

# Endotyping Chronic Rhinosinusitis Using Multiplexed Imaging

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No  
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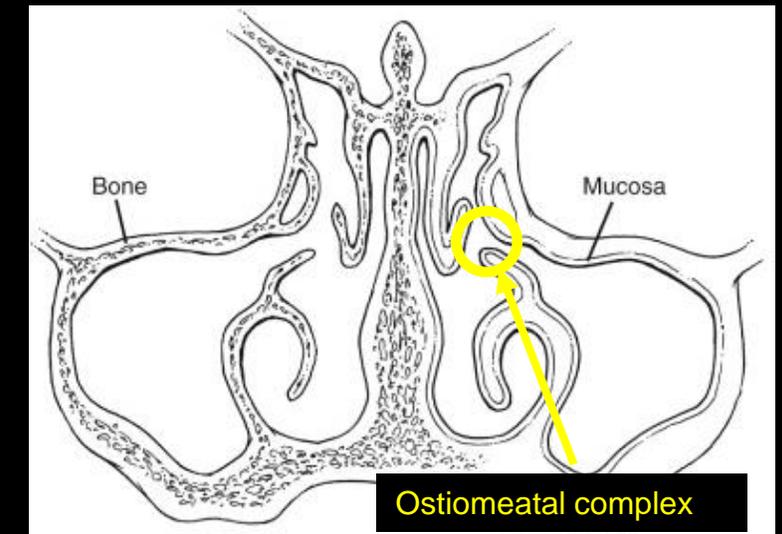
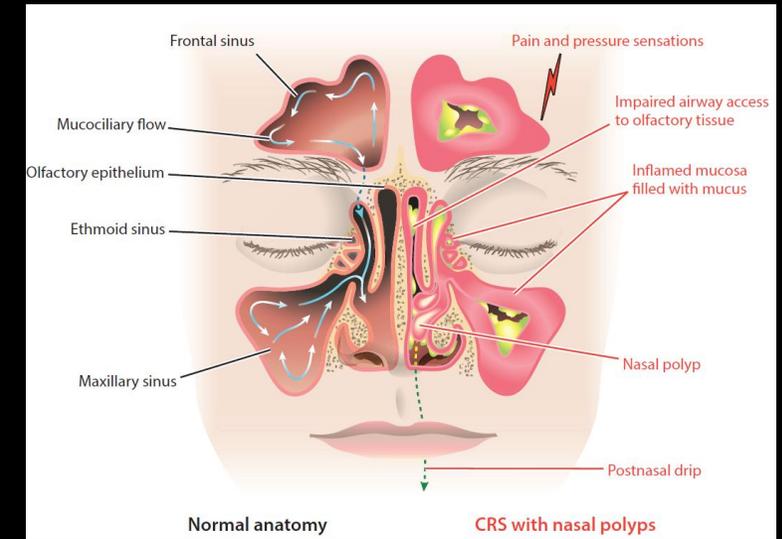
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# Outline

- Introduction
- Clinical/Molecular Characteristics of Chronic Rhinosinusitis
- Research Needs/Hypothesis
- Next Generation Immunophenotyping Tool
- Research Progress

# Chronic Rhinosinusitis (CRS)

- Debilitating, relapsing, remitting disease of inflammation in the nose and sinuses
  - Acute rhinosinusitis = infection
  - Chronic rhinosinusitis = inflammation
- Affects ~12% of the U.S. population
- Annual cost in the U.S. ~\$8 billion
- Top 10 diagnosis associated with loss of productivity



