Carnegie Mellon University

A Multi-Modal Computational Framework for in vitro Cancer Cell Analysis Based on Capacitance Sensing and Optical Imaging

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Ching-Yi Lin

Ph.D. Dissertation Committee:

Dr. Marc P. Dandin (Advisor)

Dr. Rick Carley

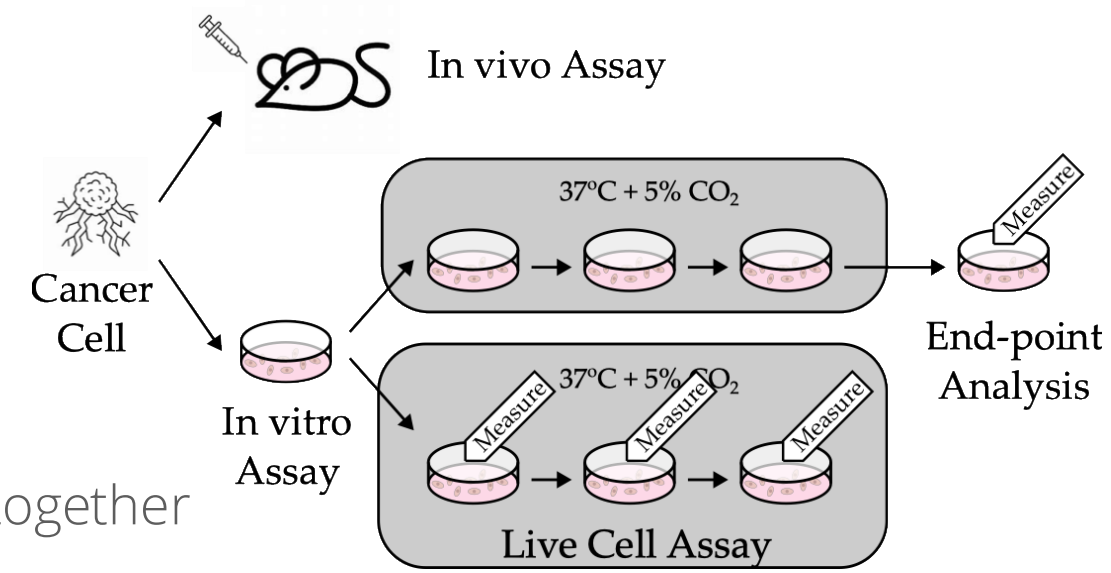
Dr. Pulkit Grover

Dr. Siyang Zheng

Dr. Elizabeth Wayne

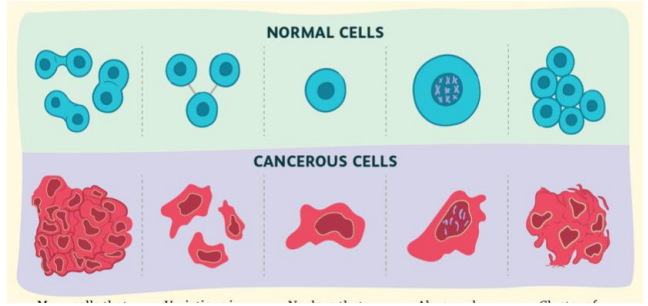
In vitro Cancer Cell Analysis

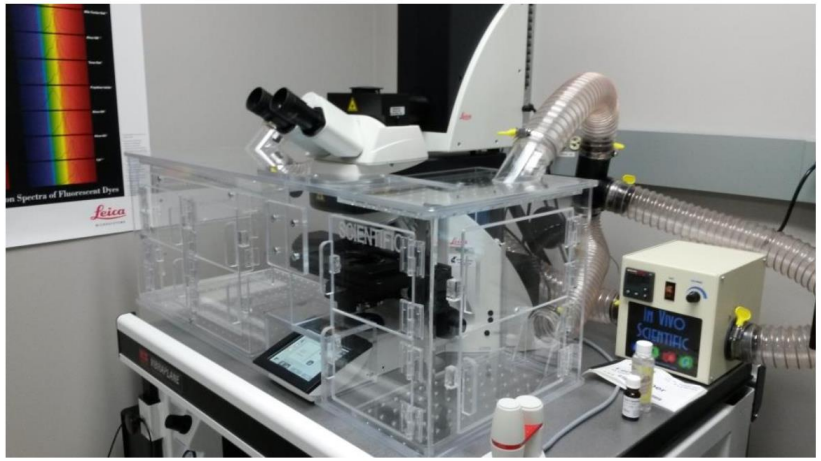
- In vivo assay
- In vitro assay
  - End-point analysis
  - Live cell assay
    - Culture and measure together
    - More data points



