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Spatially resolved, single cell atlas of human pancreatic Islets of Langerhans in type 2 diabetes, metabolically healthy obese, and normal controls

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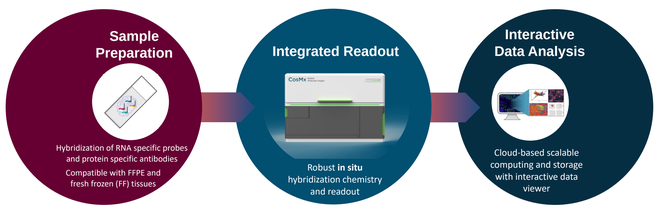
Abstract

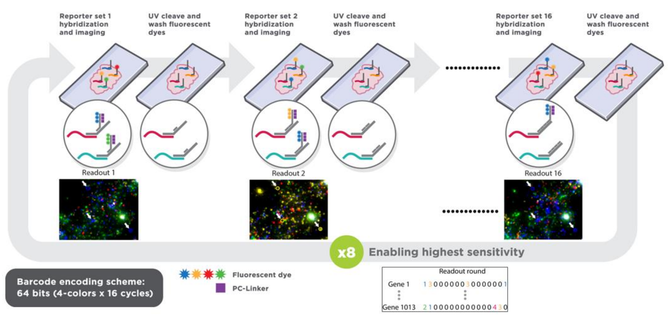
Diabetes mellitus is one of the leading causes of morbidity and mortality in the United States, and rates of type 2 diabetes (T2D) are rising. At the epicenter of the metabolic dysfunction associated with diabetes are the Islets of Langerhans, which encompass thousands of endocrine micro-organs scattered throughout the pancreas that are essential for the maintenance of normal glucose homeostasis. The majority of diabetes research has been conducted in rodent models, and few single cell transcriptomic studies on dissociated tissue have been performed on healthy or diseased human islets. Those that have revealed distinct subpopulations of alpha, beta, and delta cells, which are the major islet cell types. However, the anatomical bases of these subpopulations – and how they are altered in the setting of diabetes mellitus – remains unknown. Here we use NanoString's CosMx™ Spatial Molecular Imager (SMI) to generate a spatially resolved, single cell transcriptome of human pancreatic islet cells in type 2 diabetes mellitus (T2D), metabolically healthy obese (MHO), and normal controls. CosMx™ enabled mapping of the subpopulations of beta, alpha, and delta cells in pancreatic islets in spatial context, and, crucially, provided resolution on the relative abundance and distribution of these subpopulations in healthy controls and their re-distribution in the context of MHO and T2D. Furthermore, we characterized the abundance and distribution of immune cell populations that were present in the tissue, providing insight into the ongoing immune response in the context of tissue damage. This spatially resolved, single cell atlas of human pancreatic islets gives unprecedented insight into the functioning of these endocrine micro-organs in the context of health, and provides valuable characterization of the molecular and cellular changes that are associated with tissue dysfunction and disease.

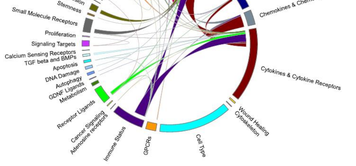
Introduction

CosMx™ SMI assay overview

CosMx™ SMI is a single instrument solution for subcellular spatial analysis







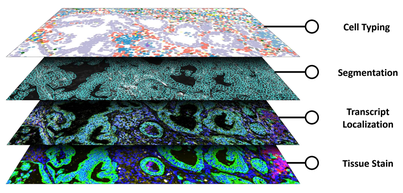
1000-Plex RNA Panel for Cell Typing and Cell-Cell Interaction Studies

Applications	# of genes
Cell typing and mapping	243 genes
Cell state and function	269 genes
Cell-cell interaction (ligand-receptor)	435 genes
Hormone activities	46 genes

Custom panel spike-in

30 genes marking islet transitional cell types[1,2]

Multi-omic layers of information key for single-cell spatial analysis



Diabetes background

Obesity and diabetes in the United States

- 1 in 11 individuals are severely obese (BMI >40)
- 1 in 10 individuals have diabetes (~90% type 2)
- Small population of metabolically healthy obese (MHO) individuals
  - Normal biomarkers (lipid, glucose, etc.)
    - Thought to be transient state

What is the link between obesity and the development of metabolic abnormalities like diabetes?

