Novel Biomechanical Analysis of Pancreatic Tumours Can Be Used for Both Diagnosis and Prediction of Short-Term Surgical Outcomes

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Background

- Around 600 people are diagnosed with pancreatic cancer in Ireland each year

- Five-year-survival for the most common type, Pancreatic Ductal Adenocarcinoma (PDAC), is around 10%

- Pancreatic cancer is currently the fourth highest cause of cancer–related mortality in Europe [1]

- By 2040, pancreatic cancer is predicted to be the second most common cause of cancer-related mortality after lung [2]

- The most common method for tissue diagnosis is endoscopic ultrasound fine needle biopsy (EUS FNB)

- Post-operative pancreatic fistulae (POPF) are intra-abdominal leaks of pancreatic fluid following pancreatic surgery

- Classified by the International Study Group on Pancreatic Surgery in 2016 [3]

- ~15% of patients develop Grade B or C POPF

- Associated with further morbidity and mortality
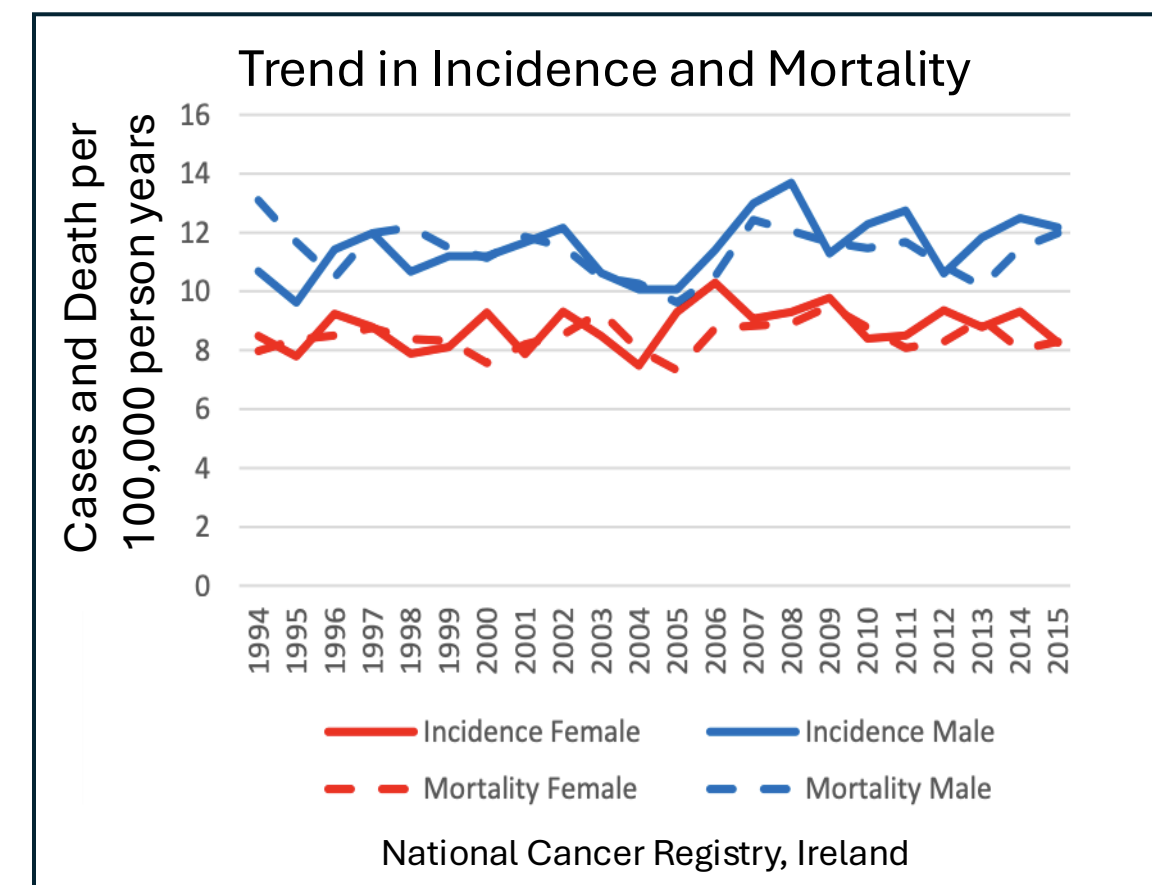
1. Joint Research Centre, European Commission; 2. JAMA Network Open (2021); 3. Surgery (2016)

Trend in Incidence and Mortality — National Cancer Registry, Ireland

Methods

Resections

- 4 μm
- Haemotoxylin & Eosin
- Regions of Interest (ROI) annotated by consultant histopathologist

- 20 μm
- Propidium iodide
- Atomic Force Microscopy (AFM)
- performed on ROIs for stiffness measurements

- 4 μm
- Picrosirius Red
- Collagen fibre analysis via polarised light microscopy