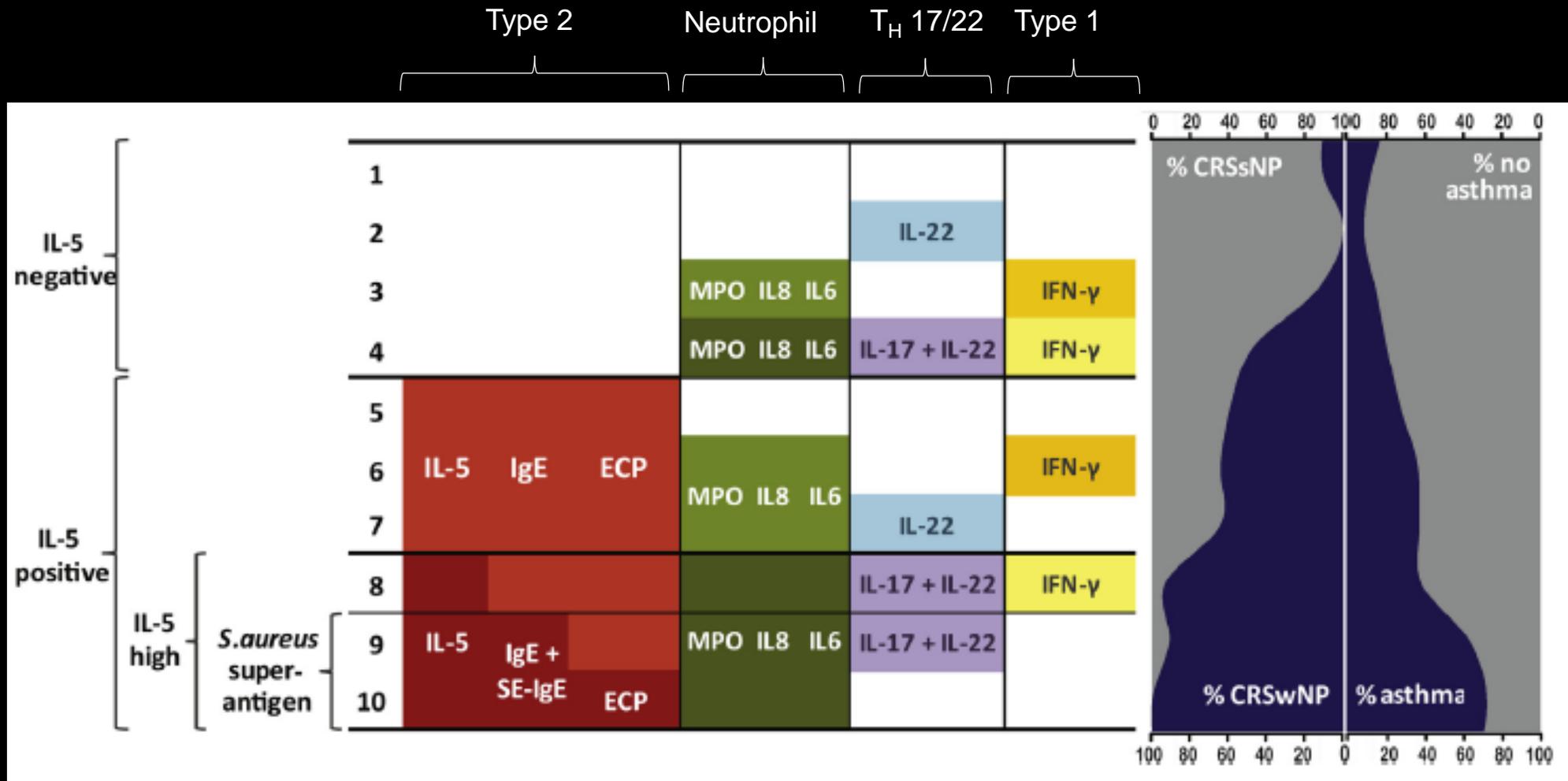
# Phenotypic Classification of CRS

	CRS with Nasal Polyps (CRSwNP)	CRS w/o Nasal Polyps (CRSsNP)
<b>Phenotype</b>		
Prevalence	~1/3	~2/3
Asthma	Higher prevalence	Lower prevalence
Recurrence	Higher	Lower
Severity	Higher	Lower

## Other Classifications:

- Aspirin-exacerbated respiratory disease (AERD)
  - 8-26% of CRSwNP
  - 90% risk of recurrence within 5 years
- Allergic fungal rhinosinusitis
- Cystic fibrosis CRS

