# Identifying the Three Unknown Components in Powder Sample #20 Using Microscopy Techniques

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## ABSTRACT

In the world, there are a lot of unknown substances made of a variety of components and in need of being identified. For instance, it is important for a herb merchant and herb customer to know what herbs are included in an herb blend or for a crime investigator to know what compounds are in an unknown substance found at a crime scene. Because of this interest and need to identify unknown substances, there are forensic scientists who are able to do the job of researching and identifying compounds found in unknown substances. With this in mind, this project is a practice of identifying components found in an unknown powder. In particular, for this project, different microscopic techniques such as brightfield microscopy, polarized light microscopy (PLM), phase contrast microscopy (PCM), and epifluorescence microscopy were put into practice to determine the components in an unknown powder sample. Using these techniques, it was determined that the unknown powder was composed of cellulose, cotton fibers, and yeast. This paper will go into the details of how, why, and what microscopic techniques were used to verify that the unknown powder was made of cellulose, cotton fibers, and yeast.

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## INTRODUCTION

Microscopy is an amazing and wonderful field of using microscopes to view samples and objects that cannot be seen with the unaided eye (Edinburgh). The most common known microscope technique is brightfield microscopy, however, the field has many different kinds of microscope techniques including but not limited to polarized light microscopy (PLM), phase contrast microscopy (PCM), and epifluorescence microscopy which will be covered, and used in this paper.

Starting with brightfield microscopy, brightfield microscopy is the technique that was developed when microscopes were first invented and generate images with contrast from differential absorption of white light by pigments in the sample (Ruzin). Using brightfield microscopy, we can generate a true color sample on a white background. Köhler Illumination is important for supplying a uniform bright background (Berkeley). Thus, this technique is not good for samples that are transparent and is good for samples that have pigment and/or can be stained with dyes such as IKI stain which is used to identify starch.

Polarized light microscopy (PLM) is a microscope technique that utilizes polarized light to investigate and identify certain crystals or crystal-like microscopic samples (Ruzin). For PLM, contrast is generated by polarized light interacting with birefringent (optical anisotropic) samples (Berkeley). Birefringent samples can be identified by using a Full Wave Retardation Plate and finding the interference color on the Michel-Levy Chart (Berkeley). A PLM microscope contains a polarizer, analyzer, and usually a retardation plate. The polarizer produces plane-polarized light to illuminate the sample. Birefringent samples create additional polarized rays that either pass through (parallel to the privileged transmittance plane) or interact at the Analyzer by wave interference. A Full Wave Retardation plate or Compensator enhances weak birefringence and induces interference colors that can be interpreted using a M-L Chart (Berkeley). When imaging

with PLM, the sample is usually colorful and the background is dark. PLM is good for analyzing crystalline samples or samples that are birefringent such as cellulose, fibers, and starch and is not good for visualizing isotropic and transparent samples.

Phase contrast microscopy also known as PCM is a microscopy imaging technique that utilizes wave interference to visualize samples by comparing the difference between sample optical path length (OPL) and background OPL (Ruzin). Contrast is generated via wave interference between rays passing through a sample vs. background. Light rays passing through the sample (higher refractive index) are retarded in phase relative to the background light and are refracted. When recombined at the intermediate image plane, wave interference occurs and contrast is generated (Berkeley). For PCM, there are two required optical components: the phase annulus (in the condenser) and the phase plate (at the objective's back focal illumination plane) (Ruzin). PCM is good for imaging tiny isotropic transparent samples. PCM is also a good microscopic technique used to identify bacteria and endospores.

Epifluorescence microscopy also known as widefield fluorescence microscopy is a technique of using intrinsic or introduced fluorescence reporter molecules to identify the presence and/or amount of target molecules in situ (Ruzin). With the idea of fluorescence, by exciting either intrinsic or applied fluorophore in a sample through a filter, we can visualize fluorescent samples on a dark background. To do epifluorescence microscopy, the components required are a high-intensity light source (laser), excitation filter, dichroic mirrors, and emission filter. Epifluorescence microscopy is good for visualizing fluorescent probe molecules and locating where target molecules are in our sample.

