SAND2015-3960C

Identification of Unknown Viral Infections by Hyperspectral Cell Sorting and Deep Sequencing

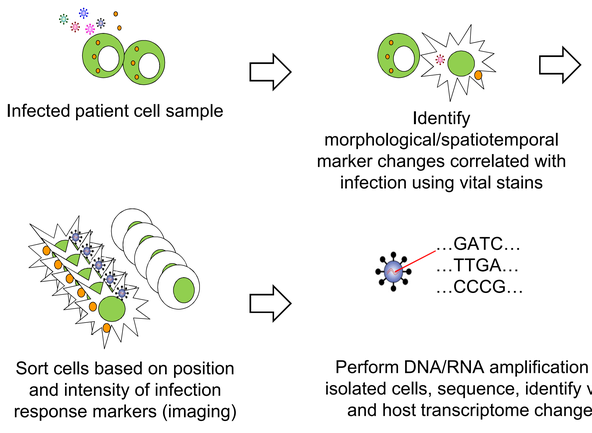
Bryan D. Carson[1†], Jerilyn A. Timlin[1], Michael B. Sinclair[1], Stephen M. Anthony[1], Matthew W. Moorman[1], Elisa La Bauve[1], Jaclyn K. Murton[1], Kelly P. Williams[1], Kunal Poorey[1], Howland DT Jones[2],

[1]Sandia National Laboratories, Albuquerque, NM and Livermore, CA; [2]HyperImage Solutions, Rio Rancho, NM; [†]Principal Investigator: bcarson@sandia.gov

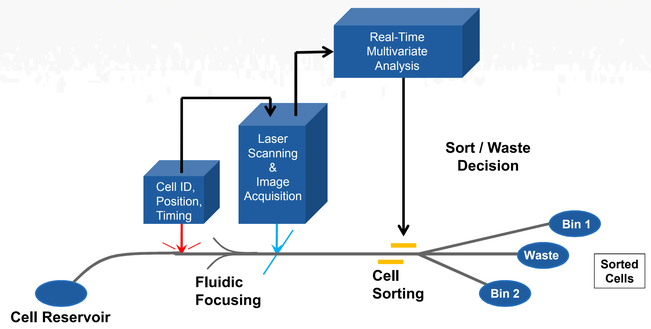
Motivation & Approach

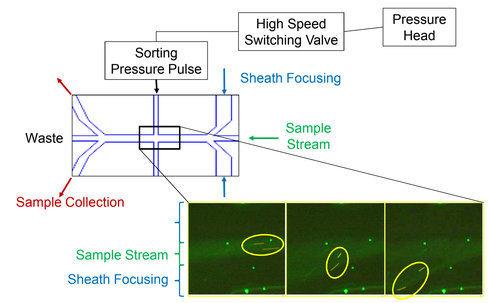
- Future infectious disease outbreaks may involve unknown, poorly-characterized, or engineered viruses.
- These pathogens are challenging to diagnose and characterize.
- Typical qPCR and immunoassay-based methods fail to do this since they only detect known agents.
- Unbiased methods that don't require foreknowledge of the pathogen are therefore needed.
- Deep sequencing alone is fraught with low signal:noise and ambiguous results due to complex microbiome background.
- We address this problem by identifying novel biomarkers of infection using hyperspectral imaging, multivariate spectral analysis, and logistic regression-based classification.
- We envision infected cells can thus be isolated from uninfected by microfluidic sorting and analyzed by deep sequencing, allowing subtraction of the patient's nominal microbiome to aid identification and characterization of the true pathogen.

Overview

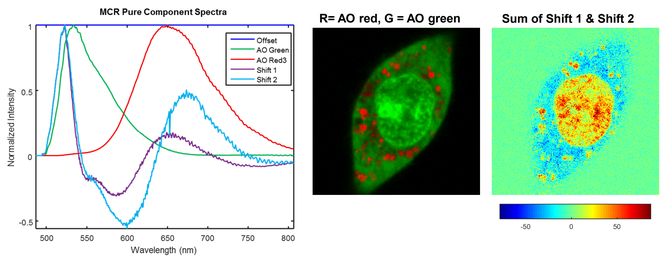


Instrumentation

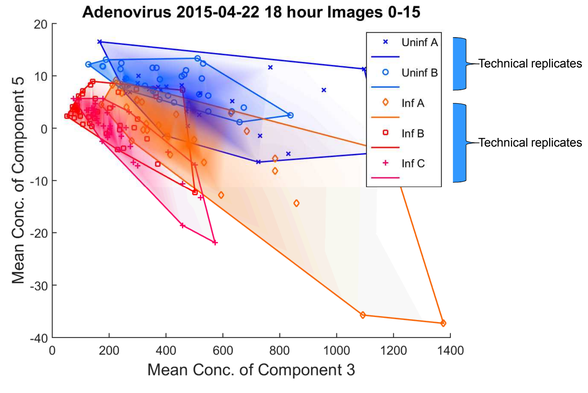




Infected Cell Classification



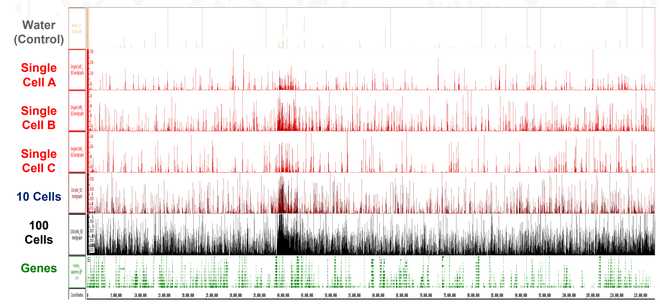
- Acridine orange (AO) is a solvato-chromatic dye
- Spectral shifts related to local environment



- Several possible classifiers were investigated
- High degree of repeatability over technical replicates

Sequencing Analysis

Low Input Transcriptome Coverage



Coordinate along 1st 120 Mbp of Human Chromosome 1

Summary & Future Directions

- Virus-infected cells can be distinguished from uninfected using vital dye staining and hyperspectral image-based classification.
- The image data shown were produced using a static imaging system, but ongoing development of a flow-based system will facilitate high throughput sorting analysis.
- Broad applications of this instrumentation include synthetic biology, directed evolution, and high content cell analysis and sorting using parameters inaccessible by current COTS technology.
- We seek sponsors interested in this and other applications of the technology

Acknowledgments

- **Anson V. Hatch (Sandia National Laboratories, SNL) aided in design of the microfluidic cell focusing and sorting apparatus.**
- **Ryan W. Davis (SNL) aided in classification analysis of spectral data to differentiate infected from uninfected cells.**
- **Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.**

National Nuclear Security Administration

U.S. DEPARTMENT OF ENERGY

LDRD LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Sandia National Laboratories