Biopsies

- EUS FNB attached to slide fresh
- AFM performed along length of biopsy
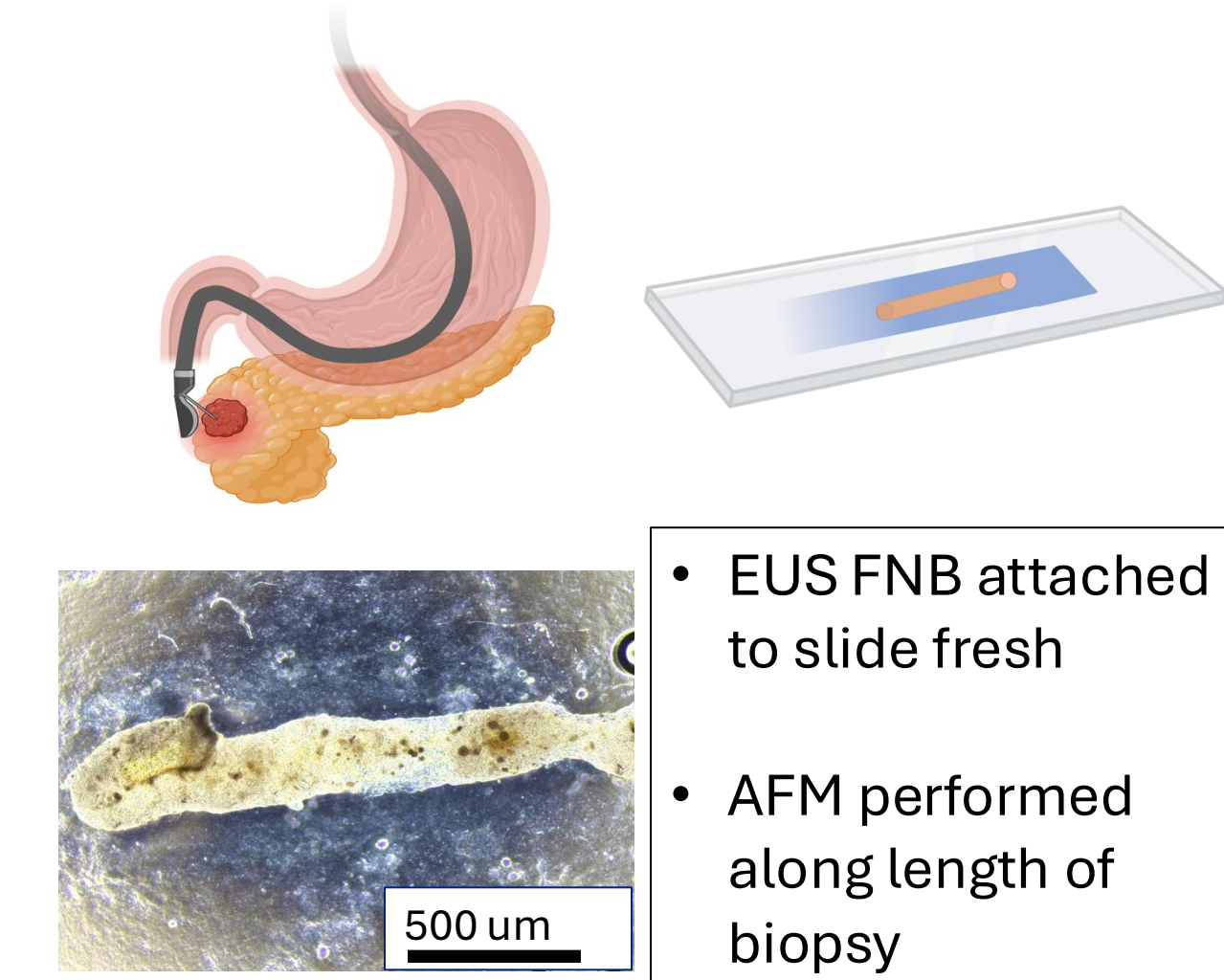
Atomic Force Microscopy

