Developmental validation of a novel approach for determining time-since-deposition of trace DNA evidence

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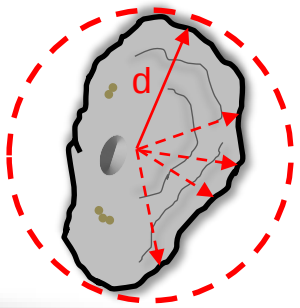
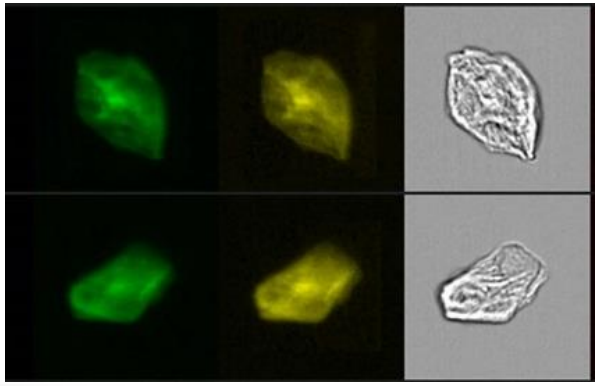
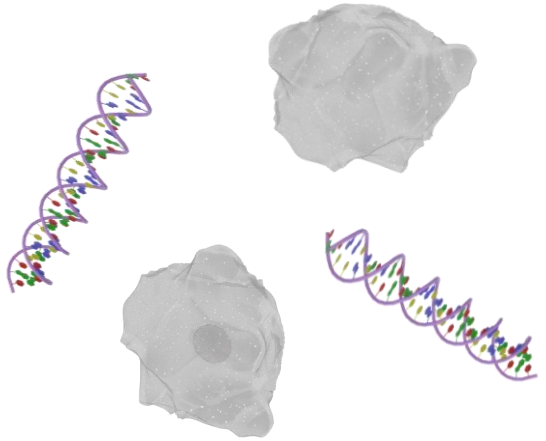




Time Since Deposition (TSD)

- One of the primary questions around DNA evidence is <u>when</u> it was deposited

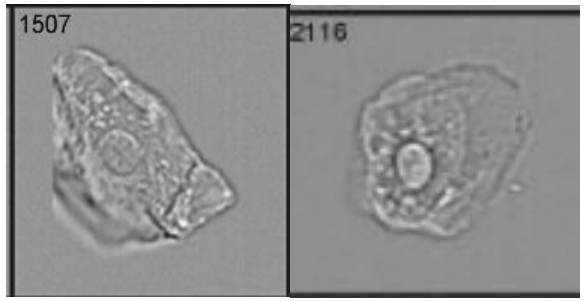
- Various methods for TSD have been proposed over the years…. none have focused on ‘touch’ biological evidence

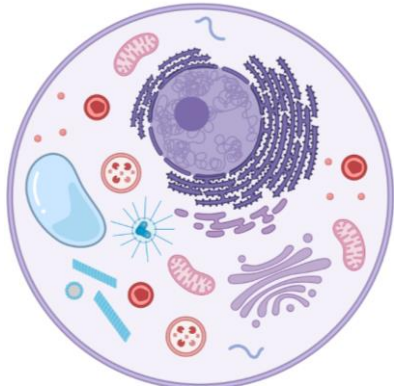


Time Since Deposition

- Epidermal cell populations are … different !

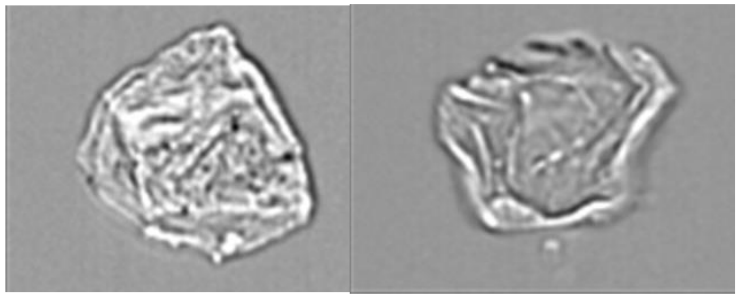
Stratified epithelial cells (saliva)

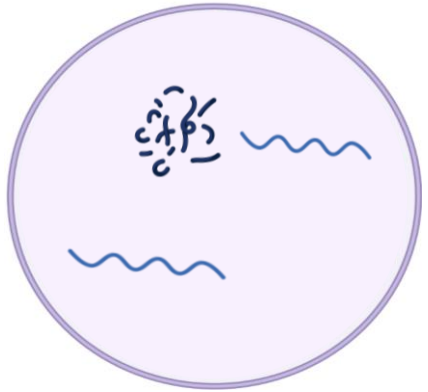




- Nucleus
- Organelles
- Vesicles
- Mitochondria
- Cytoskeleton
- Protein aggregations

Shed epidermal cells, corneocytes

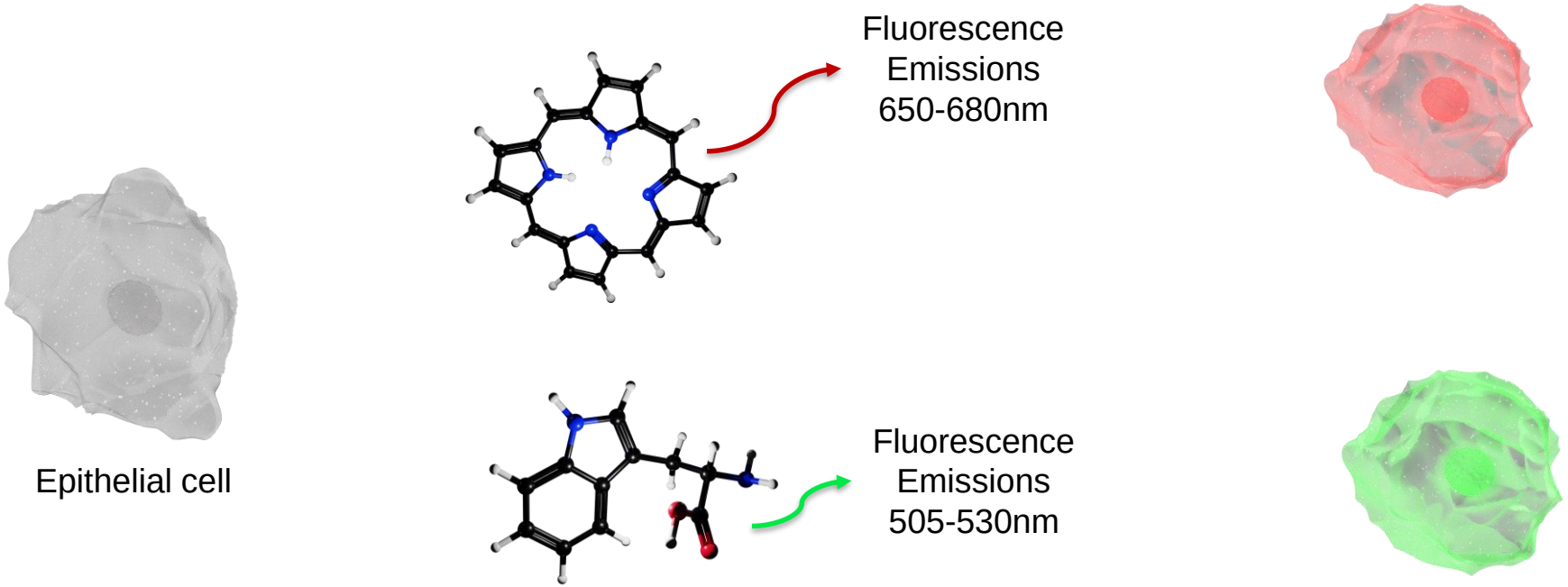




- Lipoproteins
- Keratins
- Degraded proteins, DNA

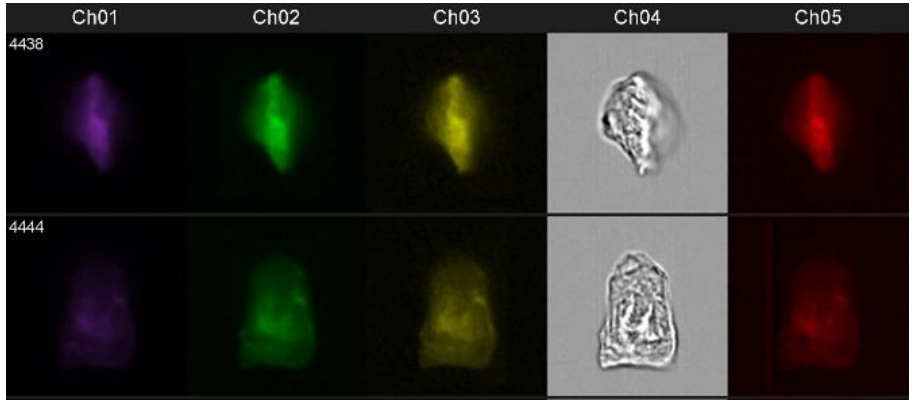
Time Since Deposition

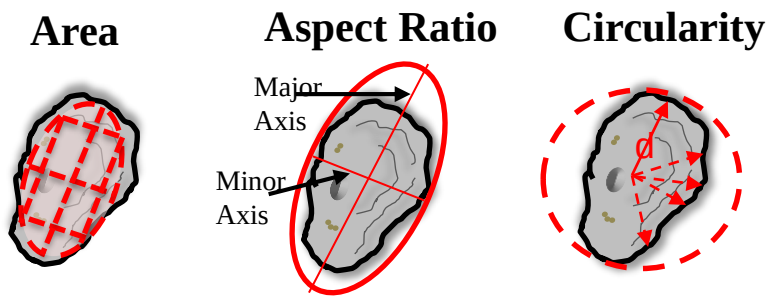
- Autofluorescence profiling: snapshot of cellular biochemistry,
- Compounds fluoresce at different wavelengths, intensity varies with compound abundance within cell



