Enabling Real-Time, Label-Free Analysis of Single Cells

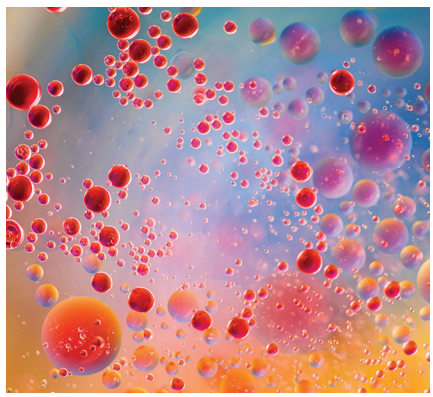
A Discussion on Analytical Needs in Cell-Therapy Manufacturing

Brian Gazaille with Renée Hart

Drug developers know well the analytical obstacles associated with cell therapies (CTs). Human cells' large size and biochemical complexity present considerable difficulties for characterization and manufacturing-process control. Because advanced therapies are still novel drug modalities, their critical quality attributes (CQAs) remain poorly defined. In the case of autologous CTs, product quality hinges on material obtained from extremely ill patients who might have undergone several previous lines of treatment. And CTs are "living products" that change over time and will participate dynamically in biological pathways. Allogeneic products introduce even more complexity for manufacturing because of inherent variability across materials from different healthy donors.

Despite incremental technological improvements over the past decade, the CT industry needs analytical methods that can account for cells' complexity and dynamism. As I learned from Renée Hart (president and chief business officer of LumaCyte), standard technologies for CT analysis require labeling of samples with antibodies, fluorophores, dyes, or nucleotides. Such techniques can influence cellular behavior and skew assay results. Equally important is that most CT analytical methods cannot generate results in real time, preventing scientists from observing cell health and quality throughout manufacturing.

Herein, Hart and I discuss ongoing analytical needs in the CT industry. She also describes her company's efforts to deliver an informative, reliable, and good manufacturing practice (GMP)–validated method that can assay cells in all their complexity without labels. To that end, she introduces the Laser Force Cytology (LFC) method, which can leverage cells' optical and fluidic "signatures" to evaluate CT quality and safety throughout manufacturing.



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Current Methods and Continuing Needs

What information do CT developers need about their products, and what factors impede its collection? A primary hurdle in CT analytics is developing reliable potency assays that truly reflect how a therapy works in a patient's body. Such assays must be sensitive and specific to measure therapeutic effects accurately.

Ensuring sterility without terminal sterilization is another challenge. Analysts need rapid, reliable, and validated tests to detect microbiological contaminants.

Variability in donor cells and other starting materials can compromise product consistency, quality, and effectiveness. Added complexity comes from ensuring compliance with continually changing regulatory requirements throughout process development and manufacturing. Comprehensive characterization of CT identity, purity, and potency is essential but technically demanding.

What methods are common in CT analysis? Several approaches are possible depending on the application. Genomic and transcriptomic analyses such as quantitative polymerase chain reaction (qPCR) and next-generation sequencing (NGS) can assess genomic stability and gene expression. For characterization, flow cytometry enables simultaneous analysis of multiple parameters at the single-cell level. But the method is generally unsuitable as a process analytical technology (PAT) for CTs, and its dependence on labels introduces repeatability challenges, preventing acquisition of new cellular insights.

For **cell-health analysis**, cell-viability assays often are performed, including dye-exclusion methods (e.g., with trypan blue). Alternatively, analysts can measure metabolic activity using adenosine triphosphate (ATP) assays or tests based on tetrazolium (MTT), formazan (XTT), or water-soluble tetrazolium salt 1 (WST-1). Dye exclusion is simple enough to be used as an in-process control, but it suffers from poor sensitivity and a lack of predictive power. Metabolic-activity

Label-free analysis preserves cells in their **NATURAL** states, reducing measurement bias and ensuring that results reflect cells' **TRUE** characteristics and physiology.

assays are more complex and generally are not used during CT production.

