

Infrared Laser Ablation Microsampling for Small Volume Proteomics of Formalin Fixed Paraffin Embedded Tissue



B. Chisom Egbejiogu; Fabrizio Donnarumma; Kermit K. Murray
Louisiana State University, Department of Chemistry, Baton Rouge, LA

Goal

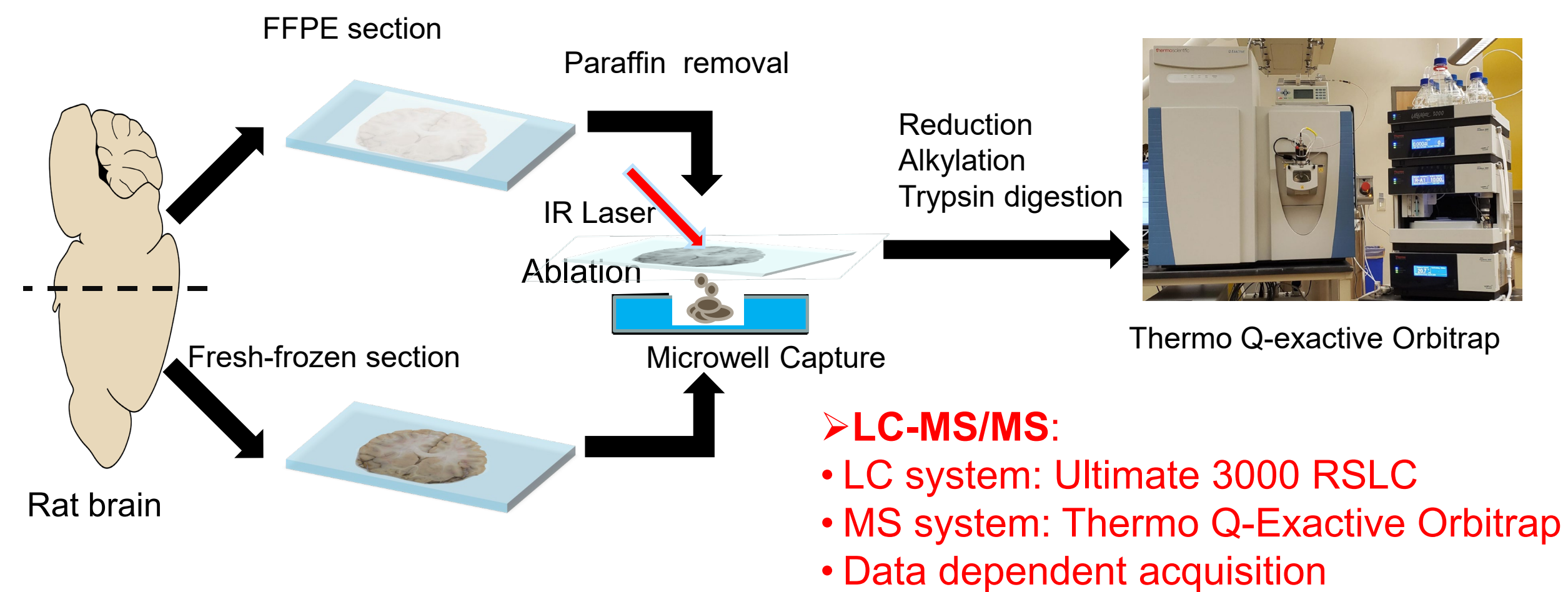
This study aims to improve localized sampling of small areas of FFPE and fresh-frozen tissues and uncover proteomic differences between the two conservation techniques for improved analytical sensitivity.

