

Tumor Microenvironment

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Tumor Microenvironment

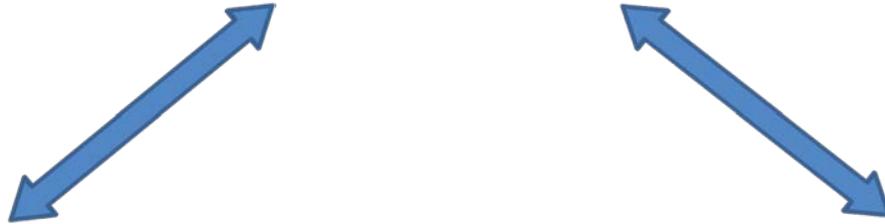
- It's not just about the cancer cells: importance of the tumor microenvironment
- It's not just about numbers: importance of spatial relationships between cells in tissue
- It's not just about the tumor: importance of tumor-draining lymph nodes (TDLNs)

Tumors are more than just cancer cells

- Around 50% of human tumors are >50% stroma
- Patients with 'stroma-rich' tumors have worse clinical outcome, which is an independent prognostic parameter in breast, colorectal, pancreatic, and other cancers
- Stromal cells provide growth and metabolic factors to cancer cells; also shown to protect cancer cells from a variety of anti-cancer drugs
- Intratumoral immune cells strongly shown to predict clinical outcome: Immunoscore

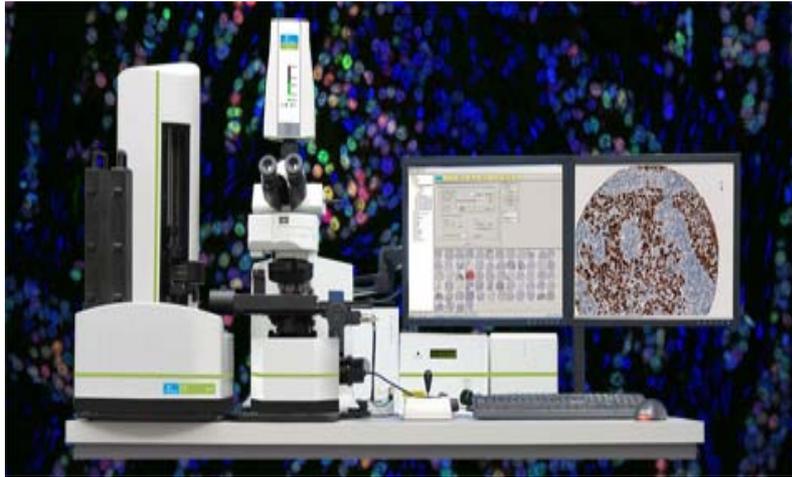
Cancer Progression & Clinical Outcome

Cancer Cells

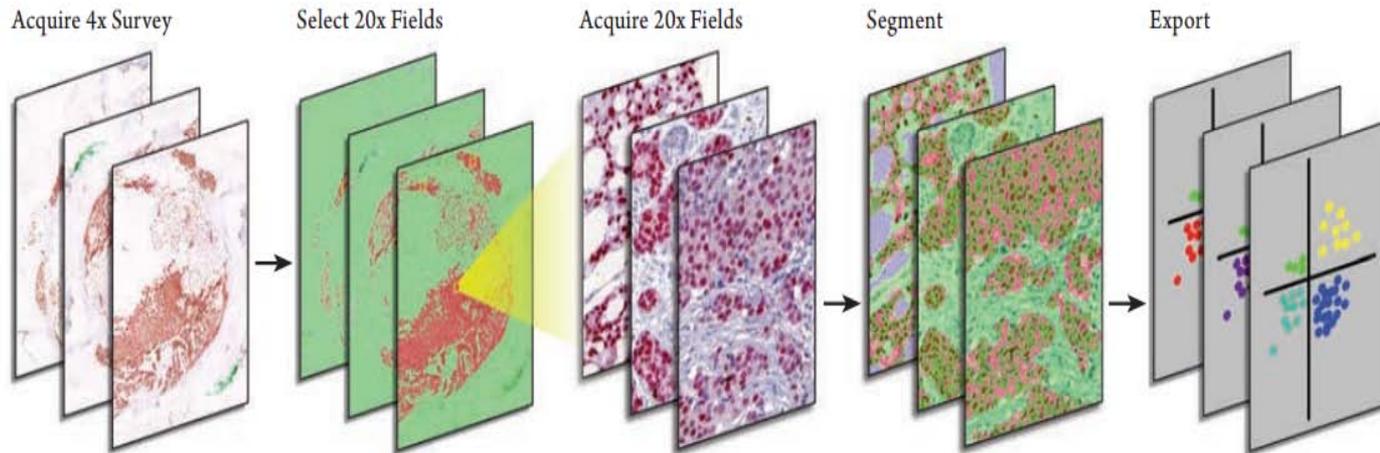


Immune Cells ↔ Stromal Cells

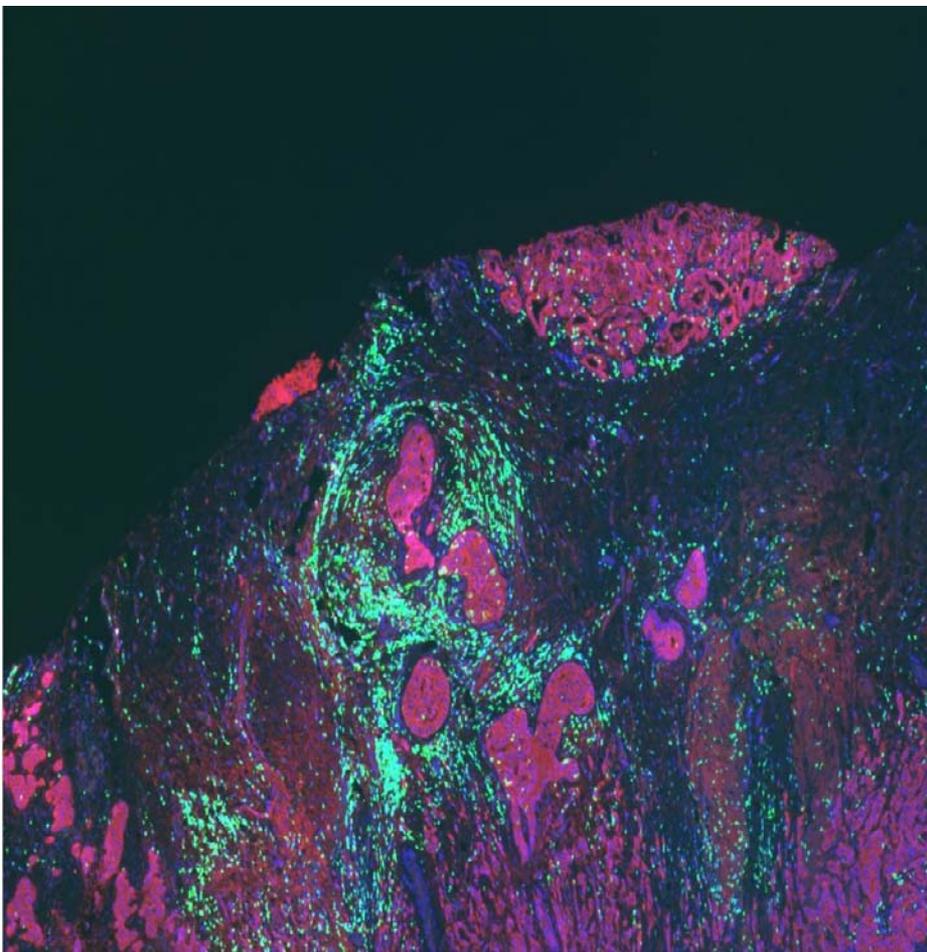
Imaging with Vectra



Multi-modal high throughput pattern-recognition based image analysis technology for biomarker quantification and morphometric analysis.



