Integrating high-content imaging with mass spectrometry (HCS+M) for high-throughput single-cell metabolomics and drug discovery

Theodore Alexandrov[1*]

[1]Structural and Computational Biology Unit, European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany
*Corresponding author: theodore.alexandrov@embl.de

ABSTRACT
Metabolomics is the latest of -omics that promises to reveal metabolic reprogramming of cells in cancer, characterize metabolic requirements of the immune cells to improve immunotherapies, and shed light on metabolic effects of drugs. Emerged single-cell transcriptomics technologies have revealed the importance of investigating biological phenomena on the single-cell level. Despite pioneering efforts showing the feasibility of single-cell metabolomics, single-cell metabolomics is still out of reach for all but a few academic labs. We have recently developed SpaceM, a method for single-cell metabolomics. Here, we aim at exploring the potential of SpaceM in drug discovery.

Keywords: single-cell metabolomics, high-content imaging, drug discovery.

1. INTRODUCTION

Metabolomics is an emerged technology able to detect and quantify metabolites, or small molecules involved in every aspect of biology. Using metabolomics is particularly important because metabolome, or the unity of all metabolites in a cell or organism, represents the final step in the flow of information from genome to phenotype, known as the "central dogma of the molecular biology". Thus, being able to detect metabolome is essential to have a precise readout for the state of a cell or organism. Moreover, metabolome is affected by various non-genetic effects such as diet, lifestyle, pollution, and microbiota. This makes metabolomics profoundly important for being able to understand any aspects of life or develop drugs which are not only effective but also cause minimal side-effects.

Despite this strong interest, metabolomics as the latest emerged omics technology was in its infancy till 2010s. Only in the past decade, the development of various experimental and computational methods as well as various metabolomics databases made it possible detecting and interpreting metabolites robustly and routinely.

In parallel, the emergence of the single-cell technologies in particular single-cell transcriptomics, has demonstrated the profound cell-cell heterogeneity and the importance of single-cell omics in both academia and industry. Science has called single-cell RNA-sequencing the Breakthrough of the Year. Timmerman Report 2019 highlighted the emergence of biotech start-ups in the field of single-cell analytics for drug R&D.

The feasibility of single-cell metabolomics was demonstrated by pioneering efforts of several academic labs in 2010s. However, this technology was out of reach for all but a few academic labs and, importantly, out of reach for drug discovery applications. In 2019, we have developed a method SpaceM for single-cell metabolomics that has several distinctive advantages compared to other single-cell metabolomics method that make it attractive for drug discovery (Rappez et al. 2019). Here, we aimed at evaluating the potential of SpaceM in drug discovery in particular for high-throughput applications.

2. STATE OF THE ART

The feasibility of single-cell metabolomics was indeed demonstrated over the past decade. Particularly successful approaches used mass spectrometry, a technology for highly-sensitive and specific molecular detection. However, despite these efforts, single-cell metabolomics is not available outside of few academic labs due to its low throughput, technological complexity, high requirements, or need for custom instrumentation. This creates a high barrier and prohibits applying it to drug discovery.

We have recently developed SpaceM, a method for single-cell metabolomics that doesn't require any custom instrumentation and can be applied to a broad spectrum

of cells (Rappez et al. 2019). The key distinctive feature of SpaceM is custom image analysis and data integration strategy that removes the need for custom instrumentation.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

Here, for the first time we are aiming to evaluate the need and promise of the SpaceM method for single-cell metabolomics to the problems of drug discovery, with the focus on the high-throughput applications and integration with high-content imaging.

4. PROJECT RESULTS

We have established that it's absolutely essential to achieve high throughput for any single-cell metabolomics method to be applied in drug discovery. The requirements to the number of samples are at least in the order of 100 and easily exceed 1000 even in the low- and medium-throughput scenarios where the use of 96-well plates or 384-well plates is common. For every sample, the number of analysed cells needs to be in the order of 1000 to be able to have enough statistical power for the computational single-cell analyses.

First, we have outlined the bottlenecks of SpaceM. For this, we have analysed various cell types and investigated both the experimental and computational rate-limiting steps.

Second, we have considered experimental and computational improvements including the use of Artificial Intelligence / Machine Learning for solving challenging and time-consuming data analysis tasks. We have considered existing solutions as well as trained our own deep learning models.

These efforts have already allowed us to successfully scal up SpaceM to tens of samples. Currently, we are finalizing an approach to scale it up to 100 of samples to make it compatible with the commonly used 96-well plates, with over 1000 single cells analysed from every well. This required developments in various aspects of the method, including cell culture, cell preparation, microscopy, mass spectrometry, signal processing as well as data management, analysis, integration and visualization (publication is in preparation).

5. FUTURE PROJECT VISION

Single-cell analyses became one of the breakthrough approaches in 2010s. The growing interest to metabolomics, as well as growing market for metabolomics solutions positions single-cell metabolomics to become a key field of science and technology, in particular in pharma and biotechnology.

Our work in the HCS+M project has provided us with the necessary experience about the drug discovery pipelines where single-cell metabolomics holds most promise. Moreover, we have performed the evaluation of the recently developed method SpaceM for integration with high-content imaging and high-throughput applications in drug discovery.

