SLACS (Spatially resolved Laser- Activated Cell Sorter) – Transforming RNA research with spatial precision

Abstract

This whitepaper focused on how Spatially resolved Laser-Activated Cell Sorting (SLACS) technology can enhance the research in spatial transcriptomics. It would emphasize the integration of spatial transcriptomics and epitranscriptomics, enabling precise analysis of gene expression within specific tissue microenvironments. The focus would be on the application of Select-seq for in-depth study of tumor microenvironments and the identification of novel biomarkers through high-resolution, full-length RNA sequencing. This abstract would underline SLACS's role in advancing our understanding of cellular mechanisms in health and disease, offering a novel perspective on the spatial and molecular complexity of biological samples.

Highlights

- SLACS is a new method that allows researchers to study the full-length spatial transcriptome and epi-transcriptome at the single-nucleotide level.
- SLACS can be used to isolate specific regions of interest from tissue samples that have been stained with immunofluorescence.
- The researchers used Select-seq to study the tumor microenvironment in triple-negative breast cancer patients and identified gene expression in different groups of microniches based on the expression of CSC markers CD44 and ALDH1.
- They also found that the A-to-I editing landscape was different in these different groups of microniches to find out novel biomarker, single nucleotide base-edited GPX4.

Introduction

In the rapidly evolving field of biotechnology, precision and specificity in cellular isolation have become paramount. Among the emerging technologies, Spatially Resolved Laser-Activated Cell Sorting (SLACS) stands out as a pivotal advancement, uniquely bridging the spatial landscape with molecular assays. Unlike other spatial omics technologies, SLACS offers the distinct advantage of directly sorting cells, providing greater flexibility in assay applications. This capability marks a significant departure from traditional methods like Fluorescence-Activated Cell Sorting (FACS), which, while effective in sorting cells based on fluorescence markers, lacks the ability to maintain and analyze the spatial context of the cells within tissues.

SLACS technology transcends these limitations, offering a novel approach that integrates the spatial arrangement of cells with high-precision molecular analysis. This integration is especially transformative in the realm of RNA research, where understanding the spatial arrangement of gene expression is crucial. Through the course of this whitepaper, we will explore concrete examples of SLACS applied in RNA research, demonstrating its superiority in maintaining spatial integrity while providing detailed molecular insights.

At its core, SLACS employs a laser-activated mechanism to precisely sort cells. This approach enables the isolation of cells based on specific spatial parameters within a tissue, a capability not achievable with traditional cell sorting methods. A key advantage of SLACS technology, particularly in the context of RNA research, is its applicability to full-length RNA sequencing. As it can sort out individual cells from the tissue, SLACS enables RNA analysis from precisely targeted areas within tissues, maintaining the spatial relationships that are critical for interpreting gene expression patterns.

The technological innovation of SLACS is not just in its precision sorting but also in its application. SLACS can isolate cells from specific regions of interest within a tissue, such as different microenvironments or niches within a tumor. This level of precision allows for a more nuanced understanding of the tumor microenvironment and the role of different cell types in disease progression. Moreover, SLACS technology integrates seamlessly with other molecular biology techniques. For example, when combined with next-generation sequencing, SLACS provides a full-length RNA sequencing offering comprehensive view of RNA expression and interactions within a tissue. This integration enables a multi-dimensional analysis of RNA, combining spatial data with molecular information. Finally, case studies utilizing SLACS in RNA research underscore its effectiveness. For instance, SLACS has been instrumental in identifying unique RNA signatures in different regions of a tumor, providing insights into tumor heterogeneity and the microenvironment. These studies demonstrate the unique insights provided by SLACS, which are essential for advancing our understanding of complex biological processes and diseases.

