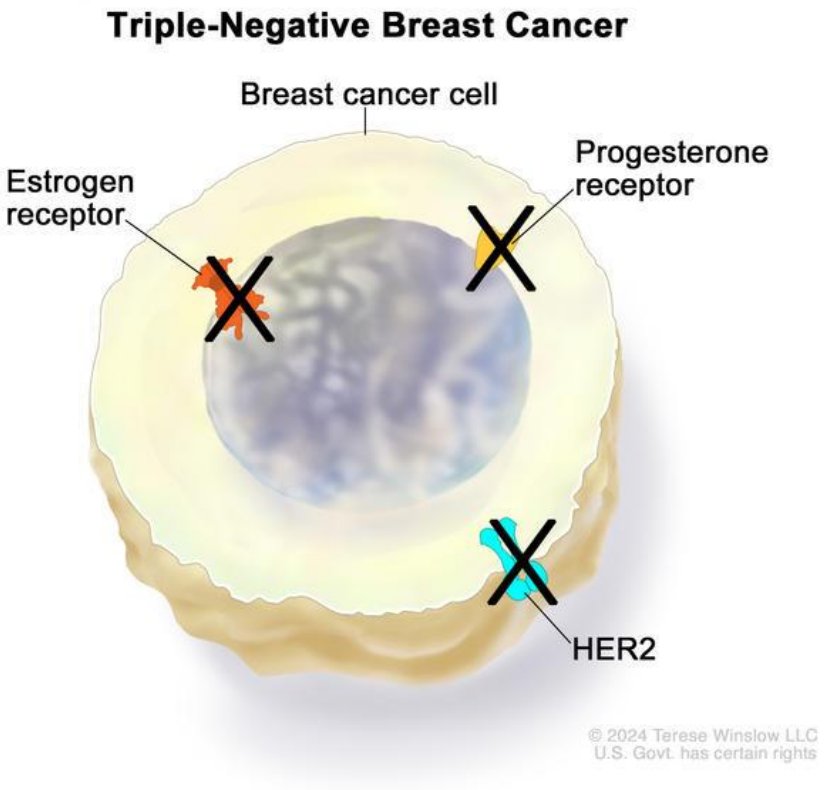
Molecular Imaging and Spatial Multi-Omics for personalized Breast Cancer diagnosis and treatment

Ph.D. End Of Year Seminar 2025/2026

Ph.D. candidate Cecilia Riani XL cycle

Supervisors: Professor Giorgio Baiocco, Professor Alessandro Lascialfari

Molecular Imaging and Spatial Multi-Omics for personalized Breast Cancer diagnosis and treatment



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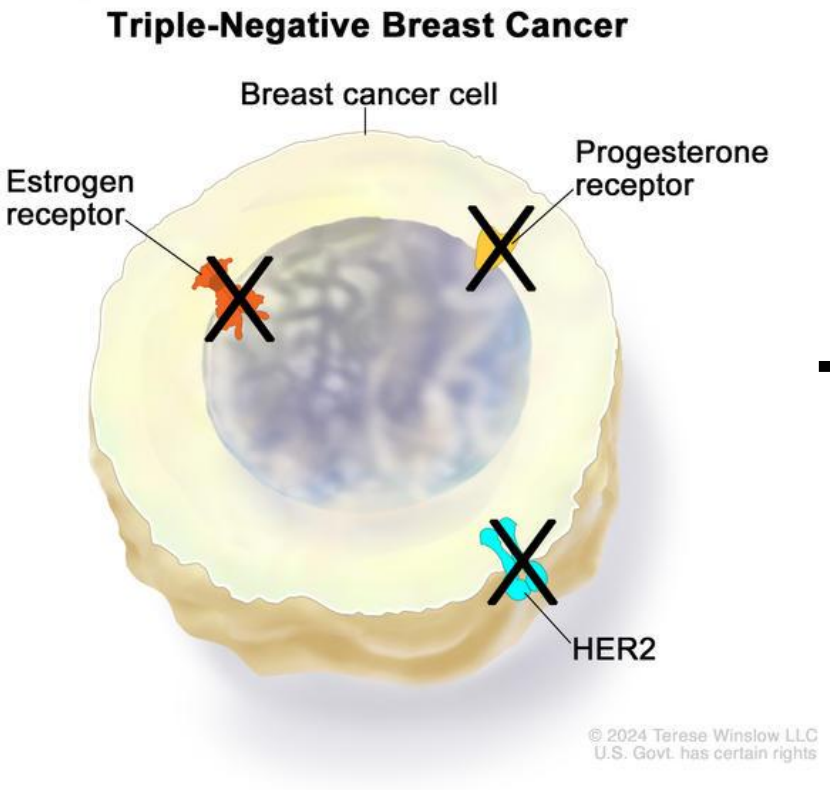

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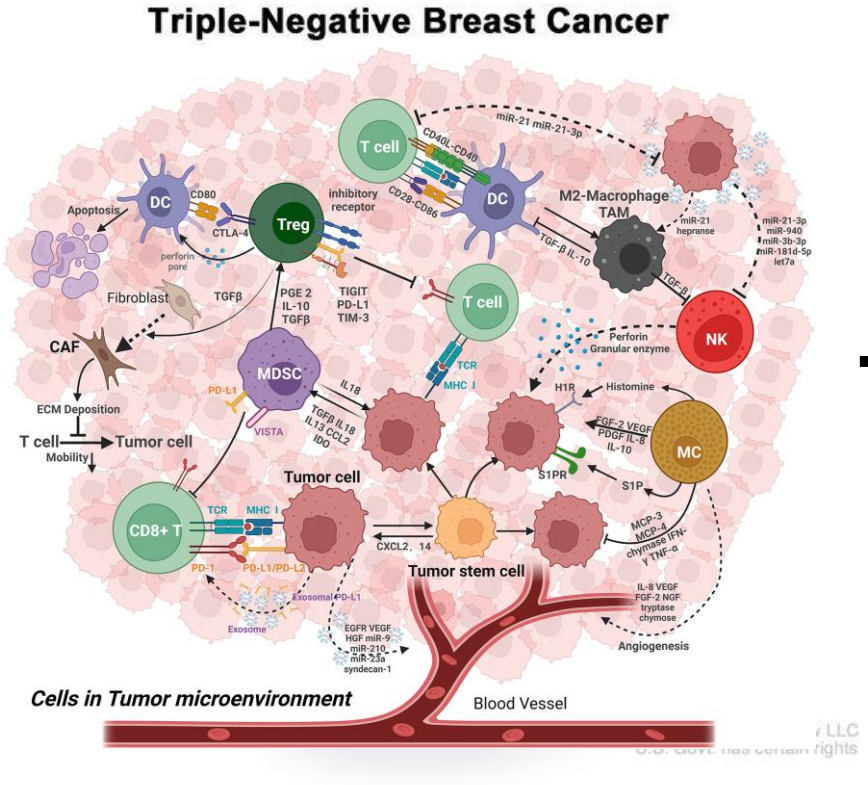
Radiotherapy

Molecular Imaging and Spatial Multi-Omics for personalized Breast Cancer diagnosis and treatment



Tumor Immune Microenvironment

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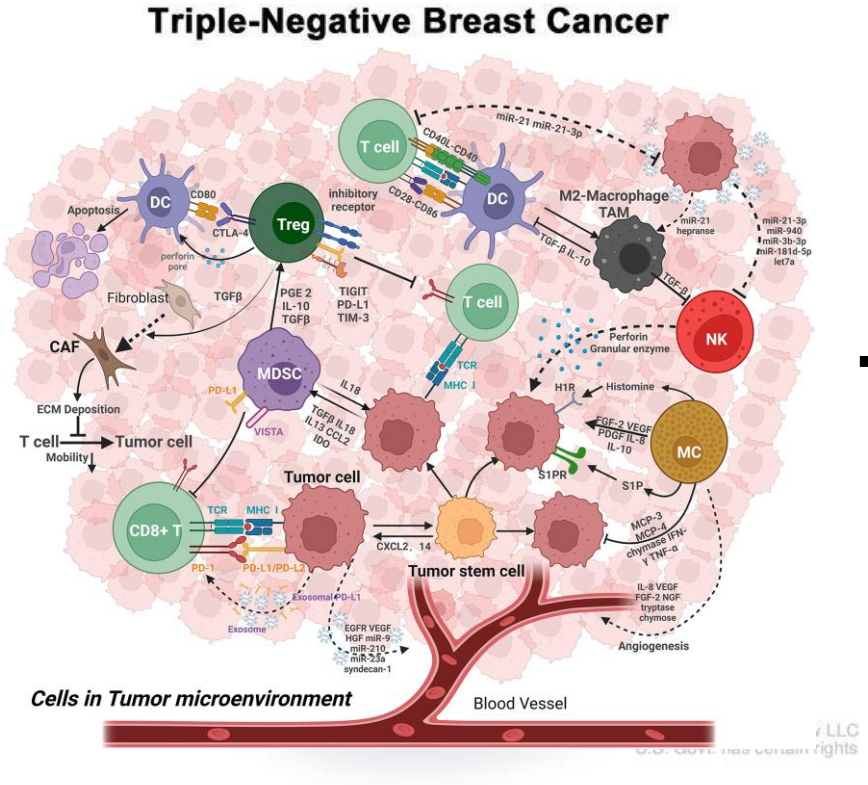

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Immune system activation

Immune system suppression

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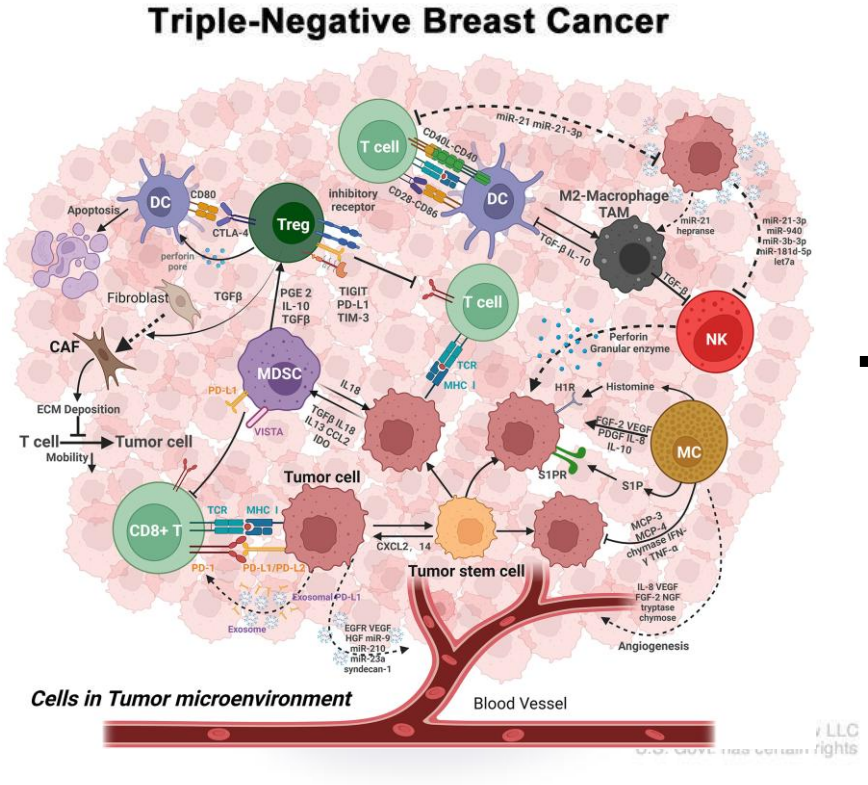

Tumor Immune Microenvironment

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Immune system activation

Immune system suppression

Identify new local molecular or cellular biomarker of treatment response

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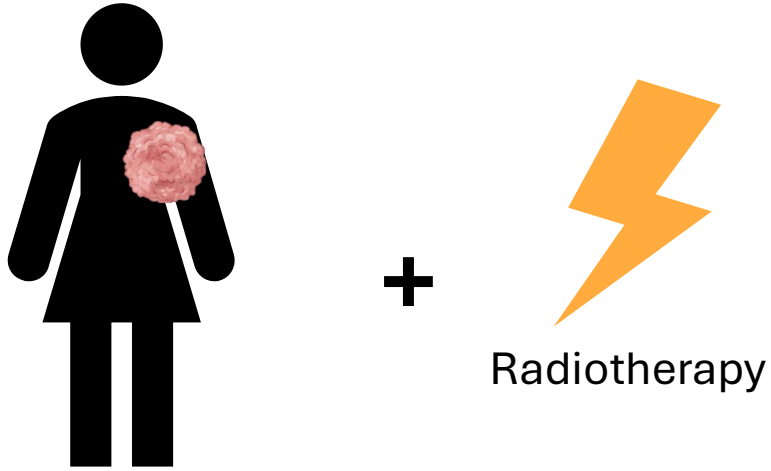

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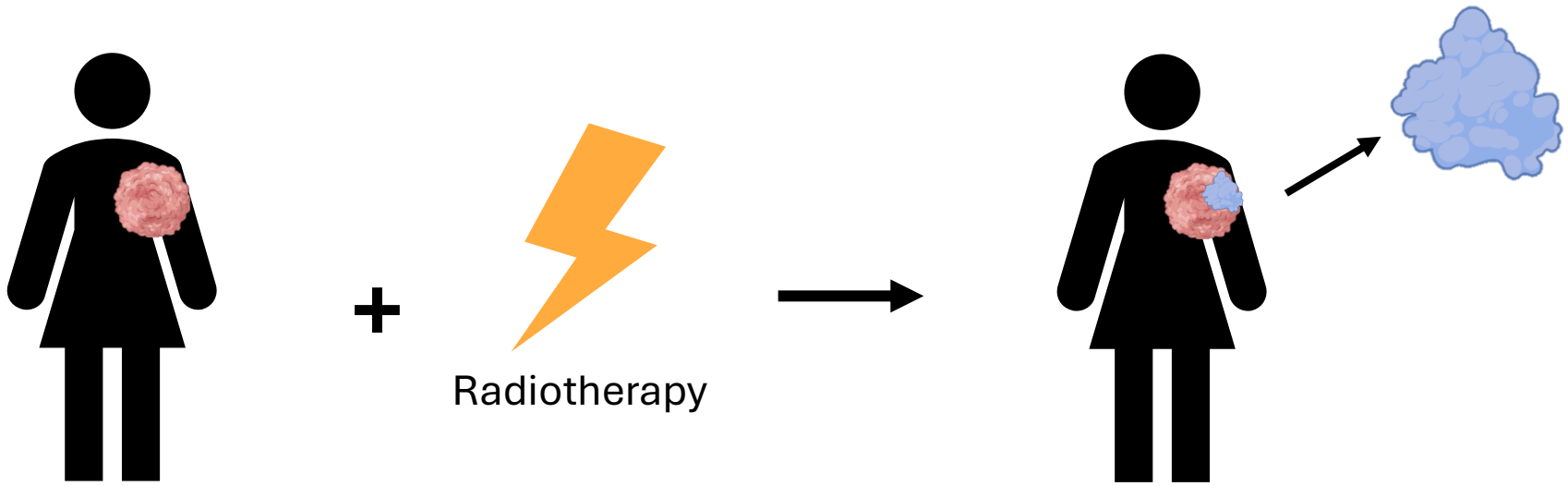

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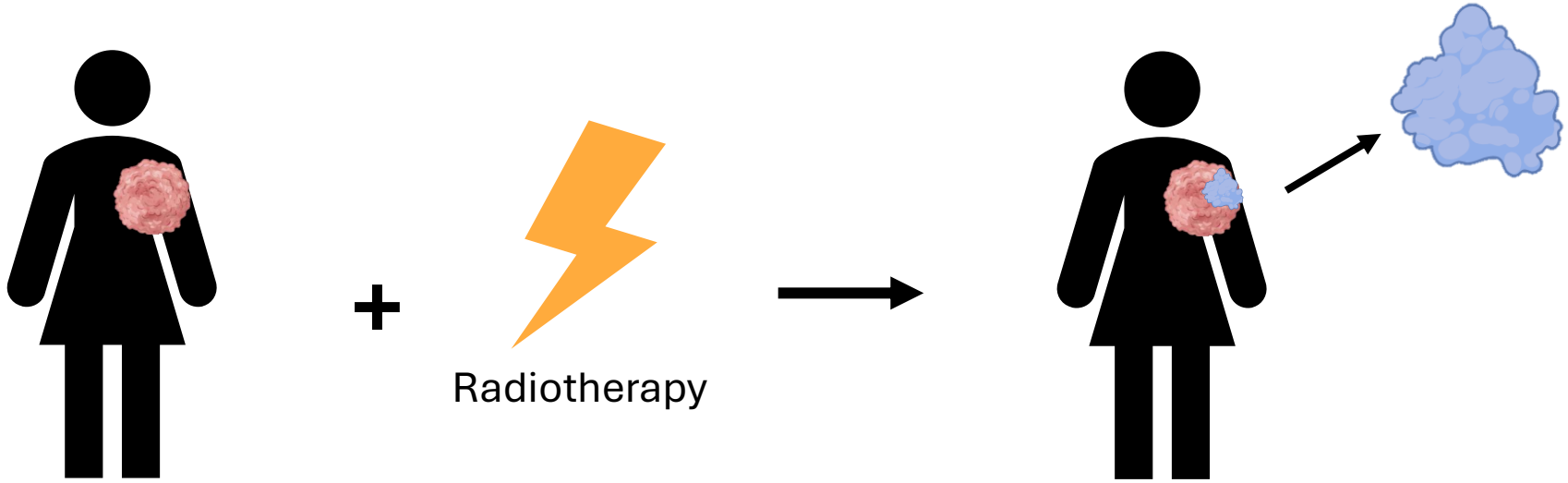

Accurate and non invasive in vivo molecular imaging system of the breast

Molecular Imaging and Spatial Multi-Omics for personalized Breast Cancer diagnosis and treatment

IMAGEOMICS : *Identifying Molecular, Cellular and Imaging Signatures of Breast Cancer Heterogeneity to Improve Personalized Therapeutic Strategies*

1. Identify new molecular or cellular biomarker of treatment response

Integration of Spatial Multi-Omics technologies for Breast Cancer imaging before and after radiotherapy

2. Accurate and non invasive in vivo molecular imaging system of the breast

Using an X-ray fluorescence Computed Tomography system with functionalized metallic nanoparticles (NPs)

X-ray fluorescence Computed Tomography for Breast Cancer imaging and treatment

- X-ray fluorescence Computed Tomography combines the high functional sensitivity of imaging system as PET, SPECT to the high resolution of Computed Tomography

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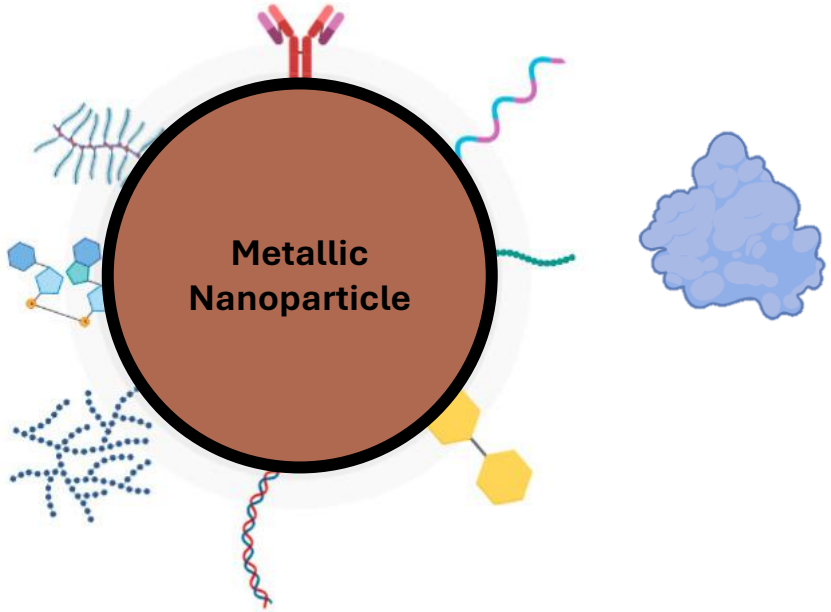

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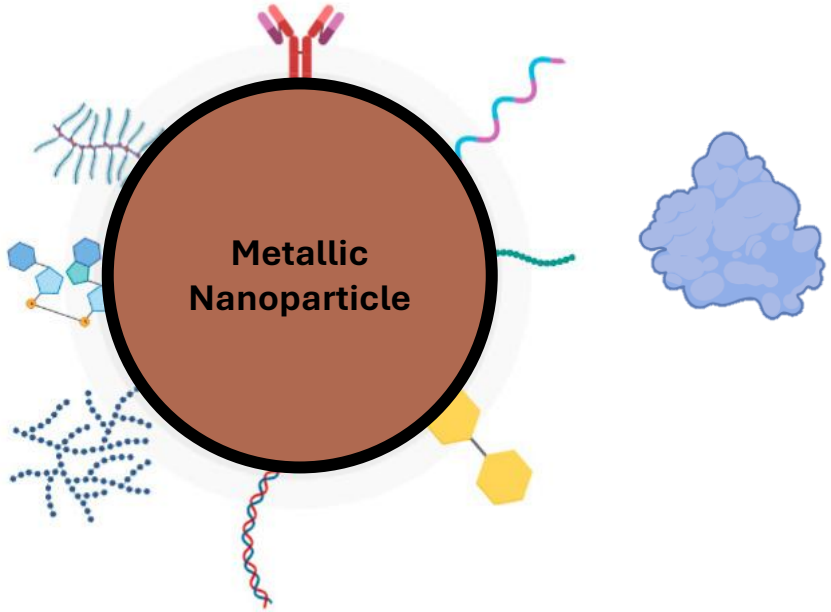
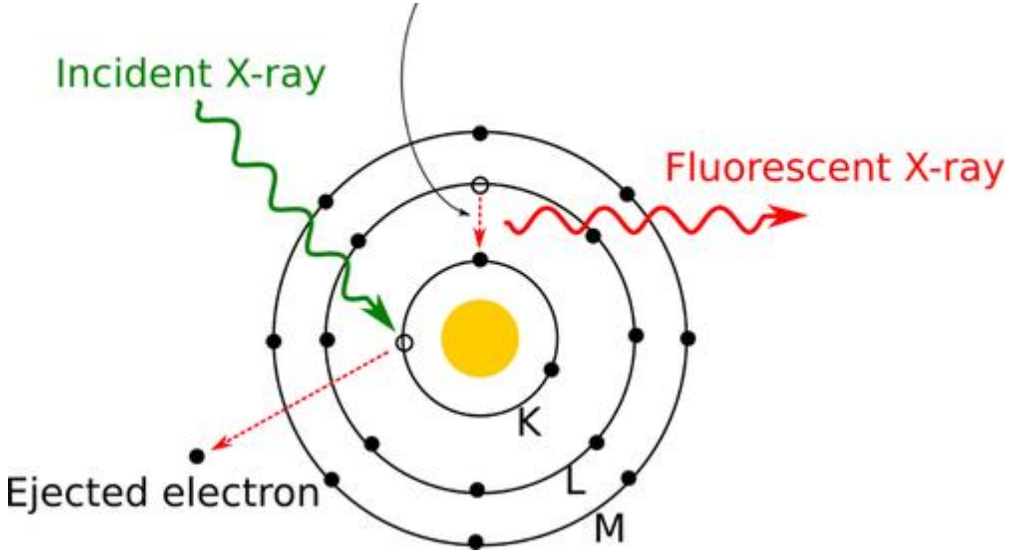


