INDUSTRIAL ADVISORY BOARD

Conclusions report

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1. INTRODUCTION

1.1. Introduction of the IAB role within HUTER

HUTER IAB is an independent board mandated with the duty to guide HUTER researchers in the development of their research and according to technological prospects and future market opportunities. HUTER IAB comprises highly qualified professionals from very diverse backgrounds. The opinions and suggestions of HUTER IAB members are non-binding. They participate in HUTER IAB sessions without remuneration and their feedback is merely taken into consideration to impact HUTER researcher's minds and to challenge them through opinions and insights more common in industrial and business settings. In general terms HUTER IAB board:

- Ensures that HUTER research priorities are aligned with future clinical needs and market
- Establishes recommendations for technical developments
- Helps to prioritise areas of research or development within the project
- Reviews HUTER Innovation strategy and provides personal feedback when needed.

1.2. Introduction to the members of IAB

The IAB has a variety of professional profiles from different industries. HUTER has recruited the following professionals to form the IAB from different sectors such as biotechnology and IT companies:

COMPANY	FULL NAME	POSITION	SECTOR
BIOPOLIS	Daniel Ramón	CSO	Biotec
NASASBIOTECH	Miguel Abal	CoFounder and Chief Scientific Advisor	Biotec
QUIBIM	Angel Alberich Bayarri	CEO	IT
GOOGLE CLOUD HEALTHCARE AND LIFE SCIENCES	Dusan Zelembaba	Software Engineer	IT
TARTU BIOTECHNOLOGY PARK	Sven Parkel	CEO	Biotech
AWS	Carlos Jouve Alons	Director of Healthcare	IT

Figure 1: List of members of HUTER Industrial Advisory Board

1.3. Calendar and activities performed

INDUSTRIAL ADVISORY BOARD HUTER PROJECT		20	21											22					
Stages	Objectives	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
1	Presentation of the project and its possible applications																			
2	After the initial introduction, questionnaire sent to the experts by email																			
3	Presentation of the questionnaire results ● Presentation of Technological issues and alienation with healthcare applications ● Consortium: Translational presentation to the market or to the patient ● Present and future needs of each HUTER partner regarding their lines of research (INCLIVA, UPPSALA, SANGER, UEA, CCHT) ● HUTER Advancements and transfer of research to the market																			
4	● Presentation of the HUTER partners on their applications ● Conclusions																			
5	IAB sends their impressions of the alignment document and the technologies presented (1-2 pages)																			
6	Conclusions																			

Figure 2: Calendar of activities performed related to Industrial Advisory Board.

1.4. State of the art of cell sequencing technologies

The aim of this section is to introduce the recent advancements of single cell technologies to the members of the IAB: Considering the different backgrounds and professional experiences involved in the board and the rapid progress of these emerging technologies, we consider essential to introduce the state of the art to them. The introduction will serve to the IAB to:

1) Know the current situation and the tendencies of single cell technologies.
2) Relate the current progress of this emerging area of knowledge with prior experiences of other rapid grow biotech/high-tech technologies and make recommendations based on their experiences.
3) Think about translational and tech transfer opportunities related to Single cell technologies, providing guidelines and recommendations based on their experiences.
4) Identify potential bottlenecks or developmental risks in the life course of these technologies and help HUTER researchers to overcome them if needed.

5) Identify synergies with other scientific areas and technological markets.

a) Introduction to single cell technologies

The cell is the fundamental unit of any living organism. The human body is composed of approximately 37 trillion of single cells, which constitutes all their tissues and organs, living harmoniously with each other.[1] This harmony may be compromised by just one cell in diseases such as cancer. Although we have increased our knowledge in genomics, transcriptomics and other molecular fields during past decades, such studies were performed using a bulk of tissue samples composed of millions of cells. Therefore, the results are based on molecular material from millions of cells which prevents from identifying molecular profiles of single cells. In this context, cell sequencing technologies are currently emerging as the solution to better analyse and understand with unprecedent the biology of cells from living organisms at single cell resolution.

Almost 20 years ago, the Next-Generation Sequencing (NGS) technologies and platforms were developed and started being commercially available from 2005, improving the traditional method called Sanger sequencing commercialized from 1986. The critical difference between Sanger sequencing and NGS is the sequencing volume. While the Sanger method was only capable of amplifying small targeted regions of the genome, NGS paved the way for sequencing millions of fragments simultaneously per run, which enabled genome-wide sequencing of DNA and RNA.[2] This high-throughput process translates into sequencing hundreds to thousands of genes at one time. NGS also offers greater discovery power to detect novel or rare variants with deep sequencing.

The vast amount of data generated by NGS and its huge potential to analyse whole genomes by new bioinformatic tools led to the establishment of new fields of molecular biology, called OMICs (genomics, transcriptomics, epigenomics, etc.). The culmination of these technologies led to the invention of the first genome-wide single-cell DNA[3] and RNA[4] sequencing methods for mammalian cells.

These first studies using genome-wide single cell sequencing promoted a new field of study in molecular biology, the single-cell sequencing field.[5] These techniques have empowered researchers to profile many diseases and biological processes at the single-cell level at the beginning of 2010 decade, such as neuron

[1] Bianconi, E., Piovesan, A., Facchin, F., Beraudi, A., Casadei, R., Frabetti, F., ... & Canaider, S. (2013). An estimation of the number of cells in the human body. Annals of human biology, 40(6), 463-471.
[2] Mardis, E. R. (2011). A decade's perspective on DNA sequencing technology. Nature, 470(7333), 198-203.
[3] Navin, N., Kendall, J., Troge, J., Andrews, P., Rodgers, L., McIndoo, J., ... & Wigler, M. (2011). Tumour evolution inferred by single-cell sequencing. Nature, 472(7341), 90-94.
[4] Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., ... & Surani, M. A. (2009). mRNA-Seq whole-transcriptome analysis of a single cell. Nature methods, 6(5), 377-382.
[5] Wang, Y., & Navin, N. E. (2015). Advances and applications of single-cell sequencing technologies. Molecular cell, 58(4), 598-609.

genomic heterogeneity,[6] carcinogenesis and tumour evolution,[7] [8] [9] circulating tumour cells (CTCs) in tumour metastases,[10] early embryo development,[11] [12] uncultivable bacteria,[13] among many others. The present and future of the technology is to improve it, covering the lack of spatial information through integration of spatial transcriptomics techniques. Spatial transcriptomics comprises combination of in situ hybridization techniques as well as spatial barcoding and RNAseq.

During the second part of the 2010-decade, new global research initiatives has born thanks to the power of the new cell sequencing technologies and spatial transcriptomics. Among them, it is important to highlight "The Human Cell Atlas initiative".[14] One of its ambitious goals is to define all human cell types to better understand their interactions as well as cellular dysregulations in human disease[15]. In this context, the cell sequencing technologies has been emerged as a solution to contribute to this complex challenge to achieve a human cell map at single-cell resolution.

In order to get new insights regarding the states or types of cells and its role in the human body, modern technologies are used by researchers to track differences on their molecular profiles. This is not only beneficial to obtain information about cells in a health context but also in disease such as cancer. Some of genetic and molecular techniques were adapted to achieve single-cell resolution. However, that protocols are not only based on NGS technologies but also some techniques use an evolution of traditional *in situ hybridization* technologies combined with state-of-art microscopes or even a combination of both approaches which provides spatial context to the molecular cell analysis. More than one hundred single-cell omics methods have been published in scientific literature. In this section, the main group of approaches will be briefly introduced:

b) Single-cell genomics: Single-cell genome (DNA) sequencing (scDNA-seq)

As it was explained in the introduction, massive DNA sequencing has been widely extended thanks to NGS technologies over cells in bulk from samples. However, it is more challenging to perform single-cell sequencing in comparison with sequencing from cells in bulk.