Live Cell Assay – Microscope-based

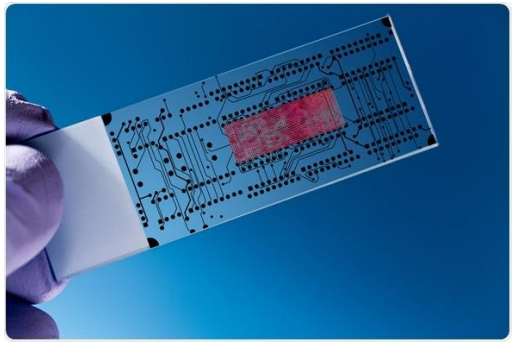
- Cell mobility and morphological change
- Requirement: Biological condition (37℃ + 5% CO2)
- Weakness: Low throughput + High labor effort





High-throughput Live Cell Assay

- CMOS Lab-on-a-Chip
  - Silicon chips are stackable
  - No focal space
  - Mature implementation



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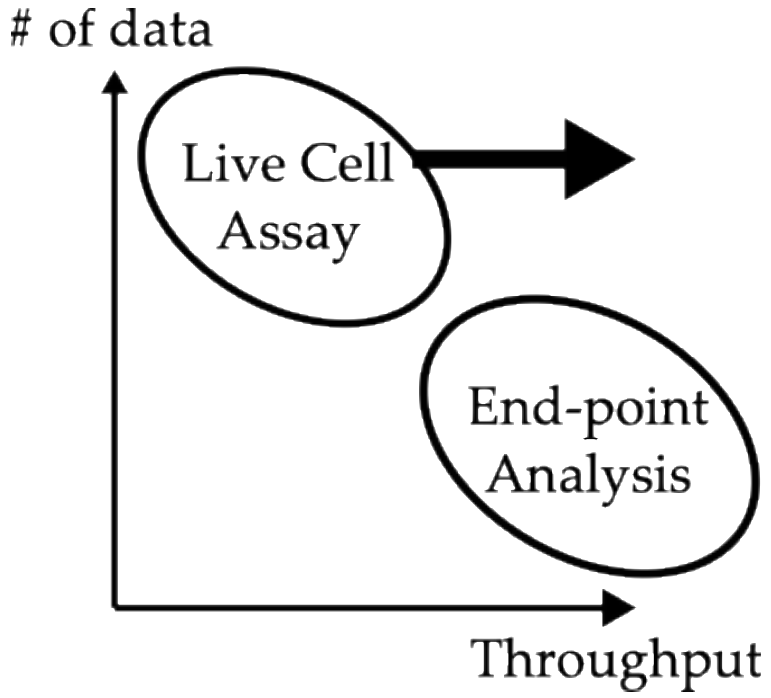
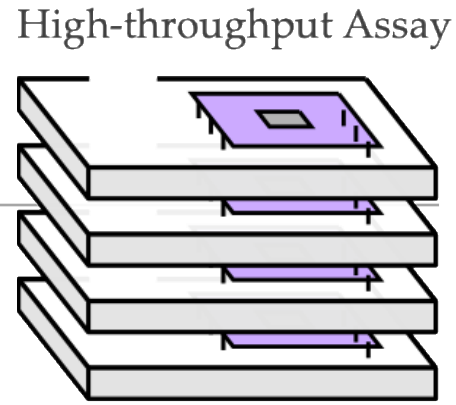
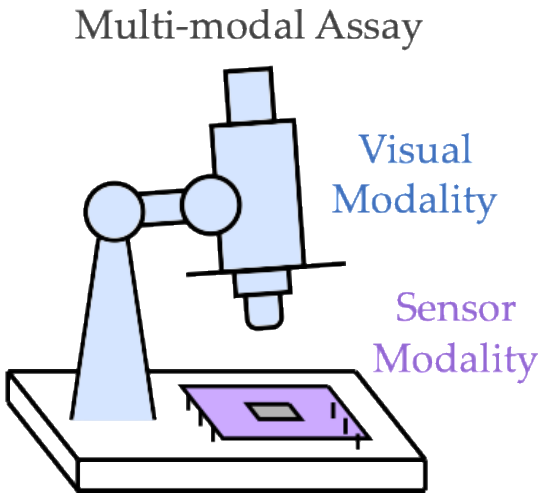


