MATANEL FOUNDATION

ACTIVITY REPORT

Program*: Summer program of the International Summer Science Institute (ISSI) of the Weizmann Institute*

□ Participation awarded as a prize in the context of the National Jonk Fuerscher contest 2022

Year: 2022

Please present your activity report according to the following lines. The whole report will not exceed 2 or 3 pages (as word document).

Name of the Program: *"Summer program of the International Summer Science Institute (ISSI) of the Weizmann Institute"*
Participation awarded as a prize in the context of the National Jonk Fuerscher contest 2022

Year of activity: 2022

Name of the report's writer: Sousana Eang

Function of the report's writer: Director

Mail: daniela@fjsl.lu
Phones: +352 621 653 158

Website / Facebook address of the organization: https://www.fjsl.lu

Number of active participants in the program: 1 - Federica Maestri

Estimated number of impacted participants: 1 - Federica Maestri

Give the actually state of the program (where the program stands at the date of the activity report, no more than ten lines):

The Matanel Foundation sponsorship enables the Fondation Jeunes Scientifiques Luxembourg to award one exceptional young laureate who participated at the national Jonk Fuerscher contest with the opportunity to participate in the ISSI's summer program, which takes place once a year, during 4 weeks (04.07-27.07.2022).

Due to the continuing uncertain situation with regards to the Covid-19 pandemic, the program in 2022, as the previous years, was regrettably again organized as an entirely virtual event, instead of an in person-event.

The virtual format however still provides a rich mix of informative and interactive science presentations, as well as social, fun and cultural activities. The exceptional

organization allowed the attendees to still participate in science projects, inspirational lectures as well as to have fun cultural exchanges, despite the virtual format.

The program's main focus is working on a group project with participants from a variety of different nations and backgrounds. The program not only allowed Federica an incredible opportunity to learn about the world of science from impressive tutors and lecturers, but also to exchange with like-minded youngsters between the ages of 18 and 20 through various social activities.

The main achievements during the last year of activity (main achievements, number of events, number of participants, etc.):

The program Federica Maestri followed at the ISSI, presented a great opportunity for personal and professional growth by having the opportunity to discuss with prestigious scientists of the Weizmann Institute, as well as meeting and collaborating with like-minded peers from around the world, and getting crucial insight into the the reality of the scientific world.

The main achievement during these uncertain times is, even if not perfect, to continue giving a Jonk Fuerscher contest winner the opportunity to deep dive into a project with spectacular mentors and like-minded participants. This year, there were around 21 participants between the ages of 18 and 21, from 9 different countries, including: the USA, UK, Germany, Luxembourg, Switzerland, India, Hong Kong, Mexico and Israel.

The evaluation (methodology, results, comparisons with the precedent year, conclusions for the future…):

The advantages if the ISSI program consist in the learning experience the participants gain both through a theoretical and a practical approach, and by additionally focusing on the importance of the social aspect of Science:

On the one hand, the participants are attending lectures by scientists of the Weizmann Institute, and can interact with them directly. On the other hand, they get to do a group project together with other participants, and whose results they present at the end of the program.

Furthermore, socializing activities allow them to get to know other participants, on a more personal and social level, build new friendships and thus underlining the importance of the social and human part of science - the team work necessary to move science forward.

Sadly, the last in person participation of the ISSI program was in 2019 due to the situation around the pandemic. In 2020 regrettably the program was canceled and in 2021 and 2022 the event has been organized as a virtual event.

As can be seen from both evaluation reports in 2021 and 2022, although the virtual format was the best possible adaptation of the program, it would be even more of an enrichment for the participants to interact directly in-person with both the scientists

and their peers. If all goes according to plan, it has been mentioned that the ISSI summer program should revert back to the standard in person event in 2023.

Provisional guidelines for the advancement of the program in the next year:

The main improvement that could be made would be, as previously stated, would be to return to an in-person format of the ISSI's summer program to enable the best possible experience for the national Jonk Fuerscher's laureate. Unless any further concerns arise, the organizers have stated that this would be the case for 2023.

Please join the Evaluation Report, the Financial Report and the list of the participants to the program (**as PDF documents**)

Please see the documents "1.Evaluation report", "2.Financial report"
The participant is: Federica Maestri

Please join photos – as **JPG files** – and any link or any other document connected to the program which seems relevant to you – as **PDF document**.

Please see the attached document "3.5 pictures & a short movie"

Please join a 5 minute movie which presents your institution and the particular project supported by the Matanel Foundation. The movie should be accessible to the philanthropic world and to other potential donors.

Short movie of the FJSL's National Jonk Fuerscher Contest & Expo-Sciences Luxembourg 2022:

https://www.youtube.com/watch?v=zs7bBpBZsVw&t=8s

Short movie of Federica Maestri's presentation at the FJSL's National Jonk Fuerscher Contest:

https://youtu.be/F7ZG6qLPfgA

Short movie of Federica Maestri's group project:

https://youtu.be/loo6ZVF1Hsc

Please see the Digital ISSI 2022 - Journal of Scientific Reports for more information on all the projects.

