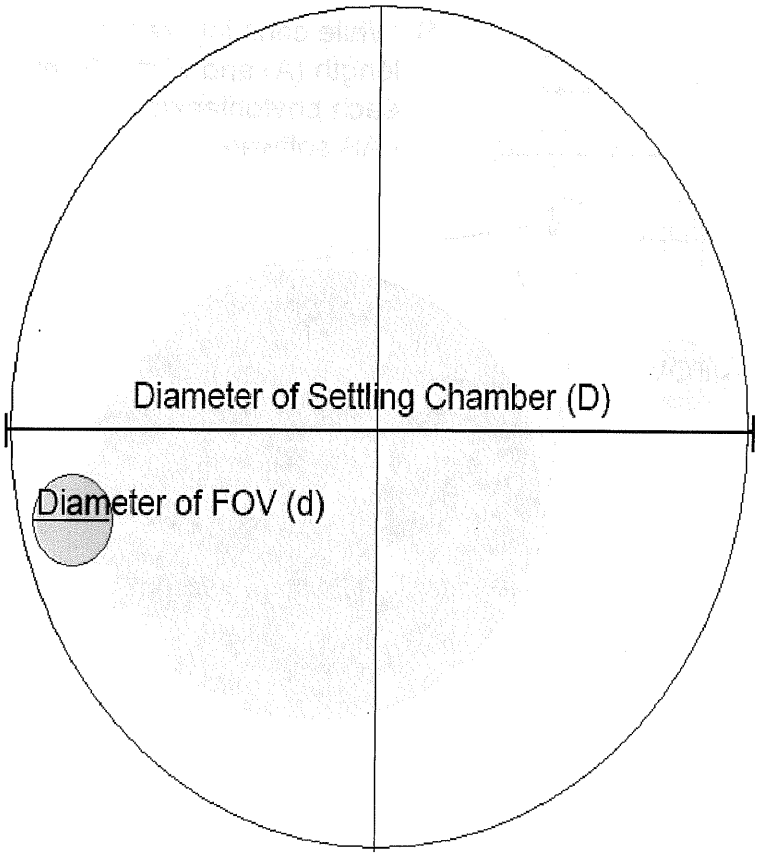
C. Disassemble the chamber and rinse the components in DI water.