# CRS is a Diverse Inflammatory Disease



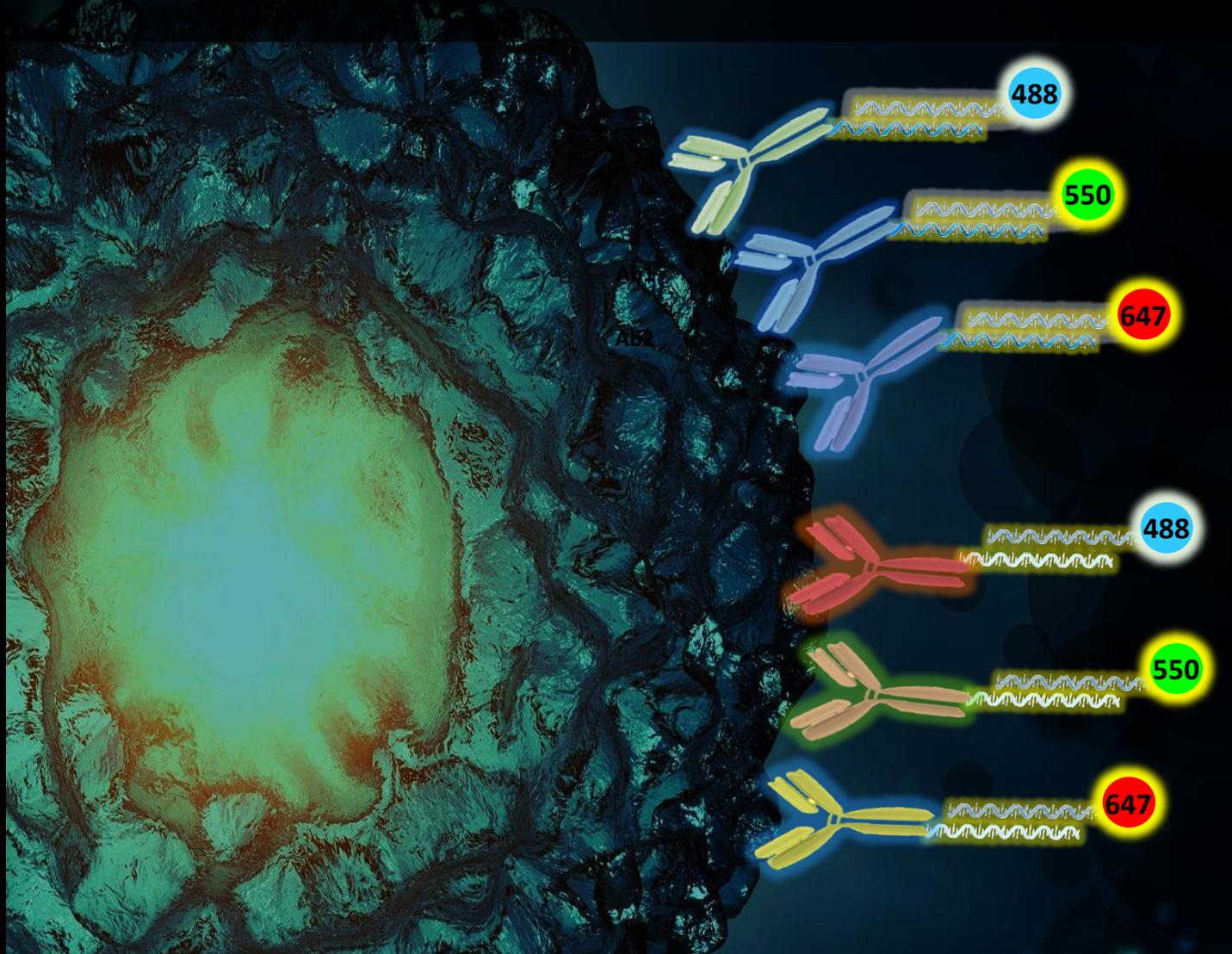
1. CRSwNP tends to be a Type 2 inflammatory disease (but not always)
2. CRSsNP tends to be a non-Type 2 inflammatory disease (but not always)

# Research Needs

- CRS is currently a “heterogeneous” disease both at the clinical level and at the molecular level.
- Current medical therapeutics are ineffective in patients with high rates of disease recurrence.
- No biomarkers have been identified to inform the extent of surgery.

A ‘signature’ of biomarkers in quantitative or spatial combination will newly define CRS subtypes and/or disease state.