Additionally, we can provide you with all the session recordings on request, as well as the links to the scientific presentation videos on youtube.

Evaluation report, analysing the pros and cons of the program and the benefits of participating.

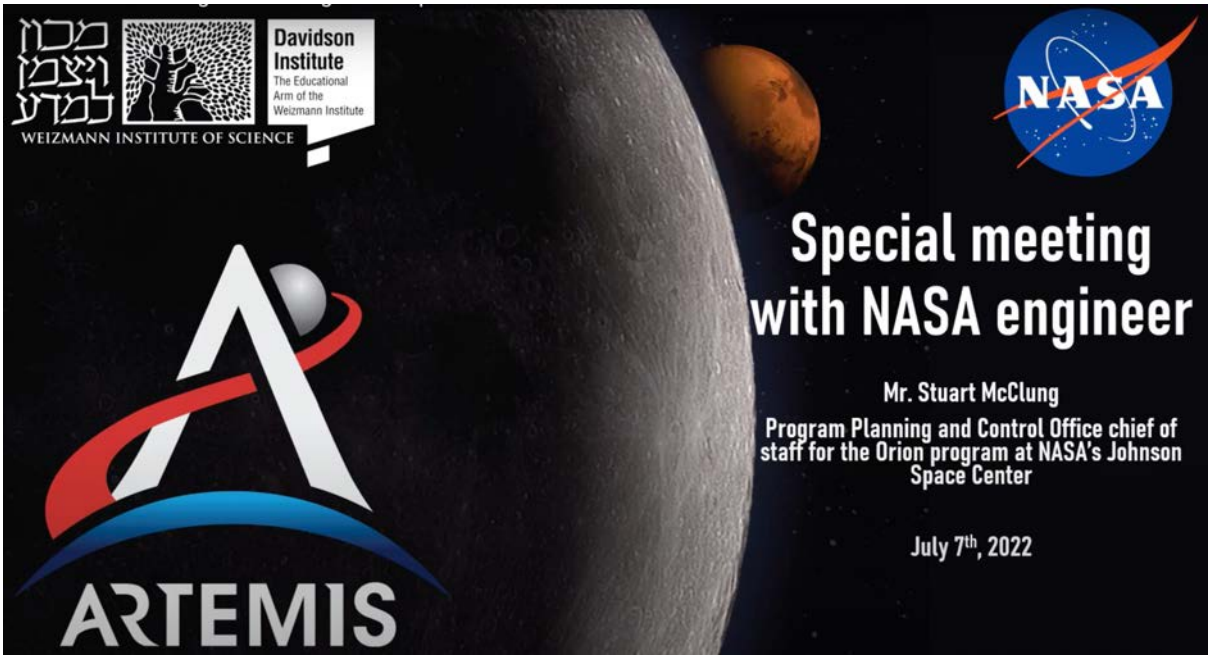
Overall, the benefits and advantages of the program outweigh the limitations currently experienced due to the virtual organisation. Below a table of Pro's and Con's:

ISSI Participation PRO's	ISSI Participation CON's
Meeting different students from different backgrounds	Virtual: social part got lost a bit
Experience on what scientific research is and looks like in one of the world's best and leading institutions in the natural and exact sciences	
Following different lectures on different topics from both academics and non-academics	
Working with really talented and brilliant mentors who are amongst the best in their field	
Team work	
Research on current topics	
Learning new skills	

Conclusion:

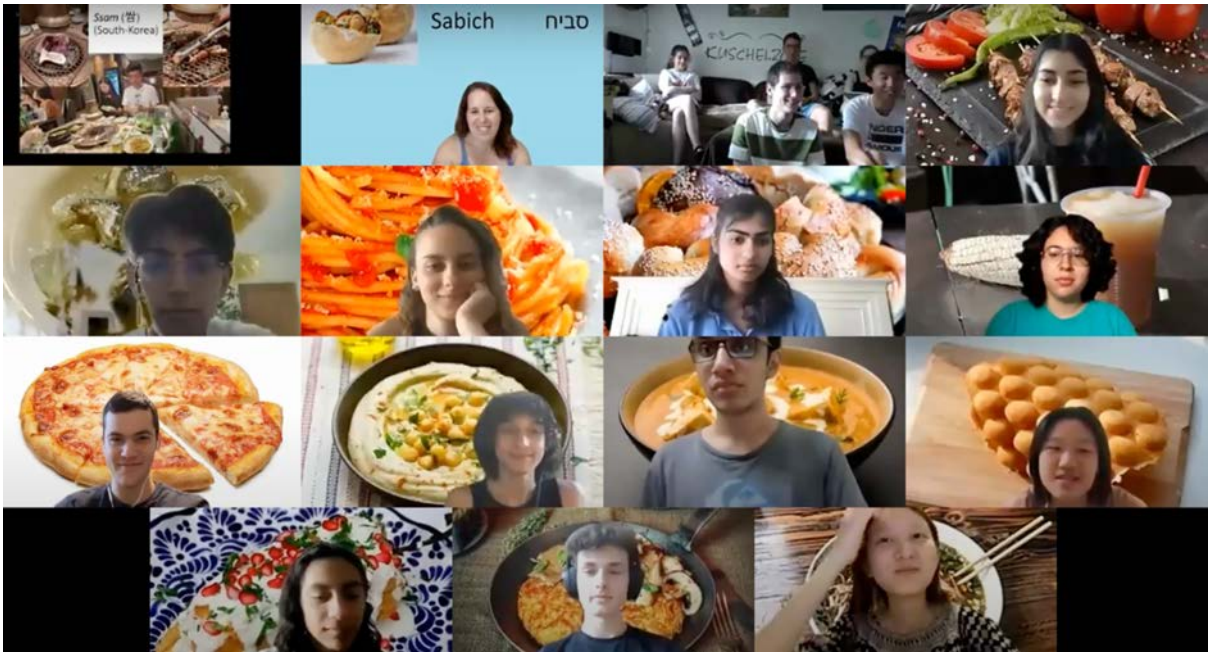
The experience and program are an incredible experience for the participants, not only on a scientific level but also on a cultural one. The participants are given the opportunity to learn from the best, to overcome their limitations and all the while meeting peers with similar interests and backgrounds - a truly cultural and scientific melting pot. As has been highlighted since the virtual format of the program, this does bring its limitation, by not allowing a true immersion into the full experience of cultural and scientific exchange, however the organisation has been excellent and has done its best to overcome this minor, but main disadvantage. An on-site experience would be favourable in the future if circumstances allow, but the excellent experience even during the virtual organisation is not to be neglected.