While scRNA-seq experiments have been performed to investigate the development of diabetes, none to date have looked at those changes in individual islets in their morphological context – or at what transcriptional changes are occurring in the islets of MHO individuals

Study design

Goals

- Spatial atlasing of pancreatic islets in diabetic and non-diabetic patients
  - Map subpopulations of beta, alpha, and delta cells in spatial context
    - Determine abundance and distribution of subpopulations in normal controls and their re-distribution in diabetes

Patient population

- 9 transplant donors – age, race, and sex-ratio matched
  - 3 normal controls
  - 3 type 2 diabetes (T2D)
  - 3 metabolically healthy obese (MHO)
- Pancreas was rejected for transplant either due to no recipient or based on visual inspection by surgeon

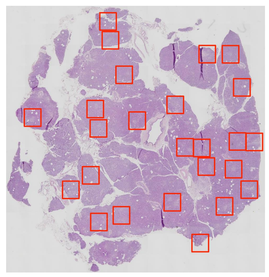
CosMx SMI reagents

- RNA 1K-plex panel + custom panel of 30 genes[1,2]
- Morphology markers: B2M/CD298, PanCK, glucagon, insulin antibodies

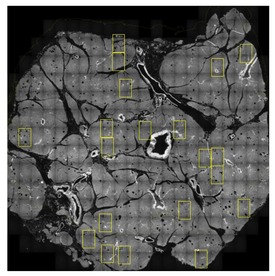
CosMx™ SMI profiling of pancreatic tissue

Selecting islet regions within pancreatic tissue

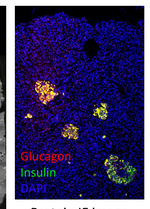
NP-3 (normal)



H&E stain for FOV selection

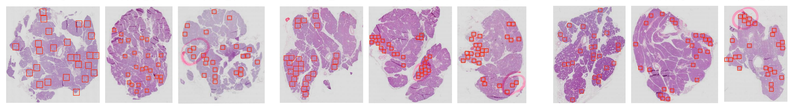


CosMx™ preview scan



Protein IF image after CosMx™ (detail)

Normal			MHO			T2D		
NP-3	NP-10	NP-17	NP-1	NP-8	NP-22	NP-5	NP-9	NP-27



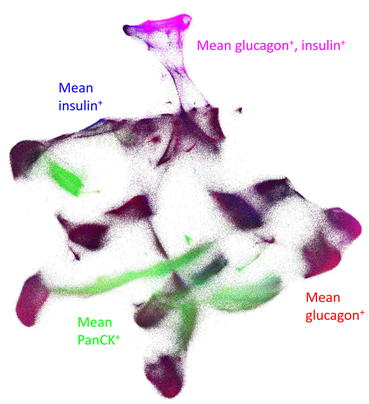
H&E stains for FOV selection

~1.2 M cells detected across nine FFPE slides

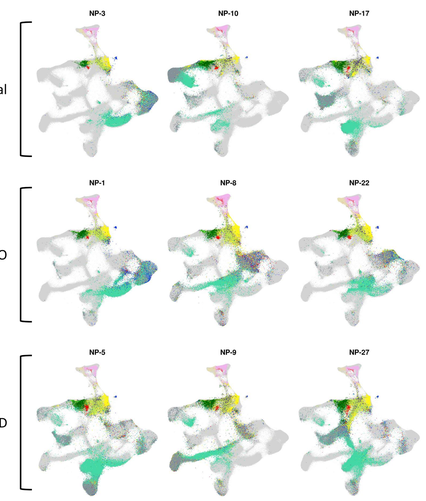
Data summary	
Tissue type	FFPE Human pancreas
Panel	960 gene targets + 30 custom genes
Number of sections in final analysis	9
Total tissue area analyzed (XY plane)	~112 mm²
Number of fields of view (FOVs)	184
Number of total cells	**1,165,105**
Number of cells passed QC	1,162,210
% of cells passed QC	99.8%
Number of genes detected	704
Transcripts detected	497,476,584
% of transcripts assigned a cell	95.9%
Mean transcripts per cell	409
Mean negative counts per cell	0.969