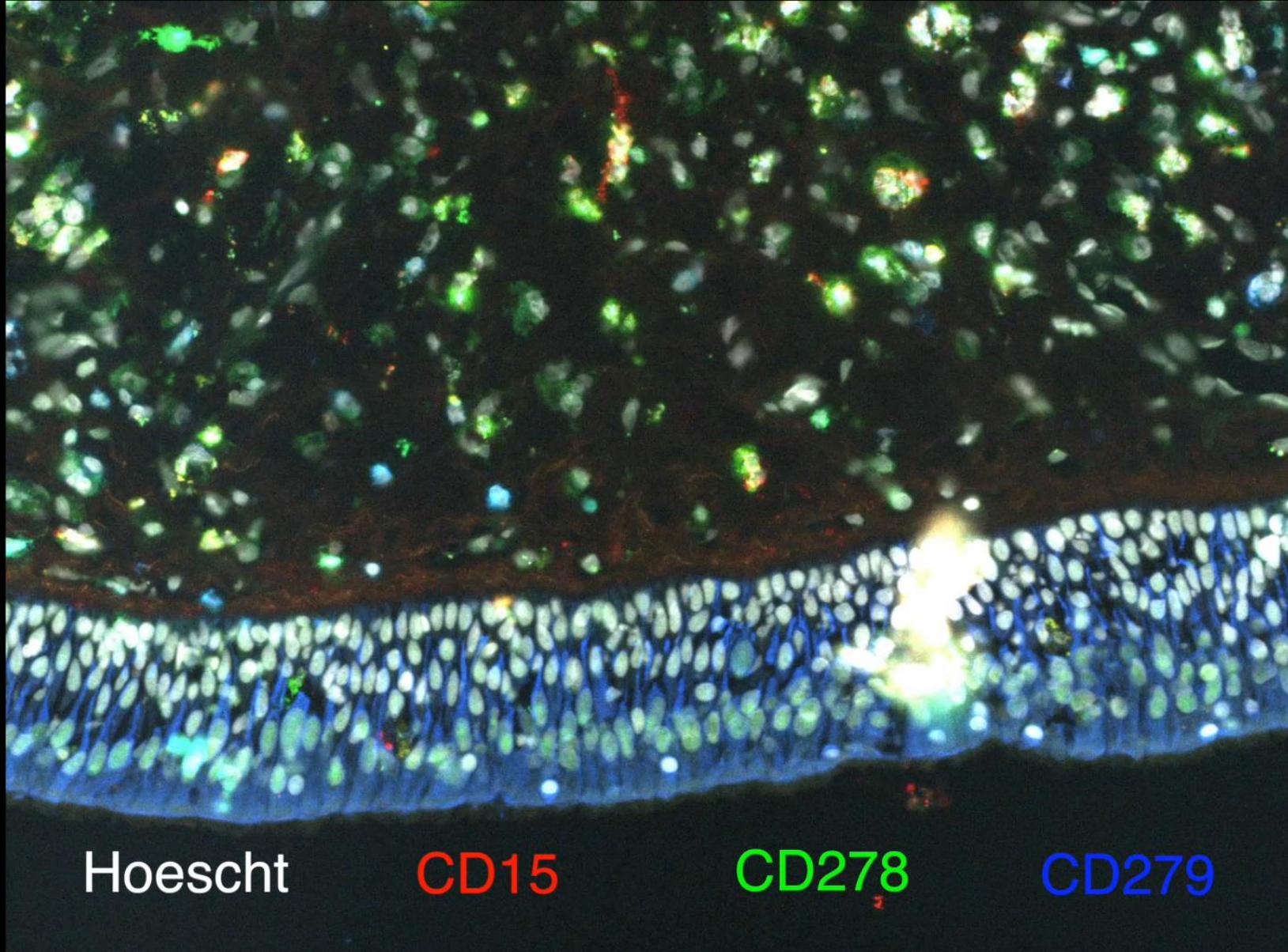
# CODEX MULTIPLEXING BY REANNEALING



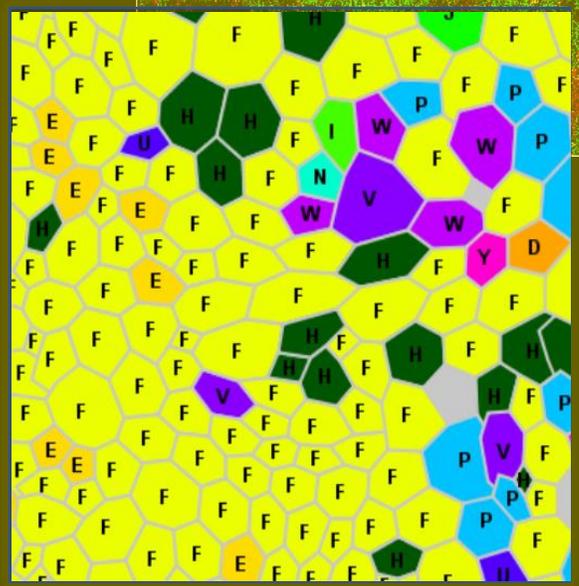
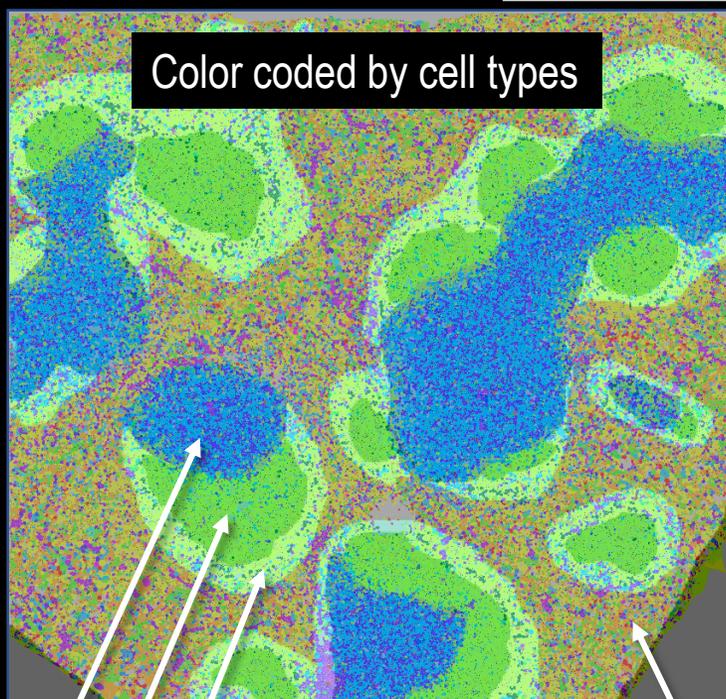
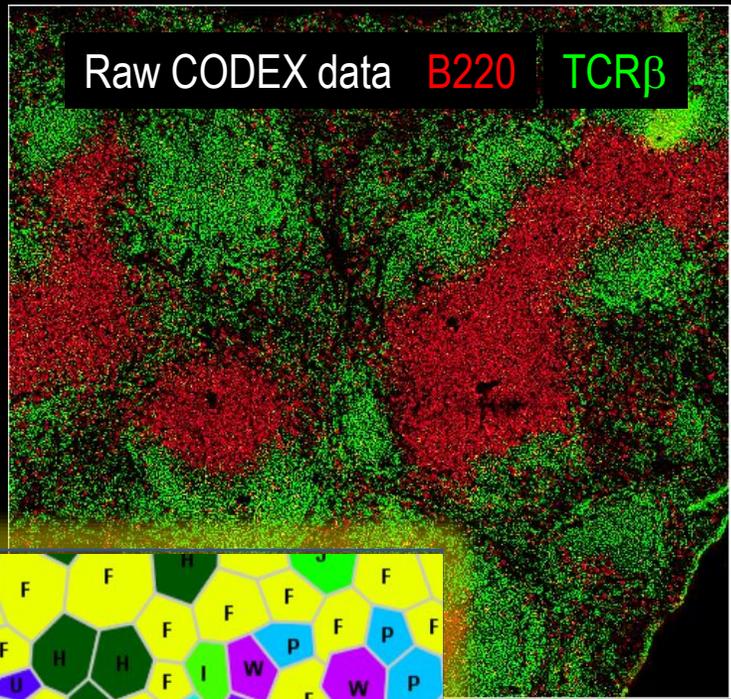
- Stain up to 50 antibodies conjugated to a single strand oligonucleotide
- Anneal anti-sense oligo-fluorophores
- Image
- Gentle Denaturation of oligos
- Repeat for all Ab/oligo sets

CO-Detection by indEXing (CODEX)