For this project, we used these microscope techniques– brightfield, PLM, phase contrast, and epifluorescence microscopy –to determine the composition of unknown sample #20. Using these microscope techniques, it was determined that the unknown powder sample was made up of cellulose, cotton fiber, and yeast.

**FINDINGS/RESULTS**

For this project, I did every microscopy technique but the bacteria test using PI probe with epifluorescence microscopy and the endospore test using SYTO probe with epifluorescence microscopy. Thus, I used stereoscope, brightfield microscopy, PLM, PCM, and epifluorescence microscopy with calcofluor and SYTO probe as well as autofluorescence. While I did do all of these microscope techniques, I did not find brightfield microscopy with IKI stain and epifluorescence microscopy for autofluorescence to be that useful in identifying the components found in my unknown sample. I will talk more about the why and what of these techniques that I did not find that useful in identifying the components in my sample in the discussion portion.

The first microscope technique that I used was with stereoscope. The stereoscope allowed me to do a gross examination of my sample. Using the stereoscope and focusing, I was able to see three sorts of things (Image 1). One thing was a thin string which made me infer that it was a fiber. Another thing was shiny and small which I inferred was something I could verify using PLM. The last thing was an off-white, grainy, sand-like substance. With this in mind, I believe that there are at least three different components in my sample.

After using the stereoscope, I used brightfield microscopy and saw four things (Image 2 and 3). One of the things was these circular cells that were slightly green and 5um wide. These circular cells were seen individually as well as in a variety of size clumps, however, I did infer that they were all the same as they were made of the same smaller individual cell. In Brightfield, I also saw rectangular-shaped, rainbowy, transparent objects which I later used PLM to verify that it was cellulose as cellulose is also rainbowy and rectangular. The third thing was dark,

multicolored grain-shaped things. The fourth was the long wire-like thing which supported my initial thought of my sample containing some sort of fiber.

With the thoughts I had in mind of samples containing fiber and cellulose due to the similar shape and color seen in BF and gross examination, I did PLM next and verified that this was true. Because PLM is good for identifying crystalline samples or samples that are birefringent such as cellulose, fibers, and starch, this was a good technique to verify that my sample did contain cotton fiber and cellulose. When I visualized my sample with PLM, I saw orange and blue french fry-shaped objects that matched with Lab 4 PLM drawing of cellulose (Image 4). I also saw rainbowy colored wires which also matched Lab 4 PLM drawing of cotton fibers (Image 4). Additionally, I did not see any bright tiny things at 100X PLM which would be a good indicator of endospores. Because of this, I did not do the endospore test using the SYTO probe with epifluorescence microscopy. With PLM, I was able to verify that my sample did contain cellulose and cotton fibers.

While I was able to verify that cellulose and cotton fibers were in my sample using PLM, I still did not know what my third component was as it did not show up in PLM. To identify the third compound, I used PCM and fluorescent probes (calcofluor and SYTO) with epifluorescence microscopy and figured out that it was yeast. With PCM, I saw spherical cells that were purple and green. Because of this, I initially thought it was *E. coli* due to its color and round cell shape, however, after thinking about it some more, I realized that it was too big to be *E. coli* which is usually 2 um in diameter while what I saw was 5 um in diameter (Image 5). With this in mind, I did not perform PI probe with epifluorescence microscopy as that helps with identifying bacteria, not yeast. With bacteria out of the picture, I probed my sample with calcofluor and SYTO separately and visualized them with epifluorescence microscopy with excitation light UV and blue light respectively. For calcofluor, I clearly saw blue spherical cells with budding scars. This verified that my sample contained yeast (Image 6). As for SYTO, my yeast cells were circular and green which matched what was seen from Lab 9 which further verified that my sample contained yeast (Image 7).

**DISCUSSION**

As I mentioned a bit in my results, I did perform brightfield microscopy with IKI stain (Image 3) and epifluorescence microscopy for autofluorescence, but did not find them useful in identifying my sample. The brightfield microscopy with IKI stain is good for identifying starch which would appear black once stained with IKI. However, when I performed this IKI stain, I did not see any black substances which verified that I didn't have starch but was not useful in identifying what my sample did have. As for epifluorescence microscopy for autofluorescence, I did not see anything noticeably autofluorescence such as seeing red things like *Spirulina* in green excitation light. Thus, this was also not helpful in identifying what was in my sample.