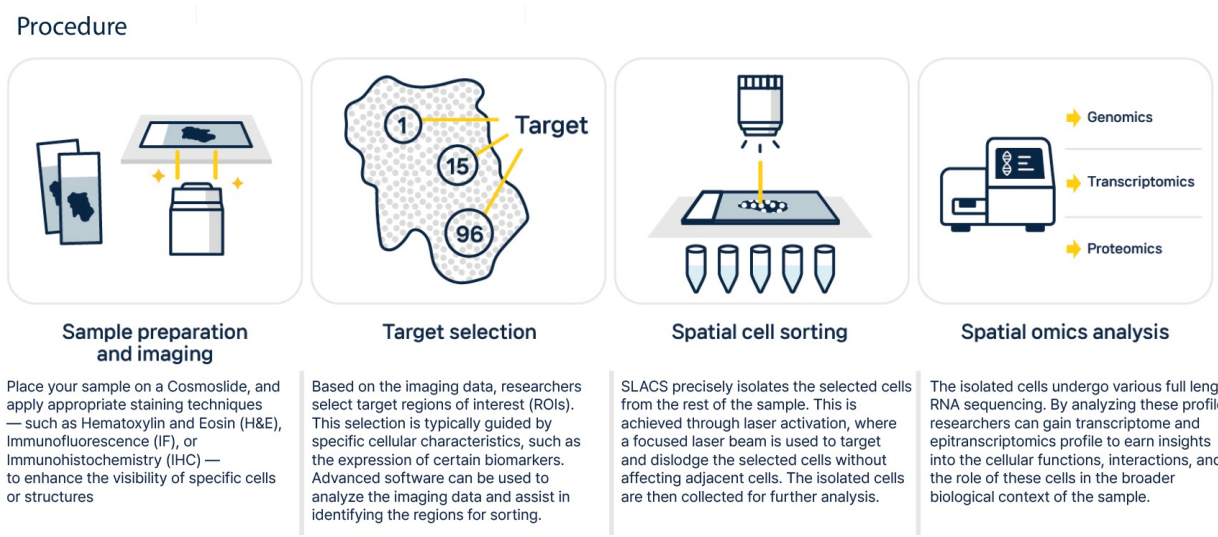

Figure 1. Procedure of using SLACS for RNA sequencing.

Advantages of SLACS for RNA research

SLACS technology transcends these limitations, offering a novel approach that integrates the spatial arrangement of cells with high-precision molecular analysis. This integration is especially transformative in the realm of RNA research, where understanding the spatial arrangement of gene expression is crucial. Through the course of this whitepaper, we will explore concrete examples of SLACS applied in RNA research, demonstrating its superiority in maintaining spatial integrity while providing detailed molecular insights.

- **Unprecedented spatial resolution:** SLACS technology offers unparalleled precision in cell sorting, allowing researchers to isolate individual cells from specific regions of interest within a tissue. This level of spatial resolution is unmatched by traditional methods like FACS, which often rely on bulk sorting and lose the crucial spatial relationships between cells. As a result, SLACS paves the way for a deeper understanding of the spatial organization of gene expression and its role in various biological processes.
- **Full-length RNA sequencing:** SLACS opens the door to analyze the full-length RNA transcripts of isolated cells, revealing valuable information beyond just the coding sequence. This comprehensive analysis enables researchers to identify and characterize novel splice isoforms, A-to-I editing events and other post-transcriptional modifications, providing a more complete picture of gene expression and its regulation.
- **Enhanced data interpretation:** By combining spatial data with molecular information, SLACS enables researchers to interpret RNA expression data in a more meaningful and contextual way. This integrated approach facilitates the identification of key genes and pathways that are specifically active in certain regions or cell types within a tissue, providing deeper insights into the underlying biological processes.

Overall, SLACS offers a powerful and transformative approach to RNA research, providing unprecedented spatial resolution, comprehensive analysis of full-length RNA transcripts, and insights into the spatial distribution of epitranscriptomic modifications. As technology continues to evolve, SLACS is poised to revolutionize our understanding of gene expression and its role in health, disease, and development.

Validating the Power of SLACS: From Cell Lines to Tissues : 1) Cell Lines

The transformative potential of SLACS extends beyond individual research studies. Its effectiveness has been validated across diverse applications, from meticulously analyzing gene expression in cell lines to dissecting the intricate spatial organization of genes within complex tissue samples.

