Envisia: Advancing antibody discovery with function-focused profiling at the single-cell level

Simon Margerison, Drew Geere, Claire Murzeau, Danai Koftori, Valentin Radu, Thomas Linford-Wood, Nicole Lederer, Scott Brouilette, Carrie Maynard and Ian Watt

Introduction

Discovery of new therapeutic antibodies is challenging, in part due to adherence to a 'binding first' approach, prioritising binding before functional assessment. We present Envisia, a next-generation platform that uses droplet microfluidics to perform highly controlled, sequential single-cell functional assays. By integrating functional screening early in the discovery process, researchers can bring function into focus and advance only the most promising candidates.

Envisia: bring function into focus

Envisia utilises droplet microfluidics, optical electrowetting-on-dielectric (oEWOD) technology, and machine learning to enable single-cell functional analysis. Soluble reagents or objects, such as cells or beads, are encapsulated into droplets of media in fluorous oil and are actively filtered based on size and content ensuring only desirable droplets are retained for analysis. Droplets are stored on chip and the history of individual droplets is logged from the point of filter until completion of the workflow. On chip, droplets are subject to an automated and flexible suite of operations including the merging of droplets and the fluorescent acquisition of assay readouts to enable complex sequential assay workflows. Droplets are then dispensed for downstream processing.

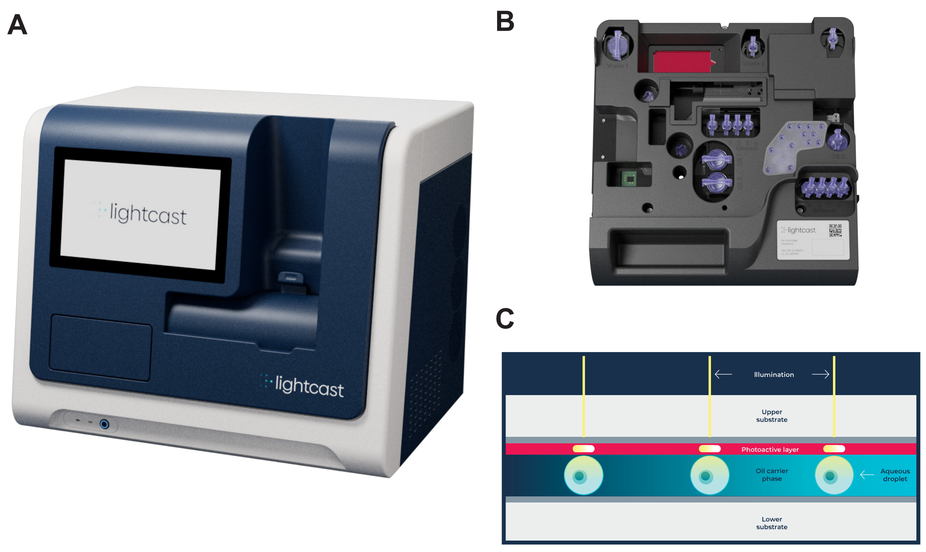

Figure 1. The Lightcast platform. *(A) Envisia instrument. (B) Cartridge-contained fluidics ensure a contamination-free workflow. (C) A cross-section diagram of an oEWOD chip outlining the core technology of the platform that enables droplets to be manipulated with projections of light.*

Rapid single B cell loading

Fast B cell loading, filtering and arraying was demonstrated with up to 12,000 single B cells loaded in less than 90 minutes. The filter function uses machine learning to actively enrich for droplets of the desired occupancy and size (Figure 2a). This ensures only desirable droplets are stored in experimental arrays for assaying and downstream processing while ensuring that a 1:1 ratio is achieved for complex sequential assay (Figure 2b). Moreover, different cell types can be loaded into droplets on the experimental array (Figure 2c).