1. **Meeting with Mr. Stuart McClung who talked about NASA Artemis Program**

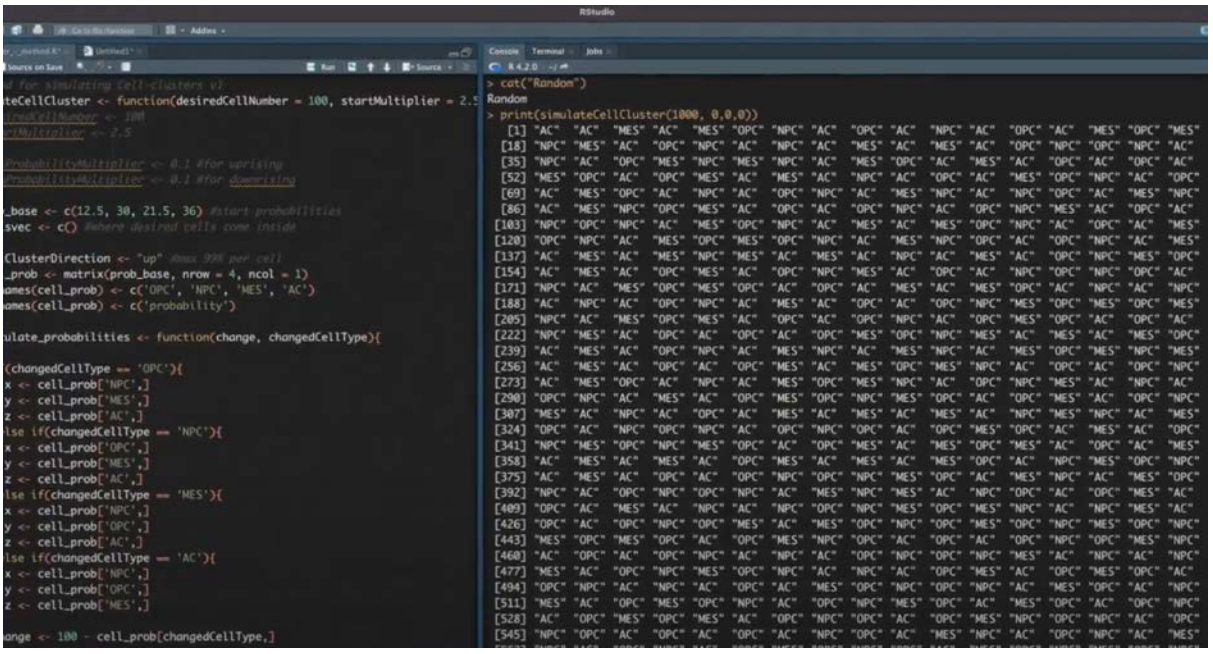


2. **Meeting with the President of the Weizmann Institute of Science, Prof. Alon Chen**

3. **Social Activity showing our favourite food from our home country and playing games**



4. **Code Snippet from our project to investigate gene expression without considering their spatial organisation within the tumour**



5. **After-Closing Ceremony with our coordinators Dr. Aya Shkedy and Dr. Dorit Granot**

Short movie of my group's project: https://youtu.be/loo6ZVF1Hsc

"Yearbook" of all ISSI 2022 participants, with more photos/ information about social activities: https://www.canva.com/design/DAFHmtdVKvw/YtDF7nlWM4zBgJytLtFIXw/view?utm_content=DAFHmtdVKvw&utm_campaign=designshare&utm_medium=link&utm_source=viewer

