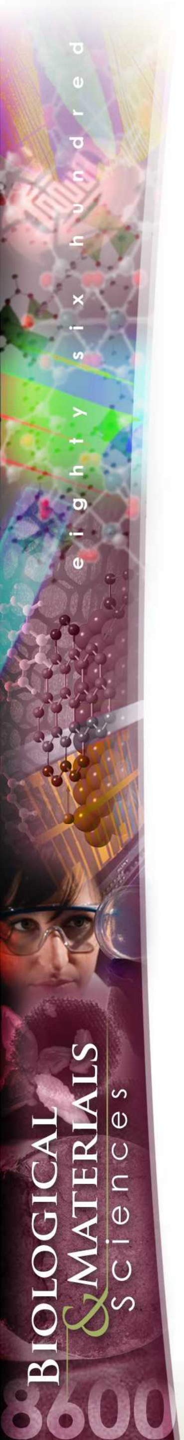
SAND2014-15628PE

A Novel Approach to Unknown Virus Identification in Clinical Samples

Sadie La Bauve, PhD
Sandia National Laboratories
Albuquerque, NM

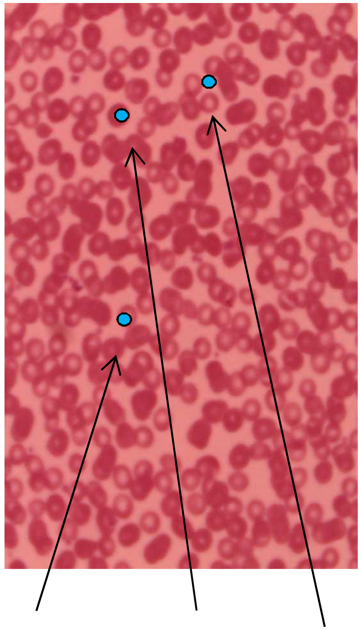


The Problem

- Unbiased Pathogen Detection System
  - Deep sequencing
- Standard diagnostic methods fail to identify unknown/unexpected viral infections
  - PCR/ELISA based methods require prior knowledge of potential pathogens
  - What about co-infections?

Deep Sequencing for Pathogen Detection

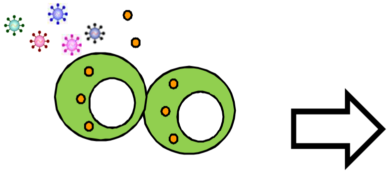
- Goal: Identification of all pathogens present in a sample - expected, unexpected and unknown
- Challenges
  - Low signal:noise
  - High human and microbiome background



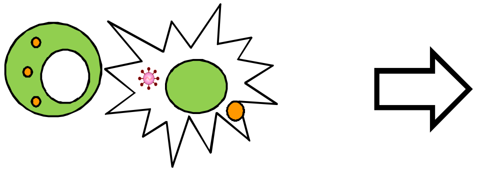
Blood Sample

Infected Cells

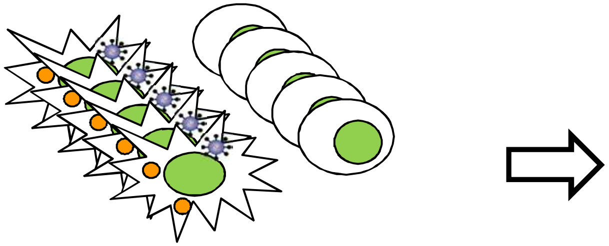
Idea: Sort Infected from Uninfected Cells Prior to Sequencing



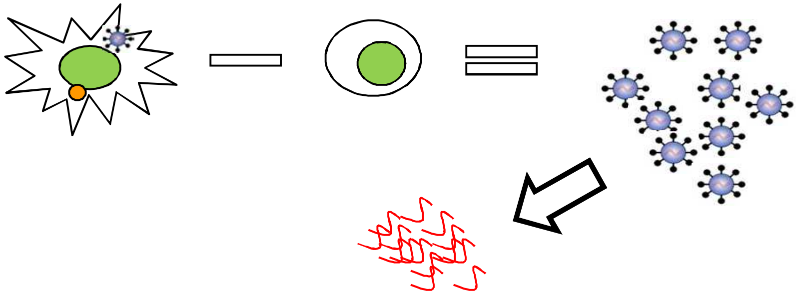
Infected blood cells



Identify morphological/spatiotemporal marker changes correlated with infection using various cell stains