Force Map
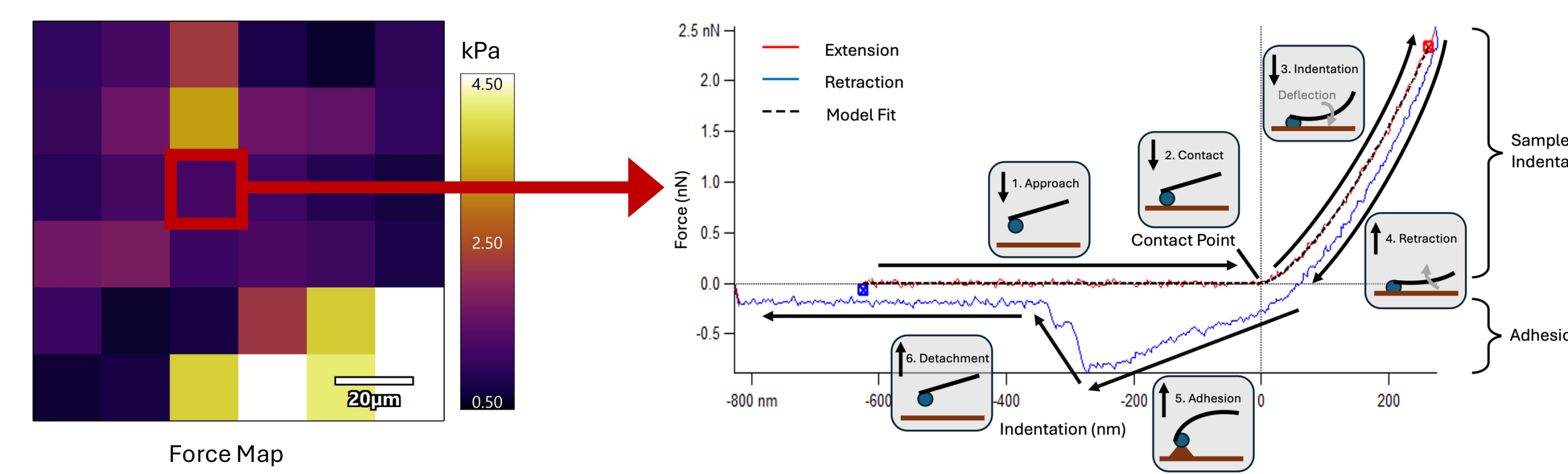
Biopsy Patients

- 7 patients
  - 5 metastatic PDAC
  - 1 non-metastatic PDAC
  - 1 neuroendocrine tumour (NET)

- Significant difference in sample modulus between metastatic and non-metastatic PDAC

- Significant difference in variance between PDAC and NET (9133889 vs 34644637, $p < 0.0001$)

Diagnosis	No. of Patients	Sample Modulus (Pa) (median, IQR)
Metastatic PDAC	5	2200 (1030 – 4640)
Non-Metastatic PDAC	1	250 (175 – 375)
Neuroendocrine Tumour	1	2000 (569 – 6410)
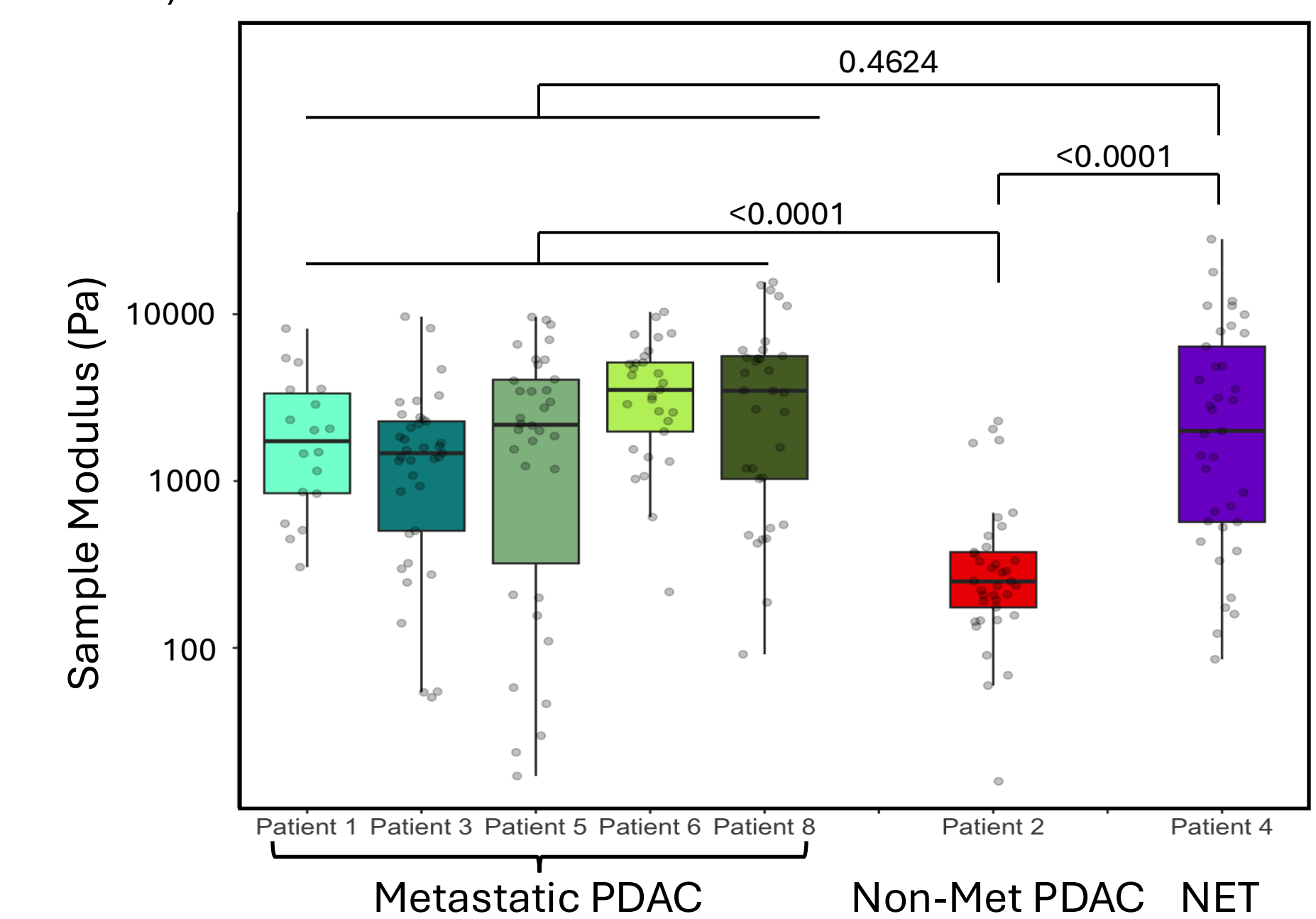
Resection Patients

