BOSTON UNIVERSITY

Sizing of Viral Pseudotypes using the Interferometric Reflectance Imaging Sensor (IRIS)

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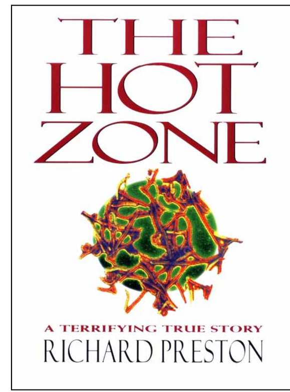
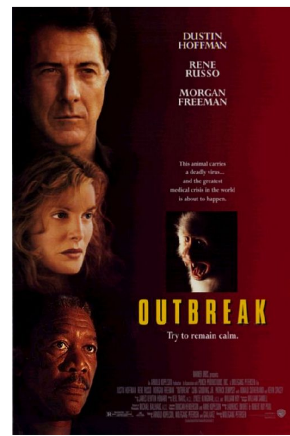
Introduction:





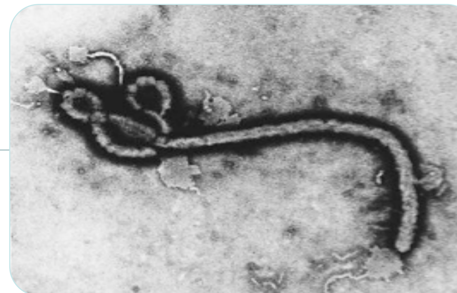
The ability to detect and study Viral Hemorrhagic Fever (VHF) viruses like Ebola and Marburg is particularly important in understanding their pathogenesis.

VHFs have a high mortality rate and are Category A pathogens (a concern as biological weapons). Cause of death is normally toxic shock, associated with fluid and blood loss into the tissues.

No FDA approved vaccines or antivirals exist against VHFs. The high mortality rate, gruesome prognosis and lack of a vaccine makes VHFs the subject of both fictional and non-fictional medical thrillers.

Currently, detection of these viruses takes many hours and is not portable. Samples must be shipped to the CDC in Atlanta. Faster, portable detection is one of many priorities when investigating Viral Hemorrhagic Fever.

EBOLA

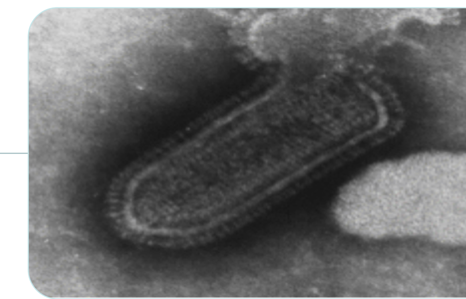


Fatal in 70-90% of all cases

Sporadic outbreaks since 1970s

Has caused lab fatalities from accidental exposure

VESICULAR STOMATITIS VIRUS (VSV)



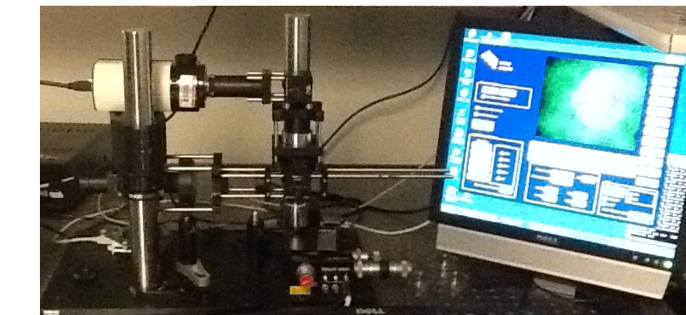
Indiana strain not a human pathogen

Outbreaks in cattle in US every 2-10 years

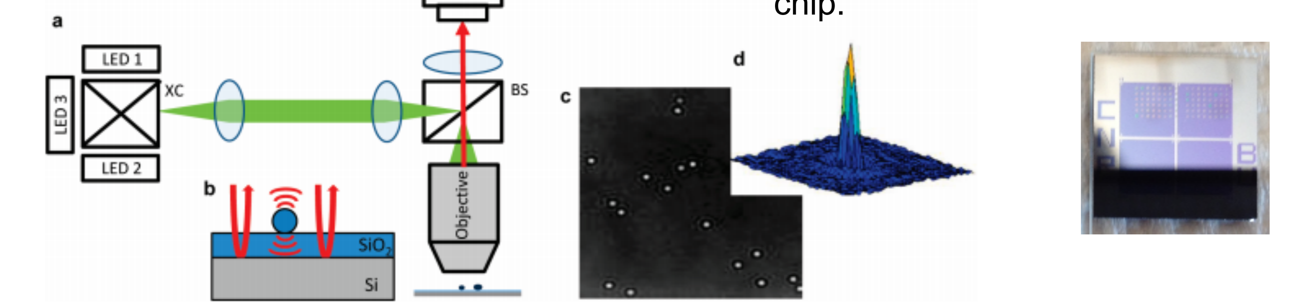
Has never caused a lab fatality from accidental exposure

Has been safely handled in 100s of labs over 50 years

How the IRIS works:



Above: The single particle SP-IRIS
Below: A schematic of the SP-IRIS
Right: A chip with antibody spots



The IRIS works 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 viruses) 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.