Time Since Deposition

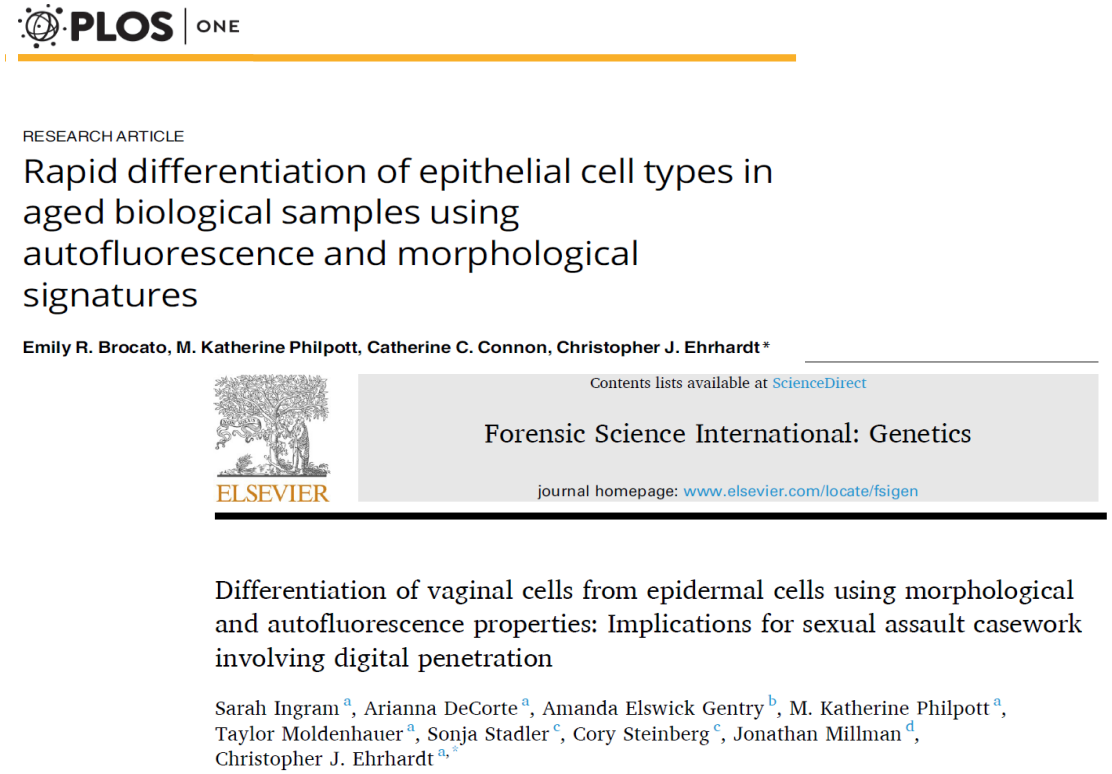
- Measure wavelengths, intensity of autofluorescence of cells…
- Combine autofluorescence with morphological measurements
- Fast and nondestructive!





Time Since Deposition

- Previous work used autofluorescence to differentiate tissue sources & identify contributor cell populations in touch DNA samples



PLOS ONE

RESEARCH ARTICLE

Rapid differentiation of epithelial cell types in aged biological samples using autofluorescence and morphological signatures

Emily R. Brocato, M. Katherine Philpott, Catherine C. Connon, Christopher J. Ehrhardt*

Contents lists available at ScienceDirect

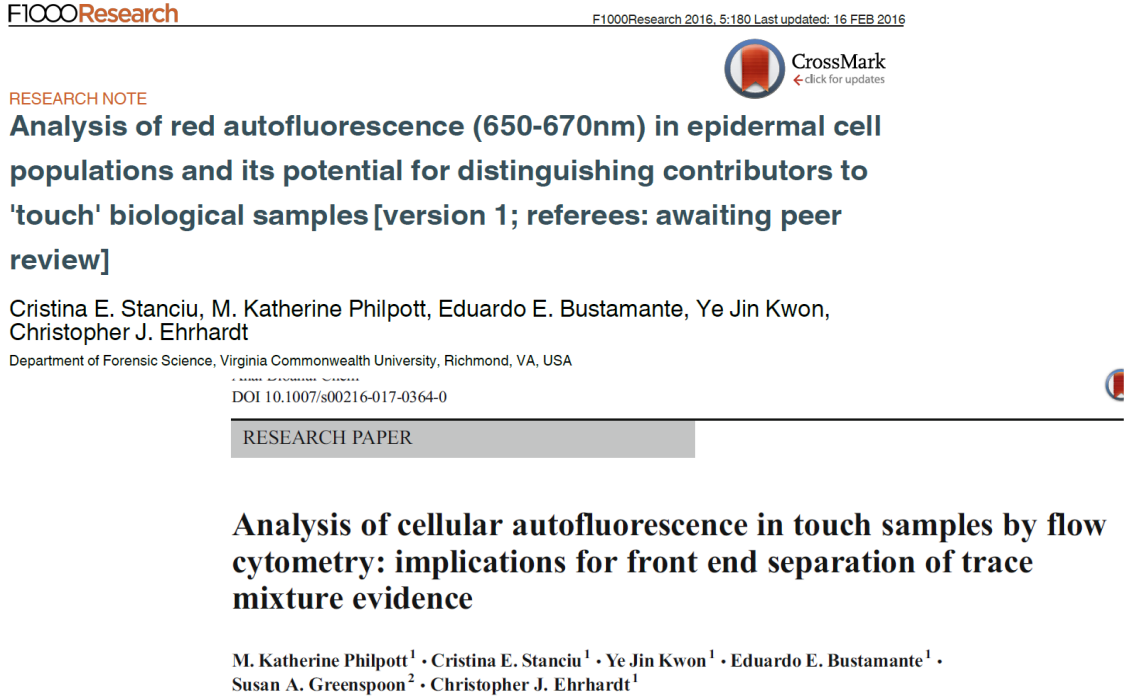
Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

ELSEVIER

Differentiation of vaginal cells from epidermal cells using morphological and autofluorescence properties: Implications for sexual assault casework involving digital penetration

Sarah Ingram [a], Arianna DeCorte [a], Amanda Elswick Gentry [b], M. Katherine Philpott [a], Taylor Moldenhauer [a], Sonja Stadler [c], Cory Steinberg [c], Jonathan Millman [d], Christopher J. Ehrhardt [a,*]



F1000Research F1000Research 2016, 5:180 Last updated: 16 FEB 2016

CrossMark click for updates

RESEARCH NOTE

Analysis of red autofluorescence (650-670nm) in epidermal cell populations and its potential for distinguishing contributors to 'touch' biological samples [version 1; referees: awaiting peer review]

Cristina E. Stanciu, M. Katherine Philpott, Eduardo E. Bustamante, Ye Jin Kwon, Christopher J. Ehrhardt

Department of Forensic Science, Virginia Commonwealth University, Richmond, VA, USA

DOI 10.1007/s00216-017-0364-0

RESEARCH PAPER

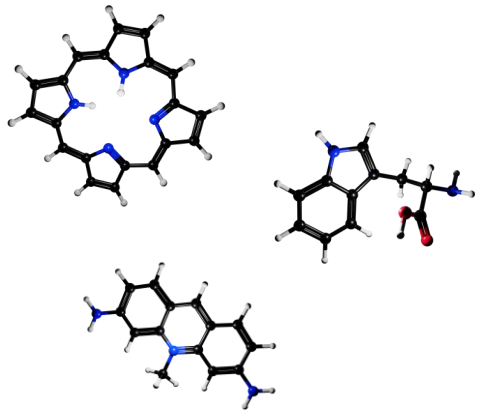
Analysis of cellular autofluorescence in touch samples by flow cytometry: implications for front end separation of trace mixture evidence

M. Katherine Philpott [1] · Cristina E. Stanciu [1] · Ye Jin Kwon [1] · Eduardo E. Bustamante [1] · Susan A. Greenspoon [2] · Christopher J. Ehrhardt [1]

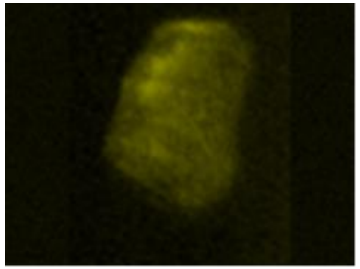
Time Since Deposition

- Even though biochemistry of touch epidermal cells are unusual, there are dozens of compounds that can autofluoresce

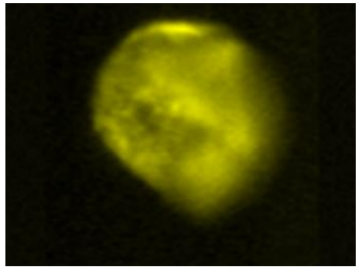
- As they degrade, autofluorescence should change with time !



