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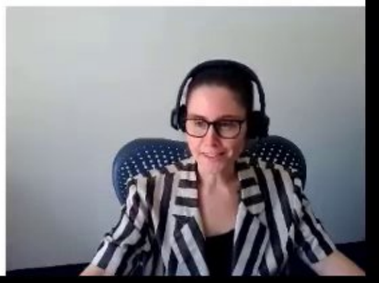
Unlocking the Lymphoid Structures with Germinal Centers: Architectural Complexity, Functionality and Clinical Relevance

Assoc. Prof. Diana Mechtcheriakova
Institute of Pathophysiology & Allergy Research
Medical University of Vienna



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MEDICAL UNIVERSITY OF VIENNA
Institute of Pathophysiology and Allergy Research

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Unlocking the lymphoid structures with germinal centers: architectural complexity, functionality and clinical relevance

Diana Mechtcheriakova
Institute of Pathophysiology and Allergy Research, Medical University of Vienna

Euro-BioImaging User Forum: Focus on Immunology
October 15, 2024

About

MSBP Molecular Systems Biology & Pathophysiology

Concept

From Systems Biology to Systems Medicine as part of the global concept of personalized medicine. “The whole is greater than the sum of its parts” *Aristotle*

Approach

Holistic for deciphering the complexity; from data to information to knowledge

Main research areas

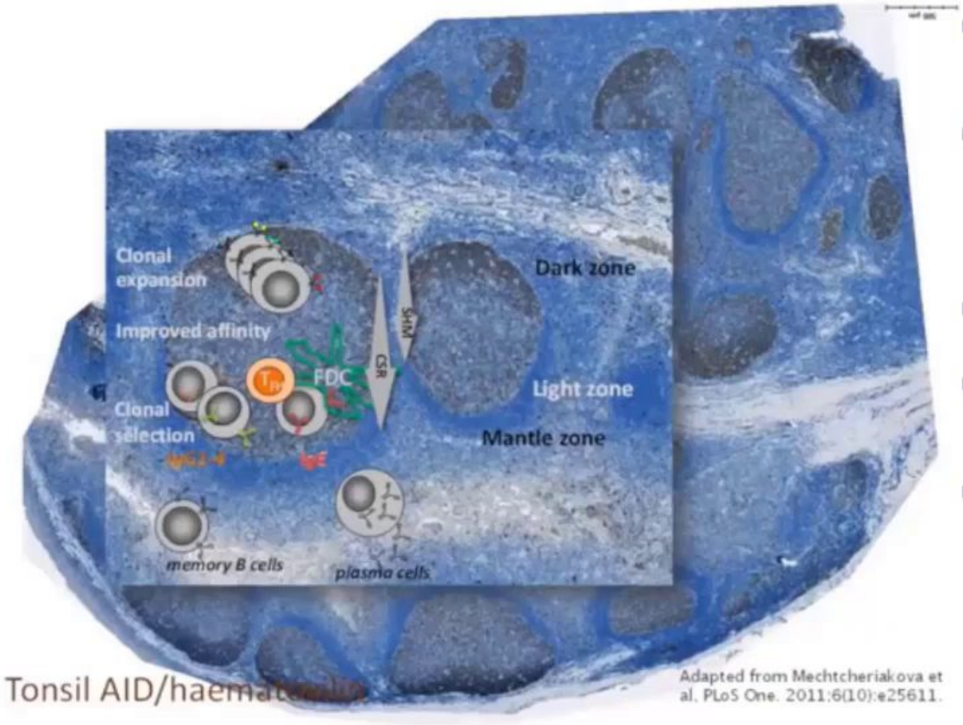
Systems biology approaches, B-cell biology, immuno-oncology, lymphoid structures, the AID/APOBEC-associated biological events and cellular sphingolipid/lysophosphatidate system – in immunity and cancer

Head of Tissue Image Cytometry Unit at Austrian Bioimaging (together with Isabella Ellinger)

Head of Basic and Translational Research Module at Austrian Platform for Personalized Medicine/ÖPPM

National Delegates Board member of International Sphingolipid Society as representative of Austria

Biology of lymphoid structures and germinal centers



Tonsil AID/haematoxylin

Adapted from Mechtcheriakova et al. PLoS One. 2011;6(10):e25611.

- **Highly organized structures** with tight packing, interaction and unique functional cooperation of various immune cell types
- When fully developed, contain **germinal centers (GC)**: functional niche where B cells proliferate, differentiate, undergo affinity maturation and switch the class of their antibodies. **AID as unique marker**
- The outcome of GC reaction: **plasma cells and memory B cells**
- **Located** in secondary lymphoid organs such as tonsils, spleen, lymph nodes, and mucosa-associated lymphoid tissues
- Additionally, **can be assembled at ectopic sites within chronically inflamed tissues including the tumor site**. In this case, they are known as ectopic lymphoid structures, **ELS** or tertiary lymphoid structures, **TLS**

Clinical relevance of B cells and ELS in cancer

Anti- or pro-tumoral role?

