Alix VENTURES

Market Deep Dive Report

High-Throughput Single-Cell Analysis & Manipulation

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1. Summary

Novel tools for analysing and manipulating individual cells hold great promise for aiding the development of the next generation of precision and personalised medicines. Searching for rare phenotypes and assessing the heterogeneity in drug responses has been previously shown to significantly improve cancer diagnostics and treatments. Together with other indications such as for stem cell therapies, the oncological applications of single-cell technologies will continue to drive the rapid growth of the market, which is expected to reach over $5.5 Bn in the next three years.

The recent technical advancements in the fields of microfluidics, optics, sequencing, and data processing can now be utilised in highly-combinatorial ways to screen billions of cells in parallel. Such cells can then be further selected or engineered to possess the desirable phenotypes in a closed-loop, fully automated fashion. The current state-of-the-art solutions on the market possess a trade-off between high throughput and high assaying capacity, and we believe that the new breakthrough technologies will address this challenge. Furthermore, decreasing the currently high cost of the single-cell bioinstrumentation will diversify the customers to include smaller biotech and researchers involved in early-stage discovery programs. The new market leaders should be able to provide full or significant discovery services and rely on easily-adaptable workflows to tailor them to the needs of their customers, be that research labs or big pharma. We believe that the combination of broadening of assaying and omic capabilities, increased throughput and cost decrease underlie the attractive investment opportunities in this space.

2. Market Overview

High-throughput single-cell (HTSC) analysis and manipulation market was valued between $2.2 Bn in 2020 and $3.1Bn in 2021 and is prognosed to expand at a CAGR of 15% from now to 2028 (Fig.1) [1,2]. This rapid growth is driven by 1) technical advances in the fields of microfluidics, 2) the rising reproducibility and cost decrease in sequencing and omics (SC analysis), and 3) implementation of high-throughput selection and amplification techniques (SC manipulation). In other words, the cutting-edge HTSC technologies combine multi-modal screening capabilities with high precision and sensitivity in cell selection, engineering, and expansion. The consequential rise in adoption in SC analysis devices for accelerating research and development (R&D) by pharmaceutical and biotechnological companies and a following

increase in wider investment into the deployment of such methods in personalised biomedicine have been continuously expanding the market.

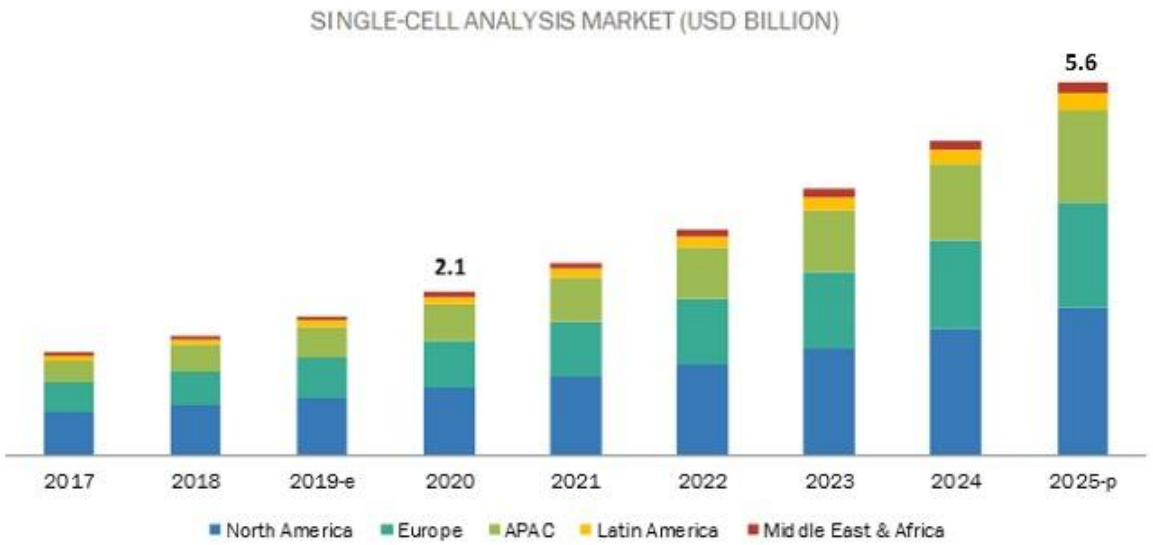

Figure 1: Single-cell analysis market: growth prediction by region. Source: Markets and Markets [2].