The accuracy and reliability of SLACS are evident in its application to cell line data. Studies have demonstrated that SLACS can accurately isolate and analyze individual cells from various cell lines, providing precise gene counts and expression profiles. This level of precision allows researchers to detect subtle differences in gene expression between different cell types or subpopulations, paving the way for a deeper understanding of cellular function and differentiation.

To ensure the reliability and accuracy of RNA sequencing after isolation, we performed a series of validation experiments using three different human cell lines (n = 152). The results are presented in Figure 2. Bulk RNA-seq data exhibited a stronger correlation with laser-isolated, PFA-fixed cells (R = 0.83) compared to methanol-fixed cells (R = 0.74). This suggests that PFA fixation yields RNA profiles that are more closely aligned with bulk RNA-seq data, potentially due to its superior preservation of RNA integrity. Comparing the FPKM values of unfixed and unstained cells with those of PFA-fixed and stained cells revealed minimal variations in gene expression patterns. This indicates that the fixation and staining steps have minimal impact on the accuracy of the full-length transcriptome data generated by SLACS.

Taken together, these validation experiments demonstrate that Select-seq offers a reliable and accurate method for single-cell RNA-seq analysis. The high correlation with bulk RNA-seq data, minimal impact of fixation and staining, and successful analysis of full-length transcripts confirm the effectiveness of this technology for capturing the true expression profiles of individual cells.