Presence of B cells and/or ectopic lymphoid structures associate with a favourable patient's prognosis in > 10 cancer types

- **Non-small cell lung cancer** (NSCLC): (Germain, 2014)
- **Small cell lung cancer** (SCLC): (Silina et al., 2018)
- **Breast cancer**: (Gu-Trantien et al., 2013; M. Lee et al., 2019; Mahmoud et al., 2012; Martinet et al., 2013; Xu, Lan, & Zheng, 2018)
- **Primary colorectal cancer** (CRC): (Bindea, 2013; Coppola et al., 2011; Remark et al., 2013; Di Caro et al.,2014)
- **Pancreatic cancer**: (Hiraoka et al., 2015; Castino et al., 2015)
- **Melanoma**: (Ladanyi et al., 2011; Garg et al., 2016)
- **Metastatic melanoma**: (Cabrita et al., 2020; Helmink et al., 2020)
- **Hepatocellular carcinoma** (HCC): (Calderaro et al., 2019)
- **Head and neck cancer**: (Van Herpen et al, 2008)
- **Colorectal cancer with metastasis in the liver** (CRCLM): (Meshcheryakova A et al, 2014; Mlecnik et al., 2018)

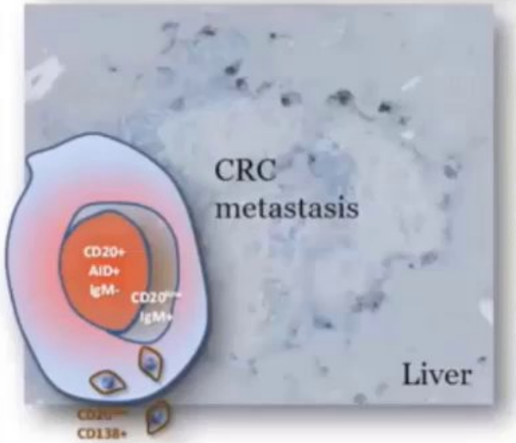
OPEN ACCESS Freely available online

PLOS ONE

B Cells and Ectopic Follicular Structures: Novel Players in Anti-Tumor Programming with Prognostic Power for Patients with Metastatic Colorectal Cancer

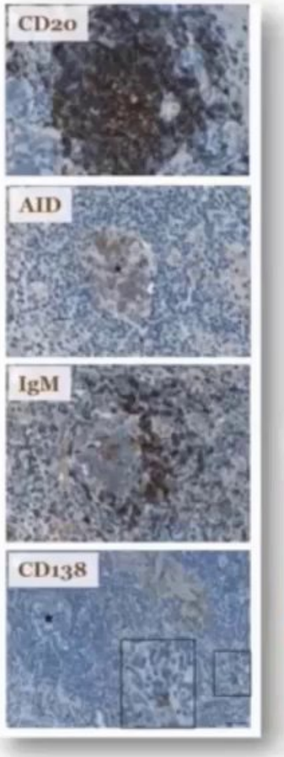
Anastasia Meshcheryakova[1☯], Dietmar Tamandl[2☯*], Erika Bajna[1], Judith Stift[3], Martina Mittlboeck[4], Martin Svoboda[1], Denise Heiden[1], Stefan Stremitzer[2], Erika Jensen-Jarolim[1,5], Thomas Grünberger[2], Michael Bergmann[2], Diana Mechtcheriakova[1*]

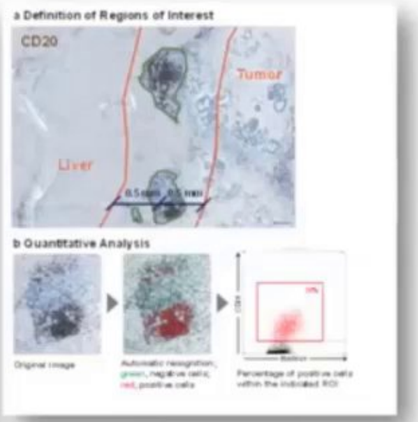
1 Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, 2 Department of Surgery, Medical University of Vienna, Vienna, Austria, 3 Department of Pathology, Medical University of Vienna, Vienna, Austria, 4 Center for Medical Statistics, Informatics, and Intelligent Systems, Medical University of Vienna, Vienna, Austria, 5 Comparative Medicine, Messerli Research Institute of the Medical University of Vienna, Veterinary University of Vienna and University of Vienna, Vienna, Austria

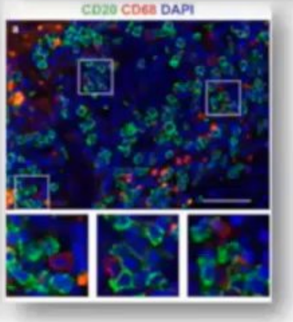


Discoveries:

- Accumulation of B cells assembled in ELS with active GCs in the liver
- ELS formed a rim around the metastasis
- Quantification using the power of TissueFAXS platform
- Higher magnitude of B cells and/or ELS – better clinical outcome in terms of survival
- Prognostic effect was superior to clinicopathological parameters