The main stakeholders include academic & research laboratories (~70% of total end users), biotechnology & pharmaceutical companies, as well as hospitals, diagnostic laboratories and other service facilities (Fig.2). The dominance of the research users can be partly attributed to the large market share belonging to consumables such as assay kits recurrently used by the research laboratories. In contrast, the high cost of the HTSC equipment limits their affordability and is ultimately considered to be the major factor hampering the market growth. The cost reduction is, thus, one of the main value-adds of the new products in the space.

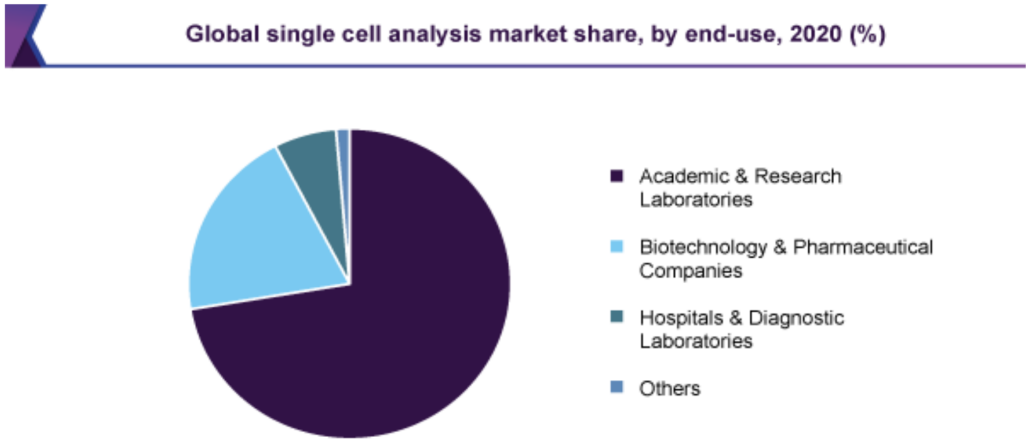

Figure 2: Global single-cell analysis market share (by end-use, 2020). Source: grandviewresearch.com [1].

The field of oncology accounts for the largest revenue share (30%) [1], and is expected to remain the main application of the market products. Analysis of individual patient cells has been demonstrated to improve the diagnostics, treatment selection and monitoring, such as upon qualitative and quantitative assessment of cellular identities and mutagenesis conditions, as well as accelerate antibody discovery and immuno-therapy development. The number of yearly cancer cases is expected to rise from 19.3 Mn in 2020 to near 30 Mn in 2040, driving the future demand for the HTSC technologies [2]. In addition to cancer, the increasing adoption of stem cell therapies for tissue and organ regeneration and transplantation will drive further demand for SC analysis methods. In research settings, there is additional applicability of SC-omics to the fields of neuroscience, immunology, and infectious diseases.

When segmented by the service suppliers, the majority of the market share (~60%) belongs to small startup companies, of which the four major players are Berkley Lights, 10X Genomics, and Fluidigm. Their technologies are discussed in detail in the following section.

3. Technology Overview

3.1 Synopsis

HTSC technologies enable precise assessment and manipulation of high quantities of individual cells. While the composition of HTSC products varies depending on the application, the general functionalities include SC-isolation, SC-sequencing, SC-omics, and SC-focused manipulation. The first two capabilities enable the majority of the products on the SC market (72%) [3]. This is partly driven by the wide range of the SC applications requiring cell library preparations. It is worth noting that cell library generation further drives the demand for reagents and kits, making consumables play a significant role in generating revenues in the SC space.

The core technologies for SC isolation include conventional methods for cell picking such as dilution-based methods, laser-based techniques (Fluorescence-Activated Cell Sorting or FACS, laser capture microdissection), as well as novel methods based on microfluidics and microwells for cell selection, isolation and following analysis, automated cell picking, and dispensing. The development of computation methods for image analysis and precise optics technologies has significantly expanded the range of SC handling capabilities in the last few years.

Once the specific cell phenotype has been examined, it often needs to be modified. The increase in biomedical applications of SC technologies has been driving the demand for advanced downstream omics analysis and following SC manipulation, which is expected to become more widely offered in the next generation of HTSC products. The main omics readouts can be broadly categorised to: genomics/transcriptomics (DNA/RNA sequencing), proteomics, secretomics, as well as the measurements of cell mechanics, both internal and external (Fig.3).

