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WHITE PAPER

Advancing drug development with function-focused profiling at the single-cell level

Keywords

SINGLE-CELL ANALYSIS | DRUG DISCOVERY | FUNCTIONAL PROFILING | DROPLET MICROFLUIDICS | MACHINE LEARNING | POISSON DISTRIBUTION

Highlights

- Single-cell analysis is transforming drug discovery by revealing cellular heterogeneity and molecular pathways, but lacks direct functional assessment, limiting insights into cellular behaviour and responses.
- Current single-cell technologies face issues like computationally inferred functions, random droplet encapsulation, and inflexible microfluidic systems that cannot support real-time functional analysis.
- Lightcast overcomes these challenges, enabling direct function-focused assessment at the single-cell level, by streamlining workflows, accelerating timelines, and driving more precise therapeutic outcomes.

Abstract

Drug discovery is a complex and resource-intensive process, with traditional bulk methods often failing to capture cellular heterogeneity and dynamic biological processes. While single-cell sequencing has emerged as a valuable tool for exploring molecular pathways and cellular behaviour, it relies on indirect measurements of function which limits its potential. Issues such as variability from encapsulation methods, random cell distribution in droplets described by a Poisson distribution, and inflexible droplet control further restrict the ability to capture dynamic cellular interactions and functional data at the single-cell level. This white paper explores how the Lightcast platform overcomes these challenges by combining step emulsification, machine learning, and optical electrowetting-on-dielectric (oEWOD) technology to enable real-time, direct functional analysis of single cells. This function-focused approach has the potential to accelerate drug discovery by providing precise functional insights, enabling the early identification of promising candidates and eliminating suboptimal targets. This could therefore drive more efficient and cost-effective therapeutic development with deeper insights into biological complexity.

Introduction

Drug discovery is an intricate and costly process, often taking years and billions of dollars to bring a new therapy to market. Single-cell sequencing has emerged as a powerful tool for drug discovery, providing key insights into cellular heterogeneity, gene expression, and molecular pathways. Unlike traditional bulk methods, which analyse average signals from large populations of cells, single-cell sequencing dissects the genomic and transcriptomic profiles of individual cells, providing a new depth of information that bulk sequencing methods cannot match. This has allowed researchers unparalleled insights into cellular behaviour, gene expression, and disease mechanisms for uncovering genetic mutations, tracking gene expression patterns, and understanding cellular responses to treatment.

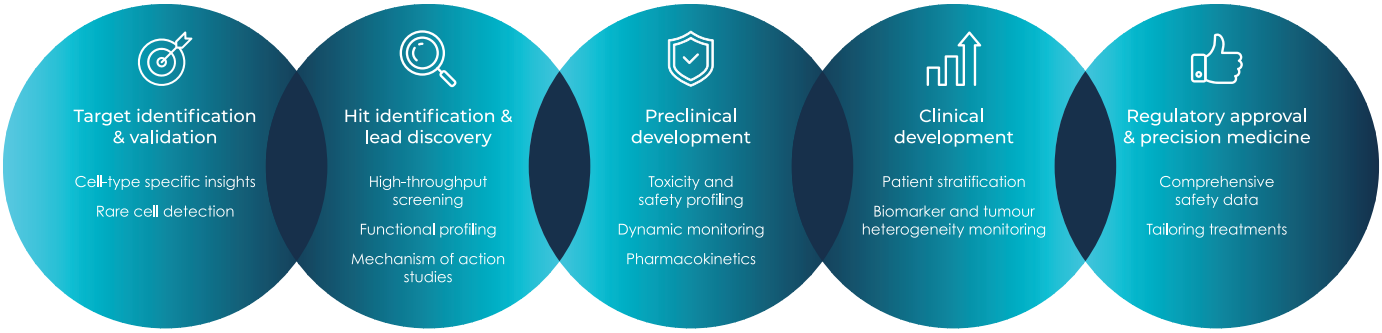

Figure 1: Applications of single-cell sequencing across the drug discovery and development pipeline.

The limitation of inferring function

While the benefits of adopting a single-cell screening approach for drug discovery are significant, a major challenge is the lack of direct functional assessment. Cellular function is a product of complex, dynamic, and context-dependent interactions across various pathways and components, such as metabolites, signalling molecules, post-transcriptional modifications, and protein activity. As single-cell read-outs are typically at the genomic or transcriptomic level, this means that cellular function is often inferred from gene expression data rather than directly measured. Without direct functional insight, researchers must rely on following up with multiple secondary screens to obtain functional data to identify high-quality leads, which significantly increases the complexity and cost of the discovery process[1,2].