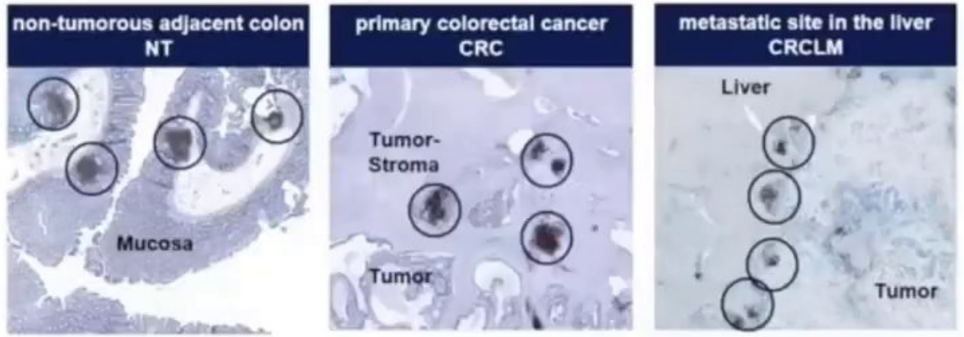






Hypothesis

The anti-tumor immune response at the metastatic site in the liver **is pre-instructed at primary CRC site and/or** even within the **non-tumorous colon mucosa** with natural **isolated lymphoid structures, ILS.**



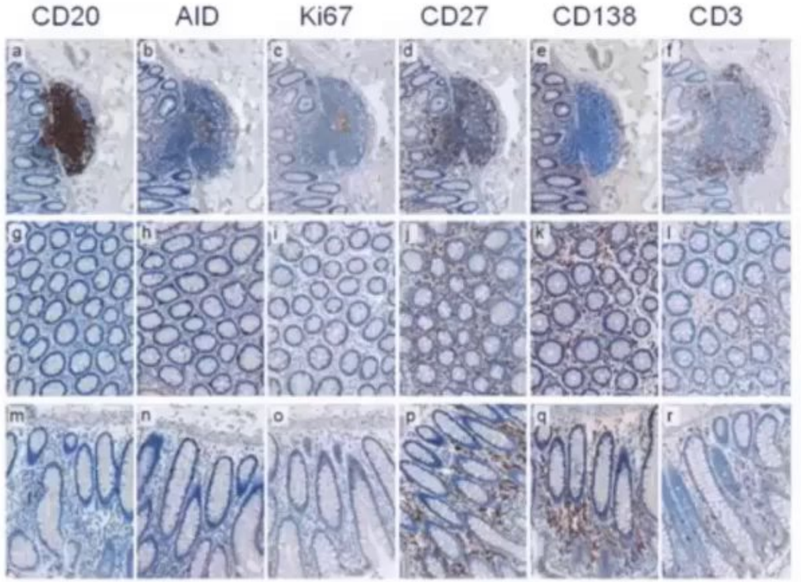
The study addressed the research question whether **specific characteristics of lymphoid structures,** established within **3 different tissues entities** – NT, CRC, CRCLM, have an impact on the **pathobiology of metastatic CRC.**

We established the multimodular **DIICO** strategy – **from Digital Immune Imaging to Clinical Outcome.**

Mungenast F & Meshcheryakova A et al, 2020

DIICO module I: the immunostaining

- **CD20** – the general B-cell marker
- **AID** – activated B cells and functionally active germinal centers
- **Ki67** – proliferating cells
- **CD27** – memory B cells and plasma cells as well as T-cell subsets
- **CD138** – plasma cells
- **CD3** – the general T-cell marker
- **Combination of markers**: CD20/CD3, CD20/CD27, CD20/Ki67, CD3/Ki67, and AID/Ki67



Consecutive NT tissue sections

➢ Applied for colon cancer specimens (n = 69) and tonsil tissues (n = 11) to address the immune cell composition and activity of lymphoid structures

DIICO module II: assessment of immunological imprint by quantitative tissue image cytometry

Staining of specimens

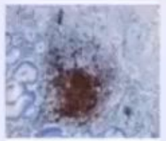
CRC:
69 x 11 markers/combinations
> 700
Tonsils: 11 x 11 = 121

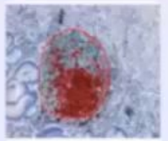
Whole-slide acquisition by TissueFAXS

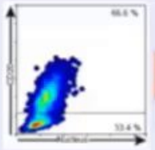
Quantitative analysis of the digital images (HQ/TQ)

Define lymphoid structures as objects
Single cell recognition-based analysis
Marker+ cell detection via marker-specific profile

Marker profile







Data sets as variables

red marker-positive cells

2022-10-19 Medicine & Science
Centre of Excellence for Quantitative Digital Microscopy

Martin Schepelmann (MedUni Vienna), Rupert Ecker (CEO TissueGnostics), sabella Ellinger, Diana Mechtcheriakova, Anastasia Meshcheryakova (all MedUni Vienna)