1 Day

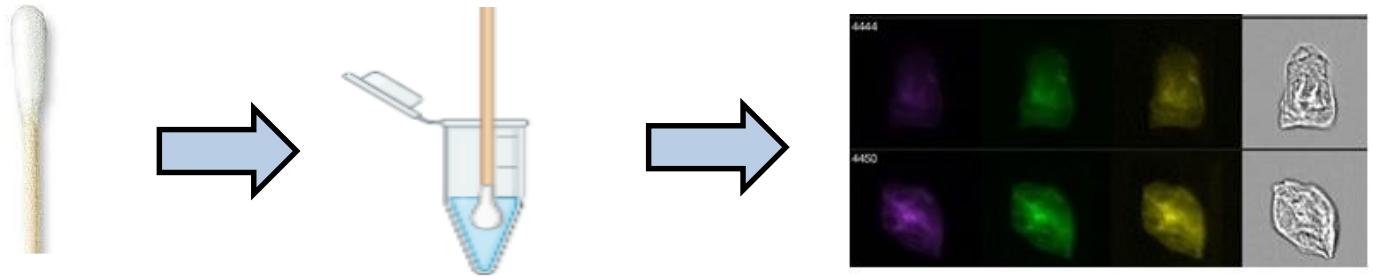


415 Days



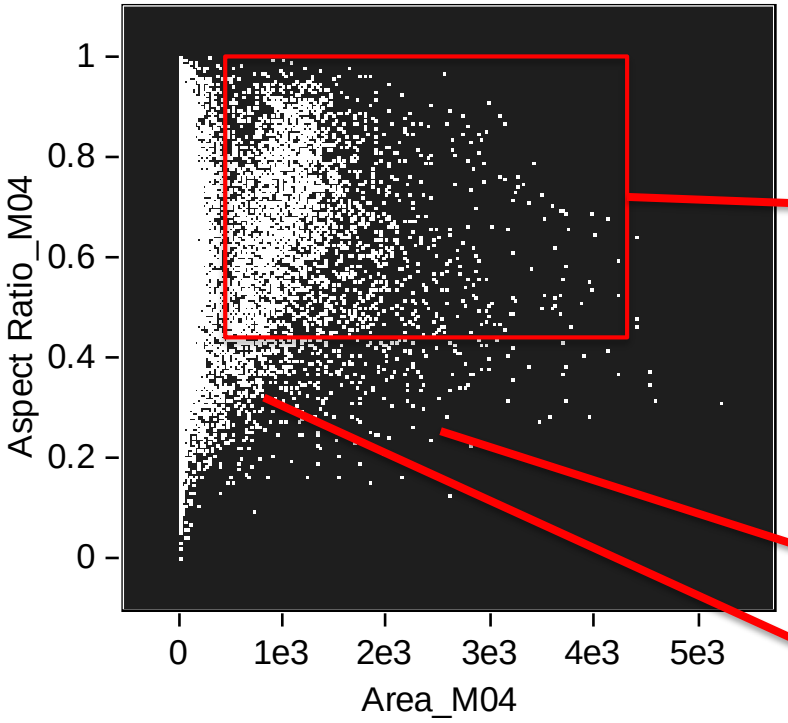
TSD Signature Development

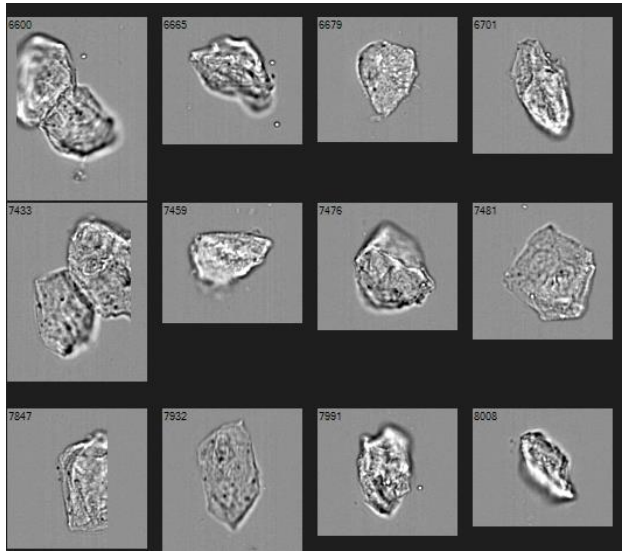
- Created time series of ‘touch’ biological samples:
  - >80 different contributor cell populations from ~50 individuals
  - Aged between 1 day and ~2 yrs, various substrates
  - Swabs eluted directly in water, cells analyzed with flow cytometry, no other steps or reagents!

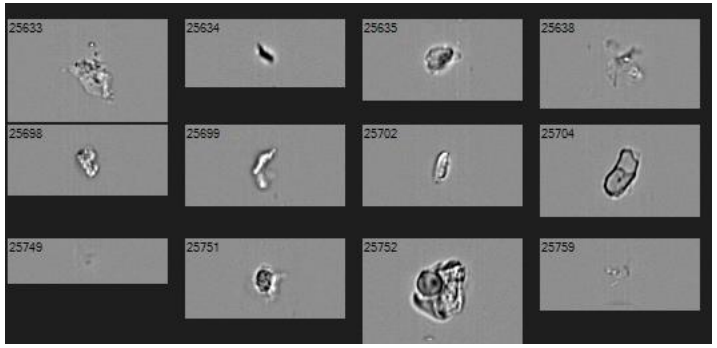


Phase I: Results

- Touch epidermal cell populations: what do we analyze?