What was useful for determining what my unknown sample was made up of was BF, PLM, PCM, and epifluorescence microscopy with calcofluor and SYTO probe. With BF microscopy, I was able to get a good idea of how many components were in my sample as well as make inferences of what those components were. For instance, with BF, I was able to see a long wire component which was most likely some sort of fiber. Thus, inferring this, I visualized my sample with PLM as it is good for identifying fibers and was able to verify that it was cotton fiber. I used this similar thought in identifying cellulose. As for yeast, it was a little more complicated. With PCM, I was able to verify that I didn't have *E. coli* as my sample did not have cells that were the same size as *E. coli*. In addition, PLM also verified that I didn't have endospores as I did not see any bright star-like things in 100x magnification with PLM. Because

of this, I was able to not perform PI probe and endospore testing. With the process of elimination, I then tested if my sample had yeast with calcofluor and SYTO probe and verified that it was indeed yeast. This was the process of thought that I did with the microscopes to identify what was in my unknown sample.

## CONCLUSION

Using stereoscope, brightfield microscopy, PLM, PCM, and epifluorescence microscopy, I was able to determine that my sample was made up of cotton fibers, cellulose, and yeast. I used Brightfield and PLM to verify that cotton fibers were in my sample. I used Brightfield and PLM to verify that cellulose was in my sample. I used Brightfield, PCM, and epifluorescence microscopy with calcofluor and SYTO probe to verify that yeast was in my sample. While I didn't do PI probe with epifluorescence microscopy and endospore test using SYTO probe with epifluorescence, I did not find it necessary as I didn't find any indication of them using prior microscope techniques. For instance, using PCM, it would be promising to find endospores in my sample if I could see bright-star like things at 100X magnification with PCM, however, I did not see anything like that which verified that I didn't have any endospores and it would be not necessary to do the endospore test. Thus, in summary, using microscopic techniques and my knowledge about them, I was able to determine that my unknown powder sample was made up of cellulose, cotton fibers, and yeast.

## REFERENCES


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**IMAGES**

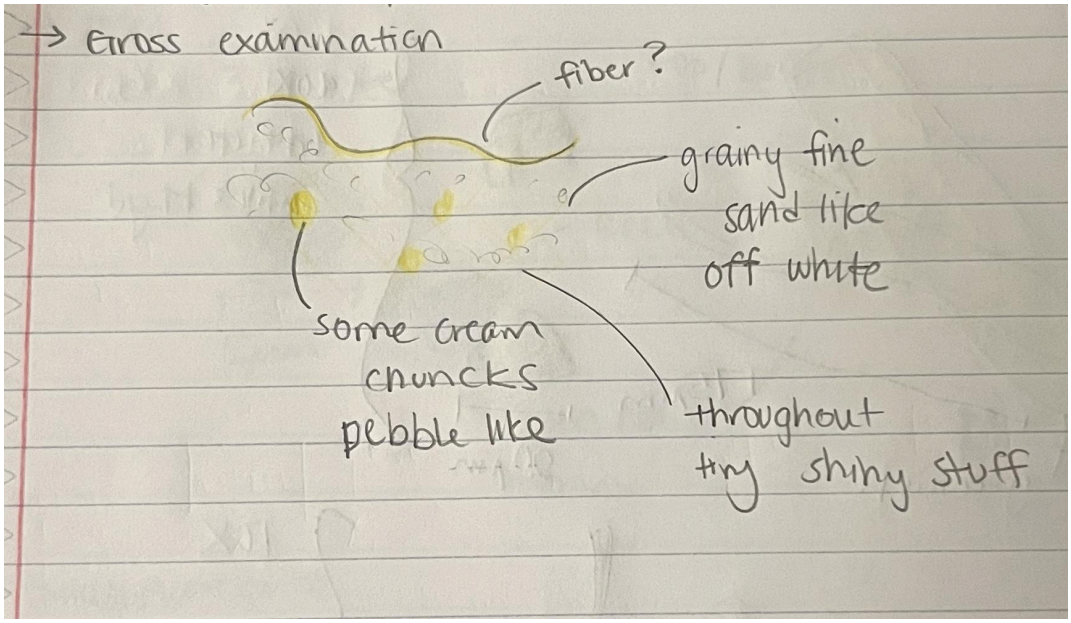


IMAGE 1. My sample #20 under the stereoscope. I identified three things: long string thing, grainy off-white sand-like powder, and something shiny and tiny.