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[6] McConnell, M. J., Lindberg, M. R., Brennand, K. J., Piper, J. C., Voet, T., Cowing-Zitron, C., ... & Gage, F. H. (2013). Mosaic copy number variation in human neurons. Science, 342(6158), 632-637.
[7] Navin, N., Kendall, J., Troge, J., Andrews, P., Rodgers, L., McIndoo, J., ... & Wigler, M. (2011). Tumour evolution inferred by single-cell sequencing. Nature, 472(7341), 90-94.
[8] Xu, X., Hou, Y., Yin, X., Bao, L., Tang, A., Song, L., ... & Wang, J. (2012). Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor. Cell, 148(5), 886-895.
[9] Potter, N. E., Ermini, L., Papaemmanuil, E., Cazzaniga, G., Vijayaraghavan, G., Titley, I., ... & Greaves, M. (2013). Single-cell mutational profiling and clonal phylogeny in cancer. Genome research, 23(12), 2115-2125.
[10] Ni, X., Zhuo, M., Su, Z., Duan, J., Gao, Y., Wang, Z., ... & Wang, J. (2013). Reproducible copy number variation patterns among single circulating tumor cells of lung cancer patients. Proceedings of the National Academy of Sciences, 110(52), 21083-21088.
[11] Ramsköld, D., Luo, S., Wang, Y. C., Li, R., Deng, Q., Faridani, O. R., ... & Sandberg, R. (2012). Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. Nature biotechnology, 30(8), 777-782.
[12] Guo, H., Zhu, P., Wu, X., Li, X., Wen, L., & Tang, F. (2013). Single-cell methylome landscapes of mouse embryonic stem cells and early embryos analyzed using reduced representation bisulfite sequencing. Genome research, 23(12), 2126-2135.
[13] Lasken, R. S. (2012). Genomic sequencing of uncultured microorganisms from single cells. Nature Reviews Microbiology, 10(9), 631-640.
[14] https://www.humancellatlas.org/
[15] Regev, A., Teichmann, S., Rozenblatt-Rosen, O., Stubbington, M., Ardlie, K., Amit, I., ... & Committee, H. C. A. O. (2018). The human cell atlas white paper. arXiv preprint arXiv:1810.05192.

An improvement in the experimental protocols of processing samples, in terms of isolation of a single cell, nucleic acid extraction and amplification, sequencing library preparation, sequencing and bioinformatic data analysis, allowed to perform NGS to single cells. These improvements led to approach a set of seemingly inaccessible problems for traditional NGS protocol by single-cell DNA sequencing, such as heterogeneous samples, rare cell types, cell lineage relationships, mosaicism of somatic tissues, analyses of microbes and disease evolution.[16]

c) Single-cell Transcriptomics:

- Single-cell transcriptome sequencing (scRNA-seq)

Traditional RNA-sequencing (RNA-seq) approach allows researchers to profile transcriptome based on NGS technology, providing a count of the number of reads associated with each gene. RNA-Seq provides a far more precise measurement of levels of transcripts and their isoforms than other methods by sequencing cDNA, obtained from retrotranscription of mRNA.[17] However, this traditional approach is limited exclusively to total RNA isolated from a bulk of cells from a tissue sample. The disadvantage of this approach and others (e.g. microarrays) emerges in the context of samples with mixed cell populations. The measurements of RNA-seq method may obscure critical differences between individual cells within these populations.[18]

The need of detecting the subtle but potentially biologically meaningful differences in transcriptomes from seemingly identical cells led to researchers to develop scRNA-seq approach. The main difference between traditional RNA-seq and single-cell approach is the successful combination of two independent techniques: the isolation of individual cells of interest from culture, tissue or dissociated cell suspensions, and the massively parallel sequencing of cDNA libraries.[19]

- Spatial transcriptomics (ST)

Although scRNA-seq technologies enable researchers to identify cell subpopulations within tissue, it does not capture their spatial distribution nor reveal local networks of intercellular communication in the tissue. In this context, recent improvements in different technologies, such as multiplexed in situ hybridization and spatial barcoding, led to the development of Spatial transcriptomics. This approach was awarded as the Method of the Year 2020 by Nature.[20]

Generally, studies based on ST can be performed in basically two ways:

[16] Nawy, T. (2014). Single-cell sequencing. Nature methods, 11(1), 18-18.
[17] Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nature reviews genetics, 10(1), 57-63.
[18] Kolodziejczyk, A. A., Kim, J. K., Svensson, V., Marioni, J. C., & Teichmann, S. A. (2015). The technology and biology of single-cell RNA sequencing. Molecular cell, 58(4), 610-620.
[19] Saliba, A. E., Westermann, A. J., Gorski, S. A., & Vogel, J. (2014). Single-cell RNA-seq: advances and future challenges. Nucleic acids research, 42(14), 8845-8860.
[20] [No authors listed] Method of the Year 2020: spatially resolved transcriptomics. Nat. Methods 18, 1 (2021).

- Transcriptomes can be read out by microscopy through in situ sequencing or multiplexed fluorescence in situ hybridization (FISH techniques).
- RNA is captured in a way that retains spatial information while sequencing is done using ex situ RNA-seq.

Although this approach provides valuable spatial information, no method provides as complete scope of the transcriptome as does scRNA-seq. For this reason, efforts are being made to integrate scRNA-seq technologies with spatial transcriptomics in order to obtain the most complete picture of the transcriptome as possible. [21]

d) Single-cell Epigenomics: Single-cell DNA methylome sequencing

Although there are several approaches in single cell epigenomics (ATAC sequencing, ChIP-seq, HI-c, etc.), herein we will introduce the single cell DNA methylome sequencing.

DNA methylation is an important epigenome mark that contributes to the definition of transcriptional and regulatory potential of genomic DNA. The carbon 5 position of cytosines (5mC) can be methylated in some regions of the genome. Methylation can be detected by NGS after bisulfite treatment on DNA, this gold standard is called bisulfite sequencing. Bisulfite treatment degrades DNA, thus the final amount of DNA with good quality to perform the rest of the experimental procedure is lower. This problem is even greater for single cell approaches where the initial amount of DNA to be modified is even lower.

A new methodology to overcome the problems of the single cell approach was reported in 2014. In this paper, single-cell DNA methylome sequencing allows to explore DNA methylation in rare cells and heterogeneous populations which cannot be analysed through the traditional approach.[22]

e) Single-cell proteomics

- Spatial proteomics

Spatial proteomics (SP) is the field of knowledge which studies the spatial organization of proteins within cells. It is important to highlight that the subcellular localization of proteins affects its function due to different molecular compositions and chemical milieus.[23] Therefore, SP allows to shed some light on the complex cell dynamics as well as to validate spatial transcriptomics data despite it is not based on previously commented cell sequencing approaches.

There are two main technical approaches for SP.

[21] Longo, S. K., Guo, M. G., Ji, A. L., & Khavari, P. A. (2021). Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. Nature Reviews Genetics, 1-18.
[22] Smallwood, S. A., Lee, H. J., Angermueller, C., Krueger, F., Saadeh, H., Peat, J., ... & Kelsey, G. (2014). Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. Nature methods, 11(8), 817-820.
[23] Borner, G. H. (2020). Spatial Proteomics: A Gateway to Understanding Cell Biology.

- Imaging-based spatial proteomics: high-throughput imaging to visualize proteins within cells or subcellular compartments.

This methodology is based on new technologies for the visualization of proteins in their native cellular environment. Thanks to this technology, researchers are realizing that populations of genetically identical cells show variability in protein expression levels and protein localization. SP has made it possible to reach this type of conclusion, which with other technologies mentioned would be impossible.

This imaging-based SP can be based on antibody protein visualization by immunofluorescence assays or fluorescent protein fusions. Therefore, advanced automated fluorescence microscopes are required to acquire the images properly, allowing time-lapse imaging, trade-off between resolution and throughput and segmentation. Good cellular reference markers are also required.

- Mass spectrometry-based organelle profiling:

This approach is based on quantitative MS to identify subcellular protein networks by organelle profiling or interactomics. MS can be used to identify proteins and quantify their abundance in complex mixtures such as organalles.[24] Furthermore, the interactome can be understood as a "local" spatial proteome because proteins must be in the same place to interact. [25]

2. HUTER CHALLENGES

The aim of this section is to go deeper into the analysis of single cell technologies, identifying the challenges that could become a priority for HUTER researchers. This section has been focused on the technologies that receive a higher attention during the development of the HUTER project (single cell genomics, epigenomics and transcriptomics). The section also reflect upon challenges more related with software and computational demands as well as the ICT infrastructures that will be necessary to scale up and deploy single cell technologies globally. The section will certainly help IAB Members to identify how these technologies or combo of technologies could be implemented in real settings with the aim to impact industries and particularly critical sectors like health.

2.1. Cell sequencing challenges

There are several limitations of cell sequencing technologies, which are common for all the approaches (scRNA-seq, scEpigenomics, scGenomics and other based on cell sequencing), that currently hinder their impact in relevant markets such as clinics.

[24] Aebersold, R., & Mann, M. (2016). Mass-spectrometric exploration of proteome structure and function. Nature, 537(7620), 347-355.
[25] Lundberg, E., & Borner, G. H. (2019). Spatial proteomics: a powerful discovery tool for cell biology. Nature Reviews Molecular Cell Biology, 20(5), 285-302.

