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

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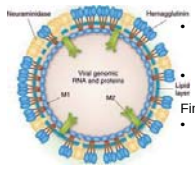
Develop protocol for capture of H1N1 influenza virus with antibodies.

Literature Search:



H1N1 Influenza Virus

Antibody Criteria:
- Responds to whole virus: Influenza A/PR/8/34.
- Is Specific to hemagglutinin or neuraminidase on viral envelope.
- Has previously worked in a solid phase ELISA assay.

Final Purchase:
- 2 different goat poly-clonal antibodies (17650-78B and 17650-05E) from US Biological.

Prepare the Si/SiO_2 chip:


Left: Si/SiO_2 chip

Below: pre-image of antibody spots on chip

- Spot antibodies on Si/SiO_2 chip previously coated with polymer.
- Prewash, wash, block, wash, & dry the chip.
- Pre-Image spots with IRIS.
- Incubate with H1N1 virus.
- Wash & dry the chip.
- Post-Image spots with IRIS.

Image the chip:


Left: low-magnification IRIS

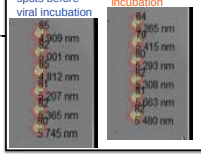
Below: high-magnification single particle IRIS

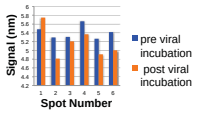
- Images taken before (pre-image) and after (post-image) viral incubation.
- Images taken with two **Interferometric Reflectance Imaging Sensors** (IRIS):
  - The low-magnification IRIS.
  - The high-magnification single-particle IRIS.

Early Low-mag Data & Results:

Pre-image: spots before viral incubation

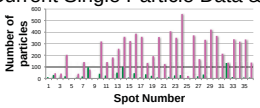
Post-Image: spots after viral incubation





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.

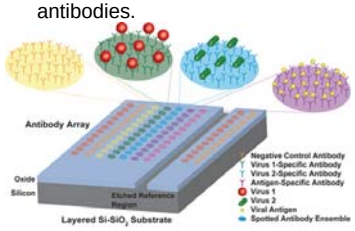
Current Single Particle Data & Results:



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.

Our Projects

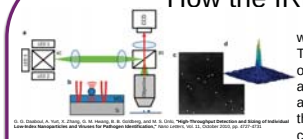
Project One: Develop protocol for capture of H1N1 influenza virus with antibodies.




The SPI
Image courtesy of Alexander Reddington

Project Two: Address the focus offset on the SPI.

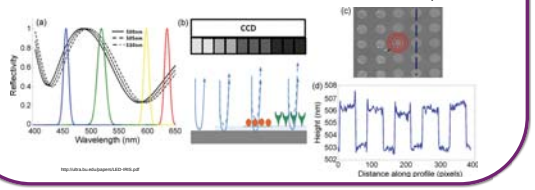
How the IRIS works



The high and low magnification IRIS work by shining LED light at a Si/SiO_2 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.

Above: High-magnifcation single particle IRIS

Below: Low-magnification IRIS

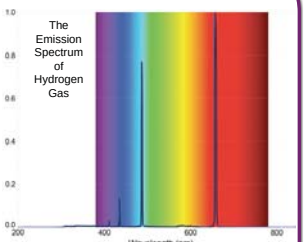


Using Light to Gather Data

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.



The Emission Spectrum of Hydrogen Gas

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.

The SPI. A Point-of-care device:

Point-of-care medicine:
- Faster results →more tests per day.
- Runs on a laptop battery → use in rural and rustic parts of the world.
- Easy to use → technician run.

The SPI (single particle IRIS) is an IRIS in a black box designed to sit in a doctor's office or rural medical clinic. A technician will place a drop of blood on a chip, place the chip in the SPI, hit a button, and patients will have diagnosis within minutes, instead of days or weeks.


SPI Si/SiO_2 chip

Layout of the SPI:

The SPI is a high-magnification single particle IRIS laid on its side equipped with a wireless router that can run on a battery.

A computer program acquires and analyzes the images, immediately confirming the attachment of nanoparticles (like viruses) on the surface of the chip.

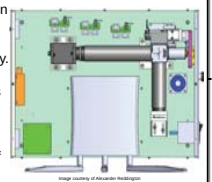
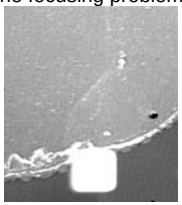


Image courtesy of Alexander Reddington

The focusing problem on the SPI:



The SPI should focus on the viral particles adhered to the spot on the chip, however it automatically focused on the Si/SiO_2 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/SiO_2) and where the focus on the virus was clearest was determined.

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 height where the SPI focused on the viral particle rather than the surface.

The hope was to find a consistent offset between the height of the auto-(background)-focused image and the height of the virus-focused image.

The data, however, showed the focus jumping all over the place.

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!

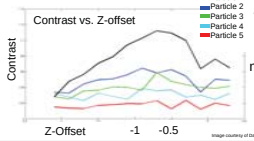


Image courtesy of David Freedman

The SPI is ready to be sent to Dr. John Connor's lab at the medical school to be used to image a number of different viruses.

Address the focus offset on the SPI.

Boston University Biophotonics Research Experiences for Teachers
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