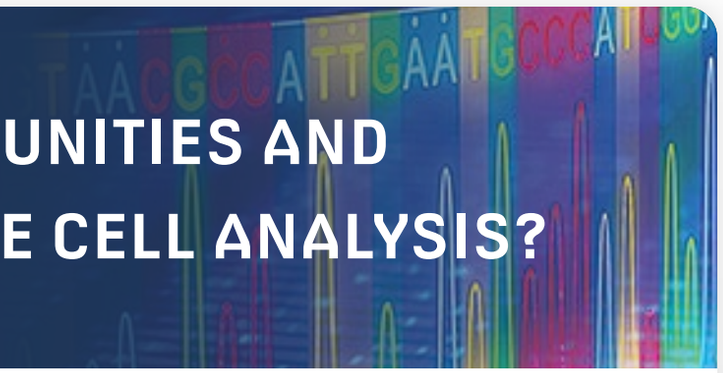
WHAT ARE THE OPPORTUNITIES AND CHALLENGES OF SINGLE CELL ANALYSIS?



As part of the NextGen Omics Series, at the Single Cell Analysis Congress, we sat down with an advisory group of leading experts in biotechnology and academia to explore their thoughts on single cell analysis – from its impracticalities today to its new applications tomorrow.

Every cell is unique. Scientists once assumed that cell populations were homogeneous, but now understand that heterogeneity is ubiquitous, existing even in the tiniest of cell populations. Measurements based on bulk cell populations, then, are nothing more than misleading averages; conclusions drawn from those samples are not unlike surveys which suggest the average family has 1.2 children – even though not a single family actually does. Taking the average cell may supply us with some information, but they cannot reveal smaller, often critical variations and changes taking place at the level of the individual cell. Such heterogeneity has wide implications for research in stem cell biology, immunology, neurology and beyond. This is where single cell comes in. The isolation of a single cell allows scientists to go much deeper, identifying phenotypic differences that are practically invisible in bulk sequencing, studying the evolution of cancer cells, and perhaps radically improving clinicians' ability to diagnose and treat disease. Single cell analysis has genuinely enormous transformative potential. In the space of only a few years, single-cell analysis has grown from a rarely used technique into a widespread technique used across many areas of biological research, including Immunology, neuroscience, developmental biology and cancer.

Indeed, the single cell arena has rapidly expanded; booming demand has already turned it into a billion-dollar market. The anticipated utility for single cell from a clinical perspective has prompted the launch of hundreds of clinical trials. 2019 in particular has been an exciting and innovative time.

This is certainly true, in late 2018, 10x Genomics acquired one of the innovators in this area,

Spatial Transcriptomics, who pioneered new technology which can integrate information from gene expression and RNA sequencing with the data offered by tissue analysis. Spatial transcriptomics offers a path towards measuring all gene activity in a tissue sample, mapping where the activity is occurring. Mike Stubbington, himself a Computational Biologist at 10x, shares such interest in spatial. However, Maryou Lambros, Senior Scientific Officer at the Institute of Cancer Research, agrees that the potential of spatial is exciting, but he is somewhat skeptical. His area is CTCs (Circulating Tumor Cells), and in that regard, he fears that using spatial would "*only…get the ones which are highly expressed genes.*"

Beyond the spatial, the other big story for our speakers is the approaching opportunity for serious clinical applications of single cell. Wilfred Van IJcken, Chief Operating Officer at the Centre for Biomics at Erasmus MC, singles out the release of a sequencing instrument from Thermo Fisher for use in the clinic. "*I think that's maybe the thing that will change in 2020, because you now see more and more – that this kind of technology is starting to enter the clinic. In the cancer field, it has already been done. There is now sequencing at least in the clinic. It's not single cell yet, but I think that will come.*" Certainly, the single cell industry as a whole is making moves towards clinical. Mission Bio is selling a single cell sequencing platform that targets clinical applications at a competitive price. Across both industry and academia, the focus has moved away from, say, identifying new biomarkers and towards the study of cancer cells and other diseases. Indeed, Jan Cools, Professor at VIB – KU Leuven, sees a possible clinical use for most of the innovative techniques emerging this year. The common goal is clinical. Maggie Bostick, Director R&D from Takara Bio agrees that going to the clinic is a goal and confirms that efforts at Takara Bio has focused on building instruments and reagents that enable the user to get more information than what they would get from a standard 3' DE RNA-seq assay. The SMART-seq full length transcriptome assay is one such assay. The Atlas projects (like Human Cell Atlas, Tumor Atlas) benefit using tools like these for building a more detailed picture that can be used for further understanding of our healthy and diseased tissues.