Potency assays are critical for measuring the biological activity of CT products. *Functional assays* mimic a therapy's mechanism of action (MoA). For instance, cytotoxicity assays can be used to gauge the potency of chimeric antigen receptor (CAR) T cells. *In vitro methods* — e.g., enzyme-linked immunosorbent assays (ELISAs) that measure cytokine production — can assess cell proliferation. *In vivo assays* can be performed to measure therapeutic effects in animal models.

Collectively, the methods that I've described help in CT characterization and quality control (QC).

How would you describe your company's LFC method? Our LFC technique provides label-free analysis of single cells. It measures each cell's biophysical and biochemical properties by analyzing optical and fluidic forces exerted upon it. The method is particularly valuable in CT applications, for which understanding of cell characteristics is critical.

During an assay with our Radiance instrument, minimally prepared samples are placed into a 96-well plate autosampler. Cells are introduced into a microfluidic channel, where they encounter a laser. The instrument measures the optical force exerted on each cell as it passes through the laser. A cell's biophysical and biochemical properties influence its refractive index. In a fraction of a second, the instrument measures 24 parameters per cell — including its size, shape, morphology, and internal structure — by discerning subtle differences in those refractive indices.

The Radiance instrument enables automated, label-free Laser Force Cytology (LFC) analysis of single cells. (HTTPS://WWW.LUMACYTE.COM)

LFC analysis enables determination of several key attributes. The method can probe deformability to detect changes in cell membranes or cytoskeletons. Viability can be determined by measuring properties that indicate whether cells are alive, dead, or undergoing apoptosis. Functional cellular properties can be evaluated, providing essential information for development of potency assays. And in another powerful application, the Radiance instrument can detect early microbial contamination in samples, ensuring CT sterility and safety.

The instrument captures real-time measurements. Sample results are available in about five minutes. The system does not require significant skill to operate and has a user-friendly interface for staff with little scientific expertise. Analysis is streamlined with our ReportR cloud-based platform, which automates data analysis and report generation.

Measuring Up with Other Methods

How does the LFC method compare with other techniques for CT analysis? One significant benefit is the LFC method's ability to perform label-free analysis. Labels such as dyes, antibodies, and fluorophores can alter cell behavior, diminishing accuracy of results. Label-based assays also are susceptible to differences in labeling efficiency, nonspecific binding, and photobleaching of fluorescent markers.

By contrast, label-free analysis preserves cells in their natural states, reducing measurement bias and ensuring that results reflect cells' true characteristics and physiology. Sample preparation is more straightforward for the LFC method than it is for label-based methods. Removing labeling steps not only simplifies workflows, but also reduces expenses.

LFC analysis also has the advantage of providing comprehensive data. The Radiance instrument measures >20 multivariate parameters for each cell in a fraction of a second. That enables scientists to examine the influences of univariate, bivariate, and multivariate parameters to uncover insights that cannot be observed with other methods. Flow cytometry, for example, requires users to know before analysis what the assay is meant to elucidate, which inherently limits the ability to garner new knowledge within a given assay.

Our method's capacity for real-time data capture also enables continuous monitoring of cell viability, biological functionality, and potency. That is particularly useful for ensuring CT quality and consistency throughout a manufacturing process. And by measuring intrinsic properties of entire cells, the LFC method has been used to

create quantitative models for prediction of process performance. Such capabilities are new in CT analysis and represent a leap forward, helping to improve processes, lower cost of goods sold (CoGS), and increase therapy quality, yields, and safety.

What are some of the Radiance system's specific applications for CT analysis? The system has several important applications throughout CT workflows, including

- donor-cell screening
- assessment of cell viability
- activation monitoring
- posttransduction analysis
- evaluation of cell expansion
- potency assays (e.g., in vitro coculture assays)
- sterility testing.

