Deep proteome mining of FFPE tissue with PASEF technology

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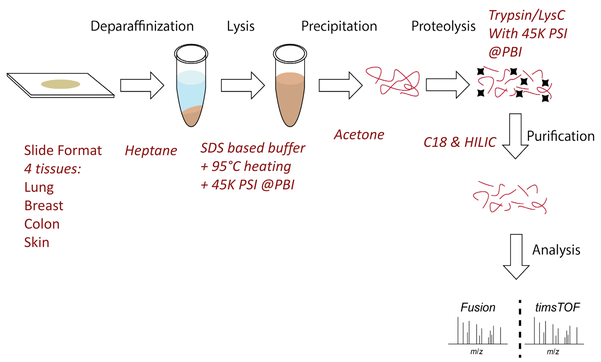
INTRODUCTION

Formaldehyde fixation and paraffin embedded (FFPE) tissues are ubiquitous in histological archives and invaluable clinical resources for diagnosis, treatment, and characterization of disease. However, FFPE tissue is a persistent proteomic challenge. Here, we define a robust, reproducible method by exploring orthogonal parameters efficiently. Of note, we tested hydrophilic interaction chromatography (HILIC) to enrich peptides from FFPE tissue following proteolytic digest in the presence of detergents and contaminants against more traditional C18-based enrichment. Further, we investigated use of the Barocycler from Pressure Biosciences Inc. (PBI) for high efficiency protein extraction and digestion. We also compare two different MS technologies, timsTOF with parallel accumulation serial fragmentation (PASEF) and Orbitrap (Fusion) to determine the advantages of orthogonal gas-phase separations. We observe increased peptide and protein depth by timsTOF with PASEF, at least in part due to the sensitivity and analysis speed of the instrument. Overall, we present our recommendations for robust analysis of tissues to open doors to information previously hidden in the large tranche of FFPE archives.

SAMPLE PREP WORKFLOW



INSTRUMENTATION

ANALYSIS CONDITIONS	TIMS TOF	FUSION
Column details	25cm sub-2uM C18 reverse phase column	25cm sub-2uM C18 reverse phase column
Fragmentation method	CID	CID
Gradient length	80 minutes	80 minutes
Injection amount & flow rate	200ng @ 400nL/min	200ng @450nl/min
Resolution (FWHM) MS/MSMS	40,000/40,000	120,000/5,000
Scan rate	120Hz	20Hz

RESULTS

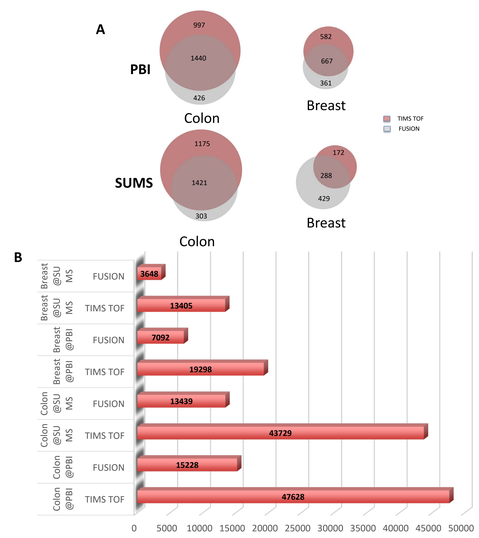


Fig. 1: (A) Protein ID comparison for FFPE samples prepared with Barocycler at Pressure Biosciences and Stanford (SUMS), **(B) 4X increase** in peptides (PSMs)