Enabling Practical Alternatives for Tumor Profiling: Spatial Transcriptomics Preserves the Cellular Resolution of Single-Cell RNA Sequencing

Adithi Adusumilli[1], Maria Fernanda Argote de la Torre[2], Sinan Arif Aramaz[3], Svenja Heß[4], Selin Kocalar[1], Federica Maestri[5], Jinho Aron Moon[4], Rohan Raghavan[3]

USA[1], Mexico[2], United Kingdom[3], Germany[4], Luxembourg[5]

Mentored by Dr. Michael Tyler, Lab of Dr. Itay Tirosh, Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel

Abstract

RNA sequencing (RNA-seq) is an extremely useful tool to study tumors and has been widely used for cellular state characterization. While in the past RNA-seq profiling could only be done by sequencing together the RNA from the whole tumor, '*in bulk*', it is now possible to profile tumors at since cell resolution with single-cell RNA-seq (scRNA-seq) by isolating and sequencing tumor cells separately. Nevertheless, this process ignores the tumor cell's spatial organization and morphology. In our research, we investigated and evaluated the role of spatial transcriptomics, where all cells and their spatial organization are retained but single-cell resolution is lost. To do this we investigated the ability to distinguish intra-tumor heterogeneity at lower resolutions by using scRNA-seq data to simulate bulk expression profiles with different sizes and spatial relationships. Our findings showed that while increasing the number of cells analyzed together does result in less distinct cell classes, spatial transcriptomics still allows for intra-tumor heterogeneity characterization.

Introduction

Transcriptomics refers to the study of RNA within biological samples and has been used extensively to study tumors [1]. In biological systems, RNA is the key molecular intermediate between genomic DNA and the proteins that DNA encodes for, which define cellular phenotypes [2]. Transcriptomic analysis allows for phenotypic trends to be defined by levels of DNA

transcription, which in cancer biology, enables the determination of various cell classes based on gene expression profiles, even within highly heterogeneous tumor microenvironments [3]. Understanding the transcriptomic activity of various cell subtypes within tumors is valuable as it can enable a deeper understanding of cancer cell biology, ultimately aiding efforts in the development of new and effective cancer therapies.

While bulk RNA sequencing of whole tumors has traditionally been used in cancer research, this approach is not suitable as it fails to provide clarity into the varying gene expression profiles of the diverse cell classes [10]. The development of single-cell RNA sequencing has partially addressed this need by allowing for the screening of individual cells [5], but the sample preparation process is both costly and biases the survival of certain cell types. As a result, single-cell RNA sequencing does not allow for accurate characterization of the tumor microenvironment and is not a widely accessible methodology due to its high costs [6].

Spatial transcriptomics, a method in which affixed tissue samples are screened in small sections of ~10 cells, poses a more promising alternative to bulk and single-cell RNA sequencing [7]. Not only does this method preserve the spatial organization and architecture of glioblastoma, but it also allows for the characterization of small clusters of cells comprising the tumor [8]. This advantage allows for further analysis of the cell classes within the tumor and the distribution of these cell classes, a key insight into the understanding of this tumor and the optimal methods of treatment. However, single-cell resolution is not achieved. Here, we seek to probe whether the loss of single-cell resolution detracts from the ability of spatial transcriptomics to elucidate the diverse cell classes within tumors.

As a test case, we use scRNA-seq data for glioblastoma multiforme (GBM), a particularly aggressive high-grade glioma and the most prevalent brain tumor. This disease's high mortality rate can be attributed to the heterogeneity of cell classes within the glioblastoma microenvironment, which makes it challenging to characterize the tumor, identify optimal treatments, and provide patients with the timely care they require. Such characteristics make it highly difficult to prevent this tumor from quickly infiltrating other parts of the brain, contributing to the low success rates of common treatments including surgery [9].

Previously, Neftel and coworkers identified 8 major cell classes through the analysis of single-cell RNA sequencing data from glioblastoma patient samples [10]. In this study, we use these results to compare cell subtype classifications between data obtained from single-cell RNA sequencing as well as simulated bulk profiles using matrix factorization while also investigating the effects of cell type proportion and spatial organization of tumors on our analysis. This dataset was selected because of both the aforementioned heterogeneity within

GBM and the well-defined cell classes Neftel and coworkers identified, which makes it ideal when it comes to comparing the scRNA-seq and simulated bulk profile results to determine the feasibility of spatial transcriptomics in preserving the resolution of scRNA-seq while efficiently characterizing glioblastoma heterogeneity.

Materials and Methods

In order to assess how cellular resolution affects detection in glioblastoma tumor RNA sequencing, the scRNA-seq gene expression dataset taken from the study conducted by Neftel et al. was investigated [10]. This study focused exclusively on the 209 cells belonging to sample MGH-100. This sample only contained malignant cells from an adult patient and contained all 4 cell states characterized by Neftel et al.

Regardless of whether it would be used as single-cell or bulk data, all sample information was normalized to TPM (Transcript Per Million) and centered. Additionally, only the 100 most expressed genes in each cell were considered during the single-cell analysis, while the top 5,000 were used in all bulk profiles. The entirety of this prep process as well as the data analysis was performed using the R Programming Language version 4.2.1.