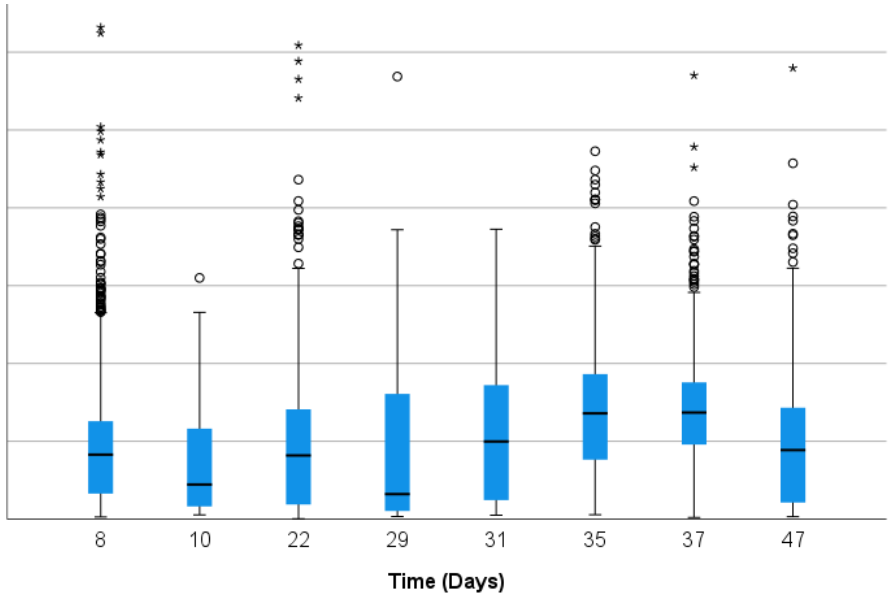


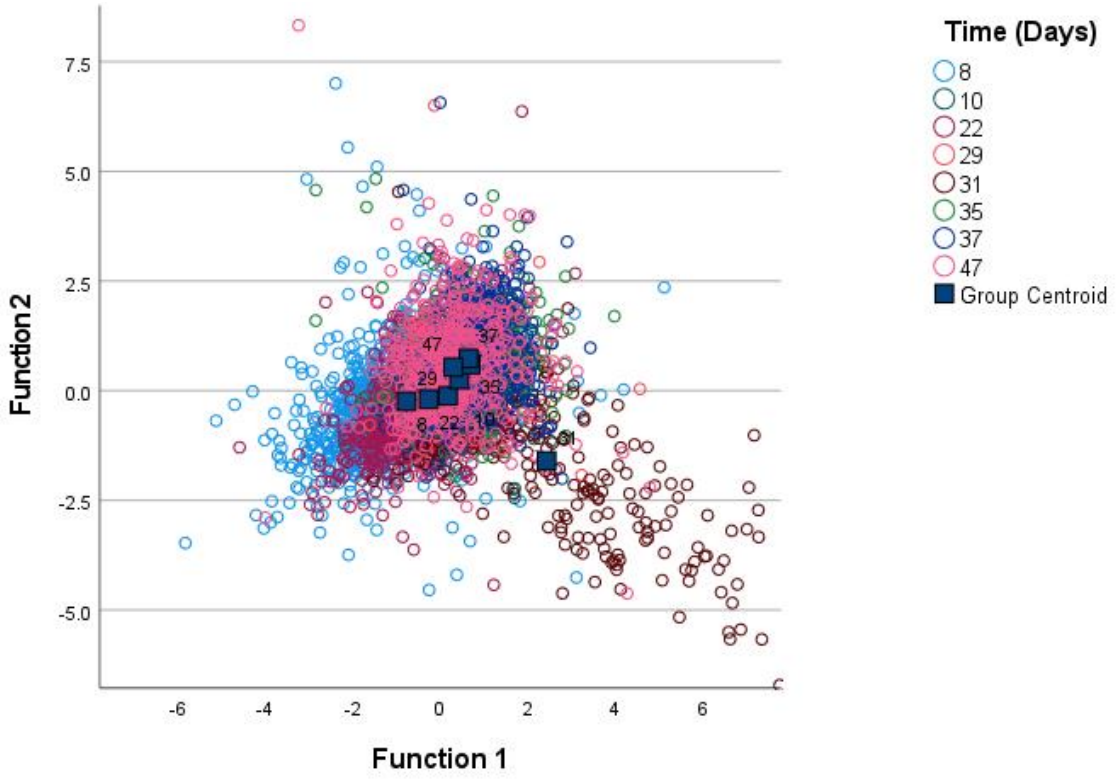


Phase I: Results

- Complex trends across entire time series….

Fluorescence Intensity (560-595nm)



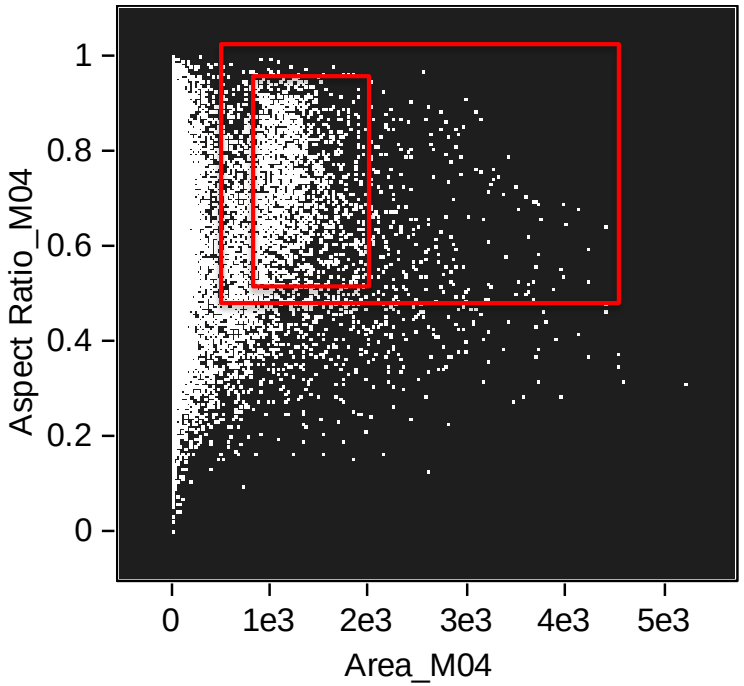


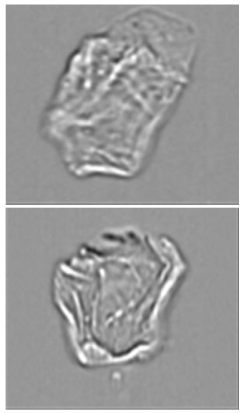
Results

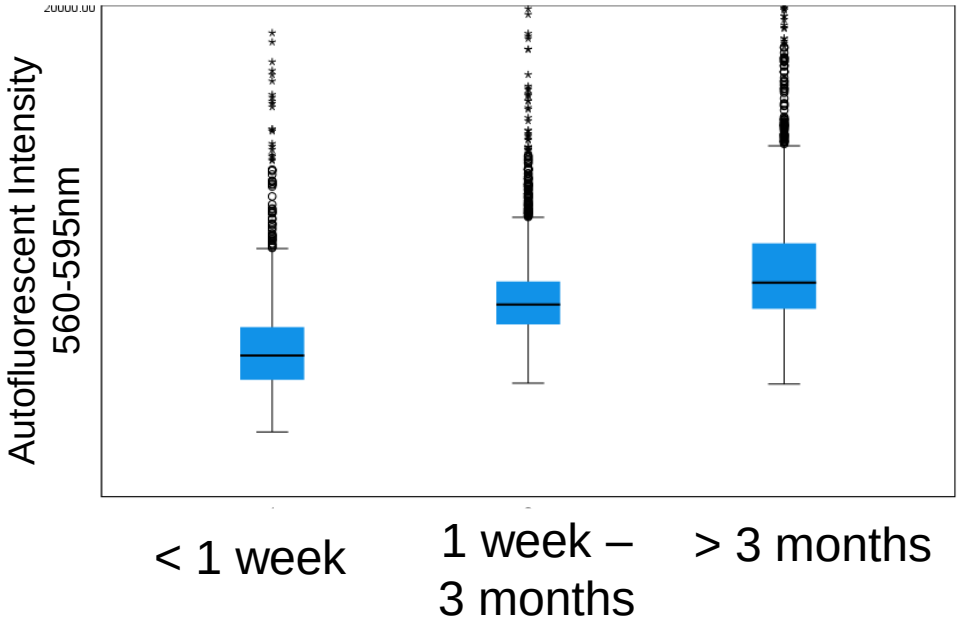
- Complete resolution of time points not likely, need a new strategy
  - Is TSD to a specific day necessary for forensics?

Phase I: Results

- Two changes: (1) modeled autofluorescence across discrete time intervals, (2) narrowed the subpopulation of cells,