D. Wash each component by hand with Dial soap two times to try and remove all traces of Vaseline/grease from each piece.

E. Rinse each component a second time in DI water.

F. Allow all parts to dry on a drying tray or dry by hand with paper towels.

Counting phytoplankton cells

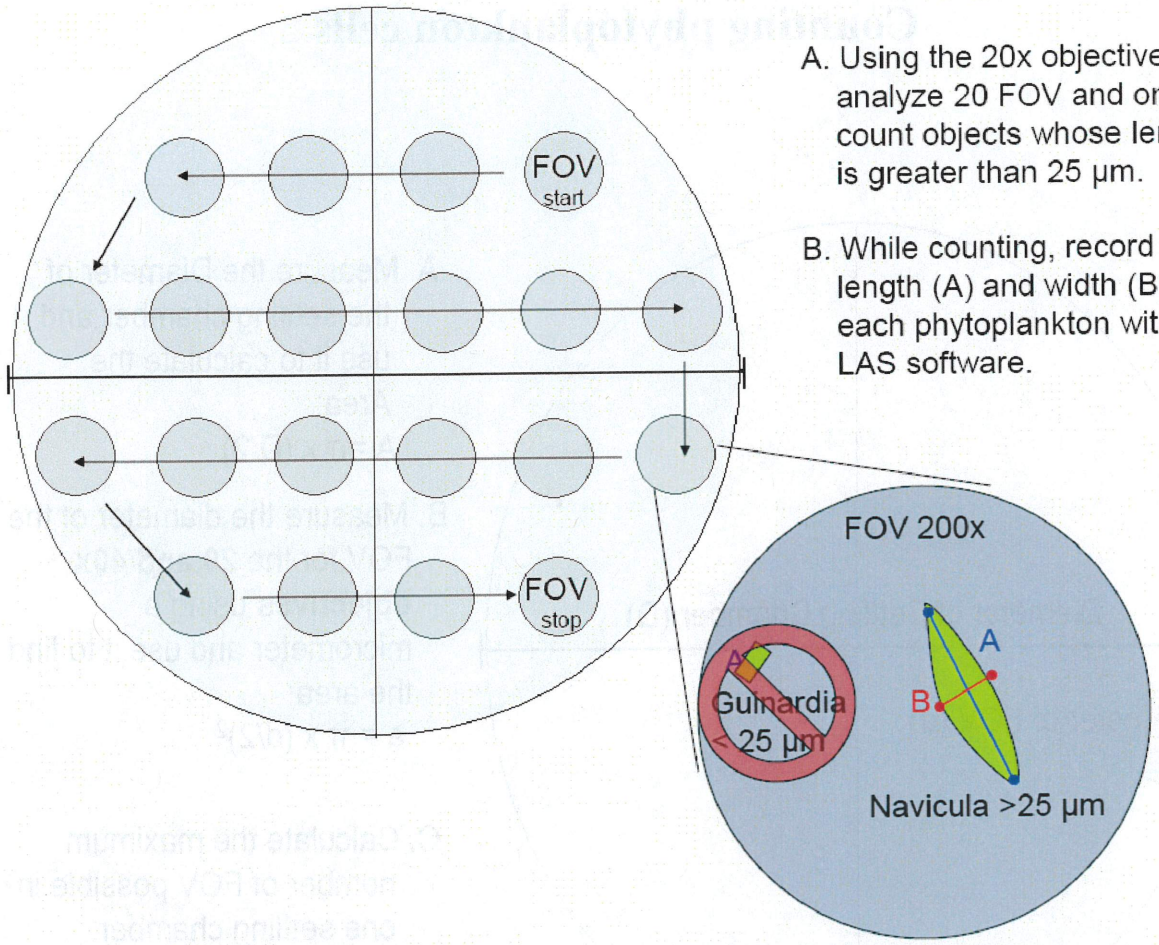


A. Measure the Diameter of the settling chamber and use it to calculate the Area:
$A = \pi \times (D/2)^2$

B. Measure the diameter of the FOV for the **20 and 40x objectives** using a micrometer and use it to find the area:
$a = \pi \times (d/2)^2$

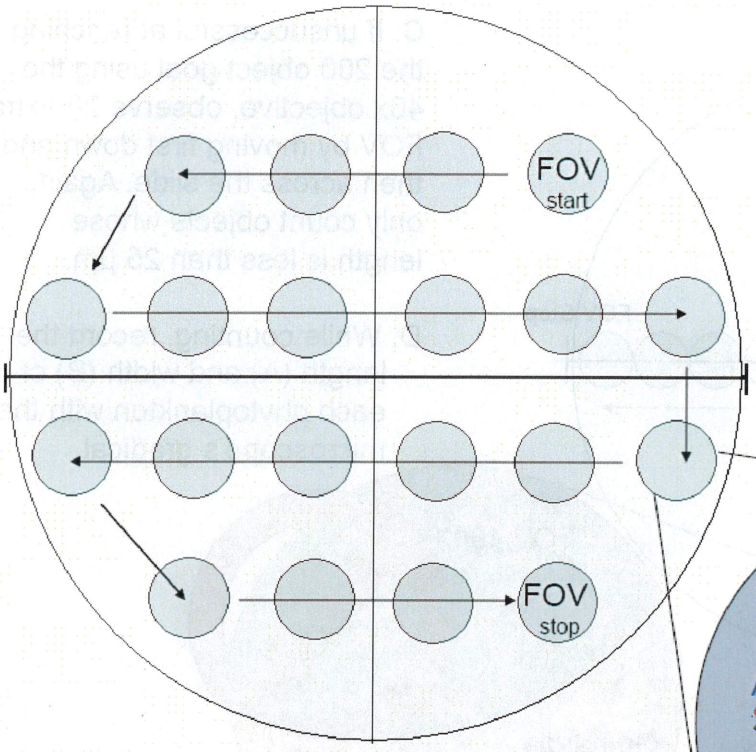
C. Calculate the maximum number of FOV possible in one settling chamber:
$FOV_{max} = A/a$