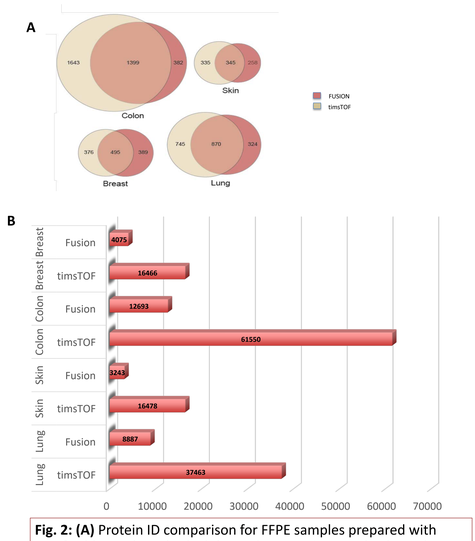


Fig. 2: (A) Protein ID comparison for FFPE samples prepared with Stanford (SUMS) methods, **(B) 4.7X increase** in peptides (PSMs) across all tissue types

Sequence coverage of Top 200 proteins

Tissue type	Fusion (%)	timsTOF (%)	Unique peptides (Fusion)	Unique Peptides (timsTOF)
Colon	37	44	2361	6327
Lung	37	52	2349	5968
Breast	28	40	1275	2309
Skin	30	41	1889	2332

2X more

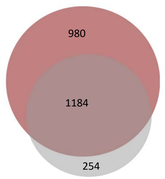


Fig. 3: Novel proteins identified from FFPE colon tissue using SAINT analysis

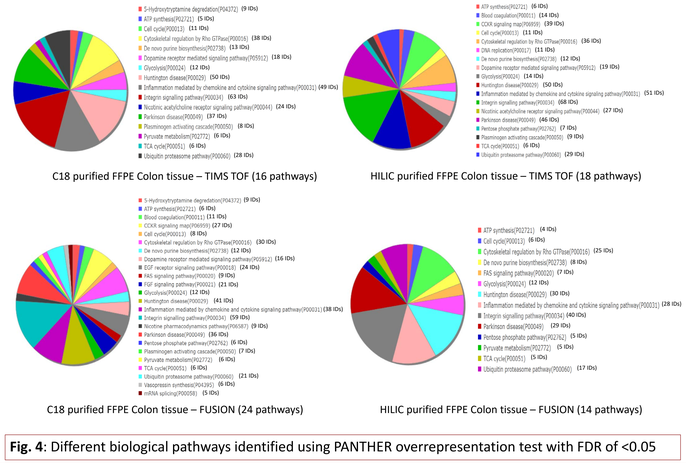


Fig. 4: Different biological pathways identified using PANTHER overrepresentation test with FDR of <0.05

DISCUSSION

- Formyl modification of K provides a quick measure of the efficiency of crosslinking reversal. Here, we observe similar low rates across various sample preparation approaches. Use of the Barocycler offers unique advantages in terms of automation, particular for larger sample sets

Sample prep method	Instrument	Modification (%)
PBI with C18 (Colon)	FUSION	1.5
PBI with C18 (Colon)	TIMS	1.1
SUMS with C18 (Colon)	FUSION	0.8
SUMS with C18 (Colon)	TIMS	1.1
PBI with C18 (Breast)	FUSION	1.5
PBI with C18 (Breast)	TIMS	2.1
SUMS with C18 (Breast)	FUSION	0.7
SUMS with C18 (Breast)	TIMS	0.6

- The increased number of observed proteins and peptides seem to be consistent across all tissue types with samples prepared from both Stanford's sample prep method and pressure cycling sample prep (Barocycler) with C18 peptide enrichment. These gains are attributed to increased speed and sensitivity observed due to the trapped ion mobility separation coupled with higher duty cycle of the timsTOF mass spectrometer

CONCLUSION

Advantages of PASEF technology:

- **5X** more peptides per protein across all tissue types
- Venn diagrams of the two instrumental platforms show a large overlap **(80%)** of proteins identified by timsTOF and Fusion
- At least **10%** more sequence coverage with **2X** more unique peptides for a higher confidence score of protein identification
- Based on SAINT probability scores, at least **30%** more novel protein identifications for FFPE colon tissue

Sample preparation strategies:

- C18 proves to be more efficient and reproducible than HILIC in terms of proteome depth and identification of biologically relevant proteins
- HILIC enrichment targeted comparable biological pathways as C18 in protein analysis using PANTHER classification system with 20% of the proteins being characterized

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