Meshcheryakova A., Zimmermann P., Ecker R., Mungenast F., Heinze G., Mechtcheriakova D. (2018) An Integrative MuSICO Algorithm: From the Patient-Specific Transcriptional Profiles to Novel Checkpoints in Disease Pathobiology. In Systems Biology: RNA Technologies. Springer, Cham

Meshcheryakova, A.(EQ), Mungenast, F.(EQ), Ecker, R., & Mechtcheriakova, D. (2021). Tissue image cytometry. *Imaging Modalities for Biological and Preclinical Research: A Compendium, Volume 1* (pp. 1.2.h-1-1.2.h-10). IOP Publishing. doi.org/10.1088/978-0-7503-3059-6ch14

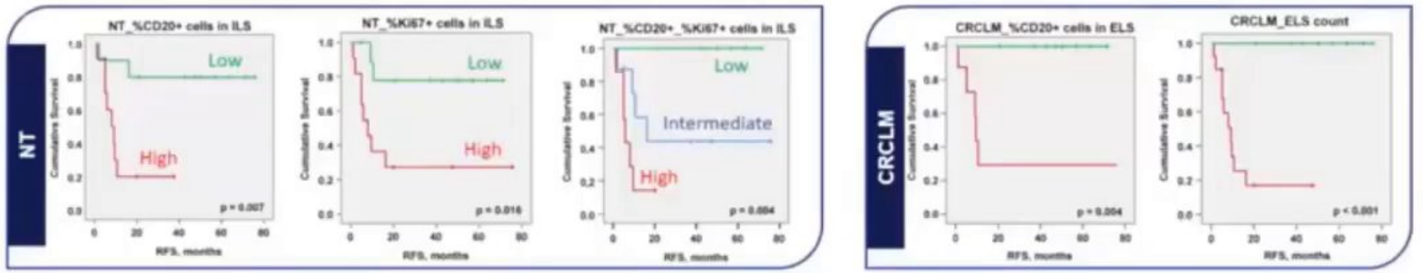
- Outcome of such analysis: transformation of the tissue encrypted information into numerical values as variables
- Anatomy- and staining-based variables exported for each tissue (NT, CRC, CRCLM) and each patient
- The variables are then used for alignment with clinicopathological parameters

Anatomy- and staining-based variables n=24

Variable	Description	Type	n
NT_ILS count	number of ILS detected within NT specimen	anatomy-based	23
NT_ILS size	mean size (mm^2) of ILS detected within NT specimen	anatomy-based	23
NT_ILS density	mean cellular density (cells/mm^2) of ILS detected within NT specimen	anatomy-based	23
NT_ILS to ILS distance	mean distance (mm) between ILS detected within NT specimen	anatomy-based	22
NT_AID$^+$ ILS count	number of AID$^+$ ILS detected within NT specimen	staining-based	23
NT_% GC size of ILS size	mean proportion of GC area to the entire ILS area (%) in NT specimen	anatomy- and staining-based	14
NT_% CD20$^+$ cells in ILS	mean % of CD20$^+$ cells within ILS in NT specimen	staining-based	23
NT_% Ki67$^+$ cells in ILS	mean % of Ki67$^+$ cells within ILS in NT specimen	staining-based	23
NT_% CD27$^+$ cells in ILS	mean % of CD27$^+$ cells within ILS in NT specimen	staining-based	23
CRC_ELS count	number of ELS detected within CRC specimen	anatomy-based	21
CRC_ELS size	mean size (mm^2) of ELS detected within CRC specimen	anatomy-based	20
CRC_ELS density	mean cellular density (cells/mm^2) of ELS detected within CRC specimen	anatomy-based	20
CRC_AID$^+$ ELS count	number of AID$^+$ ELS detected within CRC specimen	staining-based	20
CRC_% GC size of ELS size	mean proportion of GC area to the entire ILS area (%) in CRC specimen	anatomy- and staining-based	9
CRC_% CD20$^+$ cells in ELS	mean % of CD20$^+$ cells within ELS in CRC specimen	staining-based	20
CRC_% Ki67$^+$ cells in ELS	mean % of Ki67$^+$ cells within ELS in CRC specimen	staining-based	20
CRC_% CD27$^+$ cells in ELS	mean % of CD27$^+$ cells within ELS in CRC specimen	staining-based	20
CRCLM_ELS count	number of ELS detected within CRCLM specimen	anatomy-based	22
CRCLM_ELS size	mean size (mm^2) of ELS detected within CRCLM specimen	anatomy-based	17
CRCLM_ELS density	mean cellular density (cells/mm^2) of ELS detected within CRCLM specimen	anatomy-based	17
CRCLM_AID$^+$ ELS count	number of AID$^+$ ILS detected within CRCLM specimen	staining-based	17
CRCLM_% CD20$^+$ cells in ELS	mean % of CD20$^+$ cells within ELS in CRCLM specimen	staining-based	17
CRCLM_% Ki67$^+$ cells in ELS	mean % of Ki67$^+$ cells within ELS in CRCLM specimen	staining-based	16
CRCLM_% CD27$^+$ cells in ELS	mean % of CD27$^+$ cells within ELS in CRCLM specimen	staining-based	16