X-ray fluorescence (XRF) imaging



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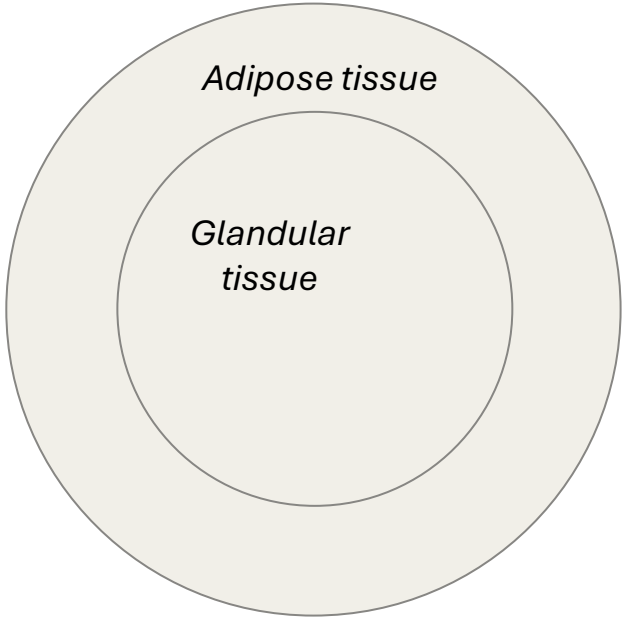
Breast tissue



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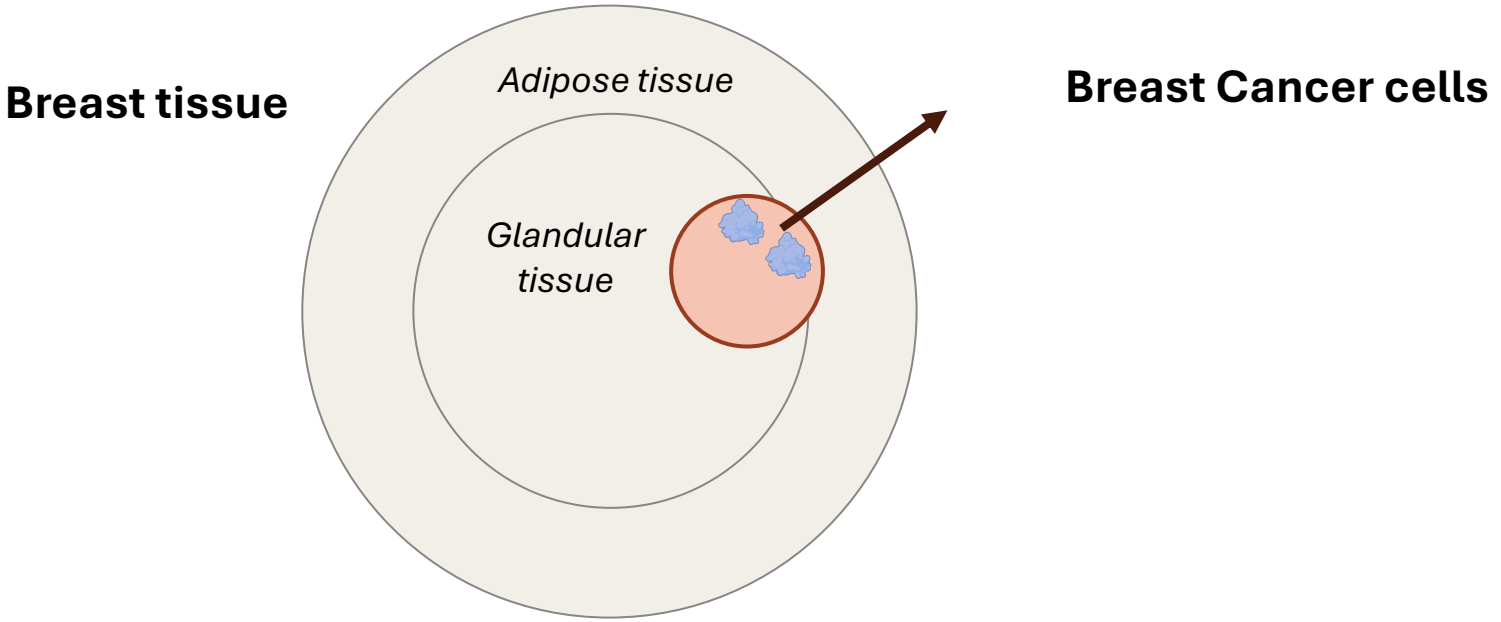

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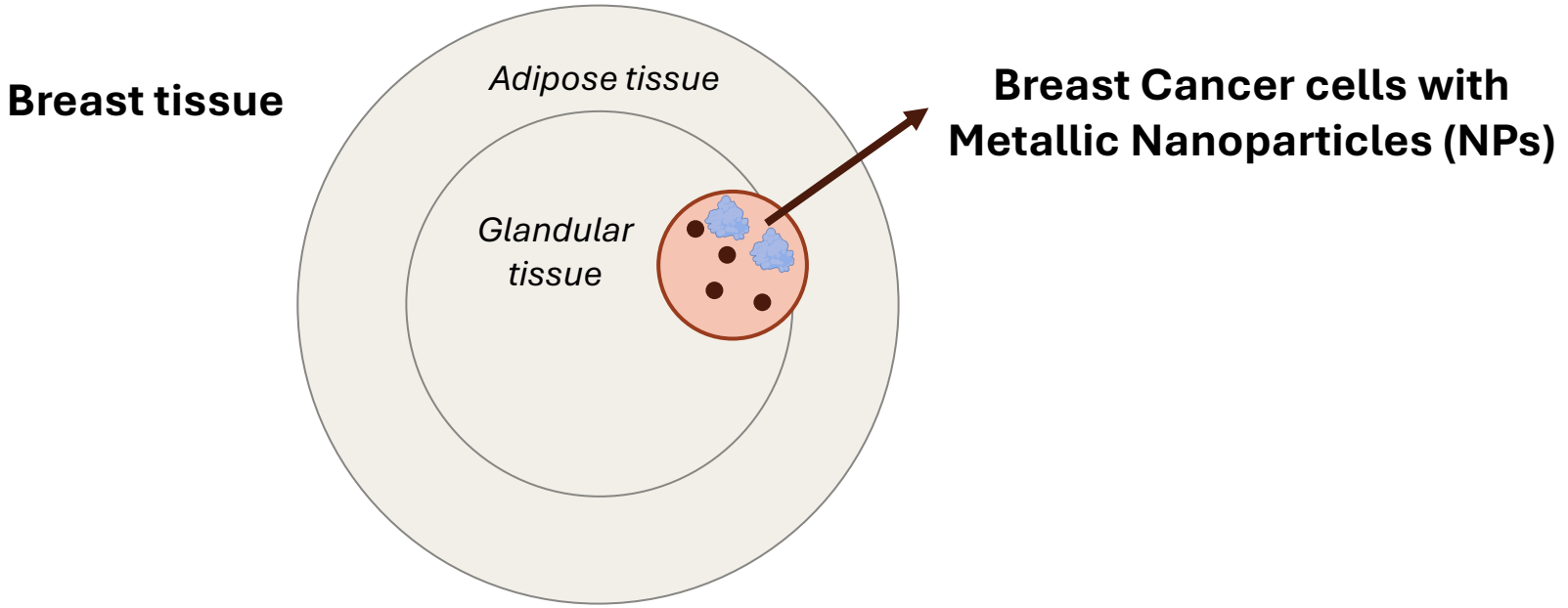

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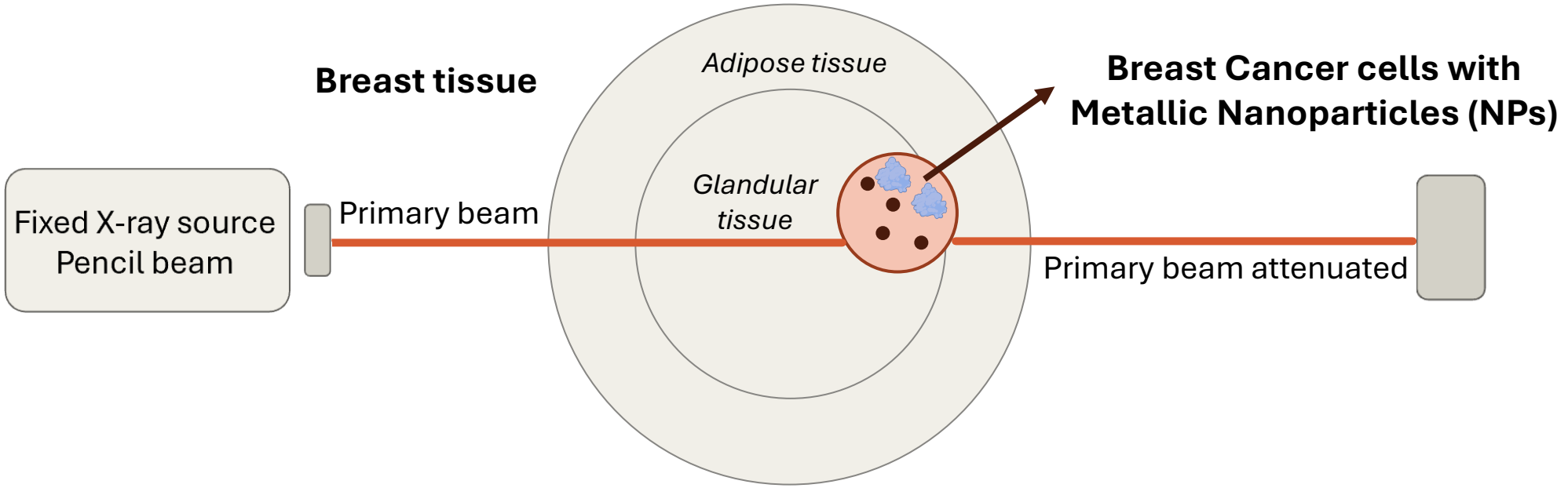

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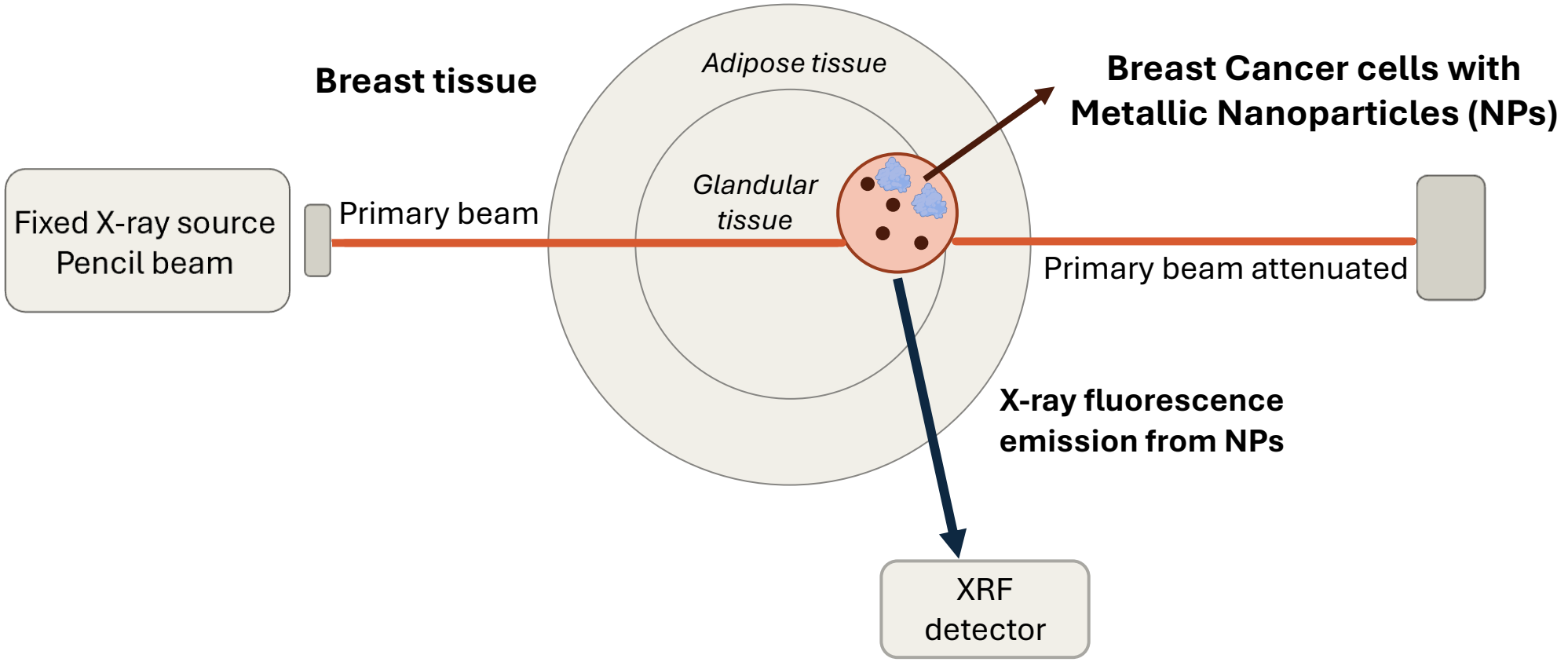

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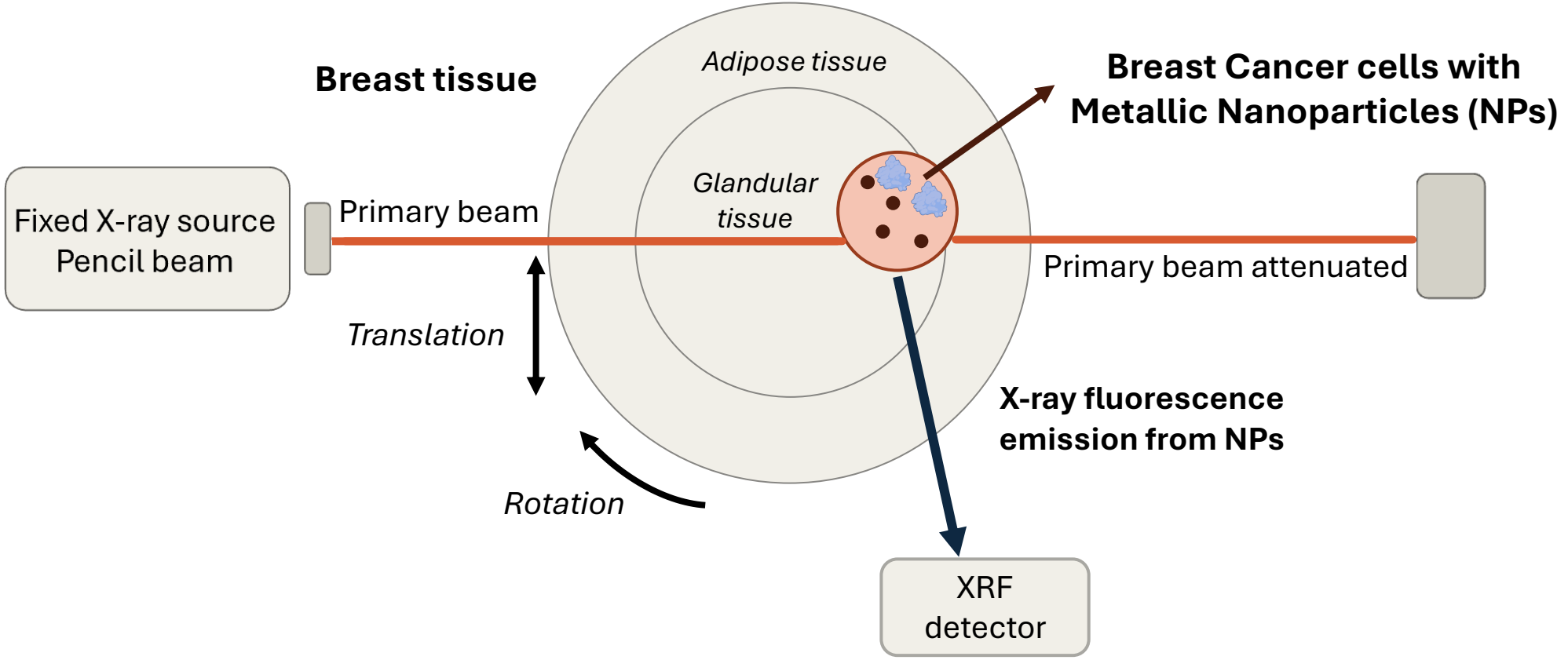

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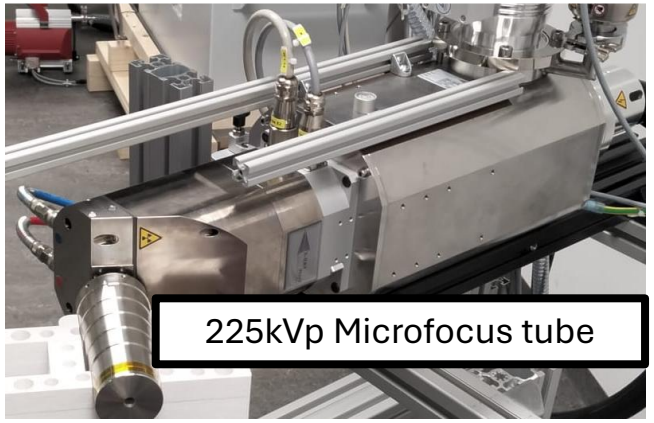

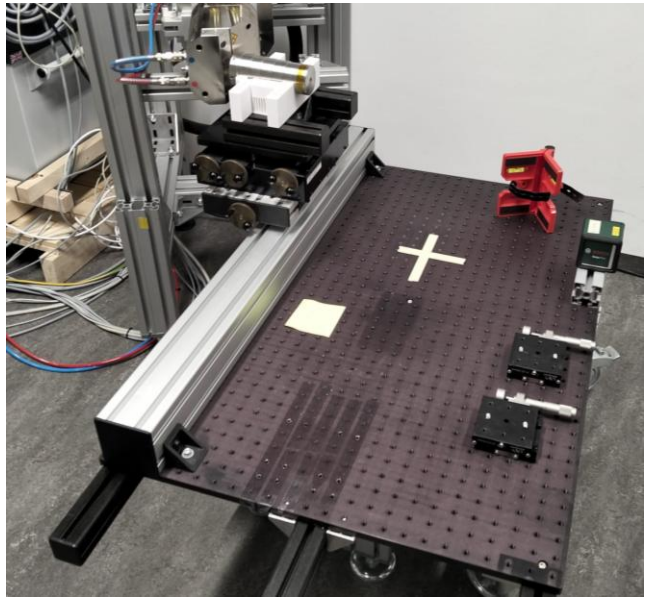

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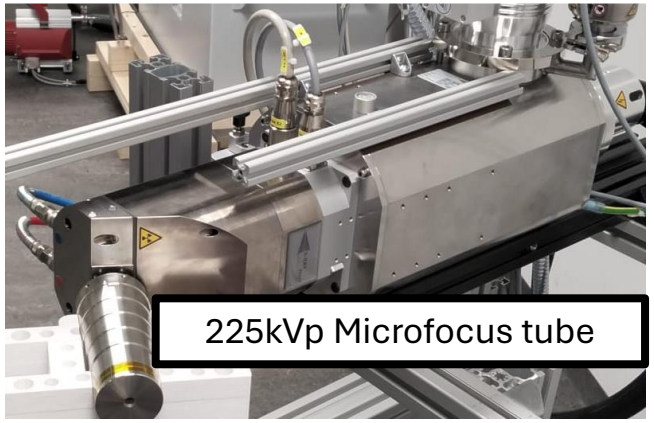

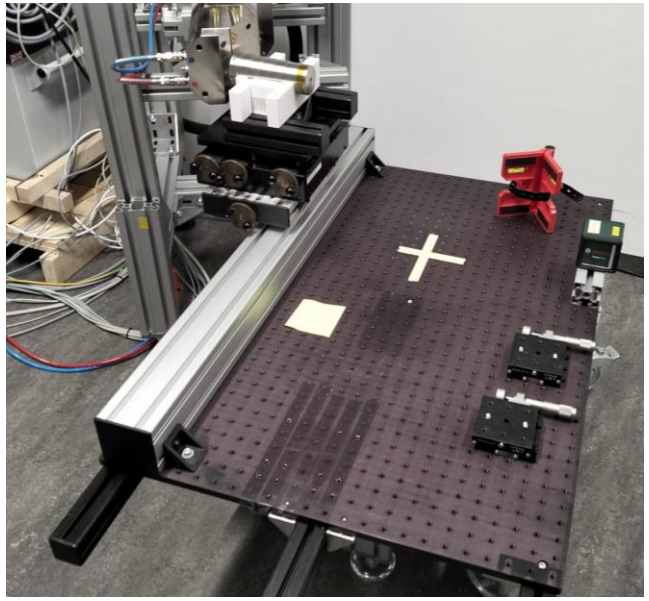

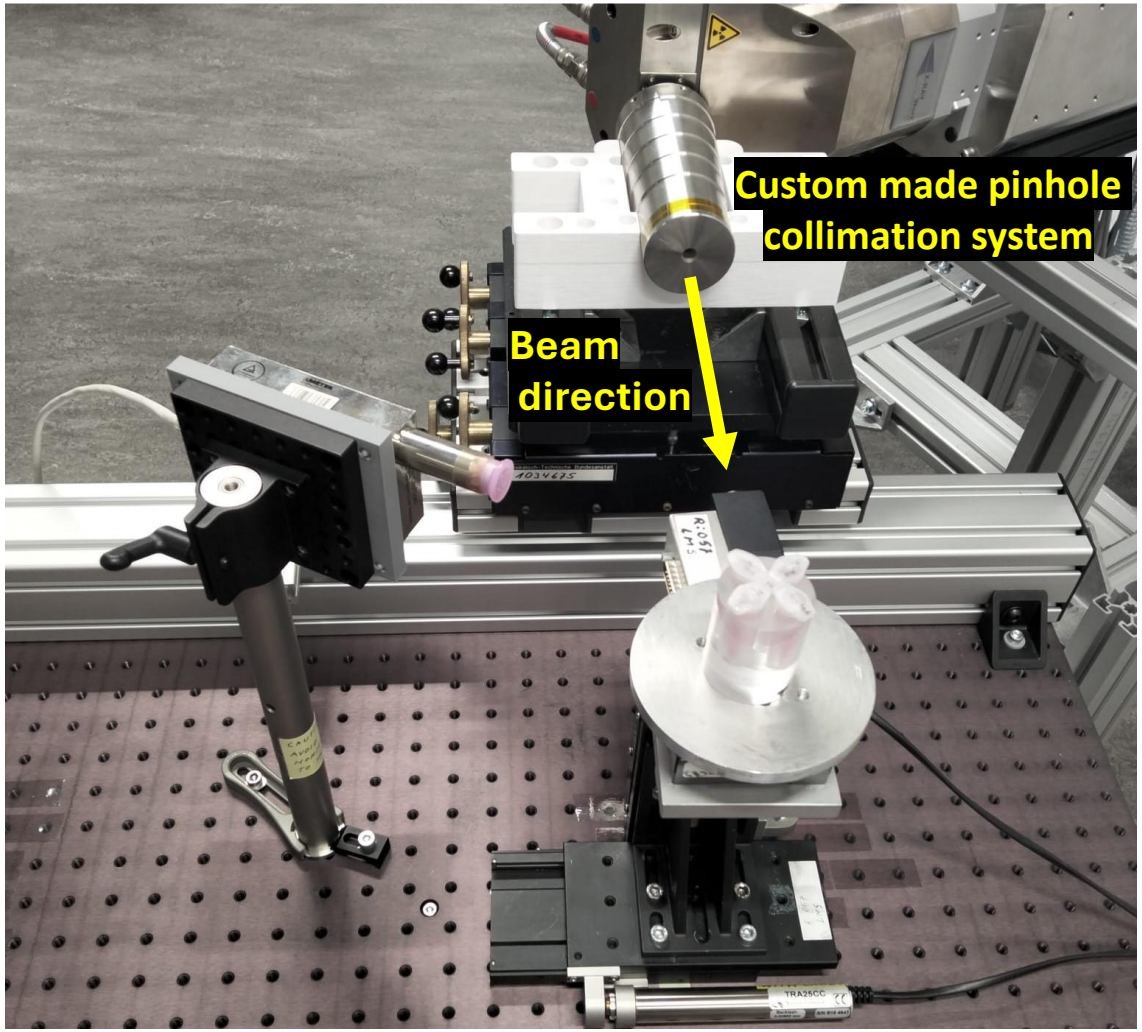

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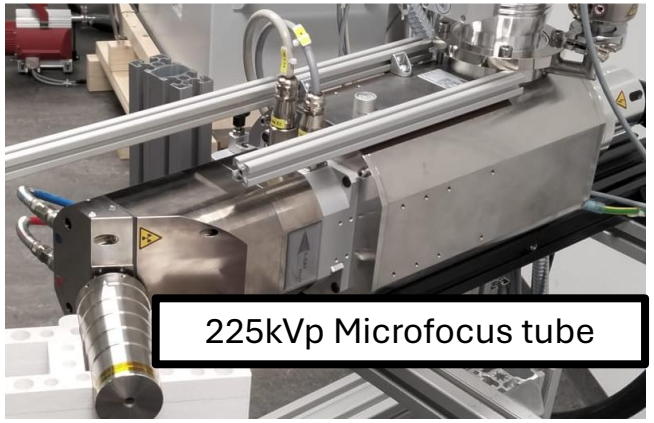