Introduction

- Microsampling for proteomics is reproducible with laser ablation
- Micro-volume sample processing for small area analysis
- FFPE capabilities improve scope of method
- FFPE tissue analysis difficult due to protein crosslinking
- New workflow needed for FFPE tissue microsampling

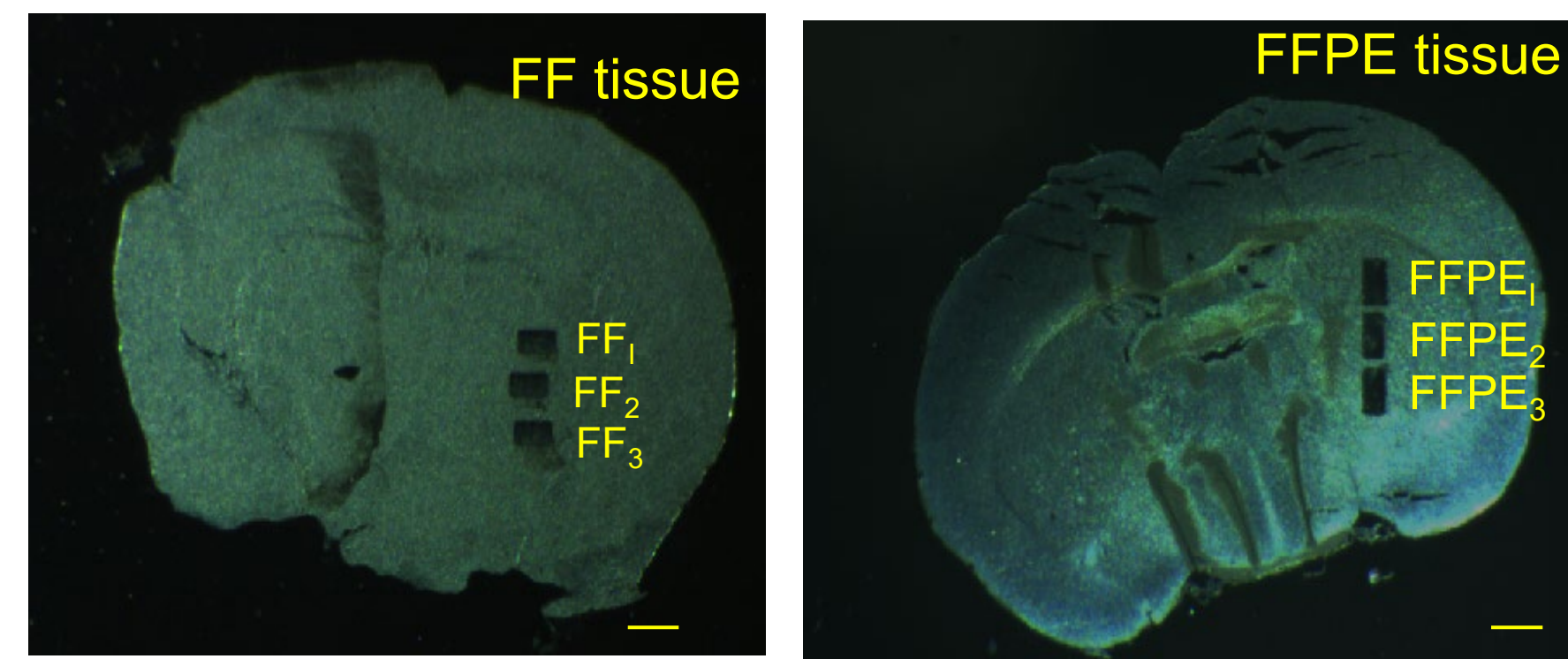
Experimental

IR Laser Ablation and Capture

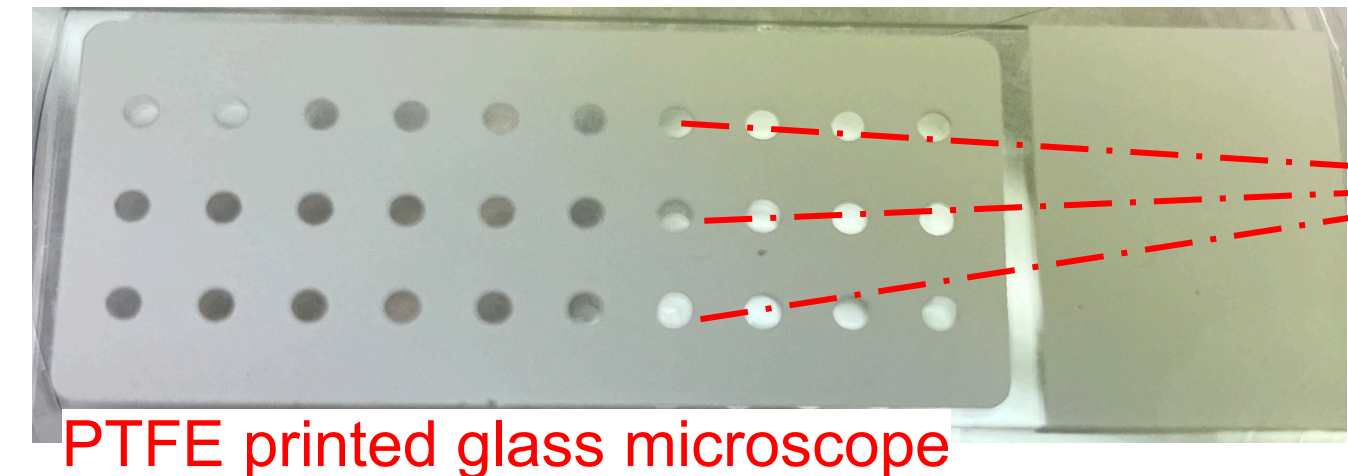


- >IR laser:**