DIICO module III: Clinical relevance of variables characterizing the ILS/ELS immune phenotype

- Kaplan-Meier estimates were created to stratify patients into risk groups; shown for RFS



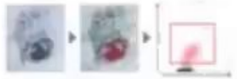
- For NT: strong prognostic power for the variables attributed to % of CD20+ cells and Ki67+ cells as well as for their combination within lymphoid structures; statistically significant stratifications of patients into low- and high-risk groups
- Major discovery to highlight: B cell-enriched and highly proliferative ILS, located within non-tumorous colonic mucosa, show a strong prognostic effect in respect of survival of CRCLM patients
- The immunological imprint of lymphoid structures within normal colon encrypts the information about clinical outcome for patients with metastatic colorectal cancer

Mungenast F & Meshcheryakova A et al, 2020

What is now, what is next:
To harness the power of synergy

Quantitative tissue image cytometry

TissueFAXS



Compendium-wide analysis of transcriptomic datasets

GENEVESTIGATOR

Spatial transcritomics

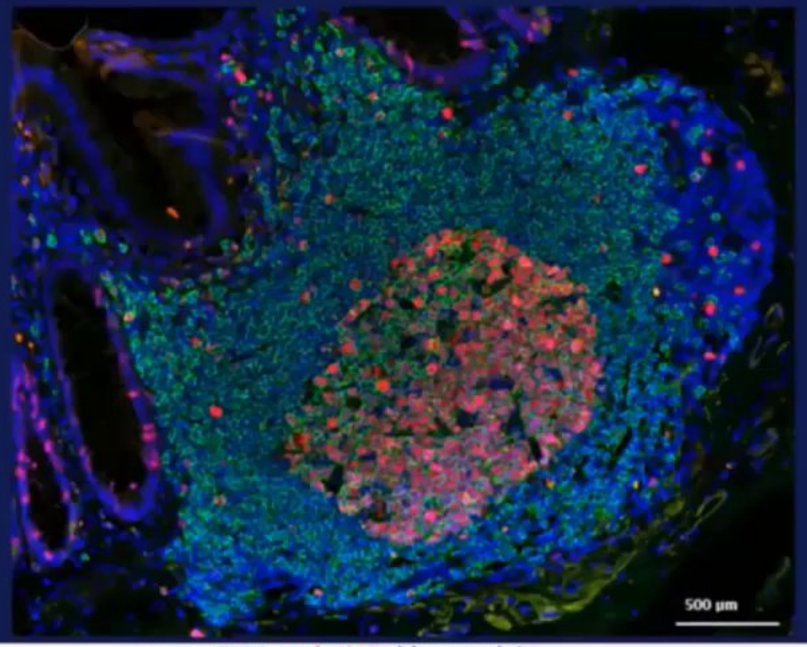
Visium Spatial gene Expression

by 10x Genomics

AI in biomedical research

LymphoidStructureMiner

Unlocking the Germinal Centers: novel biomarkers and targeting strategies



green, CD20; *red*, Ki67; *blue*, nuclei

Acknowledgements

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TissueGnostics, Vienna

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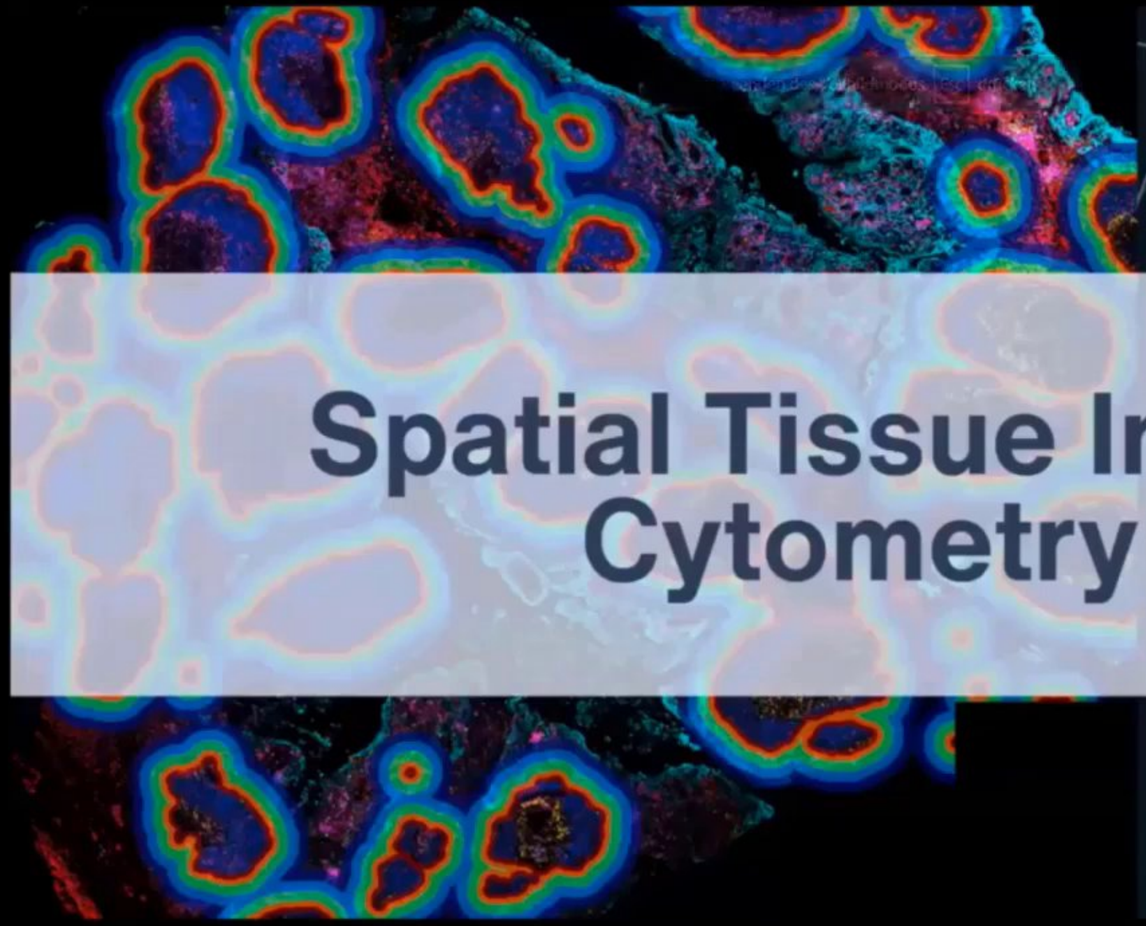
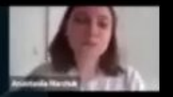
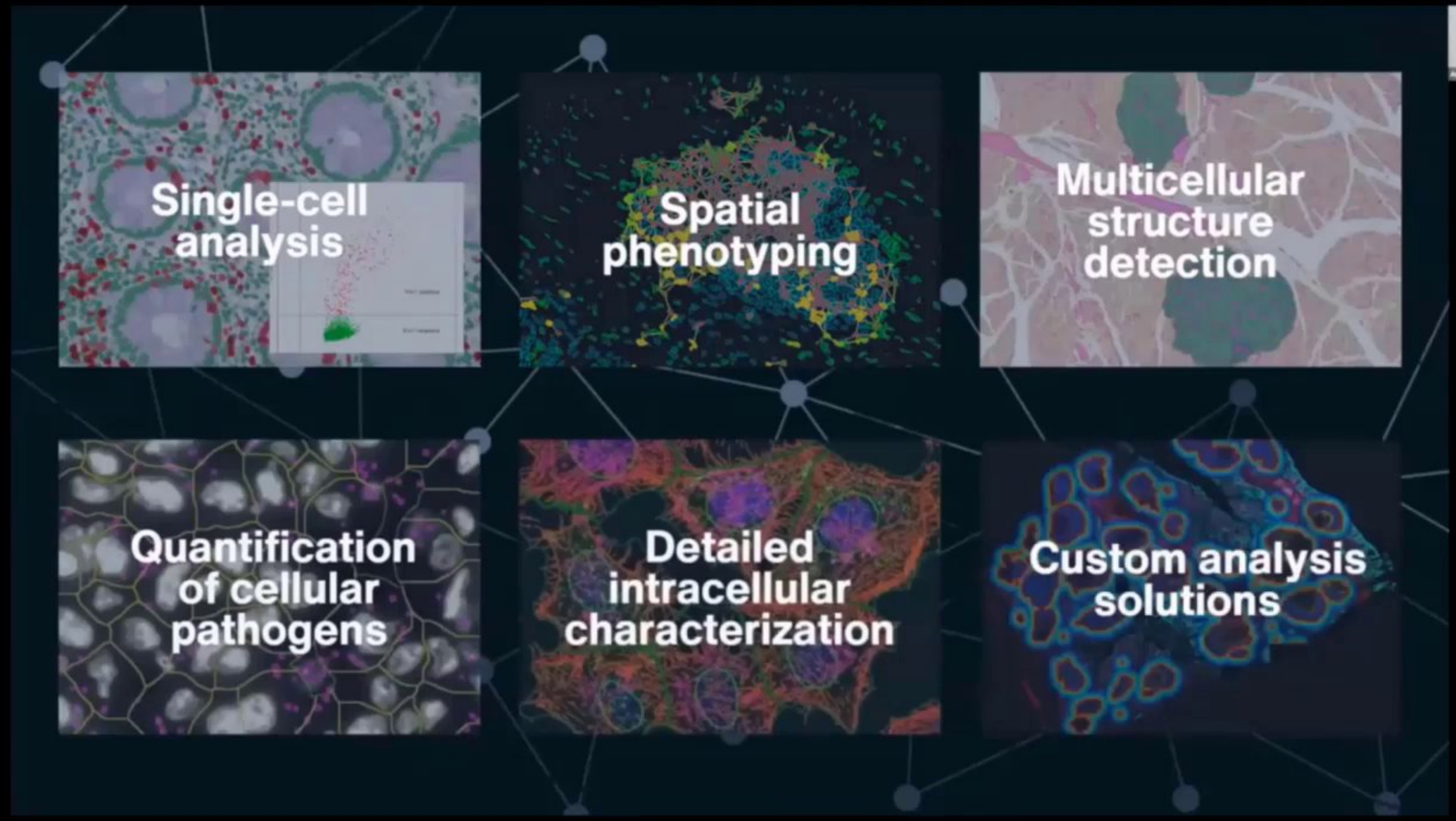