Different immune patterns within tumors

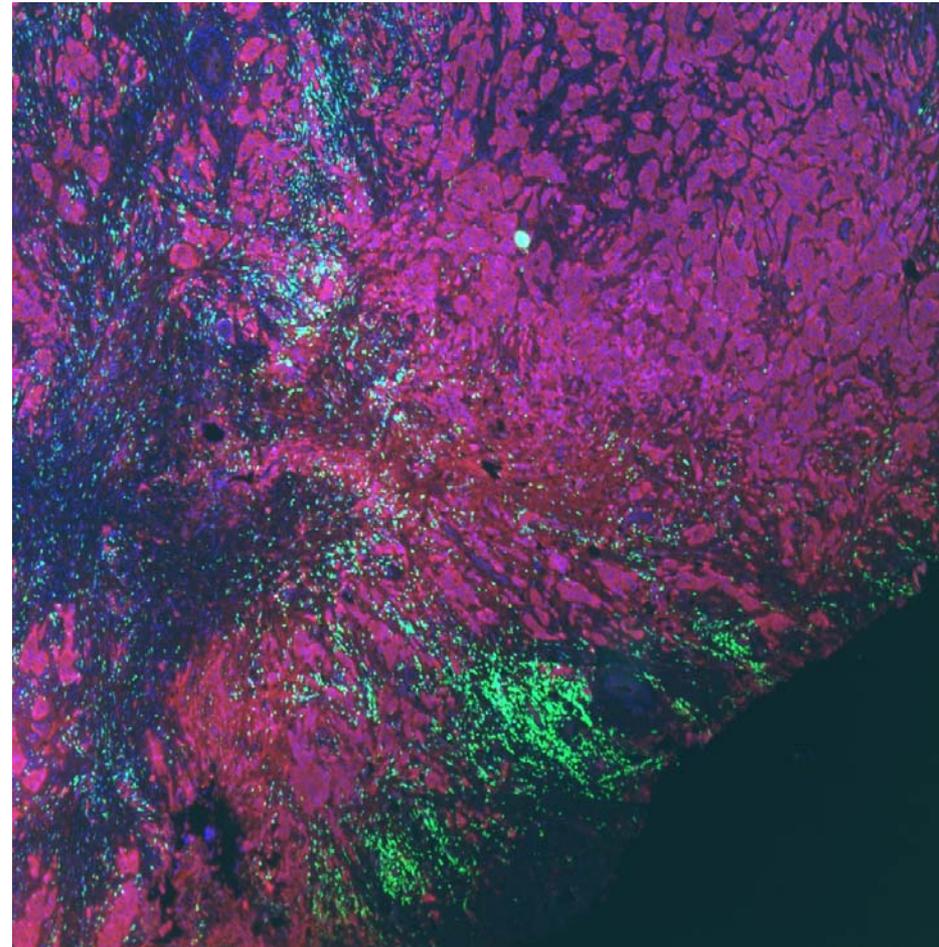


Primary breast tumors
40x images

CD3

PanCK

DAPI



Immune-cancer separation

Primary
Breast
tumor
200x
image

CD3

CD20

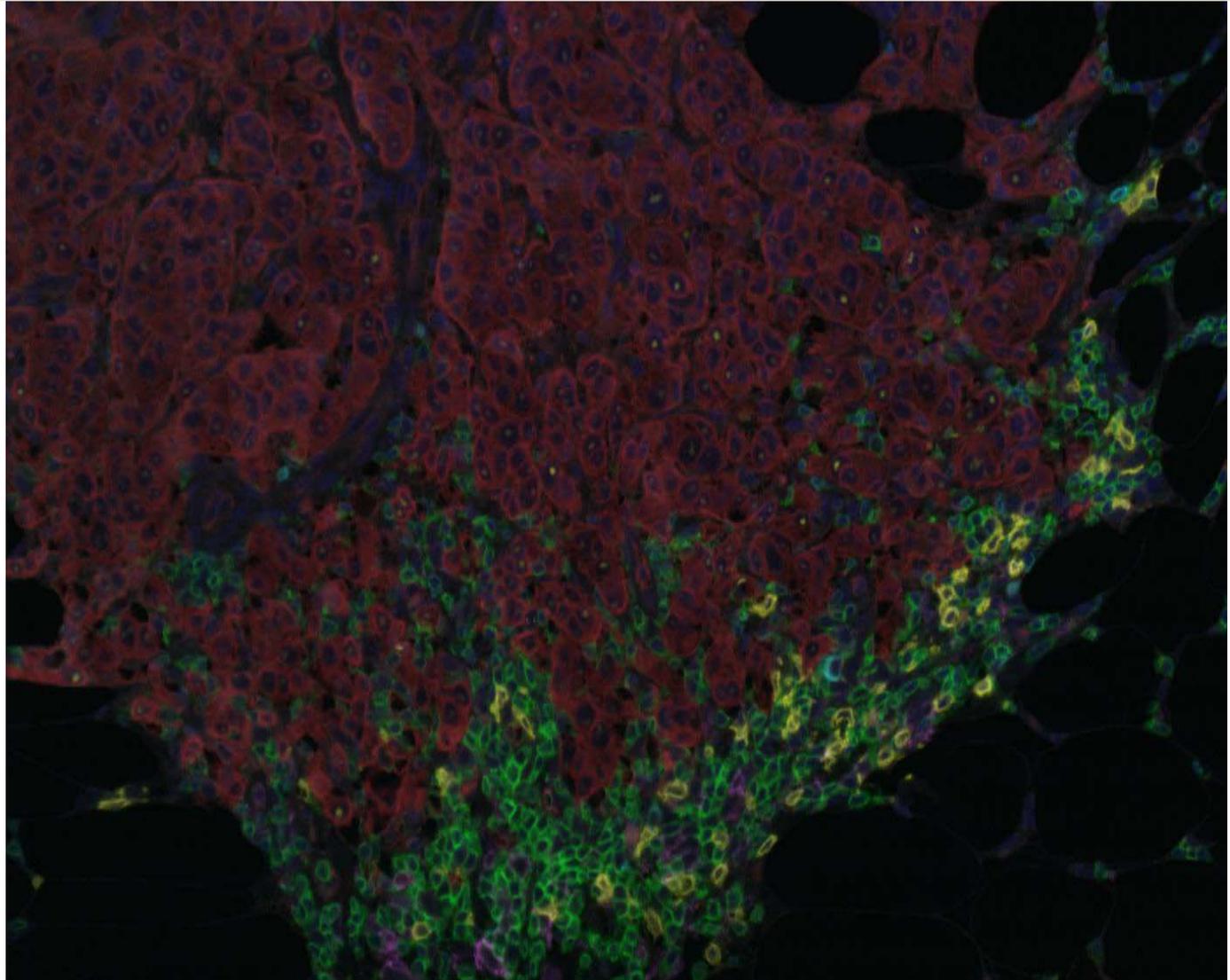
CD123

CD33

CD56

PanCK

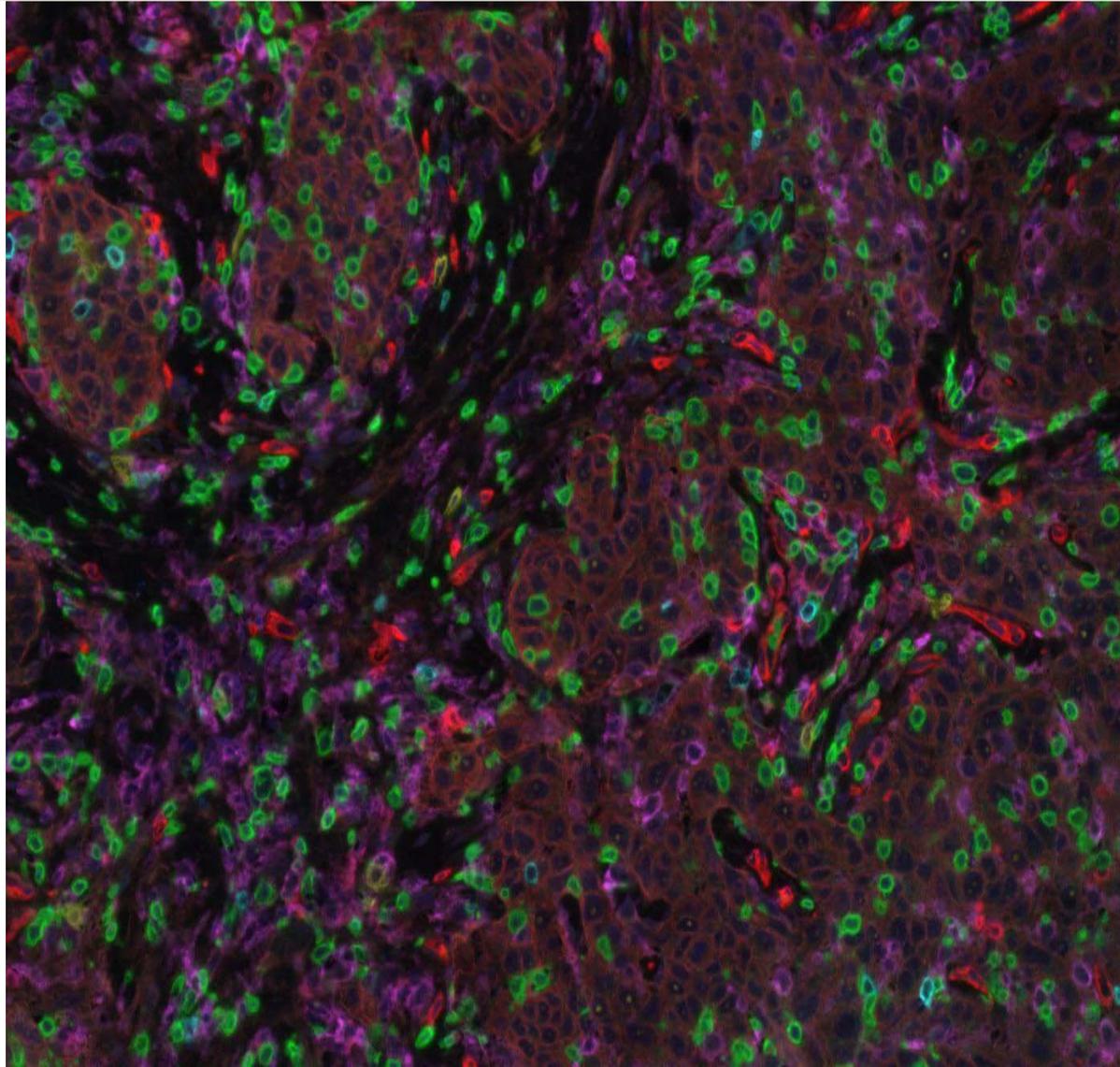
DAPI



Immune-cancer co-mingling

Primary
Breast
tumor
200x
image

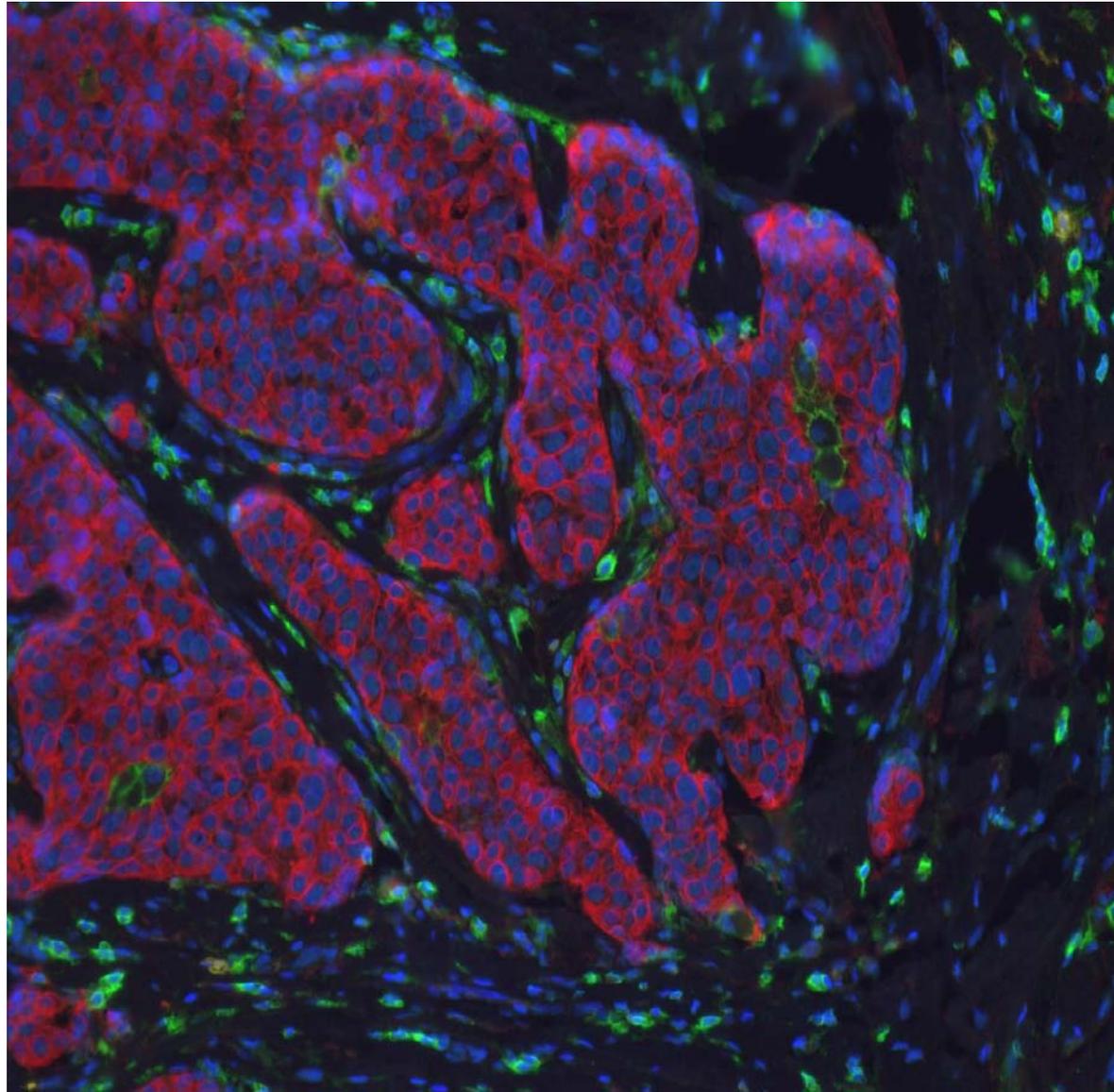
CD3
CD20
CD123
CD33
CD56
PanCK
DAPI



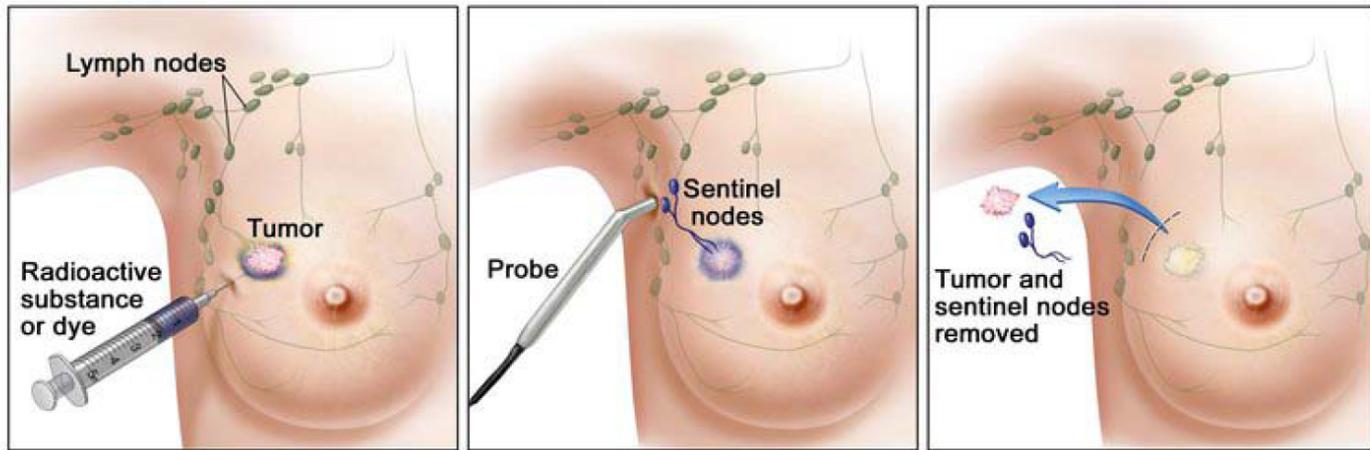
