#6619 Virtual staining enabled combined morphological and spatial transcriptomic analysis of individual malignant B cells and local tumor microenvironments

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Abstract

B-cell lymphomas are heterogeneous diseases with respect to gene expression and tumor microenvironment. Certain lymphomas, such as classic Hodgkin lymphoma, nodular lymphocyte predominant Hodgkin lymphoma, and T-cell / histiocyte rich large B-cell lymphoma, contain a minority of malignant B-cells in a largely inflammatory background. These rare malignant cells have unique interactions with the local tumor microenvironment (TME). The interaction between malignant cells and the TME may drive clinical behavior and treatment responsiveness. Recently developed molecular tools allow separate characterization of both the malignant cells and TME using spatial refinement into histologic components, and offer deeper analysis of the underlying biology in several B-cell lymphoma subtypes.
Here we have developed an integrated, single-slide workflow of virtual H&E staining using the inherent tissue autofluorescence (PictorLabs, Los Angeles, CA) of formalin-fixed, paraffin embedded lymphoma tissue sections on the GeoMx® Digital Spatial Profiler (Nanostring, Seattle, WA) combined with spatial expression analysis of 1,800 genes using the Cancer Transcriptome Atlas (CTA) panel. The H&E virtual staining allows for the assessment of B-cell lymphoma subtypes and identification and phenotyping of both malignant B-cells and the associated inflammatory milieu by a board certified pathologist. These regions of interest (ROI) are spatially profiled for transcriptional expression determined by NGS sequencing of the RNA target specific DNA oligo tags.
We benchmarked our system by analyzing classic Hodgkin lymphomas. Using virtual H&E staining on the GeoMx Digital Spatial Profiler platform, a pathologist correctly identified the diagnosis and cell subtype based on the morphology. Regions enriched in Reed-Sternberg cells could be separated from the background inflammatory milieu on virtual H&E staining, allowing subset segregation for digital transcriptional expression profiling with the GeoMx CTA panel. Analysis of the ROIs revealed distinct transcriptional profiles between areas enriched in Reed-Sternberg cells and the associated inflammatory milieu demonstrating the viability and utility of virtual H&E staining technology as a part of spatial genetic workflow.

Conclusions and Key Takeaways

- PictorLabs' digital pathology technology applies deep learning algorithms to stain tissues efficiently and accurately in silico, producing near-instantaneous virtual stains.
- Proof-of-concept data for an integrated, single-slide workflow of virtual H&E staining using the inherent tissue autofluorescence of formalin-fixed, paraffin embedded tissue sections on the GeoMx® Digital Spatial Profiler combined with spatial expression analysis.
- The H&E virtual staining allows for the assessment of B-cell lymphoma subtypes, identification, and phenotyping of malignant B-cells and the immediately proximal (within 30µm) TME regions by a certified pathologist.
- Regions of interest (ROI) are spatially profiled for transcriptional expression determined by NGS sequencing of the RNA target specific DNA oligo tags using the 1,800+ genes Cancer Transcriptome Atlas (CTA) panel.

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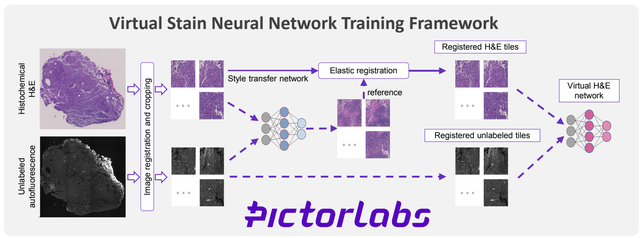
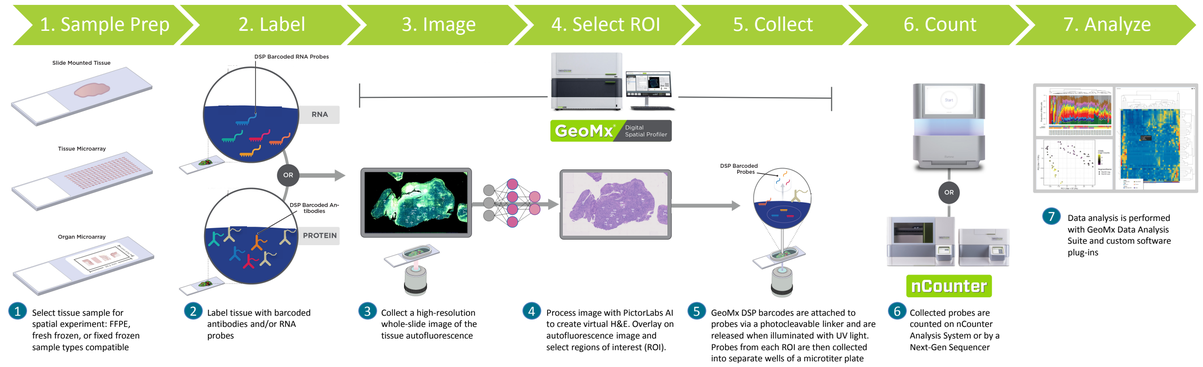
AI Training



