Spatial Single Cell characterization of SIV reservoirs in lymphoid tissues and B cell follicles in rhesus macaques

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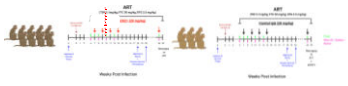
Summary

HIV cannot be eradicated by antiretroviral therapy (ART) alone. Although lifelong suppression of HIV replication with ART seems possible, side effects, need for strict adherence, resistance and cost contribute to the necessity of finding an 'HIV Cure'. The major obstacle to eradicating HIV is the persistence of viral reservoirs despite extended ART, which give rise to recrudescent infection when ART is stopped. An understanding of complex spatial host-viral interactions *in situ* that retains important contextual insight into the cellular immune neighborhoods and inflammatory landscapes, in which viral reservoirs reside, is needed to define mechanisms driving HIV persistence, which could be leveraged to develop effective cure strategies.

GeoMx profiling of 4 SIV+ lymph nodes demonstrated that Regions of Interest (ROIs) with active viral infections had significantly altered transcriptional programs when compared to uninfected ROIs. As a result, a number of different pathways in the infected cell neighborhoods were altered including Il-4, Il-17, and HLA signaling as well as interferon/inflammation and B Cell Development. The GeoMx spatial analysis allowed the discovery of >200 genes with differential expression that would have never been possible with pan-tissue or cell RNA sequencing approaches. SMI profiling of serial sections from the same samples augmented the GeoMx observations and provided further refinement into the transcriptional profile but at single cell resolution.

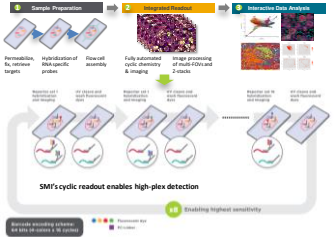
Sample and study design



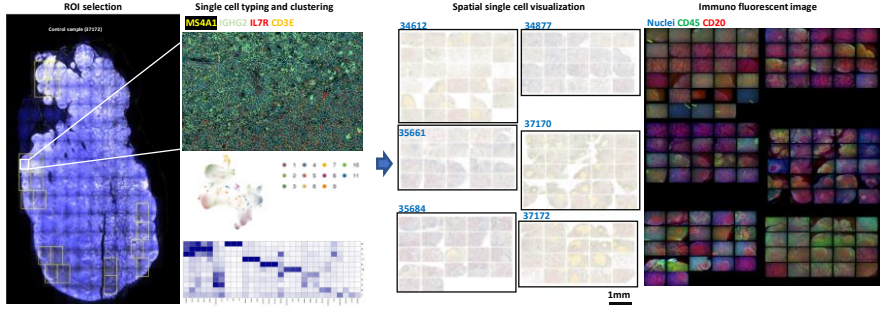
Rhesus Macaques were selected and split into two study groups (as shown above), the day 56 (week 8) post SIV infection lymph nodes were used to make FFPE blocks. Subsequently, matching 5 µm sections of the FFPE tissues were utilized for spatial transcriptomic analysis on two nanoString Technologies platforms; the GeoMx DSP and the CosMx SMI.
Samples include 6 SIV infected lymph nodes where three have been treated with αCD21 (red arrows) and three with IgG (black arrows) control antibodies. The SMI panel consisted of the RNA 1K-plex panel with a custom spike-in probe set for 9 SIV genes. The tissues were stained with a morphology kit including B2M/CD298, PanCK, CD45, and CD20 antibodies to help select applicable Field of Views (FOVs) for analysis.

SMI assay overview

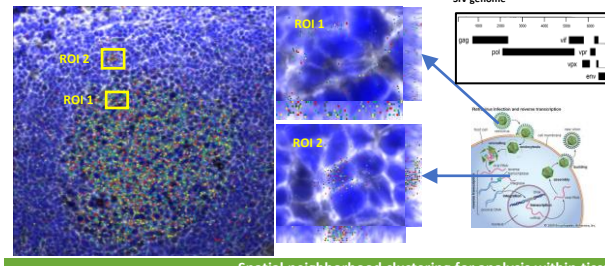
SMI is a single instrument solution for subcellular spatial analysis.