Myeloid cells surround cancer cells

Primary
Breast
tumor
200x
image

PanCK
CD33
CD3
CD20
DAPI



Tumor-Draining Lymph Nodes (TDLNs)



Sentinel lymph node biopsy of the breast

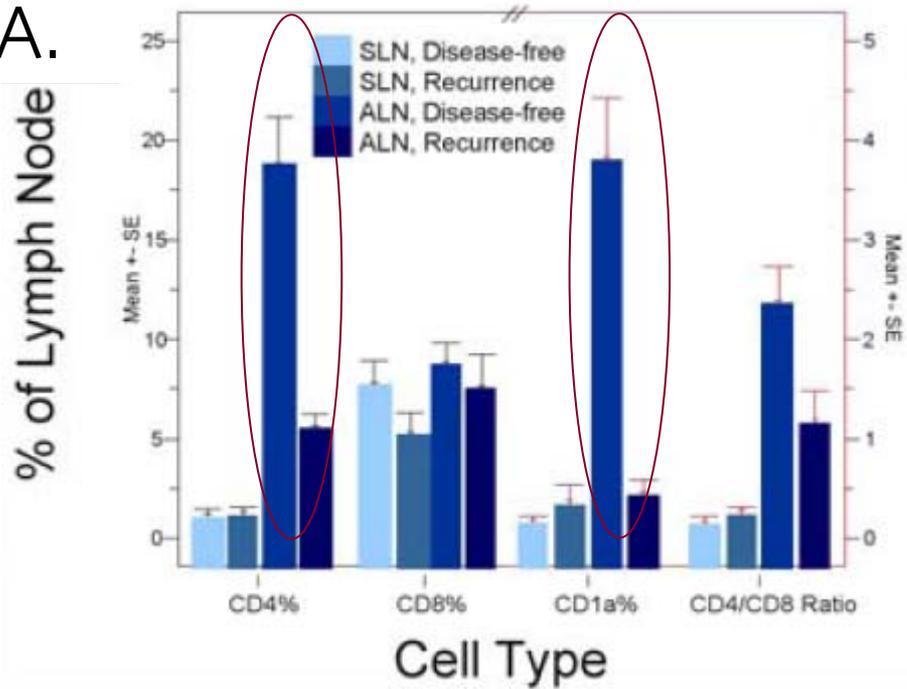
A radioactive substance and/or blue dye is injected near the tumor (first panel). The injected material is located visually and/or with a device that detects radioactivity (middle panel). The sentinel node(s) (the first lymph node(s) to take up the material) is (are) removed and checked for cancer cells (last panel).