D. Calibrate the microscope's gradical using a micrometer.



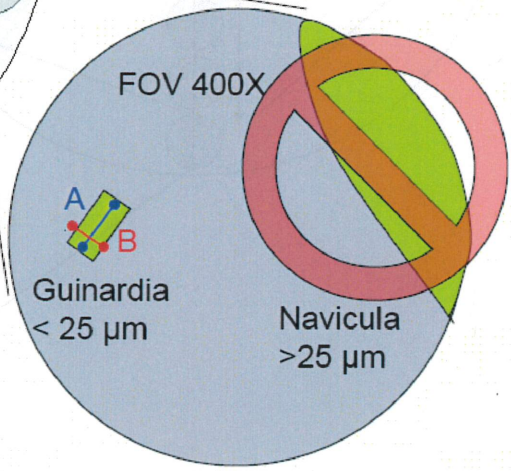
A. Using the 20x objective, analyze 20 FOV and only count objects whose length is greater than 25 µm.

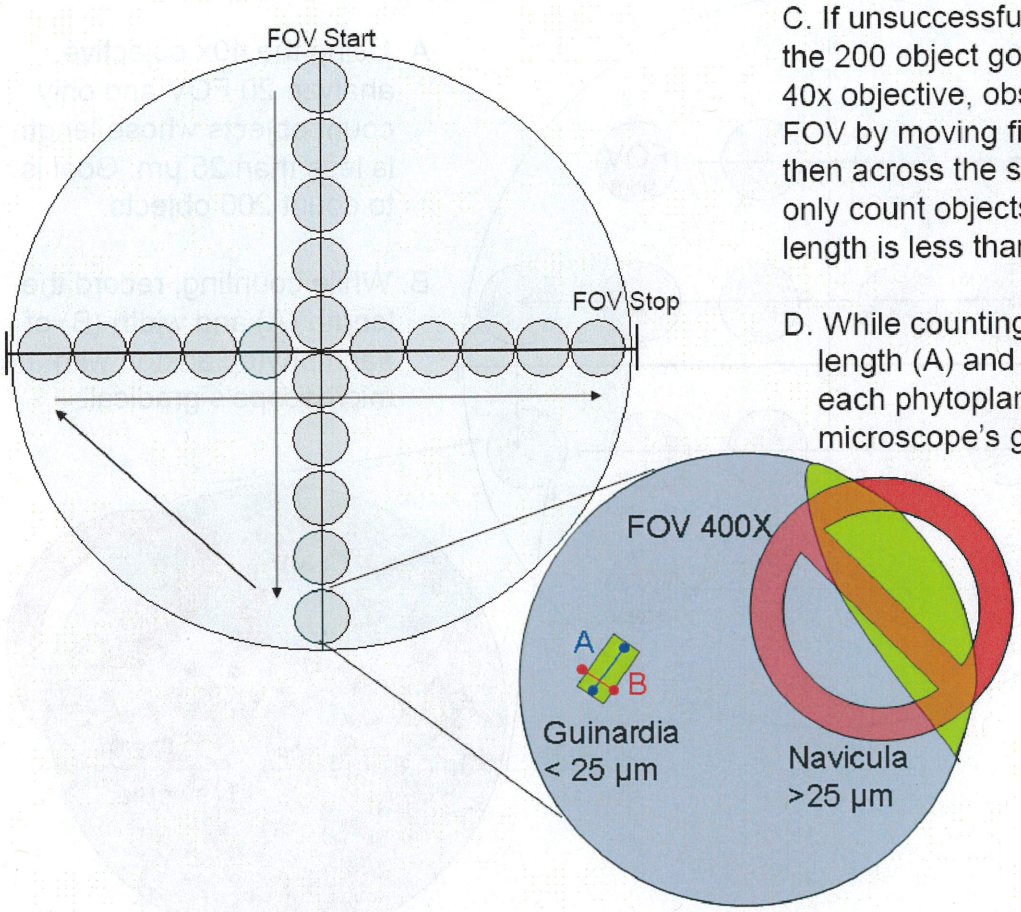
B. While counting, record the length (A) and width (B) of each phytoplankton with LAS software.



A. Using the 40x objective, analyze 20 FOV and only count objects whose length is less than 25 µm. Goal is to count 200 objects.

B. While counting, record the length (A) and width (B) of each phytoplankton with the microscope's gradical.





C. If unsuccessful at reaching the 200 object goal using the 40x objective, observe 20 extra FOV by moving first down and then across the slide. Again, only count objects whose length is less than 25 µm.

D. While counting, record the length (A) and width (B) of each phytoplankton with the microscope's gradical.