SMI visualizes spatially resolved 1K genes for 6 SIV+ NHP lymphoid tissues



SMI visualizes SIV genes in 3D



To spatially visualize SIV genes, we designed 6 custom probes targeting various locations of SIV genome (9 genes). 3D visualization of transcripts indicates that a subset of single cells have high viral gene content at the intracellular location (vRNA+), while the others are located outside of single cells (virions). ROI 1 represents gene expression from bound SIV virions retained on the follicular dendritic cell network, whereas ROI represents a productively infected T cell.

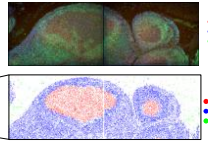
Spatial neighborhood clustering for analysis within tissue niches

	Cell#1	Cell#2	Cell#3	Cell#4	Cell#5
cell_type-1	5	60	35	5	5
cell_type-2	30	0	5	5	25
cell_type-3	20	10	5	45	10
cell_type-4	0	5	5	70	0
...	...	...	...	...	...

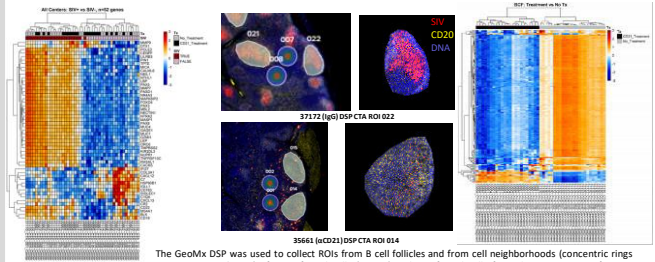
A neighborhood matrix was calculated for each cell by identifying the cell type all cells within a 100 µm radius of it. Clustering was performed on this matrix to identify a 3 distinct spatial neighborhoods which can be used to identify cells experiencing similar environments or niches.

Neighborhood/niches maps.

Comparison to the SMI morphology images confirms the accuracy of the neighborhood assignment and enables analysis of larger or smaller order tissue structures, depending on the distance used to calculate the neighborhood matrix.

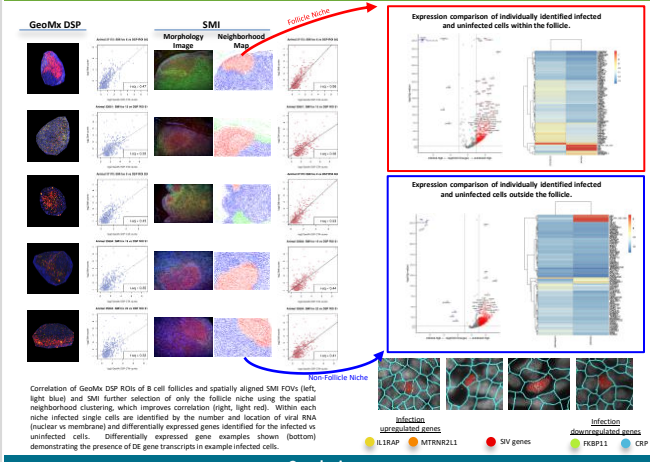


GeoMx DSP profiling of virally infected cells and B-cell follicles



The GeoMx DSP was used to collect ROIs from B cell follicles and from cell neighborhoods (concentric rings 007, 008, 002, & 001) of SIV infected cells (vRNA+) and uninfected cells from the two groups of NHPs. Heatmaps were generated showing the large transcriptional variations discovered between the SIV+ vs SIV- centers (left) and αCD21 vs IgG treated B cell Folicles (right).

GeoMx to SMI: Differential expression at single cell level within unique tissue niches



Correlation of GeoMx DSP ROIs of B cell follicles and spatially aligned SMI FOVs (left, light blue) and SMI further selection of only the follicle niche using the spatial neighborhood clustering, which improves correlation (right, light red). Within each niche infected single cells are identified by the number and location of viral RNA (nuclear vs membrane) and differentially expressed genes identified for the infected vs uninfected cells. Differentially expressed gene examples shown (bottom) demonstrating the presence of DE gene transcripts in example infected cells.

Conclusions

- **The Spatial Molecular Imager (SMI) is a single instrument solution for subcellular spatial analysis:** SMI provides sub-cellular resolution of 1000+-plex transcriptomic information and SIV viral genes.
- **Both GeoMx and SMI spatial platforms provides complementary spatial information:** GeoMx DSP can profile up to whole transcriptome while SMI gives sub-cellular resolutions of biological targets.
- The sub-cellular resolution of SMI provides the capacity to distinguish single-infected cells from virions located in the extracellular space of single cells.