Spatial Tissue Image Cytometry



Anastasiia Marchuk
Junior Product Specialist
TissueGnostics


Single-cell analysis
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Basic Imaging System: TissueFAXS

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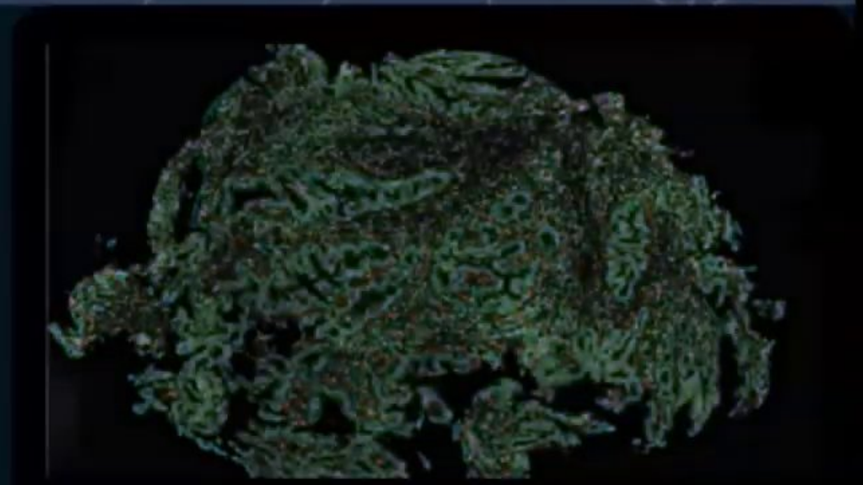
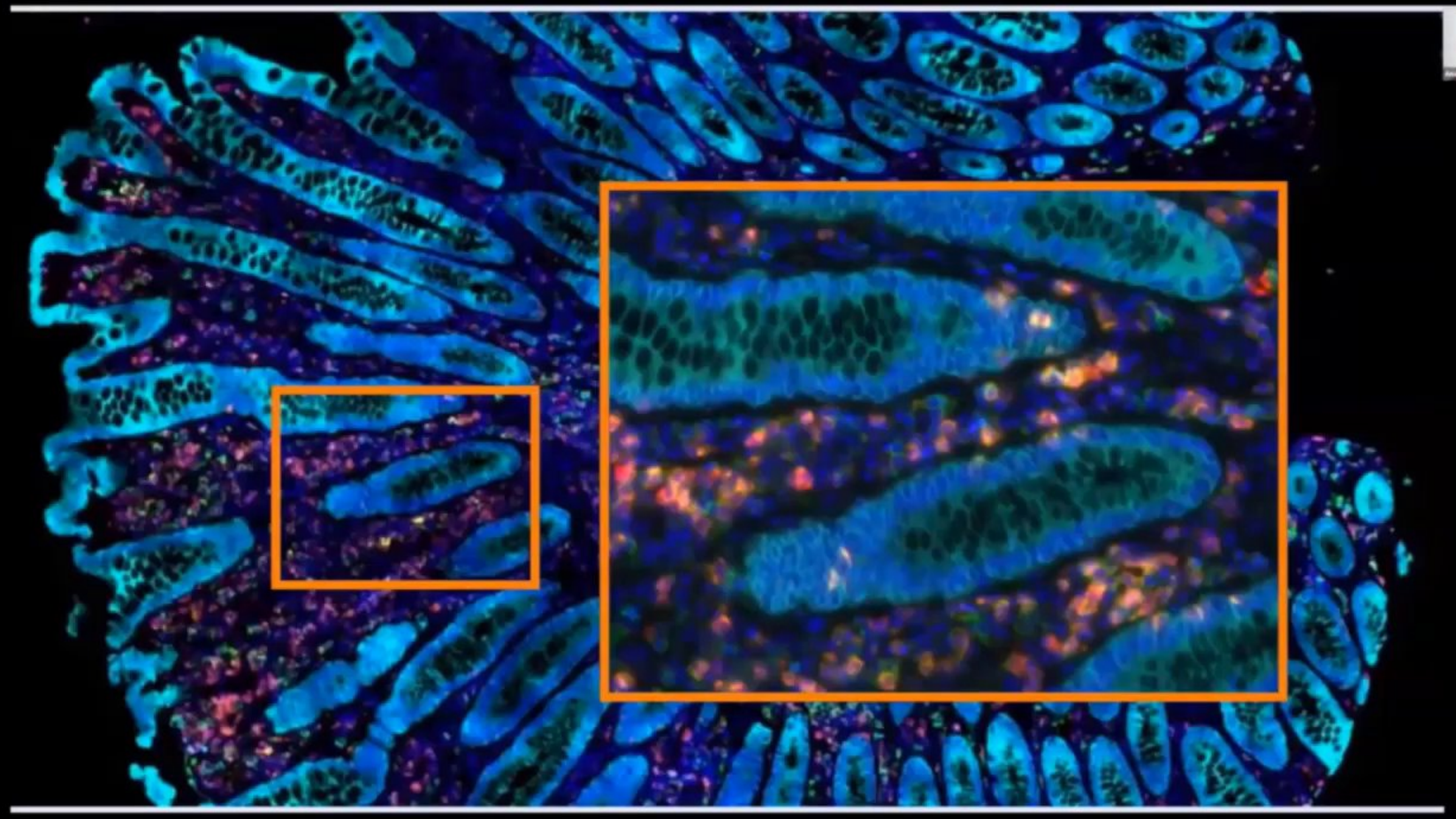
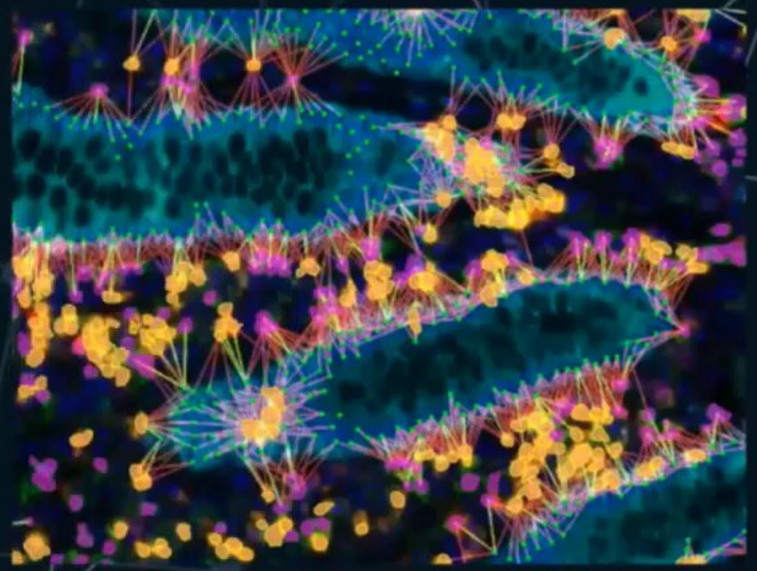