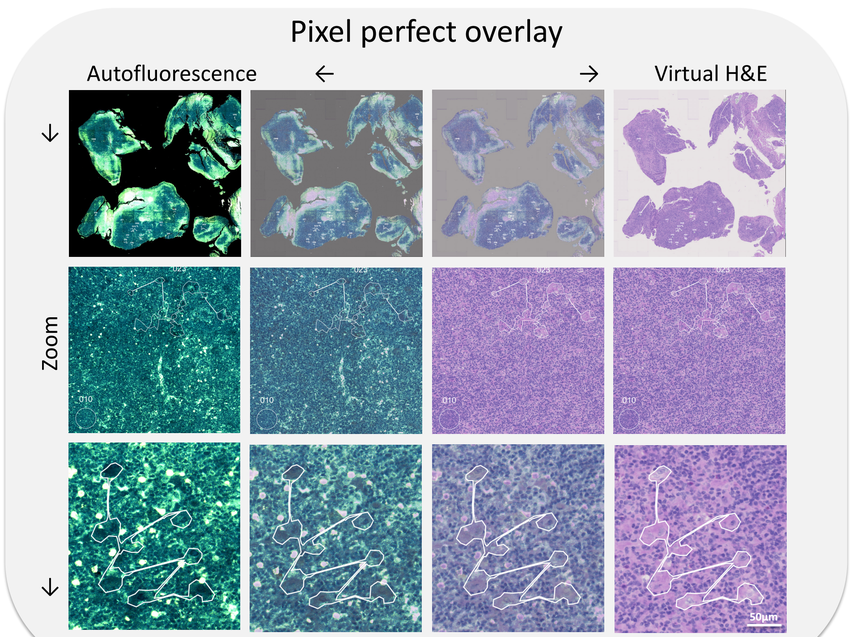
- 12 FFPE tissues: 2 blocks each of THRLBCL and NLP, 3 blocks each of DLBCL and CHL, 2 blocks of normal/reactive lymphoid tissue.
- Whole-slide autofluorescence images were captured using four fluorescent filter cubes (BGYR) on the GeoMx DSP.
- 58 total sections → brightfield imaged at ×20 (Zeiss Axioscan.Z1)
- 17 were prepared with a deparaffinization-only protocol and stained with H&E (training data only).
- 41 sections underwent an additional mock GeoMx CTA assay and stained with H&E (training, testing, and validation datasets).

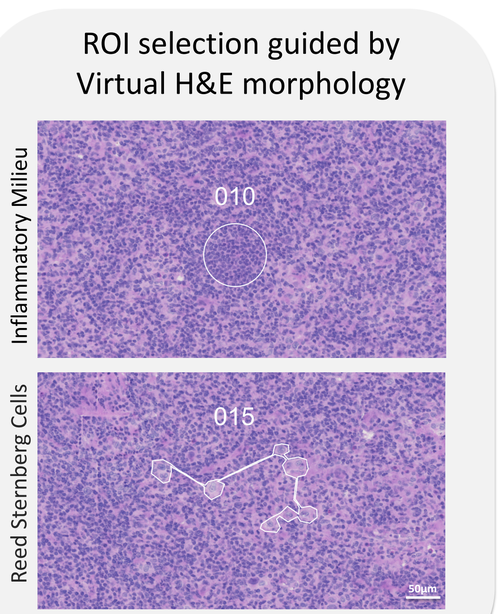
Brightfield images of 40 stained slides were accurately registered to corresponding autofluorescence counterparts . GAN-based deep learning model was utilized to learn the transformation from 4-channel autofluorescence images to corresponding stained H&E. 14 mock CTA treated stained slides validated the model's performance. Training was conducted on 2 NVIDIA RTX 3090 graphics cards and converged after ~60 hours. The best model was chosen based on L1 loss and pathologist assessment of the testing dataset. In all cases malignant cells were successfully identified, and morphologic patterns were preserved, allowing accurate diagnosis. After review of virtual stains, the presence of malignant cells and morphological features was confirmed on the corresponding H&E stained slides.

