Deep, Reproducible and High-throughput FFPE Analyses: Moving Toward Large-Scale Clinical Omics Applications

Matthew Willetts[1], Christopher Swift[1], John P Wilson[2]

1. Bruker Daltonics, Billerica, MA, USA
2. ProtiFi, LLC, Farmingdale, NY, United States

ASMS 2020, TP 130

Introduction

Formalin fixation and paraffin embedding (FFPE) tissue preparation is standard in pathology departments worldwide for diagnosis by staining and immuno-histochemistry. Analysis of the FFPE samples collected in current practice is thus a natural entry point for omics methods to inform clinical decisions. Additionally, FFPE samples are uniquely stable at room temperature, resulting in massive retrospective archives often with corresponding diagnoses and disease courses. These collections thus represent an invaluable resource for retrospective and translational studies.

However, despite this huge potential in research and medicine, proteomic analysis of FFPE samples has lagged. Crosslinked, wax-saturated samples are not in their native state suitable for MS analysis. Additionally, the myriad sample processing protocols have not in general been developed to yield flash-frozen results from paired FFPE samples, leaving question as to their output. FFPE processing must also be automatable and the resulting peptide samples must be analyzed in a time scale compatible with future large-scale deployment without a tradeoff between depth of analysis and speed.

Herein, we combine ProtiFi HYPERsol sample processing and Bruker timsTOF Pro data acquisition to prepare and analyze in one day 50 FFPE samples with great overall reproducibility and depth in an automatable format.

Methods

Cores from mouse tissues were processed using the HYPERsol protocol(1). Briefly, samples were dissolved in 5% SDS using ultrasonication and reverse crosslinked at 80 °C for 2 hrs. Samples were subsequently loaded onto S-Traps (ProtiFi) for cleanup and trypsin digestion. The resulting tryptic peptides were separated on a 20 min gradient using a 15cm C18 1.6um column (Ionopticks, Australia) and a nanoElute nano LC system (Bruker Daltonics) and analyzed on an ion mobility equipped Q-TOF (timsTOF Pro, Bruker Daltonics) operating in dda-PASEF mode. Data were processed in PEAKS X+ software (Bioinformatics Solutions Inc) and Simplifi (www.simplifi.protifi.com).

FFPE Sample Processing

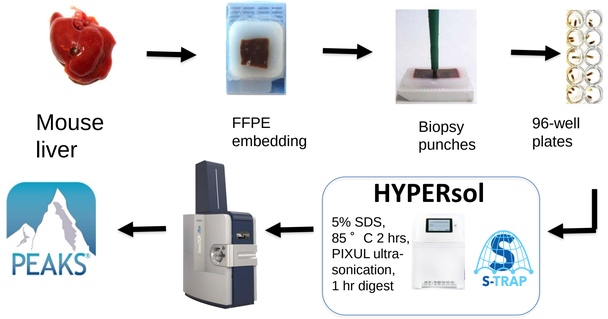

Figure 1: Experimental workflow. FFPE mouse liver samples were cored and dissolved in 5% SDS using ultrasonication on a PIXUL 96-well sonicator. S-Traps in 96 well plate format were used for cleanup and digestion. This processing methodology is fully automatable(2). Data were acquired on a timsTOF Pro using the standard short gradient method included in the software. Data were processed in PEAKS and SimpliFi (ProtiFi).

Data Acquisition

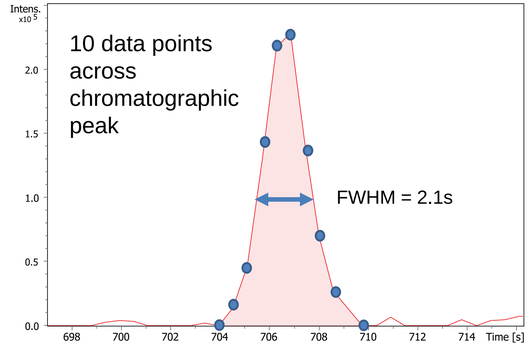

Figure 2: Median chromatographic peak width (FWHM) was 2s. The very high sequencing speed of the timsTOF Pro maintains 10 data points across the peak while acquiring MSMS spectra at an average of 70 precursors/sec.

References

1. *J. Proteome Res.* 2020, 19, 2, 973-98.
2. *Nucleic Acids Research*, Volume 47, Issue 12

Results

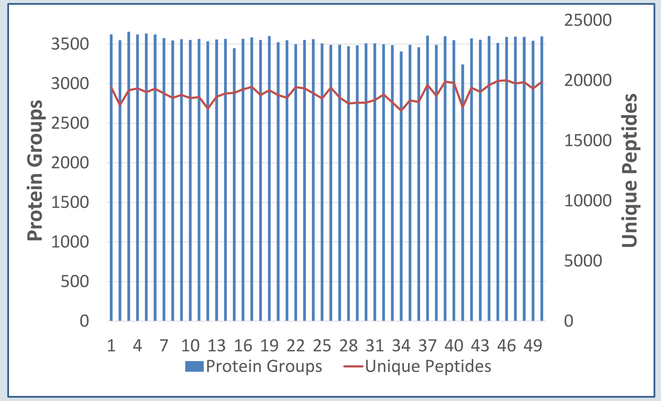

Figure 3: Deep, consistent protein and peptide identifications. A mean of 3540 protein groups and 17757 unique peptides were identified in 50 FFPE samples acquired in 24 hours. %CVs were 1.89 and 3.57 respectively.

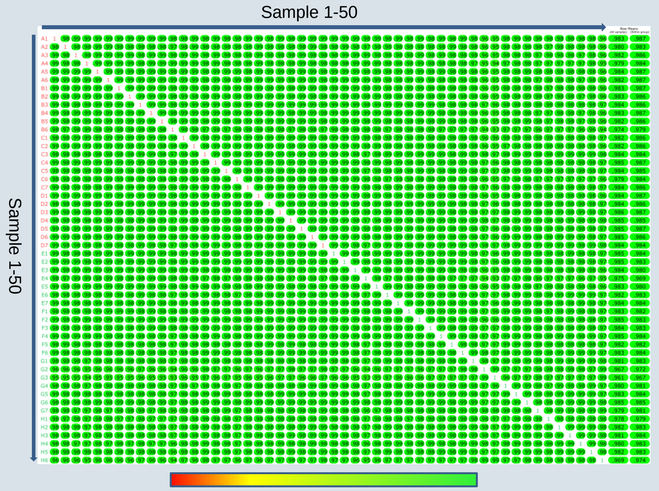

Figure 4: Person's correlation across 50 FFPE samples show excellent LFQ reproducibility (Average r = 0.981)

- **H**igh-**Y**ield **P**rotein **E**xtraction and **R**ecovery by direct **SOL**ubilization (HYPERsol), using 96-well plate ultrasonication and S-Trap plates affords direct and efficient analysis of FFEP. HYPERsol can be automated for large-scale studies.
- The high sequencing speed and sensitivity of the dda-PASEF acquisition method on the timsTOF Pro enables deep protein coverage from a very fast 20 min gradient. Allowing >50 FFPE samples to be analyzed in one day.
- An average of 3540 protein groups and 17757 unique peptides were identified across the 50 samples.

Conclusions

- The HYPERsol protocol enables highly reproducible protein identification and quantification from FFPE tissue
- Increased peak capacity via trapped ion mobility spectrometry with PASEF allows the efficient use of short gradients while maintaining excellent protein coverage.
- >50 FFPE samples can be prepared and analyzed in < 24 hrs.

timsTOF Pro