As commented before, the cell sequencing techniques offer many advantages over traditional genomic analyses, enabling the detection of complex and rare cell populations, and elucidating development trajectories of distinct cell lineages. Despite the potential to be used across diverse segments in biotechnology and medical research, this technique is still limited to early-stage research activities. This is primarily attributed to certain challenges associated with data processing and quality control.[26]

Furthermore, the lack of adapted silico tools is among the major bottlenecks to process and analyse cell sequencing data. Additionally, the high cost of these technologies and the intensive efforts required to perform their protocols are other challenges to be addressed to adopt the cell sequencing technologies. However, some initiatives such as the Human Cell Atlas project (its main aim is to create the whole transcriptome map of human cells), is considered a prominent milestone in the field of single cell transcriptomics. HUTER will help to address some of these challenges contributing to this atlas with the whole transcriptome of the human uterus.

Similarly, analyses of epigenetic mechanisms including DNA modifications, histone modifications, DNA accessibility and chromosome conformation, have emerged thanks to the last advancements in the field of cell sequencing. In this context, scEpigenomics have a big potential to improve the diagnosis and treatment of patients, and importantly in cancer patients. There are specific epigenetic profiles in the tumour cells, metastases as well as in cells after treatment that could provide valuable information to improve the patient outcomes. However, there are some current obstacles to become scEpigenomic analyses into meaningful clinical applications.[27] Those are mainly related with a common, and previously commented, problem in the clinical implementation of the cell sequencing technologies. For single cell sequencing to be performed by pathology services, robust commercial platforms must be developed, including standardized procedures for cell preparation, data analysis and interpretation of results. Nowadays, the cell sequencing technologies are essentially used in the scientific field, therefore the heterogeneity of approaches requires a standardization in terms of technology, protocols and so forth. Another bottleneck is the careful preparation of cell suspensions and reports on the proportion of cells in a sample with a particular phenotype. Due to this fact, the implementation of this technology has faced issues related to turn-around time, data analysis and interpretation of results. Finally, the clinical use of all cell sequencing technologies would require standardized and simplified reporting of complex data sets to distil clinically relevant information to be useful for clinicians and patients.

[26] Single-cell Sequencing Services and Technologies Market, 2020-2030 - Global Current Landscape and the Future Opportunities. [https://www.globenewswire.com/en/news-release/2020/12/17/2146738/28124/en/Single-cell-Sequencing-Services-and-Technologies-Market-2020-2030-Global-Current-Landscape-and-the-Future-Opportunities.html]
[27] Bond, D. R., Uddipto, K., Enjeti, A. K., & Lee, H. J. (2020). Single-cell epigenomics in cancer: charting a course to clinical impact. Epigenomics, 12(13), 1139-1151.

Spatial transcriptomics (ST) is one of the trendy and latest approach in terms of cell sequencing, and there are several new approaches under development.[28] ST is based on the idea that it can provide not only transcriptomic data but also spatial localization of that results. Despite the advancements of this technology in the last 5 years, it is in an experimental phase in the scientific field and their routine application in clinics is still far away. Before that, there are some limitations in the field of research that should be addressed to promote its take-off. The first challenge is knowing which areas to study with this technology. Without some direction into the region or cells to study, it is nearly impossible. The second challenge is interpreting the increasing amounts of transcriptional and proteomic data. This technology generates vast amounts of data that it is impossible to understand without experience and computational tools. Another of the most important challenge is the diversity of skills needed to run a successful spatial transcriptomics experiment. One kind of expertise is needed to obtain the best sample from the most appropriate portion of the tissue; another is needed to implement a long protocol; and yet another is needed to interpret the data.

2.2. Computational limitations and digital standards

Traditional NGS sequencing technologies brought with them high computational needs to perform all data processing required to analyse large amounts of data generated from a DNA sample sequencing. These technologies are already implemented in hospitals and in their genomics services. However, computational requirements have been exponentially multiplied with the arrival of single-cell sequencing technologies. Now it is not question of sequencing one mixture of DNA from a bulk of tissue, but thousands of DNA/RNA samples from thousands of single-cells in one experiment. Datasets range from 10^2 to 10^6 cells and increase in size every year. Therefore, the complex single-cell data coupled with the huge amount of data inherent to NGS data makes it a paradigm of big data. Furthermore, the scientific community seek to integrate different kind of data to identify new cell types or networks through different approaches in order to create the most complete cell landscape, such as in the HCA initiative.

Focusing on single-cell transcriptomics, the scRNA-seq technologies are improving, increasing the number of acquired cells, the amount of raw data per experiment does not grow as fast. Therefore, the pre-processing and storage stay constant, but the requirements of analytics dealing with large number of cells is crucial. With the possibility to explore bigger volumes of data, there are statistical challenges rising. For example, the Human Cell Atlas initiative is gathering a huge amount of data from different projects which requires to perform common approaches to identify interpretable cell clusters or networks. This involves the development and application of complex algorithms in order to perform complex analysis to identify patterns

[28] LeMieux, J. (2020) Spatial: The Next Omics Frontier. Genetic Engineering & Biotechnology News, October 2020 Vol. 40 No. 10 https://www.genengnews.com/insights/spatial-the-next-omics-frontier/ [Online]

and networks between cells. In this context, the high number of cells and its associated data provides and exceptional environment to the development of deep learning models.[29]

In order to help to address these data challenges, cloud platforms are emerging as powerful tools to help researchers in developing their common analyses (e.g., HCA Data coordination platform). The power of the cloud computing provides excellent tools to gather vast amount of data as well as to perform complex algorithms with high performance, best timing, and flexible scalability than traditional data centres. The joint development of algorithms and cloud platforms could provide the spread of these technologies out of the research fields, such as hospital, diagnostics laboratories, clinical trials industry and so forth.

Another challenge to be addressed is the adoption of open standards, for example in spatial transcriptomics field. State-of-the-art research equipment sometimes include their own non-open output format as default which hampers sharing data and results among researchers even from the same institution due to format incompatibilities. This incompatibility issue coupled with the fact that the scientific sector does not usually have well-stablished, defined, and adopted standard formats and protocols, raise the need of extending an open standard definition for these output data in order to enable data sharing and compatibility among the entire research community. Linked with this problem, some software linked to equipment which allows to process and analyse data, is usually a black box, without open-source alternatives which hinders innovation and research.

Finally, transfer of these technologies to other markets, out of research field, may be hindered by close formats. For instance, hospitals require high interoperability standards in their equipment in order to integrate all data within their systems (e.g., electronic health records). Adoption of open standards is a pending issue in this regard.

2.3. Challenges conclusions

The state-of-the-art of cell sequencing technologies bring us new advancements in the research field, but also helped us to detect several limitations and doubts of their potential impact across industries, and out of the scientific fields, generally due to the early stage of development.

The constitution of the HUTER IAB, with several multidisciplinary experts from the private sector, represented a valuable tool for the innovation strategy of the HUTER project. IAB Members participated in the different sessions giving recommendations. They provided feedback based on their industrial experience with the aim of developing our tasks properly and to facilitate the transfer of HUTER main research lines to the market. One of the relevant conclusions of the IAB was that HUTER partners should prioritize the development of cell

[29] Angerer, P., Simon, L., Tritschler, S., Wolf, F. A., Fischer, D., & Theis, F. J. (2017). Single cells make big data: New challenges and opportunities in transcriptomics. Current Opinion in Systems Biology, 4, 85-91.

sequencing techniques and tools in some specific sectors and industries as starting point due to the early stage of the technology. IAB members also provide very positive feedback regarding the value of the HUTER software platform and its expected functionalities, helping us to address potential limitations that could hinder its use in real hospital or industrial settings. Some of these features are related to some computational and storage limitations of cell sequencing technologies and the implementation of open standards that could be used to facilitate single cell data sharing.

Collectively, HUTER partners consider excellent and valuable all the recommendations and all the feedback received from IAB. Hopefully, it will contribute to improve future impact of HUTER results in the market and in the society.

3. IAB ANALYSIS RESULTS

After the introduction of the main technologies and the challenges, the HUTER project implemented an iterative process with IAB members to analyse relevant sectors of industry, clinical areas with greater potential, the impact of digital solutions and so forth in the particular area of single cell technologies. The process involved several virtual meetings and questionnaires to gather IAB insights about the potential applications of these technologies. The results of this collaborative work is summarized as follows.

3.1. Analysis of priority sectors for cell sequencing tools

Based on information requested from the IAB by questionnaires and the Innovation Committee Meeting with the IAB on June 21, 2021, we got some valuable information regarding the potential impact of the single cell sequencing technologies in different industries from their professional perspective.

Collectively, the information provided by the IAB suggest that the cell sequencing technologies are promising for pharma and animal (animal models) industry. However, they were considered less relevant in the short term for the human health sector (hospitals, clinics, etc.) and agriculture sector (Figure 3).