Pt	Diagnosis	Location	Operation	Stage	POPF	Grade	BMI	Pre-Op Albumin
1	Ampullary Adenoca	Ampulla	Whipple	pT1a N0	Y	A	25.9	36
2	PDAC	Tail	Distal	pT3 N2	N	-	29.6	45
3	PDAC	Tail	Distal	pT2 N1	N	-	37.4	49
4	Ampullary Adenoca	Ampulla	Whipple	pT3b N2	N	-	26.9	37
5	Ampullary Adenoca	Ampulla	Whipple	pT3a N1	Y	A	22.1	33
6	IAPN (LGD)	Ampulla	Whipple	N/A	Y	B	35.8	41
7	PDAC	Head	Whipple	ypT2 N0	N	-	30.9	37

Elastic Modulus by Tissue Type

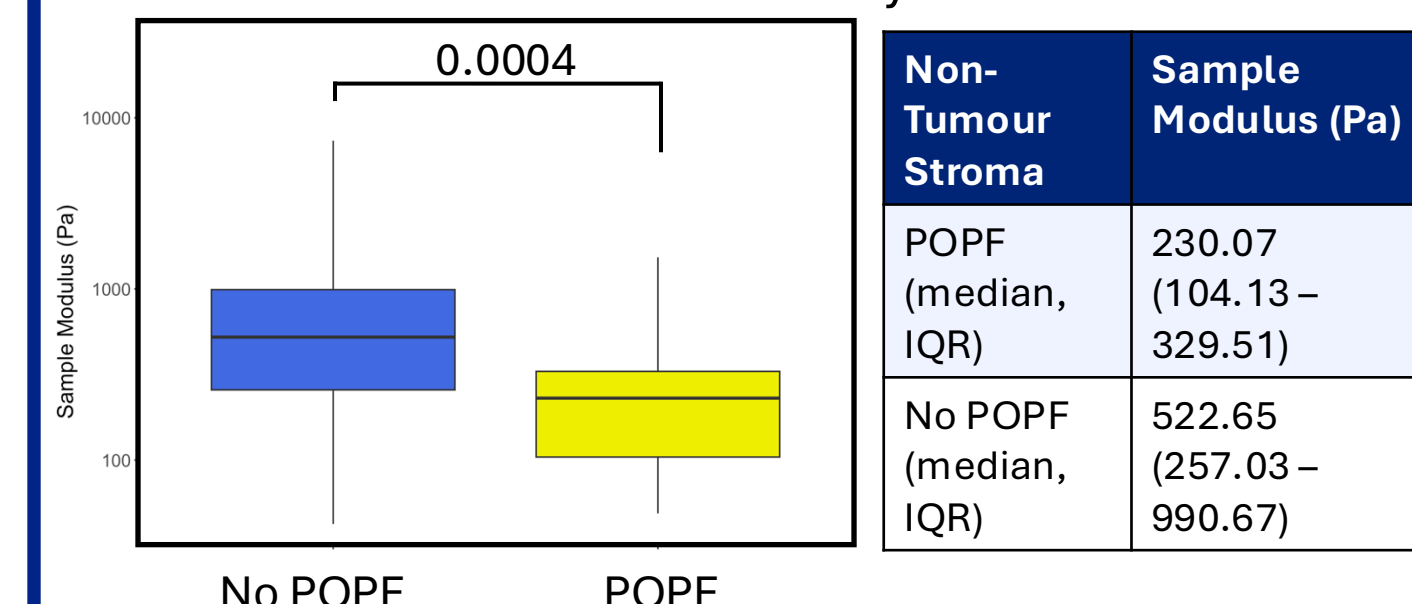| Tissue	Sample Modulus (Pa)
Non-Tumour Adjacent Epithelium (median, IQR)	99.37 (47.98 – 313.92)
Non-Tumour Adjacent Stroma (median, IQR)	507.53 (239.67 – 943.91)
Tumour Gland (median, IQR)	93.69 (39.84 – 178.59)
Tumour Stroma (median, IQR)	1275 (506.06 – 2457.50)