The ability to directly measure function at the single-cell level will not only deepen our understanding of the relationship between genotype and phenotype but also enable the discovery and targeted modulation of specific biological pathways that drive cellular function[3]. This can be achieved through function-focused measurements that assess the overall biological response of a cell or molecule, quantifying critical functional outputs, such as biomolecule secretion, cell–cell interactions, antibody blocking, internalisation, and target cell killing.

What is a function-focused approach?

A function-focused approach recognises that regardless of the therapeutic modality— be it cell therapy, checkpoint inhibitors, therapeutic antibodies, antibody–drug conjugates (ADCs), or others—all depend on the modulation of cellular functions to deliver their therapeutic effects. By capturing critical functional outputs, such as biomolecule secretion, cell–cell interactions, target cell killing, antibody blocking or internalisation, a function-focused approach provides a comprehensive understanding of how cells behave in response to various stimuli. These function-focused insights enable researchers to evaluate therapeutic potential with greater precision, reducing reliance on secondary screens and driving more efficient decision making throughout the drug discovery process.

The key challenges of current single-cell approaches

Single-cell screening relies on isolating individual cells, and improvements in droplet microfluidic technology now enables single cells to be isolated into tiny droplets, typically in the picolitre or nanolitre range. This compartmentalisation prevents the mixing of signals between multiple cells, enabling precise, individual-cell analysis, as well as allowing for the assaying of secreted products. By encapsulating cells within these droplets, researchers can control minute volumes of fluids, and capture data from each droplet as an independent mini-reaction vessel—thereby offering the potential for performing direct functional analysis. However, despite these advances, significant challenges persist across various platforms, making dynamic functional analysis at the single-cell level difficult.

DROPLET ENCAPSULATION INTRODUCES VARIABILITY TO GENE EXPRESSION PROFILES

Traditional encapsulation processes often induce stress on cells, affecting their viability and therefore reliability of sequencing data. This can be due to mechanical or shear stress in creating the droplets, stabilising surfactants, and osmotic stress, which can alter gene expression profiles, create noise in the data, and increase the risk of false positives or missed targets.

INFLEXIBLE DROPLET MANIPULATION HINDERS EXPERIMENTAL ADAPTABILITY

Traditional prefabricated channels can lack the flexibility for real-time adjustments, making it challenging to adapt the system for specific cell types or experimental conditions. The fixed design restricts the ability to customise flow patterns or alter fluid dynamics, which can be essential for isolating and analysing single cells accurately. Additionally, these channels often suffer from clogging and non-uniform flow, which can cause cell loss or inconsistencies in data collection. Scaling up for high-throughput applications is also problematic, as each channel must be individually designed and fabricated, adding time and cost constraints. These limitations reduce the versatility of prefabricated channels, underscoring the need for more adaptable microfluidic solutions that can dynamically adjust to optimise single-cell analysis.

DISTRIBUTION RANDOMNESS OF DROPLET CONTENTS IS A ROADBLOCK FOR SINGLE-CELL ANALYSIS

Random distribution of cells in droplet microfluidics arises from the statistical nature of cell encapsulation, which follows a Poisson distribution. This random distribution leads to an uneven distribution of droplets containing content, resulting in a significant proportion of droplets being either empty or containing multiple cells. This inefficiency complicates downstream analysis, as empty droplets waste resources, and droplets with multiple cells can cause cross-contamination or make it difficult to accurately attribute data to individual cells.

Relying on random distributions also hampers the feasibility of sequential assays, as this often requires the precise pairing of specific cells, molecules, or reagents within individual droplets. Due to the random and uneven distribution of contents, the likelihood of correctly encapsulating the required combinations in the same droplet is exceedingly low. This further reduces the efficiency and reliability of the workflow, making it difficult to achieve meaningful and reproducible results.

The Lightcast solution: Overcoming the key challenges to enable function-focused, single-cell profiling

Enabling functional analysis at the single-cell level during early-stage drug discovery could yield faster and more accurate identification of promising drug candidates, streamlining the path to clinical validation (Figure 2: The impact of direct functional assessment at the single-cell level on drug discovery). The Lightcast solution aims to enable direct functional analysis by addressing the key limitations of traditional technologies.