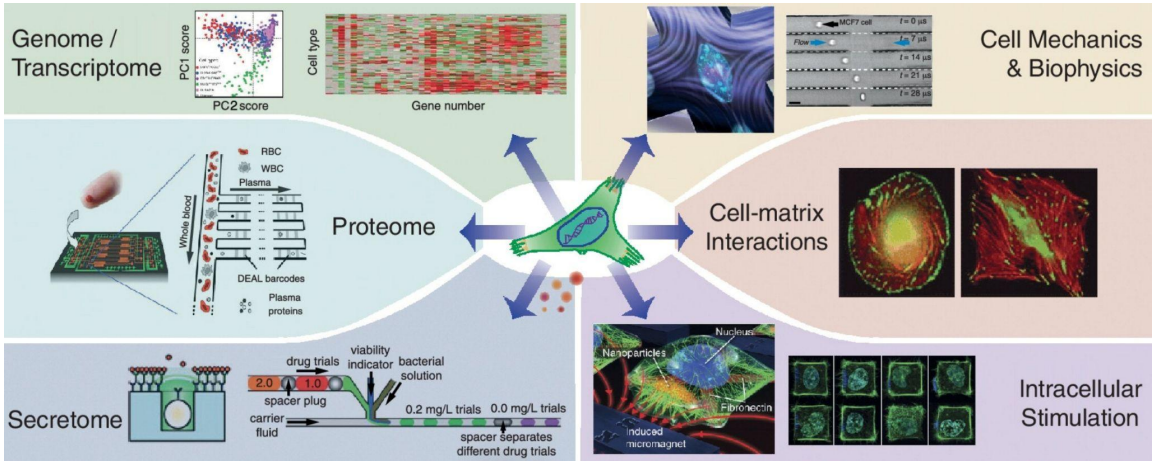

Figure 3: Advances in high throughput single cell microtechnologies. Source: Curr.Opp. in Biotechnology [4].

Together with upstream SC selection and assessment, such capabilities enable the five main application categories: 1) SC-isolation, 2) SC-sequencing, 3) cell line development, 4) protein/functional characterisation, and, ultimately, 5) drug discovery/diagnostics.

3.2 Current Techniques & Platforms

The state of art products in the field combine multiple SC analysis and manipulation techniques within one shell, while also providing high-throughput and/or a large range of live cell types the technology can be applied to. The four companies below provide examples of the best products on the current market. An overarching tradeoff between multi-dimensional analysis at low cell numbers and high-throughput analysis of a limited number of cell attributes can be observed in the following examples. We believe that the next generation of breakthrough products in this space shall be able to overcome this challenge.

Berkeley Lights (NASDAQ - BLI): Berkeley Lights uses proprietary light-activated microfluidic technology or 'optofluidics' to move and isolate millions (10^6) of individual cells. Their nano-well technology enables rapid capturing and automated assaying of small quantities of cells which would otherwise take days to be expanded to 50,000 times larger quantities prior to inspection. Combined with deep genomic profiling capabilities, this palette of technologies enables the antibody discovery, cell line development, and cell therapy services developed by the company. Amgen was one of the early customers to have adopted this technology and has now put it into practice in its antibody development work reducing the R&D time from months to days.

10X Genomics (NASDAQ - TXG): 10x Genomics combines barcoding methods and droplet-based microfluidics for analysing nucleic acids in hundreds-thousands (10^2-10^4) of individual cells in different spatio-temporal contexts. In addition to transcriptome analysis, their technology can capture the surface proteome, but lacks the cell visualisation and intracellular proteome capabilities. The setup easily fits into existing laboratory workflows and is widely used by the academic and research community.

Fluidigm (NASDAQ - FLDM): Fluidigm has historically been a veteran of the SC microfluidic technologies since releasing C1, the world's first automated solution for SC genomics, in 2012. The technology operated on a scale of 10^3 cells, making significant step up in throughput during the time of its release. Today, the main differentiating feature of Fluidigm's products is mass-cytometry, a number of methods for highly multiplexed protein detection used to assess both intracellular and surface proteomes. As an early player on the market, Fluidigm has been widely acknowledged within the research community and has its products featured in multiple peer-reviewed publications.

Isoplexis (Series D Funding): Isoplexis is a key player in the SC market focused on assaying cellular proteomes and secretomes such as in the immune cell assays. The Isoplexis platform can simultaneously assess over 30 cytokines in 10^3 cells in a fully-automated fashion. In 2021, the company was announced to expand its capabilities upon acquiring an extensive IP portfolio in nucleic acid analysis.