The *Non-negative Matrix Factorization* (*NMF)* package (Version 0.24.0) was essential. *NMF* is a standard and well-trusted dimensionality reduction method that has been used in numerous publications related to gene expression analysis. This process clusters the RNAseq data in accordance with eight well-defined cellular states the glioblastoma cells exist in. As classified by Neftel et al., these are the two MES-like, AC-like, OPC-like, and two NPC-like as well as cell cycle G1 and G2 ‘expression program’ behaviors. During the course of this study, we also referenced the meta-modules provided in the Neftel paper: a consensus set of gene lists characterizing each of these programs [10]. Using NMF, we first decomposed the Neftel et al. tumor scRNA-seq expression matrix into multiple lower-rank matrices, running it with ranks three through six in order to carry out a more in-depth analysis.

Moreover, *the Jaccard index* was used to compare different meta-modules and gene sets, processing their intersections. Using this similarity index, overlaps between gene sets could be quantified, whereby a matrix of Jaccard values could be tabulated for each pairwise intersection of gene lists. We could subsequently use *hierarchical clustering with complete linkage*, which grouped together gene sets representing the same cellular state within matrices of Jaccard values. This allowed similarities between gene sets to be visualized. Using this method, we determined which gene sets were representative of the same cell classes and which individual gene sets represented cell states not accounted for by other sets, using this

data to redefine the Neftel et al. meta-modules. *Random sampling* was initially employed in our modeling of cell aggregates, drawing from a uniform probability distribution to generally account for the high levels of heterogeneity in glioblastoma.

To simulate the random samples more realistically incorporating the cellular proportions and spatial organization of the tumor, data from Neftel et al. [10] in which they reported the frequency of the six cell classes in adult glioblastomas was used. To reduce to our four cell classes the frequencies of the subtypes were summed, leading to the probability distribution OPC: 12.5; NPC: 30; MES: 21.5; AC: 36. In our classification of cells into each expression program, we calculated the average gene expression in each cell's gene-set. The maximum was used to classify each cell in accordance with the redefined gene lists.

To simulate a clustering pattern, the probability of each preceding cell type was proportionally increased by a base multiplicative factor of 2.5, which would change with each iteration according to the user-specified function parameters and cell types selected in the previous iteration. This was representative of the noted scenario whereby cells of a certain state are more likely to occur next to other cells in the same state. More precise empirical information on the spatial relationship of glioblastoma is not yet available (07.22).

Results

Single-cell data

We initially set out to accurately represent the glioblastoma microenvironment at single-cell resolution, creating a 'ground truth' model from which comparisons could be drawn in accordance with the aim of our experiment. Having run the NMF algorithm and created a single matrix composed of the 18 resulting columns or gene sets, we mapped the intersection between all 18 of them (Fig 1A). Afterward, we re-ran this same intersection analysis, only this time comparing the gene sets to the previously mentioned meta-modules defined by Neftel et al. to determine which cells (and as such, which genes) within the MGH100 sample most closely resemble each cell class (Fig 1B).

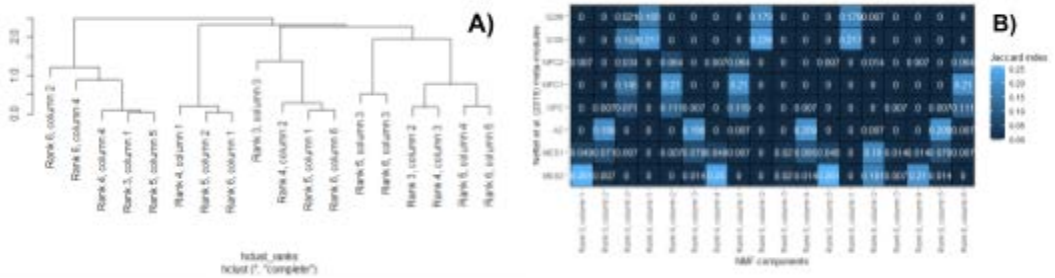


Fig 1: Intersection between MGH100 samples.

Based on these two figures, we were able to determine our ‘ground truth’ and define four gene lists: NPC, OPC, Mes, and AC as described in the Materials and Method section. The purpose of redefining the Neftel et al. cell classes and tailoring them to the MGH100 sample was to discard the genes that were not relevant to this study and thus minimize noise within the data. An important thing to note in Figure 1B is the relationship between the NPC and the OPC lists. As we can see, when a gene set intersects with either of them it will most likely also intersect with the other, albeit at a different level. As such, for the purposes of this study, while these two lists will be represented independently, during data analysis they will be examined together.