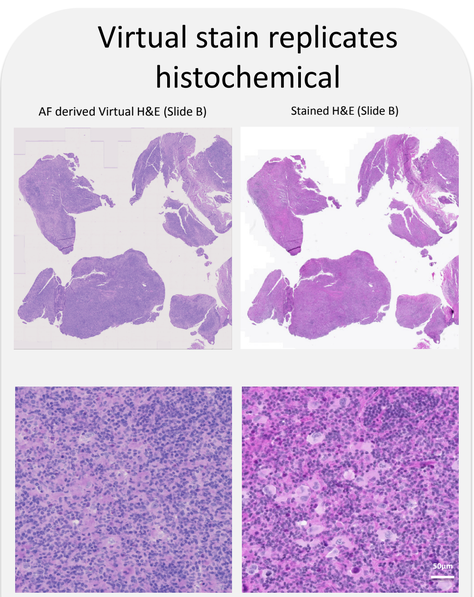
Spatial Profiling Workflow Using Virtual H&E



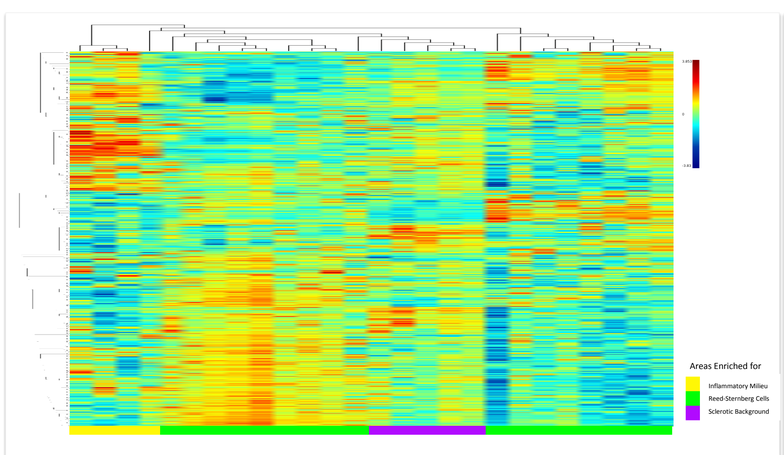
Virtual H&E Validation on Classic Hodgkin Lymphoma

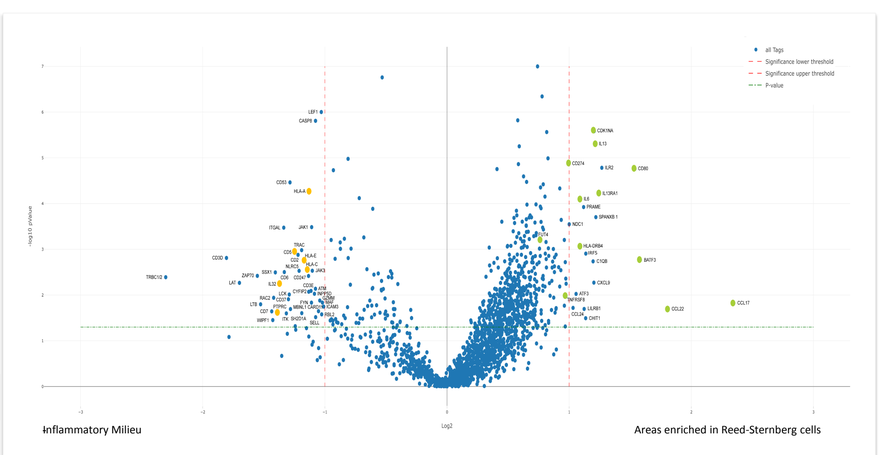






Spatial transcriptomics with GeoMx Cancer Transcriptome Atlas confirms cell type segmentation with H&E guided custom ROI





Unsupervised hierarchical clustering analysis of detected RNA expression profiles in H&E guided cell subsets in Classic Hodgkin Lymphoma. 812 genes detected in > 10% of ROI sampled

Differential expression analysis between inflammatory milieu and Reed-Sternberg cell enriched custom ROIs in Classic Hodgkin Lymphoma. Volcano plot resulting from the differential expression analysis between these areas for RNA (●) analytes.