TDLNs in Breast Cancer

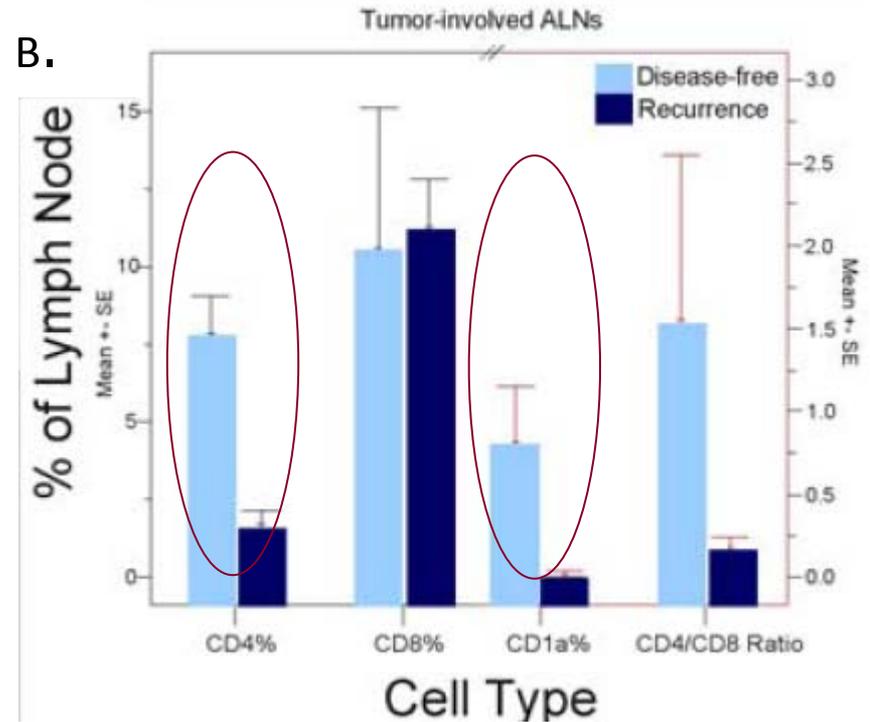
- Lymph Nodes are Immune Organs!
- Hypothesis:
 - Cancer alters the immune cells and function of draining lymph nodes
 - Extent of immune alterations in TDLNs may determine clinical outcome
- Questions:
 - Are immune cell populations (T cells, B cells, and dendritic cells) altered in TDLNs?
 - Does TDLN immune profile predict clinical outcome?

Higher % CD4 T cells and CD1a DCs in TDLNs from Disease-Free vs. Relapsed Breast Cancer Patients

A.



B.



