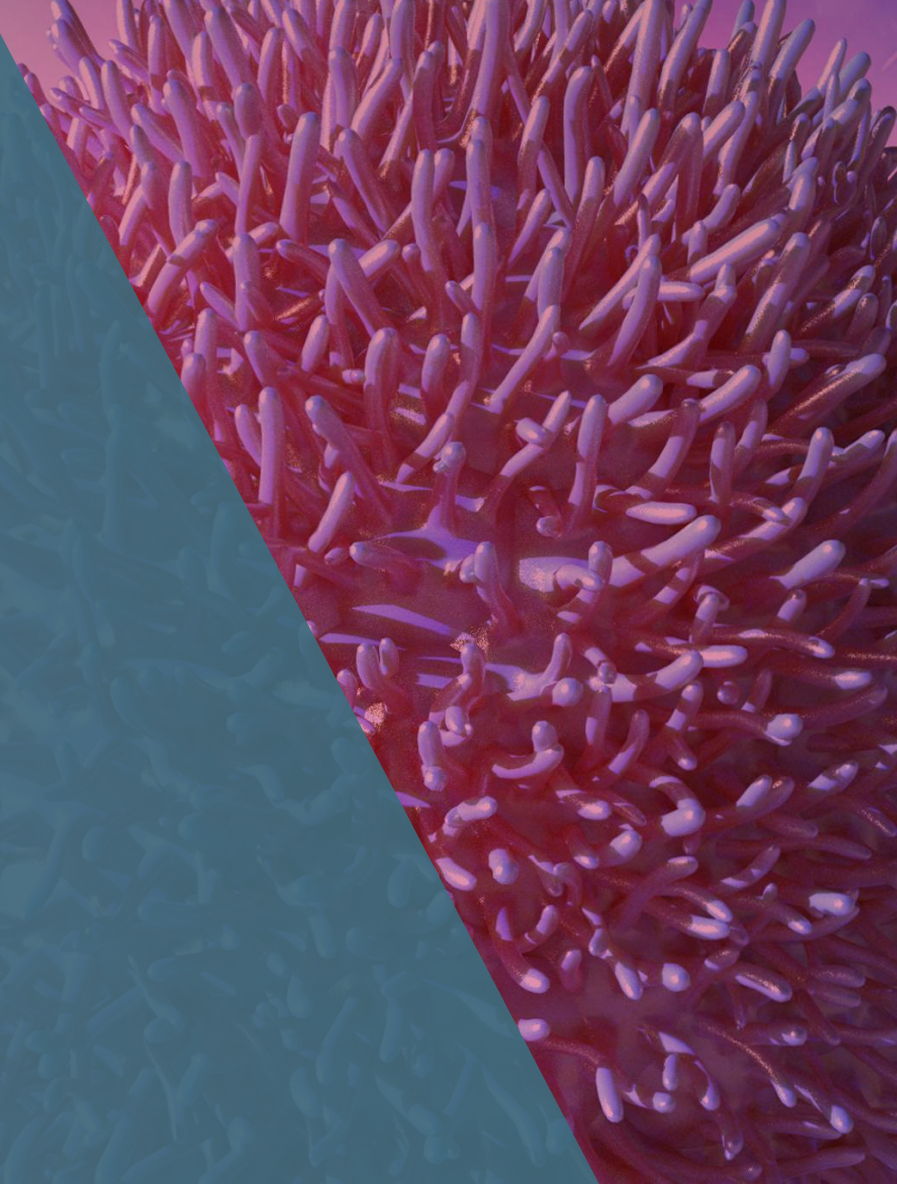




LASER FORCE CYTOLOGY™

Quantitative Cellular PAT Analytics Driving Improvements in Advanced Therapy Process and Production Outcomes

March 2025



Biomanufacturing Life Saving Cell & Gene Therapies and Vaccines is Complicated

Starting materials are complex, variable, and difficult to characterize, reducing process and product consistency.

Delayed and imprecise analytics result in lost process and production efficiency, delaying delivery of life saving treatments.

