Integrating fluorescence-based post-mortem interval estimation into forensic DNA/RNA extraction workflows

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Introduction

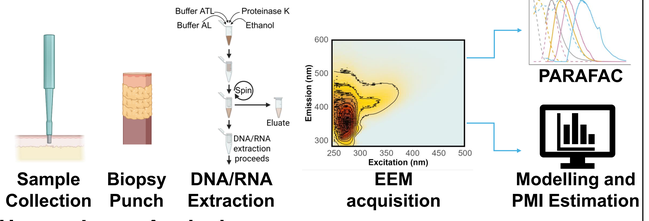
- **Post-mortem interval (PMI):** Time elapsed between physiological death and discovery of the deceased person[1]
  - Accurate and reliable PMI estimates could establish timelines and contextualize other physical evidence
- Soft tissue degrades post-mortem due to **autolysis** and **putrefaction,** leading to macromorphological changes[2,3]
- Recent studies have shifted focus from macroscopic observations to **time-dependent degradation of different biomolecules** to estimate PMI[4]
  - Biotic and abiotic factors, deposition substrate, and inter-donor variability remain complicating factors when estimating PMI
- Analyzing different types of **biomolecules with varying degradation kinetics** could increase model predictivity[4,5]
- **We devised a novel, multi-instrumental workflow for the analysis of different biomolecules within the same sample**
  - Integrated PMI analyses directly into various DNA/RNA extraction protocols
  - Eluates (typically discarded materials) were analyzed using fluorescence spectroscopy, which has previously shown promising post-mortem trends

1. Comparative analysis between pigs and humans
- Fluorescence from pig tissue samples with varying PMIs were compared to that of humans to evaluate its suitability as a mammalian analogue

2. Comparative analysis between human tissue layers
- Uncover and analyze the different degradation mechanisms occuring simultaneously in various tissue types (i.e., muscle, adipose, skin)

Methods

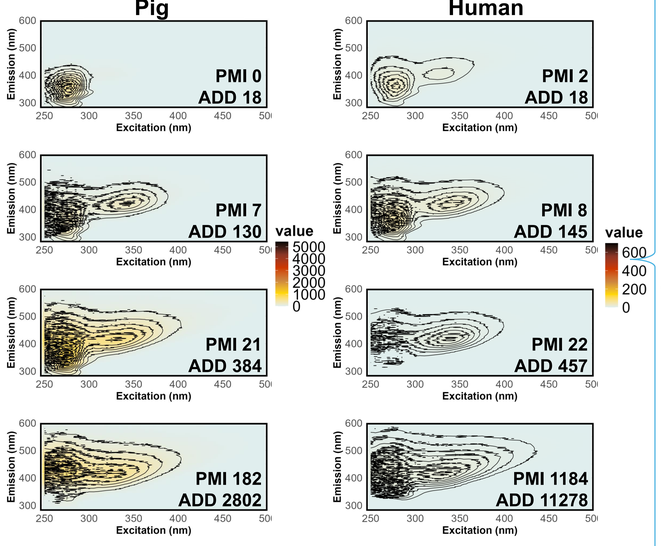
Pig vs Human Comparison:
- Pig: **21 biopsies from 2 pigs** placed at Ontario Tech University, Oshawa, PMI of 0-182 days (ADD 16-2802)
- Human: **50 biopsies from 15 donors placed at REST[ES]** (Recherche en Sciences Thanatologiques [Éxperimentales et sociales], Trois-Rivières, PMI of 2-1184 days (ADD 10-11278)
- Performed **DNA extractions**: Qiagen Blood & Tissue Kit[6] (pig) and Qiagen MagAttract HMW DNA Kit (human)[7]
  - First eluate in extraction (pig) and supernatant (human) retained
- An **excitation-emission matrix (EEM)** was acquired for each eluate, excitation of 250-500 nm and emission of 280-600 nm
  - Parallel Factor Analysis (**PARAFAC**) used for deconvolution