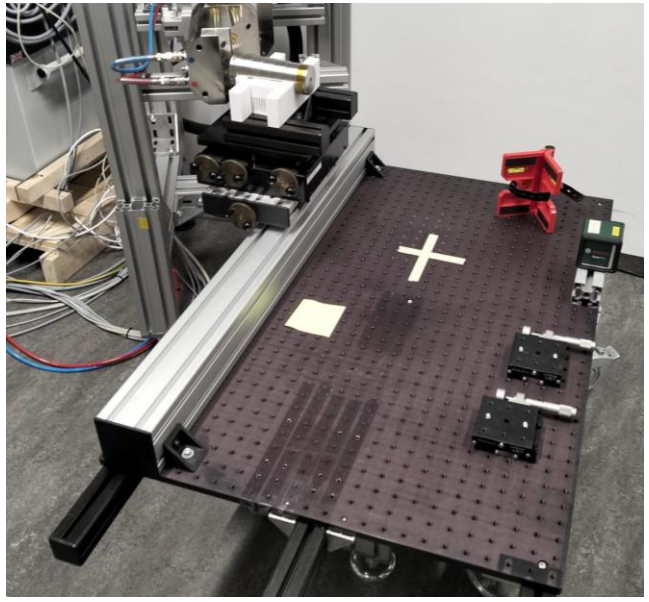

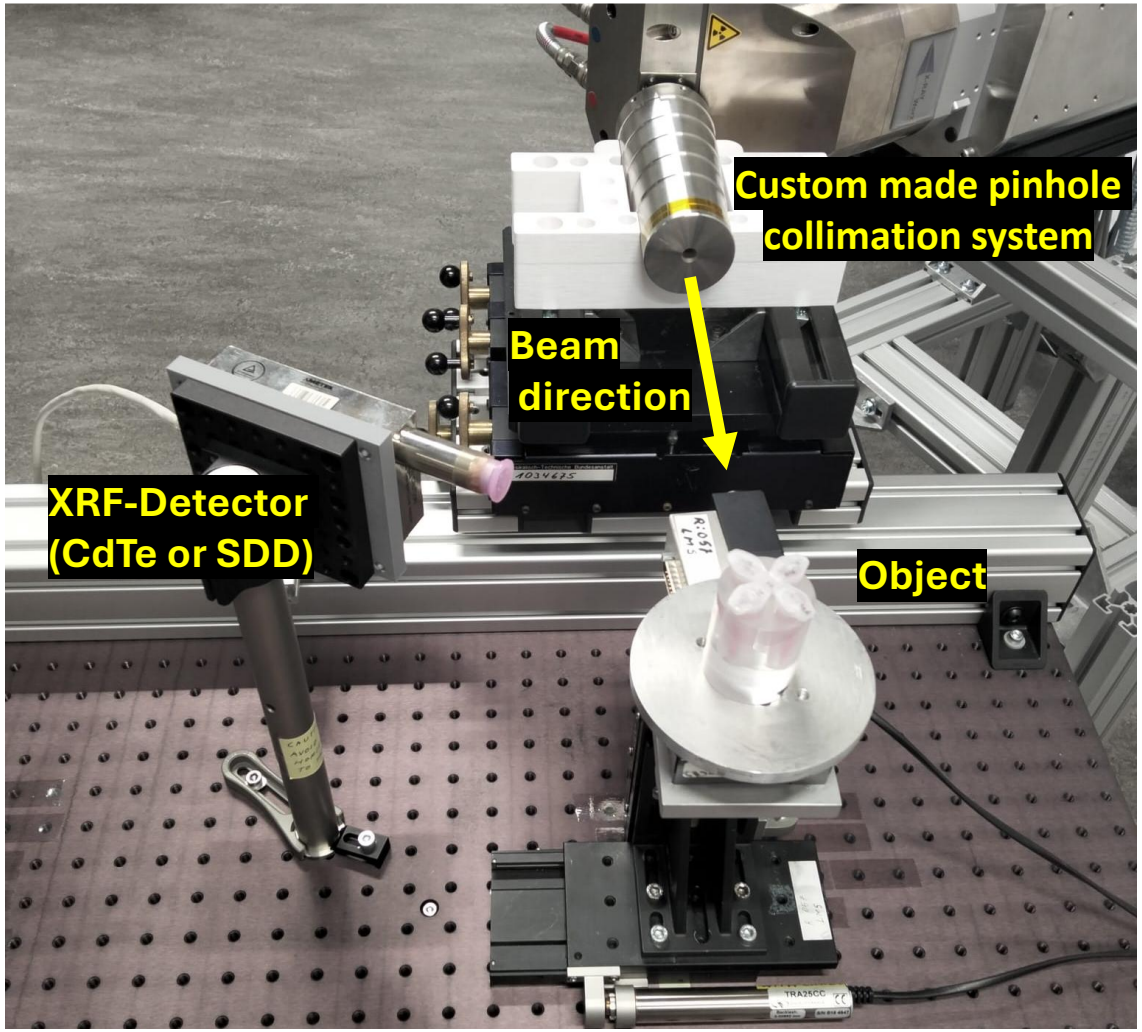

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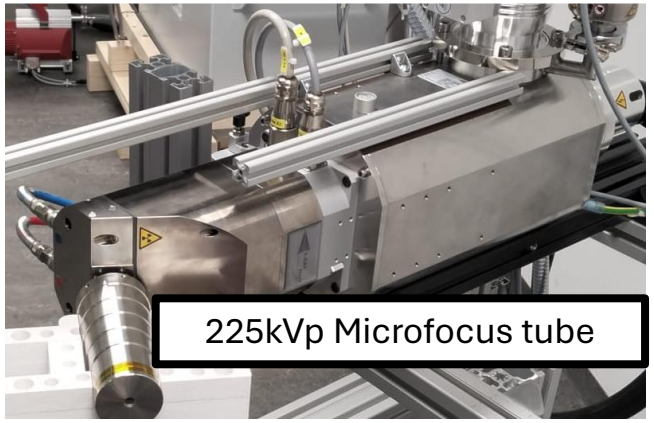
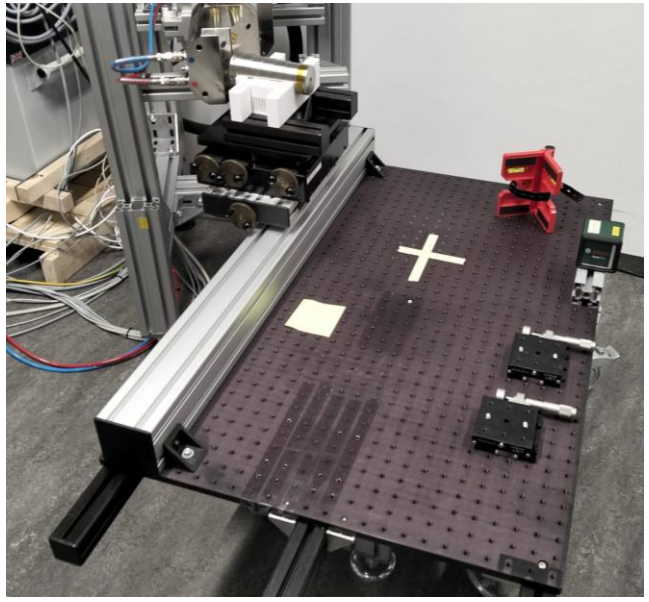
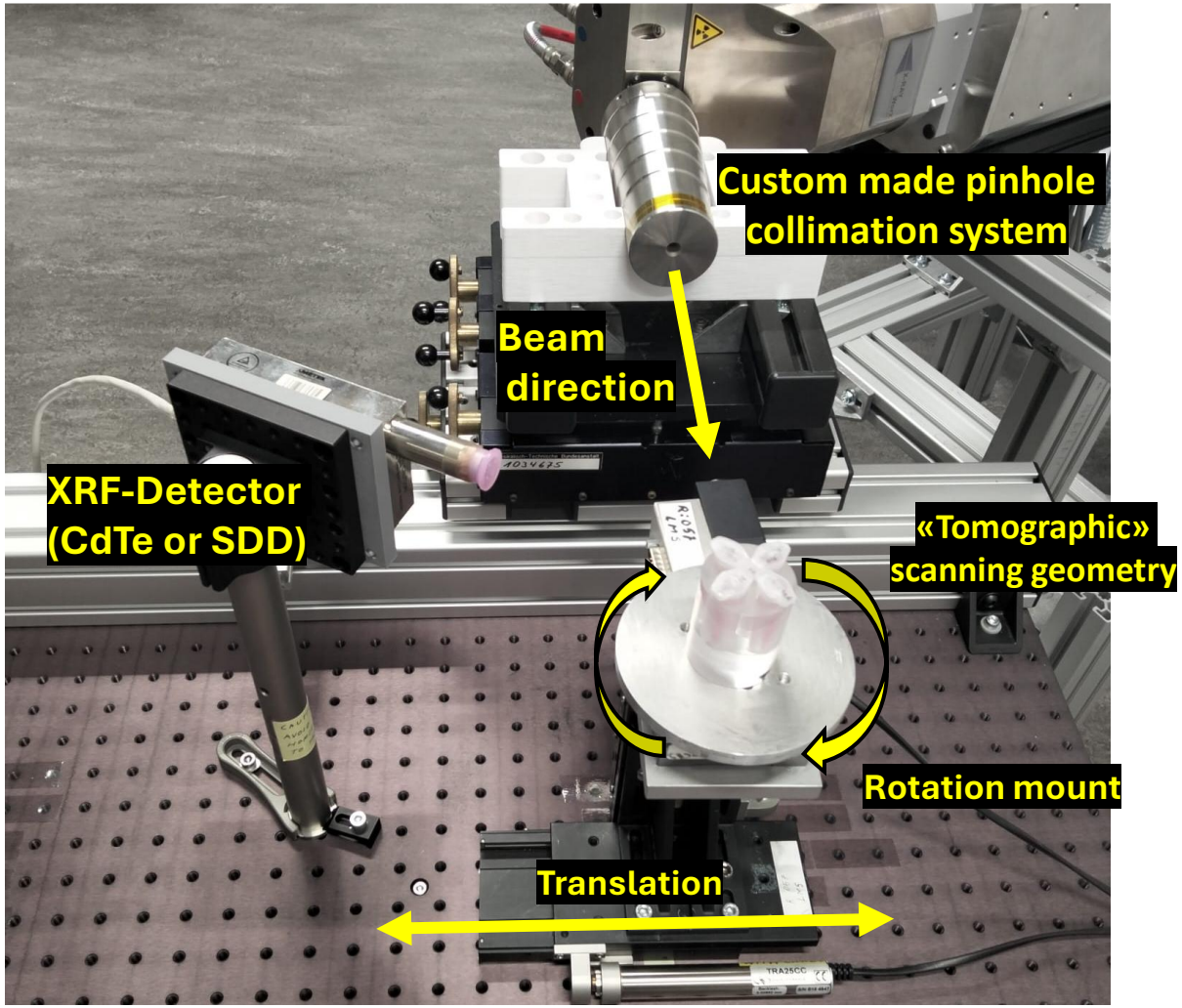




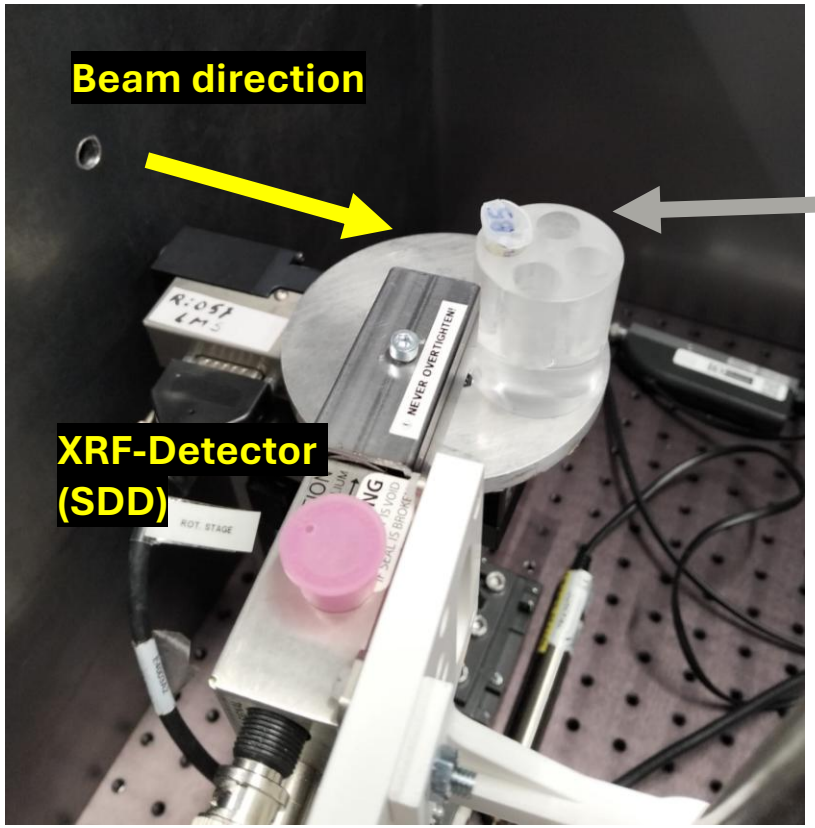


Signal detection from UpConversion Nanoparticles embedded in hydrogel

UpConversion (UC) Nanoparticles Yttrium

Signal detection from UpConversion Nanoparticles embedded in hydrogel

UC NPs concentrations tested:
[50,2000] µg/mL



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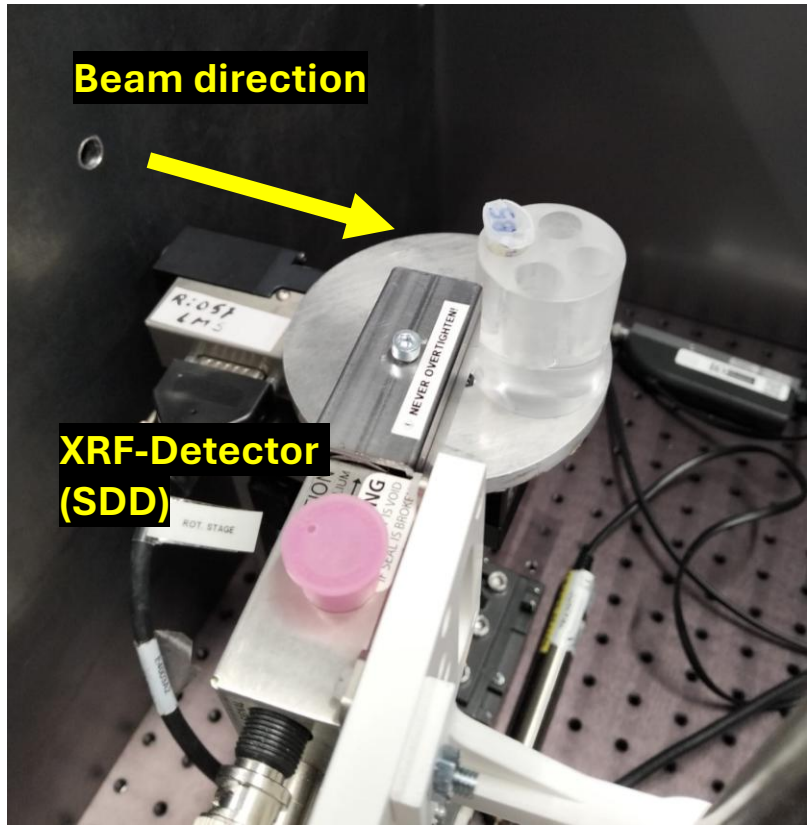
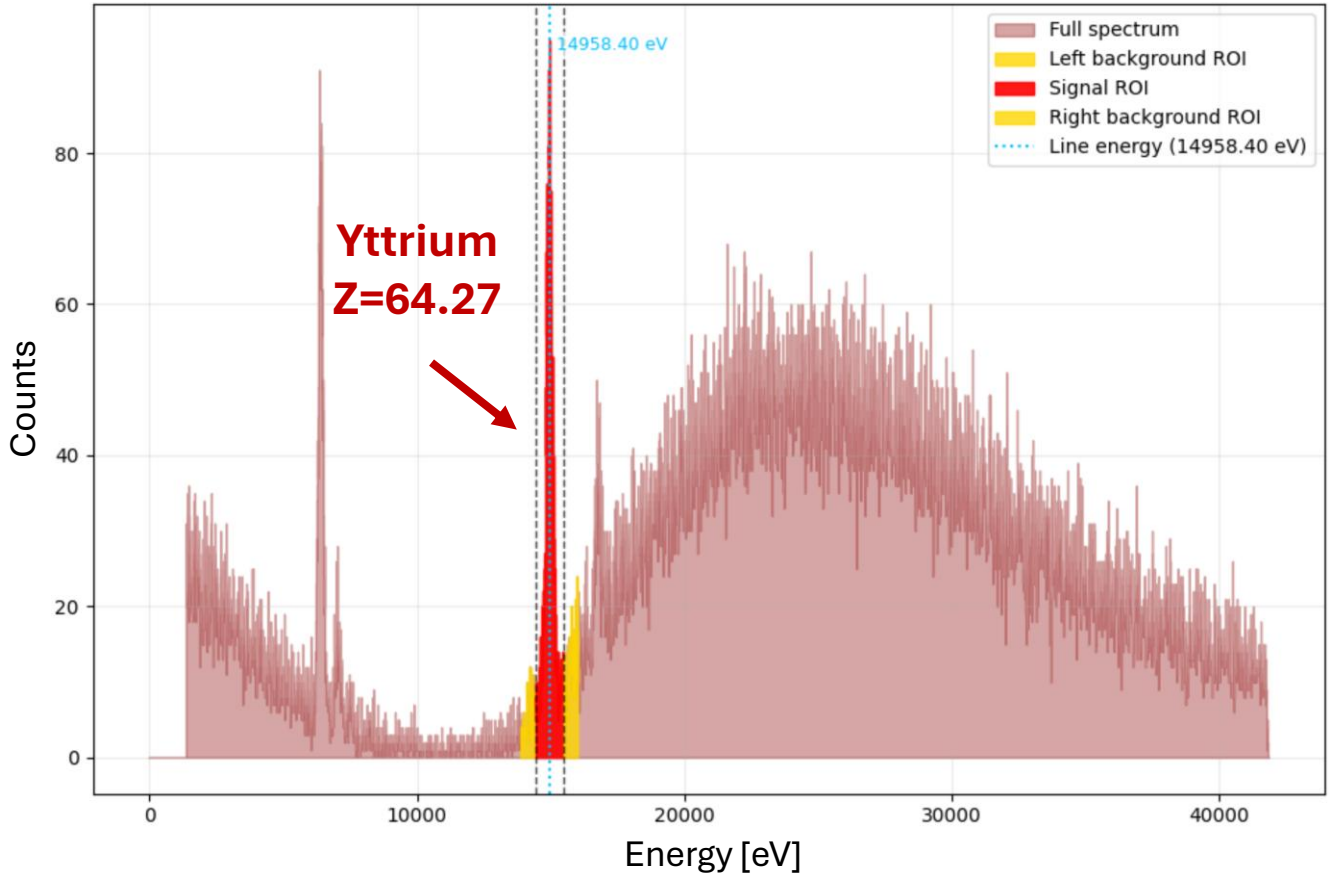


Y(kα1) E=14.958 keV **2000 µg/mL**



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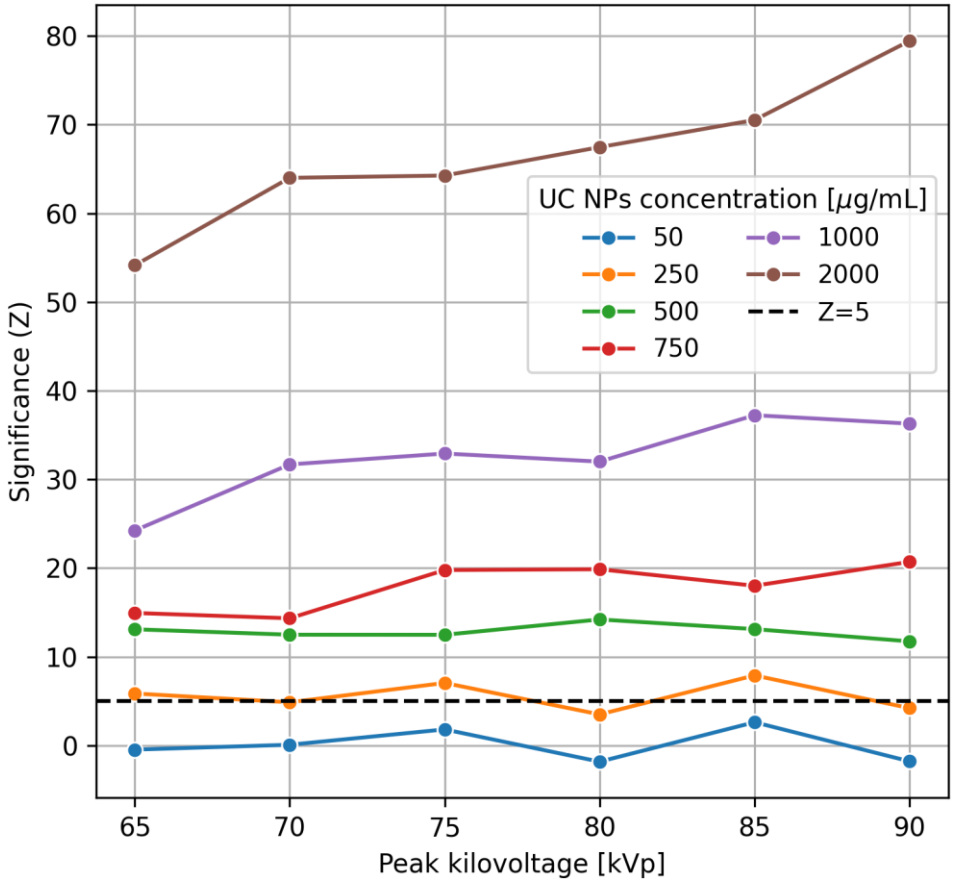