Large biological variability throughout manufacturing processes results in batch-to-batch variability and potential for OOS/OOT and batch failure events.

Current Analytics ➡ Slow + Highly Variable + Labor Intensive + Costly

Increasing Demand for Life Saving Advanced Therapies
Is Driving the Need for Speed and Innovation



**Laser Force
Cytology™**

Real-time Label-free Single Cell Analysis Provides Solutions

- **Label-free cellular analytics** reduce the need for antibodies, provide unbiased measurements and enable the sensitive measurement of cellular changes
- **Accurate, precise and non-subjective** data comprehensively characterize complex starting materials and products
- **Real-time analytics increase process knowledge**, enable improved process controls and optimization, and maximize product quality, yield, and shelf life
- **Quantitative precision PAT analytics allows biological evidence** to be carried forward from early process development, production and QC streamlining tech transfer across resources and sites
- **Reduced labor and waste** significantly lowering costs and accelerates time to patient



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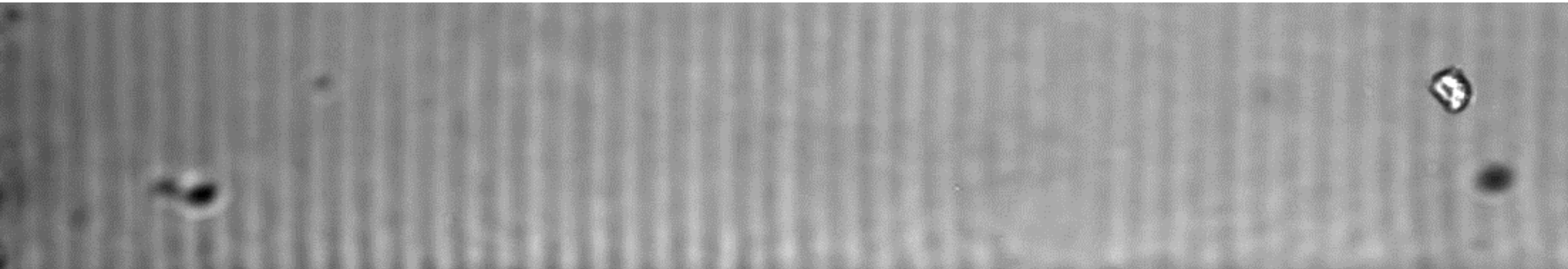
FDA
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Laser Force
Cytology™

Label-free, single cell analysis based on **intrinsic biochemical & biophysical properties** using a balance of optical and hydrodynamic forces in a microfluidic channel

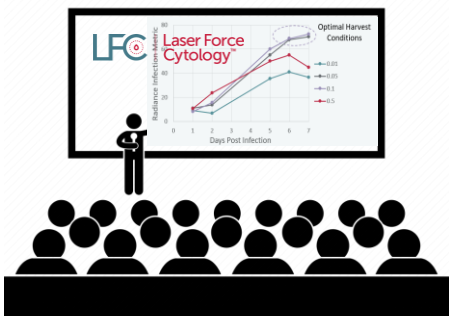


Virally infected Vero cells

Laser (Optical Force) \longrightarrow $\text{Velocity} \propto F_{\text{Optical}}$ \longleftarrow Fluid Flow (Drag Force)

- An **optical force** is generated when a **laser beam reflects and refracts through a cell**
- Laser Force Cytology™ measures velocity (optical force) and other parameters to detect **subtle phenotypic changes in cells**, rapidly measuring **quantitative early indicators** of cellular response **to viral infection, activation, transfection and differentiation**
- Applications in **cell and gene therapy, vaccine development, and biomanufacturing**

REGULATORY SUPPORT FOR NOVEL CELLULAR PAT ANALYTICS



Approach for Establishing Novel and Fit-for-Purpose Cell Viability Methods to Support Cell Manufacturing Process Monitoring

MATERIAL MEASUREMENT LABORATORY

NIST

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Introduction

Cell viability is a critical measurement for cell-based products where it is routinely used for in-process monitoring as well as for release testing. Novel measures of cell health are being developed to improve process monitoring including modalities that can be incorporated into in-line or online methods (e.g. label-free and non-destructive approaches).