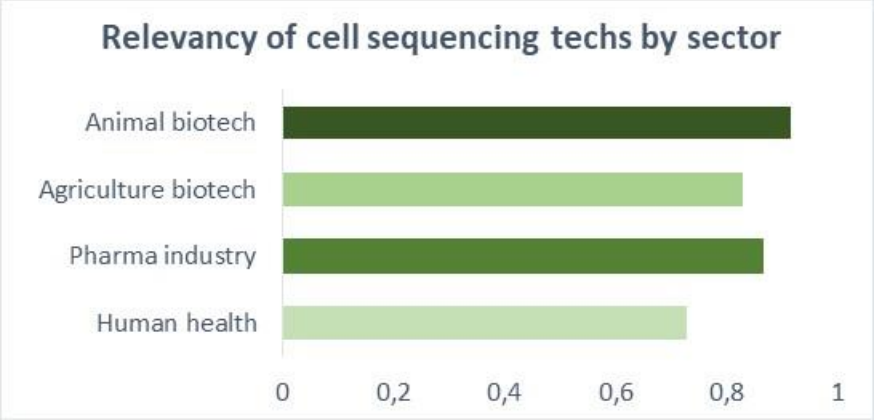

Figure 3: Relevancy of cell sequencing technologies by sector (average collected from IAB questionnaires from 0 to 1)

The cell sequencing technologies have a variety of approaches as commented before. The analysis of IAB consultations suggests that single-cell sequencing (sc Genomics) and scRNA-seq (sc Transcriptomics) could be the most relevant for the different sectors. The rest of the approaches have also a high score, therefore, cannot be discarded their future relevance in the market (Figure 4).

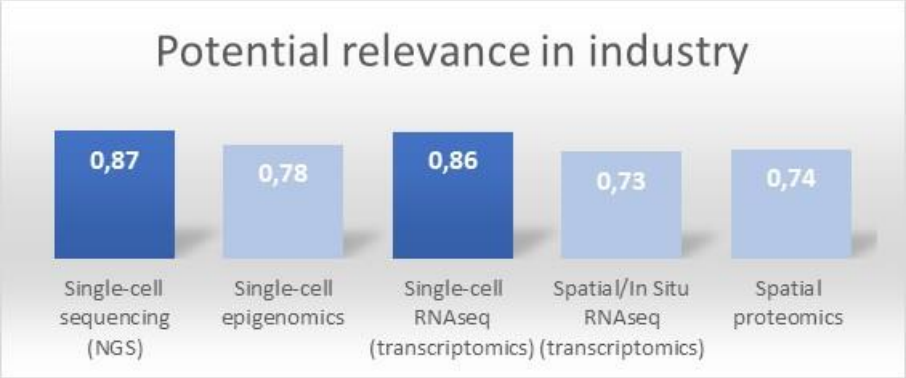

Figure 4: Potential of cell sequencing technologies in industry (average of IAB members from 0 to 1)

Due to the high relevance of the different approaches, IAB feedback was collected regarding the potential relevance of each approach in different relevant fields such as clinical trials industry, medical devices and diagnostic technologies, pharma industry/drug development and human health sector. In this context, single-cell sequencing based on NGS, was considered useful and promising for all sectors (first of all technologies) (Figure 5). However, the results also suggested that this approach is less relevant for the pharma industry/drug development sector (but it also obtained a high score).

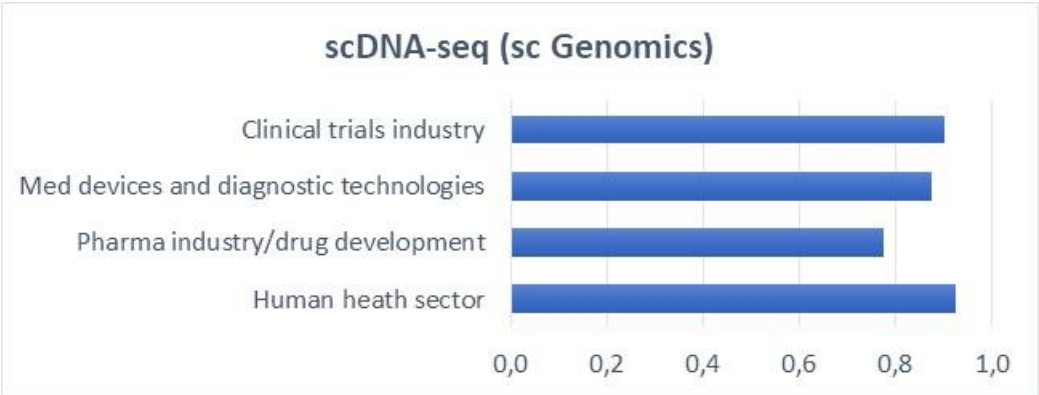

Figure 5: Potential of single-cell sequencing (NGS) approach in different sectors (average of IAB members from 0 to 1)

The scRNA-seq approach was the second most relevant for all the sectors considered. The trend is similar to single-cell sequencing based on NGS, being considered less relevant to the clinical trials industry than other technologies. However, the score still being high (Figure 6).

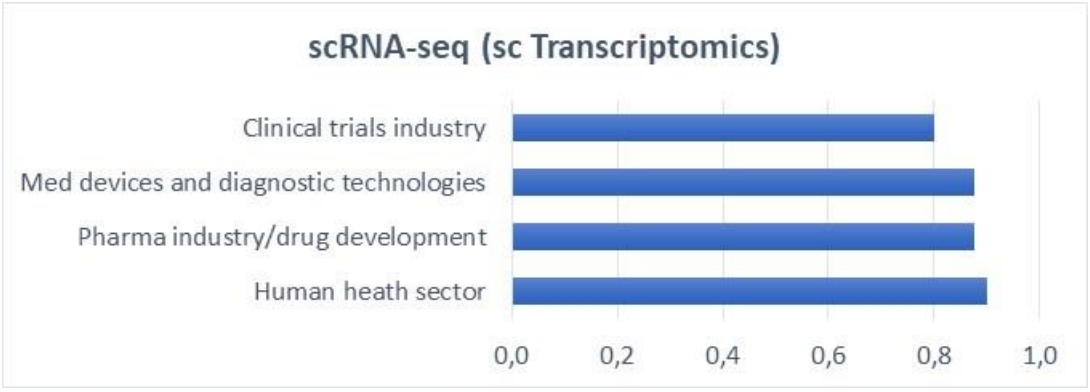

Figure 6: Potential of scRNA-seq approach in different sectors (average of IAB members from 0 to 1)

scEpigenomics ranks third in terms of greatest potential among all sectors. Interestingly, the analysis of IAB feedback suggest that medical devices and diagnostic technologies sector is the best to apply scEpigenomic approaches (Figure 7). It may be due to the fact that there are several scientific publications in the field of epigenomics and biomarkers for the early diagnosis of diseases such as cancer.

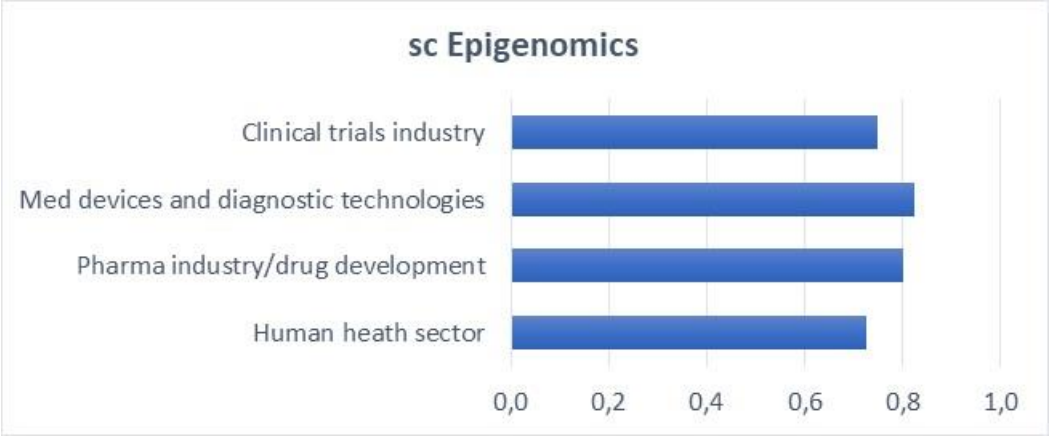

Figure 7: Potential of sc Epigenomics approach in different sectors (average of IAB members from 0 to 1)

Imaging-based spatial proteomics is another approach that was ranked as fourth in terms of greatest potential among all sectors. It is important to highlight that this technology has received low score in clinical trials industry. This may indicate that the technology is still in an early phase and its potential was underestimated (Figure 8).