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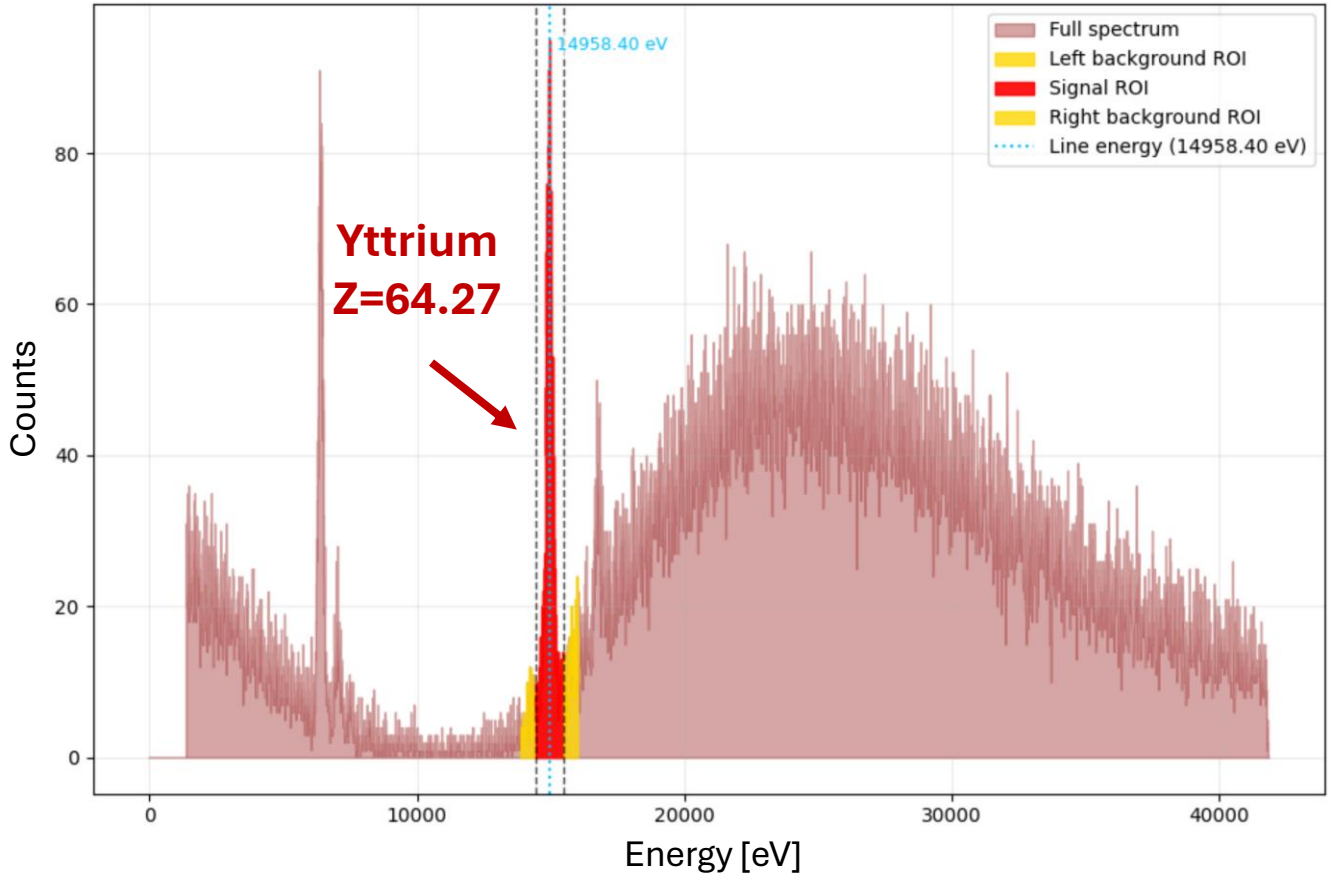

Localising UpConversion-Nanoparticles in 3D Breast Cancer Model by X-ray fluorescence imaging

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- MDA-MB-231 Triple Negative Breast Cancer (TNBC) cell line
- UpConversion NPs are spiked at 500 µg/mL in TNBC cells embedded in hydrogel

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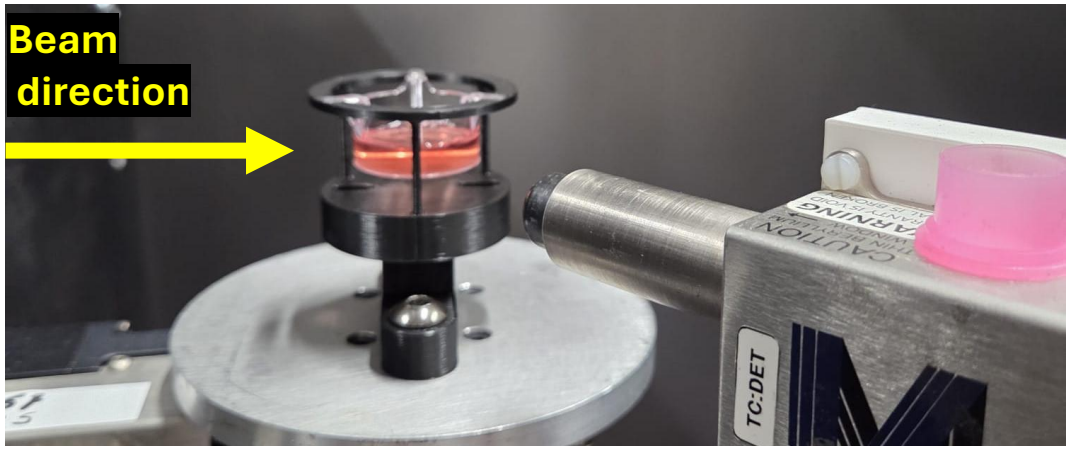

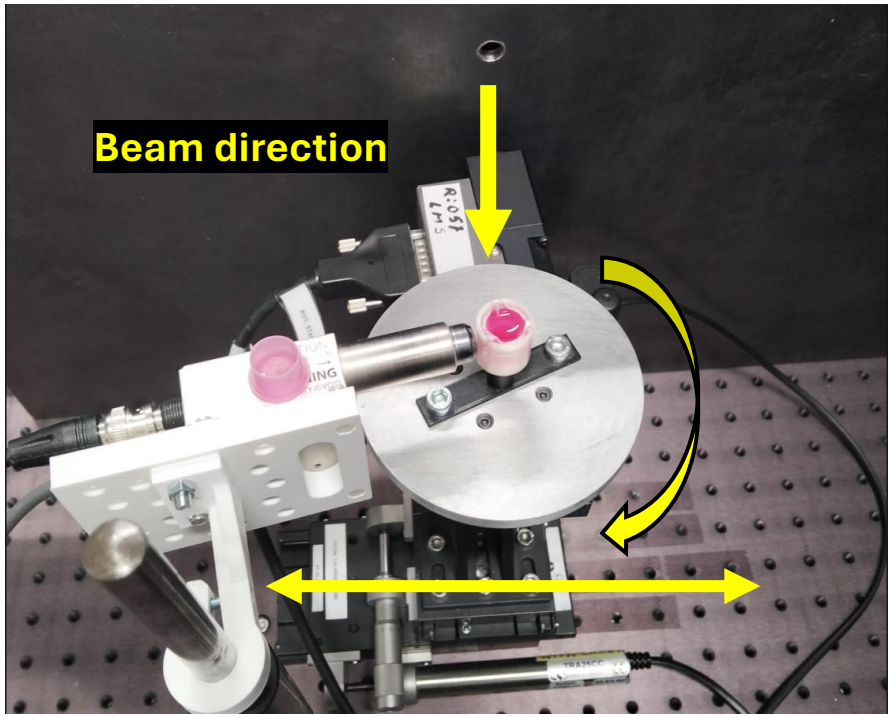

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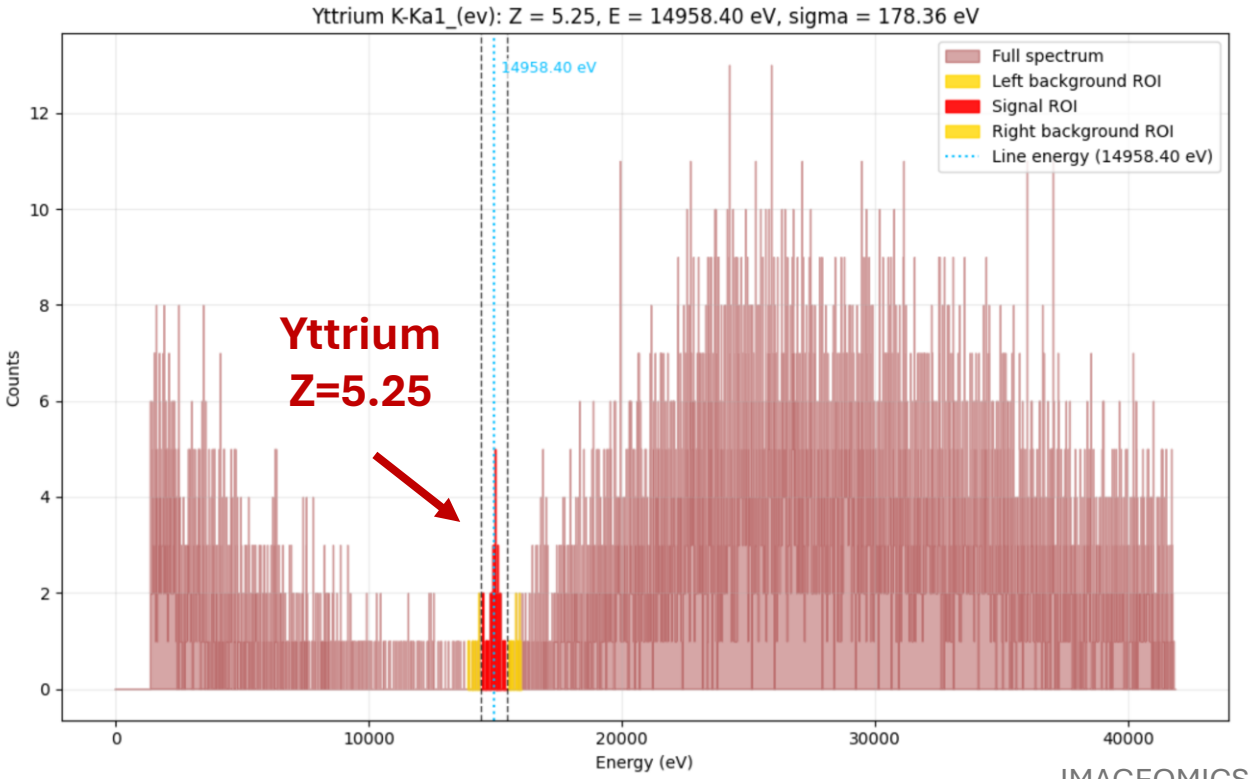
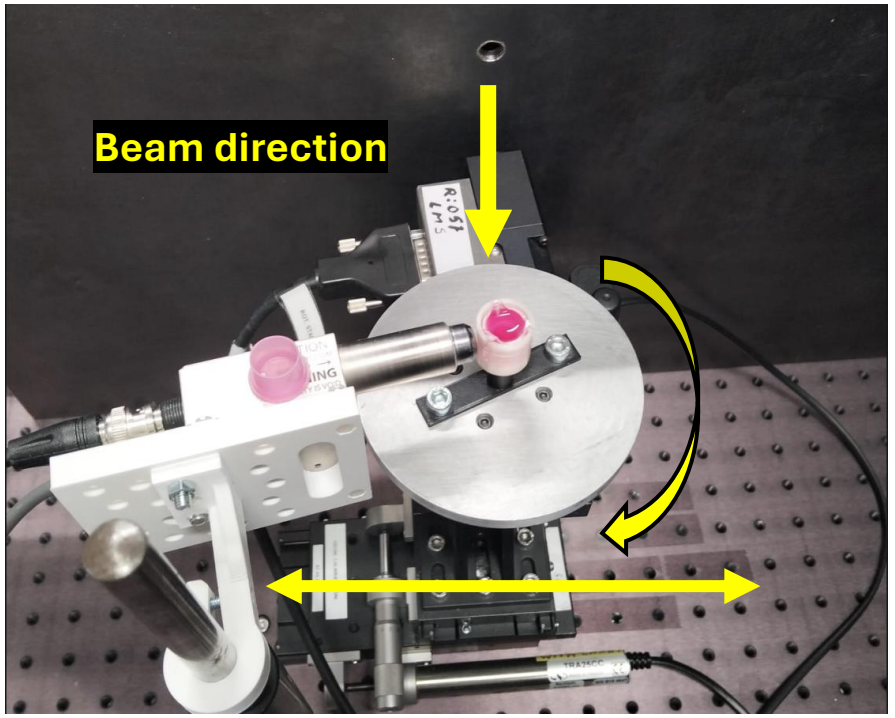
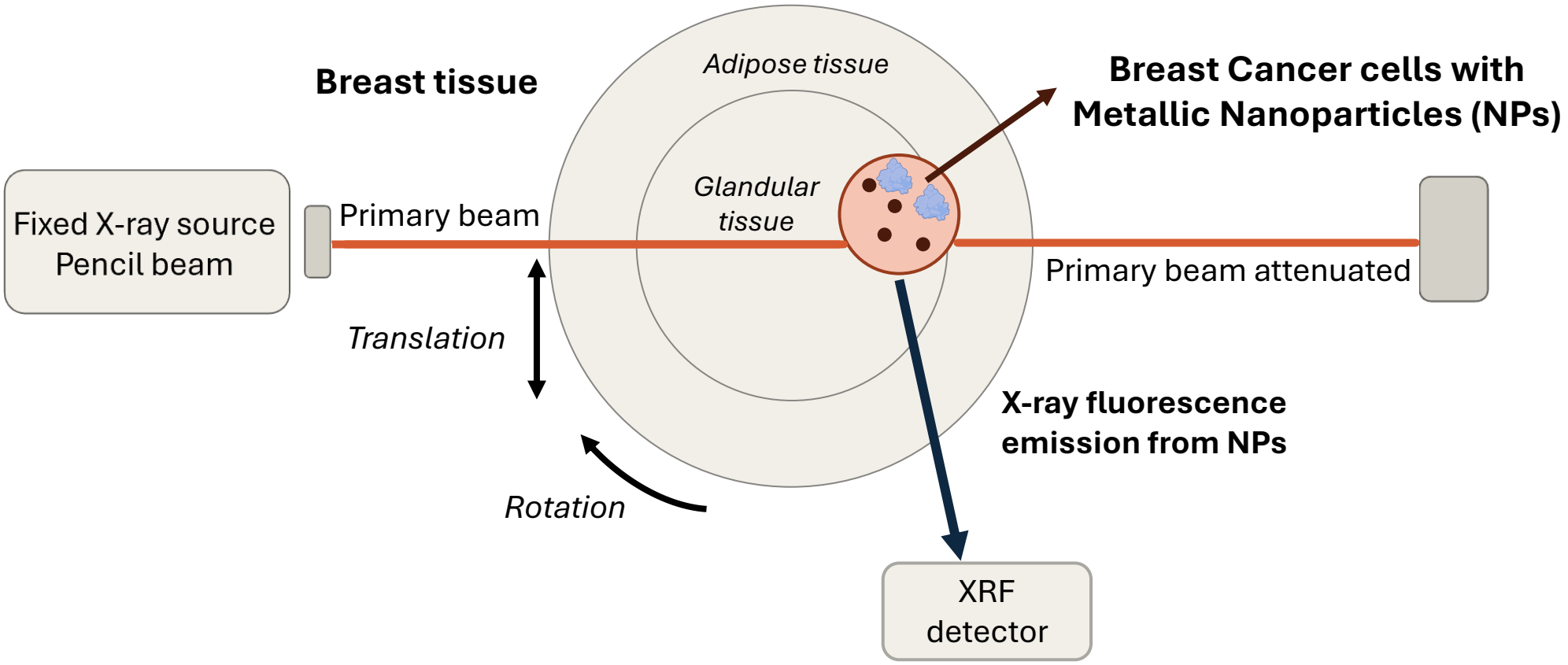


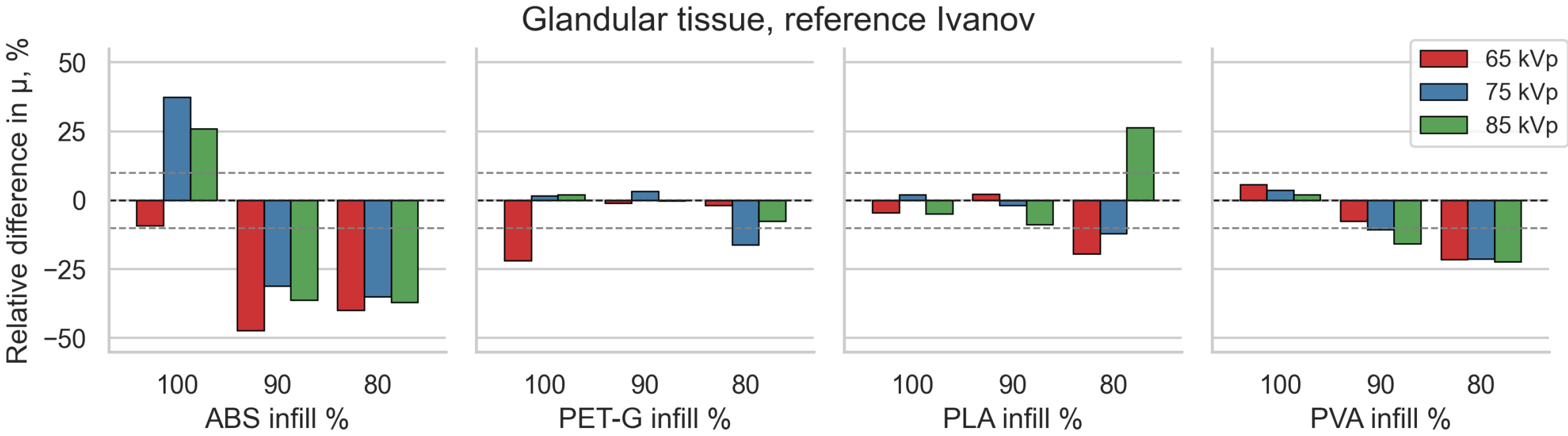


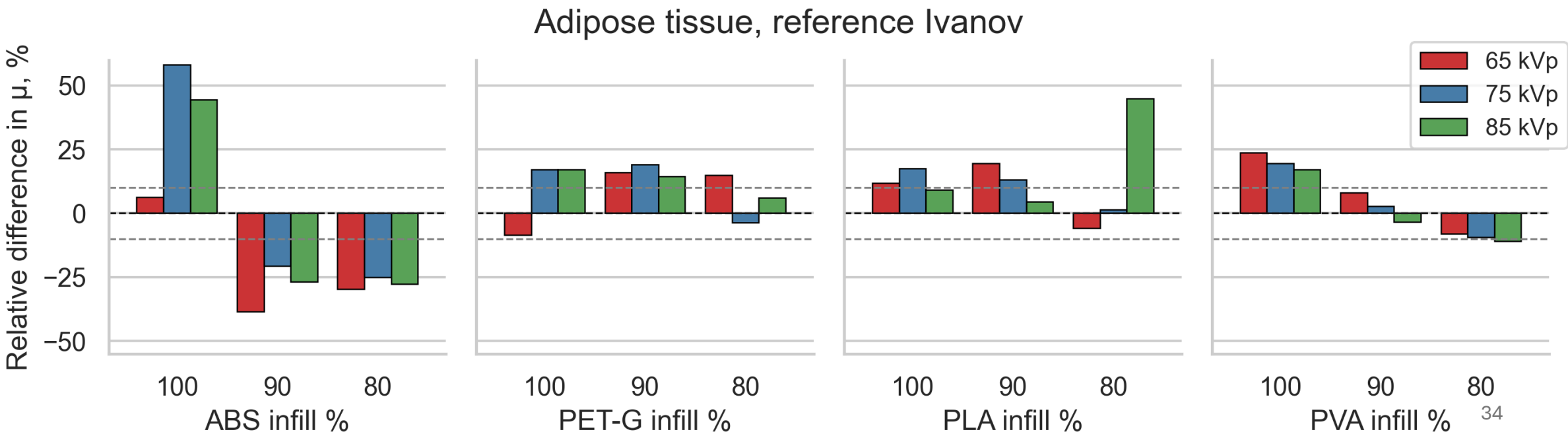
Localising UpConversion-Nanoparticles in human size Breast by X-ray fluorescence imaging



Localising UpConversion-Nanoparticles in human size Breast by X-ray fluorescence imaging

- We want to recreate the radiological properties of the different mammary tissues components;
- We can mainly describe mammary tissue as adipose and glandular tissue;
- We tested 4 filaments: PLA, PVA, ABS, PET-G and three infill densities (80-90-100%)


Glandular tissue, reference Ivanov
Relative difference in μ, %
50
25
0
−25
−50
65 kVp
75 kVp
85 kVp
100
90
80
ABS infill %
PET-G infill %
PLA infill %
PVA infill %


Adipose tissue, reference Ivanov
Relative difference in μ, %
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0
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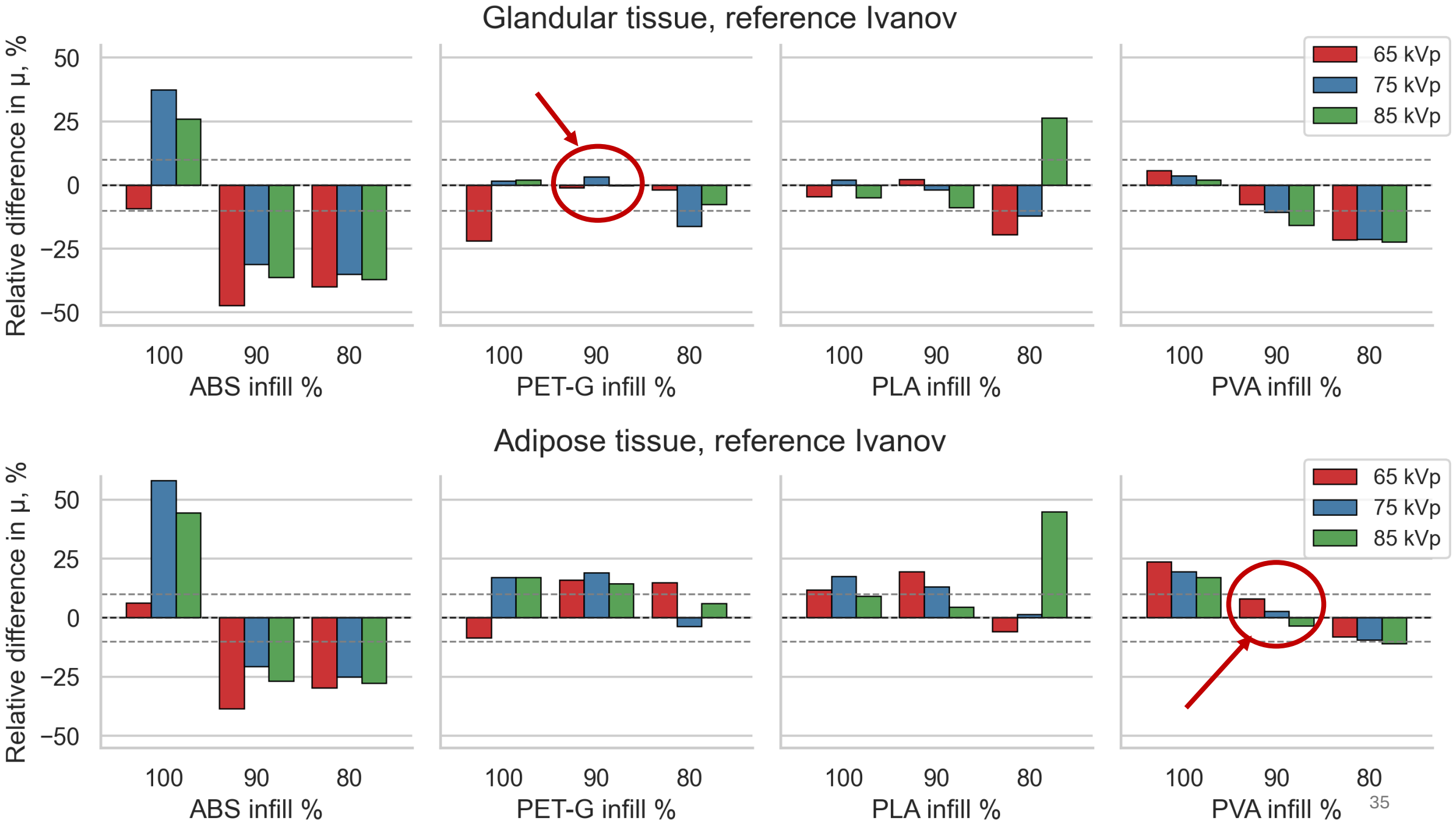
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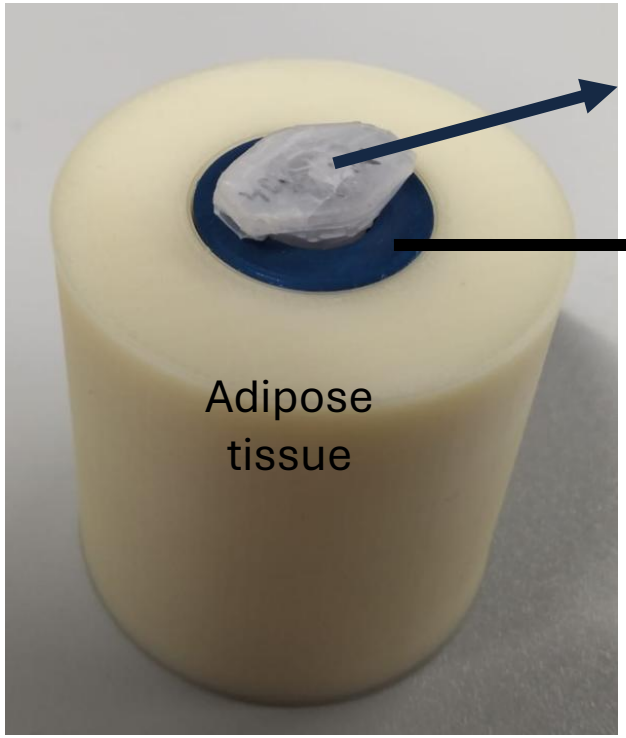
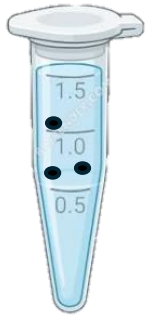
3D printed Breast Phantom