Multimodal Assay Application

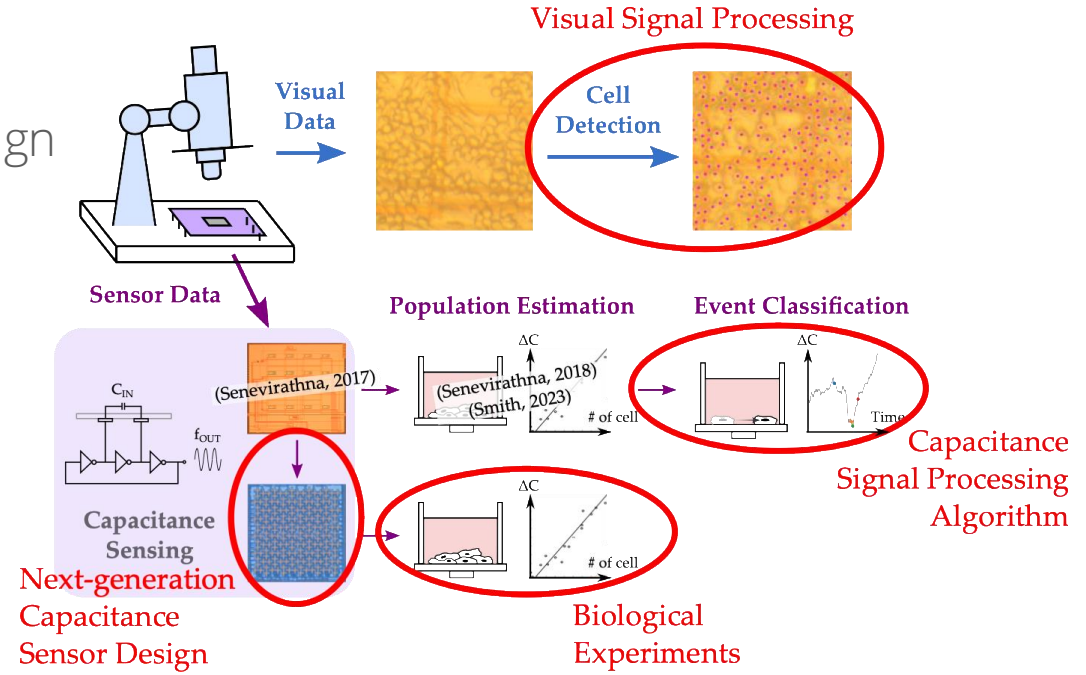
- Lab-on-a-chip can be
  - High-throughput assay
    - No microscope
    - Fast and stackable
    - (Validate with visual groundtruth)
  - Multi-modal assay
    - Provide extra modality for microscope assay