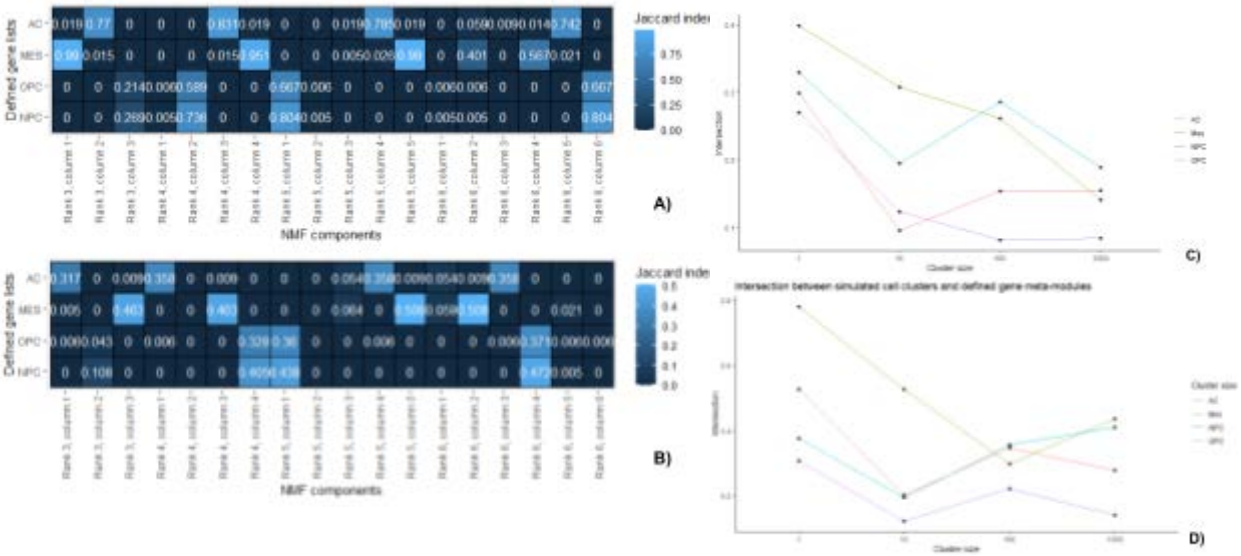
Simulating bulk profiles with a uniform distribution

To gauge the glioblastoma microenvironment at a lower resolution like the one observed through spatial transcriptomics, we simulated aggregates of 10, 100, and 1000 cells from the data and carried out the same process we did for our single-cell data. We initially did so by sampling from a uniform distribution, whereby 500 aggregates of random cells in the sample could be generated for each varying aggregate size. These could then be tabulated against the overall expression of each gene per aggregate. After normalizing and processing this data through NMF as before, we could then compare the resultant gene sets to our ground truth model once again using the Jaccard index. Hierarchical clustering was used, as above, to define and classify expression signatures using different cell resolutions for each expression program. This data subsequently examined the concordance between aggregate expression signatures and our ground truth model. Hence, we could determine the limiting cellular resolution for accurate tumor representation and thus detection. We also decided to discard the G1/S and G2/M classes due to the fact that they are not tumor-specific and that Neftel et al. Figure 3B shows cells in any other state can also be cycling. Figure 2A and Figure 2B show the intersection between our single-cell and bulk data and the new gene lists.

The following two figures (Fig 2C and Fig 2D) show the comparison between these three bulk profiles, our single-cell data, and the defined gene lists from our ‘ground truth’ with slight variations in how the intersection means were calculated. Figure 2C took into account both hierarchical clustering and the intersection between each gene set and the four gene lists, resulting in a mean that considered all non-zero values for each gene list. Figure 2D, on the other hand, only takes the cell class each gene list or column most intersects with into account, with only OPC and NPC allowed to occur simultaneously. All the data used to plot these figures was obtained from heatmaps such as Figures 2A and 2B. These plots show that the ability to

capture the meta-modules indeed decreases with larger cluster sizes, but the rate of decrease seems to slow or even plateau, suggesting that even relatively large cluster sizes are still sufficient to capture these meta-modules. In some cases, the intersection appears to increase along with cluster size, which is likely due to the randomness in cell sampling and may not truly reflect the improved performance of the NMF method or that larger clusters are better at capturing the meta-modules.

Figure 2



A) Intersection between MGH100 samples and the defined gene lists. B) Intersection between MGH100 uniformly sampled 100-cell clusters and the defined gene lists. C) Intersection between MGH100 randomly sampled cell clusters and the defined gene lists considering all intersection values above zero. D) Intersection between MGH100 randomly sampled cell clusters and the defined gene lists only considering each column's highest expressed cell class.

Inclusion of spatial relationships while sampling

It is important to outline that there are many potential spatial relationships and consequently, to simplify things, we considered only the scenario where cells of each type are likely to occur next to other cells of the same type, which should be sufficient to simulate the overall degree of spatial segregation or "*compartmentalization"*. In order to simulate spatial relationships, and therefore investigate cell type proportion and spatial organization, we introduced a control multiplicative factor to avoid unrealistic cluster sizes. We used the already defined gene lists also used in the uniformly sampled analysis.

Through the algorithms we designed, we sample each cell sequentially, with the probability distribution changing depending on the preceding cell's type. If the first cell is chosen, the standard probabilities (outlined by Neftel et al.) will be chosen.