A fit-for-purpose strategy is needed to support the development of meaningful cell viability assays for specific applications. In this study, we demonstrate an approach for establishing novel cell health monitoring measurements based on the evaluation of test samples with systematically varied cell health and growth profiles and single cell optical and hydrodynamic force measurements. We apply this approach to label-free, single cell optical and hydrodynamic force measurements.

Multi-Parametric and Label-Free Single Cell Analysis based on Laser Force Cytology™ (LFC)

LumaCyte's Radiance™ instrument uses a combination of optical and hydrodynamic forces to interrogate the biochemical and biophysical properties of individual cells in a label-free manner (Laser Force Cytology™ (LFC)).

An optical force is generated when a laser beam reflects and refracts through a cell

Laser (Optical Force) → Velocity & Force → Fluid Flow (Drag Force)

The detected changes in cell response to optical force and fluid flow may reflect:

- Velocity [μm/s]
- Size [μm]
- Elasticity/Modulus
- Optical Force Index (OFI)
- Minor Axis [μm]
- Major Axis [μm]
- Minor Axis Deformability
- Eccentricity/Deformability
- Elongation Factor
- Elongation Index

Machine Learning for Prediction of Cell Treatment using LFC

We applied machine learning analysis for multi-parametric, single-cell data based on linear support vector machines (SVMs) to quantify LFC differences in Jurkat cell populations and associate them with different culture conditions that systematically impact cell health. (2 replicate studies).

SVM classifiers are designed to find the optimal classification boundary that separates data points in the multidimensional parameter space. The analysis was conducted at a multi-cell level, where a "supercell" method used average measurements of small groups of single cells to account for heterogeneous populations and microenvironment.

Large supercells (e.g. 89 cells) can lead to highly variable classifiers, while small supercells (e.g. 1 or 5 cells) can result in classifiers with poor accuracy.

Chen D, Sarkar S, Camp C, Lowry M, Pierce L, Varisco D, Hebert C, Evans Z, Hart S. Machine learning based methodology to identify cell shape phenotypes associated with microenvironmental cues. *Biomaterials*. 2019; 201: 1-14. 104-119.

Analytical Procedures for Viral Vectored Vaccine Quality

Draft guidelines

Accelerating product development through a common understanding of quality

GENWebinars

Rapid Bioprocess Analytics Needed to Drive Improvements in Product Consistency and Quality

Peter Marks, MD, PhD
Director of the Center for Biologics Evaluation and Research, FDA