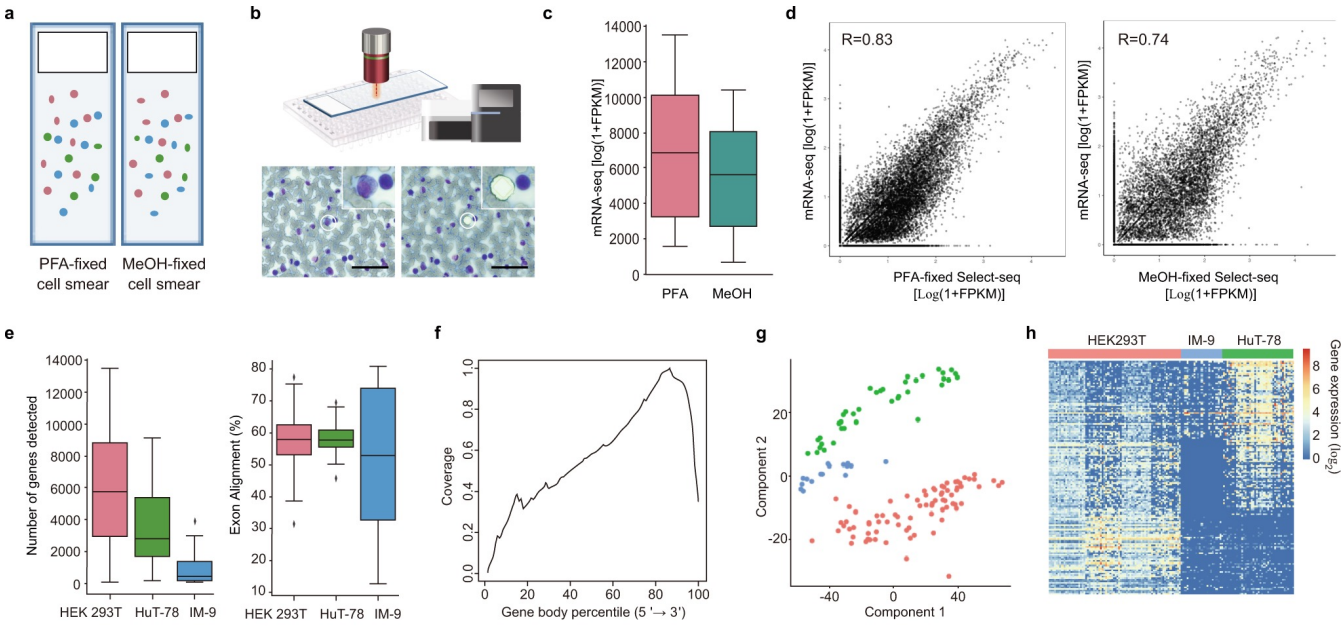

Figure 2. Validation in cell lines. a Experimental design and an example of single-cell isolation. b Single-cell isolation of the SLACS device. Scale bar, 100 µm. c Fragments per kilobase of transcript per million mapped reads (FPKM) values for the paraformaldehyde (PFA)- and methanol (MeOH)-fixed cells (n = 60 biologically independent cells examined over 2 independent experiments). Interquartile range (IQR) of boxplot is between Q1 and Q3 and centre line indicates median value. Whiskers of boxplot is extended to the maxima and minima. Maxima is Q3 + 1.5*IQR and minima is Q1 – 1.5*IQR. d Correlation between the mRNA sequencing profiles from bulk mRNA-seq and Select-seq with ten types of PFA-fixed cells and Select-seq with ten types of MeOH-fixed cells. e Number of genes detected (FPKM) (left) and exon alignment percentage in three different cell lines fixed with PFA (right) (n = 92 biologically independent cells examined over 3 independent experiments). Interquartile range (IQR) of boxplot is between Q1 and Q3 and centre line indicates median value. Whiskers of boxplot is extended to the maxima and minima. Maxima is Q3 + 1.5*IQR and minima is Q1 – 1.5*IQR. f Representative 3′ end bias of the full-length transcriptomes. g Principal component analysis (PCA) and h unsupervised clustering heatmap of the cells analyzed with Select-seq. i Representative transcript isoform diversity from two samples.

Validating the Power of SLACS: From Cell Lines to Tissues : 2) Tissue samples

The validation of SLACS extends to complex tissue samples, ensuring its applicability in a wider range of biological contexts. Just as it has proven effective in cell line experiments, SLACS demonstrates a high degree of accuracy and reliability when applied to tissues. By isolating and analyzing regions of interest within tissues, SLACS provides detailed insights into the spatial organization and gene expression profiles, critical for understanding tissue-specific biology and disease pathology. This rigorous validation in tissues further confirms SLACS as a versatile and dependable tool for advanced biological research.

We applied Select-seq to the immunofluorescence(IF)-stained tissue to obtain full-length transcriptome data at single-base resolution. We investigated triple-negative breast cancer focusing on microniches that harbor cancer stem cell-like characteristics to see the tumorigenesis markers. targeted regions stained for CD44 and ALDH1, markers associated with stemness, to understand the intricate molecular environment. This approach allowed for a detailed exploration of how these microniches contribute to tumor behavior and heterogeneity.

We were able to verify the expression of cancer stem cell-like. Immunosuppressive gene expressions in actual stem cell regions, and additionally, we could observe the RNA diversity including alternative splicing variants, A-to-I editing events, of the transcriptomics expressed in those locations as well as BCR/ TCR sequences. The comprehensive RNA diversity profile revealed by SLACS in these microniches provides a deeper understanding of the molecular repertoire employed by cancer stem cells.