But not all is rosy in the single cell world. There are obstacles that need to be surmounted. Qi Liang is the Senior Director of Antibody Discovery at Kymab, and she believes one of the most significant challenges is simply the matter of communication. "*The people who know how to analyze the data are not really lab-based scientists. They don't really know the limitations of the lab-based technology.*" Wilfred Van IJcken concurs: "*Single cell was just the technology that let it explode – because the data increased. There was more metadata, so therefore it became more complex.*" This, our participants contend, makes the communication between those in clinical and those in research much harder, as they speak different languages. To Lang: "*…the*

research stage is easier, because the partners involved in the discussion are limited. But later on, through the preclinical and clinical, it is so complicated, because everyone is a boss…somebody has to make the decision." A lack of knowledge from some clinical directors, Lang suggests, makes product development difficult, because those same directors are ultimately the customers.

Conversely, some have taken steps to improve knowledge sharing in an ambitious way: the Human Cell Atlas, for instance, is a cell-making initiative which aims to achieve nothing so much as a comprehensive reference map of all human cells, creating a foundation of understanding to guide researchers in understanding human health and treating disease more capably than ever before. The scientific community is calling for open access to data and data sharing resources, and the complexity and abundance of information in the era of single cell surely necessitates it.

For Maggie Bostick, HCA was a huge contributor in 2019 in improving our fundamental understanding of all human health.

> ***"HCA has gone from sequencing a few cells to many million cells globally from multiple organs".***

One problem is the exorbitant cost of single cell analysis. Maryou Lambros despairs at one adverse impact of rapid innovation on research labs:

> ***"You have a lot of machines, which within two or three years have become obsolete. You've done a project and you cannot use it anymore. So, the next time you want to buy something, you have to be very careful!"***

He continues: "*…when you add up the DNA sequencing behind it and everything, it reaches between £40-95 per single cell depending on the sequencing depth and number of cells used.*" Indeed, that's without even factoring in the labor costs and the bioinformatics. *"The more cells you put in, the more you find, the more challenging in bioinformatics, the more challenging in cost."* Most of the instruments required for single cell sequencing are highly priced, so any laboratory choosing to incorporate single cell sequencing is making a significant investment. Some laboratories are proving adaptable, however – it is becoming popular to offer single cell sequencing and analysis as a paid service. After all, the costs of research have to be

recouped somehow. Mike Stubbington believes that the price will drop down in the future as single cell analysis becomes more normal, but for now – *"...I think it comes down to: is the cost worth it to answer the question that you have, and is single cell the only way to really do it?"* But aside from matters of cost and communication, there are problems with the actual data analysis itself. Maggie Bostick expresses her concern:

> ***"Right now, many of the methods haven't been scaled very well... the scalable assays aren't as good, or as robust, or as well-tested as just doing what we used to with bulk – this is why in R&D at TBUSA, we are focusing on ever improving cell preparation, library preparation and data analysis methods."***

She has a point. The rapid progress of the single cell industry has been hindered by mechanisms which damage cells. In addition, undertakings in single cell analysis often fail to capture enough cells to represent the population in a sample accurately. Cell damage and capture efficiency are two of the big practical problems that have yet to be solved. The popular flow cytometry technique is cost-efficient, yet cytometry approaches can easily harm or even kill cells. Newer tools for single cell RNA sequencing workflows can be helpful, but their cell capture rates are only as high as 65% and can be as low as 7%. Such inefficiency means that rare populations of cells can be missed entirely. For single cell analysis to develop even further, the isolation processes for cells need to be gentler, the capturing more efficient, the tools more affordable – and the communication clearer.