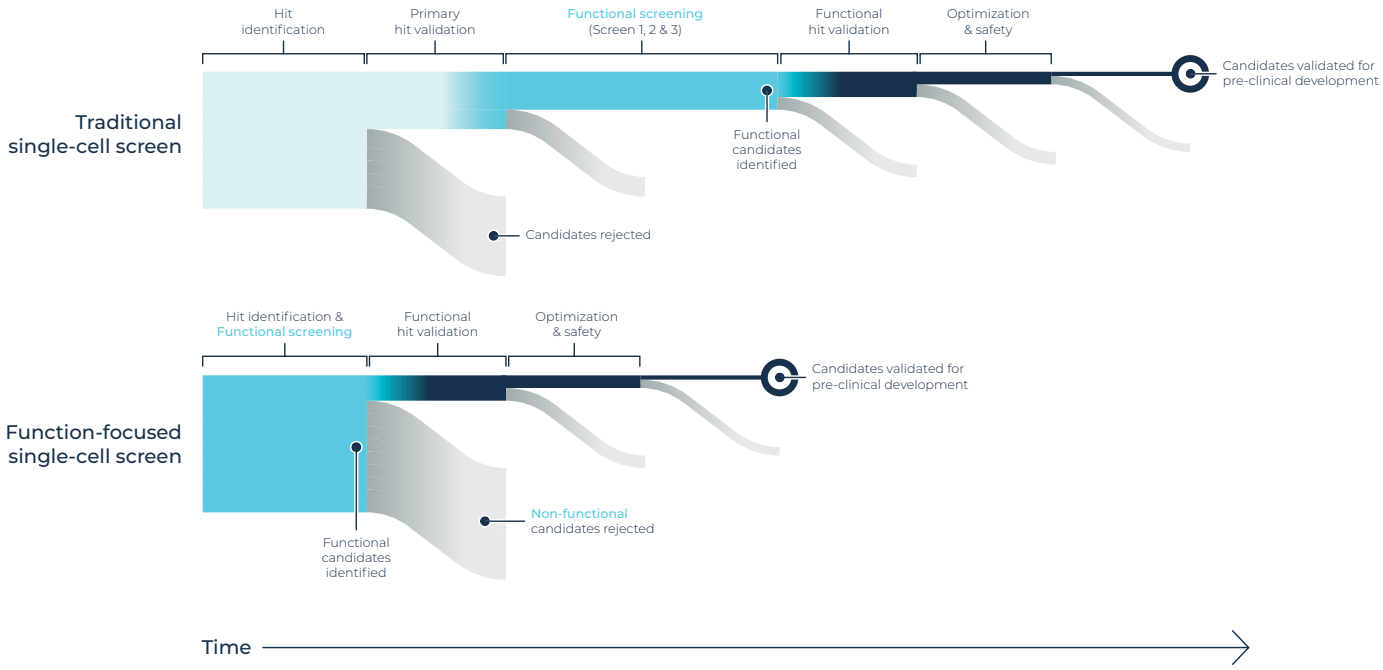

Figure 2: The impact of direct functional assessment at the single-cell level on drug discovery

Traditional single-cell screening involves multiple sequential stages and attrition points to identify candidates for pre-clinical development. After hit identification and validation, multiple functional screens are required to identify functionally validated candidates. This complex and lengthy process can advance non-functional candidates, increasing costs and delaying development timelines. A function-focused approach streamlines drug discovery by integrating hit identification and functional screening into a single step. Non-functional candidates are rejected earlier, reducing attrition points, accelerating timelines and decreasing resource demands.

1. STEP EMULSIFICATION TO REDUCE SHEAR STRESS

Step emulsification is a highly efficient technique for droplet generation, which significantly reduces shear stress during encapsulation and preserves cell viability and functionality. One of the primary benefits is its ability to produce droplets of uniform size, which is crucial for consistent single-cell encapsulation and analysis. This uniformity ensures that each cell is subjected to the same reaction conditions, leading to more reliable and reproducible results. Reducing shear stress improves cell viability and increases the throughput of valid, useful cell–cell interactions. The ability to encapsulate cells whilst preserving cell viability and functionality in highly uniform droplets with the required reagents therefore enables the parallel processing of tens of thousands of cells.