Dissertation Overview

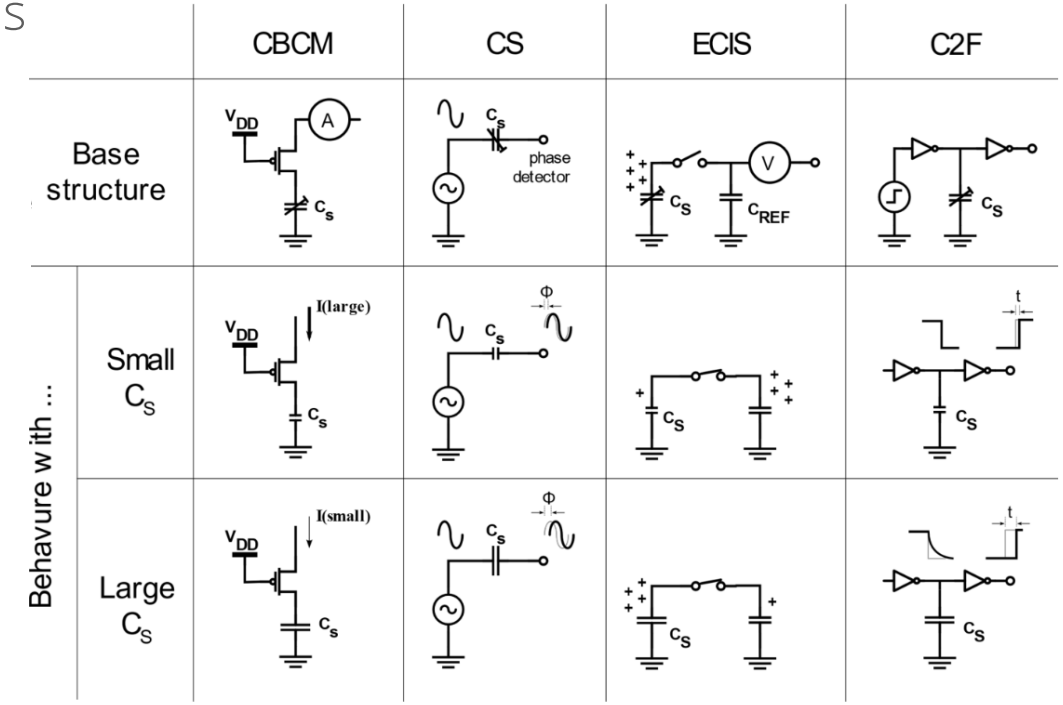
- Visual Signal Processing Algorithm
- Capacitance Signal Processing Algorithm
- Next-generation Capacitance Sensor Design
- Biological Experiments



Capacitance Signal Processing Algorithms

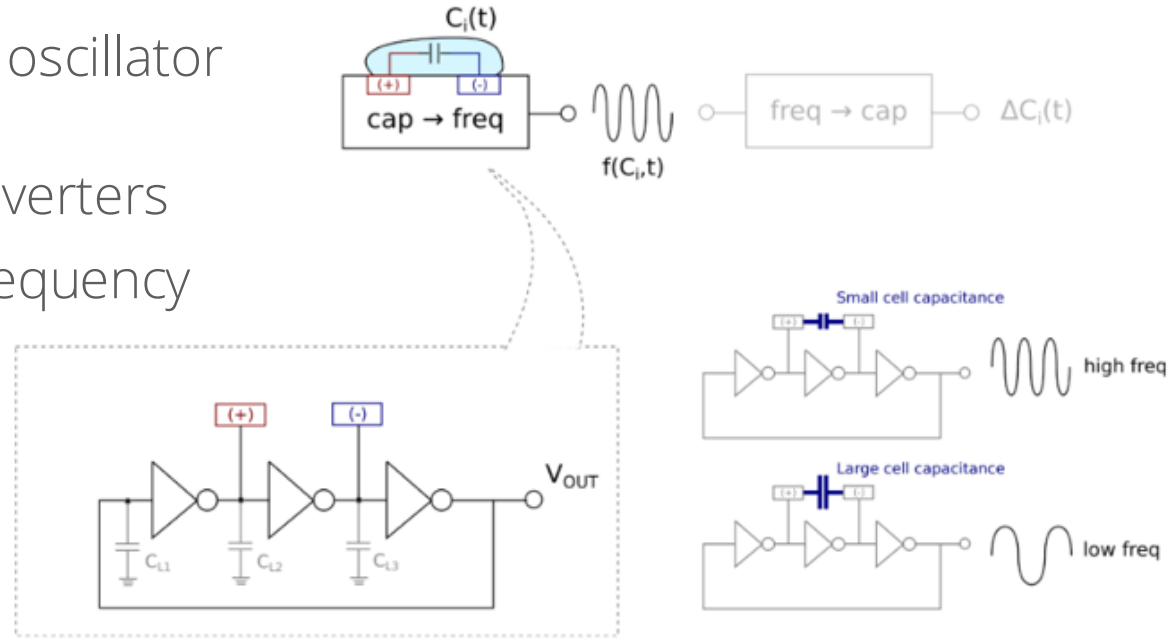
Capacitance Sensing (Lin, 2022)