While the exact prices of above mentioned products are not disclosed, the price range they fall into varies from $600,000 - 700,000 (10x Genomics, Isoplexis, Fluidigm) to $1Mn (Berkeley Lights) which limits their affordability within the early-stage biotech companies and small research organisations. Apart from high cost, additional customer challenges include non-trivial combinations of multiple data outputs often obtained from

different instruments. All these call for a cheaper alternative integrating the existing strengths and throughputs of current leading products within one comprehensive platform. As such bioinstrumentation would likely be used in the early stages of R&D, another crucial factor would be the ease of its integration within other laboratory workflows.

4. Historical Context, Key Trends, & Future Development

Over the last few decades, the field of SC analysis made a leap from a dispersed arsenal of low-throughput technologies irreversibly perturbing the cells into a number of high-throughput multi-modal products with almost 100% cell viability. While the initial analyses were so disruptive that there were no cells to be manipulated afterwards, researchers are now able to not only precisely change their types and behaviours but also re-introduce them back into the organisms of origin.

At the same time, the realisation of the importance of cell heterogeneity in personalised and precision medicine has led to the increasing use of HTSC products beyond the research labs and in the development of next-generation therapies. The rapid evolution of the cell processing capacity uncovering such heterogeneity is also accompanied by an increase in the devices' sensitivity. Today, even small samples of rare/hard to obtain cell types can be reliably assessed – this is yet another factor driving the biomedical use of such technologies. Technological advancements of microfluidic technologies and increasing uptake of omic technologies and big data analysis in drug development will continue to further drive the HT SC market.

Finally, from the business perspective we anticipate the verticalization of the future platform technologies in this space. The most ambitious companies operating in the HTSC field are aspiring to provide complete drug discovery services to pharmaceutical partners with the ultimate goal of transitioning into fully-integrated pharmaceutical companies themselves. While only a minority of these startups will develop to this stage, we think that many of them can still become novel service providers diversifying the R&D opportunities for lesser-funded organisations through receiving upfront payments even if the royalties will not always be available.

5. Opportunities

5.1 Startups to Watch

Zafrens: Zafrens is a stealth-mode biotechnology company working on combining multiple proprietary SC technologies, while significantly increasing the screening throughput, and simultaneously reducing the cost of their platform product. The Alix team believes that such technology could revolutionize the market by 1) unmasking very rare cellular phenotypes and 2) providing access to the new product users due to significant cost decrease. The platform will be initially applied towards developing small molecule drugs that perturb RNA binding proteins (RBPs), which are expected to significantly expand the druggable proteome space (currently ~5%). More information on the company is available in the respective Investment Memo.

Sphere Fluidics: Sphere Fluidics is an England-based microfluidics company which develops affordable benchtop bioinstrumentation for single-cell screening within picodroplet compartments. Their Cyto-Mine® technology can process up to 40 Mn mammalian cells and is primarily used for antibody discovery and cell line development.

Lightcast Discovery: Lightcast Discovery is developing a new light-driven technology for precise and independent manipulation of individual cells and reagent-containing microfluidic droplets which can be assays in a highly combinatorial fashion. The interest to this Berkley lights competitor comes from their primary focus on live image analysis which enhances the potential design space for their microfluidics. Lightcast is currently engaged in undisclosed partnerships with pharmaceutical companies to develop their POC studies.

Sestina Bio: is a Foresite Labs-raised synthetic biology company aiming to develop a closed-loop automated toolkit for SC genotyping, phenotyping, and machine learning-driven experimental design. Thanks to the diversity of input cell types the Sestina technology can work with, the wide range of its potential applications includes not only healthcare but also clean-tech and food-tech sectors.

5.2 Emerging Technologies & Applications

As mentioned in the Synopsis (3.1), the novel products on the market increasingly incorporate multiple capabilities for downstream analyses and cell manipulation based on the screening outcomes. While some analysis methods have successfully

transitioned from the fundamental research to the translational settings, there are a number of SC assays yet to enter the biomedical market. Examples include new modalities such as epigenetic analyses or cell morphology assessments, as well as new combinations of existing methods such as spatial transcriptomics or single-molecule detection tools in live cells.

Additionally, the rapid development of precise genomic manipulation tools such as CRISPR will significantly expand the range of downstream workflows HTSC technologies can be integrated with. The increasing applicability of such methods in the fields of neuroscience and infectious diseases will drive the expansion of cell type inputs and further incorporation of the field-specific methods in the SC analysis and manipulation workflows. We believe that the next generation of HTSC products should possess the flexibility in the device architecture and accompanying analysis software which would allow for modular incorporation of novel technologies to the new versions of the products.