G. G. Daaboul, A. Yurt, X. Zhang, G. M. Hwang, B. B. Goldberg, and M. S. Ünlü, "High-Throughput Detection and Sizing of Individual Low-Index Nanoparticles and Viruses for Pathogen Identification," Nano Letters, Vol. 11, October 2010, pp. 4727-4731.

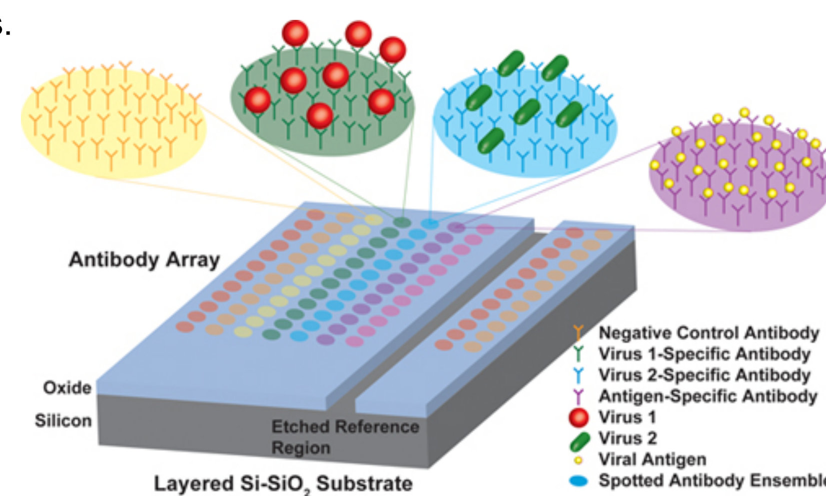
Long-term Goal:

Use the Interferometric Reflectance Imaging Sensor (IRIS) as an easy-to-use, portable, multiplexed viral detector

Viruses will be captured onto a small chip by antibodies.
-Antibodies are a well-established, high affinity way to capture viruses.

Visible light will detect virus binding.
-Visible light is cheap and uses very little power.

This is analogous to a small, label-free ELISA.



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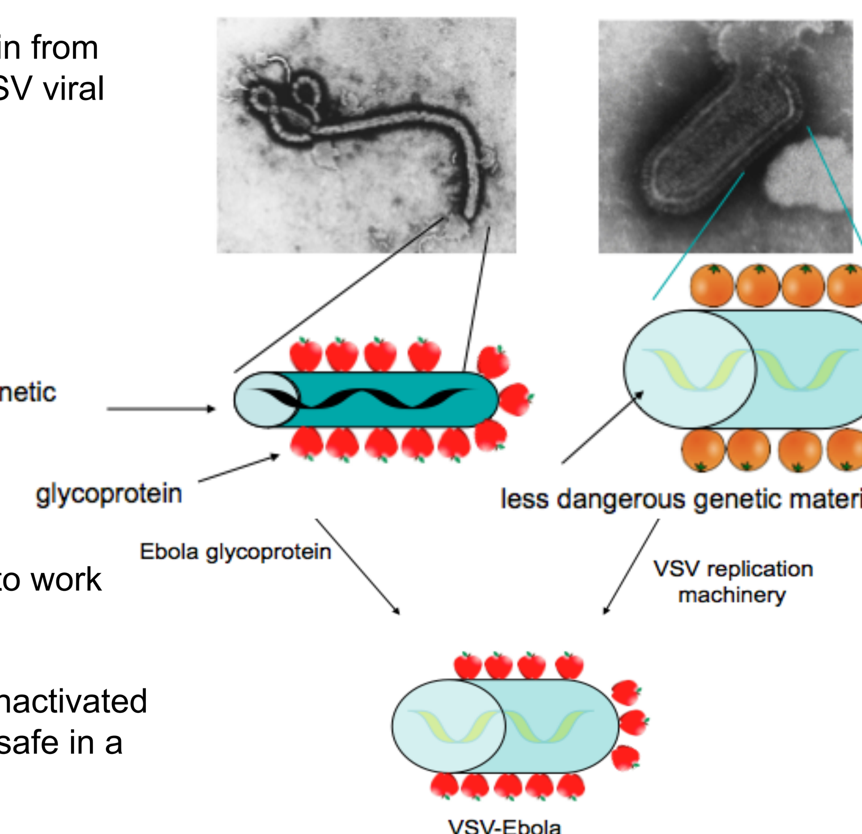
Solution: Viral Pseudotypes

Take the outside glycoprotein from Ebola and place it on the VSV viral particle.

The result looks a bit like Ebola and binds to antibody like Ebola, but this resulting pseudotype virus <u>does not contain any other part of Ebola!</u>

Viral pseudotypes are safe to work with at BSL2!

The pseudotypes are then inactivated with UV light to make them safe in a normal laboratory setting.