TDLN Immune Profile Correlates with DFS

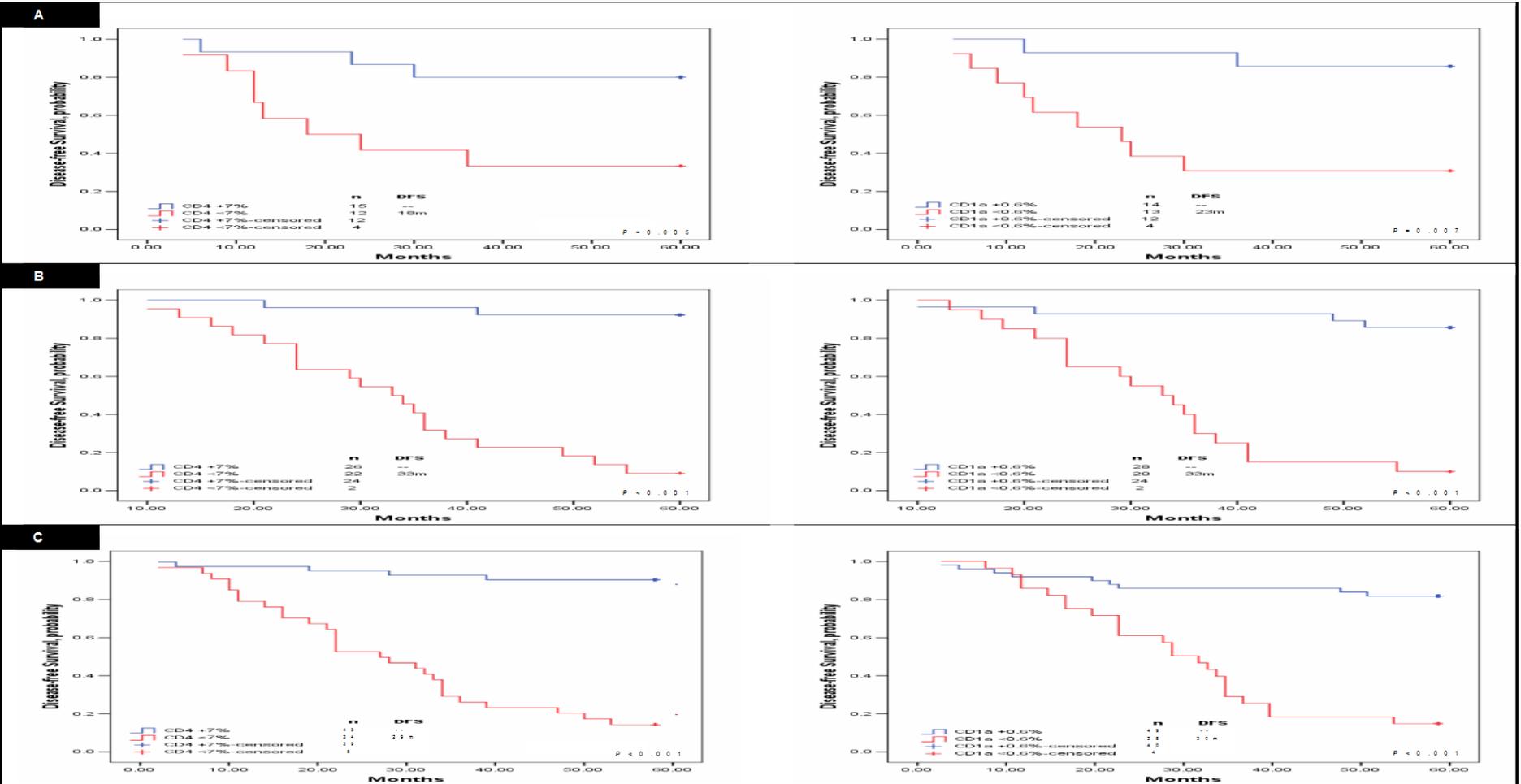
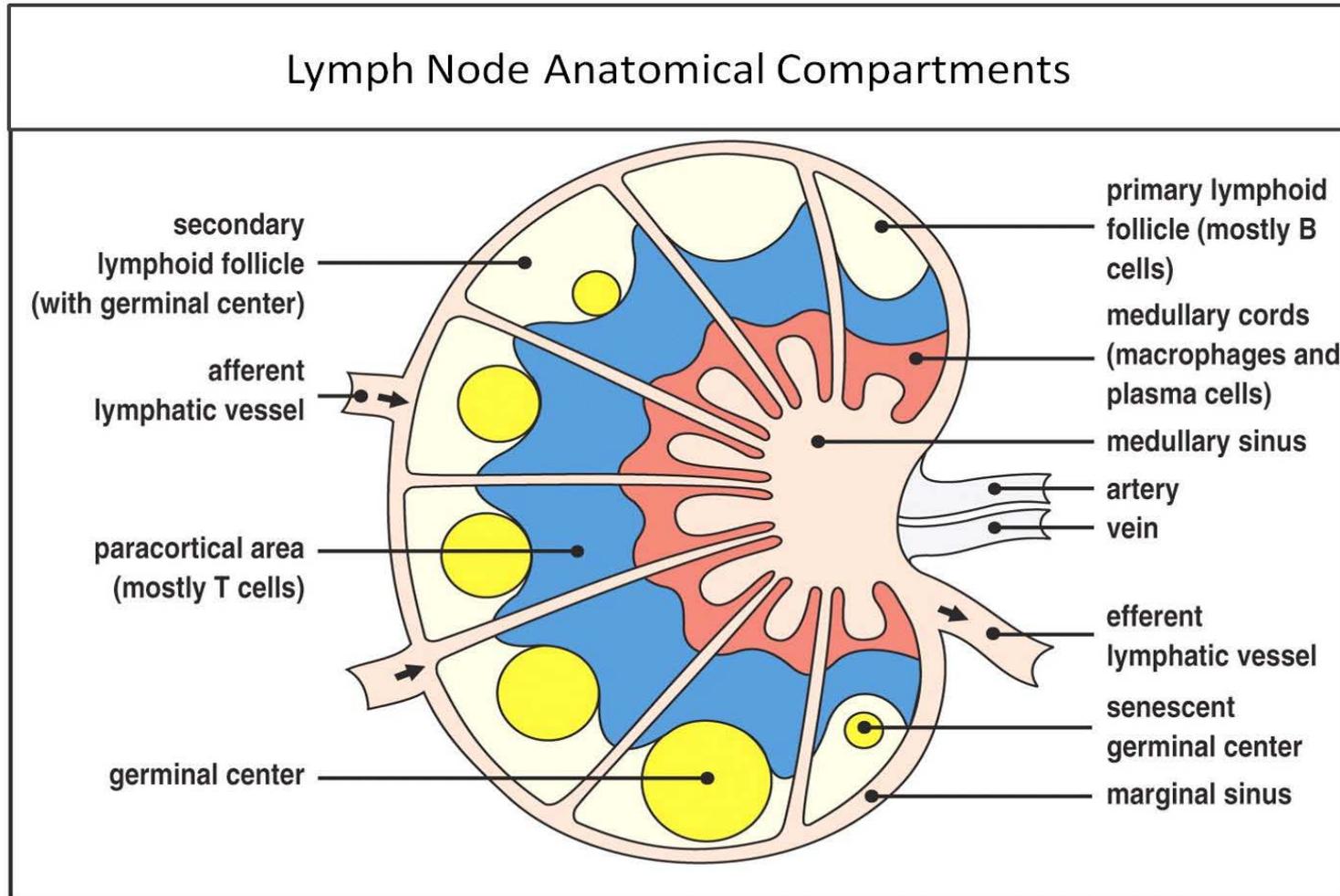
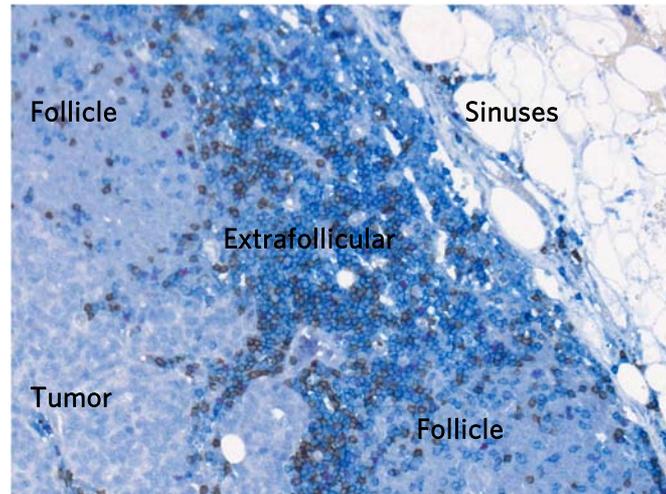
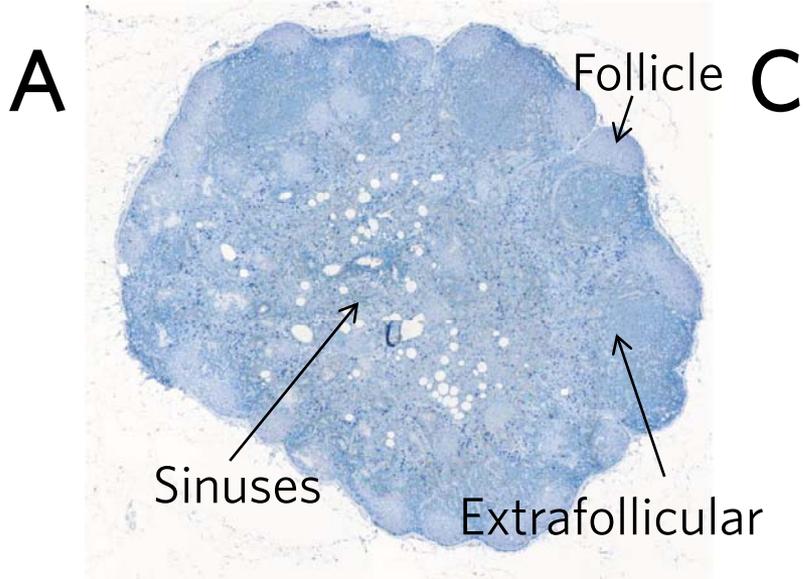