- Sense electrical characteristics from cells
- Four primary ways
  - Charge-based Capacitance Measurement (CBCM)
  - Charge sharing (CS)
  - Electric cell-substrate impedance sensing (ECIS)
  - Capacitance-to-frequency (C2F)



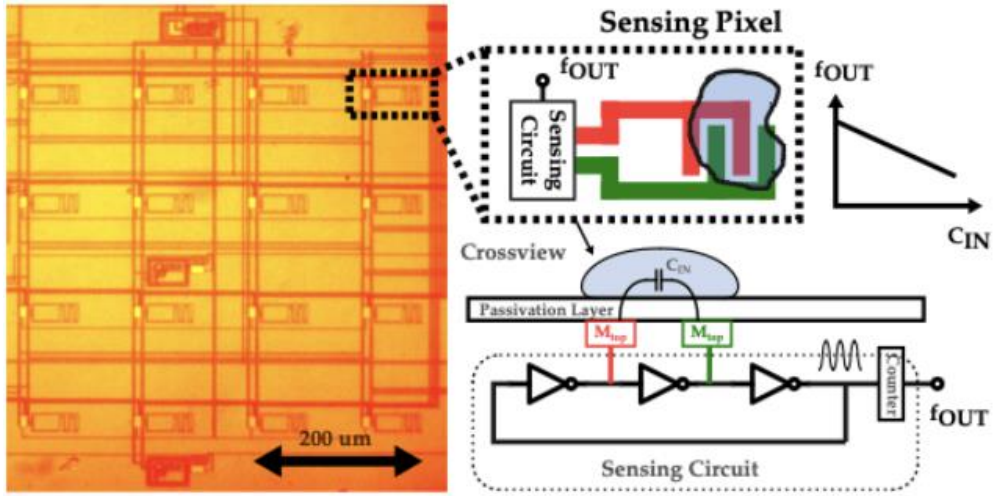
Capacitance-to-Frequency (C2F)

- Encode capacitance to oscillator frequency
- Capacitance slows down the oscillator
  - 3-stage oscillator using inverters
  - More capacitance, less frequency



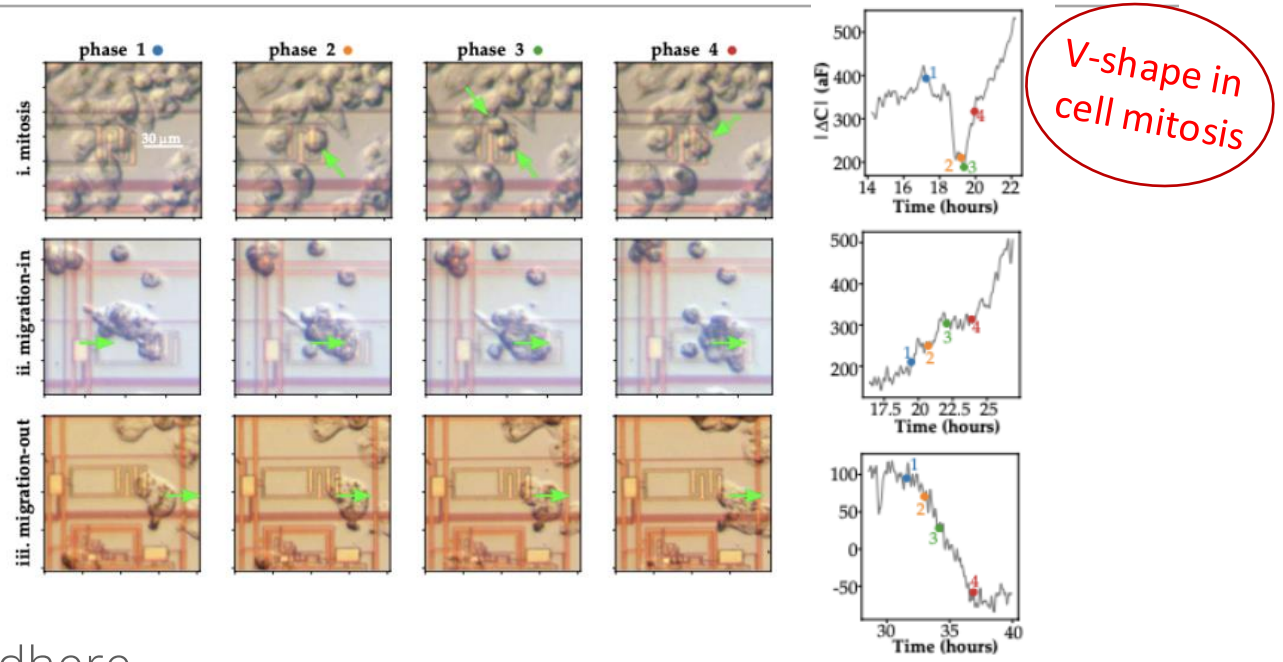
Capacitance Sensing for Cell Event Detection