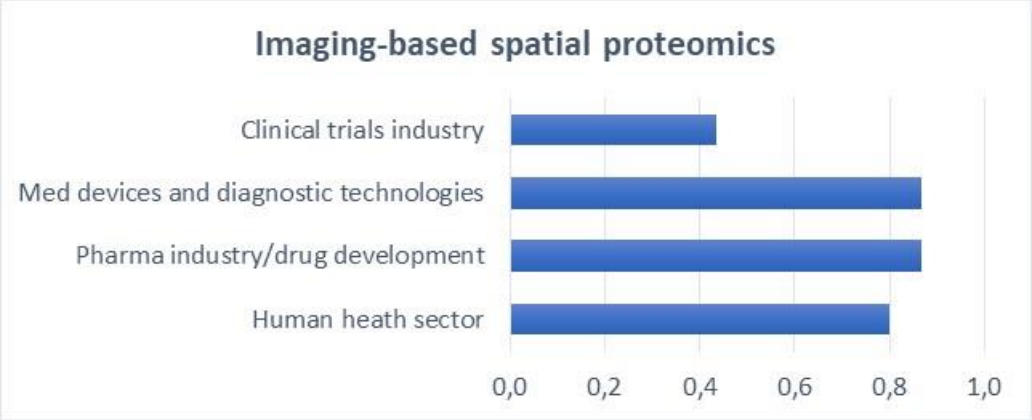

Figure 8: Potential of imaging-based spatial proteomics in different sectors (average of IAB members from 0 to 1)

Finally, the Spatial/in situ RNAseq approaches were ranked in the last position in terms of potential relevance among all sectors. This result could be directly related to the fact that these approaches are the most in an early phase (Figure 9).

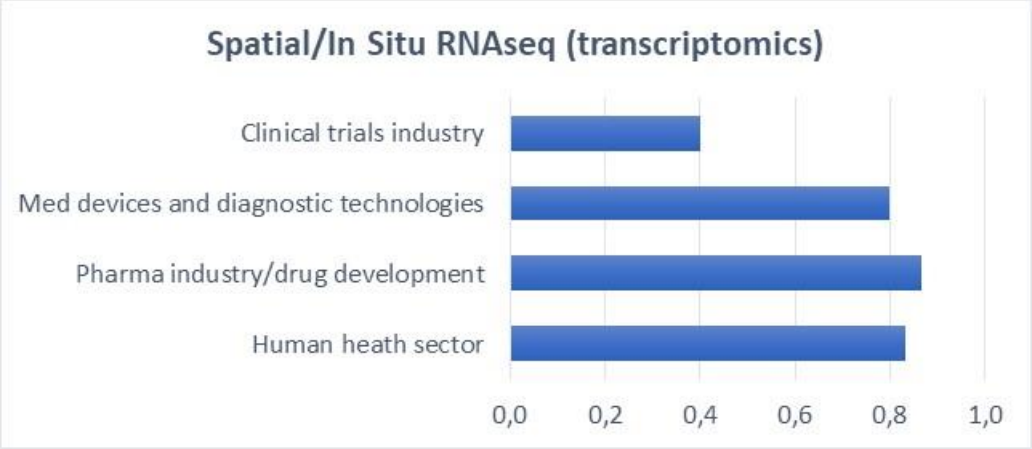

Figure 9: Potential of Spatial/In situ RNAseq in different sectors (average of IAB members from 0 to 1)

Additionally, IAB provided some information regarding the HUTER platform and the potential interest of the industry in this regard. Particularly, the impact in different clinical sectors of hospitals were addressed. The results showed that digital platforms could be relevant in the main clinical areas, such as Cardiology, Endocrinology, Oncology, Pediatric diseases, Reproductive Medicine, Rheumatology, Urology (Figure 10). Neurology has also been identified as a potential field for digital platforms by one IAB member.

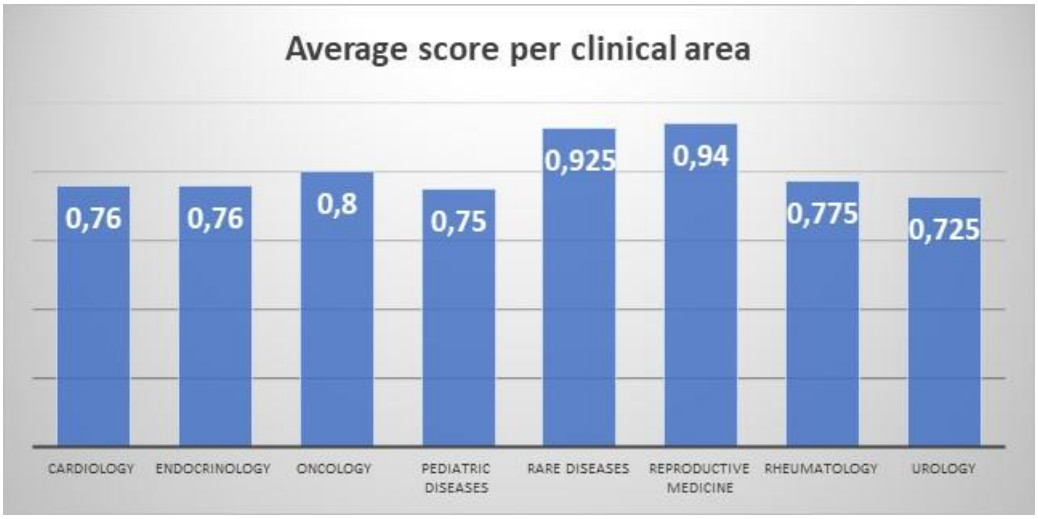

Figure 10: Relevance of digital platforms for analysing, hosting and visualizing data and microscopy images in clinical areas based on IAB feedback (average of IAB members from 0 to 1)

Collectively, all this data suggest that digital platforms could have an important relevance in markets related to rare diseases and reproductive medicine. However, the scores of the rest of clinical areas were also high and cannot be underestimated.

IAB was also asked for feedback regarding their opinion regarding features and functionalities for the HUTER platform. Valuable information was gathered not only about functionalities, but also regarding open standards, best target sectors, knowledge about other online tools for omics data, and so forth. We summarized here the 5 most relevant messages obtained from the answers of the IAB questionnaires:

- Digital platforms were considered promising to analyse/exploit data in clinical trials and new therapies.
- IAB agrees that algorithms to transform private formats into open standards would be useful for HUTER platform.
- The most well-known standards are DICOM, HL7, SNOMED and CDA.
- Most of OMICs portals for data analysis/process are unknown to IAB experts (Terra, UCSC Xena…).
- Conversion algorithms, advanced visualization tools, synchronized data store and dynamic storage were considered the 4 most interesting functionalities for HUTER platform.

4. Brief technological vigilance analysis

In parallel and with the aim of confirming that IAB feedback is well-aligned with the trends of cell sequencing technologies, the HUTER Innovation committee carried out a brief technological vigilance analysis. This study intended to gather information from public scientific databases to turn it into knowledge and to make

decisions with less risk and to be able to anticipate the changes. The present technological vigilance analysis carried out in the HUTER project should be consider as is a basic element of the HUTER innovation strategy, as it will allow to prioritize and focus on those research lines that could be critical or have more potential for the partners involved in our consortium and discard those of less strategic importance. At the same time it also helped us to open our minds to design new collaborative projects well aligned with the technological evolution of single cell technologies. Additionally, the study served to start discussions about future funding of opportunities of the new Horizon Europe programme.

In this context, and as a complement to the IAB's conclusions, the brief technological vigilance analysis presented here has been performed with the following objectives:

1) Analyse external and independent sources such as papers or patents to compare and validate these tendencies against the previous opinions gathered from IAB members.
2) Identify trends and opportunities to be shared with the IAB, challenging them to get their personal opinion about the technological pace and penetration of these technologies.
3) Discuss about new areas of development that are beyond the HUTER objectives like cancer diagnosis.
4) Identify relevant research groups and innovate companies working in this domain.

The main conclusions of this short analysis is presented in the following sections.

4.1. Current status of cell sequencing technologies in the market

The potential of cell sequencing technologies in a variety of fields has been addressed by the IAB in previous sections. Herein, a brief analysis of the number of patents as well as the number of papers per field will also be commented (using *worldwide.espacenet.com* and *lens.org* public databases respectively) in order to confirm the information provided by the experts of IAB.