Figure 3.

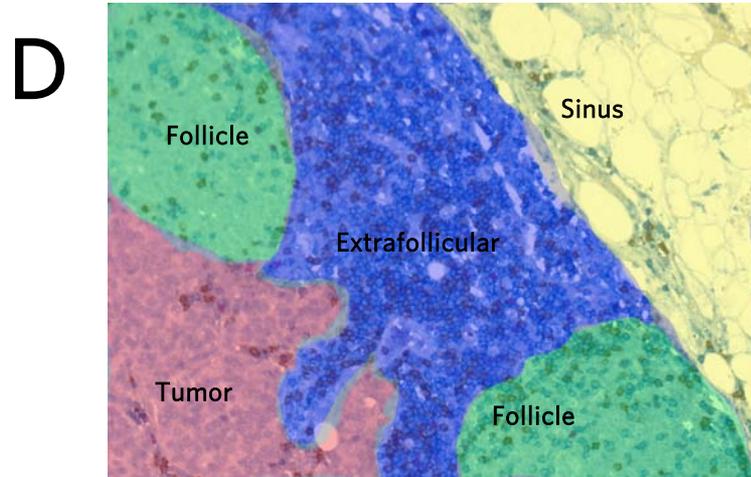
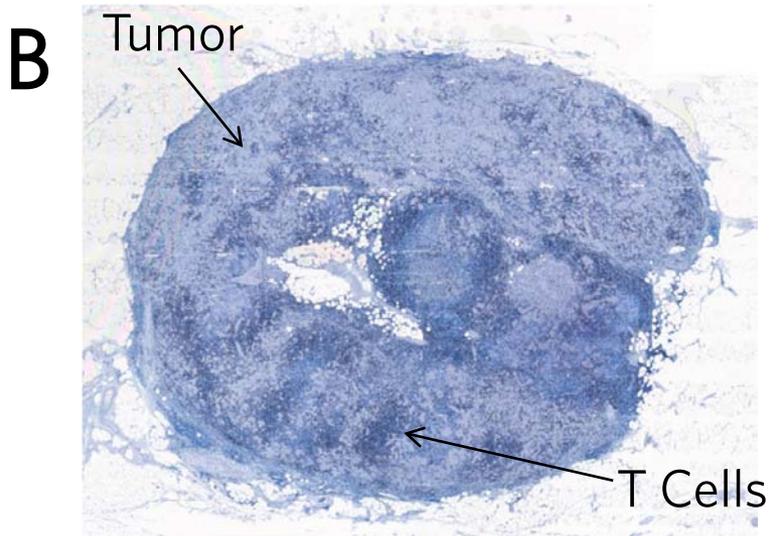
Anatomical Compartments of the Lymph Node



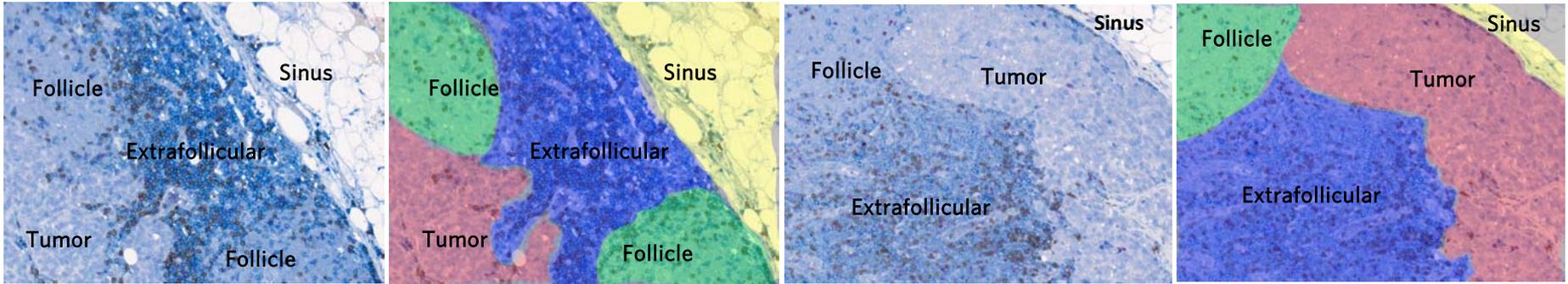
Tissue Segmentation



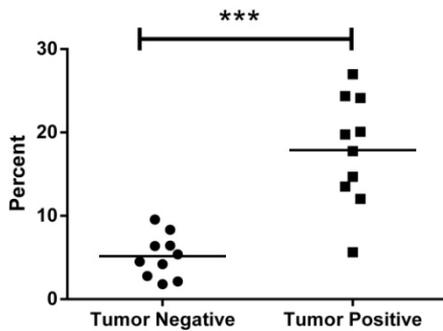
A. 40x magnification of a tumor negative lymph node. **B.** 40x magnification of a tumor positive lymph node. **C.** 200x magnification of one cube of an RGB. **D.** Tissue segmentation of the image cube in C using InForm™.