Importantly, we have established a network of partners in pharma and biotechnology with whom together we are ready to go to the next steps. First, we will develop drug discovery-ready and AI-powered single-cell metabolomics. Second, together with our partners, we will integrate this approach into existing drug discovery pipelines.

5.1. Technology Scaling

Currently, the method is at TRL 4. We have submitted a preprint (Rappez et al. 2019) as well as performed evaluation for many more cell types and biological questions.

The steps necessary to move to TRL 5: 1) addressing the rate-limiting steps formulated in this project in both experimental and computational part of the method, 2) integration of AI/ML approaches for image analysis, 3) implementation of software for streamlined data analysis.

To move beyond TRL 6, together with the partners and academic collaborators we will perform validation trials as well as publish applications of the method for drug discovery.

5.2. Project Synergies and Outreach

As the method development lab, we will seek the complimentary expertise in particular in drug discovery. We will evaluate synergies with ATTRACT projects focused on imaging and software to address the outlined bottlenecks. We will go beyond the ATTRACT network to attract strong world-leading partners in the field of drug discovery and biotechnology to work together on the scaling of the method and its applications.

We have already established collaboration with the key players of the future consortium and by enacting our academic dissemination activities worldwide in particular supported by our other projects funded by ERC, EU Horizon2020 ICT, NIH NIDDK, NIH NHLBI, as well as industrially-funded projects. Our broad and international network of partners and collaborators as well as the strong interest to single-cell analysis and metabolomics will ensure the success of the outreach activities.

5.3. Technology application and demonstration cases

We will address the key gap in the area of *Health, demographic change and wellbeing*. Namely, we will develop a Europe- and world-wide-unique technology for single-cell metabolomics in drug discovery. This will provide a general solution that aims to significantly speed up the process of drug discovery across various (bio)pharma areas and to more accurate predict the off-target and side effects of drugs and drug candidates early in the pipeline of drug discovery.

Similar to our project METASPACE, funded by EU Horizon2020 HEALTH PHC program in 2015-2018, that led rise to several EU projects (ERC Consolidator project METACELL, ID 773089; EU Horizon2020 ICT project CloudButton, ID 825184), NIH-funded projects (NIH NIDDK KPMP, NIH NHLBI LungMap2) as well as a project funded by a industry-public partnership OpenTargets, we expect this project to multiply the benefits and give rise to various other projects, in the fields of omics, imaging, AI, software, and drug discovery.

5.4. Technology commercialization

Single-cell metabolomics technology, alike other single-cell technology currently available or being developed, is very attractive for commercialisation due to the emerging market and the lack of full commercially-available solutions.

We are actively exploring the commercialization opportunities provided within the EU Horizon2020 funding scheme, as well as provided by commercialization-focused industry-led partnerships and third-party foundations.

5.5. Envisioned risks

We envision a number of risks in the development of this technology. However, with the help of preliminary results in particular achieved in this project, as well as by capitalizing on our experience in developing methods and “one-click” software solutions in the field of metabolomics, we have formulated contingency plans.

5.6. Liaison with Student Teams and Socio-Economic Study

We will establish a pool of tasks, in particular in the field of AI, where the collaboration with MSc Level student teams would be mutually beneficial. Similar to our efforts in the EU Horizon2020 project METASPACE, where we have established an open platform for community AI, we will engage students in the field of technology and computer science to solve well-formulated problems with the data carefully selected by us (Alexandrov 2020).

By engaging in the cutting-edge areas of single-cell biology and AI-driven drug discovery, and by establishing a strong and world-wide visible position, our project will have a high impact not only on the economy of Europe but will also attract many talented people, scientists, engineers, and students to explore this field of R&D. Moreover, by providing a missing and essential tool to speed up drug discovery and development, our project will help significantly improve health and wellbeing of the population in Europe and worldwide.

6. ACKNOWLEDGEMENT

We thank Luca Rappez, Andreas Eisenbarth, Sergio Triana, Shahraz Mohammed, Mans Ekelof, Veronika Saharuka, Luisa Abreu who developed the SpaceM method and contributed with their expertise to the efforts in this project. We thank Lachlan Stuart, Vitaly Kovalev, and Richard Goater for their work on the METASPACE cloud software that was used in this project. We thank collaborators in particular Mathias Heikenwalder and Mira Stadler for providing cells and expertise in biology that helped us outline the rate-limiting steps. This project has received funding from the ATTRACT project funded by the EC under Grant Agreement 777222, as well as from the ERC Consolidator grant 773089.

7. REFERENCES

Alexandrov, Theodore. 2020. “Spatial Metabolomics and Imaging Mass Spectrometry in the Age of Artificial Intelligence.” *Annual Review of Biomedical Data Science* 3 (July): 61–87.

Rappez, Luca, Mira Stadler, Sergio Heli Triana Sierra, Prasad Phapale, Mathias Heikenwalder, and Theodore Alexandrov. 2019. “Spatial Single-Cell Profiling of Intracellular Metabolomes in Situ.” *BioRxiv*. https://doi.org/10.1101/510222.