- Optical parametric oscillator (OPO)
 - 2.94 μ m
 - 300 kJ/m² fluence

Tissue Ablation and Capture



- Rat midbrain (triplicates)
- Area: 0.4 mm²
- < 5 minutes ablation time
- Ablation tissue volume:
 - 0.004 mm³
- Scale bar: 1mm

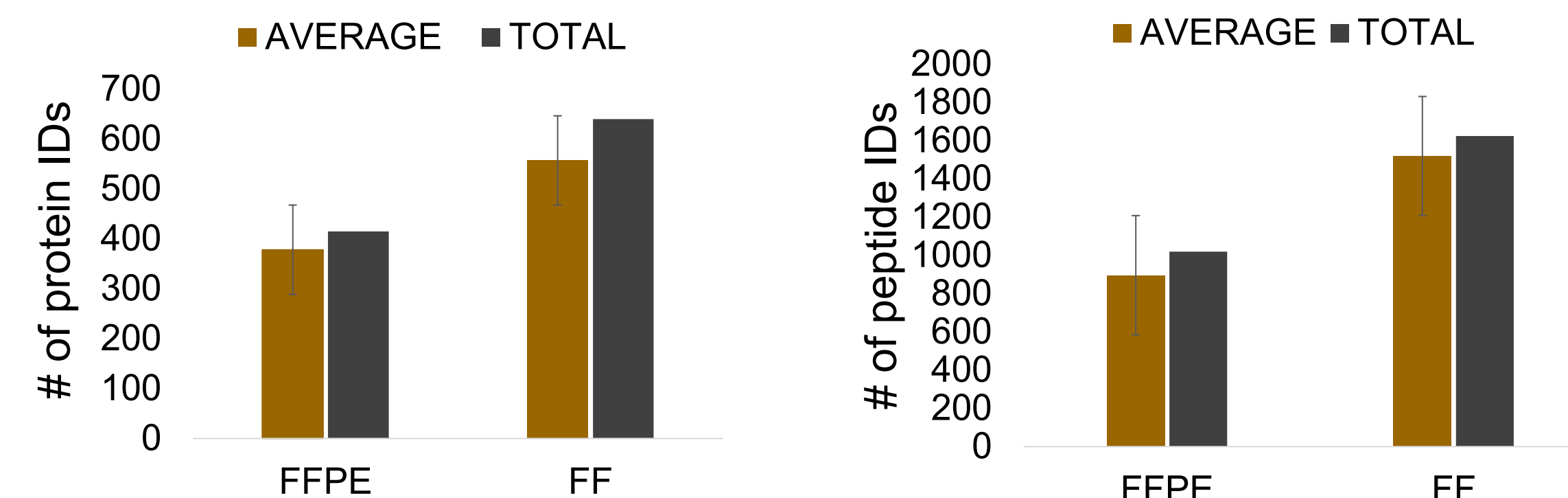


Microwells containing DMSO for capturing ablated tissue.
PTFE printed glass microscope
DMSO in microwells improved capture efficiency with minimal solvent evaporation.