Post-Operative Pancreatic Fistulae

Elastic Modulus of NTA Epithelium by Fistula Status
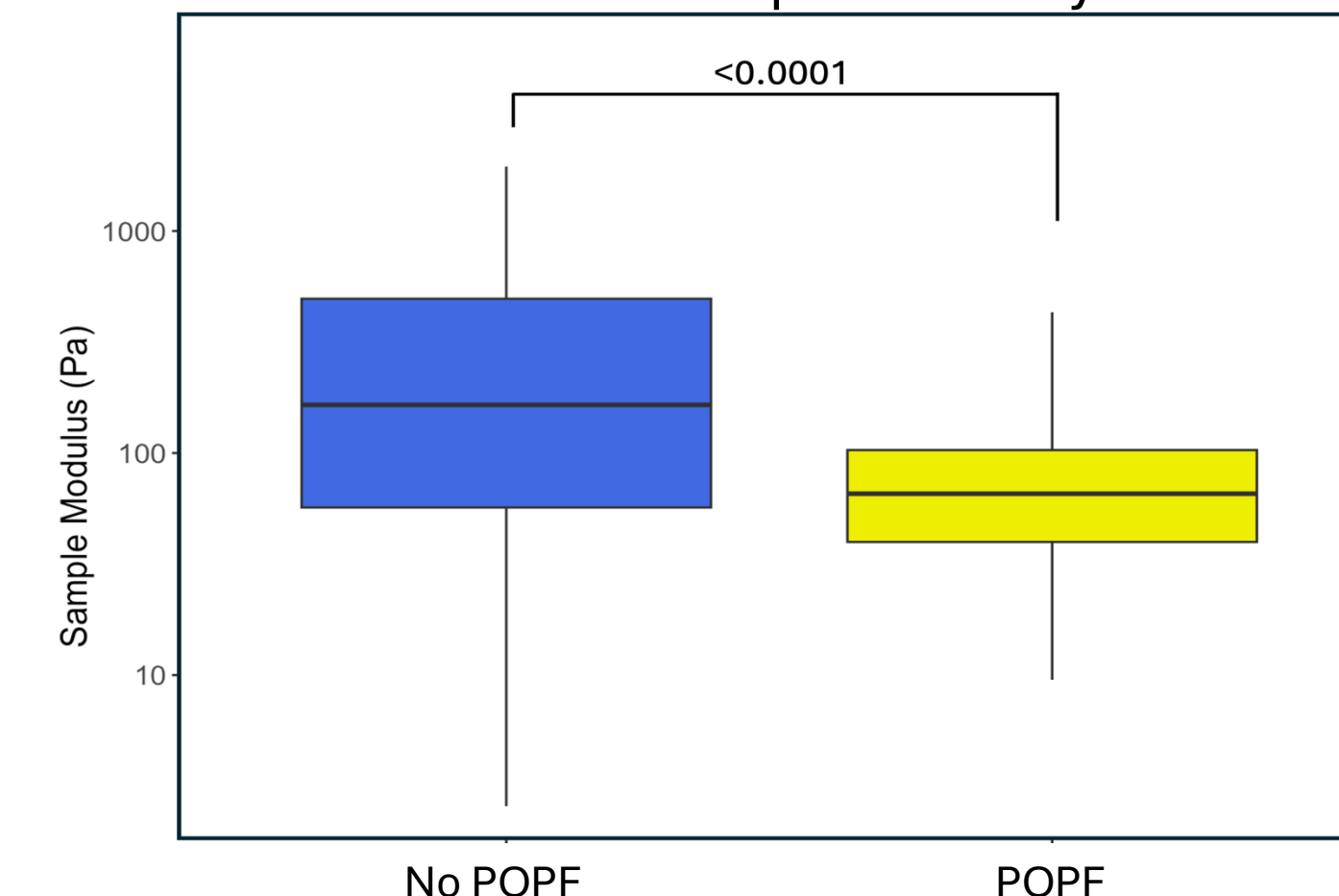
| Non-Tumour Adjacent Epithelium	Sample Modulus (Pa)
POPF (median, IQR)	65.59 (39.78 – 103.14)
No POPF (median, IQR)	164.81 (56.86 – 495.42)

Elastic Modulus of NTA Stroma by Fistula Status

Non-Tumour Stroma	Sample Modulus (Pa)
POPF (median, IQR)	230.07 (104.13 – 329.51)
No POPF (median, IQR)	522.65 (257.03 – 990.67)