2. FROM RANDOM TO REPRODUCIBLE: USING MACHINE LEARNING TO OVERCOME CELL DISTRIBUTION VARIABILITY

The Poisson distribution of cells into droplets poses a significant challenge in droplet-based single-cell analysis, often leading to droplets containing multiple cells or, conversely, empty droplets. Empty droplets are especially problematic when working with low cell concentrations, such as precious primary cells. The Lightcast solution overcomes this limitation by utilising machine learning algorithms to precisely eliminate any droplets with unwanted contents in real time, thus enriching for desired single-cell occupancy in droplets. This approach enables more reliable single-cell analysis and isolation for downstream applications.

3. OPTICAL ELECTROWETTING-ON-DIELECTRIC (OEWOD) TECHNOLOGY FOR ENHANCED DROPLET CONTROL

Optical electrowetting-on-dielectric (oEWOD) technology offers significant advantages for single-cell analysis over traditional prefabricated channels by providing enhanced flexibility, integration, and efficiency. Unlike static prefabricated channels, oEWOD enables precise control of droplet movement through reconfigurable paths defined by virtual electrode actuation, allowing dynamic isolation, merging, dispensing, and collection of single cells. This adaptability overcomes the fixed and constrained flow paths of traditional channels, whilst being economically and environmentally favourable, requiring minimal sample and reagent volumes.

4. REAL-TIME DROPLET IMAGING TO MEASURE DYNAMIC CELLULAR RESPONSES

Traditional single-cell technologies provide only a single snapshot in time, failing to capture the dynamic changes of individual cells. In contrast, the Lightcast imaging platform enables multi-snapshot monitoring of droplet contents over time, allowing for the assessment of cellular dynamics. This ability to track cellular behaviour over extended periods provides a more comprehensive understanding of cellular processes, offering insights that static methods cannot reveal.

A SINGLE PLATFORM TO ENABLE MULTI-STEP WORKFLOWS AND SEQUENTIAL ASSAYS

A unique feature of the Lightcast platform is its ability to perform sequential assays through precise droplet merging, allowing controlled interactions, such as cell–cell communication, cell–molecule binding, or serial killing (Figure 3: The Lightcast solution facilitates efficient droplet merging for sequential assays). By integrating a sequence of merges, the Lightcast platform delivers function-focused insights while simplifying complex workflows and reducing reliance on multiple separate assays. This innovative capability allows researchers to study dynamic biological processes—such as signalling cascades, enzymatic activity, or serial killing—with precision and minimal resource use. By analysing multiple functional outputs on a single instrument, the platform also enables the identification of highly functional cells or antibodies with unparalleled efficiency.