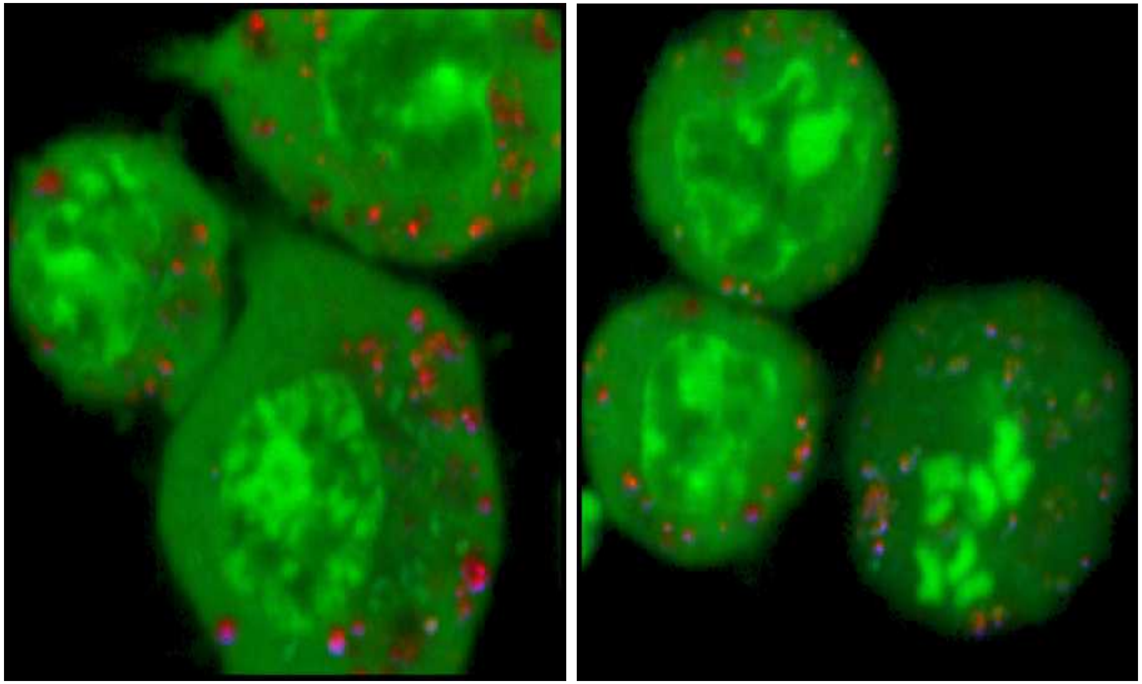


Sort cells based on position and intensity of response markers (imaging)



Perform DNA/RNA amplification on isolated cells, sequence, identify virus and host transcriptome changes

How To Identify Infected Cells?