Human Layer Analysis:
- 9 PMIs (33 samples; **9 composites, 24 layer samples**)
  - Donor 24, placed at REST[ES] in summer 2023
- AllPrep DNA/RNA Mini co-extraction protocol[8]
  - Used ~ 25 mg of each tissue layer
  - Compared to a composite sample ('All') consisting of all layers
- EEM acquired as above and PARAFAC performed on layers

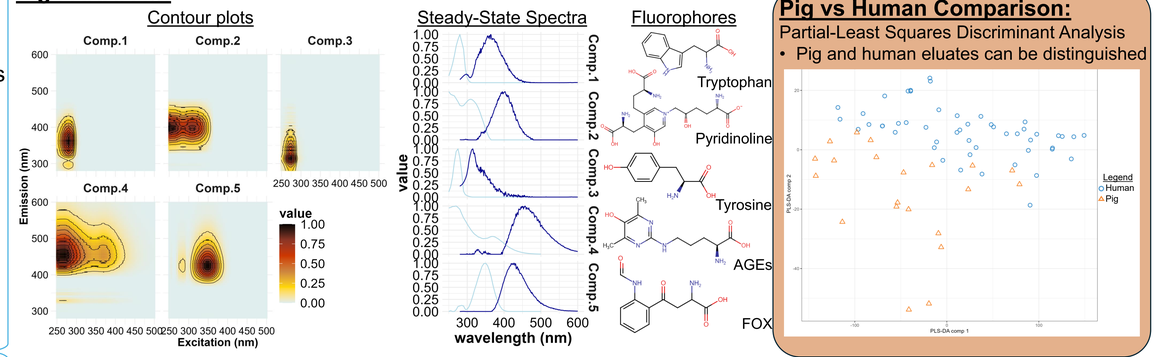
Results

Pig vs Human Comparison:
- Subset of EEMs shown below for pigs and humans
- **Increase in intensity and broadness** of fluorescence between 400-500 nm as PMI/Accumulated Degree Days (ADD) increases



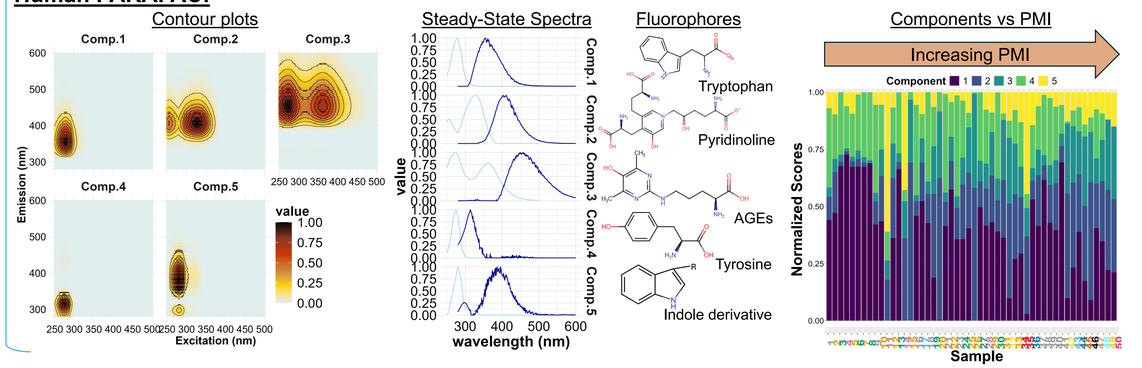
- Broadening in fluorescence attributed to formation of **fluorescent oxidation products (FOX)** and **advanced glycation end products (AGEs)**

Pig PARAFAC:

