Poly-L-Lysine

- Cationic charges electrostatically binds anionic surfaces of glass and specimen
- Poly-D-Lysine may be used if there are proteases in the sample
- Use PLL stock containing polymers in the range of 70-150 kDa
- If creating own stock, make 1% (w/v) solution with ddH_2O
- Specimen may flatten and morphology may be affected depending on cell

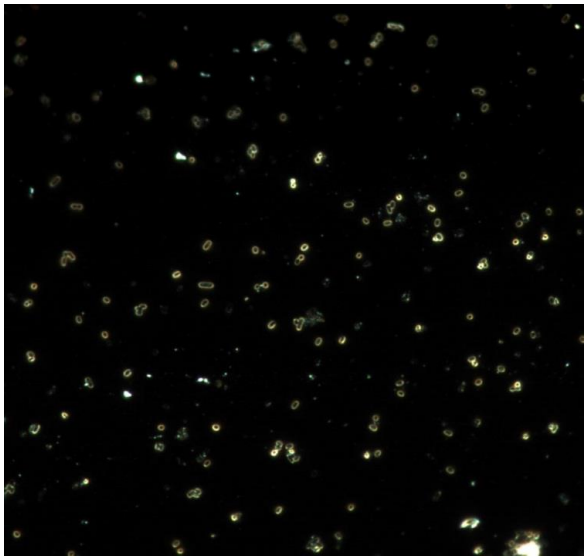
To make poly-L-lysine coated slides:

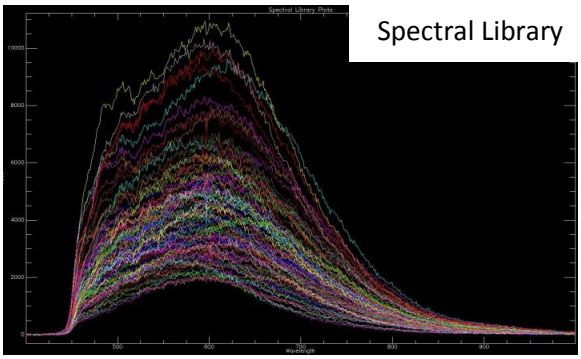
1. Make a .1% (v/v) working solution of poly-L-lysine (PLL) with ddH_2O or PBS. It's best if this solution is made immediately prior to use. Keep stock in -20ºC for up to 2 years.
2. Coat cleanroom slides thoroughly (shaker would be ideal) with PLL solution and let air dry for about 5 minutes at room temperature. Let dry in a low-dust environment, such as a laminar flow hood. Slides will stay good stored at 4ºC for up to a year.
3. Depending on the sample, it may also be desirable to coat coverslips with PLL as described above.
4. Retrieve slides from 4ºC immediately prior to use. The fixative appears to be less effective if the slides are left out at room temperature.
5. Vortex sample, drop 2.0 µL or less onto the PLL-coated slide, and add a coverslip. Depending on the sample, you may need to wait from 30 seconds to 10 minutes for the specimen to adhere.
6. Once all movement has ceased, sample is ready for optical and hyperspectral imaging.

*Note: PLL-coated slides and coverslips are commercially available, however are usually more expensive than homemade slides. It is advantageous to purchase these slides if possible. It is easier to contaminate the homemade slides with dust and particulates during the drying process, and the homemade slides typically don't last as long as the commercial slides.

E. coli Example

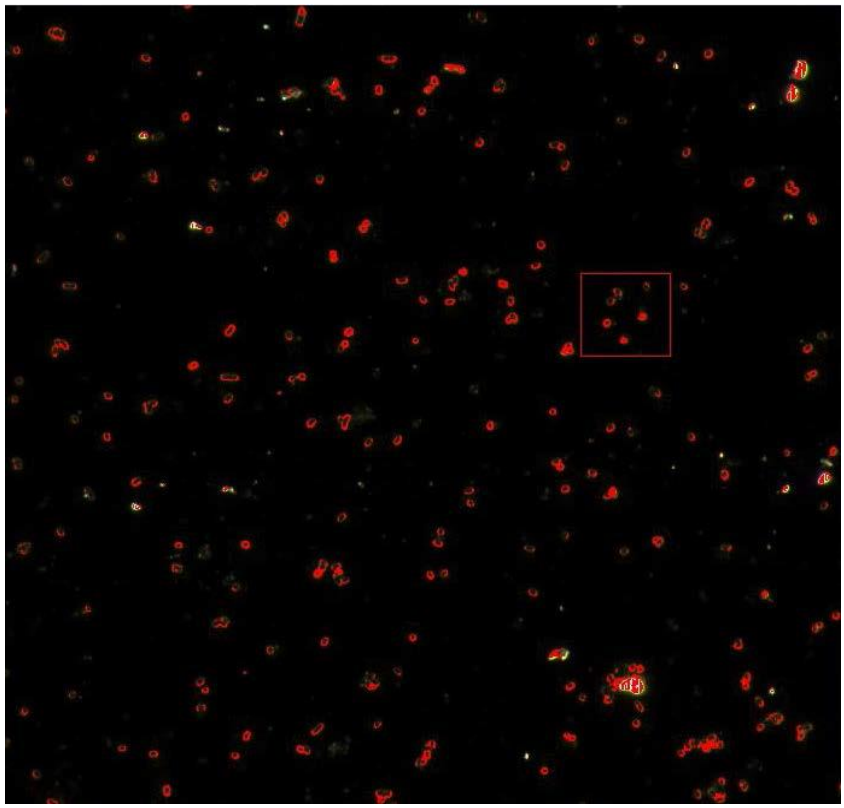
Optical Image





Spectral Library

Hyperspectral Image with Spectral Angle Mapper Overlay



This is an example of the efficacy of poly-L-lysine (PLL) in the imaging of live bacteria. These *E. coli* (live) were plated on a PLL-coated slide and allowed to settle for about 5 minutes. Ordinarily, live bacteria are very active which poses implications for hyperspectral imaging (HSI). The sample must be completely still during HSI or else the image will come out blurry. PLL coated slides/coverslips can alleviate this issue.

Every spectrum in the *E. coli* spectral library (left) was compared to the spectra in the image, and all pixels whose spectra matched were mapped red, indicating the presence of *E. coli*. The spectral libraries may be modified to identify bacteria of a certain phase, serovar, etc.