Uninfected

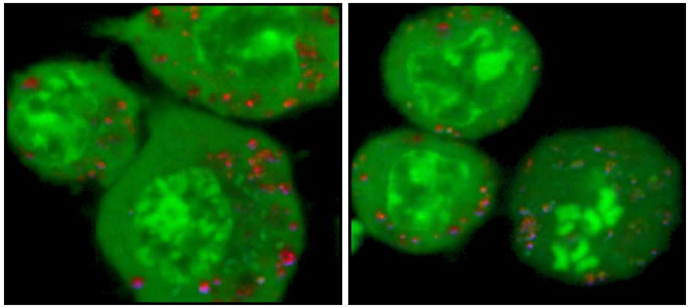
Sendai Virus Infected
18hrs

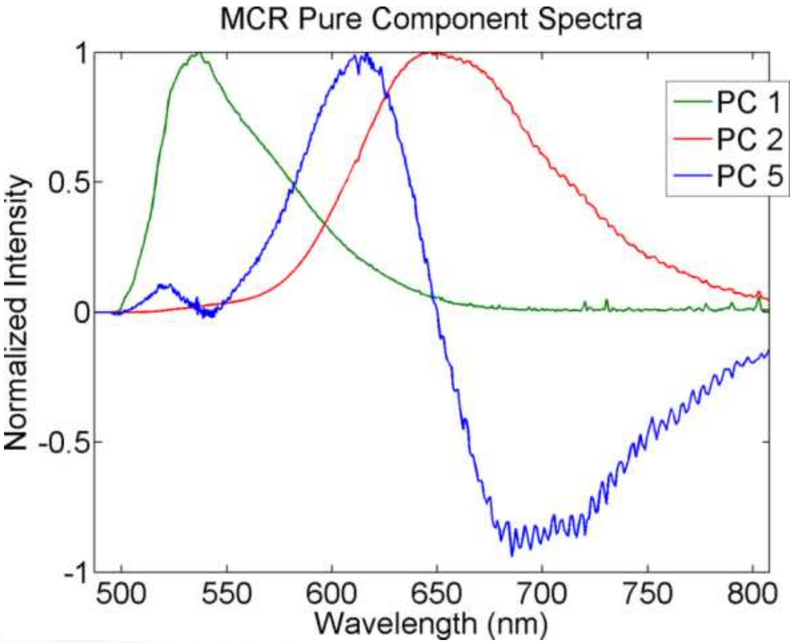
Acridine orange stained P388D1

Hyperspectral Analysis

Uninfecte**d**

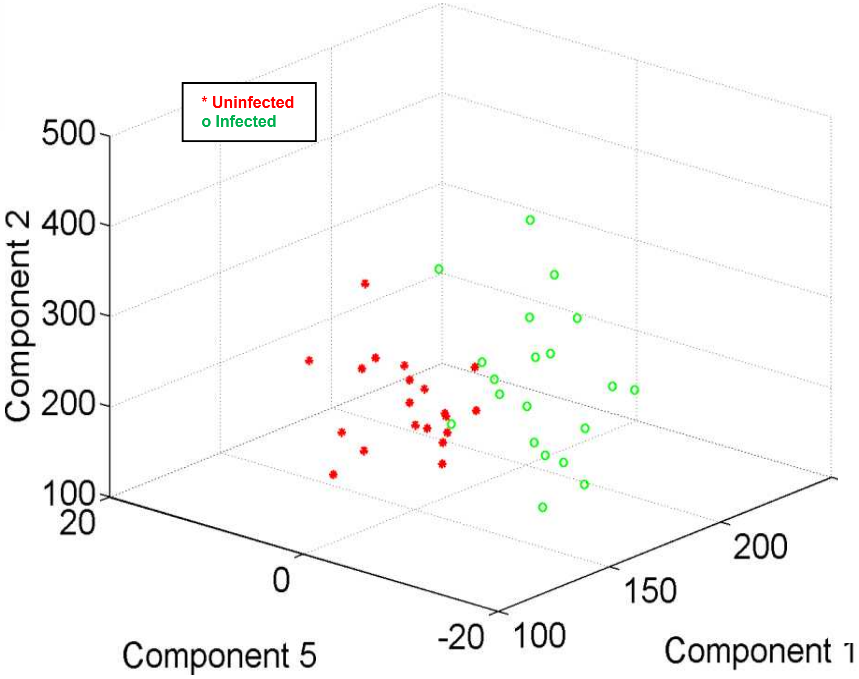
Sendai-Infected 18hrs

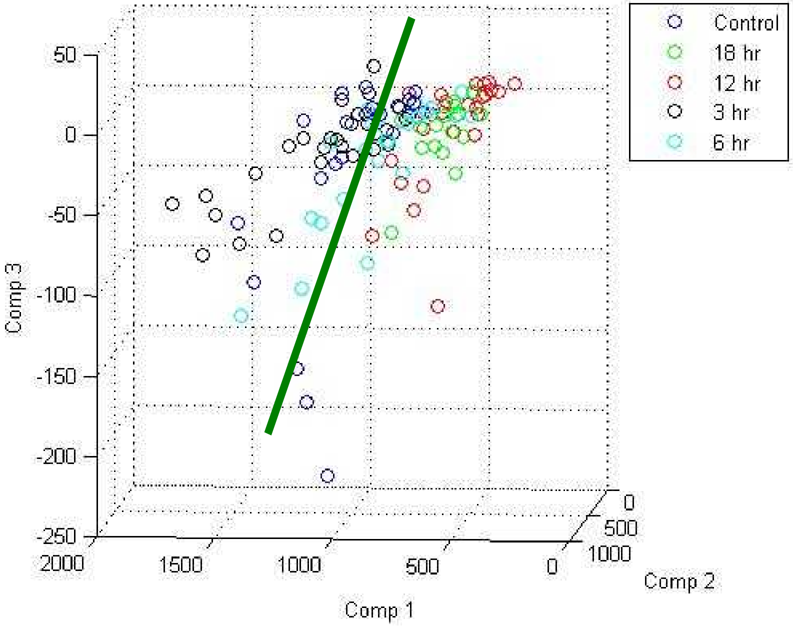




- Multivariate curve resolution
  - Isolate spectral components
- Spectral components change upon infection
- Spatial and spectral resolution allows for separation of infected and uninfected cells