Nanoparticles in hydrogel

Glandular tissue



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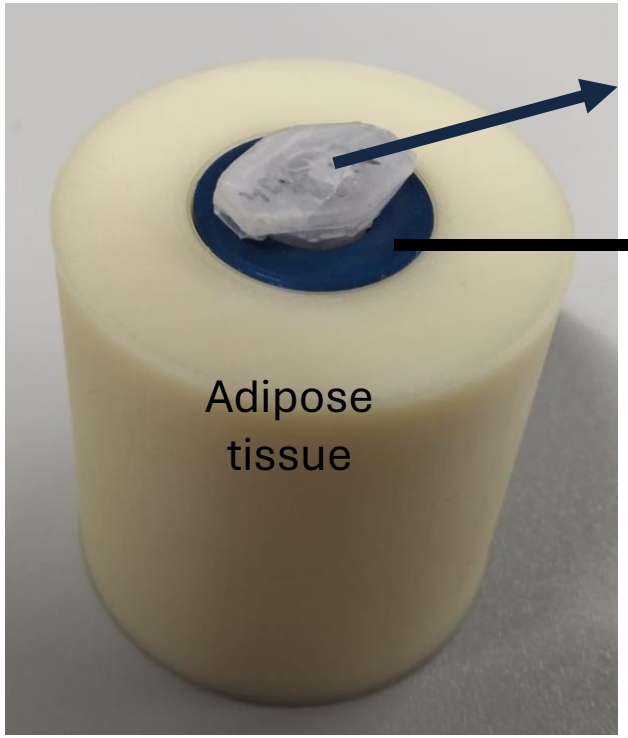

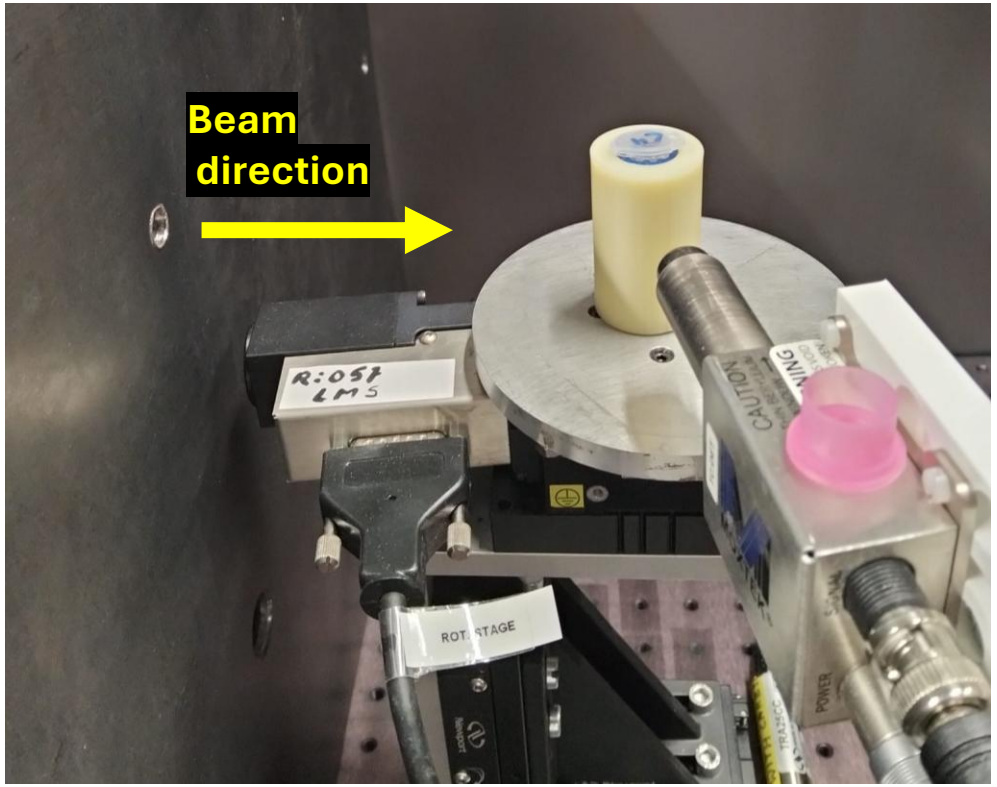

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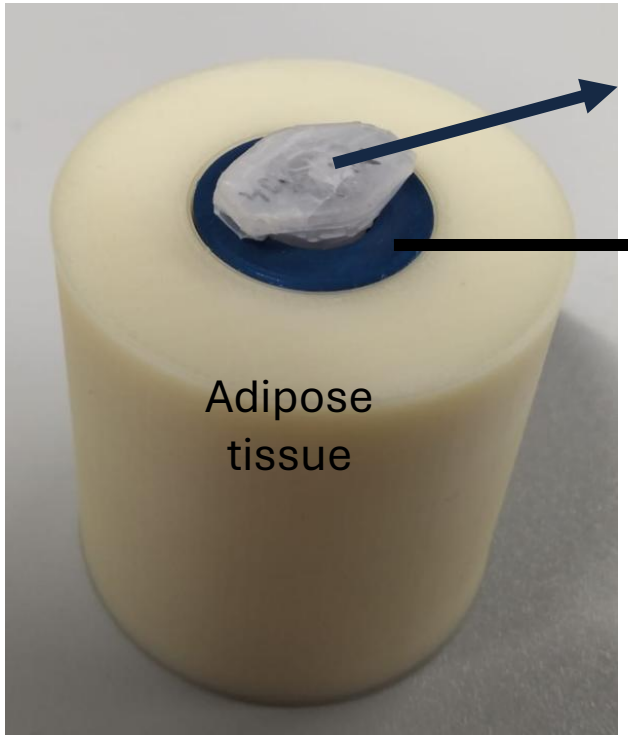

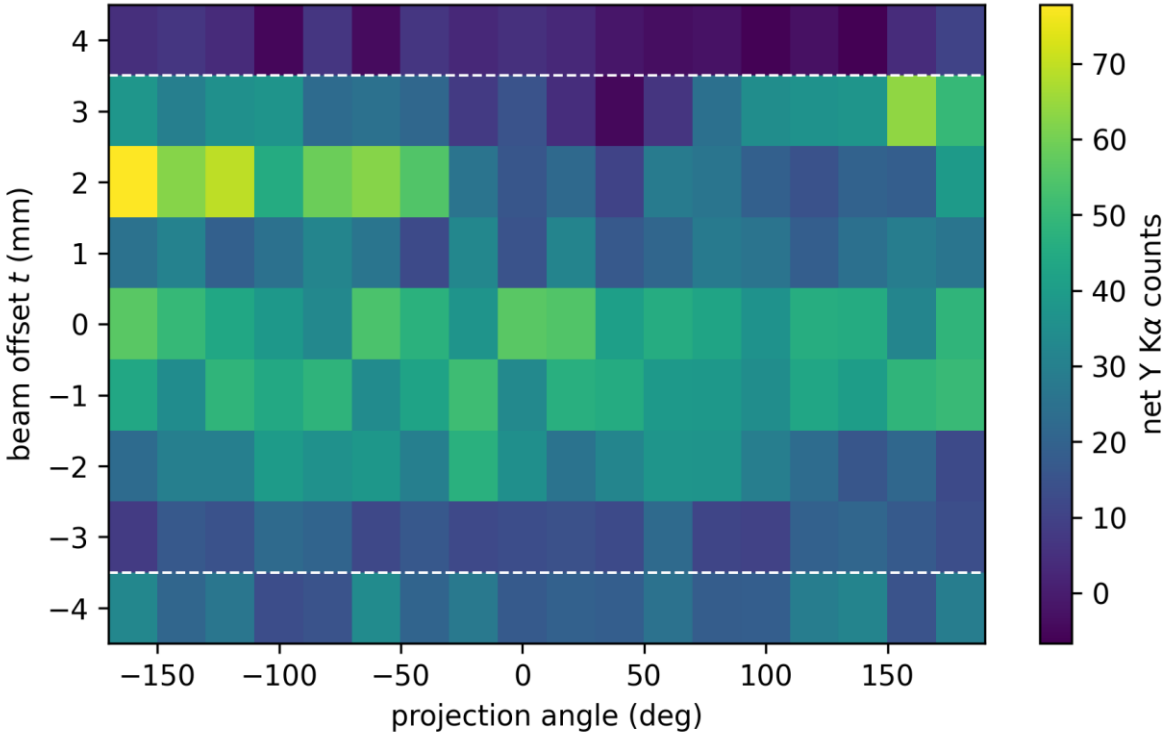

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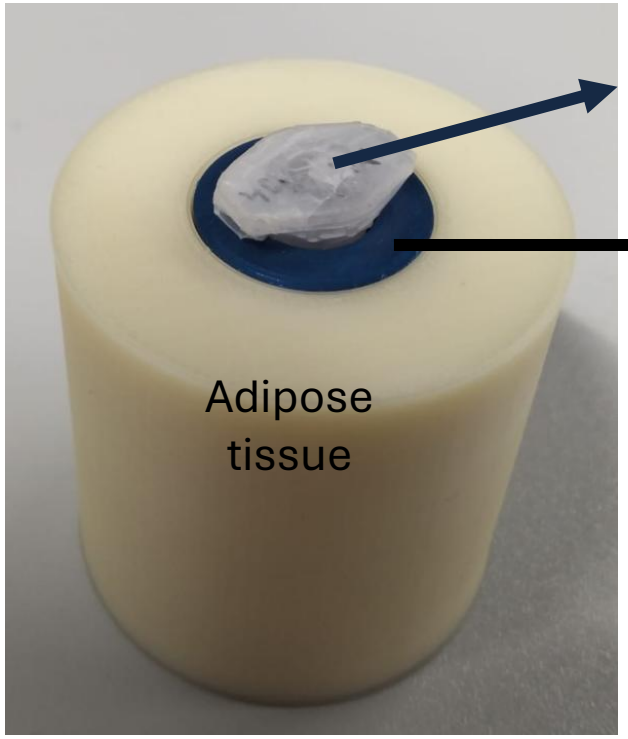

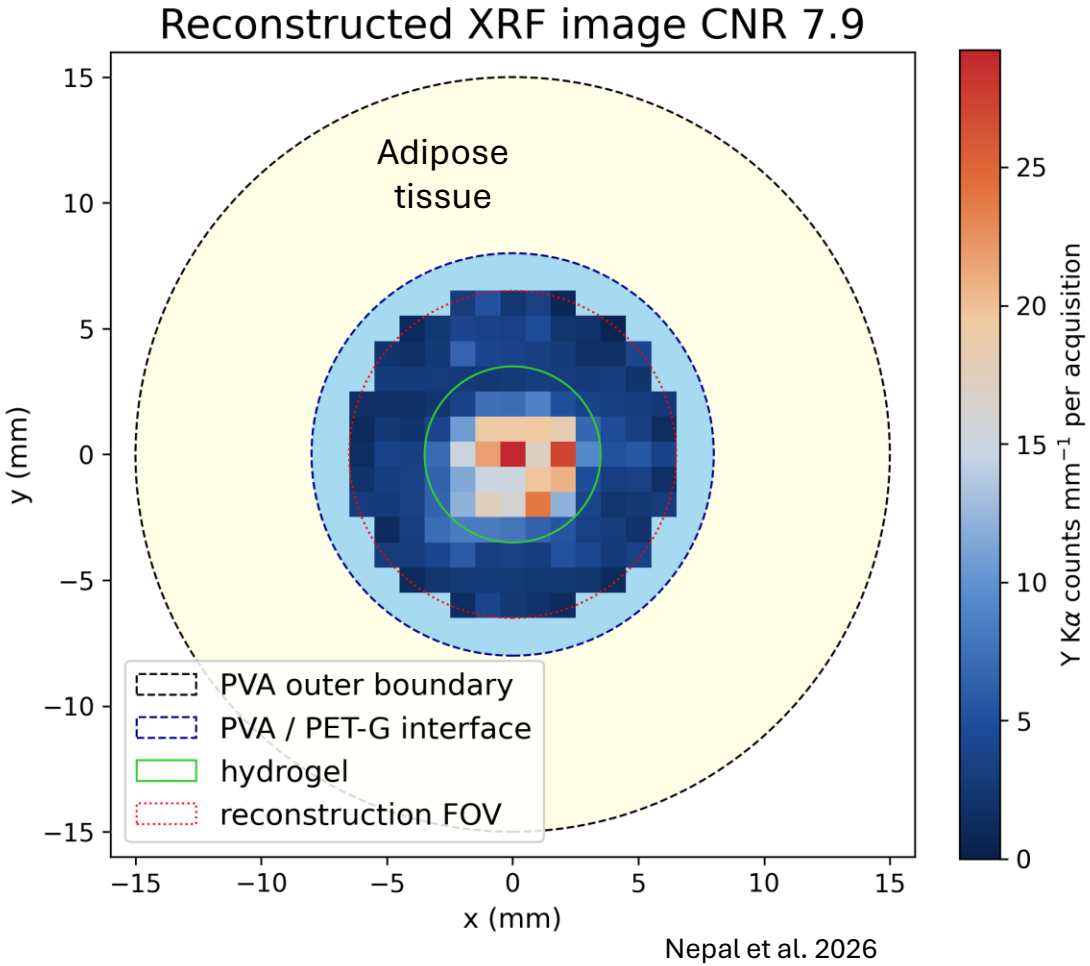

Nepal et al. 2026

Conclusions and outlook

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Ex-vivo Breast Cancer tissues for Spatial Multi-Omics

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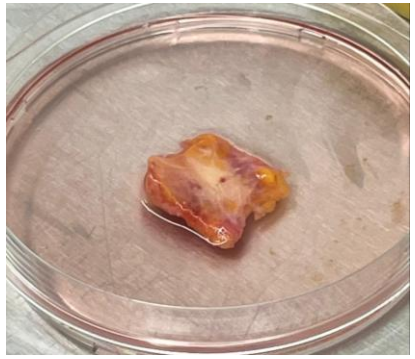
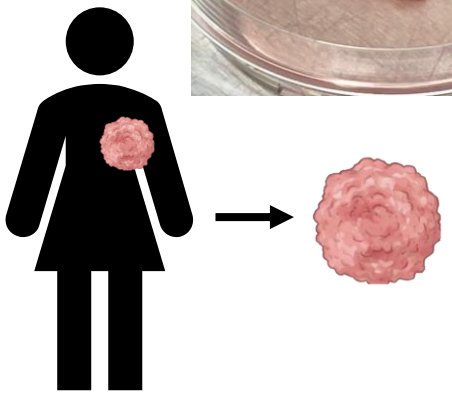
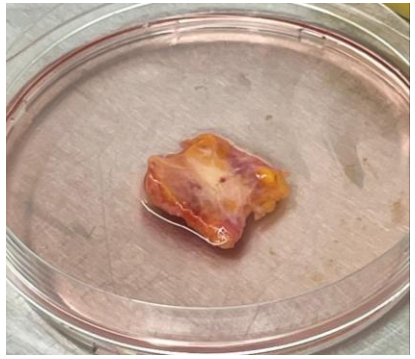


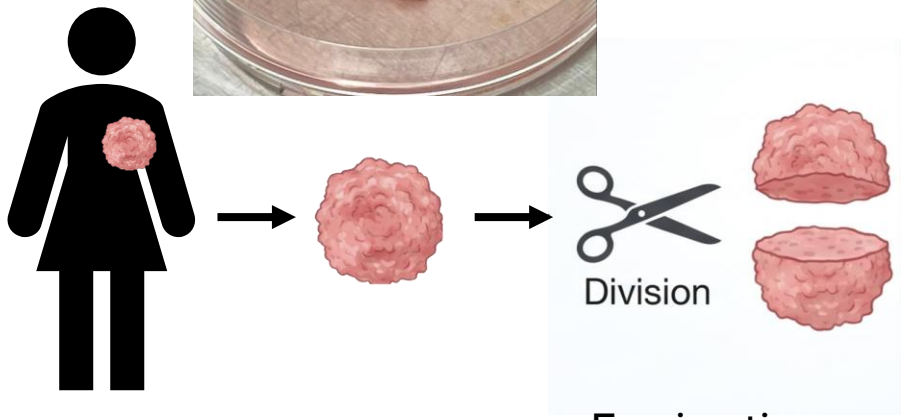


Breast Cancer
patients

Breast resection
Breast Surgery Unit

Ex-vivo Breast Cancer tissues for Spatial Multi-Omics





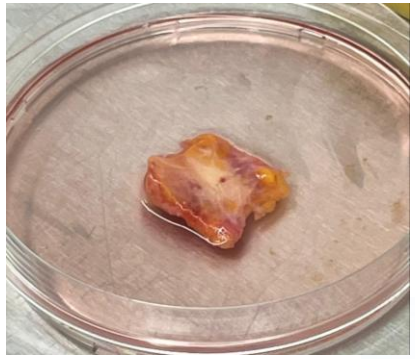
Breast Cancer
patients

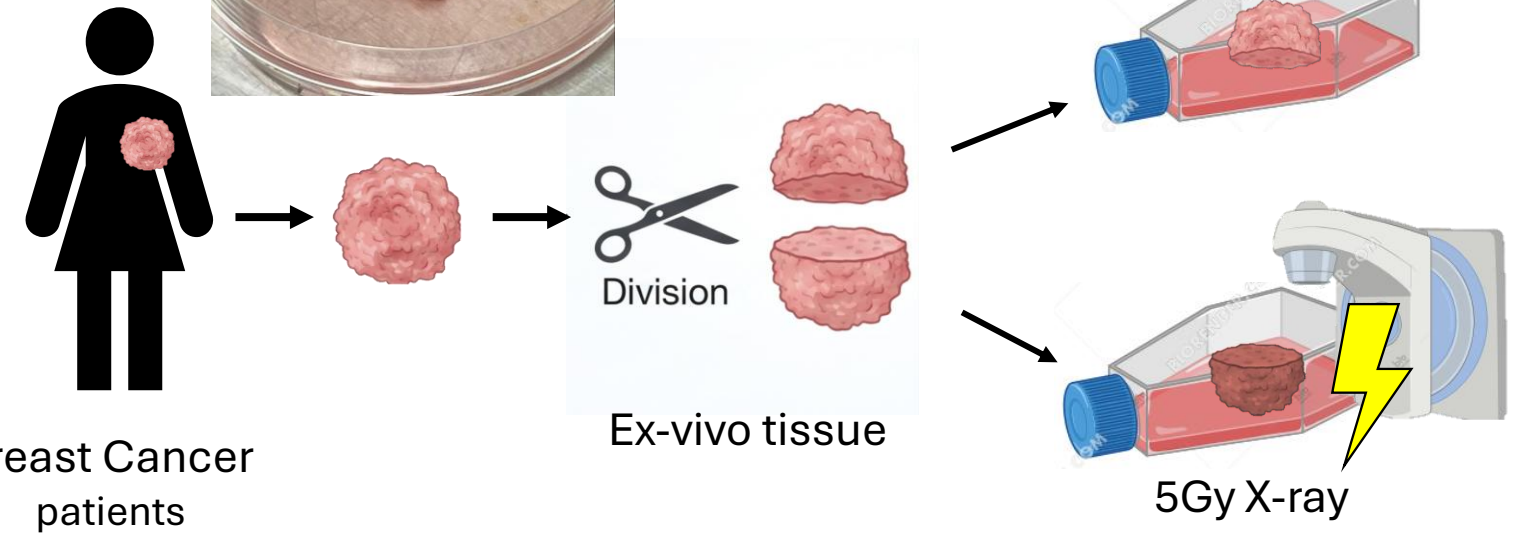
Ex-vivo tissue

Breast resection
Breast Surgery Unit

Tissue sections preparation
Pathological Anatomy Unit

Ex-vivo Breast Cancer tissues for Spatial Multi-Omics





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Breast resection
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Tissue sections preparation
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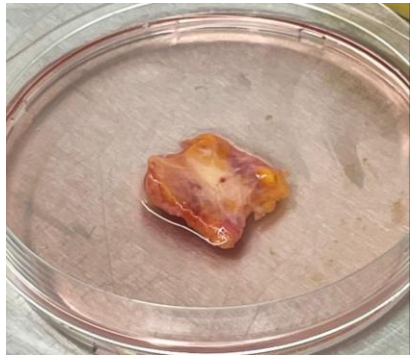
X-ray irradiation Radiotherapy
+ Medical Physics Units

Ex-vivo Breast Cancer tissues for Spatial Multi-Omics



Breast Cancer
patients

Division

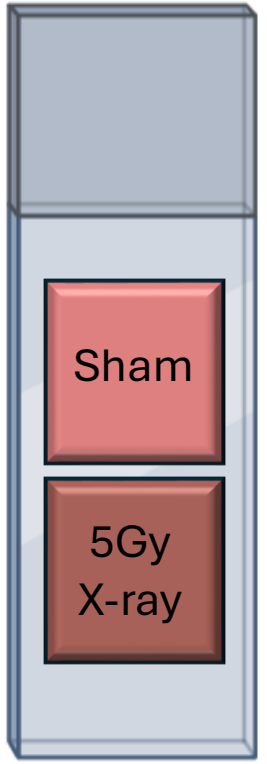
Ex-vivo tissue

5Gy X-ray

$10\mu m$ *tickness*

Sham

5Gy X-ray



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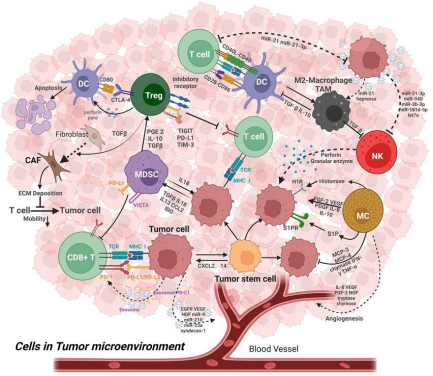
Spatial Multi-Omics technologies

Targeted Spatial Proteomics
Akoya phenocycler

Untargeted Spatial Transcriptomics
VISIUM HD



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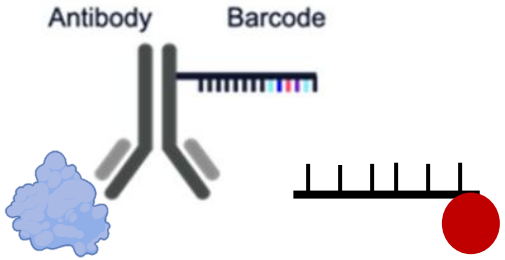
27 biomarkers