4.1.1. Patents related to single cell sequencing technologies

The number of patents related to “single cell sequencing” terms has been collected in the public browser of worldwide.spacenet.com. The analysis of the number of patents in recent years shows that the trend has been an increase in the number of patents over the last 6 years. This upward trend suggest that the number of patents related to single cell sequencing will continue increasing in the incoming years (Figure 11).

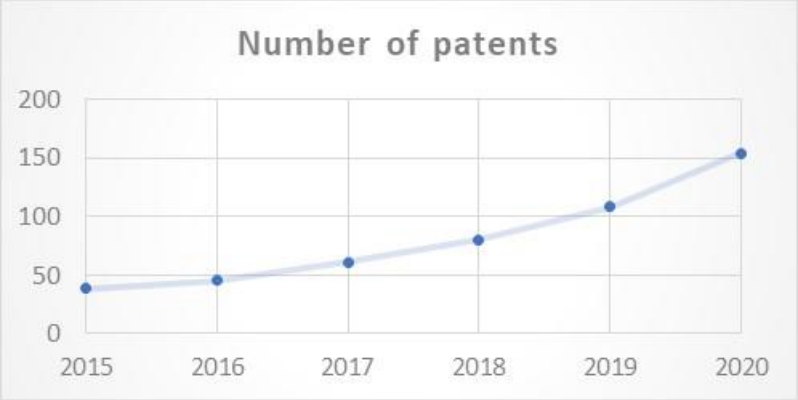

Figure 11: Number of patents single cell sequencing (information extracted from worldwide.espacenet.com)

4.1.2. Bibliography analysis related to single cell sequencing and fields of interest

In order to try to predict the future relevance of cell sequencing technologies in different fields of industry, an analysis of the current number papers in those fields has been performed. Specifically, the number of papers during last 6 years provided a trend of the use of these technologies by the research community in specific fields. For this purpose, a search of the terms "single cell sequencing" and the field of knowledge (such as human health, drug development, agriculture/plants and animals), using *Lens.org* (a public browser) were performed. It provided a number of papers per year and field.

- **Human health**

The analysis performed for human health found a total of 235 papers in the last 6 years:

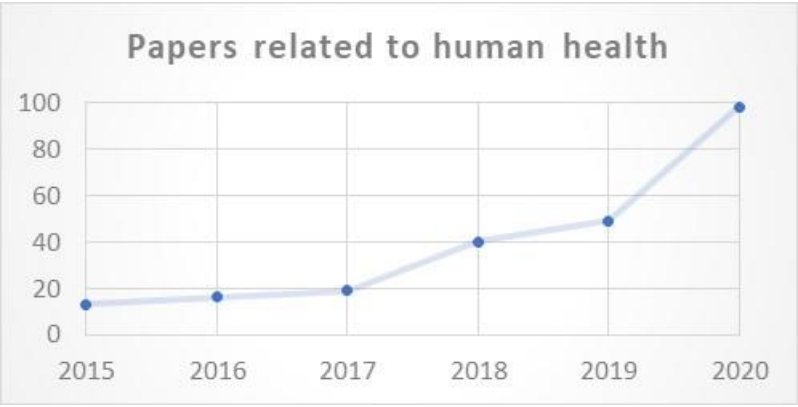

Figure 12: Number of papers of single cell sequencing related to human health (information extracted from lens.org)

The trend suggests an increase of single cell sequencing papers related to the field of human health in the incoming years (Figure 12). In fact, COVID19 research may have a positive impact in the use of these new technologies to better understand how the virus affect single cells.

- **Drug Development**

The analysis performed for Drug Development found a total of 100 papers in the last 6 years:

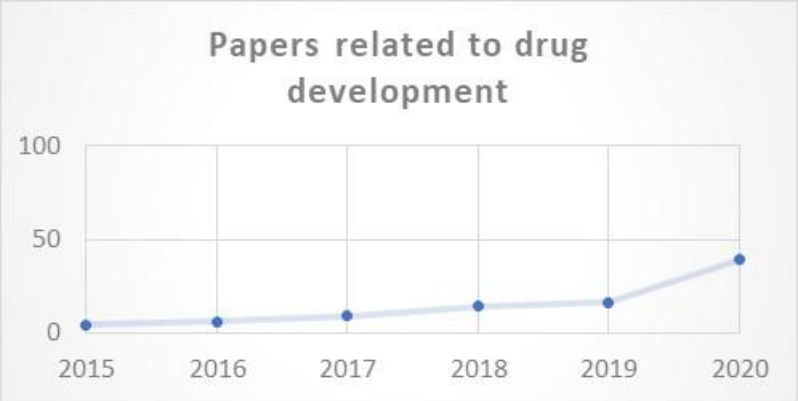

Figure 13: Number of papers of single cell sequencing related to drug development (information extracted from lens.org)

The trend also suggests a slightly increase of papers in the drug development field in the incoming years. This could be because the technology is still in an early phase (Figure 13).

- **Agriculture/plants**

The analysis performed for Agriculture/plants found a total of 10 papers in the last 6 years:

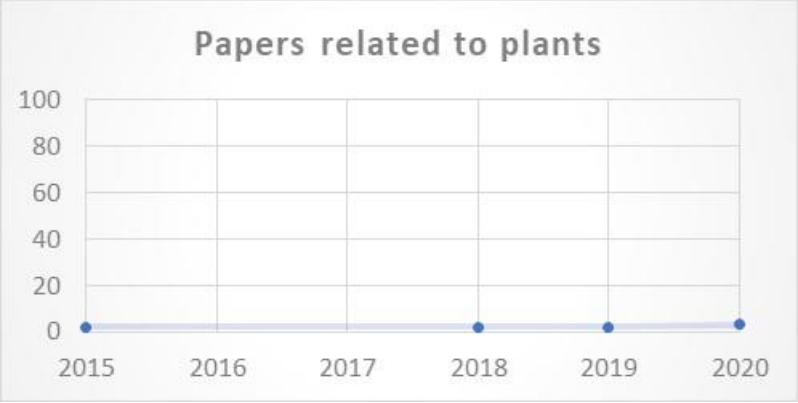

Figure 14: Number of papers of single cell sequencing related to plants (information extracted from lens.org)

Papers related to agriculture and single cell are significantly lower than other sectors (human or drug development). This may be due to the fact that the plant cells required different adaptations of the cell sequencing approaches to overcome some barriers such as the cellulose wall (Figure 14).

- **Animals**

The analysis performed for Animals found a total of 21 papers in the last 6 years:

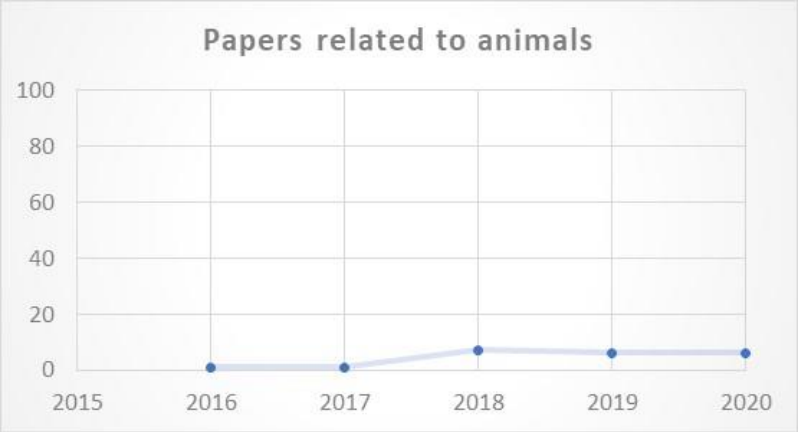

Figure 15: Number of papers of single cell sequencing related to animals (information extracted from lens.org)

Papers related to animal health field and single cell are higher than agriculture field but still low. This may be since state-of-art discoveries are firstly applied to human health (Figure 15).

4.2. Current single-cell sequencing applications in research resulted from the brief technology vigilance analysis

As commented before, single-cell sequencing has a wide spectrum of potential applications including those out of global initiatives such as HCA. Herein, some relevant examples of applications of single-cell sequencing technologies in different fields are included and briefly explained in order to highlight the potential of this technology in diverse scenarios.

- **Fertility and regenerative biology**

Endometrium tissue undergoes transcriptomic changes during human menstrual cycle. Despite its importance on human fertility, little was known regarding the tissue homeostasis. New discoveries related to the transcriptomic changes during the menstrual cycle were reported based on scRNA-seq analysis in endometrium cells.[30] Authors guarantee that this new knowledge provides a better understanding of the physiological process of endometrium cells and will contribute to develop new therapies to improve fertility in women.

