**MCB2010L –Microbiology Lab**

**Week 2: Darkfield and Phase Microscopy**

* Darkfield Microscopy
	+ Simple yet effective technique used to observe live and unstained specimens
	+ Only light scattered by the specimen goes on to produce image, whereas directly transmitted light is omitted (giving the appearance of a dark background)
	+ How to prepare scope: remove blue filter, attach dark field adapter
	+ Make wet mount of pond water, observe
* Phase-Contrast Microscopy
	+ Able to differentiate the transparent protoplasmic structures and enhance the contrast between a cell and its surroundings without staining
		- Staining usually results in cell death
	+ Utilizes phase-shift of light’s wavelength
* Both of these microscopic techniques are used to observe motility in live specimens

**Motility**

* Motility Determination
	+ Major organelles of motility in bacteria are flagella (some have it, some do not)
		- Allows cells to move toward nutrients or away from harmful substances
	+ Motility and arrangement of flagella around the cell are important taxonomic characteristics that are useful in characterizing bacteria
		- Differentiating between true swimming motility and Brownian motion
			* Brownian motion is movement that is due to molecular bombardment or currents under cover glass of slide, not true movement
	+ 2 methods we will use today:
		- Wet mount
			* Look at slide immediately so it doesn’t dry out



* + - * Focus – make sure condenser is down, focus on edge of air bubble, focus on 40x, bacteria will be right outside air bubble
			* Bring condenser up, push the phase-contrast adapter into condenser
			* Advantage: immediate; Disadvantage: can dry fast, may have to handle pathogenic bacteria
		- Culture method
			* Using semi-solid agar
				+ Be sure not to shake and disturb agar
				+ Inoculate agar with specimen (straight in, straight out)
				+ Incubate at **room temp**
			* Advantage: don’t have to handle wet mount of possible pathogenic bacteria; Disadvantage: takes time to incubate

**Activitites**

1. Learn how to change from Brightfield to Darkfield and Phase Contrast.
2. Making wet mount slide using pond water, observe and compare between Brightfield to Darkfield and Phase Contrast.
3. Inoculate *Staphylococcus aureus* and *Pseudomonas aeruginosa* into motility agar. Observe the result the following week.
4. Making wet mount slide with *Staphylococcus aureus* and *Pseudomonas aeruginosa* and observe the motility using Phase Microscopy.