User guide for biofilm growth for SPIM imaging and FCS

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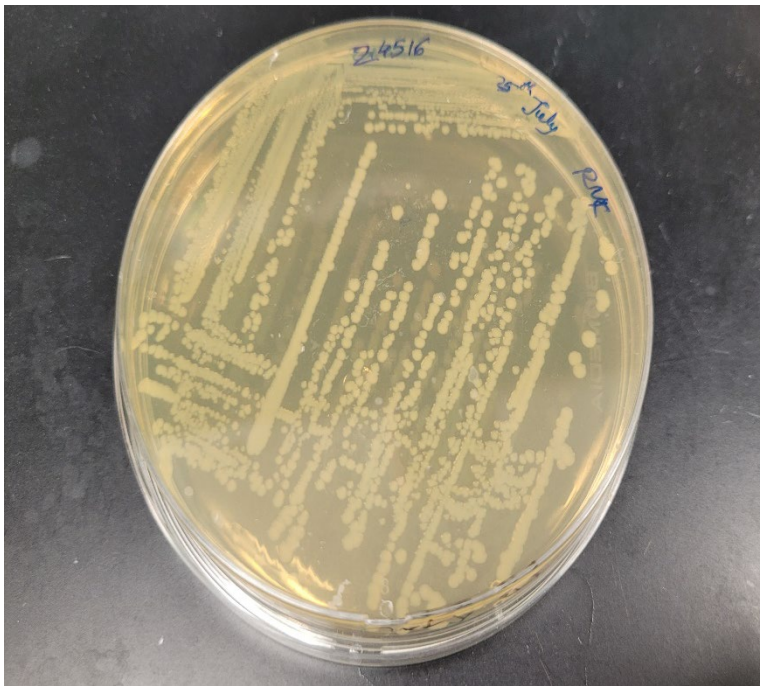
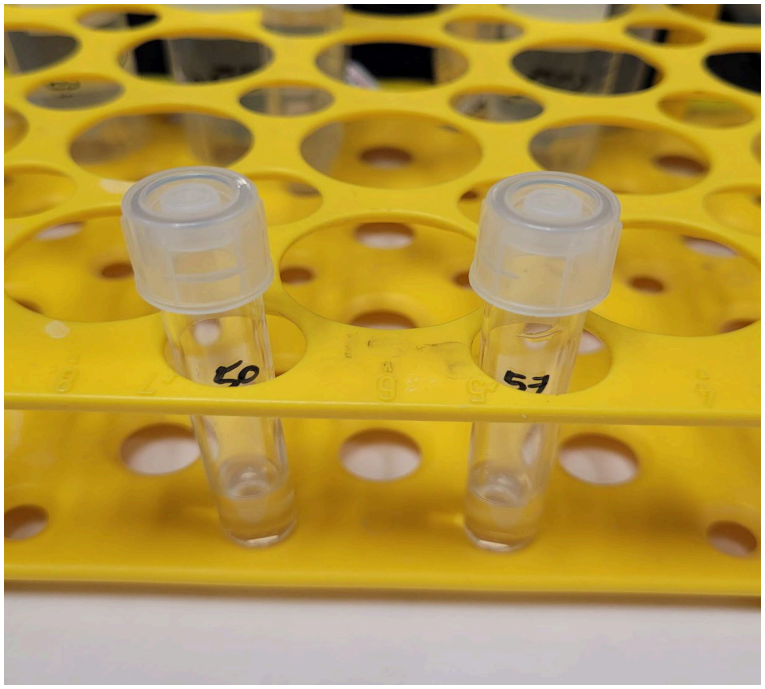
Step 1: Streak bacterial strains from glycerol stock on LB Agar plates. Incubate overnight so that isolated colonies are obtained.