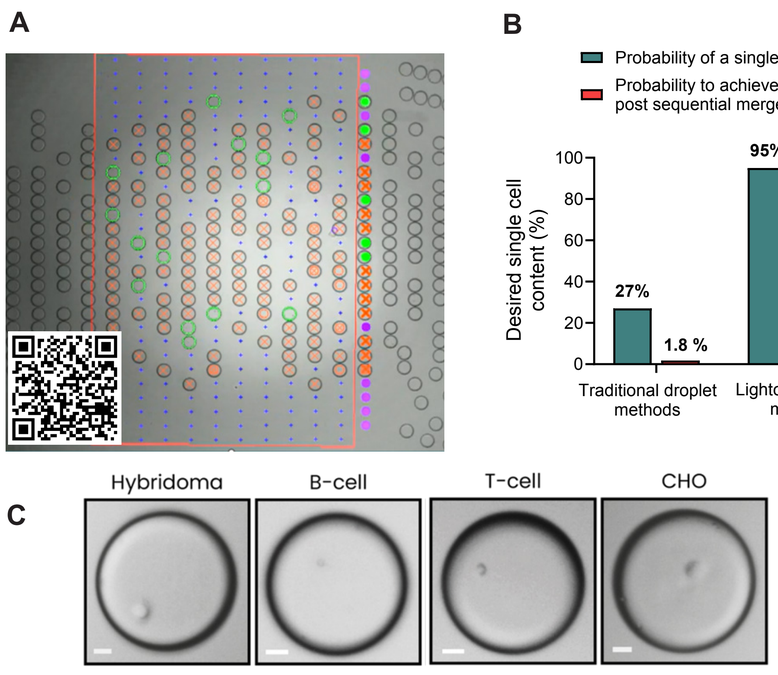

Figure 2. Cell loading. *(A) A still from a video demonstrating the filter function enriching for droplets containing single B cells. (B) Probability of single cell droplet content occupancy using traditional methods compared to the Lightcast method. (C) Still images of different cell types loaded onto platform in droplets including hybridoma, B cell, T cell and CHO cell*

Peer-reviewed Publication

Scan here to access our recent publication in *Lab on a Chip* where we demonstrate additional workflows applicable to antibody discovery and infectious disease research.

lightcast

Lightcast Discovery Ltd, Broers Building, 21 JJ Thomson Avenue, Cambridge, United Kingdom, https://www.lightcast.bio/

Spiking case study: detecting rare population of antibody-secreting cells

A mixture of a low percentage (1%) of hybridomas secreting antibodies against a relevant TNF-alpha target and a large percentage (99%) of hybridomas secreting antibodies against an irrelevant target were dropletised and loaded, then merged with droplets containing target antigen-coated beads and a PE-conjugated secondary antibody. Fluorescence was measured at 1 hour intervals to find those rare hybridomas secreting the relevant target antibody, presumably 1% of all droplets. Prior to load, the relevant secretors were stained with CellTracker Deep Red (CTDR) while irrelevant secretors were stained with CellTrace Violet (CTV). As demonstrated below, a positive "hit" would be defined by a merged droplet containing a CTDR+/CTV- cell, and a bead with increasing PE intensity over time.