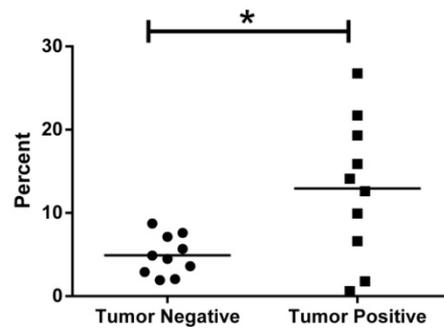
Regional distributions of FoxP3+ cells within TDLNs



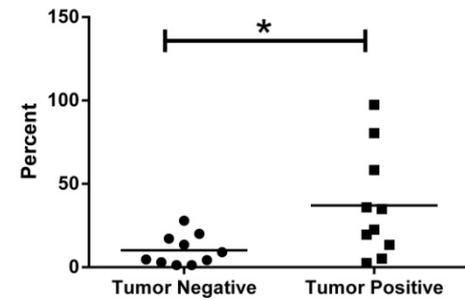
Foxp3+ Cells per Total T Cells



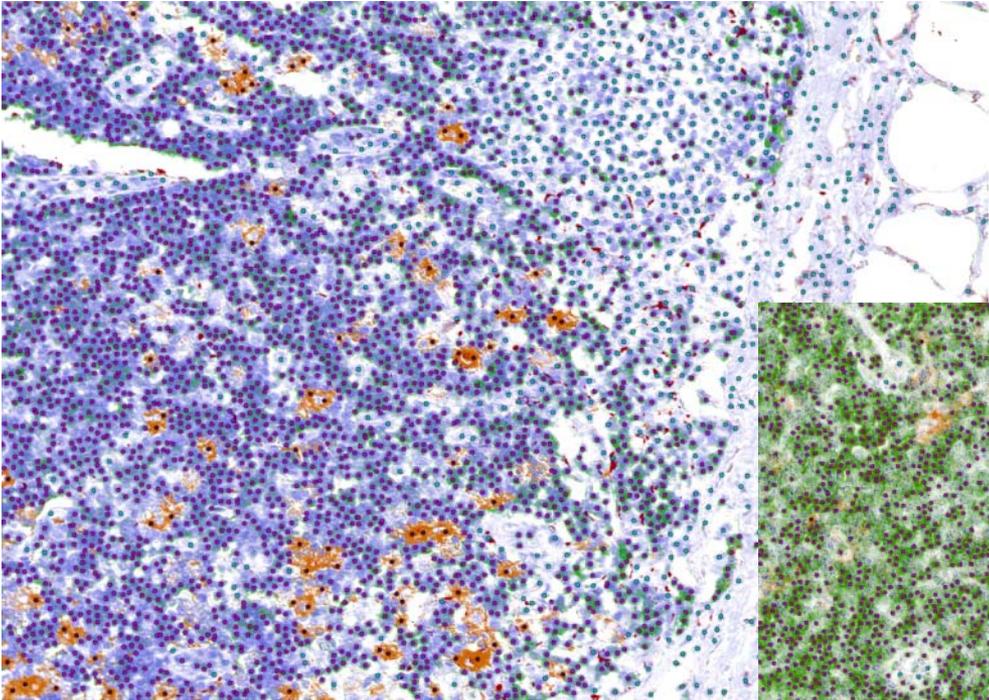
Extrafollicular Region Foxp3+ Cells per Total T Cells



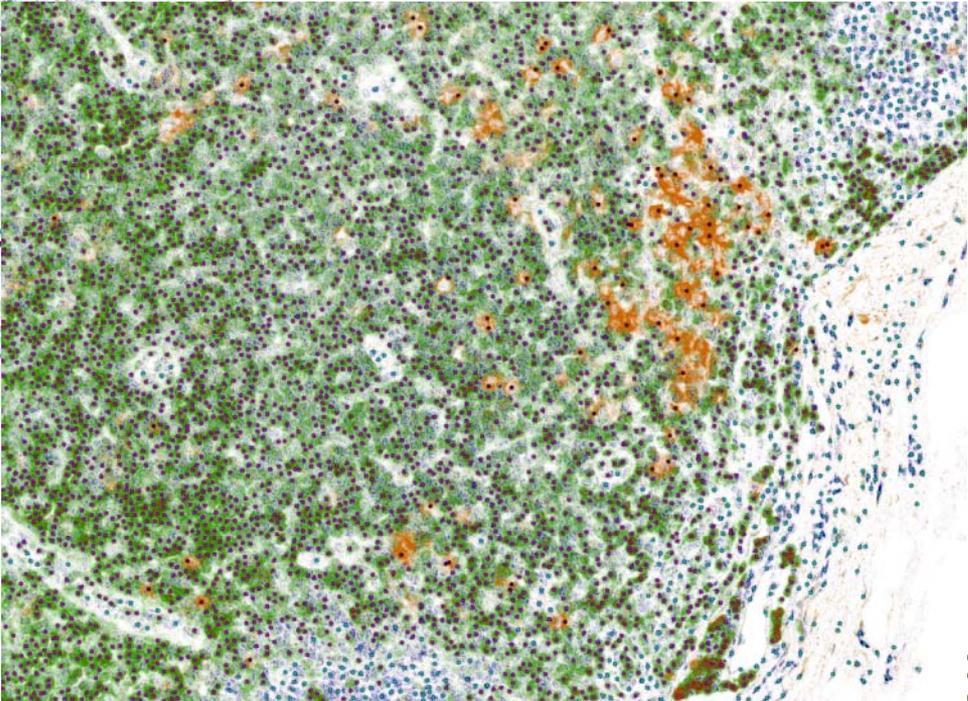
Follicular Region Foxp3+ Cells per Total T Cells