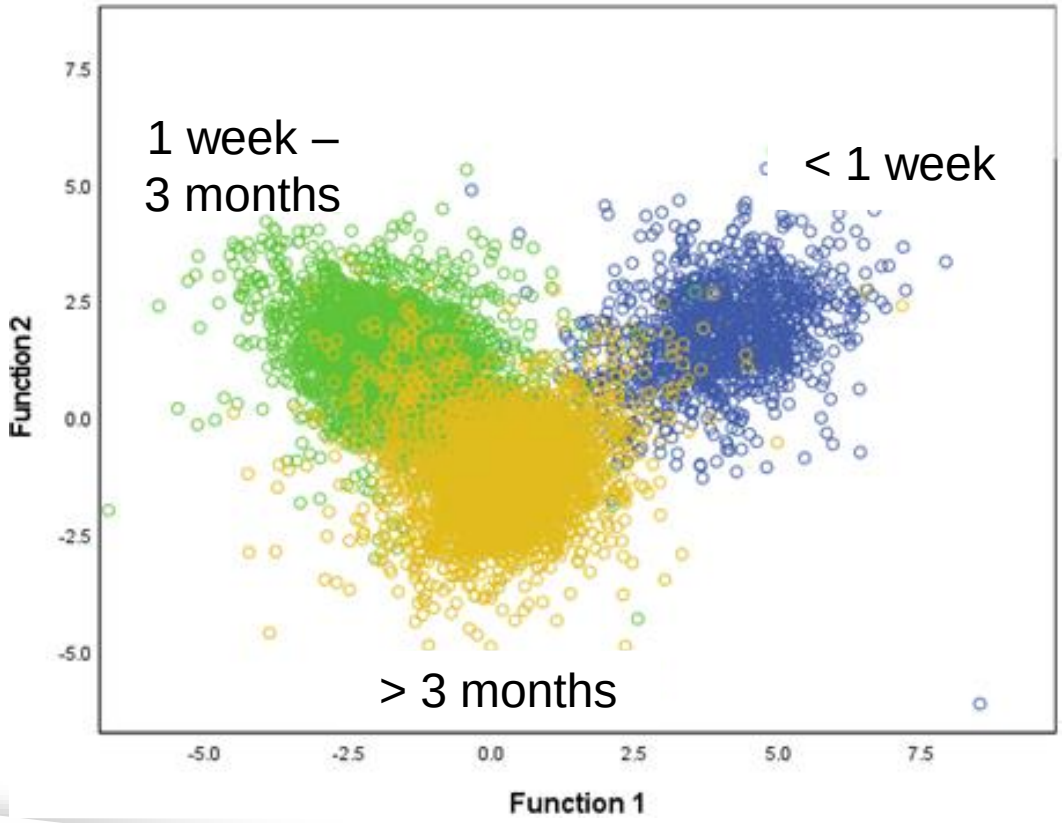






Phase I: Results

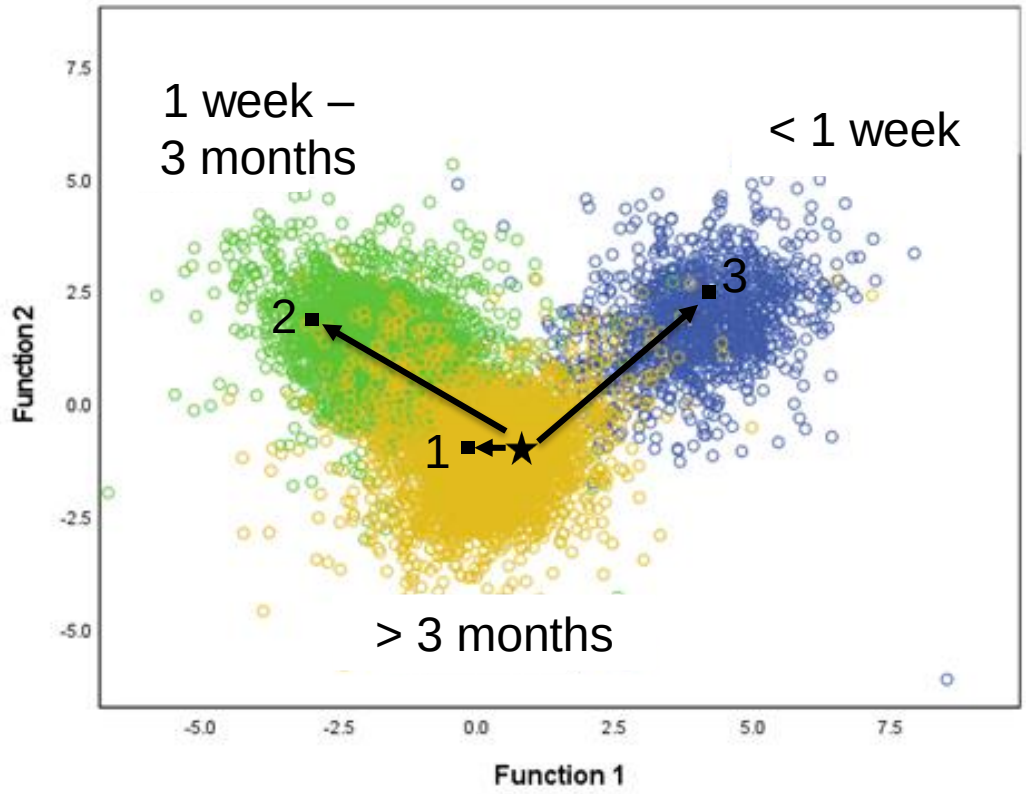
- Multivariate modeling of time intervals (Linear Discriminant Analysis)



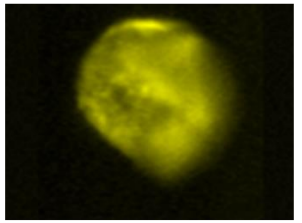
Wilks lambda =0.18,
p<0.001

Results

- Estimate TSD of individual cells within sample based on multivariate distances, also calculate posterior probability of tissue ID



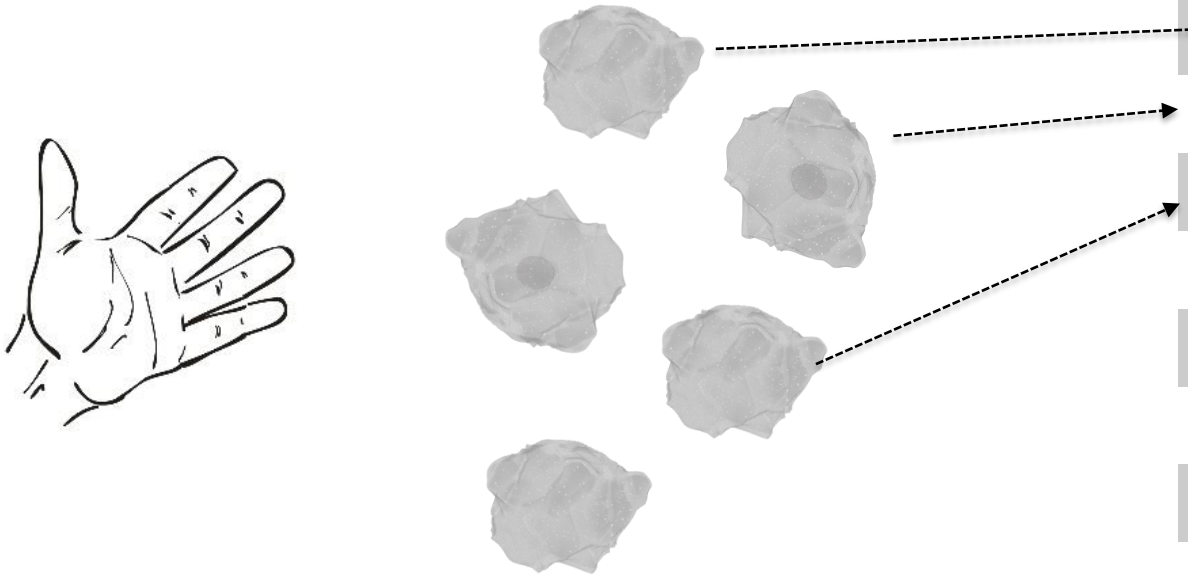
$$Probability = \frac{D_j^{-\frac{1}{2}} e^{-X^2/2}}{\sum_{j=1}^{g} D_j^{-\frac{1}{2}} e^{-X^2/2}}$$



Prob
0.98, < 1 week

Results

- Tabulate TSD and probability for *every* cell in a given sample:



Cell	TSD	Post Prob
1	<1 week	0.98
2	< 1 week	0.95
3	< 1 week	0.78
4	< 1 week	0.99
5	< 1 week	0.90
6	~ 2 months	0.56
7	< 1 week	0.82
...	...	...
300	< 1 week	0.96

Results

- For one cell population, metrics can be combined

Contributor ID	Total Cells	Time Since Deposition (Actual)	Time Since Deposition (Predicted)	Posterior Probability (Average)
B32	93	22 days	1week-3months	0.93
J72	192	22 days	1week-3months	0.98
L12	312	22 days	1week-3months	0.99
K47	67	29 days	1week-3months	0.97
H21	271	31 days	1week-3months	0.95
I66	38	35 days	1week-3months	0.76

Results

- ~80 samples classified into three time intervals: < 1 week, 1 week to 2 months, >3 months
- Correct TSD every time the sample had >75 cells & the average posterior probability >0.90 !
- TSD possible for ~50% of touch samples

			Highest Group		
Sample ID	Total Events	Time/Group	Total Events	Group	Posterior Probability
C58	219	1 day / 1	218	1	0.999
L49	39	1 day / 1	36	1	0.999
N90	156	1 day / 1	114	3	0.892
P22	184	1 day / 1	180	1	0.995
D68	3	3 days / 1	2	1	1.000
I66	15	3 days / 1	15	1	0.999
P22	83	3 days / 1	75	1	0.970
T04	12	3 days / 1	11	1	0.957
C58	331	4 days / 1	329	1	0.998
E10	54	4 days / 1	49	1	0.957
P22	180	4 days / 1	178	1	0.997
B32	27	8 days / 2	20	2	0.855
H71	18	8 days / 2	14	2	0.837
I66	76	8 days / 2	69	2	0.953
J72	856	8 days / 2	826	2	0.962
V73	61	8 days / 2	57	2	0.953
H21	56	10 days / 2	51	2	0.964
A24	129	22 days / 2	120	2	0.927
B32	93	22 days / 2	89	2	0.938
J72	192	22 days / 2	188	2	0.984
L12	312	22 days / 2	307	2	0.994
K47	67	29 days / 2	65	2	0.972
H21	271	31 days / 2	232	2	0.954
I66	38	35 days / 2	18	2	0.759
J72	707	35 days / 2	626	2	0.904
B32	779	37 days / 2	655	2	0.941
L12	49	47 days / 2	26	3	0.821
V73	186	47 days / 2	105	2	0.856
L12	35	59 days / 2	29	3	0.883
B32	12	72 days / 3	10	3	0.985
A24	8	75 days / 3	7	3	0.858