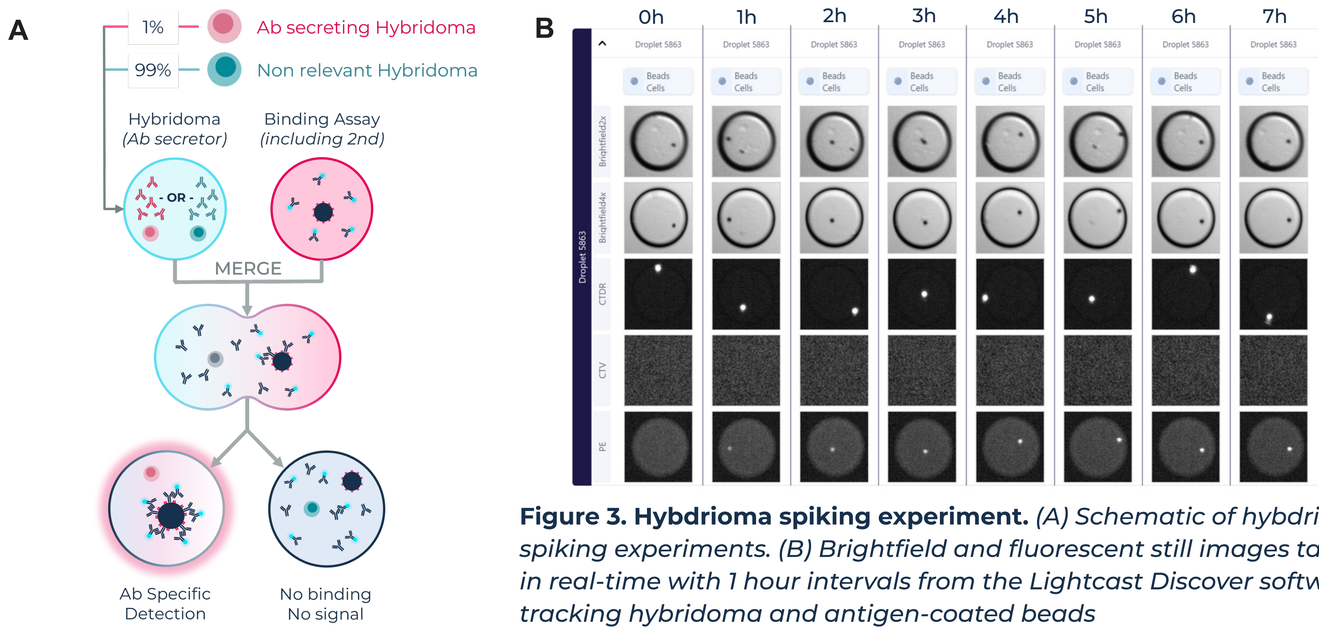

Figure 3. Hybdrioma spiking experiment. *(A) Schematic of hybdrioma spiking experiments. (B) Brightfield and fluorescent still images taken in real-time with 1 hour intervals from the Lightcast Discover software tracking hybridoma and antigen-coated beads*

Of the 1624 cells loaded, 15 hits were identified (out of an expected 16), within just 3 hours post droplet merging (Figure 4a). This resulted in a spike recovery of 0.9%, therefore demonstrating the platform's ability to detect rare populations of secretor cells. Fast hit identification, combined with rapid loading and hit recovery into well plates, enables end-to-end antibody discovery in under a day - significantly faster than other available platforms. Each droplet containing a single cell is assigned a unique ID, allowing dynamic tracking of hits over time (Figure 4b). This enables the detection of increasing fluorescent signals for kinetic insights, distinguish true hits from false positives, and ensure only the most promising candidates are selected for further analysis and recovery.

