Using Interferometric Reflectance Imaging Sensor (IRIS) to Image Viral Particles

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Literature Search:
Antibody Criteria:
- Responds to whole virus: Influenza A/PR/8/34.
- Specific to hemagglutinin or neuraminidase on viral envelope.
- Has previously worked in a gold phase ELISA assay.
- 2 different goat poly-clonal antibodies (17650-78B and 17650-05E) from US Biological.

Prepare the Si/SiO₂ chip:
- Spot antibodies on Si/SiO₂ chip previously coated with polymer.
- Prewash, wash, block, wash, & dry the chip.
- Pre-Image spots with IRIS.
- Wash & dry the chip.
- Post-Image spots with IRIS.

Image the chip:
- Images taken before (pre-image) and after (post-image) viral incubation.
- Images taken with two Interferometric Reflectance Imaging Sensors (IRIS):
  - The low-magnification IRIS.
  - The high-magnification single-particle IRIS.

Early Low-mag Data & Results:
Most spots lost height after the viral incubation, therefore virus must not have adhered to the antibody and some of the antibody itself may have been washed away.

Current Single Particle Data & Results:
More particles appear after incubation, but the binding is non-specific. Particles did not coat the entire spot, nor adhere to every spot. Another experiment is underway to minimize the non-specific binding and hopefully bind virus.

How the IRIS works:
The high and low-magnification IRIS work by shining LED light at a Si/SiO₂ chip. The light reflects off of the different layers on the chip causing interference. When additional materials (for example antibodies and antigens) are adhered to the chip, the resulting height difference changes how the light interferes. The CCD camera images the difference in how the light interacts, thereby indicating how much biomass is adhered to the chip.

Our Projects
Project One: Develop protocol for capture of H1N1 influenza virus with antibodies

Project Two: Address the focus offset on the SPI.

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Using Light to Gather Data:
In chemistry class light is used to identify elements through spectroscopy. Elements, when heated, release only certain wavelengths of light (a line spectrum) rather than the entire continuous spectrum. Therefore, by observing which colors of light are released, one can identify which element is being observed.

Students are going to observe the line spectrum of excited hydrogen gas and record the wavelengths of the light produced. Using these wavelengths they will calculate the energy of the photons emitted and their frequencies.

By the end of the unit students will be able to perform calculations relating wavelength, frequency, and energy, as well have have a good understanding of how these three variables relate and what colors of visible light are associated with them. Students will then work to connect this understanding to Bohr’s model of the atom.

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Data Analysis 1:
The first analysis compared the contrast between the intensity of specific particles to the intensity of the background at several height increments along the z-axis. The image with the most contrast is the background-focused image and the height of the virus-focused image.

Data Analysis 2:
The inconsistencies were caused by the stage moving unexpectedly along the z-axis. Once fixed, the contrast appeared to peak consistently at 1 to -0.5 microns away from the autofocus.

The SPI should focus on the viral particles adhered to the spot on the chip, however it is automatically focused on the Si/SiO₂ base of the chip. By analyzing the focus at several increments along the z-axis, the offset between where the SPI auto-focused (on the Si/SiO₂) and where the focus on the virus was clearest was determined.