For all that such challenges frustrate the experts, they were not regarded as insurmountable, and there was an optimistic consensus for the future. The most energetic parts of the discussion concerned the multitude of possible future applications of single cell analysis. Assuming the technology costs went down, Maryou Lambros sees ample scope for single cell analysis to be utilized for CTCs. Indeed, the acquisition of knowledge in the field of single cell omics of CTCs is markedly slower than in other fields. CTCs are scarce and frail, and the stressful cell isolation methods which single cell technology presently involves only exacerbates the problem. Frailty can mean viability disappears, and analysis is useless. Lambros notes how the CTC genomic changes resemble the tissue biopsies from the same patient reflecting not only the localised disease but also the systemic disease at genomic level. He found in the same patient some CTC resemble similar genomic changes

to the diagnostic biopsy and some CTC resemble the metastatic lesion. If those CTC could be understood at the individual cell level and the heterogeneity appreciated, the progression of the disease could be much better understood. In many ways, liquid biopsy and single cell analysis could provide a better and safer approach to investigate tumour heterogeneity.

New microfluidic devices to isolate cells, or droplet technology combined with barcoding, could recognize the DNA, RNA, proteins and metabolites belonging specifically to single CTCs, and identification and analysis could be much more fortuitous. And earlier this year, a team from the University of Michigan developed Hydro-Seq, a new system to trap individual CTCs in a blood sample via high-throughput single-cell RNA-sequencing. The analysis confirmed that even within a single patient, the cancer cells often behave very differently.

Jan Cools, meanwhile, singles out immunotherapy as an area where single cell sequencing can continue to make advances.

> ***"One of the advantages of single cell sequencing, especially on the RNA level, [is that] you can identify exactly which are the tumor cells, which are the T cells, the different subsets involved there. Putting that data together with the response to immunotherapy is a great challenge – to know exactly why some cancers respond to immunotherapy, and why others don't respond."***

Immunotherapy is the treatment of a disease, such as cancer, by activating or suppressing the immune system. There has been recent single cell work on immunotherapy; the use of passive immunotherapies depends on a clear understanding of the tumor composition, which is beset by the recurring issue of heterogeneity. Single cell analysis technologies are proving to be the answer. Akos Vertes, Professor at George Washington University, linked the development in single cell proteomics to immunotherapy, as proteins are major players in immune response.

The overriding consensus of the meeting was a sense of excitement and opportunity. Despite some challenges currently slowing down their work, the experts in biotechnology and academia were excited by the prospect of new applications of single cell, from CTCs to immunotherapy, to – as was pondered briefly Mike Stubbington and Maggie Bostick – use in neurons. Clinical is the next step, and it is approaching fast. Single cell analysis can, it seems, be used almost anywhere.

A THANK YOU TO OUR EXPERT SPEAKERS, CONVERSATIONS WITH WHOM INSPIRED THIS PIECE AND HELP US CONTINUE TO PROVIDE YOU WITH HIGH-QUALITY, INDUSTRY-LEADING EVENTS

Wilfred Van IJcken, Chief Operating Officer at the Centre for Biomics, Erasmus MC

Wilfred received his PhD in Molecular Sciences from the Wageningen University in 2001. In 2002 he joined as a postdoc the group of prof. Grosveld at the Erasmus MC in Rotterdam. Here, he initiated and started a Genomics Core facility. In 2008 he was appointed as assistant professor and implemented a wide range of genomics technologies including arrays and NGS. He is cofounder of a startup in methylation sequencing. Wilfred teamed up with an national and international research group which has yielded over 200 publications in peer-reviewed journals.

Qi Liang, Senior Director of Antibody Discovery, Kymab

Dr.Qi Liang is the Senior Director of Antibody Discovery in Kymab Limited. With private financing from Wellcome Trust, Bill & Melinda Gates Foundation, Kymab has been supported to establish a mature and long-term vision for success in tackling some of the most challenging diseases. Dr Liang is a founding scientist of Kymab. She is one of the major contributors to invent and establish Kymab's IntelliSelect® Transgenic platforms capable of rapidly generating an exceptionally broad diversity and high quality of fully human antibodies against challenging disease targets. Her team also developed the IntelliSelect® Screening technology combining single-cell sequencing, genomics and proprietary bioinformatic algorithms to prioritize and select antibodies generated by our IntelliSelect® Transgenic platforms that have the most desirable drug-like properties.