Elastic Modulus of Tumour Stroma by Fistula Status
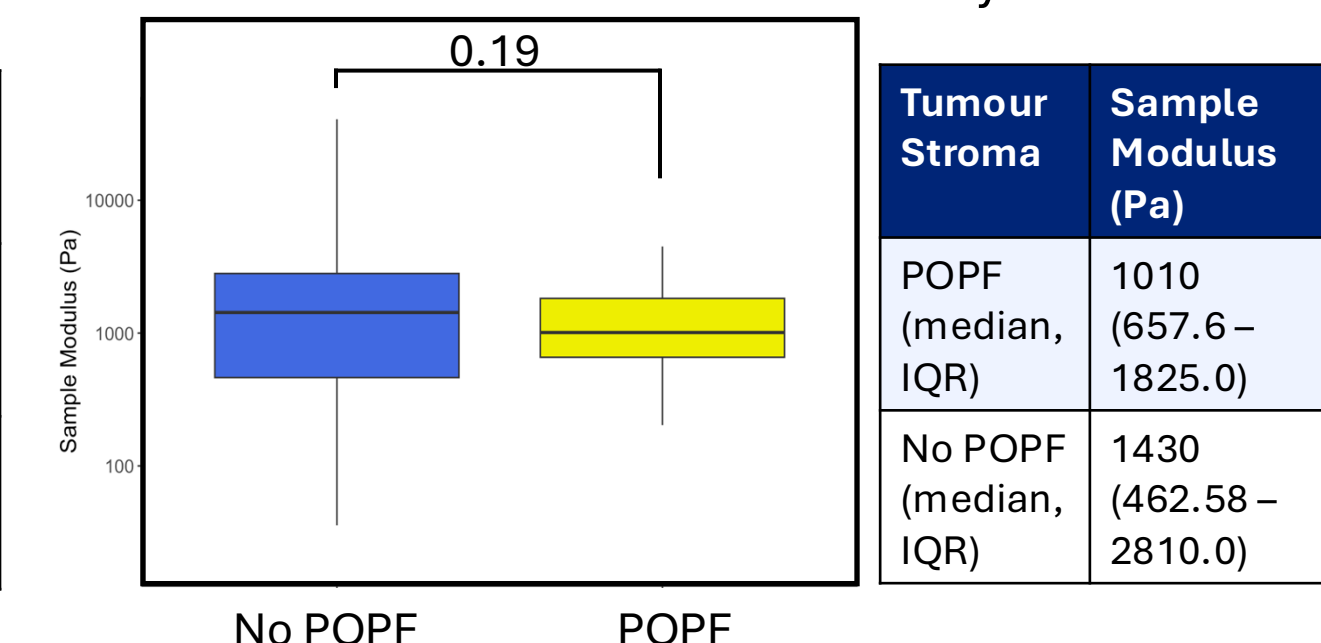
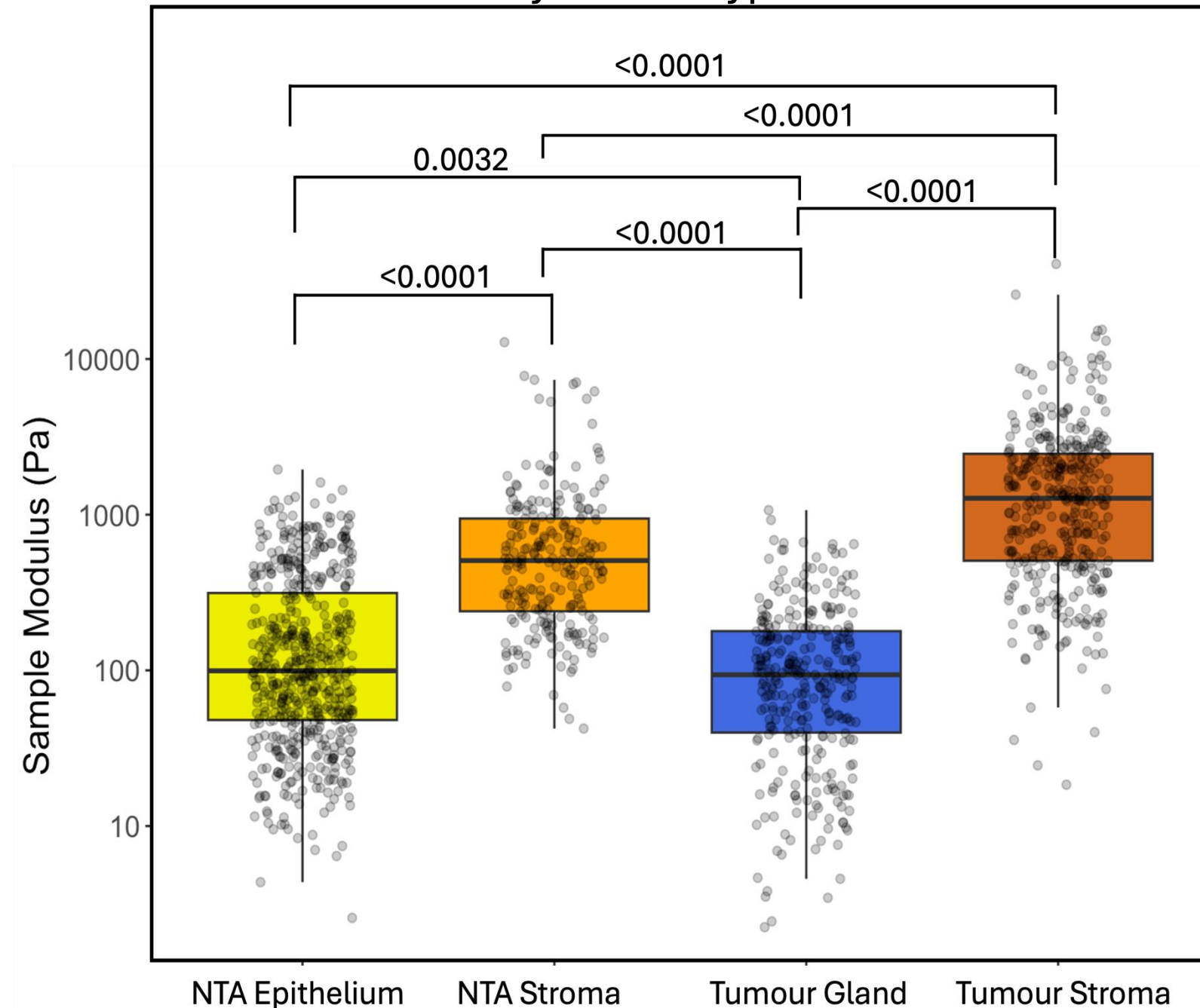| Tumour Stroma	Sample Modulus (Pa)
POPF (median, IQR)	1010 (657.6 – 1825.0)
No POPF (median, IQR)	1430 (462.58 – 2810.0)