Infected vs. Uninfected Cells Cluster Distinctly

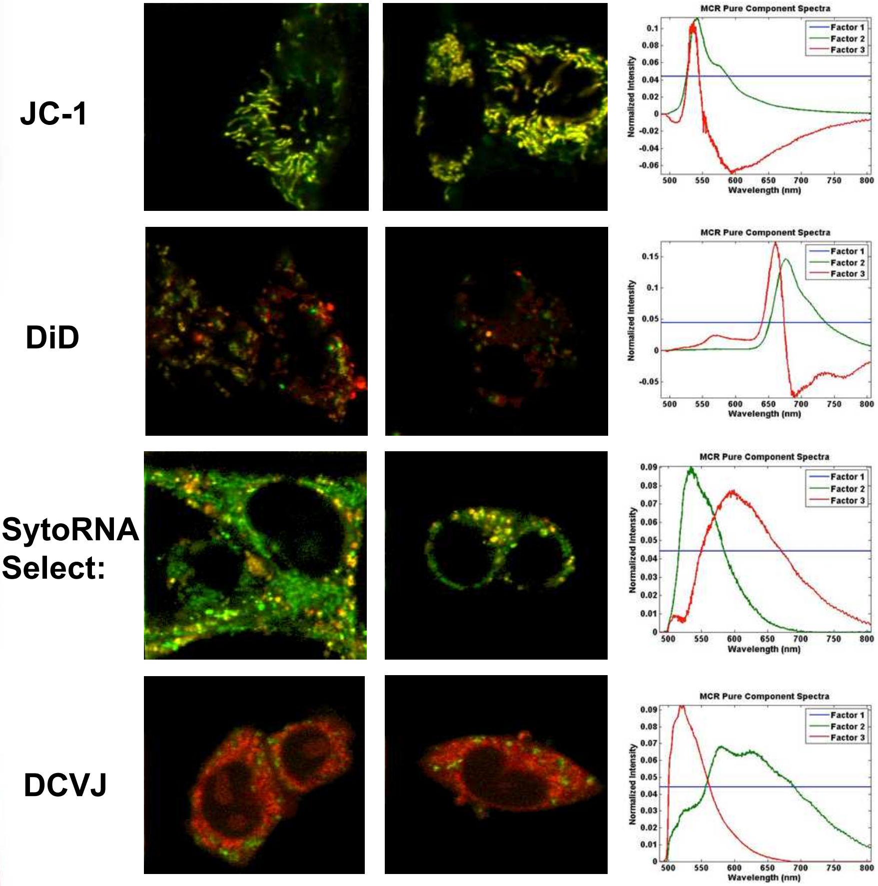




Each point represents a single cell

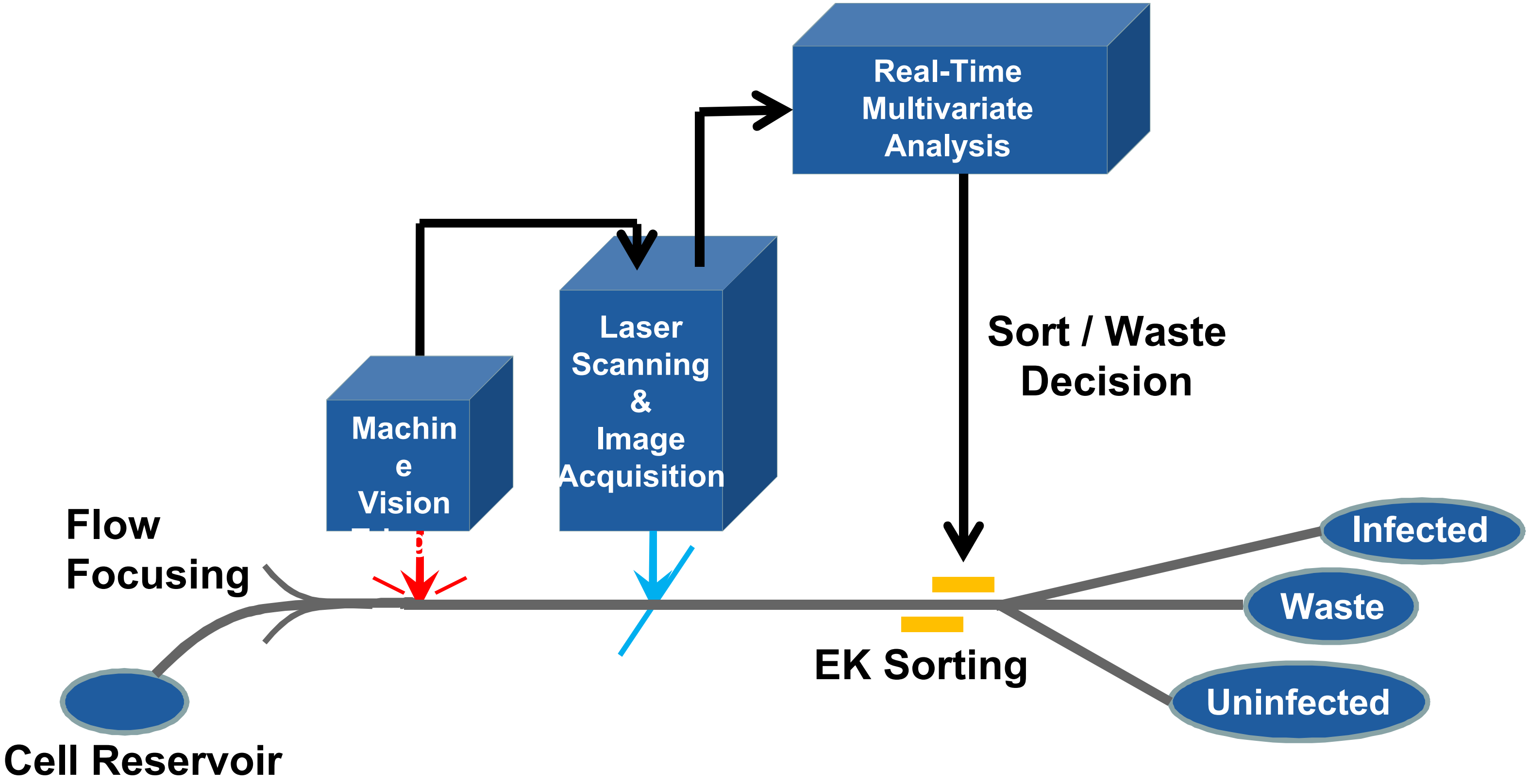
H.D.T. Jones, et al. 2012. Journal of Chemometrics and Intelligent Laboratory Systems

Combination Multiple Cell Stains



- Multiple cell stains
  - Targeting various cell cycle mechanisms that could be affected by viral infection
- Most complete infected cell profile