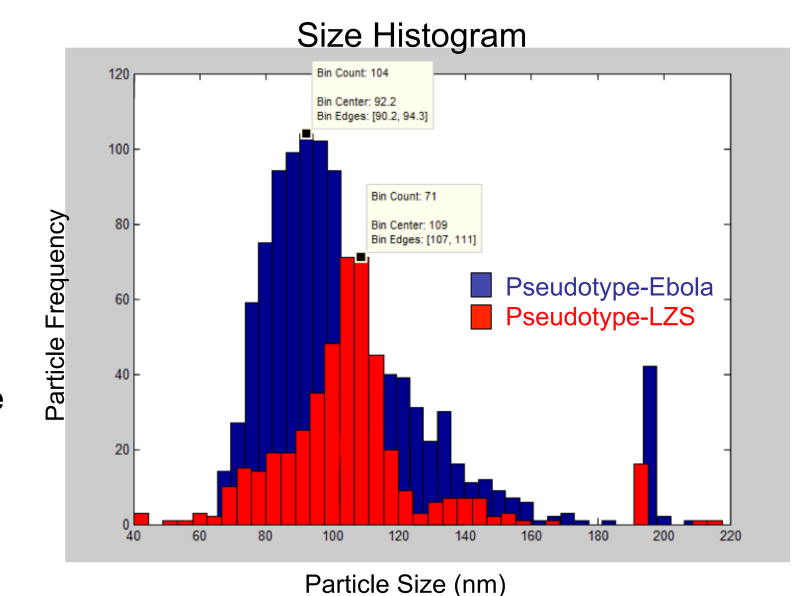
Results & Analysis:

This histogram shows the frequency of particles detected vs. the particle size.

The blue plot is pseudotype-Ebola.

The red is pseudotype-LZS.

The majority of pseudotype-LZS particles detected are larger in size than the pseudotype-Ebola particles. Since pseudotype-LZS expresses the glycoproteins of more VHFs the size difference makes sense.



The number of particles detected at a range of sizes which may be due to a focusing issue on the SP-IRIS so repeat experiments are being measured on a new SP-IRIS set-up that has more uniform focusing capabilities. Experiments are also being repeated with a number of different pseudotype combinations.

Hurdle:

Studying viruses that cause hemorrhagic fever requires biosafety level 4 (BSL4).

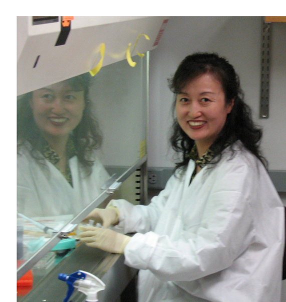
These are researchers in the BSL4 suits needed to work with VHF viruses.

Research done at BSL4 requires extensive protection including showering on the way in and out of the lab.

Progress can be slow and not all instrumentation is available in a BSL4 lab.

By using modified Vesicular Stomatitis Virus (VSV) to create a viral pseudotype we can study VHF viruses in a BSL2 environment, allowing for more collaborative research.

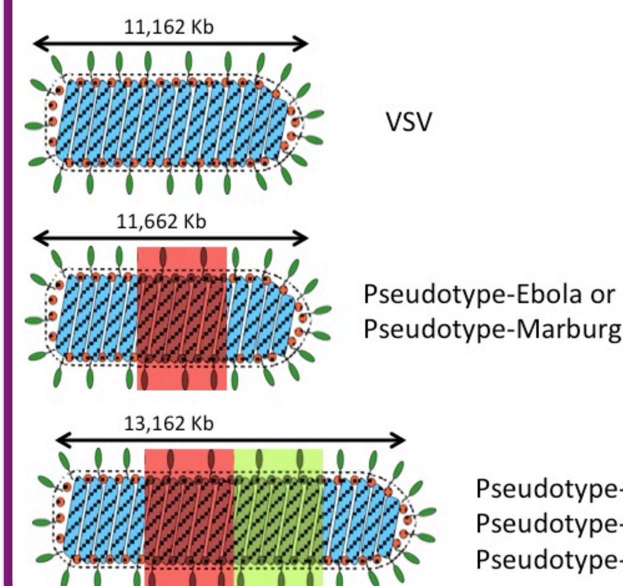
Irradiating the pseudotype with UV light inactivates it, allowing work to develop a quicker point-of-care assay with the IRIS in an normal laboratory environment.



In BSL2 the researchers need to wear gloves and lab coats but do not need full protective biohazard suits.

Experiment:

Sizing of viral psuedotypes using IRIS



Viral pseudotypes are different sizes depending on the glycoproteins they have been designed to express. Pseudotype-Ebola or Pseudotype-Marburg is slightly larger than VSV and Pseudotype-Ebola-Zaire-Sudan is larger still. The IRIS was used to detect these size differences.

Si/SiO_2 chips were spotted with antibodies to Ebola, Marburg, VSV, and a negative control. Pre-incubation images were taken using the IRIS.

The chips were incubated with a variety of inactivated viral pseudotypes. Post-incubation images were taken using the IRIS and the images were analyzed for size of viral particles adhered.

Acknowledgements:

Thank you to Dr. George Daaboul and Jyothsna Chinnala for teaching me and letting me take part in your important research for the past two summers.

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