Elastic Modulus of Tumour Gland by Fistula Status
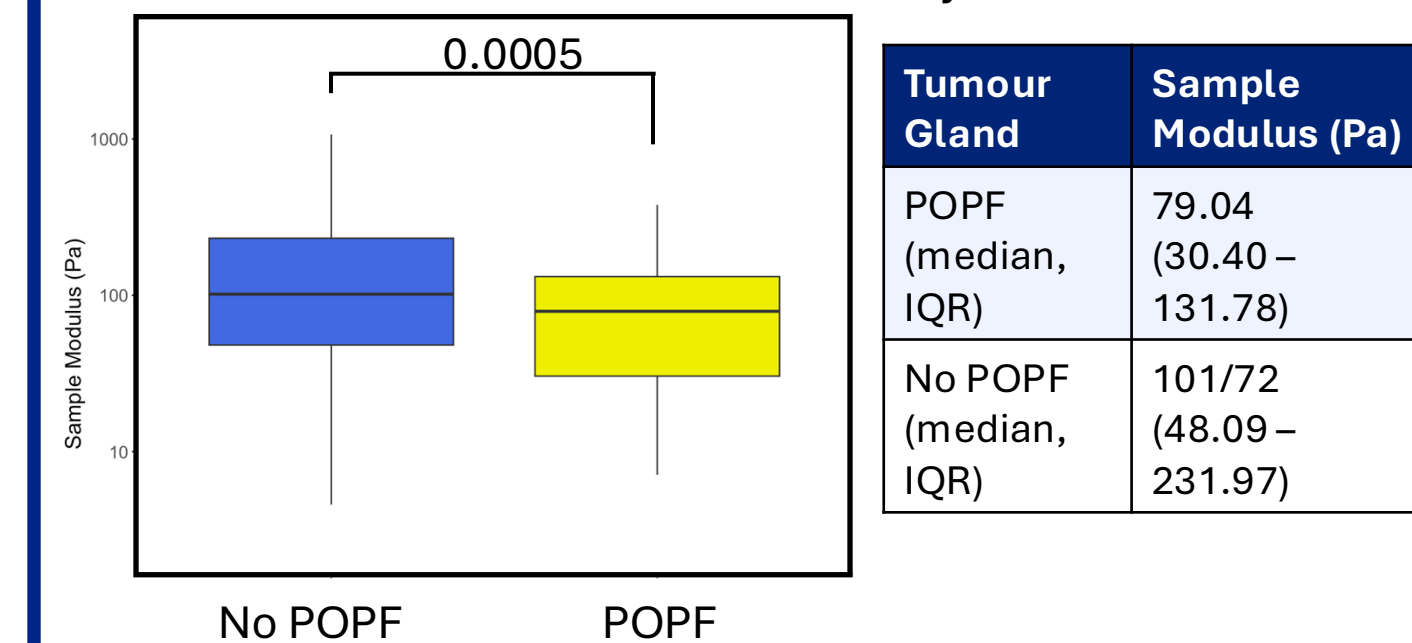
Tumour Gland	Sample Modulus (Pa)
POPF (median, IQR)	79.04 (30.40 – 131.78)
No POPF (median, IQR)	101/72 (48.09 – 231.97)