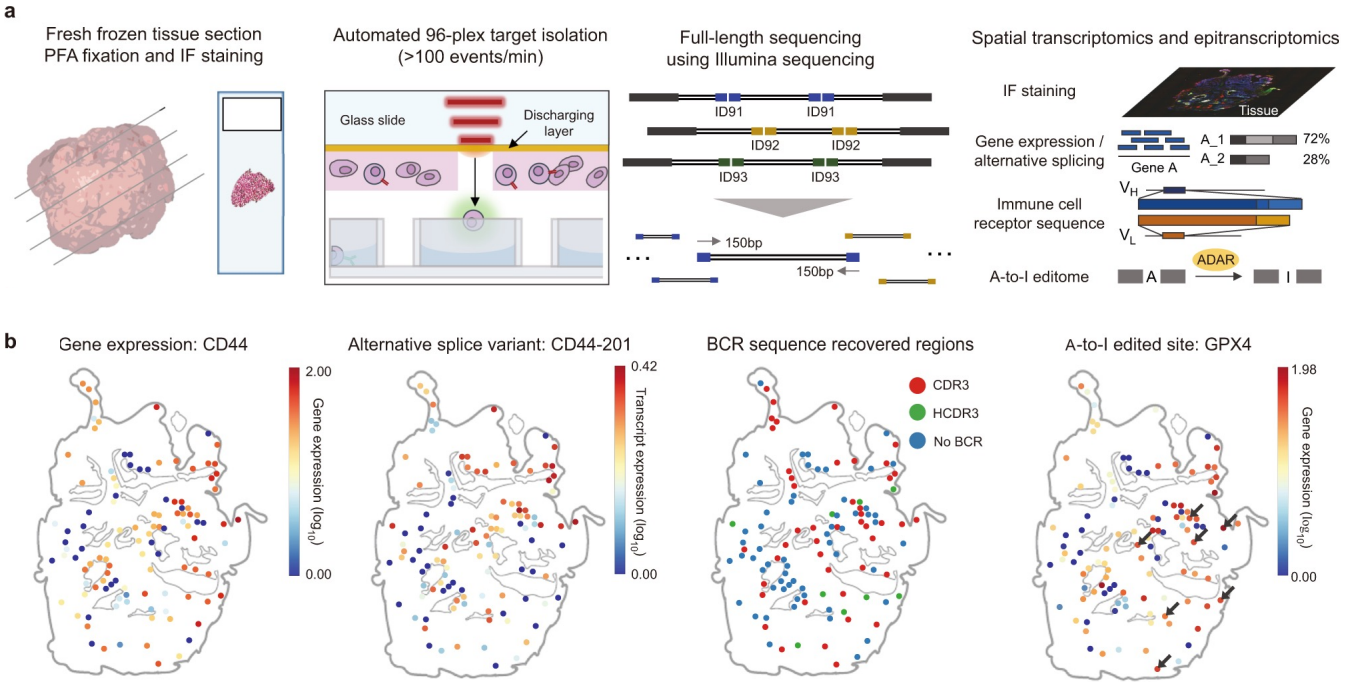

Figure 3. Validation in tissue samples. a Schematic of the Select-seq protocol. Selective isolation of target regions in tumour sections was performed using a near-infrared pulsed laser following immunofluorescence staining. Full-length transcripts extracted from each targeted region are tagged with barcodes used for tracking the target region. Multi-modal analysis of the full-length transcriptome is connected with the spatial and staining information using barcodes included in the sequencing data. b Each transcriptomic and epitranscriptomic data point was mapped to the tissue based on the barcodes.

Application : Therapeutic target discovery

By isolating and analyzing regions of interest, the team observed significant A-to-I RNA editing events, particularly in GPX4 transcripts, which are linked to ferroptosis, a form of programmed cell death. This discovery provides valuable insights into the post-transcriptional modifications occurring within cancer stem cell-like microniches and their potential role in TNBC progression.

The findings from this study not only deepen our understanding of the complex molecular landscape of TNBC but also highlight the potential for novel therapeutic strategies. By pinpointing specific RNA editing events within cancer stem cell microniches, researchers can target these areas for future treatments, aiming to disrupt the tumor's microenvironment and halt its progression. The study's methodical approach and detailed analysis pave the way for further research into the epitranscriptomic mechanisms of cancer and their implications for patient care.