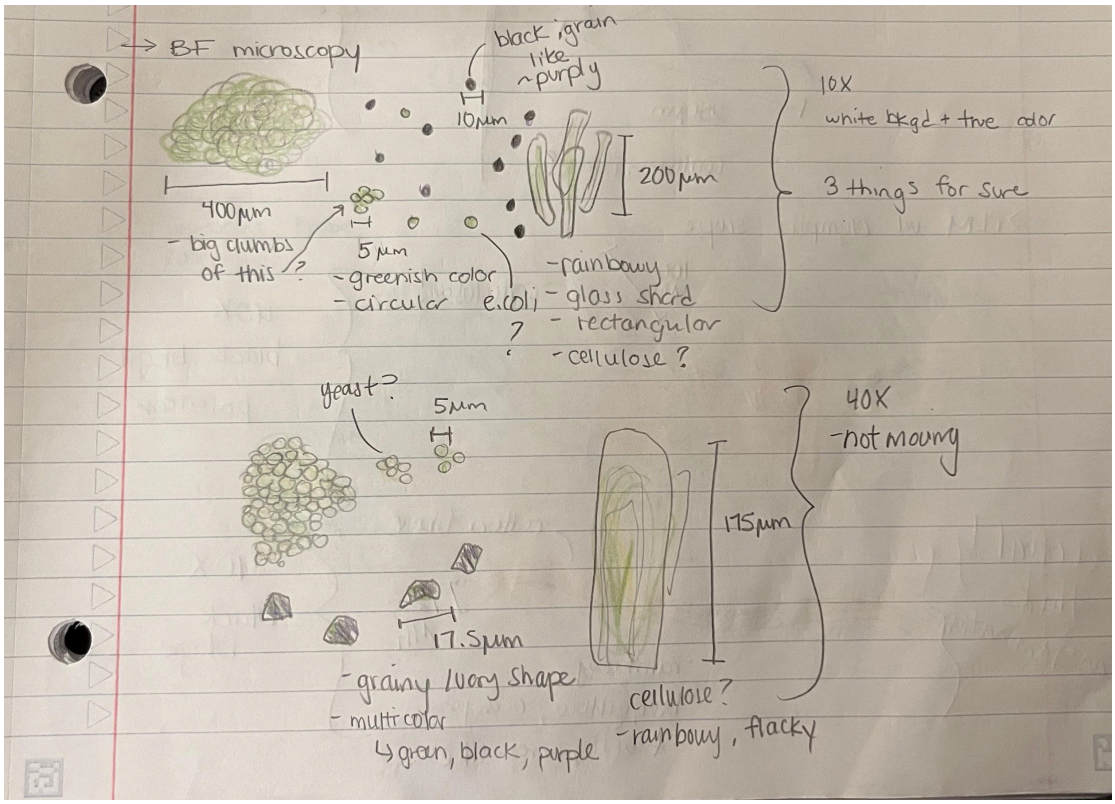


IMAGE 2. Brightfield microscopy at 10x and 40x magnification.