Each ablated tissue region was collected and digested in situ prior to LC-MS/MS analysis.

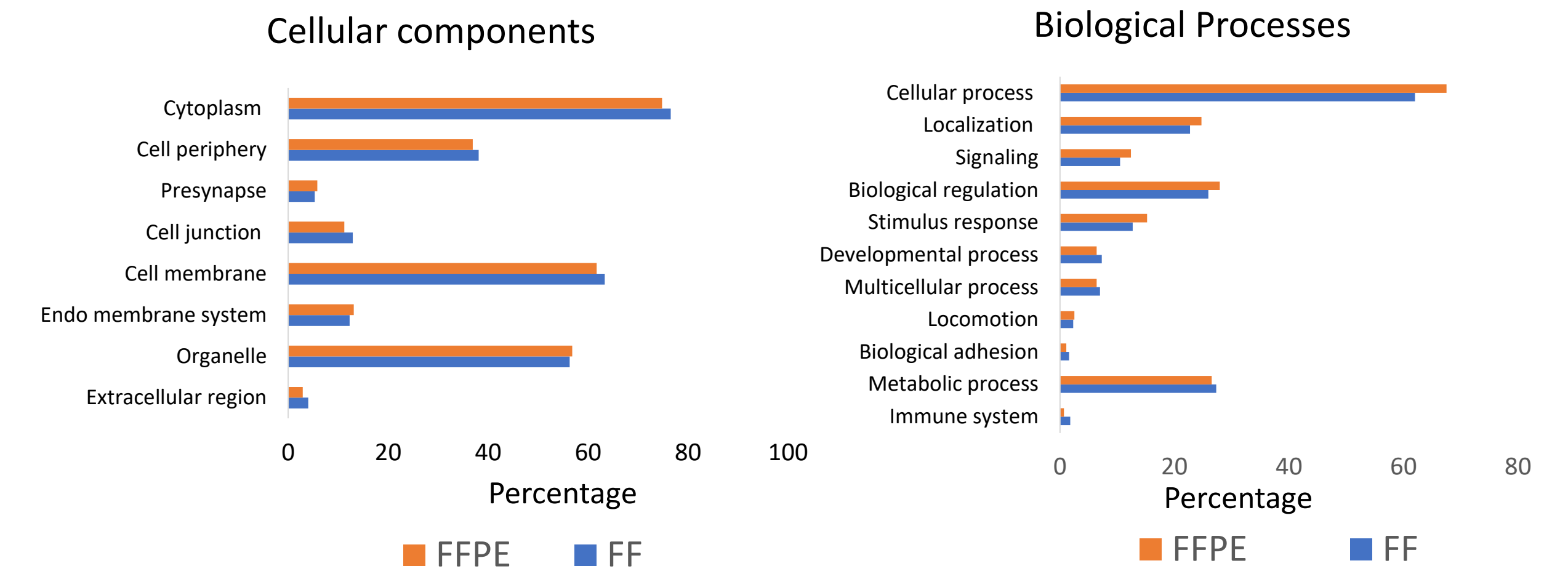
Protein/Peptide Identification

- >Region: Midbrain
- >Reproducible protein identification with an average CV <12% for # of peptides and proteins



Results

Gene Ontology



- Gene ontology analysis (Panther GO) of all proteins identified for each type of conservation technique.

Discussion

- Efficient ablation and capture from both FFPE and FF tissue
- >400 proteins and >900 peptides identified from mm² area
- Comparable cellular components and biological processes FFPE vs. FF

Conclusions

- Simple micro-volume workup adapted for FFPE microsampling
- Efficient proteomics down to 0.4 mm² area containing <5 ng protein

Future work

- Sample clinically relevant tissue (FFPE cancer biopsies)
- Sample smaller areas of FF and FFPE tissue
- Laser ablation sampling of stained tissue

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