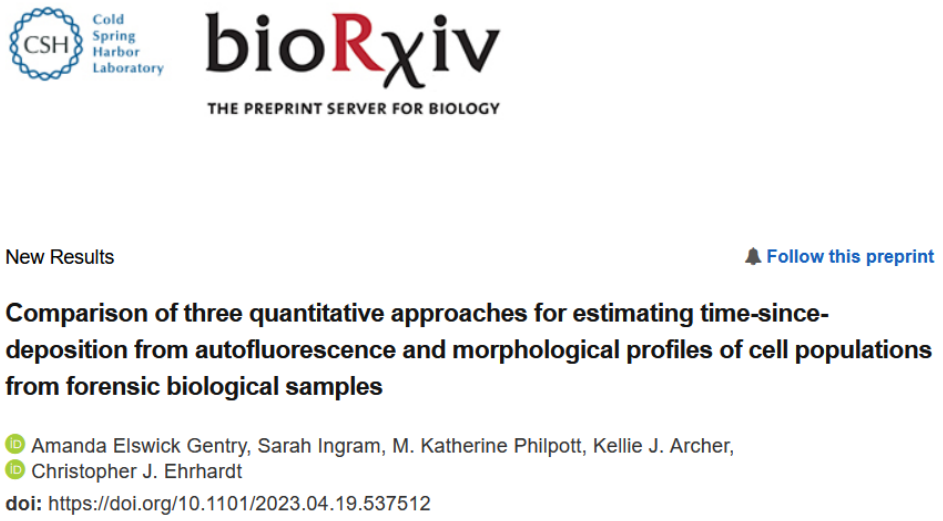
Results

- What about samples with less than <75 cells?
- Tested other frameworks:
  - Generalized Linear Mixed Model (GLM),
  - Gradient Boosting Machine (GBM),
  - Ridge Regression Model (RRM)
- Binary TSD estimates (i.e., less than or more than a week old, …)

Results

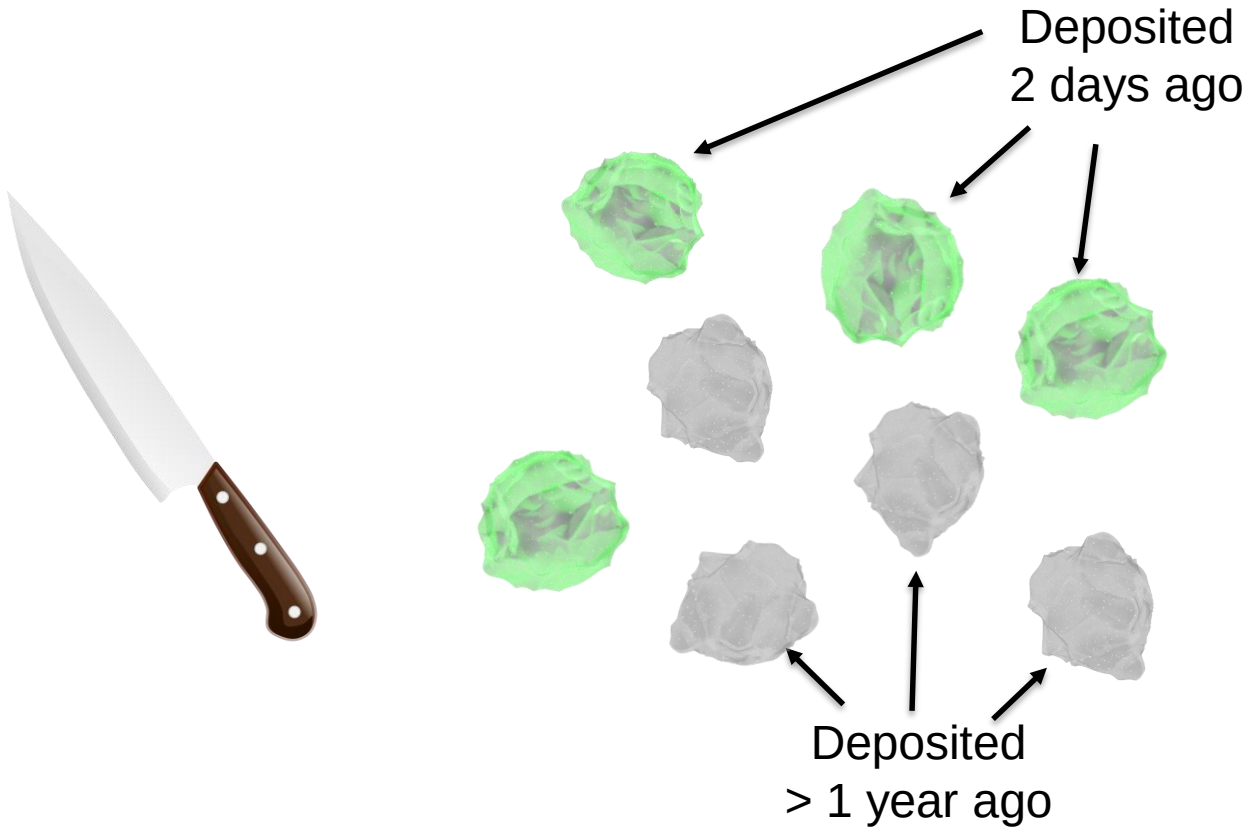
- GLM >99% accuracy with TSD without minimum number of cells

TSD Estimate	Generalized Linear Mixed Model
< 7 days	0.996
< 30 days	0.913
< 60 days	0.919
< 90 days	0.847
< 120 days	0.891
< 180 days	0.961



Results

- What about mixture samples with multiple TSDs ?



Cell	TSD	Post Prob
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2	< 1 week	0.95
3	> 6 months	0.78
4	< 1 week	0.99
5	> 6 months	0.90
6	> 6 months	0.56
7	< 1 week	0.82
...	...	...
300	< 1 week	0.96

Results

- Question for mixture samples: “Are there fresh cells present ?”

Vacant Laboratory

		Cell Counts		
Substrate	**TSD**	**< 1 week**	**1 week-2 mos**	**> 3 months**
Door Knob 2	~ 1 year			
Door Knob 3	~ 1 year			
Airhood 1	~ 1 year			

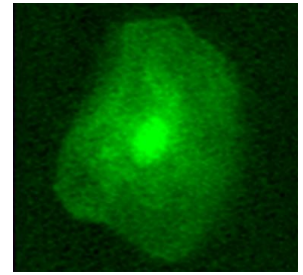
Results

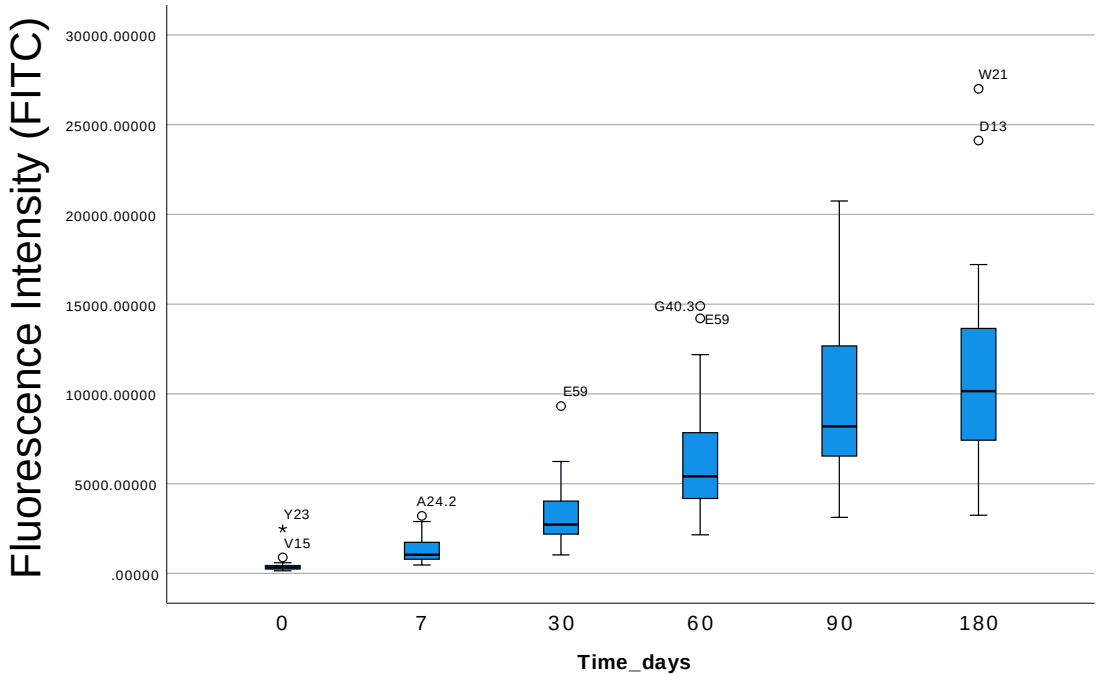
- Question for mixture samples: “Are there fresh cells present ?”