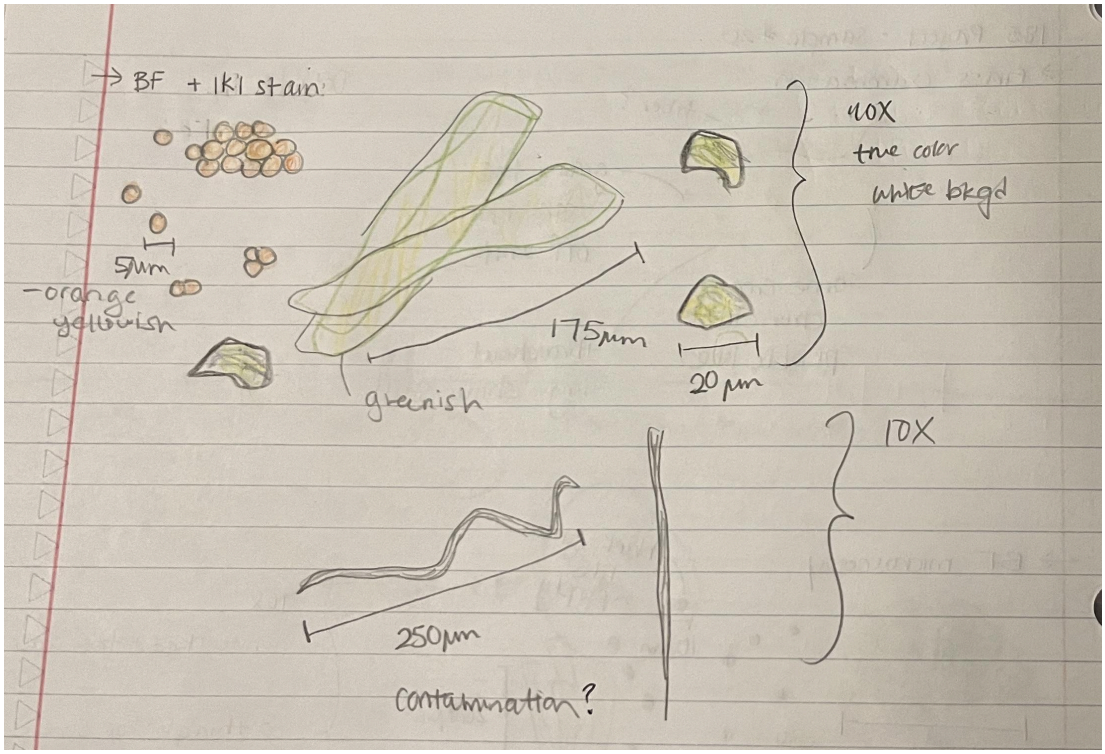


IMAGE 3. Brightfield microscopy of the sample with IKI stain. Saw no black stuff aka no starch in my sample. Also saw long wire things that were later verified to be cotton fiber using PLM.