# CODEX CRS Tissue Section



# AUTOMATED ANNOTATION OF TISSUE

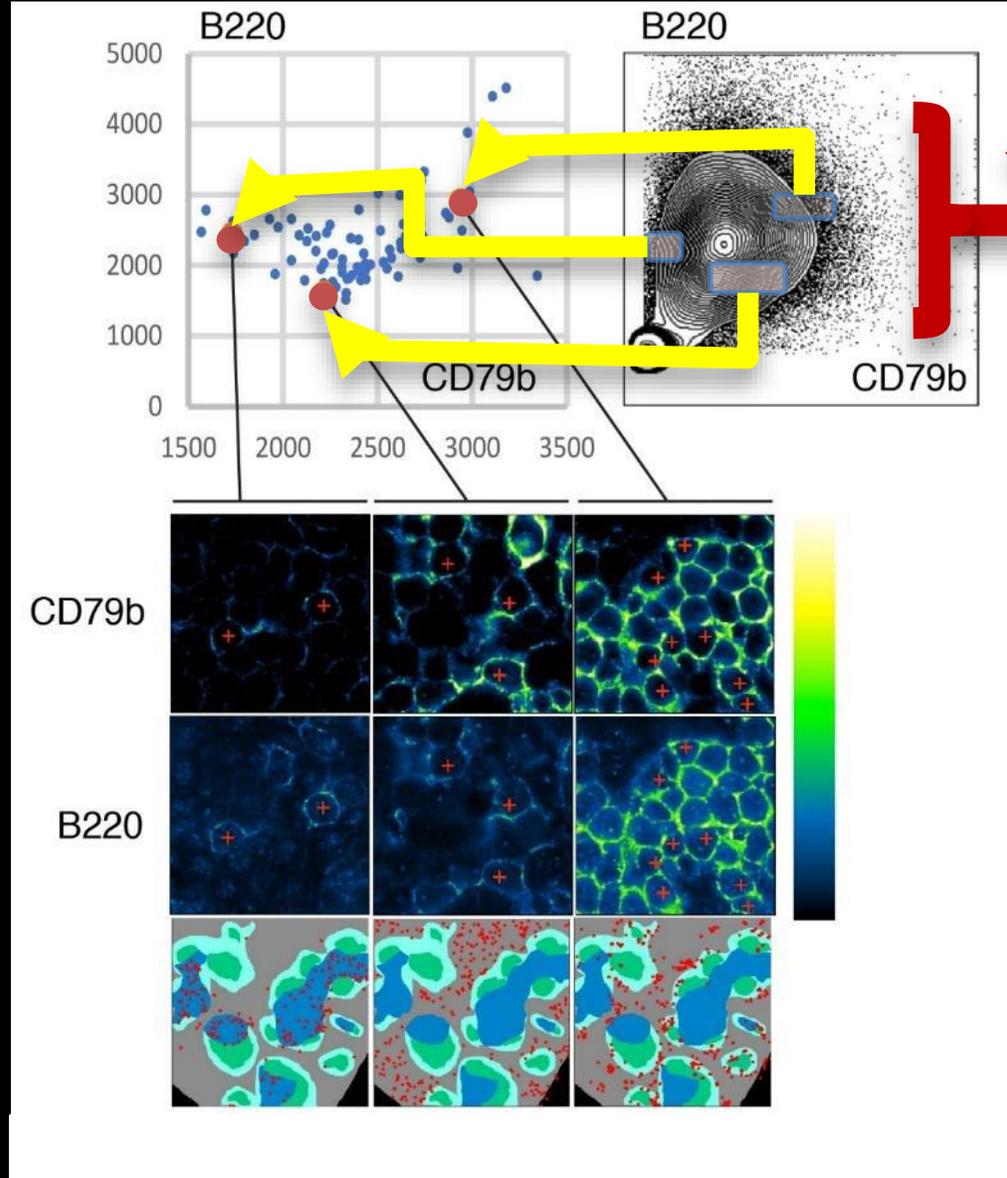


PALS (T cells)  
 B-cells follicle  
 Marginal zone

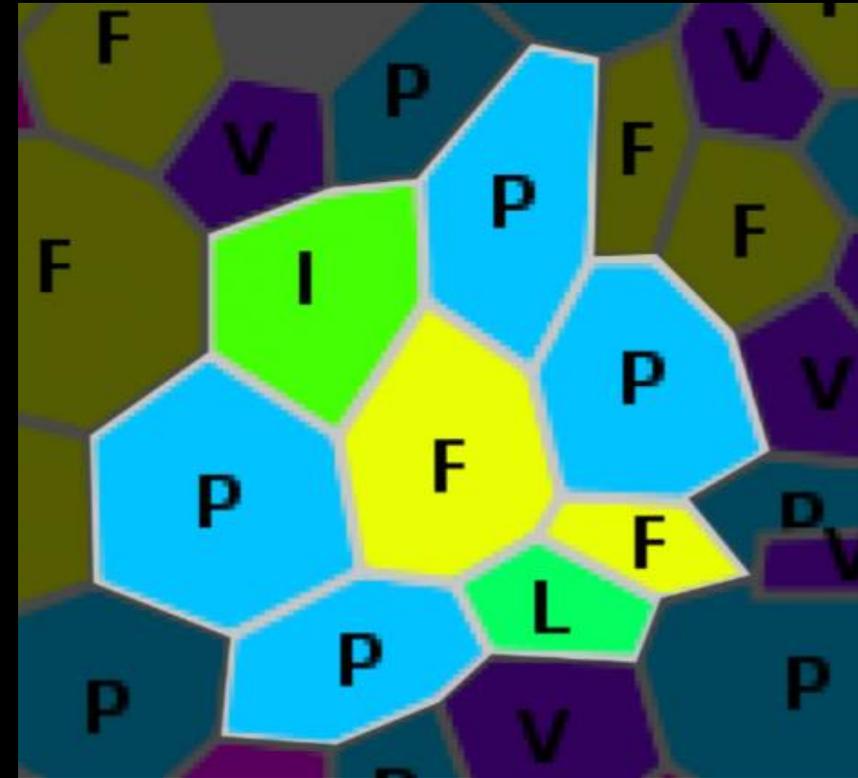
Red pulp

- A** 9587, NK-cells
- B** 9590, CD4+ CD8- cDC
- C** 9591, B220 pos DN Tcells
- D** 9595, F4/80+ CD16/32+ CD106pos macrophages
- E** 9597, follicular dendritic cells
- F** 9600, B-cells
- G** 9601, capsule
- H** 9602, marginal zone macrophages
- I** 9607, erythroblasts
- J** 9608, CD106+ CD16/32+ CD31+ stroma
- K** 9609, granulocytes
- L** 9611, CD8+ CD4- cDC
- M** 9614, CD106- Ly6C+ CD16/32- CD31+
- N** 9615, megakaryocytes
- O** 9617, CD106- Ly6C+ CD16/32+
- P** 9619, CD4 T cells
- Q** 9626, plasma cells
- R** 9628, CD4hi only
- S** 9629, Vasculature
- T** 9632, CD3 only
- U** 9635, CD106+ CD16/32+ CD31- stroma
- V** 9637, CD8 T cells
- W** 9638, ERTR7-hi stroma
- X** 9639, CD106+ CD16/32- Ly6C+ CD31+ stroma
- Y** 9643, CD4+ CD8+ CD16/32+ cDC