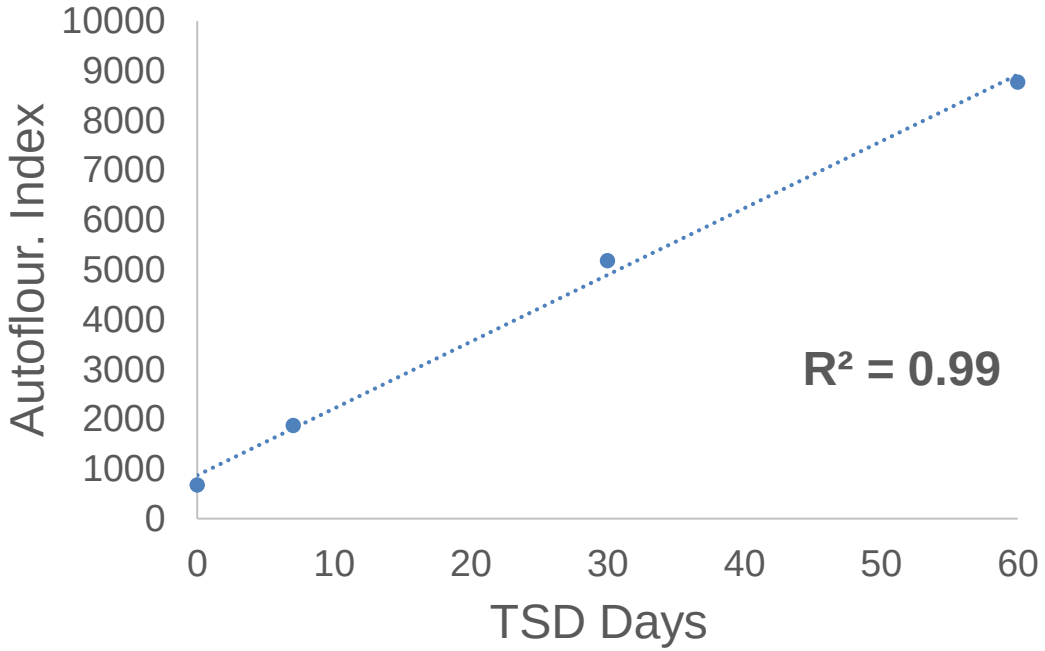
		Cell Counts		
Substrate	**TSD combination**	**< 1 week**	**1 week-2 mos**	**> 3 months**
Computer Mouse	~2 weeks / 1 day			
Gas Cap 1	> 1 year/ 1 day			
Gas Cap 2	> 1 year / 1 day			
Gas Cap 3	>1 year / 1 day			

Results

- Will this also work for other cell types, e.g. saliva ?

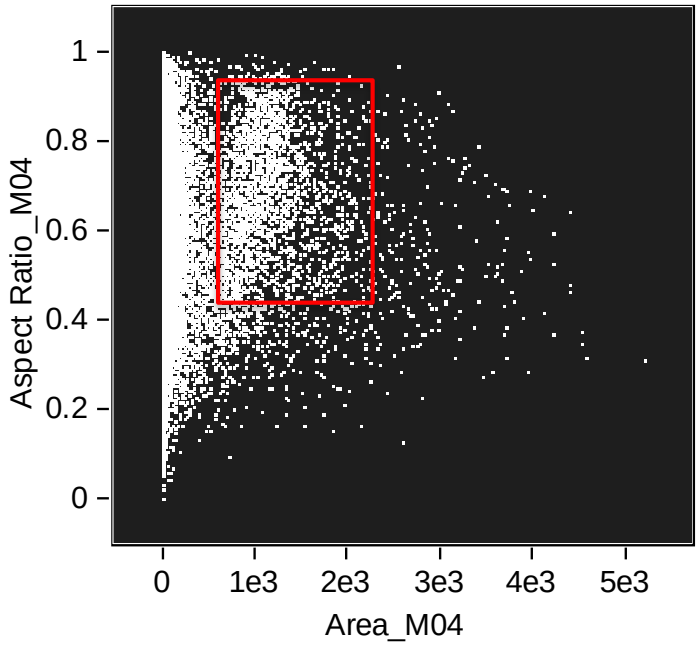


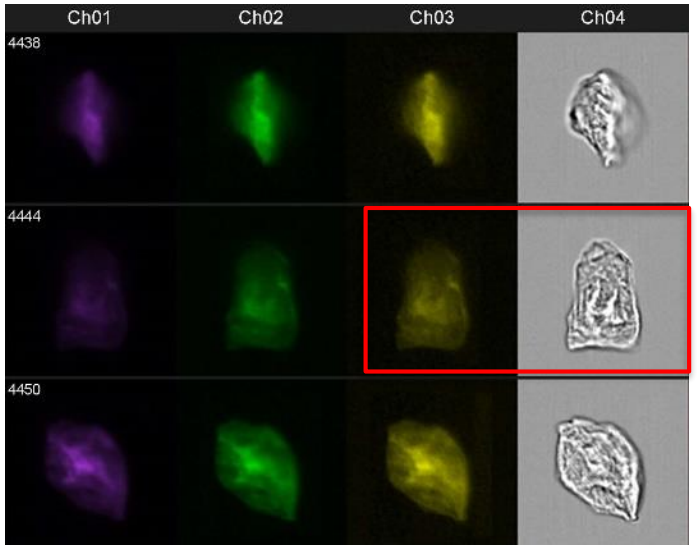


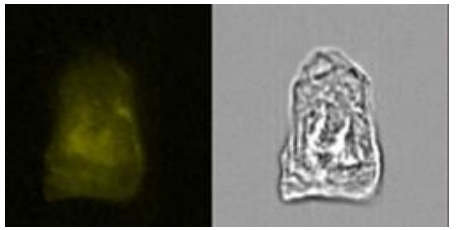


Future Directions

- Research focus has been signatures themselves (not the instrument) & quantitative framework for interpretation



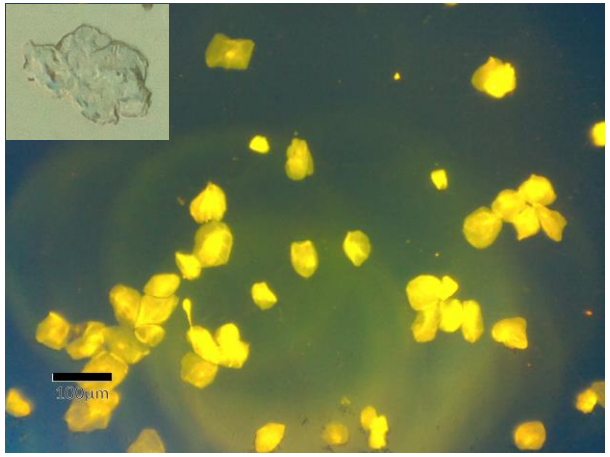




Future Directions

- TSD signatures can be obtained with **any** microscope
- Challenge is ‘segmenting’ cells out of the image for analysis

Zeiss Axioscope A1
(~$10K)