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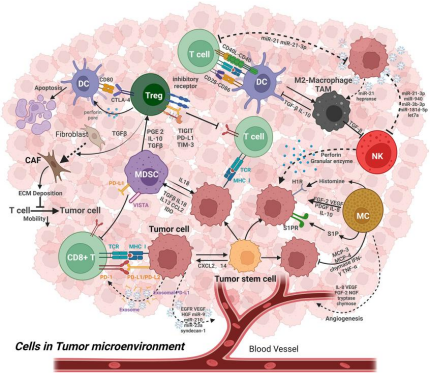

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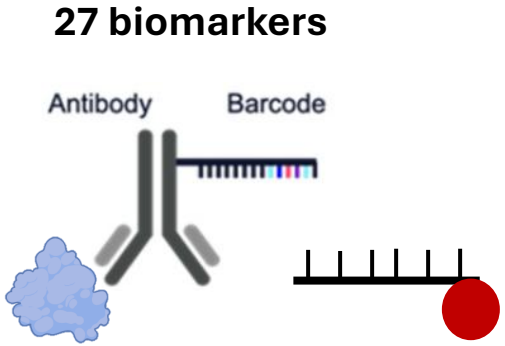

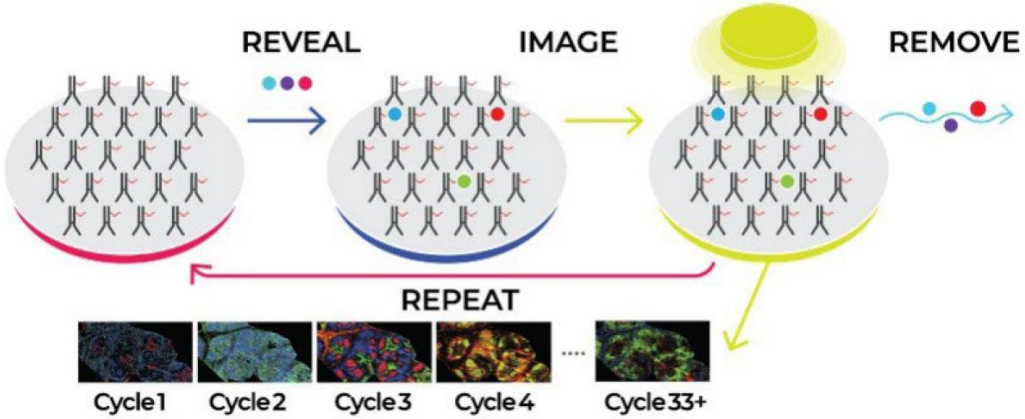

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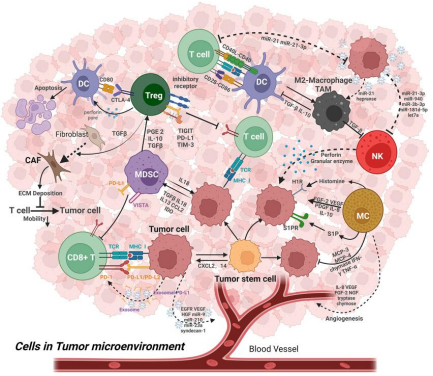

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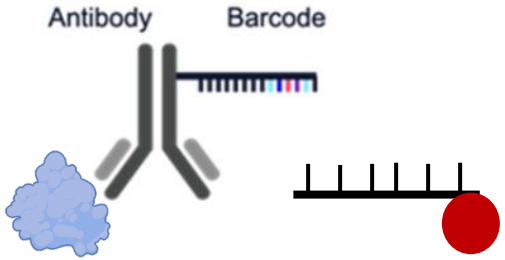

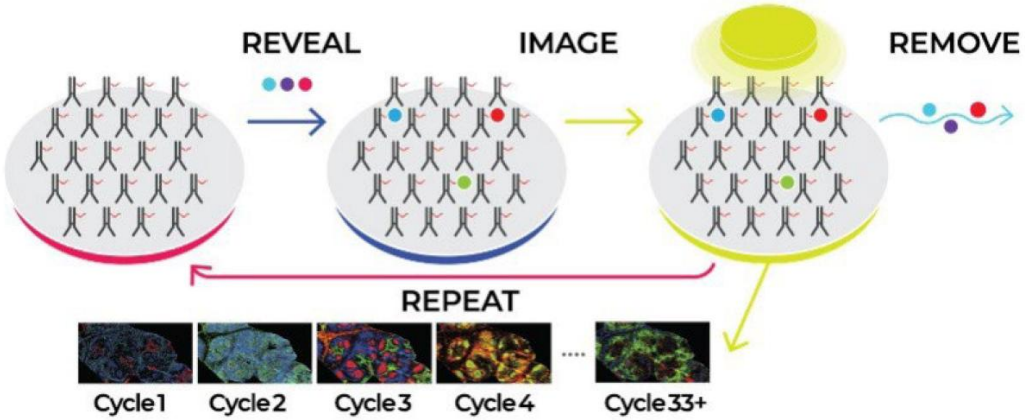

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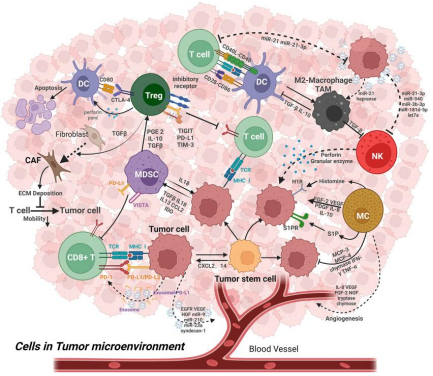

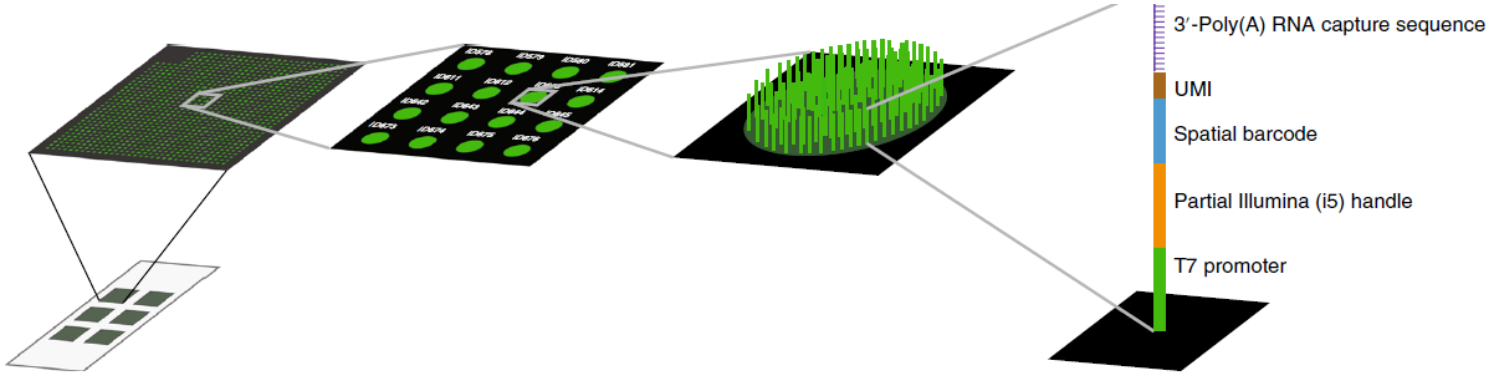

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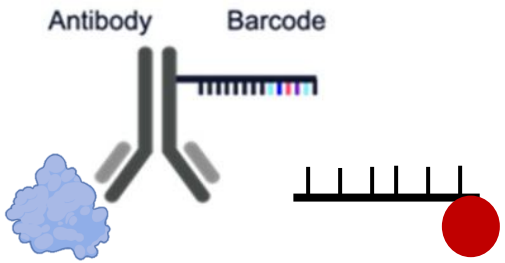

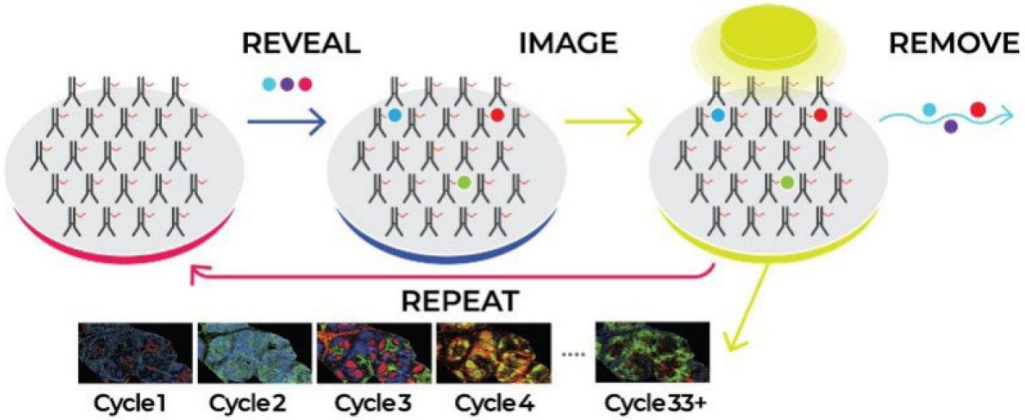

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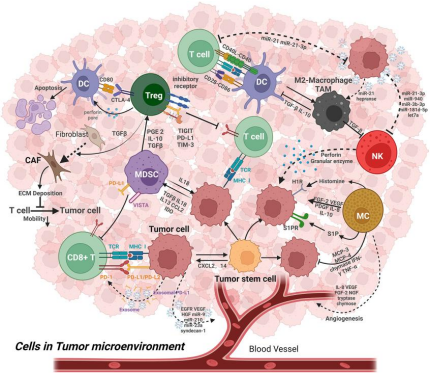
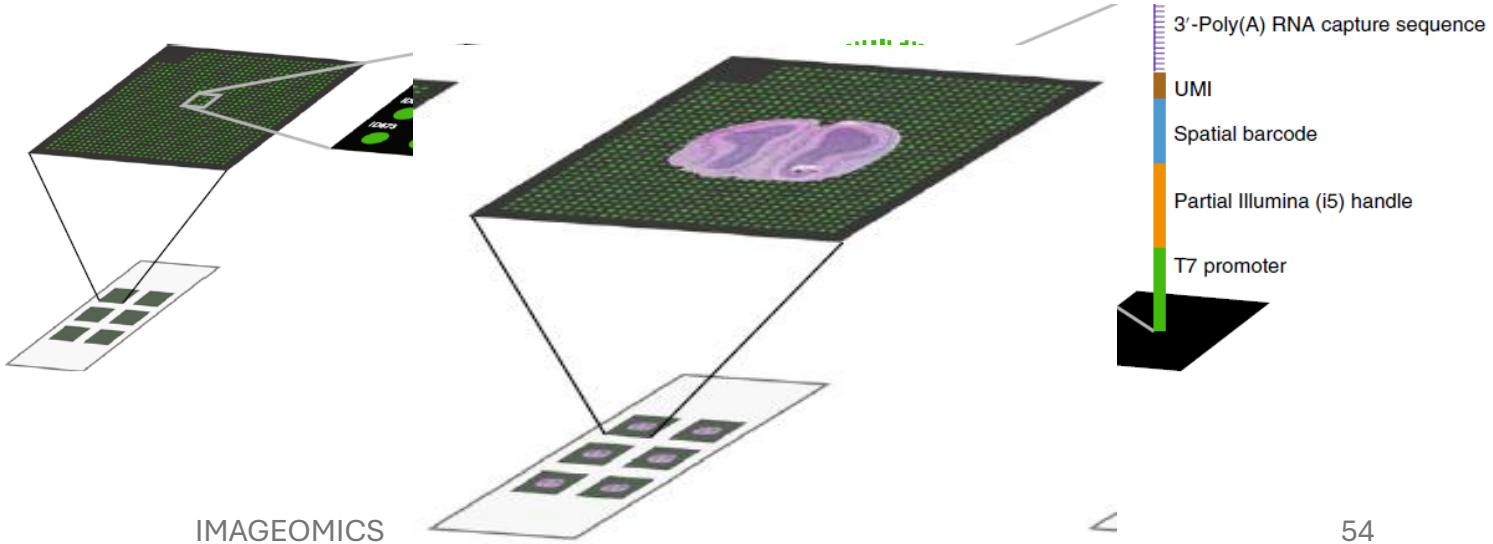
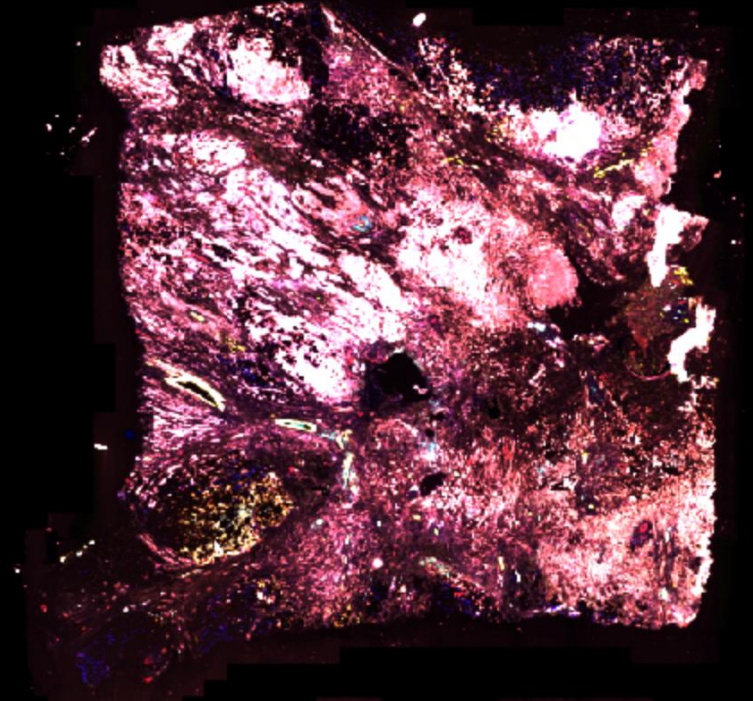




Spatial Proteomics markers

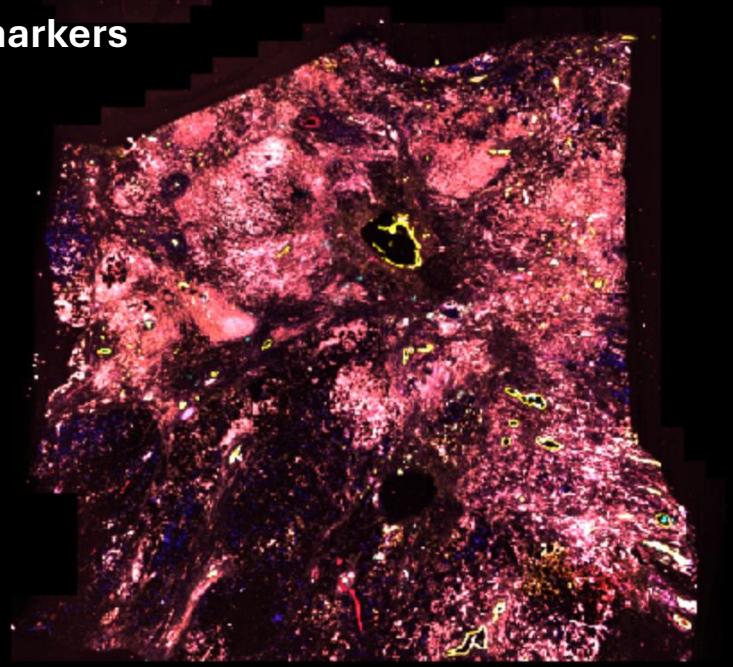
Category	Marker	Biological function
T $CD8^+$ cytotoxic cells	CD8, CD45, CD107a, Granzyme B, ICOS, PD-1	Cytotoxicity, immune memory, exhaustion
T regulator cells	FOXP3, ICOS, CD45RO, PD-1	Immunosuppression
Natural Killer cells	CD57, CD107a, Granzyme B	Cytotoxicity, immune surveillance
Macrophages (TAMs) cells	CD68, CD11c, iNOS, Galectin-3 (Mac2)	Pro- or anti-tumor, immunoregulation
Dendritic cells	CD11c	Antigen presentation
T helper cells	CD45, ICOS, PD-1	Support for the immune response
Tumor cells	E-cadherin, CK5, CK8, CK14, Catenin beta-1	Tumor identity, invasiveness
Cancer stem cells	CD44, SOX2	Self-renewal, resistance
Proliferation	Ki-67, PCNA	Active division
Immune evasion	PD-L1, PD-1	Suppression of the T-cell response
Ipoxia / Metabolism	HIF1A, iNOS	Metabolic adaptation, inflammation
Matrix / Strom	Collagen IV, Podoplanin	Structure, invasion, drainage

SHAM



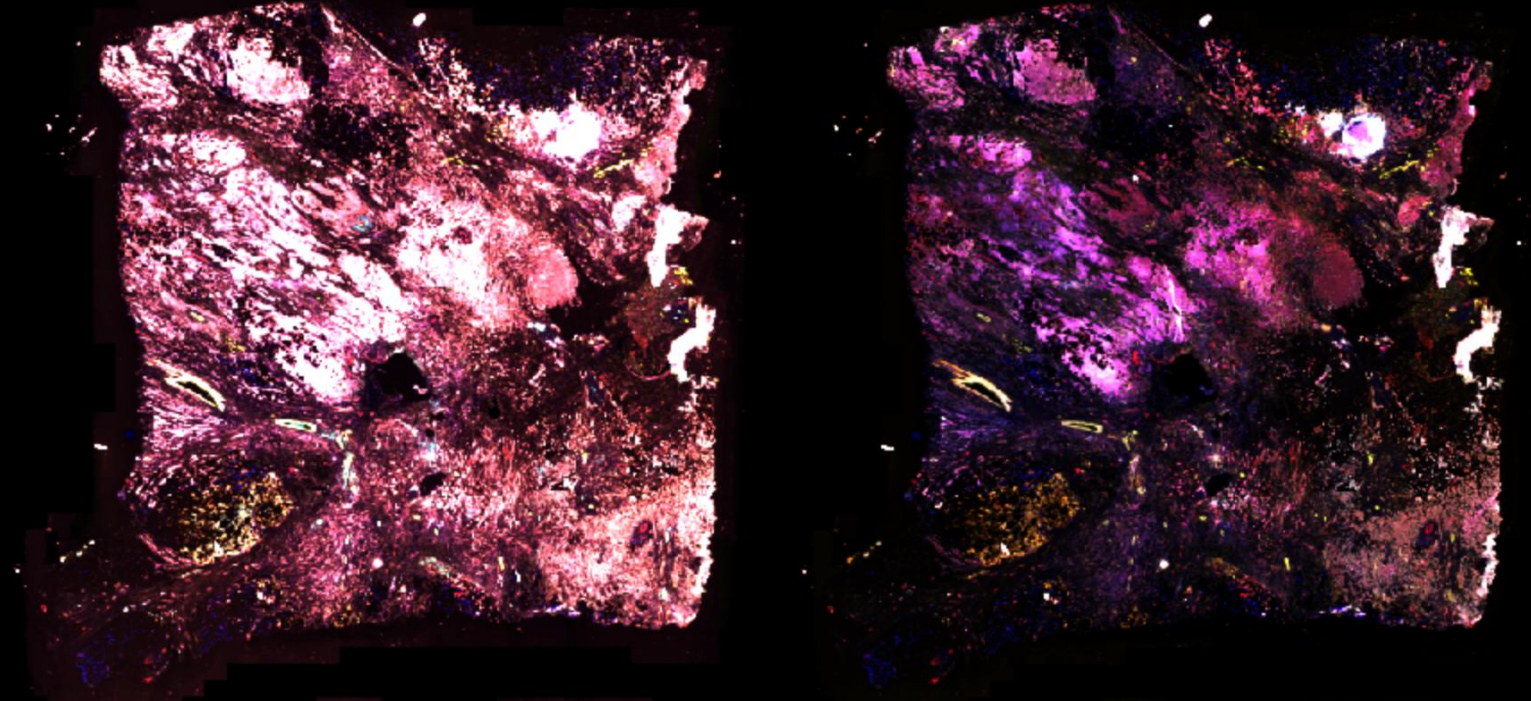
27 biomarkers

5Gy
X-ray



5 mm

SHAM



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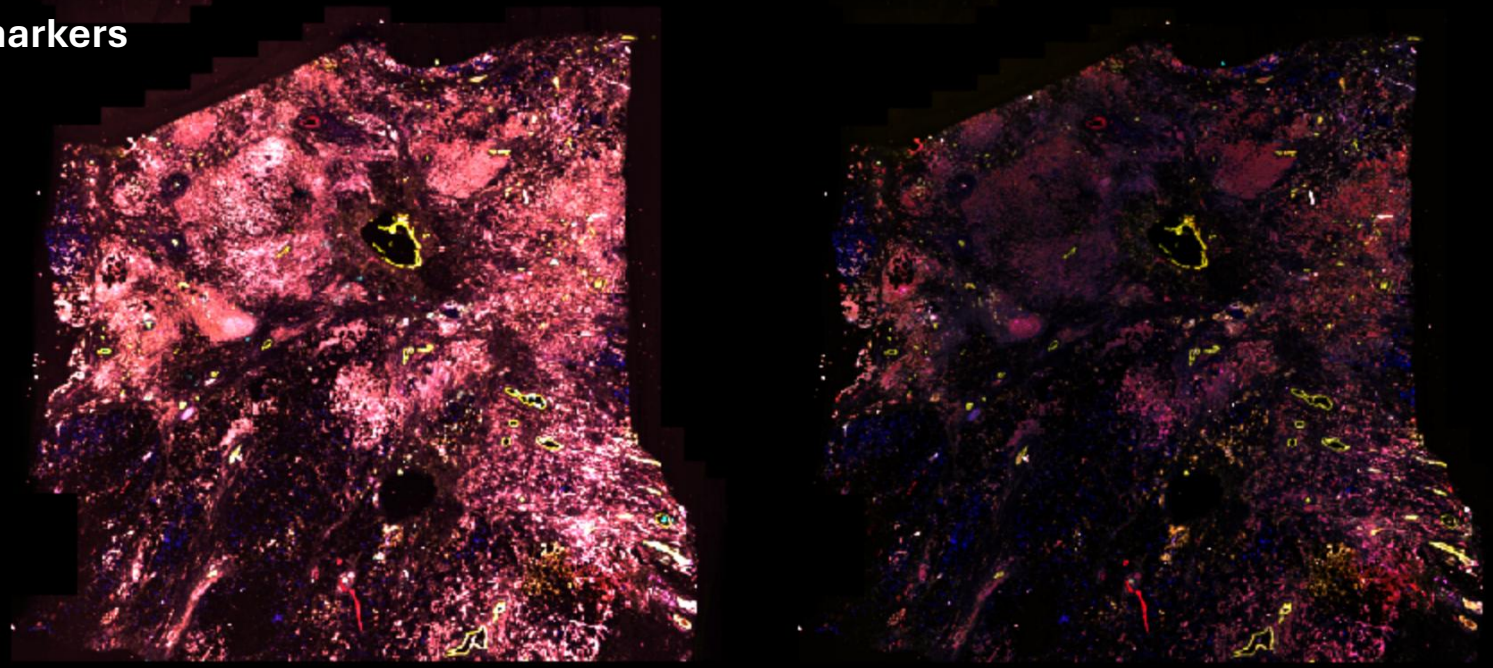
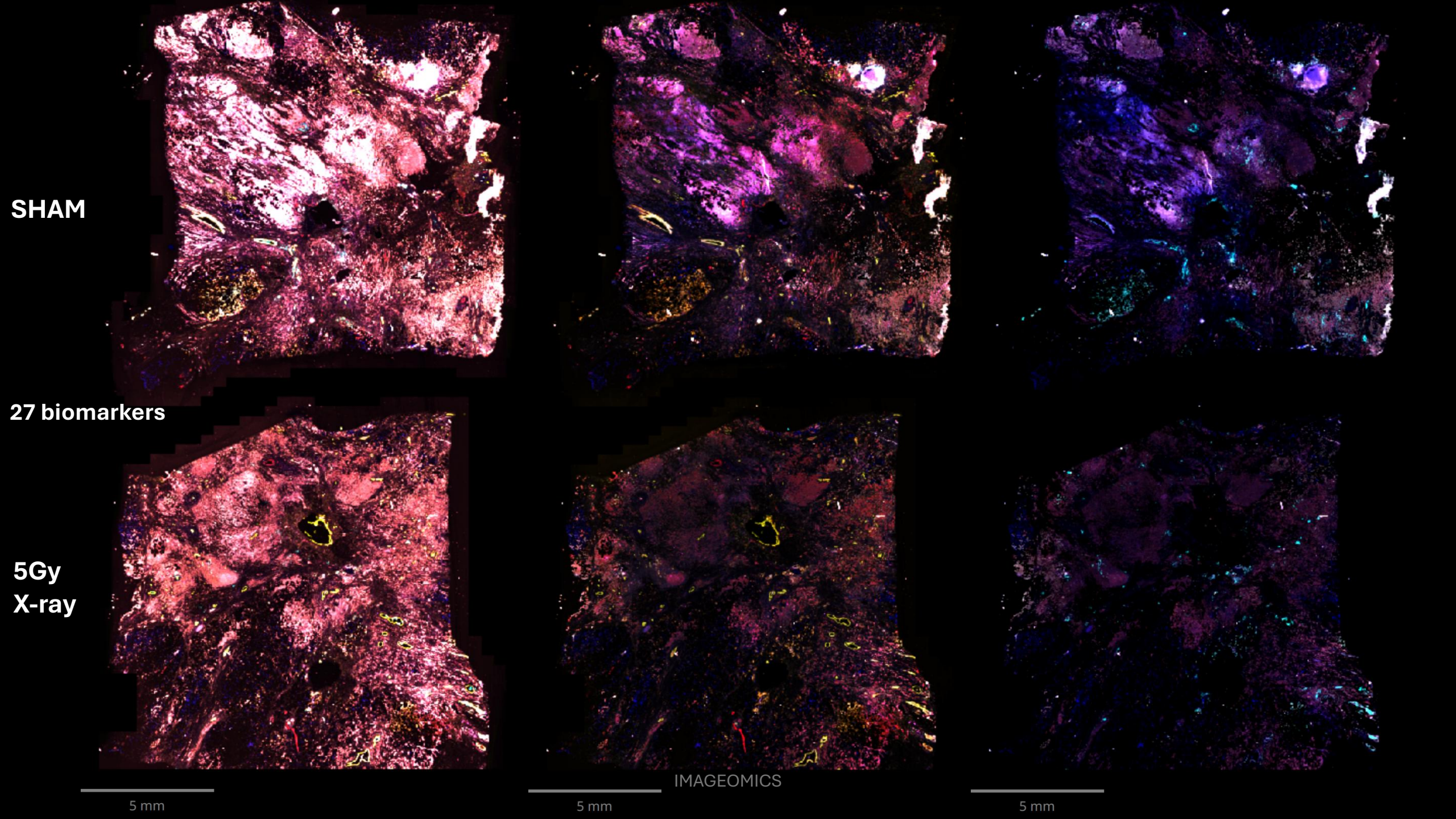