Maryou Lambros, Senior Scientific Officer, Institute of Cancer Research

Maryou works as senior scientific officer with professor Johann DeBono in the cancer biomarkers team in the ICR, Sutton, UK. His research focus is on circulating tumour cell, single cell analysis and AR splicing in metastatic castration resistance prostate cancer (mCRPCs). Maryou also worked with professor Jorge Reis Filho at the breakthrough breast cancer centre and with professor Alan Ashworth in the tumour profiling unit at the ICR. He is experience in many molecular techniques such as CISH, FISH, aCGH, NGS and CTC enrichment technique.

Maggie Bostick, R&D Director, Takara Bio

Dr Magnolia Bostick received her bachelor's degree in Biochemistry from Louisiana State University in 1997 and doctorate in Biochemistry and Molecular Biology from University of California, Davis in 2005. She continued her training with a postdoctoral fellowship at University of California, Los Angeles. She started at Clontech Laboratories (now Takara Bio USA) in 2011 and over the last 8 years, she has been involved with the development and launch of numerous NGS-RNA products for tubes and automation platforms.

Stuart Edelstein, Professor, University of Geneva

Stuart J. Edelstein received a Ph.D. in Biochemistry from the University of California (Berkeley). Following a post-doctoral year at the Pasteur Institute in the laboratory of Jacques Monod, he joined the faculty of Cornell University where he became Professor and served as Chairman of the Section of Biochemistry, Molecular and Cell Biology. He later moved to the University of Geneva, and was director of the Department of Biochemistry until 1994. He returned to the Pasteur Institute for a sabbatical leave in the laboratory of Jean-Pierre Changeux, with whom he continues an active collaboration. He held the International Chair at the Collège de France in 2002-03. In 2007 he joined the Computational Neurobiology group at the European Bioinformatics Institute, following the groups' move to the Babraham Institute. Stuart is cofounder of Scipio bioscience, a Paris-based startup in the field of single-cell RNA sequencing.

Akos Vertes, Professor, George Washington University

Akos Vertes is a Professor of Chemistry and a Professor of Biochemistry and Molecular Biology at the George Washington University in Washington, DC. His research interests encompass the development of new analytical techniques applicable in diverse fields of chemistry, biology, and medicine. Research areas include high throughput and ultrasensitive methods in systems biology, proteomics and metabolomics, new methods for molecular imaging of biological tissues under native conditions, and single cell and subcellular analysis. One of his major accomplishments, a new ionization method called laser ablation electrospray ionization (LAESI), received several awards, including a "Top 10 Innovations of 2011" award from The Scientist magazine, and a "2012 R&D 100 Award" from the R&D Magazine. His research has been presented in over 160 peer-reviewed publications, and in two books. He is a co-inventor on 17 patents and several pending patent applications. He was elected Fellow of the National Academy of Inventors.

Mike Stubbington, Computational Biologist, 10x Genomics

Mike Stubbington gained his undergraduate degree in Natural Sciences from the University of Cambridge and then spent five years working as a research scientist in high-containment microbiology for the Health Protection Agency (now Public Health England). Mike then returned to Cambridge to undertake a PhD in molecular immunology at the Babraham Institute. Mike left the bench for a postdoctoral position in computational biology at EMBL-EBI and then the Wellcome Trust Sanger Institute. Mike then led the Human Cell Atlas team at Sanger from the very early days of the HCA up until April 2018 when he moved to a position at 10x Genomics with a particular interest in the application of single-cell technologies to questions in immunology.

Jan Cools, Professor, VIB-KULeuven

Jan Cools obtained his PhD degree in 2001 from the KU Leuven with a study on chromosomal defects in leukemia. From 2001 to 2003 he continued his research on the genetic causes of leukemia at Harvard Medical School (Boston, USA). After return to Belgium, he was promoted to assistant professor in 2005 and to full professor in 2009 at KU Leuven. In 2008, Jan was also appointed as group leader of VIB, a life sciences institute in Flanders. His research team studies the genetic complexity of acute lymphoblastic leukemia (ALL) and uses that information to develop novel models of leukemia development and novel treatment strategies. For these studies the team is now using single-cell DNA and RNA sequencing to unravel the heterogeneity of ALL at diagnosis and during chemotherapy treatment. He has served as a board member of the European Hematology Association and has been the editor-in-chief of the open access journal Haematologica from 2012 to 2017 and is now editor-in-chief of a new open access hematology journal: HemaSphere (journal of the European Hematology Association).