Fixing The Potholes in CGT Manufacturing

Dr. Pyodhar Umay

Peter Marks, MD, PhD

Chris Spivey

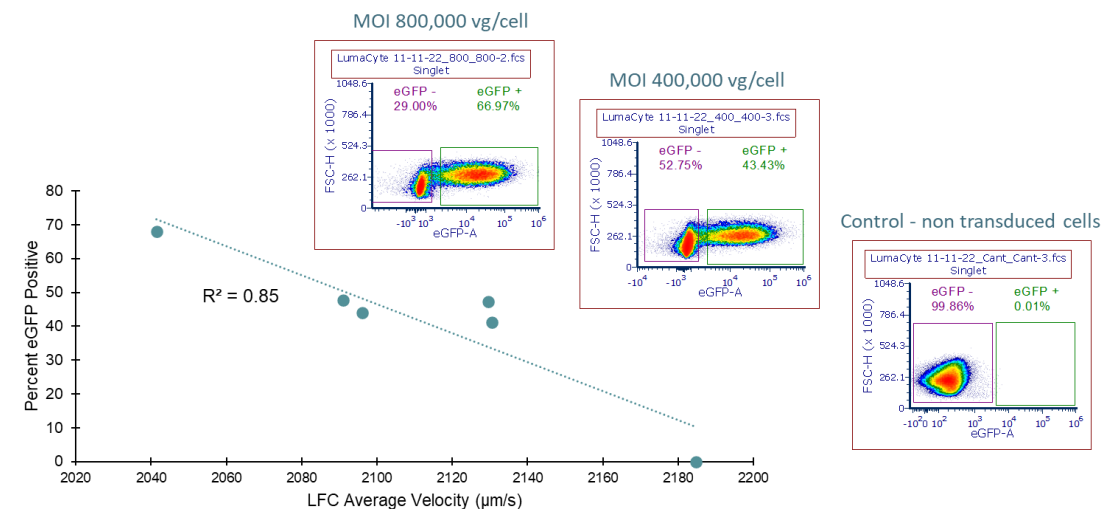
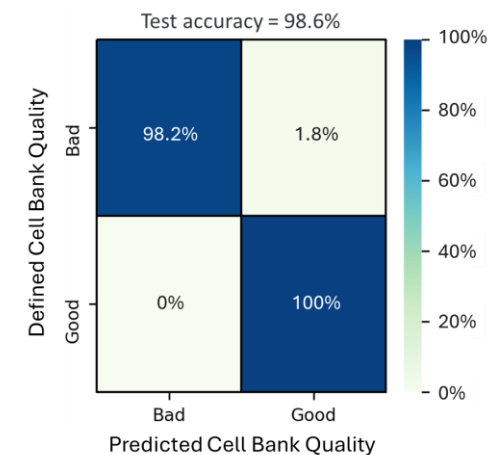
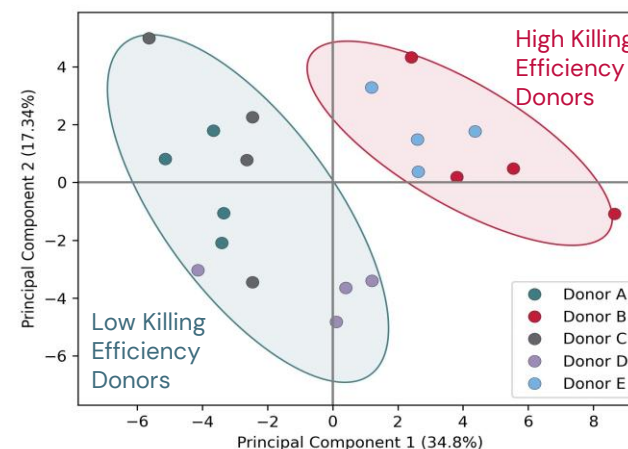
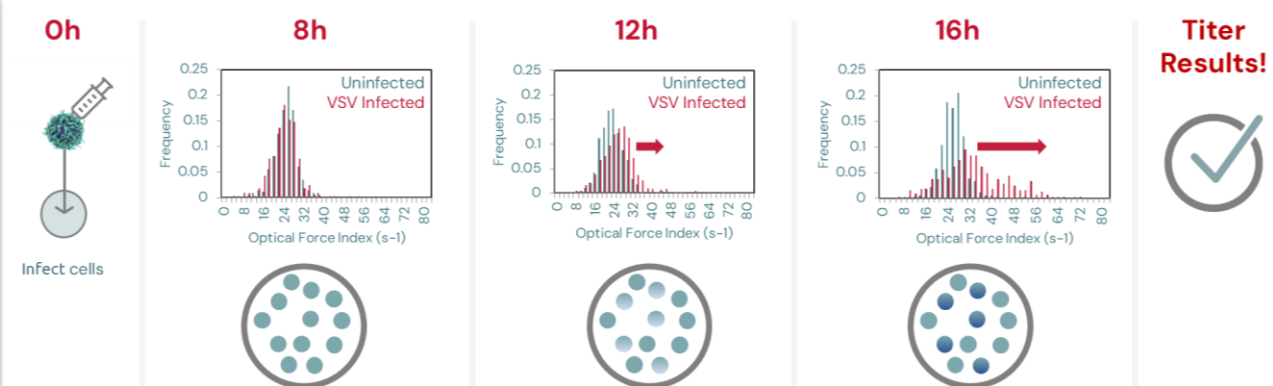
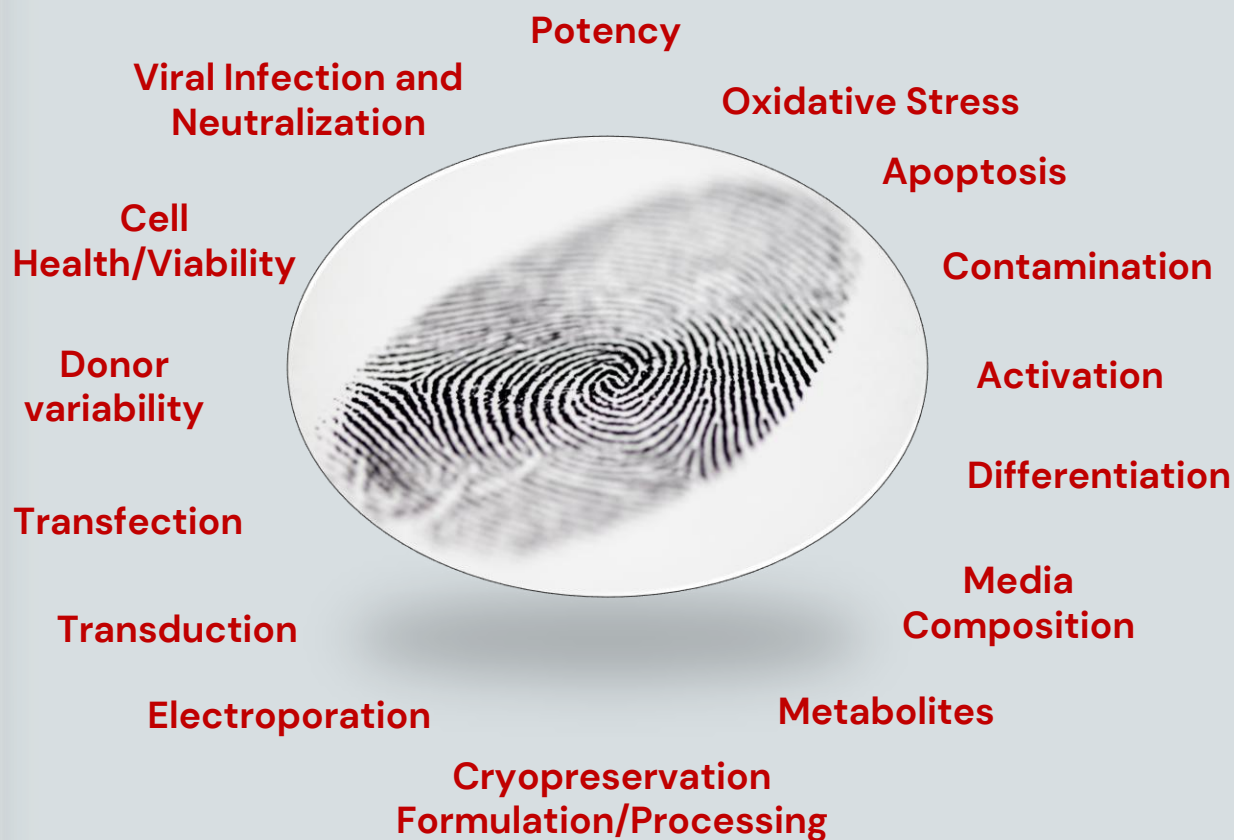
Dr. Fabian Gerlinghaus

Dr. Sean J. Hart



Cellular Fingerprinting

Biological/Cellular Processes Affecting the LFC Fingerprint



Cellular Fingerprinting
Biological/Cellular Processes Affecting the LFC Fingerprint

Single Analytic Spanning Every Stage of Production



Accelerate Development



Enable Discovery



Ensure Process Consistency



Streamline Tech Transfer