5 mm

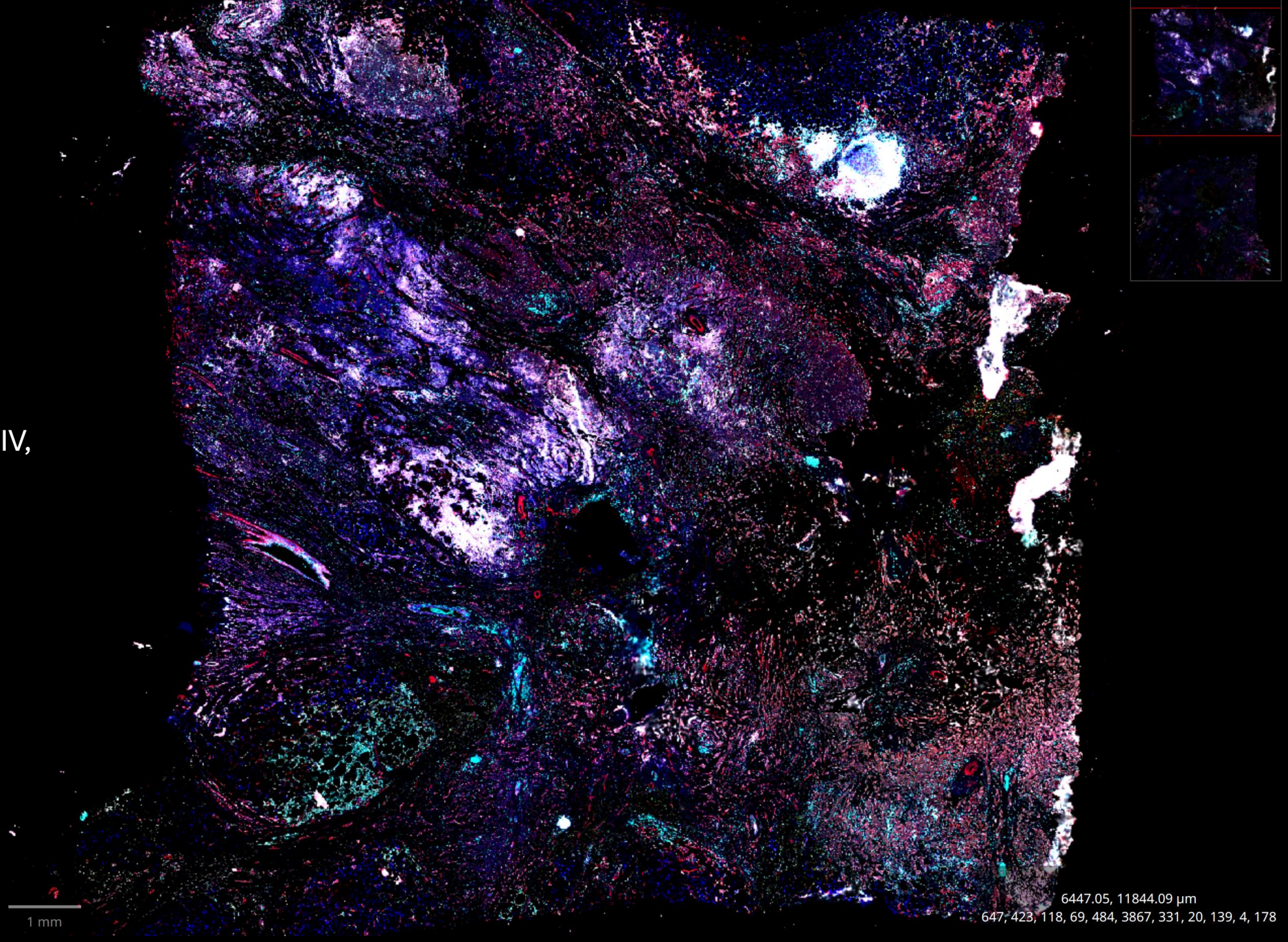
5 mm

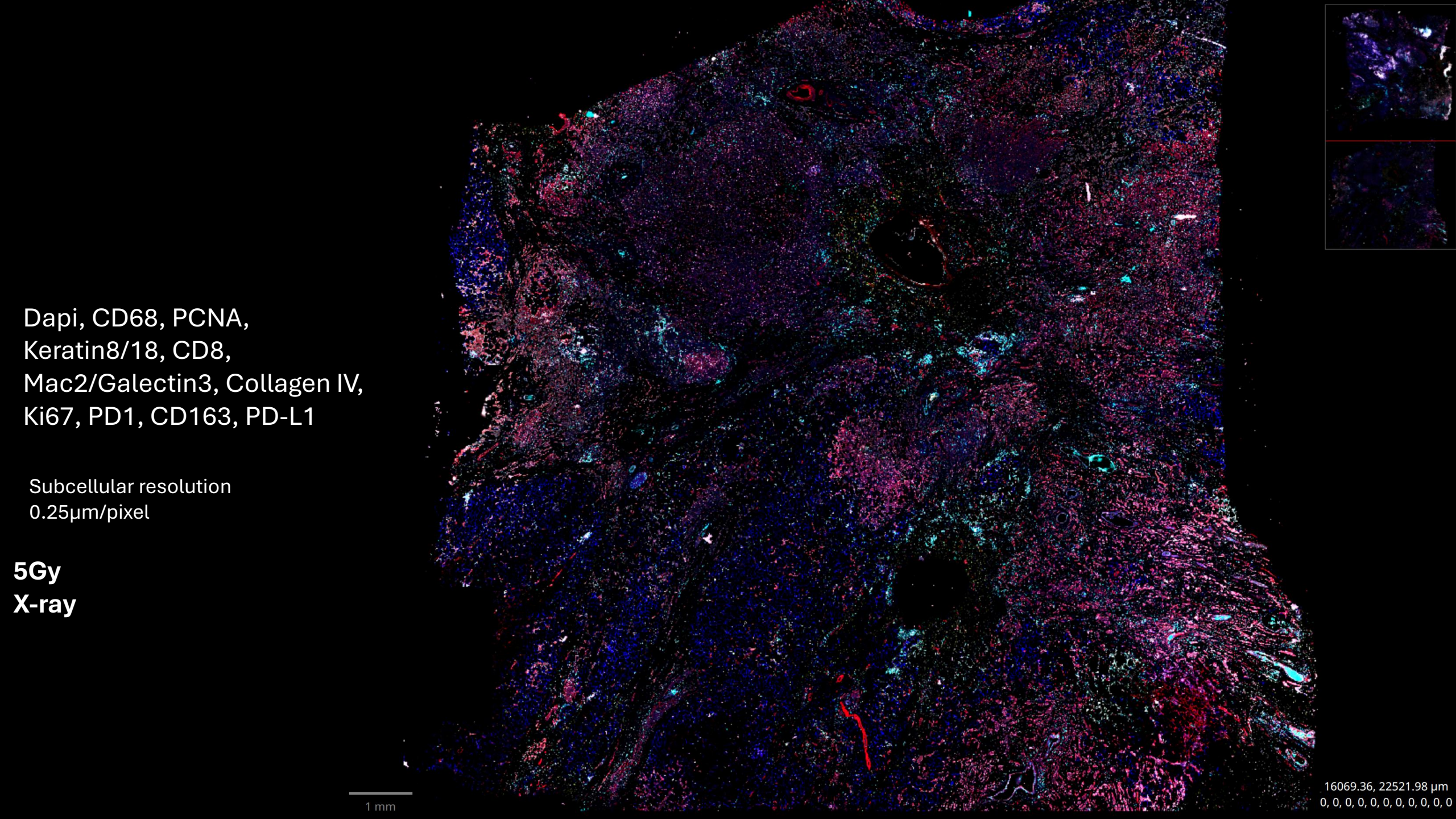

SHAM
27 biomarkers
5Gy
X-ray
IMAGEOMICS
5 mm
5 mm
5 mm

SHAM

Dapi, CD68, PCNA,
Keratin8/18, CD8,
Mac2/Galectin3, Collagen IV,
Ki67, PD1, CD163, PD-L1

Subcellular resolution
0.25µm/pixel

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Subcellular resolution
0.25μm/pixel
5Gy
X-ray
1 mm
16069.36, 22521.98 μm
0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0

Spatial Proteomics

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- **Spatial organisation and niche identification:** detection of spatially distinct tumour–immune niches
- **Cell–cell interaction analysis:** characterisation of tumour–immune cellular interactions
- **Spatial heterogeneity analysis:** assessment of molecular and cellular differences across tissue regions
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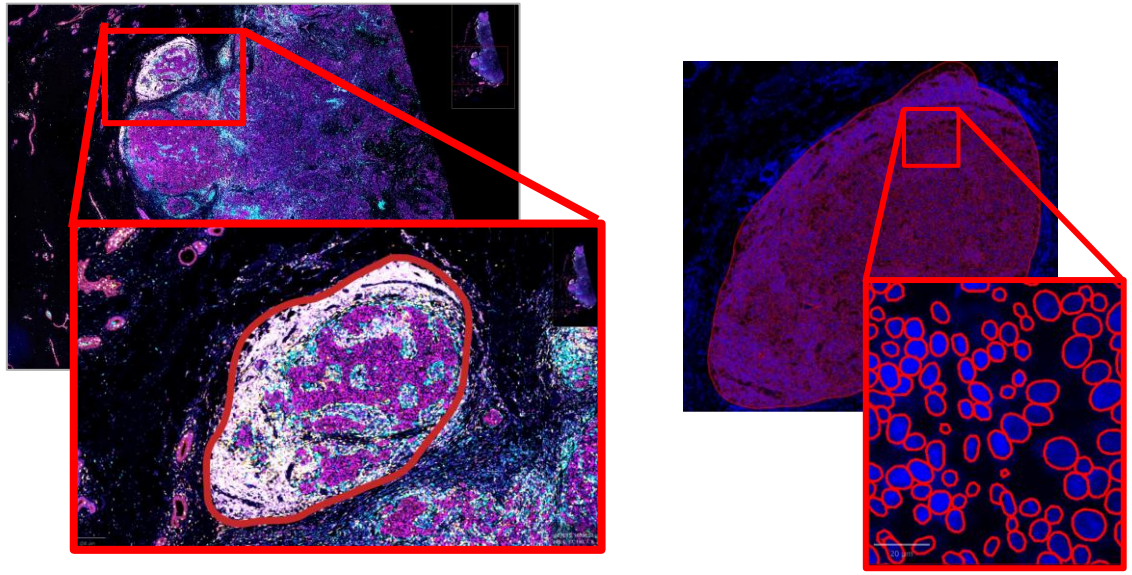

Cell Segmentation and signal extraction

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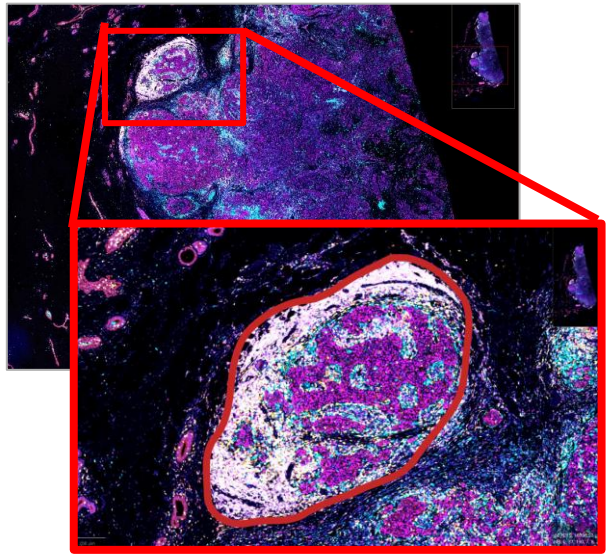

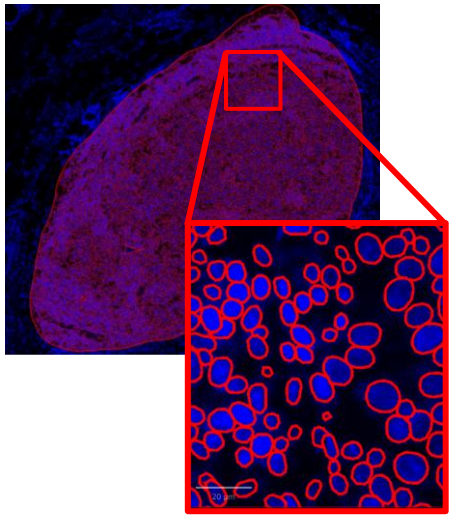

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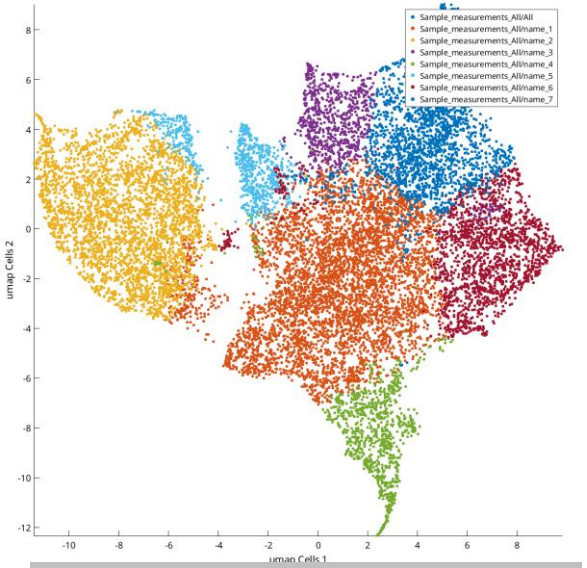

Dimensionality reduction and clustering from marker expression

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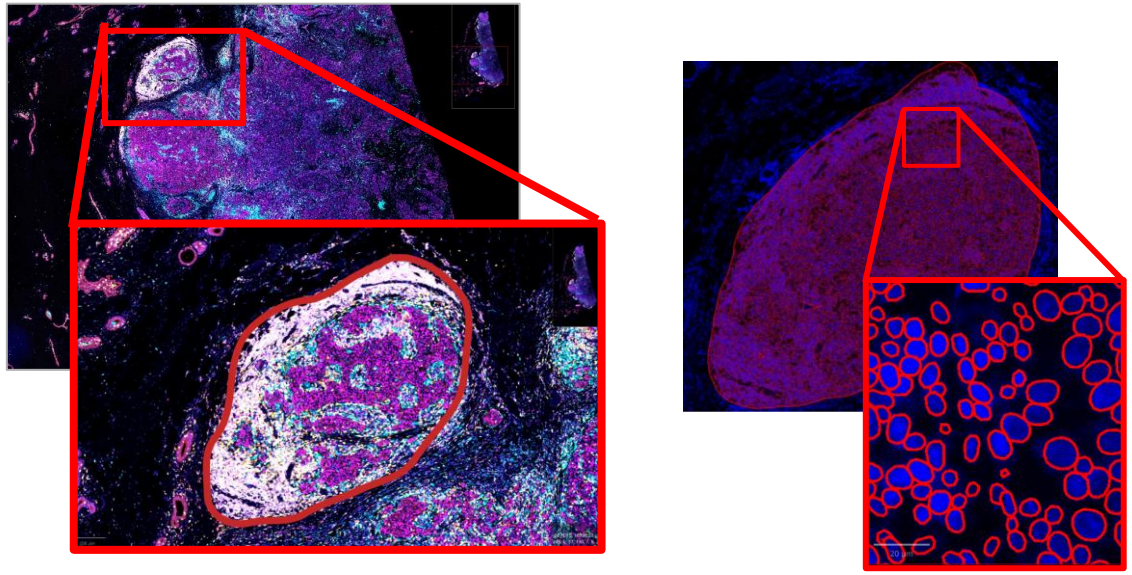

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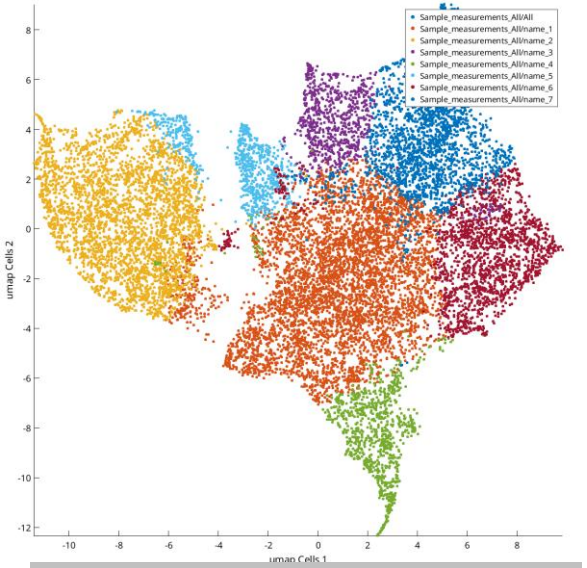