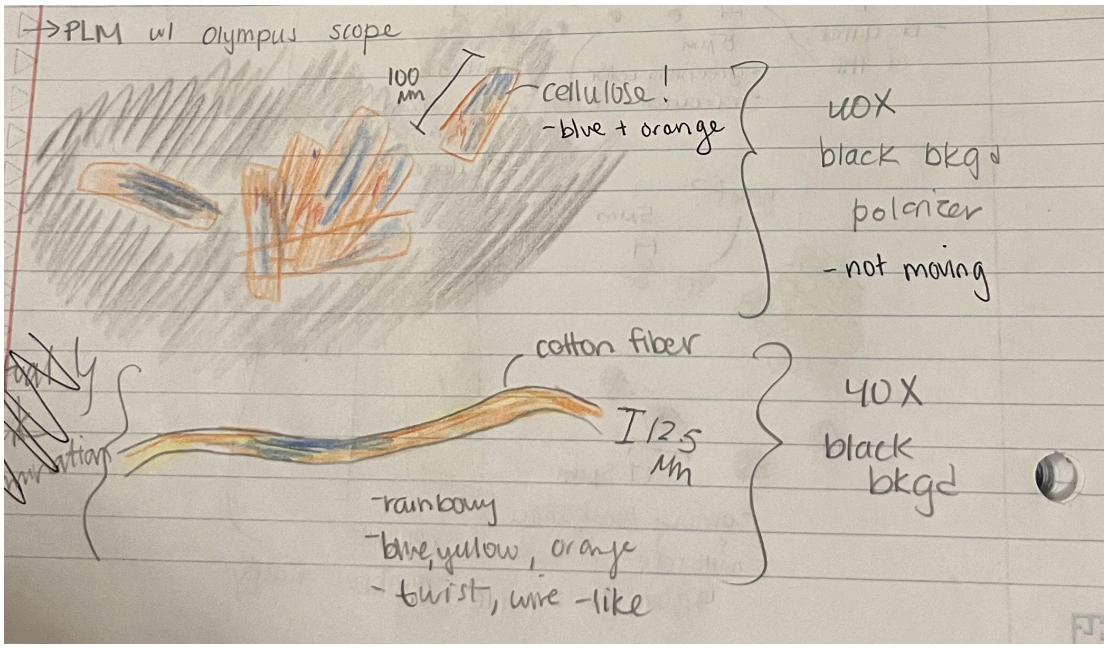


IMAGE 4. My sample under PLM verified that my sample contained cotton fibers and cellulose as what I saw matched with the descriptions of cotton fibers and cellulose from Lab 4 using PLM.

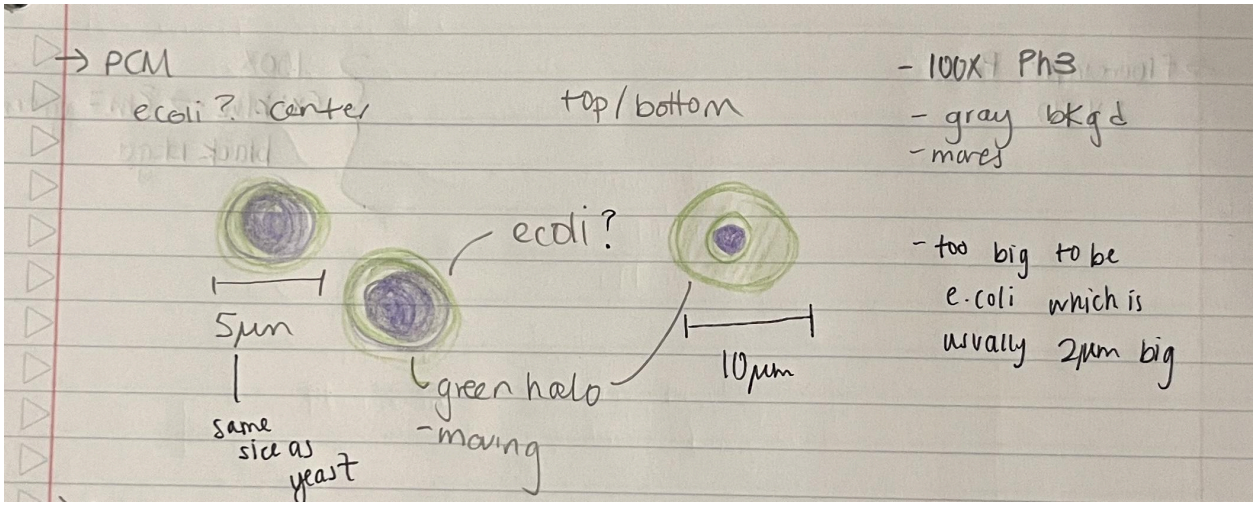


IMAGE 5. Sample visualized with PCM. Saw circular cells that were green and purple like *E. coli* from Lab 5, however, the size of the cells didn't match up. In the sample, the cells were 5um in diameter vs 2um in diameter for *E. coli* in PCM from Lab 5.