There are three different parameters: the base and control factors already explained above and the multiplicative factor. With each sampled cell, if the cell previously selected is the same type as the one currently selected, the probability for that cell type is multiplied by a multiplicative factor. However, if a different cell is chosen, all the probabilities are reset.

By keeping the cluster size constant (100 cells), and varying the multiplicative factor (0.1 and 1), we were able to clearly outline a difference, having the 1 multiplier give the best clustering. The results (Figures 3A and 3B) suggest that a higher degree of spatial segregation increases the ability to distinguish the expression programs, even at a low cellular resolution (100 cells). This suggests that lower resolutions could be tolerated for more spatially organized tumors.

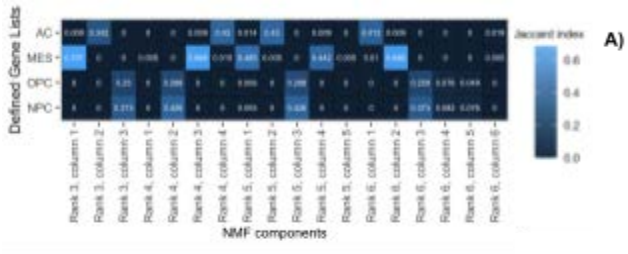


Fig 3A: Intersection of NMF gene signatures with meta-modules for 0.1

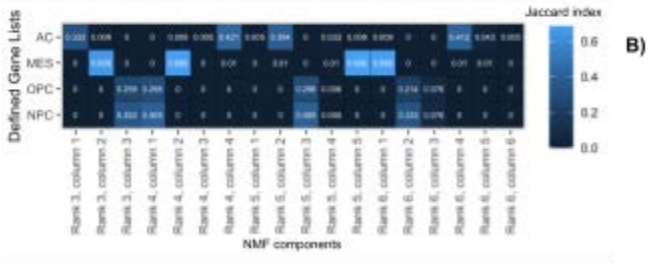


Fig 3B: Intersection of NMF gene signatures with meta-modules for 1

Discussion

Ultimately, through this study, we validated that spatial transcriptomics preserves the cellular resolution of single-cell RNA sequencing, and, thus, that spatial transcriptomics can be used as an alternative method for efficient determination of glioblastoma heterogeneity. Not only does this finding enable a more insightful approach to RNA sequencing as spatial transcriptomics provides insight into the architecture of tumors, but it also demonstrates that these additional insights can be achieved while maintaining the cost-effectiveness of the methodology itself. In the future, spatial transcriptomics may become the standard technique for studying different cancer types, more specifically in our case glioblastomas, over single-cell RNA sequencing given these benefits. It is also worth mentioning that spatial relationships could be relevant for decision-making in the clinic. For instance, a clinician can assess under a microscope how spatially segregated a tumor is, and decide accordingly what resolution is needed for RNA-sequencing. Our results also suggest even lower resolutions (100 or 1000

cells) to be sufficient to characterize tumors. Hence, we could imagine more cost-effective sequencing methods such as overloading droplets as in scifi-RNA-seq or a simple dissection approach.

Due to limitations in experimental parameters, it would be essential to also look at non-malignant cells in addition to malignant cells during the clustering process to most accurately recapitulate the native tumor environment. Additionally, the computational findings should be compared to and repeated with experimental data obtained from spatial transcriptomic analyses to verify that the *in situ* results corroborate with what is achieved experimentally. Moreover, our findings could be used to optimize a computational sampling method in order for spatial relationships to be inferred using only single-cell or bulk RNA-seq data, without *in situ* methods. This program could be employed on large scales to gain further insight into pre-existing single-cell RNA sequencing data. In fact, by applying this method to study other cancer types, we can better understand its applicability to different scenarios of varying heterogeneity. Finally, other cancer types should be investigated to assess the generality of the results.

Understanding the complex tumor microenvironments that result in large numbers of mortalities each year necessitates the availability of inexpensive, practical, and easy-to-analyze laboratory techniques. By demonstrating the high precision of spatial transcriptomics, we pave the way for such methodologies to be harnessed and applied toward ensuring safer prospects for cancer patients.

Acknowledgments

We would like to thank our mentor, Dr. Michael Tyler, for inspiring us, providing continuous feedback, support, encouragement, and constant motivation to dig deeper into our research. A special thanks to the Tirosh Lab and the Department of Molecular Cell Biology at the Weizmann Institute of Science for virtually having us and providing the opportunity of investigating such interesting topics. Lastly, we would like to thank the coordinators of the ISSI, Dr. Dorit Granot, Dr. Aya Shkedy, and Ms. Nirit Alon, for organizing all the different activities and giving us the possibility of meeting such talented and brilliant people.