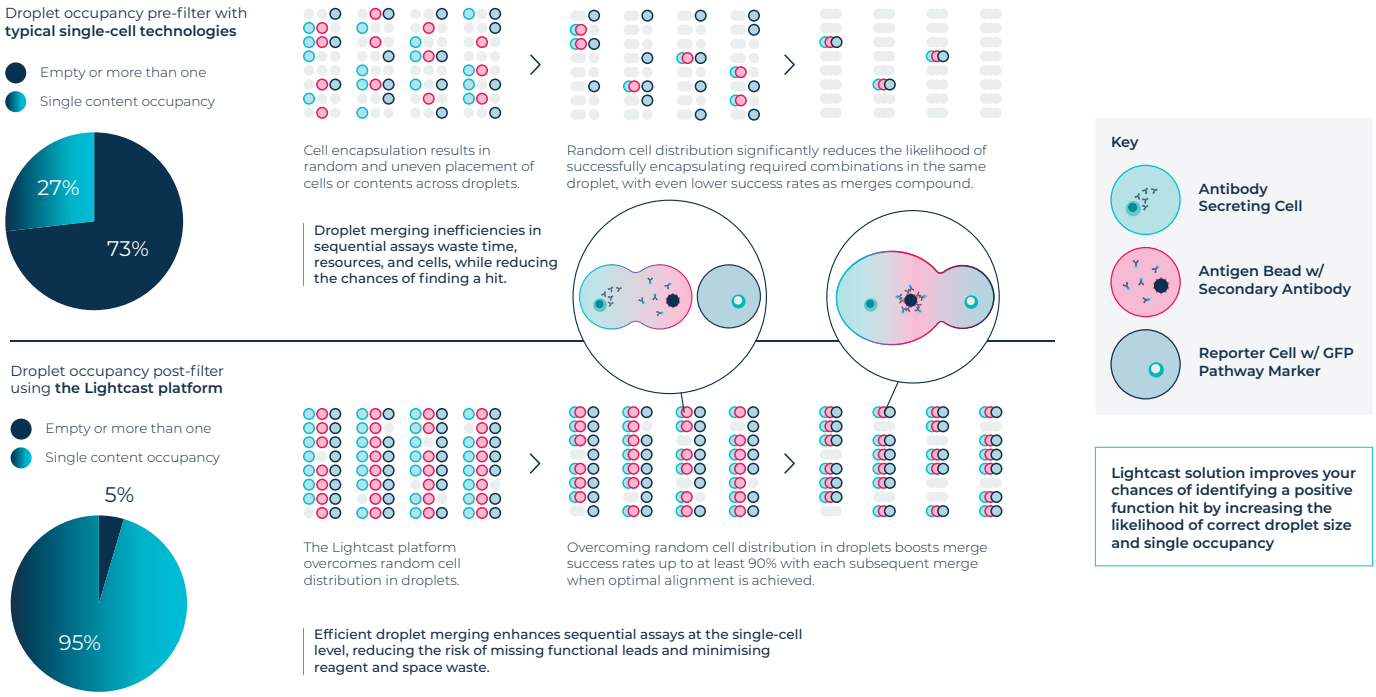

Figure 3: The Lightcast solution enables efficient droplet merging for sequential merges.

In single-cell technologies, typically only 27% of droplets will contain the required contents due to random distribution described by Poisson statistics. With the Lightcast machine learning filter applied, 95% of droplets are correctly populated[4]. Without filtering, many droplets lack the required components which prevents sequential assays following successful merging of cell contents to identify positive functional hits. The Lightcast solution ensures efficient and reliable sequential merges, streamlining processes and improving the likelihood of identifying functional hits.

An innovative function-focused single-cell platform

Direct functional analysis at the single-cell level provides a more complete understanding of dynamic biological interactions, offering critical insight into cellular and molecular pathways. The Lightcast solution is a novel platform that seeks to address the major challenges of utilising single-cell technology, combining droplet microfluidics, oEWOD, and machine learning to achieve high-throughput, complex manipulation, and efficient analysis of single cells. A unique filter function overcomes the limitations of Poisson distribution, ensuring that each droplet contains the desired contents and maximises chip space for significantly increased assay throughput. The versatile oEWOD manipulation enables a range of workflow modules, such as merging droplets, which when combined with filtering supports complex, sequential assay workflows involving multiple merge events. Droplets are assigned a unique ID, with each droplet's history tracked throughout the workflow, allowing real-time analysis of single-cell biological functions.

Enhancing the drug discovery pipeline with single-cell functional profiling

Addressing the major limitations of traditional single-cell technologies could enable direct functional analysis of single cells in real time, with the potential to transform early-stage drug discovery. The Lightcast solution offers the ability to perform complex, sequential single-cell workflows with automated tracking and data collection, enabling high-throughput, scalable single-cell screening for a more comprehensive understanding of biological complexity. This innovation could therefore accelerate the drug discovery process, providing researchers with a powerful tool to better understand cellular responses and identify promising drug targets earlier in development. This direct functional profiling capability paves the way for more informed decision making and streamlined validation, ultimately improving the pipeline of therapeutic candidates and enhancing the development of effective treatments.

REFERENCES

1. Hughes, J.P., Rees, S., Kalindjian, S.B. & Philpott, K.L. Principles of early drug discovery. Br. J. Pharmacol. 162(6):1239–49 (2011).
2. Fox, S. et al. High-throughput screening: update on practices and success. J. Biomol. Screen. 11(7):864–9 (2006).
3. Usaj, M.M., Yeung, C.H.L., Friesen, H., Boone, C. & Andrews, B.J. Single-cell image analysis to explore cell-to-cell heterogeneity in isogenic populations. Cell Syst. 12(6):608–621(2021).
4. Welch, L.G. et al. A programmable and automated optical electrowetting-on-dielectric (oEWOD) driven platform for massively parallel and sequential processing of single cell assay operations. Lab on a Chip 24(16):3763–3774 (2024).

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