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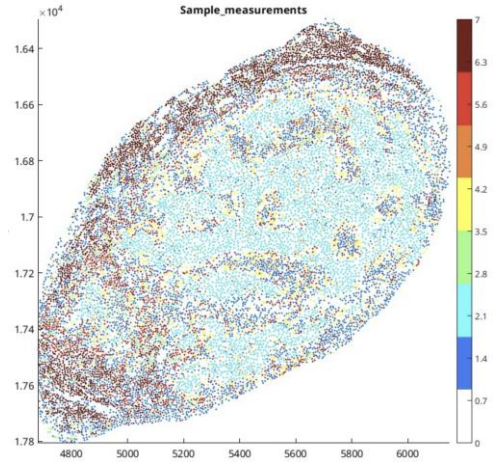
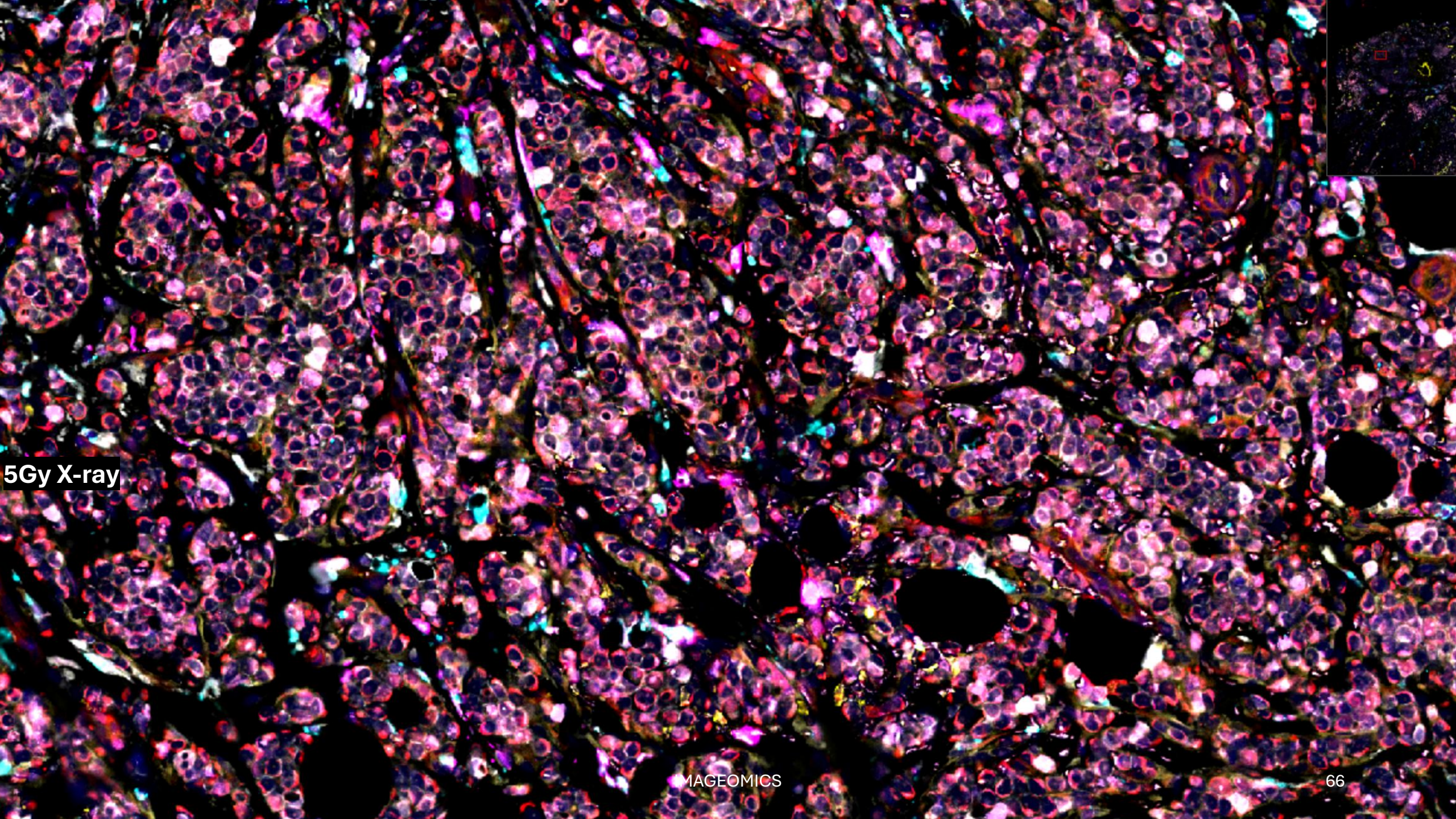
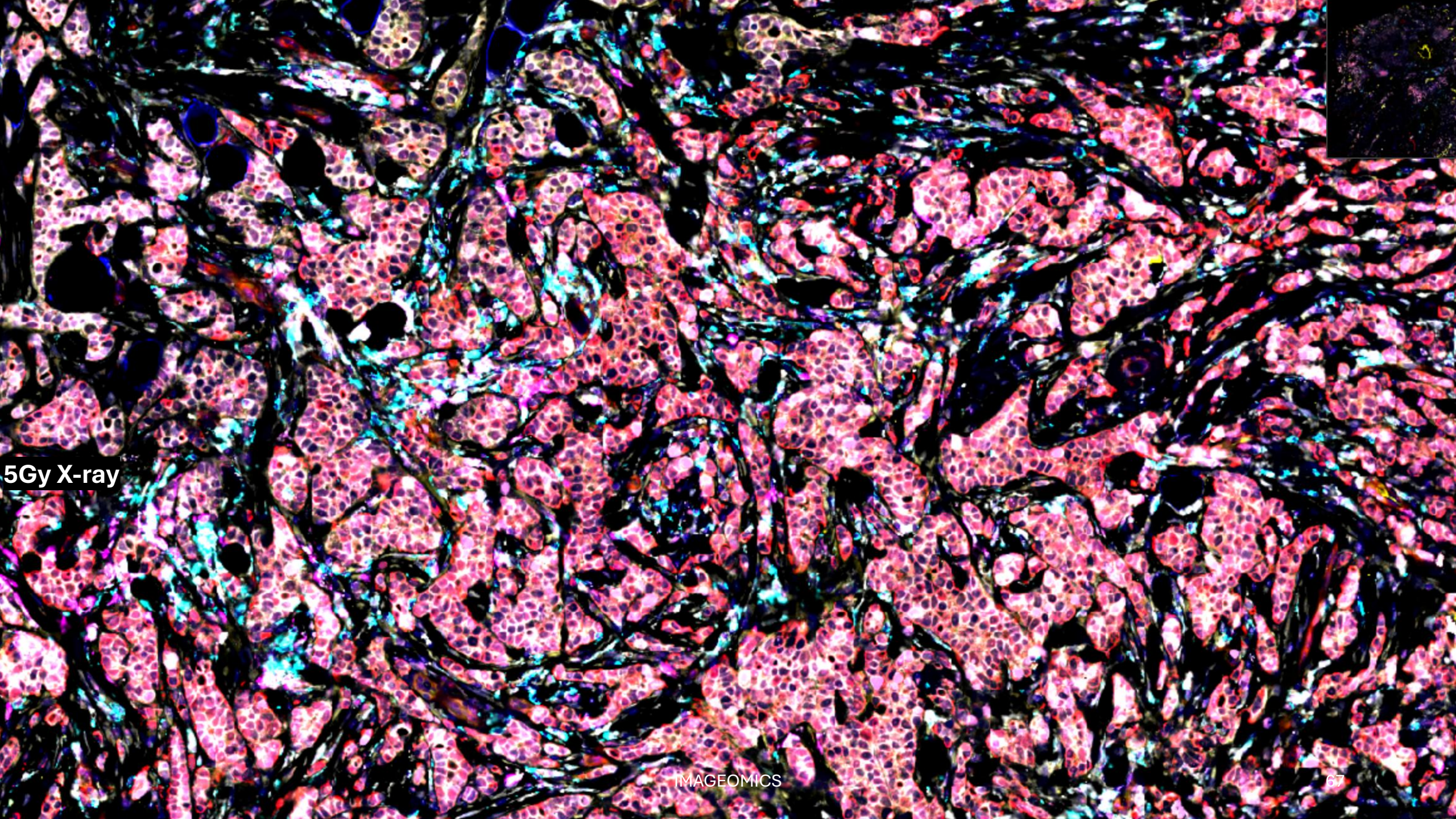


Spatial coordinates integreted with cluster annotation


5Gy X-ray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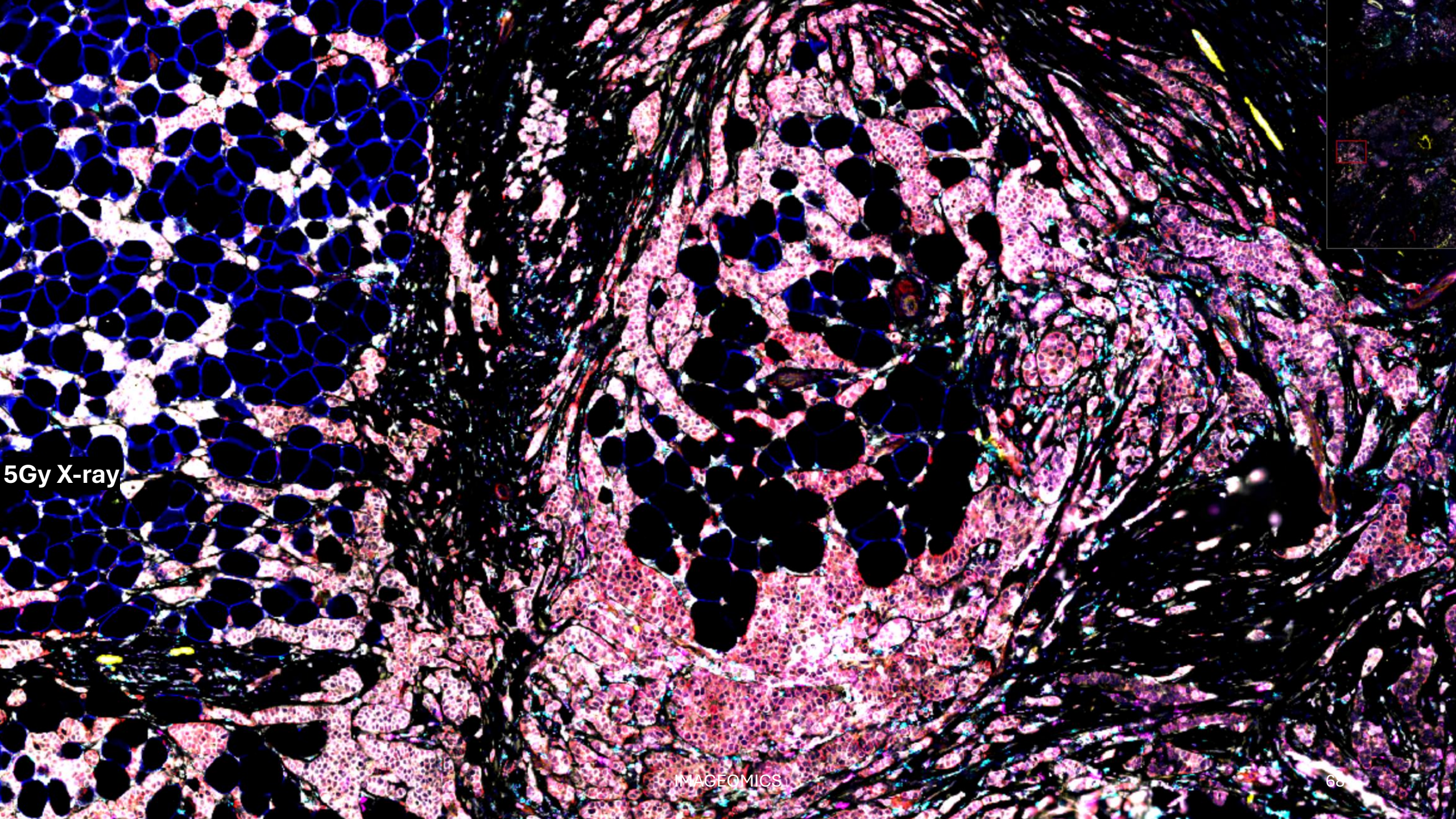
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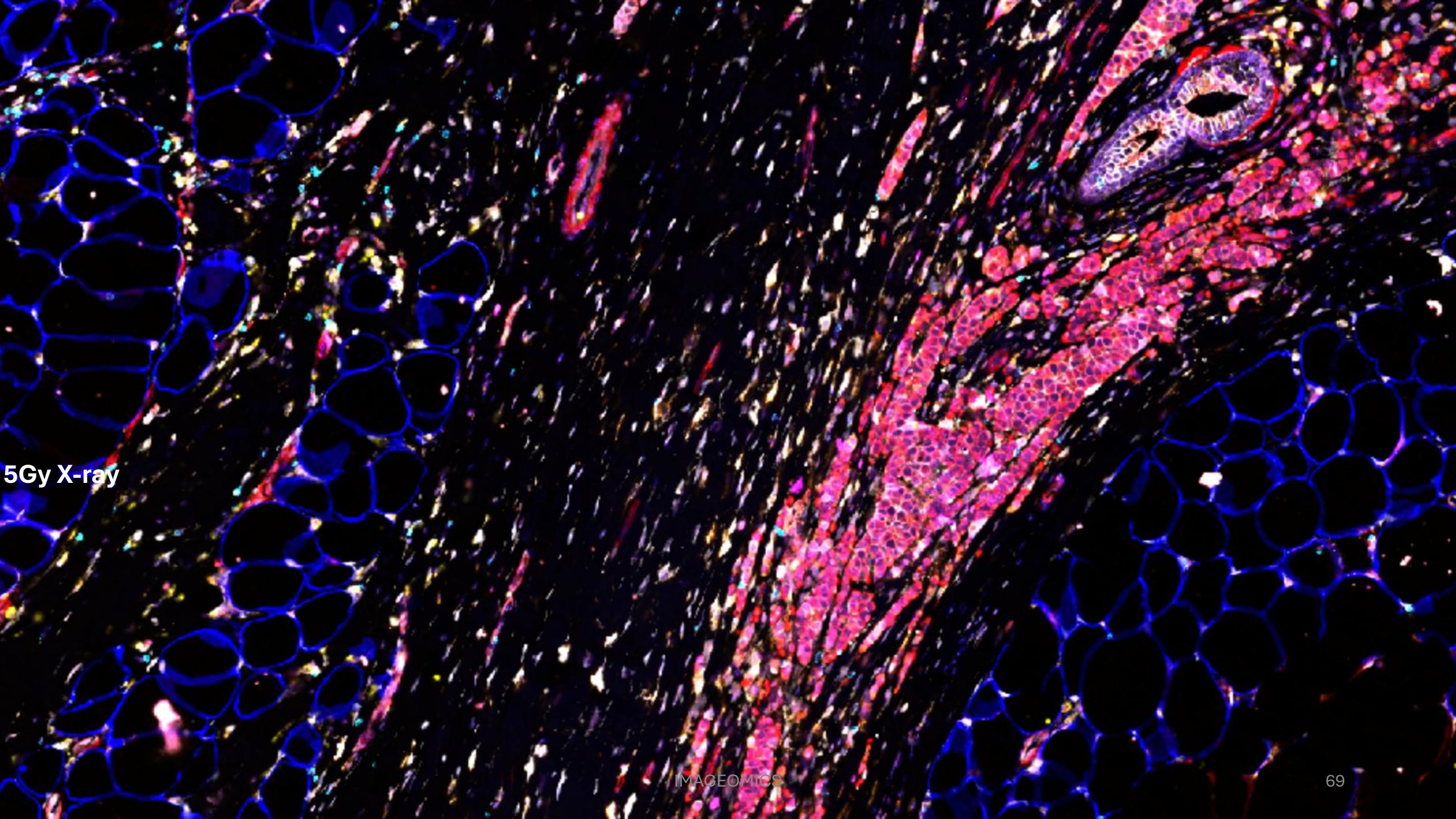
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Conclusions and outlook

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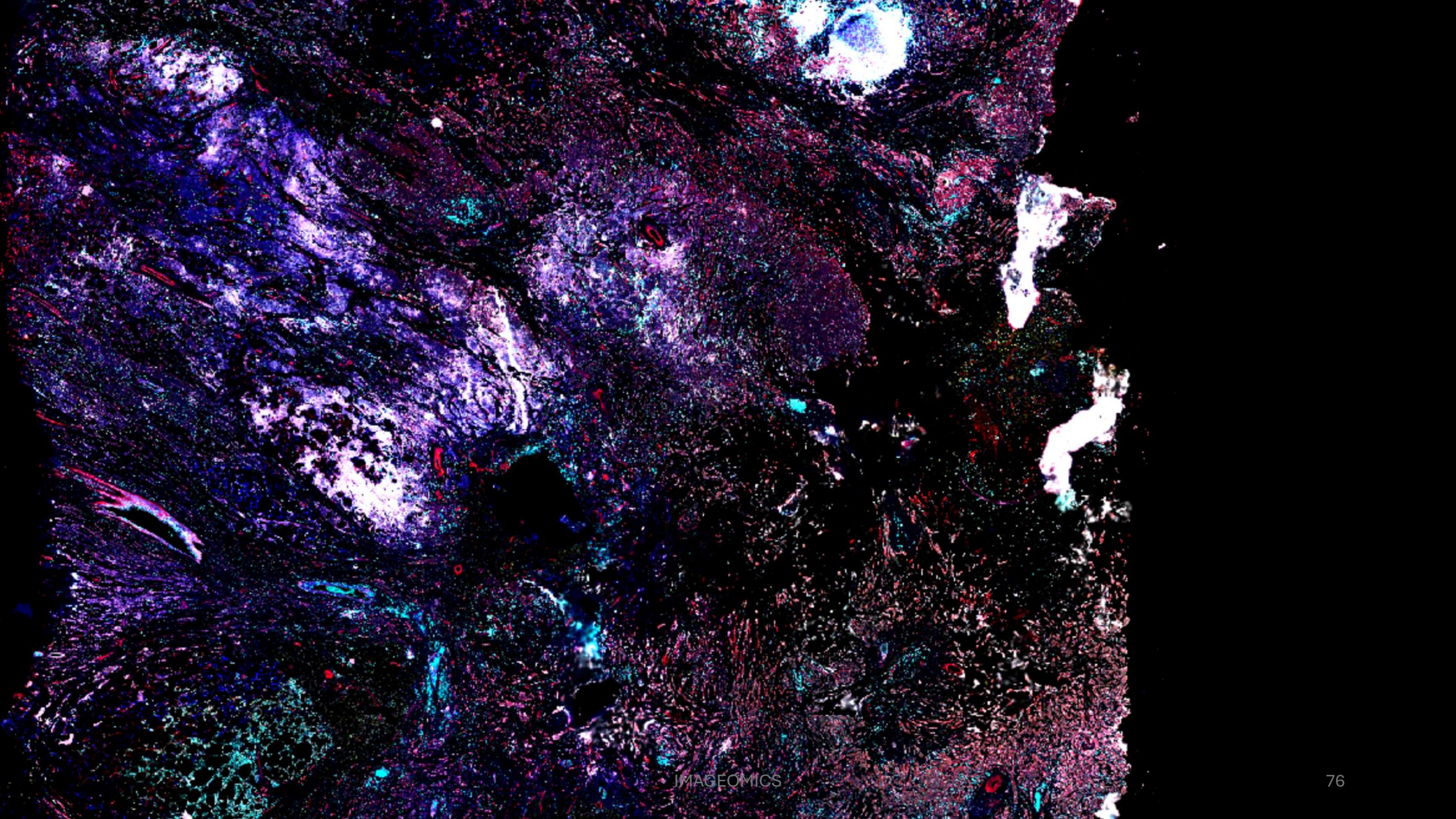
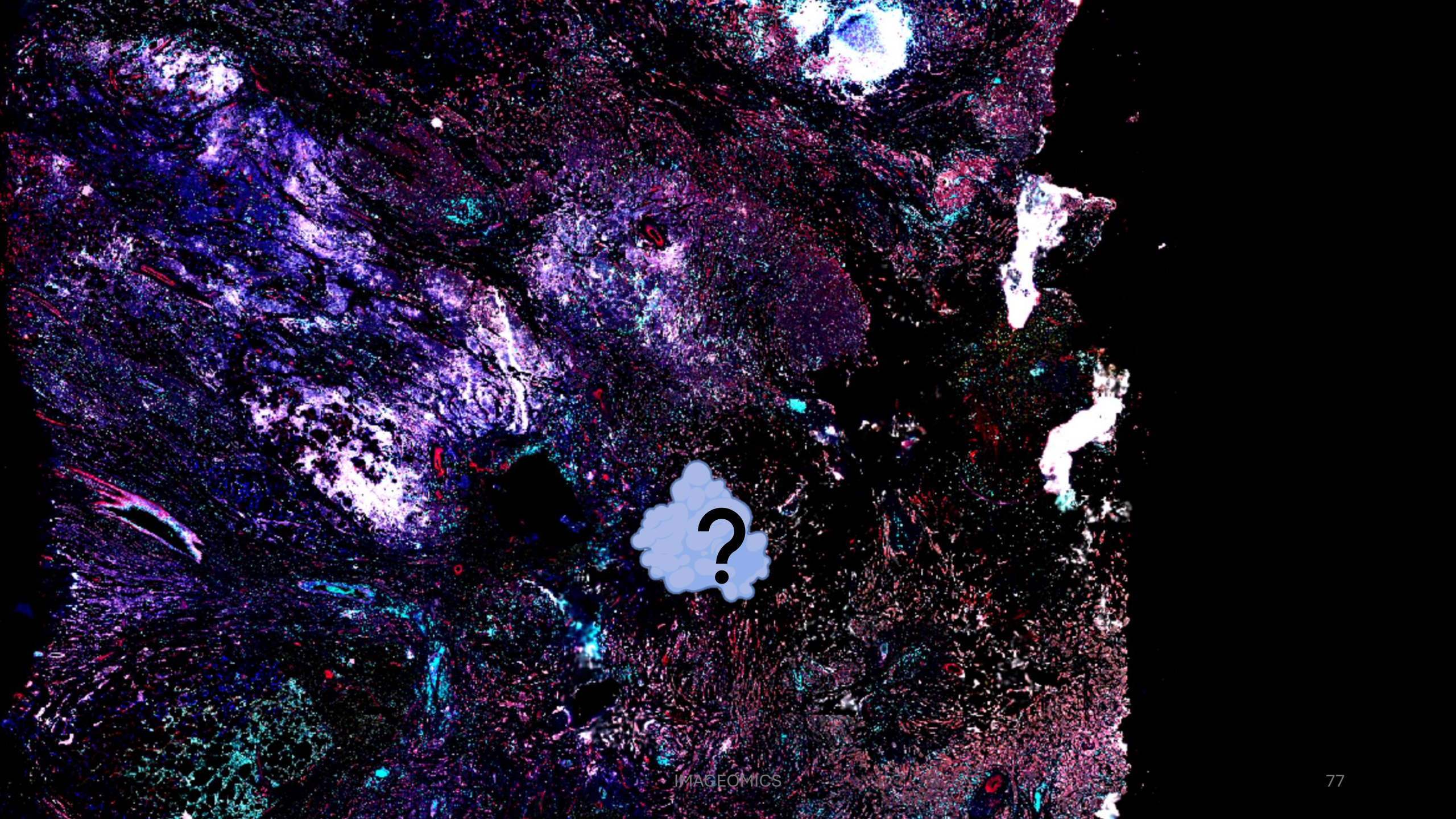
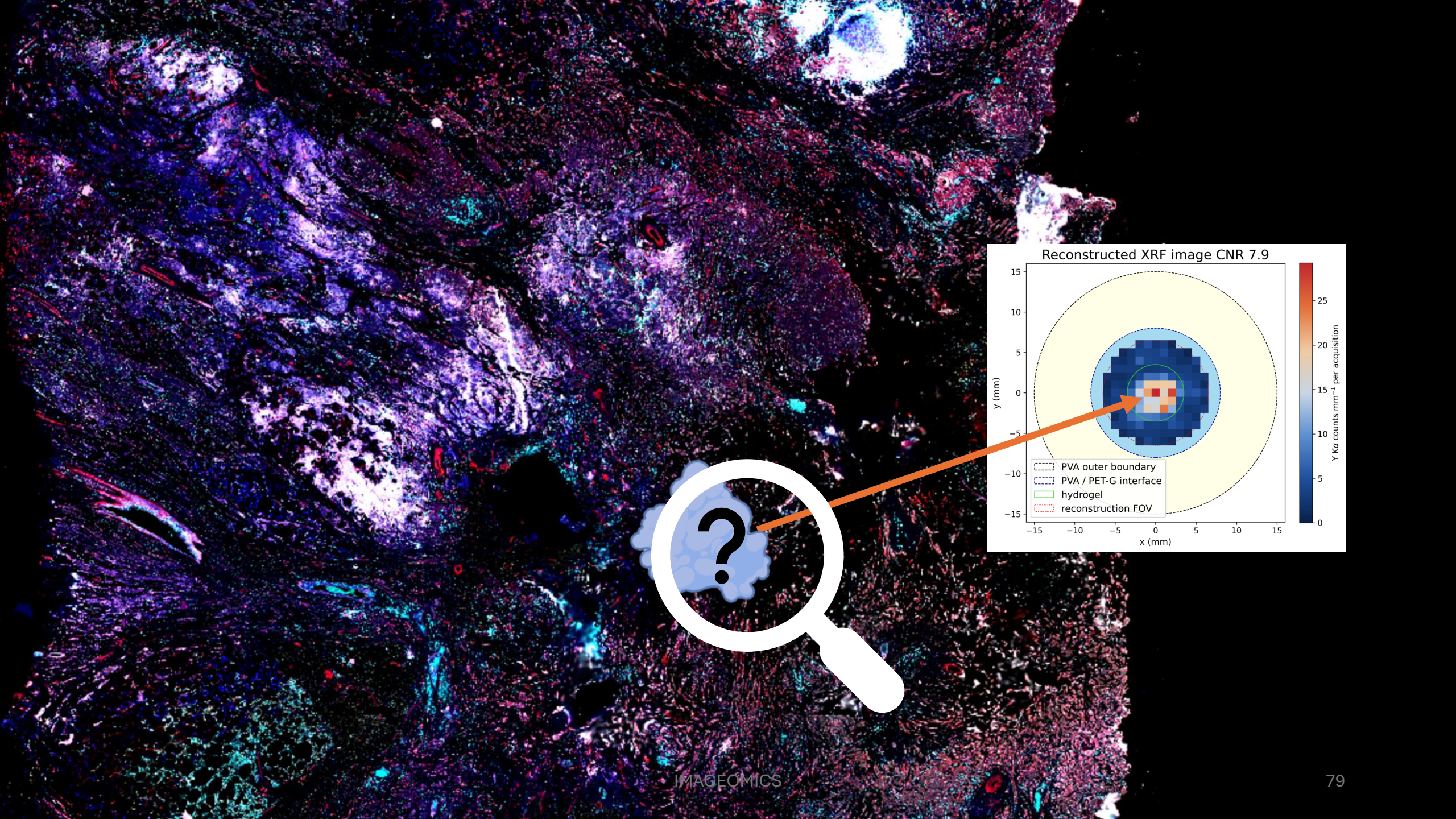
- Analysis of Spatial Proteomics dataset for the identification of new marker of treatment response
- Data integration with Spatial Transcriptomics dataset






Reconstructed XRF image CNR 7.9
PVA outer boundary
PVA / PET-G interface
hydrogel
reconstruction FOV
x (mm)
y (mm)
Y Kα counts mm⁻¹ per acquisition