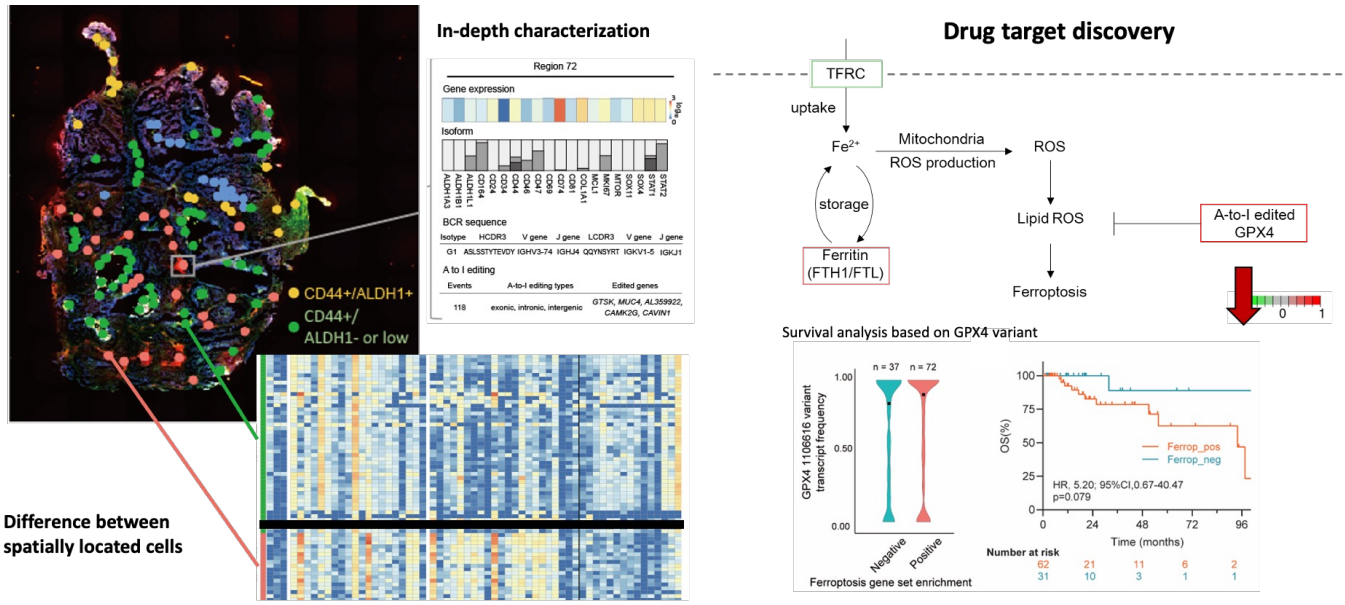

Figure 4. Validation in tissue samples. a Schematic of the Select-seq protocol. Selective isolation of target regions in tumour sections was performed using a near-infrared pulsed laser following immunofluorescence staining. Full-length transcripts extracted from each targeted region are tagged with barcodes used for tracking the target region. Multi-modal analysis of the full-length transcriptome is connected with the spatial and staining information using barcodes included in the sequencing data. b Targets selected from a triple-negative breast cancer (TNBC) patient tumour section. The tissue was stained with Hoechst dye and anti-ALDH1 and CD44 antibodies (scale bar, 100 μm). We obtained the above results from a single tissue section. c Transcriptomics and epitranscriptomics of target region 72 containing 5–30 cells at single-nucleotide resolution and its gene expression profiles, transcript isoforms, B cell receptor sequences, and adenosine-to-inosine (A-to-I) editing events.

Conclusion

Automated cell sorting using SLACS (Spatially-resolved Laser Activated Cell Sorting) enables high-throughput spatial analysis of neurodegenerative disease pathology. Fluorescently-labeled cells can be precisely targeted and isolated based on their spatial phenotypes using automated cell sorting.

In the 5xfad mouse model of neurodegenerative disease, we identified biomarkers by comparing the gene expression and isoform information of microglia near and far from amyloid beta plaques using automated cell sorting for spatial analysis. The resulting data can provide valuable insights into the spatial distribution and heterogeneity of pathological features in neurodegenerative diseases, which may help inform the development of new therapeutic approaches.

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- **Gene expression levels:** Quantify the precise expression levels of individual genes, revealing subtle differences in activity across different tissue areas.
- **Alternative splice variants**: Identify and characterize alternative splicing events, providing a deeper understanding of protein diversity and function within specific compartments.
- **BCR /TCR sequences:** Analyze B-cell receptor sequences within immune cells present in the targeted tissue, offering insights into the adaptive immune response in specific locations.
- **A-to-I editing events:** Map the landscape of A-to-I RNA editing, a form of post-transcriptional modification that can alter the coding potential and function of RNA, providing crucial clues into regulation and cellular behavior within the targeted tissue region.

References

1. Lee, A.C., Lee, Y., Choi, A. et al. Spatial epitranscriptomics reveals A-to-I editome specific to cancer stem cell microniches. *Nat Commun* **13**, 2540 (2022). https://doi.org/10.1038/s41467-022-30299-3