Analysts can leverage insights from LFC analysis to optimize CT process development and manufacturing. Real-time data inform immediate process adjustments to maintain optimal conditions and reduce the likelihood of manufacturing variability and batch failures. Continuous monitoring supports high product consistency and quality, which are essential for meeting regulations and ensuring patient safety. By streamlining production and minimizing required resources, LFC analysis can reduce CoGS significantly for CTs, increasing their accessibility and improving vein-to-vein time. Real-time measurements also can support scale-up, which will be critical for CTs to treat large patient populations.

Frequently Asked Questions

What questions do Radiance users ask you about the system? Users often ask about the system's capabilities, applications, and operational details.

What types of cells can be assayed? The instrument has been used on both adherent and suspension cells, and it is cell-type agnostic. To date, users have applied the instrument on >45 cell types, from homogenates to patient primary cells and engineered cell lines.

How are data analyzed and reported? LumaCyte's software automatically analyzes each cell in real time without user intervention. After data collection, our automated and cloud-based ReportR platform receives and processes that information according to predefined methods to generate reports, which ultimately are emailed to analysts.

How do you operate the system? The system is simple to use and is designed for unattended operation. Each morning, the Radiance instrument automatically performs a calibration routine to maintain measurement accuracy. During application, user involvement is minimal and requires no specialized knowledge or skill. Operators simply load samples, enter metadata, and begin the fully automated analysis.

Using design-of-experiments (DoE) methodologies helps to evaluate systematically the effects of different parameters on assay performance, optimizing the assay and ensuring robust and consistent data outputs. Assay variability is removed, so users can be confident that shifts observed in their data relate to the biology of their samples and not to the assay.

Regular calibration and maintenance of the instrument are essential to ensuring consistent performance. Developing and adhering to detailed standard operating procedures (SOPs) for sample preparation, instrument operation, and data analysis can ensure assay precision and consistency. Providing comprehensive training for all users, regardless of their scientific expertise, ensures that a system will be operated correctly while providing users with robust outputs and easy operation.

Implementing a robust cellular PAT across process development, manufacturing, and QC workflows ensures connection of data outputs, reducing difficulties surrounding comparability and helping to streamline compliance with important regulatory requirements. Health agencies are laser- focused on comparability concerns, and in their guidances, they are asking CT manufacturers and technology developers to "build with the end in mind" and thereby design consistency into extremely complex processes.

Leveraging Real-Time Analysis

What is the "next frontier" for CT analytics, and how might the LFC method fit into it? The next step is likely to involve integration of advanced technologies that enable real-time, label-free analysis of therapeutic cells. Such advancements will enhance CT developers' ability to monitor cell health, viability, and functionality continuously. In turn, companies can ensure the quality and consistency of cellular products throughout manufacturing while reducing batch variability in both product yields and clinical efficacy. Learning about innate cellular responses and integrating those insights into production workflows will be pivotal to refining processes and achieving consistency across manufacturing stages.

With LFC technology, LumaCyte also hopes to further prescriptive analytics, helping CT developers to anticipate manufacturing success and failure. Having demonstrated the ability to predict process and production performance based on donor-cell attributes, we are working with customers to make that critical capability widely available. If a patient's cells are of poor quality and may not yield a successful therapy, then what can be done differently to achieve a successful therapeutic outcome? LumaCyte's advanced analytics might hold the key to enabling transformative results for CT production and clinical outcomes.

Given such capabilities, we have created a new service offering for CT manufacturers and suppliers of cellular starting material. By shipping us frozen peripheral blood mononuclear cells (PBMCs) or T-cell isolates with manufacturing results — e.g., orthogonal measurement of target-killing efficiency from a coculture killing experiment — we can use the Radiance instrument to generate a multivariate predictive model. From that, future donor cells can be evaluated, and CT developers can predict the likelihood of manufacturing and ultimately clinical success.

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