Non-spatial UMAPs

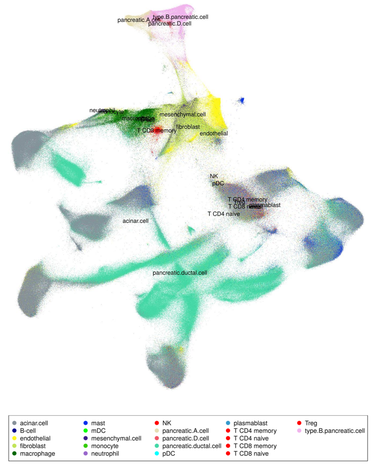
Immunofluorescence UMAP



Cell type UMAP - by patient

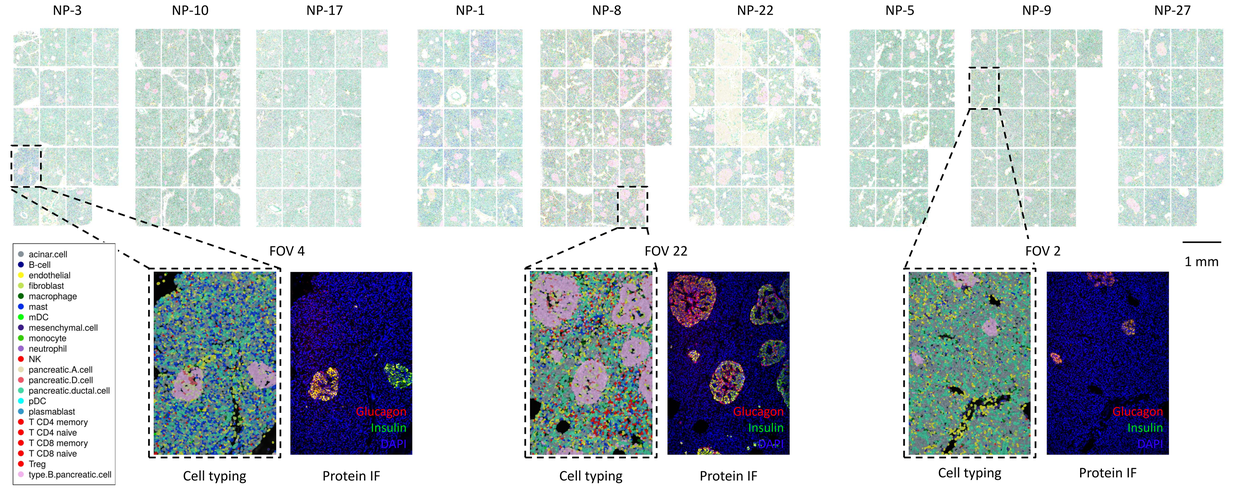


Cell type UMAP - all patients

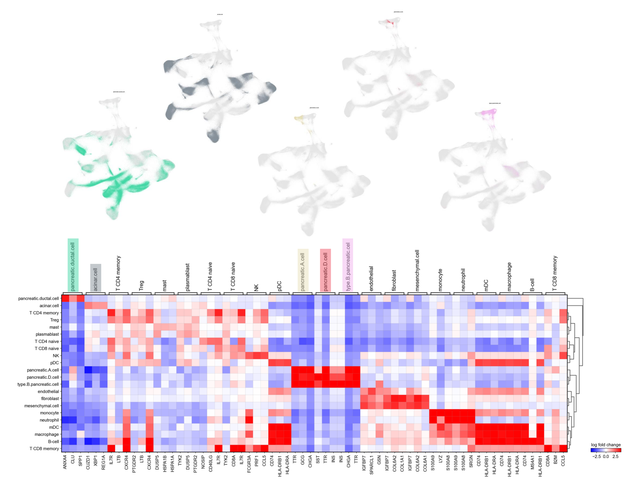


Spatial map of cell types

Normal	MHO	T2D
NP-3	NP-1	NP-5
NP-10	NP-8	NP-9
NP-17	NP-22	NP-27

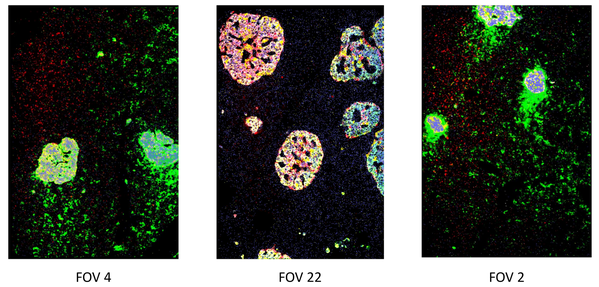


Heatmap of marker genes + cell type clustering

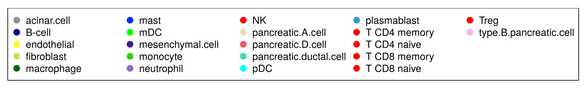


Spatial mapping of marker gene transcripts

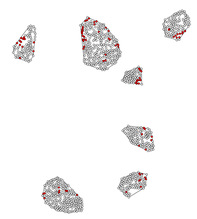
Normal	MHO	T2D
NP-3	NP-8	NP-9



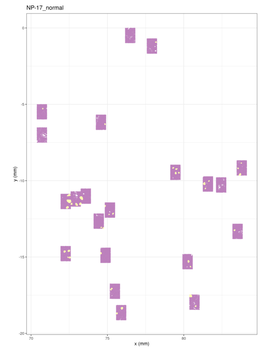
Protein IF: Glucagon Insulin Transcripts: GCG INS SST



Islets defined by dense clusters of endocrine cells



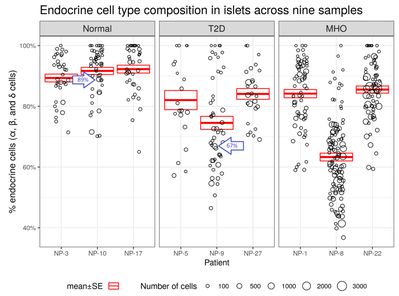
Example islet identification
NP-17 (normal)
39 islets



1. Identify dense clusters of α, β, and δ cells
2. Define convex hull of these cells as islet region
3. Assign any other cells in the enclosed space to the islet

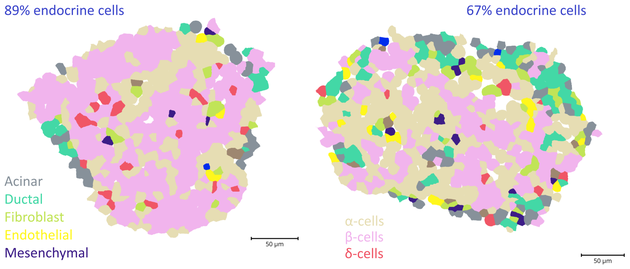
463 islets identified across nine samples

Endocrine cell type composition in islets across nine samples

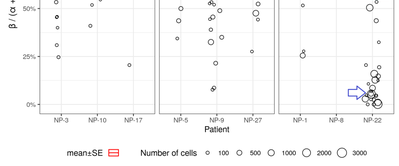


NP-10 (normal)
89% endocrine cells

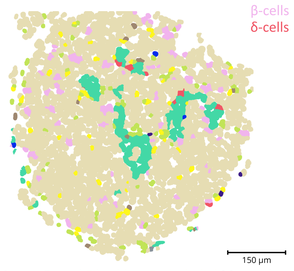