Finally, the multiplicity of omics analyses available will generate the new non-trivial insights into cellular phenotypes. Developing proprietary AI/machine learning tools to create predictive models driving further experimental design will become a key service offered by the HTSC technology providers as a part of their drug discovery toolkit.

5.3 Industry Challenges

As stated above, the high cost of next-generation HTSC analysis & manipulation equipment has been significantly impeding the growth of the associated market. Since the majority of the potential customers cannot afford owning a tool incorporating multiple techniques in one box (e.g. Berkeley Lights products), they are further facing the challenge of integrating the data obtained from multiple sources and processed with multiple software types. Building the standardised data generation and analysis workflows will be, thus, of help in attracting new product users to the space.

Another challenge comes from the high cost of human capital currently associated with operating HT SC devices. Today, some of the most skilled workers are needed to demo and operate such platforms, which further limits their use in the early-stage discovery context. Full automation of such products will further diversify their accessibility and enable on site assessment of the cellular samples which are otherwise too fragile to be transported to discovery sites away from the hospitals.

6. Conclusion

6.1 Overall Summary

Despite the recent disconnect between the cell analysis and manipulation tools in the past, the current HTSC technologies market has matured to incorporate multiple techniques under one shell. The increasing understanding of the role of rare cell phenotypes and cellular heterogeneity in personalised and precision medicine and, in particular, in the spaces of oncology and regenerative medicine will be driving the demand for biomedical applications of such tools in the future. We believe that novel technologies in the space of HTSC analysis and manipulation have a potential to trivialise drug discovery and, ultimately, lay the foundations for emergence of the new, fully-integrated, pharmaceutical companies operating in the space of precision medicine. Below we summarise the particular strengths and weaknesses underlying our investment theses in this space.

6.2 Vertical Strengths

- **Serving a Rapidly Growing Market**: HT SC manipulation is a rapidly growing market with huge potential in personalised medicine that we expect to continue driving growth for several years.

- **Potential to Significantly Expand the Market:** Through building collaborations with users with different input material and building versatile tools handling variable and heterogeneous samples

- **Limited Downside Risk**: Through collaborations with pharmaceutical companies able to pay upfront and then using the earnings to verticalize the assets into a full drug discovery platform.

6.3 Vertical Weaknesses

- **Currently Limited Customer Space:** High cost of SC manipulation technologies is a major factor limiting routine use of multimodal SC bioinstrumentation beyond existing workflows of wealthy research labs and biopharma. While the cost decrease is expected to come in the next decade, it might not be sufficient to open up the market to the new categories of users.

- **Market Fragmentation to Crowded Sub-Markets:** While not many companies can provide all services at once, there exists significant competition in the separate domains of single-cell isolation, manipulation, and various assessment methods. Unless the value-add of combining all services in one box is justified and aligned with the product price, potential customers might choose to opt out for more niched service providers.

6.4 Investment Theses

- **Ultra-Throughput Power:** We are interested in achieving the increase in throughput from 100s to Mns of cells to rare phenotypes previously missed in less extensive SC screenings. Combined with novel data analysis/AI tools, a further increase to a Bn-scale throughput in SC analysis will enable the discovery of new rare phenotypes and has high potential for curing some of the most devastating diseases.

- **Cell Type Versatility:** We are looking for platforms that allow for extended screening of cells to analyze phenotypic responses over a period of time allowing for improved accuracy over approaches that limit analysis to a single snapshot of a cell.

- **Broad Based Assays & Omic Capabilities:** We are focused on platforms that can perform a wide range of assays and omics testing, allowing researchers to tie genotype to phenotype across millions of cells in parallel. We are particularly interested in platforms that can clone chips, allowing for terminal assays to be performed without losing cell lines.

- **Rapid & Low Cost Asset Development**: The platforms that can reduce the cost and time of drug discovery, while still developing novel drugs against new and difficult to hit targets are of key interest to us.

- **Early Partnership & Services Opportunities:** We are looking for single cell platforms that have a wide range of potential uses and have ample opportunity to develop early stage partnerships and research agreements with pharma to fund pipeline or platform development. We are also interested in companies who recognize the value of this approach.

7. Sources & Additional Reading

- [1]https://www.grandviewresearch.com/industry-analysis/single-cell-analysis-market
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