References

1. Liang, Kung-Hao. “Transcriptomics.” *Bioinformatics for Biomedical Science and Clinical Applications*, Woodhead Publishing, 27 Mar. 2014, https://www.sciencedirect.com/science/article/pii/B9781907568442500036.
2. Milward, E.A., et al. “Transcriptomics.” *Encyclopedia of Cell Biology*, Academic Press, 20 Aug. 2015, https://www.sciencedirect.com/science/article/pii/B9780123944474400295.
3. Crick, Francis. “Central Dogma of Molecular Biology.” *Nature News*, Nature Publishing Group, 8 Aug. 1970, https://www.nature.com/articles/227561a0.
4. Jiang, Zhihua, et al. “Whole Transcriptome Analysis with Sequencing: Methods, Challenges and Potential Solutions.” *Cellular and Molecular Life Sciences: CMLS*, U.S. National Library of Medicine, Sept. 2015, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6233721/.
5. Zhang, Yijie, et al. “Single Cell RNA Sequencing in Cancer Research - Journal of Experimental & Clinical Cancer Research.” *BioMed Central*, BioMed Central, 1 Mar. 2021, https://jeccr.biomedcentral.com/articles/10.1186/s13046-021-01874-1.
6. Hong, Mingye, et al. “RNA Sequencing: New Technologies and Applications in Cancer Research - Journal of Hematology & Oncology.” *BioMed Central*, BioMed Central, 4 Dec. 2020, https://jhoonline.biomedcentral.com/articles/10.1186/s13045-020-01005-x.
7. Burgess, Darren J. “Spatial Transcriptomics Coming of Age.” *Nature News*, Nature Publishing Group, 12 Apr. 2019, https://www.nature.com/articles/s41576-019-0129-z.
8. Yoosuf, Niyaz, et al. “Identification and Transfer of Spatial Transcriptomics Signatures for Cancer Diagnosis - Breast Cancer Research.” *SpringerLink*, BioMed Central, 13 Jan. 2020, https://link.springer.com/article/10.1186/s13058-019-1242-9.
9. Davis, Mary Elizabeth. “Glioblastoma: Overview of Disease and Treatment.” *Clinical journal of oncology nursing* vol. 20,5 Suppl (2016): S2-8. doi:10.1188/16.CJON.S1.2-8.
10. Neftel, Cyril, et al. “An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma.” *Cell*, ScienceDirect, 8 Aug. 2019, https://www.sciencedirect.com/science/article/pii/S0092867419306877#mmc2.

Enabling Practical Alternatives for Tumor Profiling: Spatial Transcriptomics Preserves the Cellular Resolution of Single-Cell RNA Sequencing,

Luxembourg (together with US, UK, Mexico and Germany)

<table>
<tr>
<td>Quote: “Understanding the complex tumor microenvironments that result in large numbers of mortalities each year necessitates the availability of inexpensive, practical, and easy-to-analyze laboratory techniques. By demonstrating the high precision of spatial transcriptomics, we pave the way for such methodologies to be harnessed and applied toward ensuring safer prospects for cancer patients.”

Origin of the quote: Concluding statement of our group project report, highlighting the importance of spatial transcriptomics and the role it could be playing in the future.</td>
<td>Photo of the Project
Please do not copy the picture here- send it separately, in .jpg format</td>
</tr>
<tr>
<td>Photo of projector
Please do not copy the picture here- send it separately, in .jpg format</td>
<td>Vision:
To investigate and evaluate the role of spatial transcriptomics, where all cells and their spatial organization are retained but single-cell resolution is lost.</td>
</tr>
<tr>
<td>Bio of projector:
Federica graduated from the International School of Luxembourg in May 2021. She is now studying Computer Science at the University of Luxembourg. In March 2022, she</td>
<td>Activity:

RNA sequencing (RNA-seq) is an extremely useful tool to study tumors and has been widely used for cellular state characterization. While in the past RNA-seq profiling could only be done by sequencing the RNA from the whole tumor, ‘in bulk’, it is</td>
</tr>
</table>

participated in the National Jonk Fuerscher Contest (FSJL) and was awarded with the participation in the ISSI, thanks to her project on ALS and its possible causations.
Federica is strongly fascinated by health informatics, bioinformatics and similar fields. She is intrigued by the role they have been and will be playing in the future. The use of AI in recognizing neurons which are about to die or are already dead 100 times faster than humans can, studying tumors using spatial transcriptomics, are just some examples highlighting what can be achieved through the combination of different subjects. Healthcare is an enormously complex part of the global economy, however she believes the world's sustainability will strongly depend on the importance and priority we will give to new scientific breakthroughs.

now possible to profile tumors at single cell resolution with single-cell RNA-seq (scRNA-seq) by isolating and sequencing tumor cells separately. Nevertheless, this process ignores the tumor cell's spatial organization and morphology.

In our research, we investigated and evaluated the role of spatial transcriptomics, where all cells and their spatial organization are retained but single-cell resolution is lost. To do this we investigated the ability to distinguish intra-tumor heterogeneity at lower resolutions by using scRNA-seq data to simulate bulk expression profiles with different sizes and spatial relationships. Our findings showed that while increasing the number of cells analyzed together does result in less distinct cell classes, spatial transcriptomics still allows for intra-tumor heterogeneity characterization.

Project website address: https://youtu.be/loo6ZVF1Hsc