Dendritic Cell Clustering in TDLNs

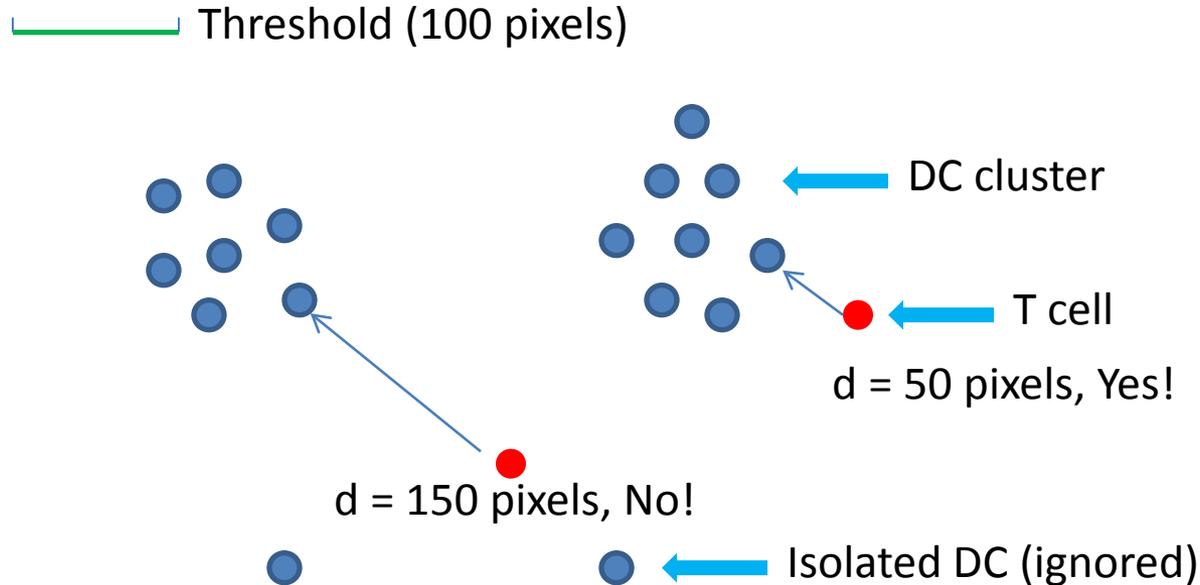


Relapsed



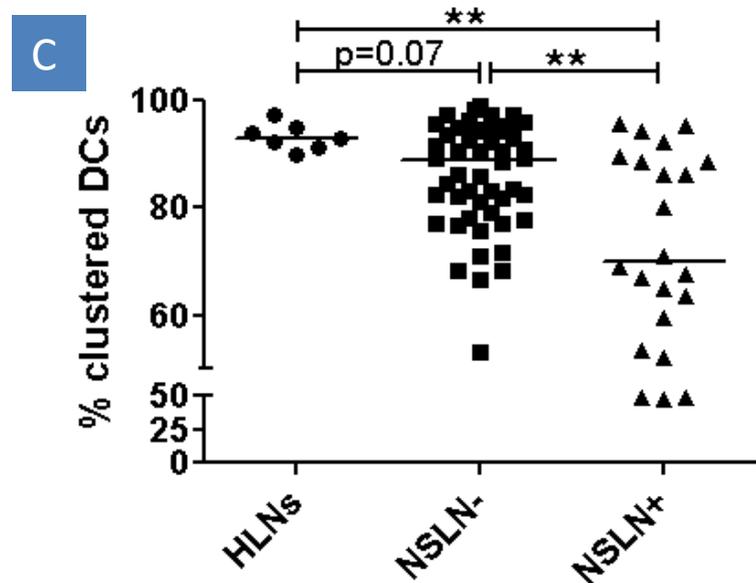
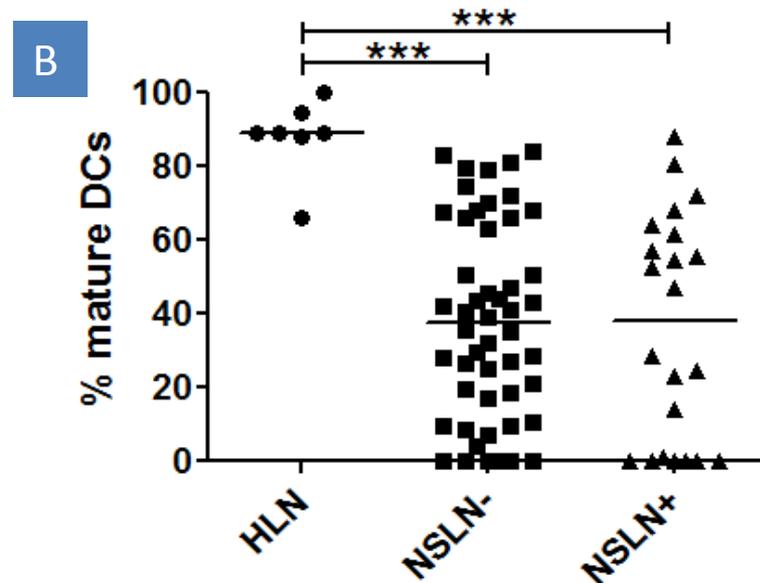
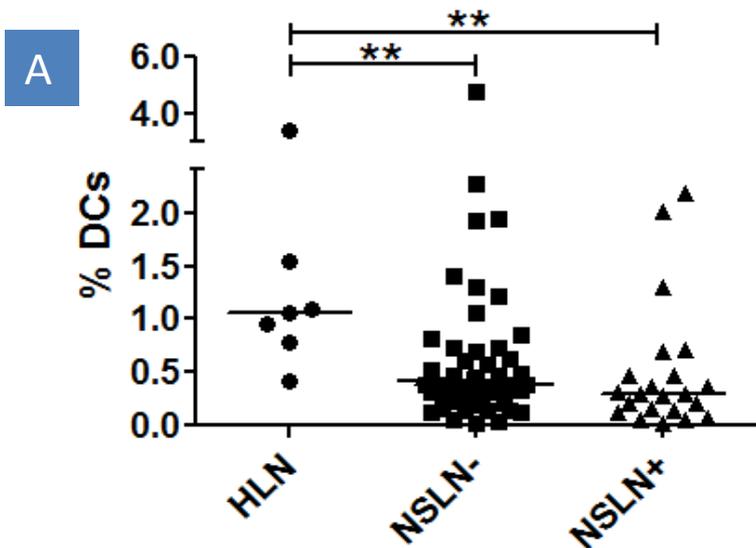
Healthy LN

Analyzing DC-T cell Interactions



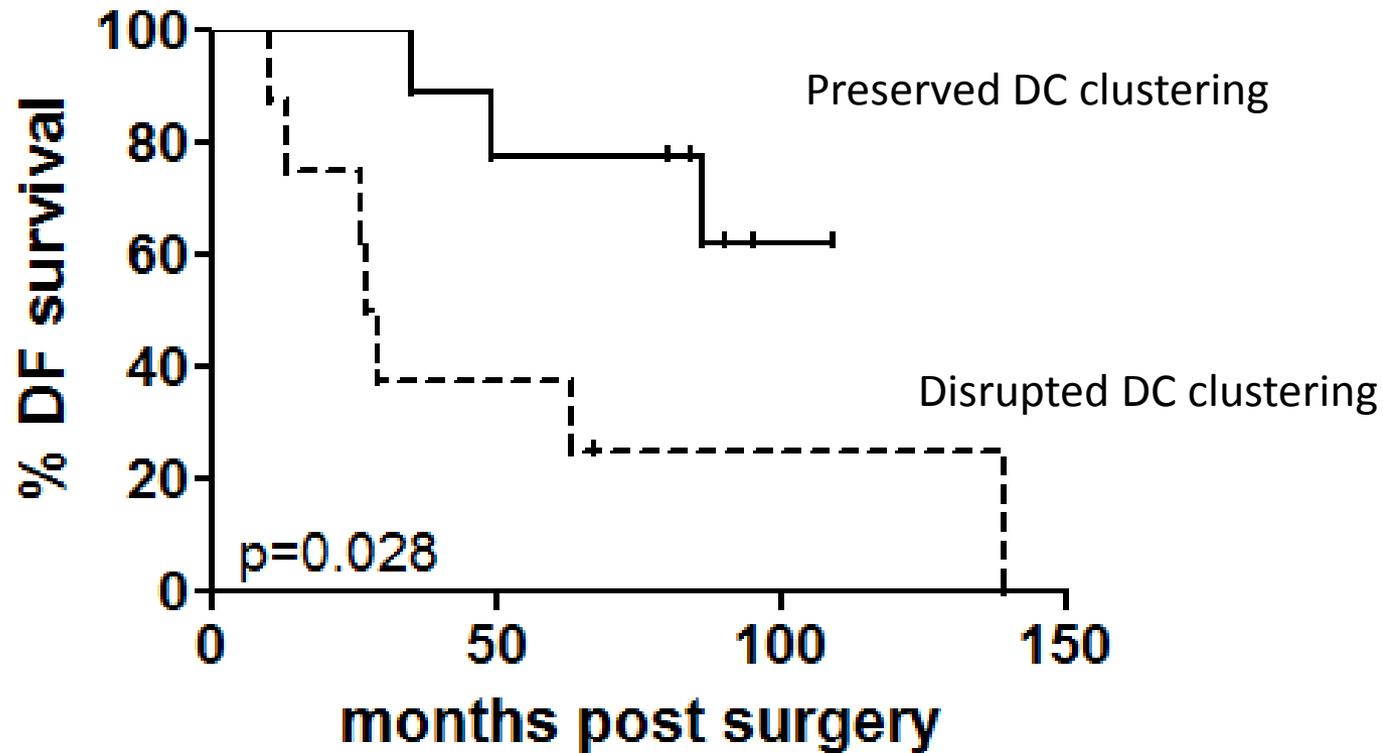
- Step 1: identify DC clusters
- Step 2: for each T cell, look for its nearest DC cluster
- Step 3: if the distance exceeds the threshold, ignore it; otherwise, consider it as clustered

Decreased DC %, Maturity, and Clustering in Tumor-Invaded Lymph Nodes



Chang, J Trans Med 2013

DC Clustering in TDLNs Correlates with Clinical Outcome



Summary

- Tumors are complex, heterogeneous collections of cancer, stromal, and immune cells.
- Reciprocal interplay between these cell populations drive progression, metastasis, response to therapy, and clinical outcome.
- Numerical and spatial relationships between immune cells are altered within tumors and TDLNs that correlate with clinical outcome in cancer.
- Quantitative, spatial image analysis of tumors and TDLNs will be highly informative in understanding how therapies modulate the balance between cancer and host immune responses.

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In memory of Holbrook Kohrt, MD, PhD