- **Oncology and drug discovery**

Melanoma is the skin cancer with the highest mortality rate. Some studies described how treatment against one tumour cell subpopulation, in some cases, provides space and support for uncontrolled growth of more aggressive subpopulations. In a recent study, researchers identified different cell populations with different

[30] Wang, W., Vilella, F., Alama, P., Moreno, I., Mignardi, M., Isakova, A., ... & Quake, S. R. (2020). Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle. Nature Medicine, 26(10), 1644-1653.

drug resistance patterns through scRNA-seq analyses.[31] This advancement in the field of melanoma could provide potential improvements in targeted and immunotherapies.

- **Immunotherapies**

The immunotherapy uses our immune system to fight cancer. One example is anti-PD1 treatment. It works by helping the immune system recognize and attack cancer cells. However, in some cases, subsets of patients do not respond to immunotherapy but others yes. A report based on scRNA-seq analysis focused on immune system cells identified various immunophenotypes and gene sets correlated with T cell expansion following anti-PD1 treatment.[32] Therefore, cell sequencing techniques allowed to identify the processes that underlie heterogeneity in treatment response to immunotherapy. This opens a research line to study the differences between cells in order to improve the therapies.

- **Clinical trials**

During clinical trials in cancer, sometimes, patients do not respond to treatment in a similar way due to different resistances patterns of their tumours. Tumours are very different between patients, despite of being the same disease. This phenomenon is called “heterogeneity of cancer”. In this way, there are reports that incorporates the cell sequencing technologies to clinical trials in order to identify the differences between tumours after treatment. For example, multiple myeloma has heterogeneity, different cell populations with different treatment response. In a recent report, scRNA-seq analysis identified a signature of highly resistant myeloma patients and new therapeutic targets for these tumours.[33]

- **Microbiota**

Every human being harbours anywhere between 10 trillion and 100 trillion microbial cells in a symbiotic relationship. This benefits both the microbes and their hosts, as long as the body is in a healthy state. Estimates vary, but there could be over 1,000 different species of microorganism making up the human microbiota. Cell sequencing technologies in bacteria has lagged relative to in eukaryotes because of their though bacterial cell walls, low messenger RNA content, among others. However, recent scientific efforts are trying to adapt single cell approaches, such as scRNA-seq, to bacteria.[34] This advancement will allow gene expression analyses in bacteria communities such as human microbiota. Therefore, this cell sequencing technique will enable a potential improvement of human health through understanding microbiota states in subpopulation of cells.

[31] Izar, B., Tirosh, I., Stover, E. H., Wakiro, I., Cuoco, M. S., Alter, I., ... & Regev, A. (2020). A single-cell landscape of high-grade serous ovarian cancer. Nature medicine, 26(8), 1271-1279.
[32] Bassez, A., Vos, H., Van Dyck, L., Floris, G., Arijs, I., Desmedt, C., ... & Lambrechts, D. (2021). A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer. Nature Medicine, 27(5), 820-832.
[33] Cohen, Y. C., Zada, M., Wang, S. Y., Bornstein, C., David, E., Moshe, A., ... & Amit, I. (2021). Identification of resistance pathways and therapeutic targets in relapsed multiple myeloma patients through single-cell sequencing. Nature medicine, 27(3), 491-503.
[34] Kuchina, A., Brettner, L. M., Paleologu, L., Roco, C. M., Rosenberg, A. B., Carignano, A., ... & Seelig, G. (2021). Microbial single-cell RNA sequencing by split-pool barcoding. Science, 371(6531).

- **Liquid biopsy**

During the last decade, liquid biopsy became in an emerging field in oncology. This is the analysis of tumours using biomarkers circulating in fluids such as the blood. The ability to detect and characterize tumours in such a minimally invasive and repeatable way could have considerable clinical implications. In this context, researchers are using cell sequencing technologies to better understand tumours through liquid biopsies, such as circulating tumour cells (CTCs). It was recently reported in a breast cancer research that single-cell genome (DNA) sequencing can be used to detect genomic alterations in circulating tumour cells (CTCs) from liquid biopsy associated with different survival rates.[35] The cell sequencing technologies allowed to create a validated tool for the identification of patients with a higher risk of relapse, including those diagnosed with breast cancer. These new discoveries could lead to an improvement in clinical decisions and healthcare in patients with breast cancer as well as other potential cancer types.

- **Animal health**

The increasing cases of zoonotic (infectious disease that has jumped from a non-human animal to humans) and chronic diseases are the key factors driving the market for veterinary medicine. In this context, better understand the animal health is very important to them as well as to human health indirectly. Cell sequencing technologies has also been applied to this field. Recently, scRNA-seq approach was used to better understand monkey infected cells with SARS-CoV-2 (virus that caused COVID19 pandemic).[36] This has led to an identification of SARS-CoV-2 infection course in specific cells from lungs of monkeys and to an improvement in the understanding of the disease. This approach can be extrapolated to other animals (such as dogs, cats, cows, horses, etc.) and animal/zoonotic diseases in order to improve their care.

- **Plant biotechnology**

Transcriptomics and other traditional molecular approaches were used to better understand plants development and other issues such as response to different stresses include nutrient deficiency, pathogen attack, exposure to toxic chemicals etc. Transcriptomics applied to cash crops including barley, rice, sugarcane, wheat and maize have further helped in understanding physiological and molecular responses in terms of genome sequence, gene regulation, gene differentiation, posttranscriptional modifications and gene splicing. On the other hand, comparative transcriptomics has provided more information about plant's response to diverse stresses. Thus, transcriptomics, together with other biotechnological approaches helps in development of stress tolerance in crops against the climate change.[37] In this context, cell sequencing

[35] Rossi, T., Gallerani, G., Angeli, D., Cocchi, C., Bandini, E., Fici, P., ... & Fabbri, F. (2020). Single-cell NGS-based analysis of copy number alterations reveals new insights in circulating tumor cells persistence in early-stage breast cancer. Cancers, 12(9), 2490.
[36] Speranza, E., Williamson, B. N., Feldmann, F., Sturdevant, G. L., Pérez-Pérez, L., Meade-White, K., ... & De Wit, E. (2021). Single-cell RNA sequencing reveals SARS-CoV-2 infection dynamics in lungs of African green monkeys. Science Translational Medicine, 13(578).
[37] Imadi, S. R., Kazi, A. G., Ahanger, M. A., Gucel, S., & Ahmad, P. (2015). Plant transcriptomics and responses to environmental stress: an overview. Journal of genetics, 94(3), 525-537.

technologies are beginning to be applied to the field of plants. A recent report performed a transcriptomic characterization of cell types from root tips of two agronomically important rice cultivars world-wide using a new scRNA-seq approach.[38] Roots are essential plant growth and serve nutrient and water uptake as well as providing firm anchorage to the soil. The applications of cell sequencing technologies and these results open new avenues to study cell type specification, function, and evolution in plants as well as to improve crops.

5. OVERALL Impressions and recommendations for HUTER

After several fruitful Innovation Committee meetings and interactions with the IAB, the members of this board provided some recommendations and impressions regarding the project and technologies involved in HUTER. Herein, the main suggestions are summarized per industry and preserving the identity of each contributor.

5.1. Impressions from Biotech sector

The technologies applied within HUTER project could arise synergies with other fields of research. Particularly, one IAB member would expect synergies in terms of (i) the possible transfer of the technology generated to other areas of knowledge or (ii) the study of the knowledge generated to define origins or consequences of the biological problem under study. Another member states that as far as HUTER is dedicated to generate an atlas of the uterus by single cells technologies, the synergy with related diseases like embryo implantation, endometriosis or uterus carcinoma are clear. Additionally, there is a synergy with research on immunotherapy where the crosstalk between the tumour and the microenvironment is critical. Likewise, there is a synergy with research on CAR-T and CRISPR-Cas9 technologies for gene-based and cell-based therapies. Finally, the digital platforms and single cell technologies synergizes with preclinical modelling, especially in the case of complex 2D-3D co-culture systems like organoids derived from patients, or small in vivo models like zebrafish.

Regarding market potential, the IAB member commented that single cell sequencing technologies could have market relevance in the microbiology-related industry in a long term. He would consider very interesting to analyse responses of individual cells to “microbiome stimuli”. The IAB member also commented that he believes that the technology and knowledge generated in HUTER can be applied in any area of biomedicine. However, this will depend on the reduction of the costs of these technologies, in the opinion of a member of the IAB. If the cost would be much cheaper than the usual analysis cost for other methods these days, the technology could find use in many areas. As long as the sequencing costs more, it has to provide some kind of

[38] Liu, Q., Liang, Z., Feng, D., Jiang, S., Wang, Y., Du, Z., ... & Gu, X. (2021). Transcriptional landscape of rice roots at the single-cell resolution. Molecular Plant, 14(3), 384-394.

other value. And on many occasions, if the test has to provide a clear and simple answer, the additional value is just not needed.