# MARKER EXPRESSION DEFINES THE NICHE



● Expression levels of proteins relates to  
Neighbors  
Tissue locale



# Future Directions

- Correlate CRS inflammatory niche with clinical characteristics (severity scoring, recurrence, comorbidities, medications, etc).
- Endotype CRS in East Asia and compare/contrast with CRS in the U.S.
- Develop an algorithm to determine CRS subtypes and optimal therapy.
- Provide individualized care based on the unique clinical and molecular profile of the patient.

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- Nayak Lab Funding

# Nayak Lab/Tissue Team



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# Stanford Sinus Center



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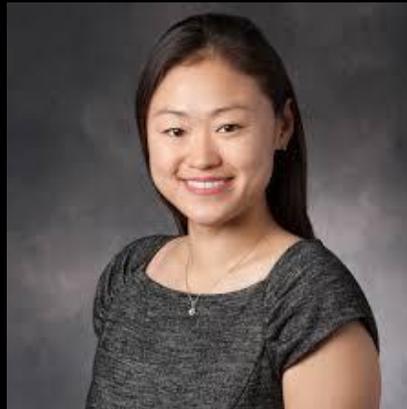
Jayakar Nayak



Peter Hwang



Zara Patel



Jennifer Lee



Carol Yan



Matt Tyler

# International Team



東京慈恵会医科大学



# Thank you!

- Questions/suggestions?

# Polyps have a variable extent of inflammation