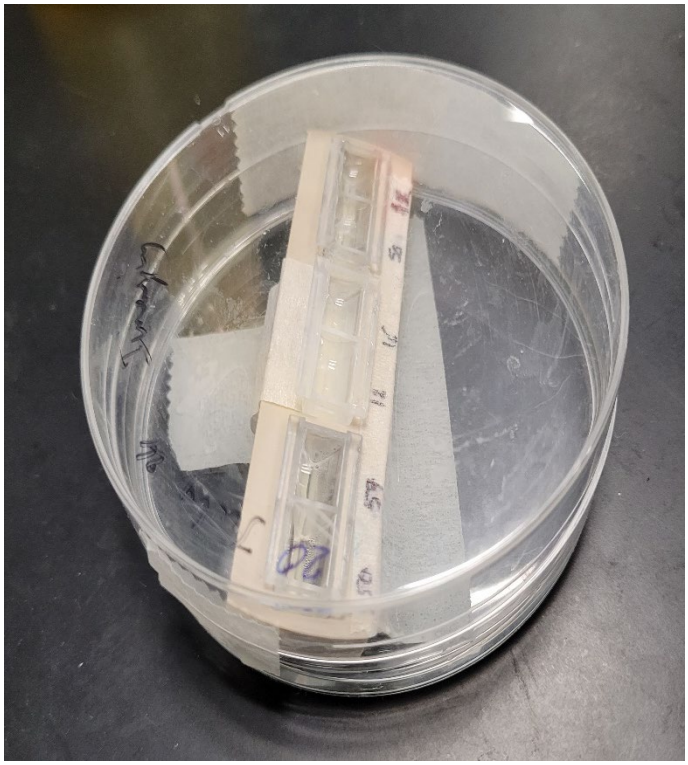


Step 2: Inoculate a single bacterial colony in 1 ml broth in culture tube.



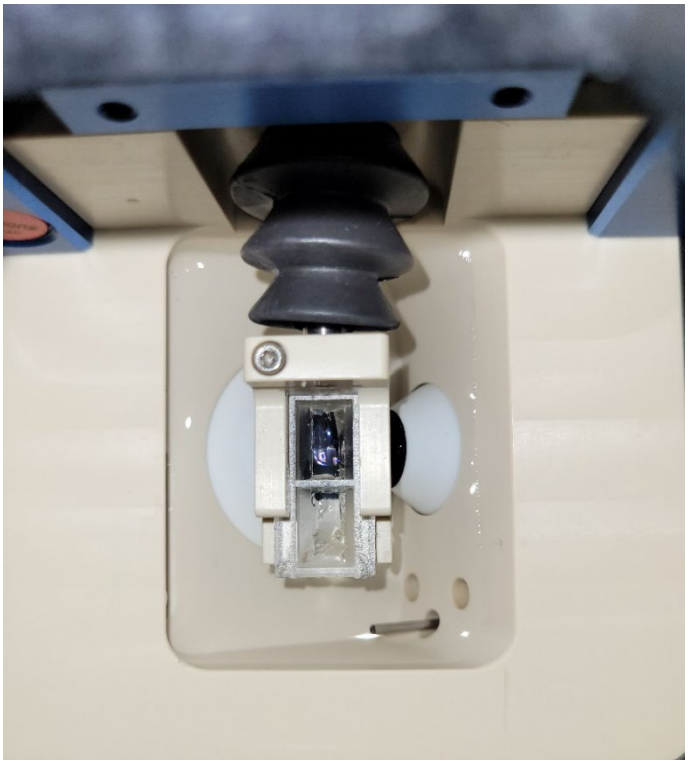
Step 3: Incubate the tubes in shaking incubator (180 rpm) for overnight growth of 14 to 18 hours, with temperature settings as per requirement for bacterial strain.

Step 4: After checking OD (~1.5 to 2 usually), dilute the cultures to about 0.1 OD (10x dilution) in the following sample chamber for 3 days. Before incubating, make sure the sample holders are kept tilted for the biofilms to grow on one side only.



Step 5: After 3 days, remove the excess broth and wash the chambers once with 1x PBS solution, to get remove excessive planktonic bacteria. Add fresh 1x PBS solution (~ 200µL).

Step 6: Mount the sample chamber using the sample holder as shown below. The side with biofilm growth should be kept towards the illumination objective to obtain better visualization.



Refer to the CBIS user manual for the microscope for imaging procedures and FCS measurements.