Infected Cell Isolation: Hyperspectral Imaging Cell Sorter



Real-Time Multivariate Analysis

Laser Scanning & Image Acquisition

Machine Vision

Sort / Waste Decision

Flow Focusing

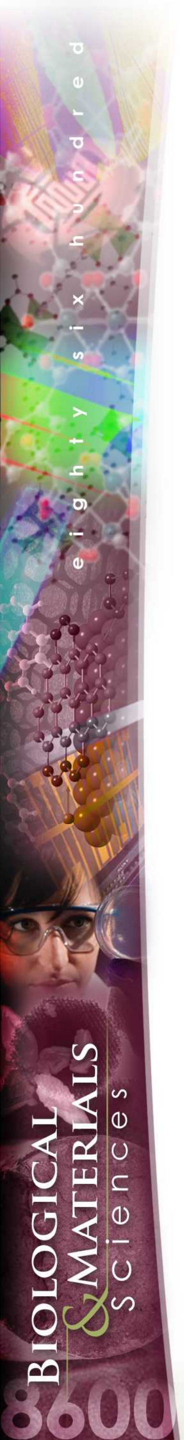
Cell Reservoir

EK Sorting

Infected

Waste

Uninfected

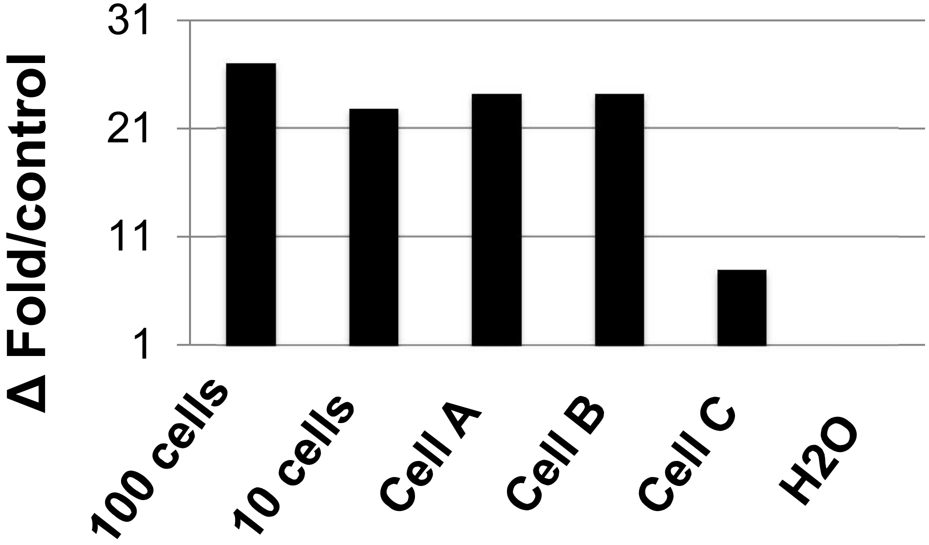


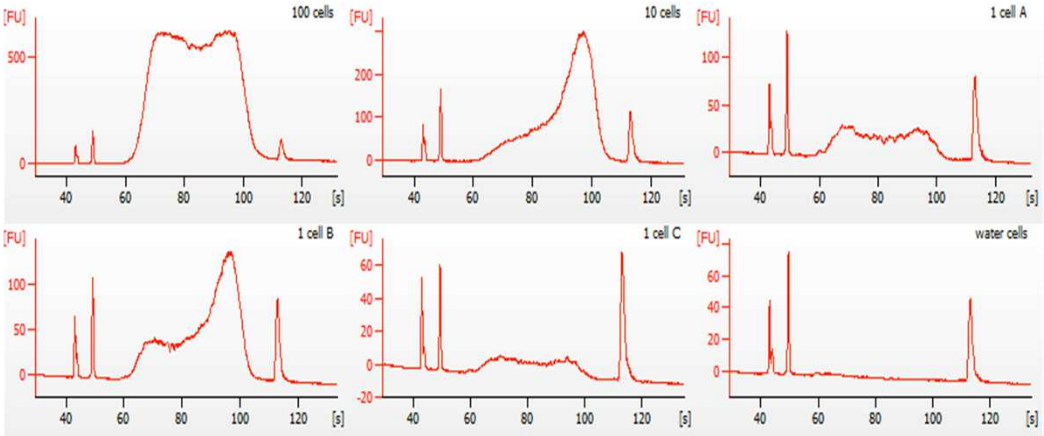
Favorable Single Cell Illumina Library Prep Yields

Sample	Yield
100 cells	607.2 ng
10 cells	228.2 ng
Single Cell A	38.4 ng
Single Cell B	78.2 ng
Single Cell C	13.8 ng
Water	3.2 ng