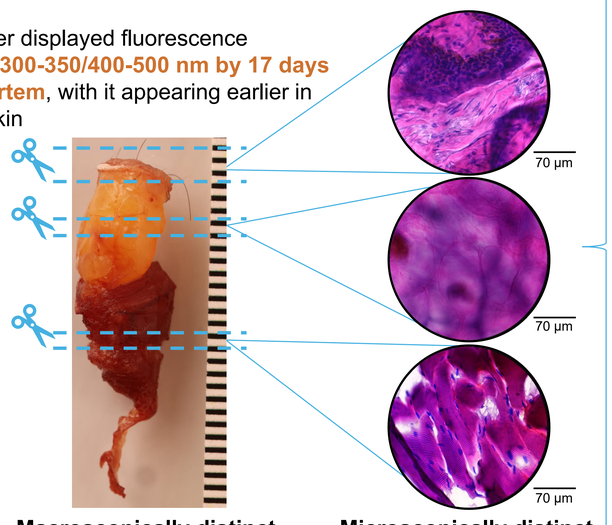


Pig vs Human Comparison:
Partial-Least Squares Discriminant Analysis
- Pig and human eluates can be distinguished

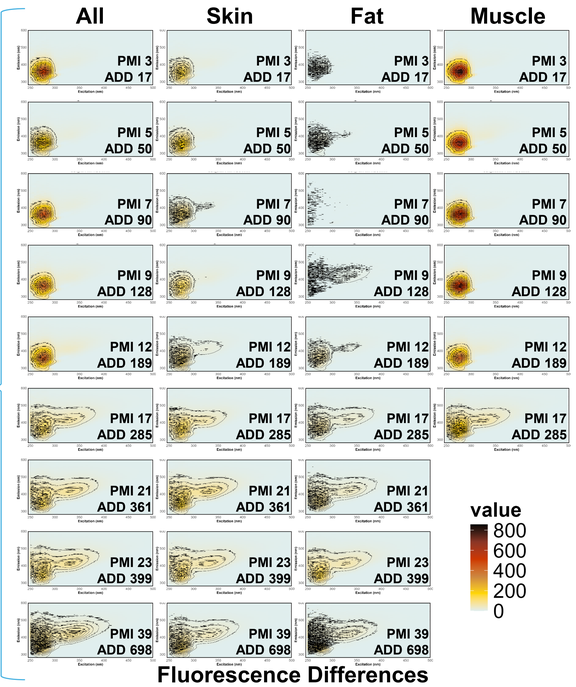
Human PARAFAC:



Human Layer Analysis:
- **PMI- and layer-dependent** fluorescence were observed
- **Differences in fluorescence** between layers at earlier PMIs are **intensity driven**, as shape of emission is similar between layers
- Each layer displayed fluorescence between **300-350/400-500 nm by 17 days post-mortem**, with it appearing earlier in fat and skin

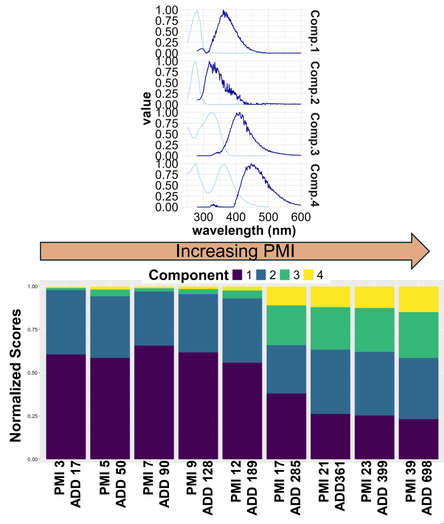


Macroscopically distinct **Microscopically distinct**



Fluorescence Differences

PARAFAC for 'All' samples (i.e., composite sample):
- The **4-component model** provided the best fit
- **Component 1 decreases** with increasing PMI, while **components 3 and 4 increase** over time



Future Work

- Complete tissue processing (~50 samples remaining) and build chemometric models to estimate PMI
- Propose degradation mechanisms for each tissue layer based on fluorescence and mass-spectrometry data
- Determine if and how post-mortem redistribution is influencing fluorescence of different tissue layers

References

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