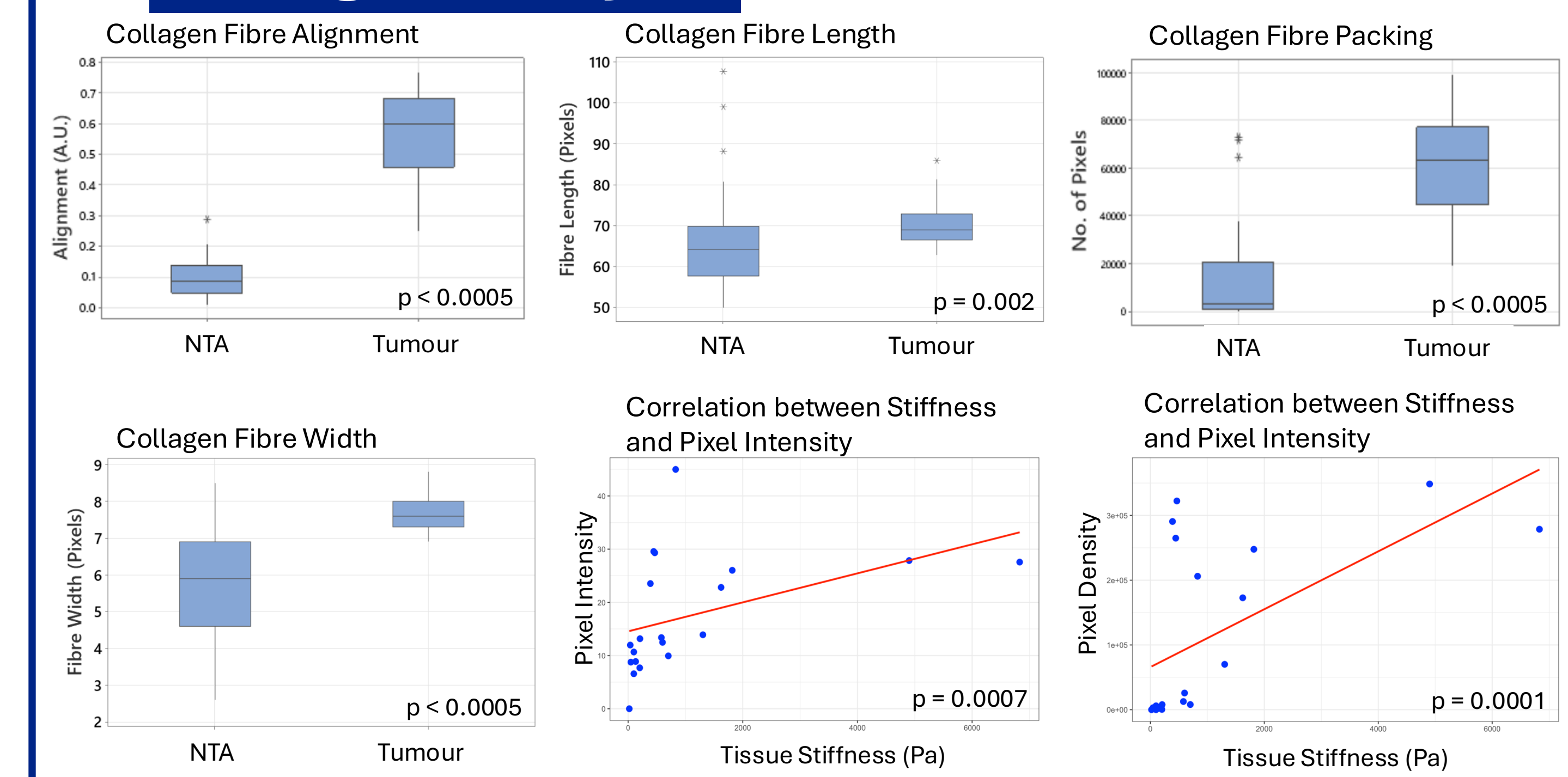| Length of Stay	Days
POPF (median, IQR)	16 (15 – 18.33)
No POPF (median, IQR)	11 (10 – 15)

Other Complications:
- Delirium x 2 (both a/w POPF)
- DGE grade B x 1 (a/w POPF)
- LRTI + Ileus (not a/w POPF)

Collagen Analysis

Collagen Fibre Alignment — $p < 0.0005$

Collagen Fibre Length — $p = 0.002$

Collagen Fibre Packing — $p < 0.0005$

Collagen Fibre Width — $p < 0.0005$

Correlation between Stiffness and Pixel Intensity — $p = 0.0007$

Correlation between Stiffness and Pixel Intensity — $p = 0.0001$

Conclusions

- This is the first time biomechanical analysis has been performed on pancreatic biopsies
- In our small sample size, non-metastatic PDAC tumours are softer than their metastatic counterparts, in keeping with in vitro studies
- The correlation between POPF and soft pancreata has been demonstrated for the first time using AFM
- Different tissue types in and surrounding pancreatic tumours have different elastic moduli
- Increased tissue stiffness is associated with more collagen fibres
- Tissue biomechanics has the potential to both diagnose tumours, identify distinct tissue types, and predict those at a higher risk of developing POPF