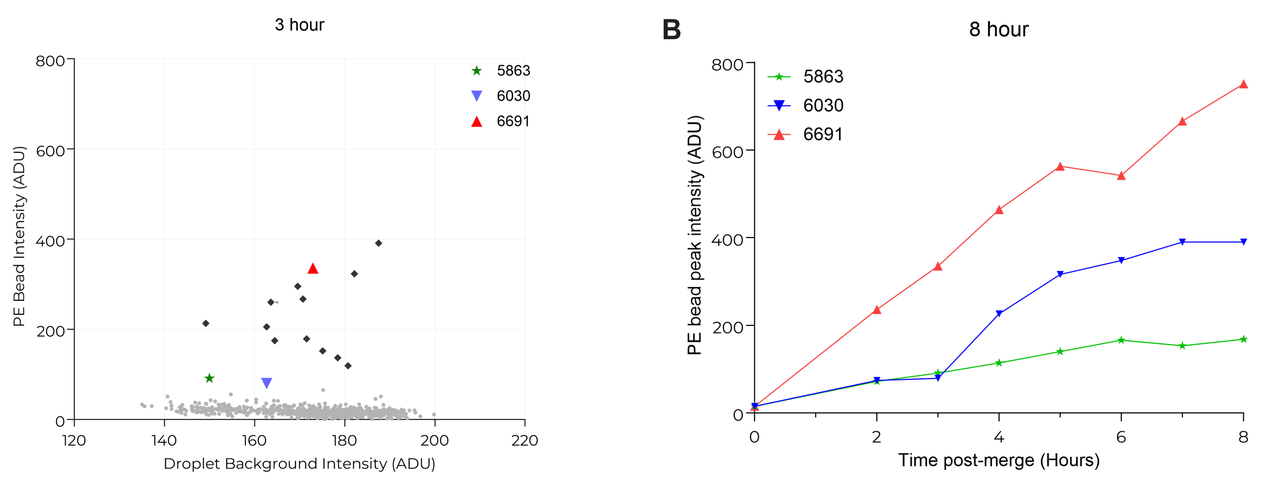

Figure 4. Single-cell hit identification and dynamic tracking *(A) Postive hit identification from peak PE bead intensity taken at 3 hours after droplet merging. (B) Dynamic cellular tracking of three positive hits across an 8 hour time period post merge.*

Fast hits, no crosstalk: antibody discovery workflow in 1 day

Through its controlled oEWOD technology, Envisia ensures that individual antibody-producing cells are singulated and analysed with minimal crosstalk (Figure 5), thereby reducing variability of cellular functional readouts compared to open assay environments (where "fluidic drift" has been reported to risk compromising data quality).

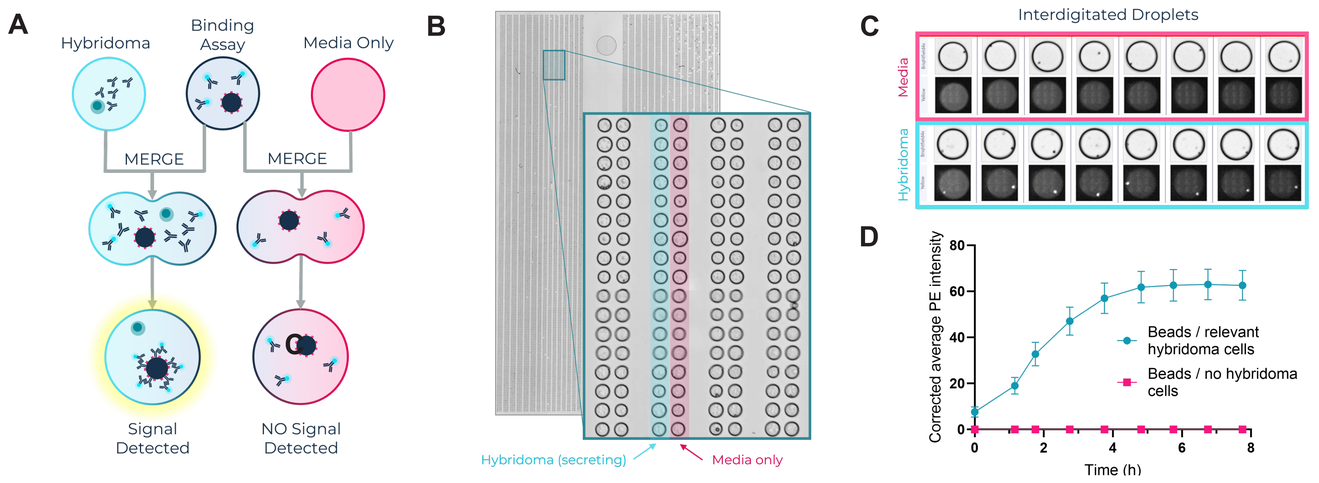

Figure 5. Minimal crosstalk in a droplet microenvironment. *(A) Schematic workflow of an antibody binding assay merged with media only or hybridoma containing droplets . (B) Brightfield array images of control droplets next to droplets containing hybridoma secreting cells. (C) Droplet images in flourescence and brightfield at 1 hour intervals. (D) Corrected average PE fluorescence intensity over time.*

Envisia enables fast & reproducible single-cell loading, ensuring reliable assay pairing for accurate hit identification. With no crosstalk during detection, true hits are confidently distinguished from negatives. Finally, efficient hit recovery into well plates for off-platform sequencing allows end-to-end antibody discovery in less than a day.