In Oncology, an improved sensibility/specificity in the detection of tumour driver mutations achieved by single cell sequencing, could represent an added value for those technologies that are already in the market (NGS, target panels, ddPCR…). For instance, for the identification of specific mutations associated with response to target therapies, or mutations associated with resistance to therapy. If this improved sensibility/specificity can be achieved by single cell sequencing, these technologies could be translated to other clinical settings with a broad market, like early cancer detection and cancer screening programs. One member of IAB expects an impact on the characterization of tumour heterogeneity in liquid biopsy leading to a clear description of the different tumour subpopulations in circulation that will eventually result in micrometastasis; this could impact in a more appropriate selection of therapies specially at progression disease. Likewise, the characterization of the tumour subpopulations in circulation in sequential liquid biopsy samples (at diagnosis, at first progression, at second progression, etc) could provide valuable information about the evolution of the disease; oncology professionals go always behind the tumour and if they could anticipate how this tumour is going to evolve, this would be extremely valuable. Finally, the possibility to capture and disaggregate the gene-expression profile of different cell subpopulations in liquid biopsy also applies to the interaction of tumour cells and other cell types of the tumour microenvironment, like immune cells and CAFs; this could impact on the identification of biomarkers of response and selection of patients to immunotherapy.

Regarding the possibility to transform digital platforms into market solutions for other biotech sectors, one of the IAB members suggest that it would be possible if a clear and scalable business model is developed. However, he has doubts regarding the application of these platforms out of basic research and field of science. Finally, regarding the impact of digital platforms (similar to HUTER platform) in fields such as drug development or diagnostic devices, the IAB member states that this kind of platforms can strongly support basic research in finding leads if made publicly available. The IP aspect must be kept in mind, as companies, which could be using the database, do not want to share the IP.

5.2. Impressions from IT sector

a) Advanced microscopy techniques potential in the medical sector

One IAB member made some comments regarding the advanced microscopy techniques, currently in the research field, and their potential application in the medical sector. He states that advanced microscopy techniques are expected to impact clinical setting in the coming years if they are appropriately standardized. There is still a big unmet need in making advanced instruments available not only in large reference clinical sites but also in primary care to deliver health, such as in remote areas and far from large cities. These techniques will allow for increased point-of-care resources and diagnostic capabilities. For this, affordable high

throughput microscopy technologies (grouping automated microscopy, robotics and quantification by image analysis) must be evolved and their costs reduced to be provided as diagnostic test.

Complete biological systems such as the immune system can be visualized with the use of advanced microscopy techniques, with a previously unattainable understanding of dynamic cell to cell interactions during immune responses of T-cells. Novel techniques such as light-sheet microscopy and Swept, Confocally-Aligned Planar Excitation (SCAPE), allowing for 3D imaging with dramatically high temporal resolutions (48 frames/second) are also a challenge for the creation of processing tools that aid in the development of image analysis pipelines that provide results to key clinical questions.

b) Artificial intelligence for diagnostics recommendations

Regarding the potential of artificial intelligence applied to imaging, herein represented by deep learning techniques or convolutional neural networks (CNN) applied to medical images have completely outperformed traditional computer vision algorithms. Any type of image in which cells or tissues must be characterized by their identification and extraction of properties are highly suitable for CNN. The challenge in the application of CNN is the need for large expert annotated datasets that allow to train new AI models in combination with the availability of data scientists with 2D-3D skills and access to high-performance computing capabilities with graphics processing units (GPU). With regards to data, being the bigger challenge, one member of IAB strongly recommends to align the imaging techniques used in HUTER as well as to complement the data collection efforts with the ones from already existing open datasets such as the Image Data Resource (IDR) and the Cell Image Library, that can be used to pre-train AI models before they are trained to characterize uterus cells.

c) Digital platforms on the field of diagnostics and healthcare

Regarding the impact of digital platforms for OMICs data and medical imaging on the field of diagnostics and healthcare, the IAB member expects an integration of advanced microscopy techniques and associated image analysis pipelines within in-vitro diagnostic medical devices (IVD). This will allow for the integration of high-throughput microscopy in the clinical setting. These devices will require the management and analysis of the data not only at a local level but also through cloud (due to the large volumes of data). In this regard, software platforms able to link advanced microscopy images (unstructured information) and their associated findings (structured information) with clinical data and other omics will be also made available to healthcare organizations. Instead of analysing the data locally, these digital platforms will integrate artificial intelligence models that will analyse the images in the cloud, allowing the users to accept, amend or reject the analysis of AI at any time. The management of annotations in the digital platform is also useful in gathering new annotated cases that can be used for model improvement in new future versions of the IVD. Local continuous learning workflows (where a specific user from a site annotates the cases for improvement of the algorithm only at a single centre) are not expected to be used for the AI models within the CE mark or FDA cleared IVD, since these techniques have the risk of inducing forgetting of the AI algorithms, biasing the model behaviour

to resemble the expert criteria from the centre in which it is deployed. Continuous learning of AI models is expected to be managed centrally by the providers of the IVD and the digital platforms.

d) Value of open standards

Another IAB member states that Open standards allow for easier sharing and replication of results. By using and promoting open standards like DICOM, a wealth of existing tools and applications can be used to view, share and analyse the data. Many of these tools are open sourced themselves and actively maintained by research communities, so it also allows consuming of any data produced by future third party researchers much easier.

Open standards also promote the eventual commercialization of results. DICOM is far and away the standard of choice for exchanging medical imaging data within hospitals and even nascent technologies like digital pathology are quickly converging on it. This foundation means that almost any clinical applications will be expected to consume and operate on DICOM images. In general, these technologies will help unlock new capabilities within these fields. Taking medical pathology as an example, by increasingly digitizing these practices new assistive capabilities like early triage, lesion detection, and similar case search can start to be deployed. Adopting industry standards promotes a whole ecosystem of vendors and tools that can work together seamlessly instead of having each hospital lock into a single vendor for all of their digital pathology capabilities.

e) Cloud computing benefits

Several IAB members also commented the benefits of cloud computing for digital platforms with the aim of improving the healthcare and research. They state that cloud computing has a lot of benefits for research, such as being able to only pay for what you use and have availability of any amount of computing resource immediately, researchers can also benefit from sharing repositories with information such as open data or genomics data that can be used fast without having the need to download from other organization. At the end this will lower the cost of research and accelerate the time to science.

This main benefits also apply to general healthcare practice, with a broader view, it is not an option for each hospital in the world to create a Machine Learning algorithm to screen breast cancer for example. They will use already existing products from the market. If you see this example at scale, including all the possible clinical practices, the only platform that will enable partners to create, implement and constantly update this big amount of innovations is cloud computing. It is the only way to ensure the patients have access to the latest technology and research under their point of view.

They listed the following benefits:

1. Access to instant, almost limitless compute and storage.

Researchers and healthcare institutions no longer have to have multi month lead times to provision new clusters or increase storage capacity which increases execution speed.

2. Easier reproducibility of results.

Researchers can not only share data & analytic code, but also the exact configurations and machine setups used to produce results.

3. Access to novel healthcare specific capabilities from Cloud providers.

Examples like Google's Healthcare API or Azure FHIR API or the various public datasets provided by cloud providers provide powerful tools for working with health data.

4. "Bring your workflows to the data" not the other way around.

Instead of researchers struggling to copy around petabyte sized datasets for research purposes, they can instead do their research and analysis directly in Cloud sandboxes and avoid bandwidth and duplicate storage costs.

One IAB member commented that the HUTER platform is currently focused on understanding uterus cells, but the next phase should be to include new capabilities to automate and accelerate the analysis over the cloud, such as Machine learning. He quoted one example from the Munich Leukaemia Lab, that has created an automatic leukaemia diagnosis pipeline using Machine learning over Amazon Web Services.

f) Barriers to be overcome in terms of digital platforms in hospitals

One IAB expert make some suggestions in order to overcome the barriers to transfer digital platforms from research to hospitals. The first things that should be considered when planning to enter into production at scale in the healthcare technology market is to think on the future from the beginning, cloud computing will enable you to be more agile and secure, to scale faster and to include new innovations in your product faster, but all the compliance and scalable solution should be implemented from the beginning and considering international markets as regulations differ from one country to another, if not, implementing it while entering a new market will delay significantly the innovation in the product as all resources will be dedicated to compliance.

Second, the tool should integrate seamlessly with the current applications of the hospital and should improve a complete patient view not creating silos.

Third, it should be as standard as possible, so every investment is made to increase innovation and not to personalize for a specific need.