Oil Immersion Microscopy Protocol (Biotech Series)

• Utilizing aseptic technique, smear a loopful of a bacterial sample from a pure culture onto the center of a clean slide. If loops are not available a toothpick works fine. You only need a small sample; a tiny bit of broth from a liquid culture or a light touch from a single colony on an agar surface contains more than enough bacteria. If the sample is from a slant or appears very thick, add a tiny drop of water.

• Smear the sample in a circle on the slide, while being careful to stay within the center 1/3 of the slide. Particularly with the moistened sample from the agar slant, care must be taken to see that the bacteria from the colony are separated from each other so they may be observed individually. This may take rather vigorous action with the loop as you smear onto the slide surface. **If you see dense particles, or a very turbid smear, you have chosen too large a sample.**

• Mark an "X" with a grease pencil to show the side of the slide with the bacteria. You would be surprised to know how many students turn the slide over during the procedure and inadvertently stain the wrong side, or try to observe the wrong side with the microscope. The oil immersion lens will not focus of the bacterial cells if you have placed the slide on the stage smear side down. If you are not sure, you can scratch the "X" mark to determine if you are on the correct side with staining or observing procedures. You should not be able to see the smear itself. If frosted slides are available, you can easily write the name of the organism on the frosted section.

• Let the bacterial preparation on the slide dry for a few minutes. Then, hold the slide (smear-side away from the heat) near the tip of the Bunsen burner flame for a second or two, passing the slide over the top of the flame in sweeping motions to avoid overheating. Caution: A greater length of time would heat the slide to the breaking point. This procedure is known as **"heat-fixing."** It kills the bacteria and causes them to adhere to the slide.

• Let the slide cool. During this time obtain the appropriate stains for the slide depending on the staining technique you are using.

Gram Stain				
Step	**Reagent**	**Time**	**Gram +**	**Gram -**
Primary Stain dH_2O Rinse	Crystal Violet	1 min	Purple	Purple
Mordant dH_2O Rinse	Lugol's Iodine	1 min	Purple	Purple
Decolorize dH_2O Rinse	95% Ethanol	≤ 15 sec	Purple	Clear
Conterstain dH_2O Rinse	Safranin	2 min	Purple	Red or Pink

• To perform a Gram stain follow use the reagents listed above in the order presented. Put 2-3 drops of a stain on the smear and let it sit for the time intervals indicated. Rinse very *gently* with distilled water or 95% Ethanol as indicated. Make sure not to squirt your rinse directly on the smear, instead squirt it at the top of the slide and allow the rinse to gently flow over the sample to rinse.

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• Blot your smear dry between sheets of bibulous paper. Be careful not to use too much pressure as this will break the slide; a “wiping” motion will remove the smear, simply blot up and down on the sample with light pressure.

Oil Immersion (background)**:** Viscosity of immersion oil is mostly a matter of individual preference of the microscopist. Low viscosity oils are more likely to creep if applied over-abundantly. The low viscosity is preferred by some when the distance from the cover glass to the objective is very small. Low viscosity oils are less apt to retain small bubbles. Higher viscosities are the most commonly used oils. The higher viscosity oils fill the larger gaps more satisfactorily and are also reusable in that a second slide can be positioned and swung into place and contact made with the oil drop remaining on the objective lens. The high viscosity gives more latitude with oiled condensers since it will fill a larger gap without "breaking" and making re-oiling unnecessary. You will need to choose which viscosity works bests under different conditions. Both types are available for you.

* Locate sample with the lowest power objective, and work your way up through the various magnifications, making sure to focus your field on your sample with each lens. Once you have adequately found and focused on your sample with the 40x objective lense, move on to the oil immersion lens.

*Swing the oil immersion lens halfway to its final position, so that an open space between the lenses is over your sample.

• Place a drop of immersion oil (low or high viscosity) onto the coverslip of the slide where the objective will be positioned. Please see **figure 1** before moving to the next step.

• Slowly rotate the oil immersion objective to come in contact with the bead of oil on the slide. (If there are air bubbles or the image is poor, there may be air caught between the lens and the slide. Rotate the objective back and forth to remove the captured air from the front lens).

• **Focus on the specimen using the fine focus only**. Do not use the coarse focus. The slide and lens are very close and contact between may damage the oil immersion lens.

• If you want to change slides rotate the nosepiece to the lowest power objective or empty slot before removing the slide.

* When you are finished, remove the slide as in the previous step. To clean the lens, take two sheets of lens paper, add **very small** amount of turpentine (or another acceptable solvent) to wipe extra oil off of the lens. With the dry portion of the lens tissue, dry off any excess turpentine.

• The oil immersion lenses need to be cleaned to prevent oil from attracting dust, or evaporating and leaving crystals on the lens.

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• Take great care to not rotate the 10x, 20x, or 40x non-immersion objectives into the oil. The oil will damage the optical quality of the dry objective lenses.

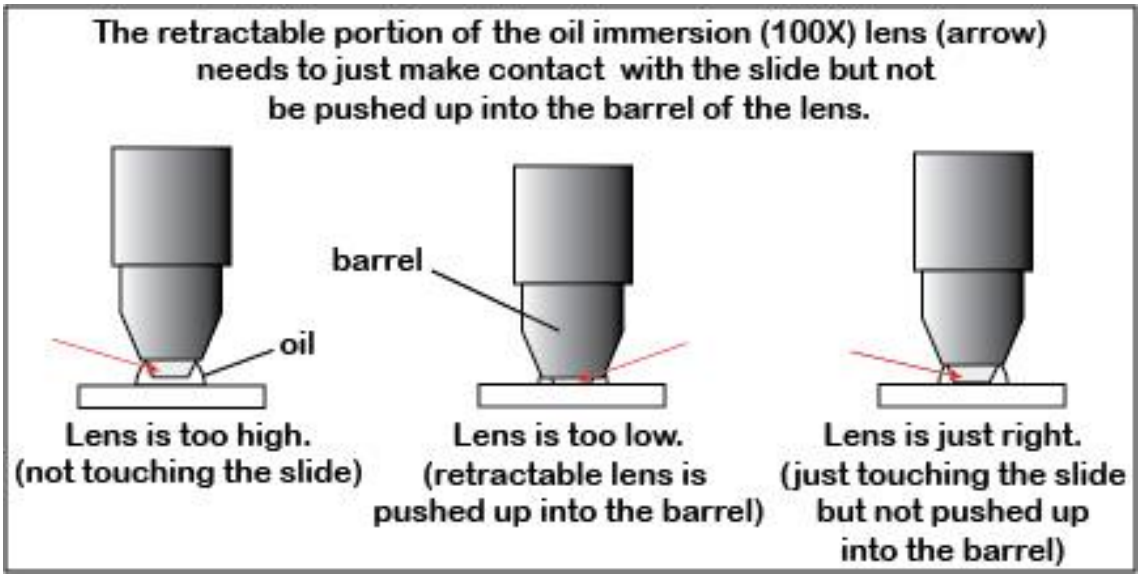

Figure 1: Schematic diagram illustrates the correct method and position for oil immersion microscopy.