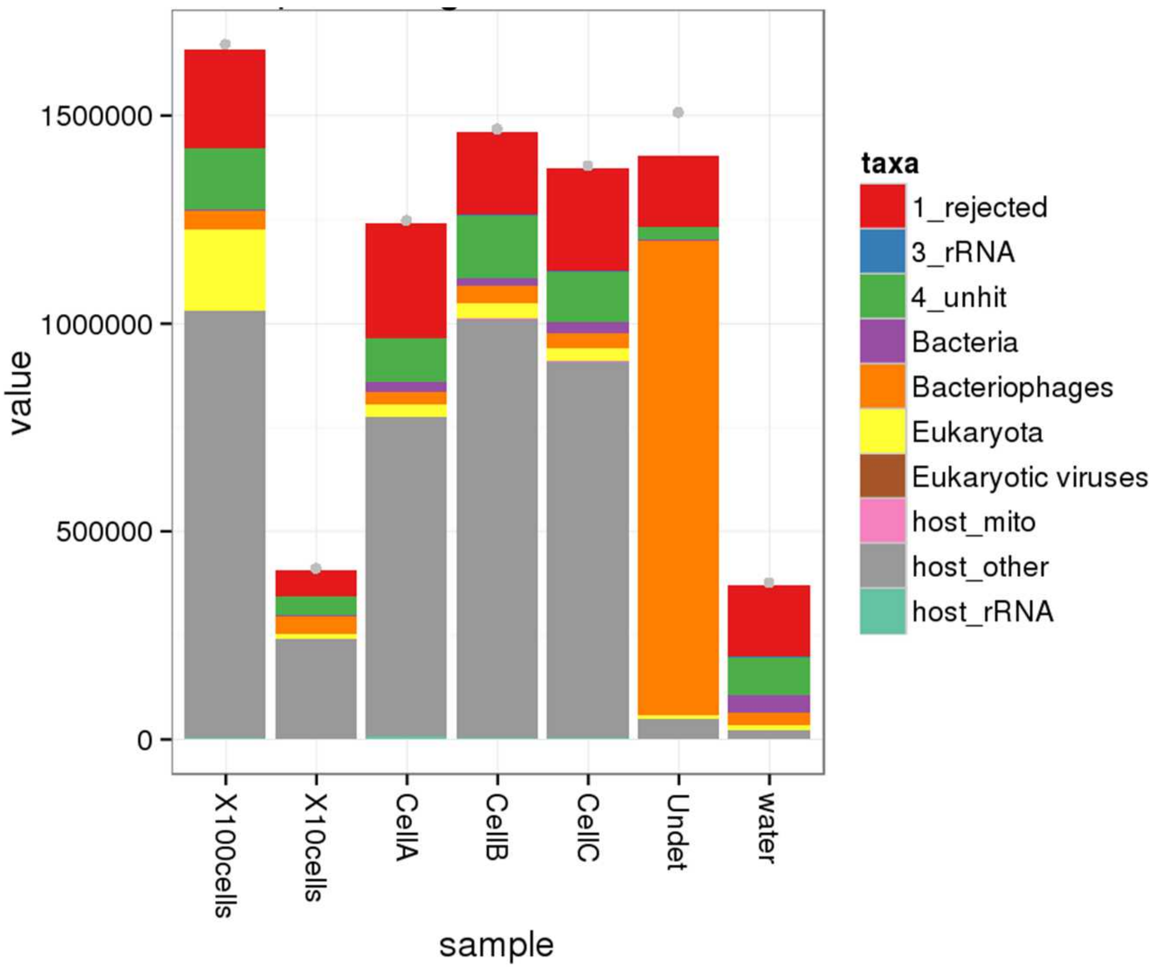
Library Output

18S qPCR





Metagenomic Results of Single Cell and 10 Cell Libraries

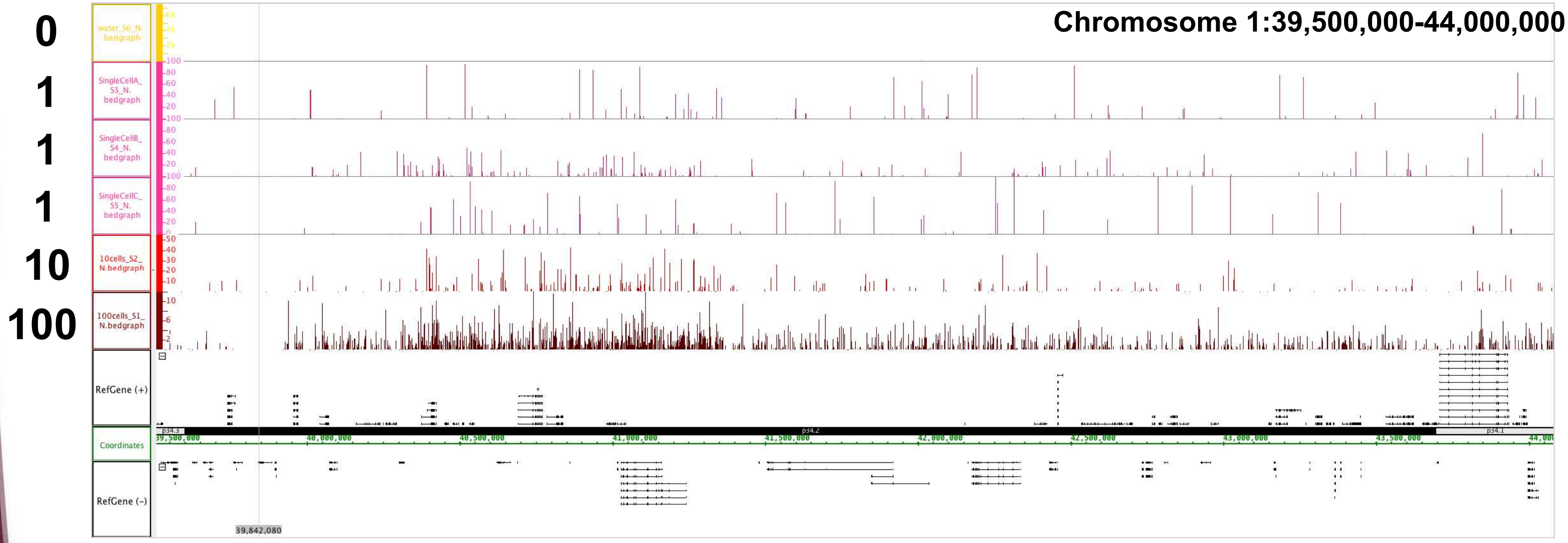


Transcriptomics Results

- Detailed analysis in progress
- As expected, single cell data are noisy and show lower coverage than with 10-100 cells.

#cells

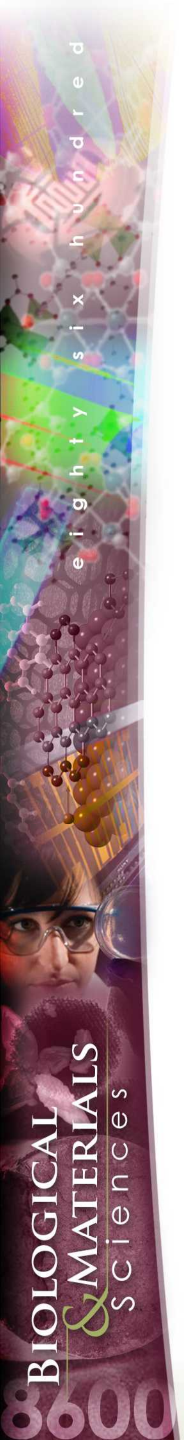
FPKM (expression Level)



Infected Low Input Library Preps

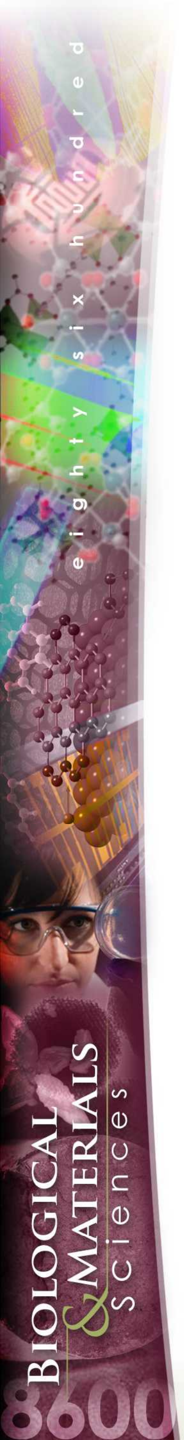
- Currently working on creating low input/single cell libraries with infected cells
  - Determine if we can identify virus in low input libraries

Sample	Yield
Infected 10 Cells	452.5 ng
Infected Single Cell A	82 ng
Infected Single Cell B	38 ng
Infected Single Cell C	58 ng
Uninfected 10 Cells	106.5 ng
Uninfected Single Cell A	39 ng
Uninfected Single Cell B	20.4 ng
Uninfected Single Cell C	147.5 ng
Media	11.5



Summary and Next Steps

- Hyperspectral imaging to separate infected from uninfected cells
  - Additional Stains
  - Human blood samples
  - Other viruses
- Low input sequencing
  - Infected vs Uninfected cell sequencing (in progress)
  - Begin single/100 cell sequencing on human blood samples



Acknowledgments

- PI: Bryan D. Carson
- Imaging
  - Howland T. Jones
  - Michael B. Sinclair
  - Jeri A. Timlin
  - Ryan Davis
- Bioinformatics
  - Kelly P. Williams
  - Kunal Poorey
- Microfluidics
  - Anson V. Hatch
  - Conrad James

Funding:
Laboratory Directed Research and Development Program (LDRD)

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.
SAND No. 2011-XXXXP