NP-9 (T2D)
67% endocrine cells



Balance of β- and α-cells in islets across nine samples

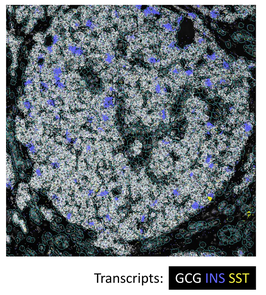


NP-22 (MHO)
α-cell dominant islet



Acinar Ductal Fibroblast Endothelial Mesenchymal

NP-22 (MHO)
α-cell dominant islet (detail)



Transcripts: GCG INS SST

Conclusions

- Profiled ~1.2 million cells across nine FFPE pancreatic tissue slides with NanoString's CosMx™ SMI
  - Mapped subpopulations of beta, alpha, and delta cells in spatial context
- The percentage of endocrine cell types in the islet is higher for normal individuals than type 2 diabetic (T2D) or metabolically healthy obese (MHO)
- Islet cell type composition varies across disease and tissue type
  - For instance, the islets of one MHO individual (NP-22) were dominated by alpha cells

References

[1] Matsuoka et al. Mafa Enables Pdx1 to Effectively Convert Pancreatic Islet Progenitors and Committed Islet α-Cells Into β-Cells In Vivo. Diabetes. 2017 May;66(5):1293-1300. doi: 10.2337/db16-0887.

[2] Wei et al. Antagonistic Glucagon Receptor Antibody Promotes α-Cell Proliferation and Increases β-Cell Mass in Diabetic Mice. iScience. 2019 Jun 28;16:326-339. doi: 10.1016/j.isci.2019.05.030.

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