Visualize your Results



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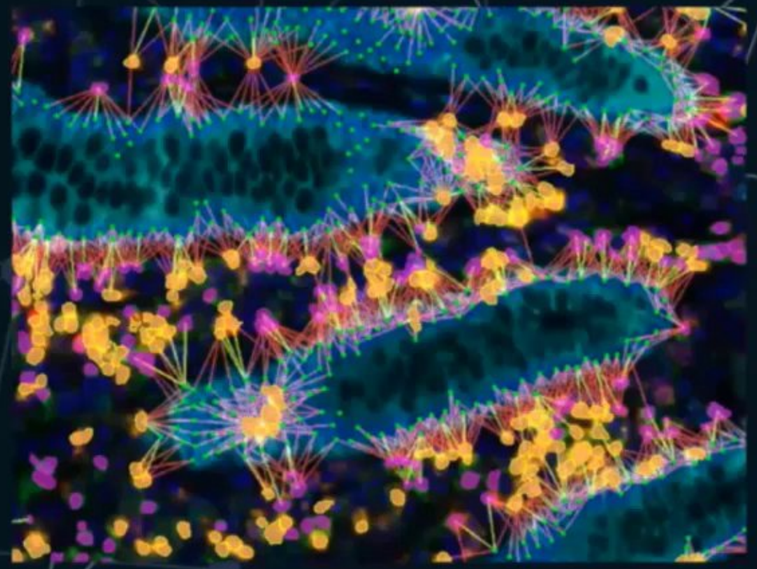

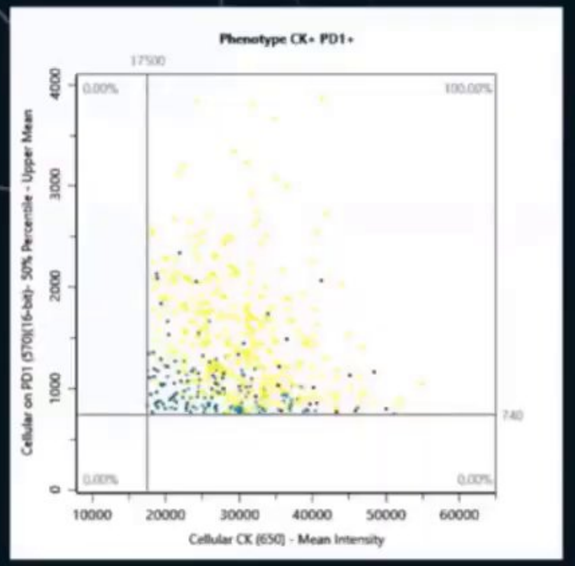


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