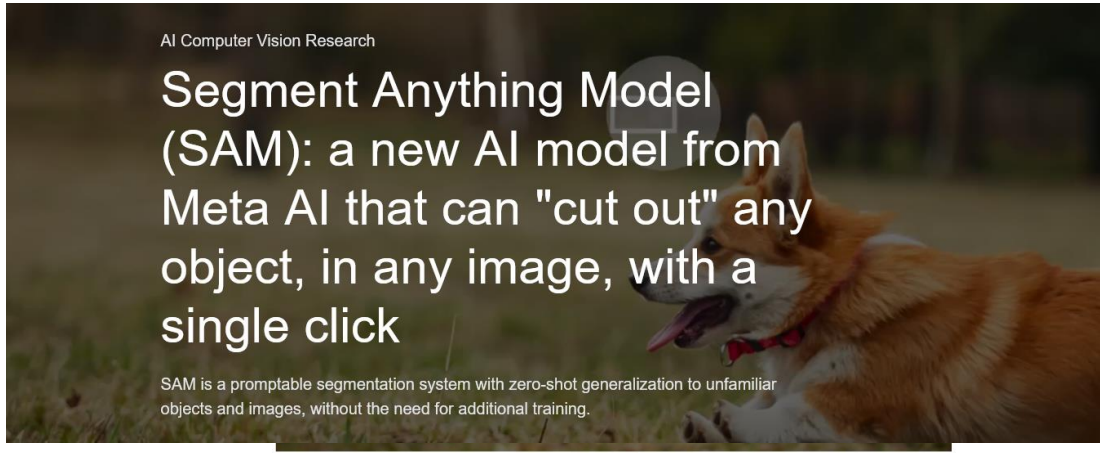


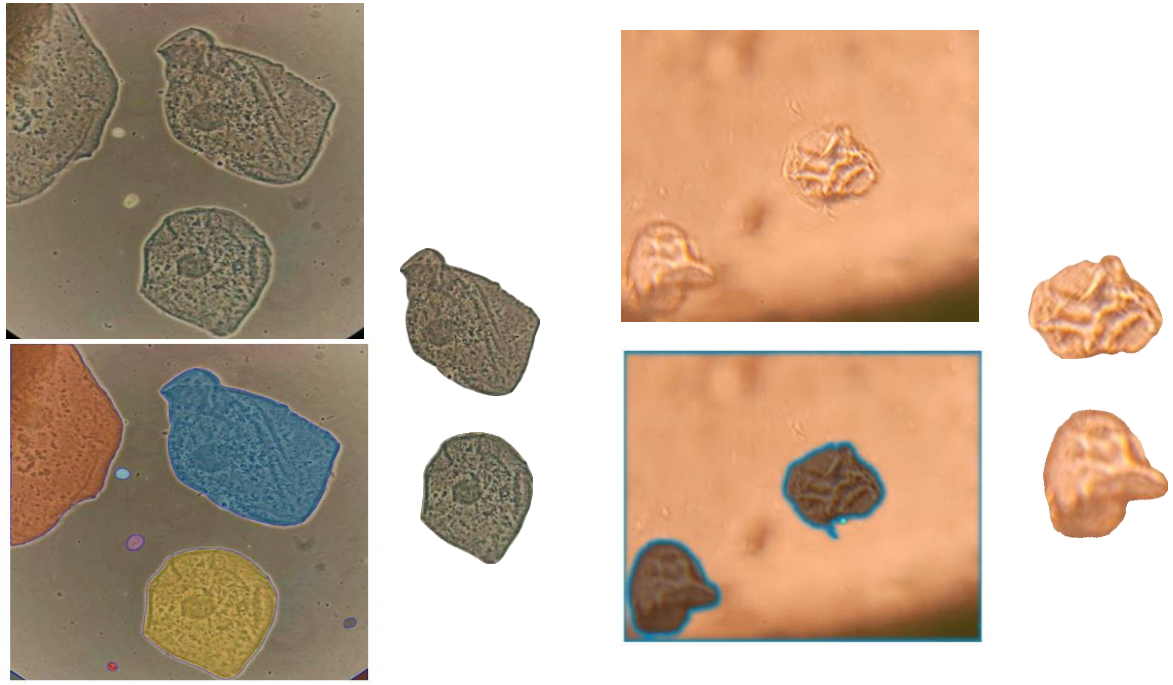
Handheld fluorescent
microscope
($800)

Cell phone portable
microscope ($17)

Future Directions

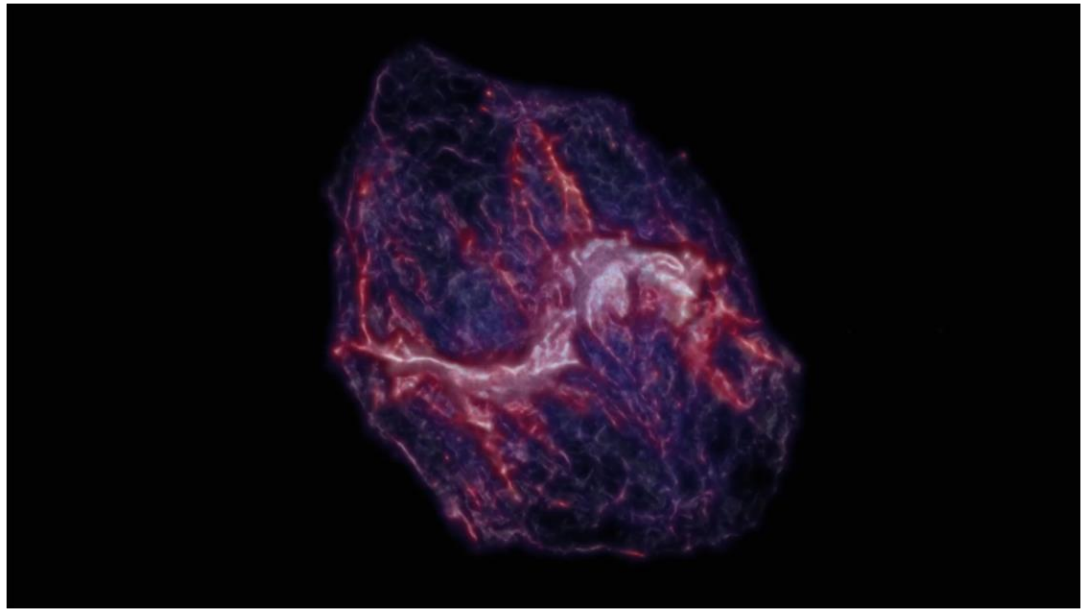
- Meta’s image segmentation tool ….

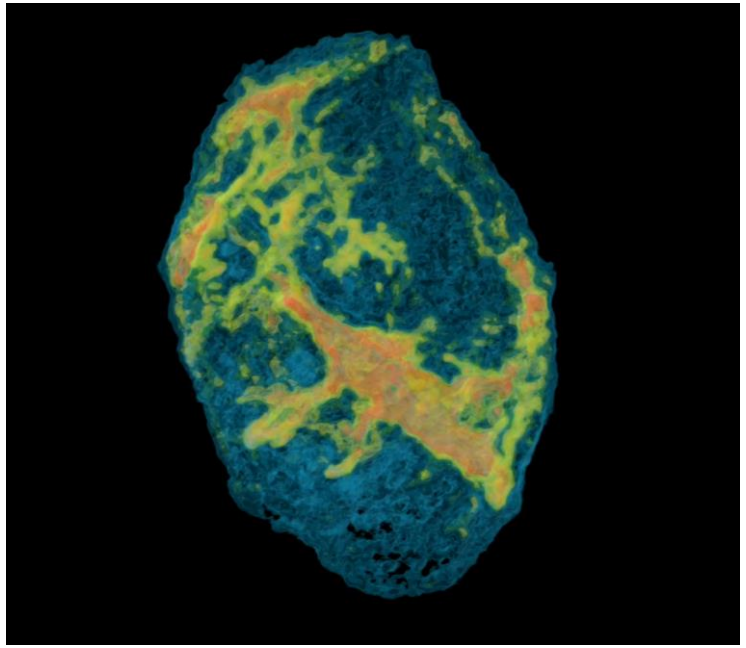




Cellular Autofluorescence

- Figuring out which compounds are causing autofluorescence and driving TSD signatures an ongoing challenge !





Laser Confocal Scanning Microscopy

Acknowledgements

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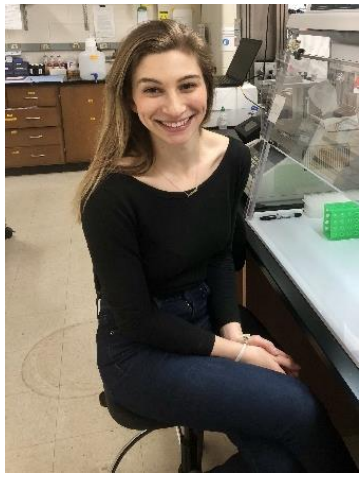
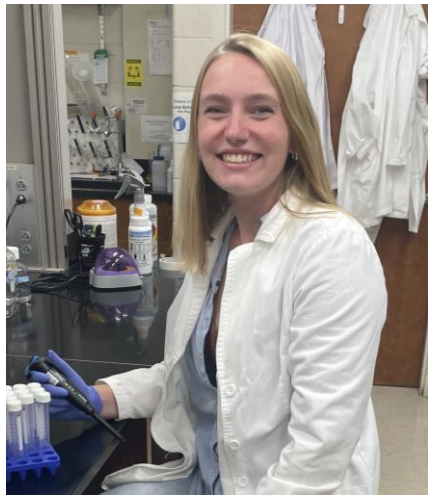
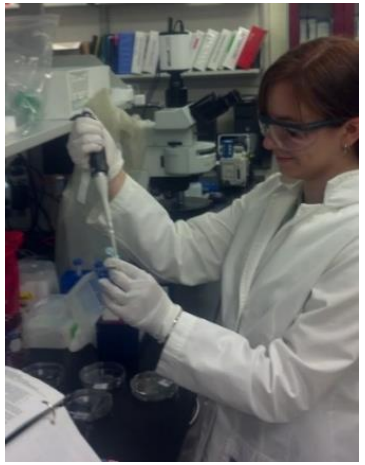
Collaborators

Jon Millman; Ontario Centre for Forensic Sciences
M. Katherine Philpott
Susan Greenspoon, VA-DFS
Amanda Gentry, VCU-MCV

CENTRE OF FORENSIC SCIENCES







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