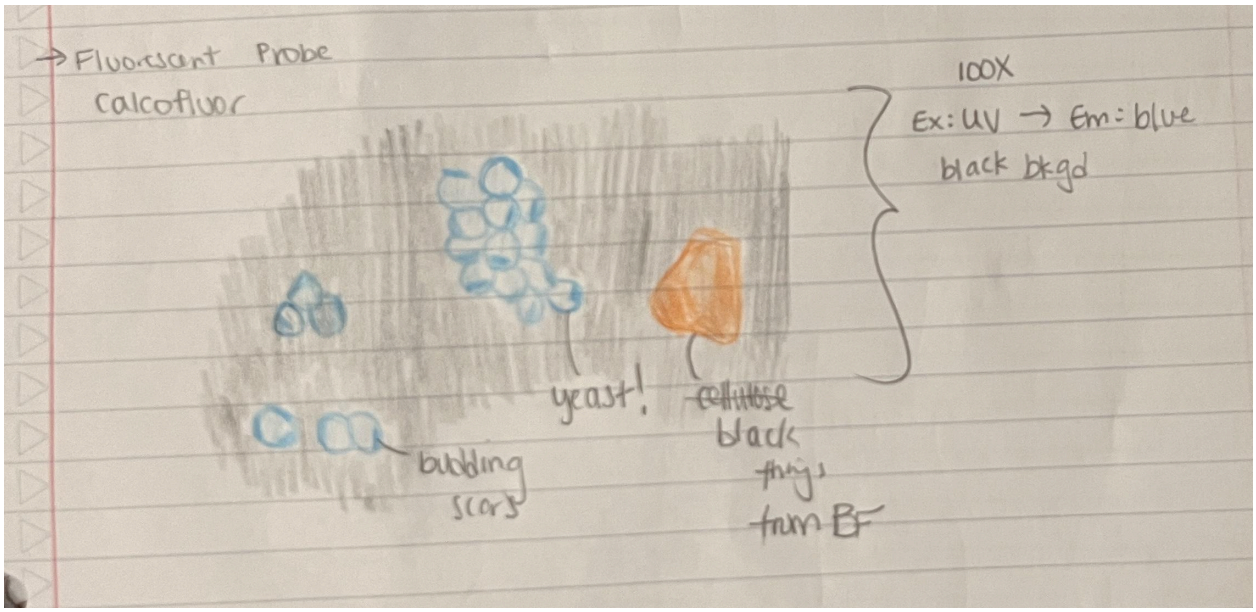


IMAGE 6. Sample probed with calcofluor and visualized with epifluorescence microscopy. This verified that my circular cells were yeast as they looked blue and had budding scars like yeast does when probed with calcofluor.

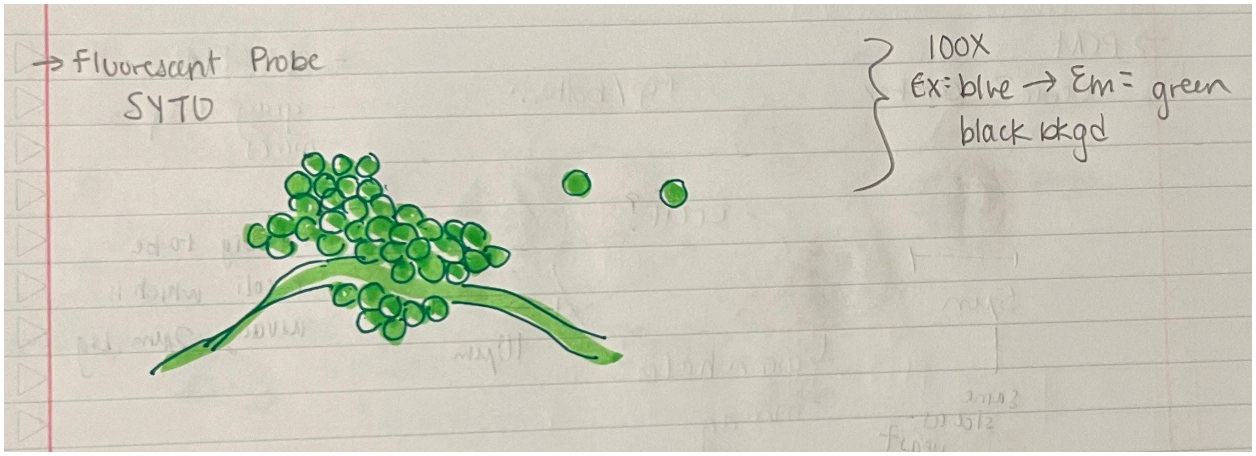


IMAGE 7. Sample probed with SYTO and visualized with epifluorescence microscopy. This also verified that my circular cells were yeast as they looked green and circular like yeast does when probed with SYTO from Lab 9. The long green thing was probably the cotton fiber fluorescing green.