- Capacitance Sensor Used (Senevirathna, 2018)
  - 4x4 array interdigitated electrode
  - Pre-paired mutual capacitance
  - Limit of detection (LOD): 17.5 aF
  - Spatial resolution: 180um



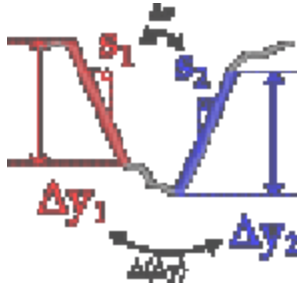
Cell Event Sensing Example (Lin, 2024)

- Sensors respond to
  - Larger overlap
  - Stronger attachment
- Cell events
  - Mitosis
    - Detach -> Divide -> Adhere
    - Weaker adhesion -> (Division) -> Stronger adhesion
  - Migration



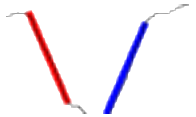
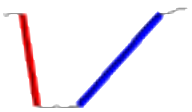
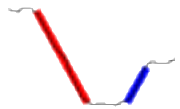
Motivation for Event Classification

- Differentiate V-shape pattern from normal slope
- Define 2 features for slope pair symmetry



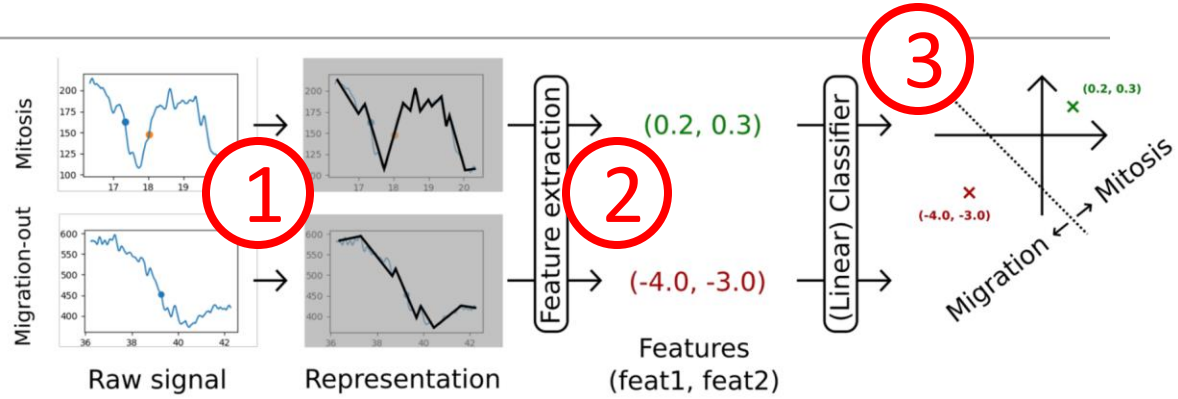
- Slope distance Δs
- Amplitude distance $\Delta(\Delta y)$

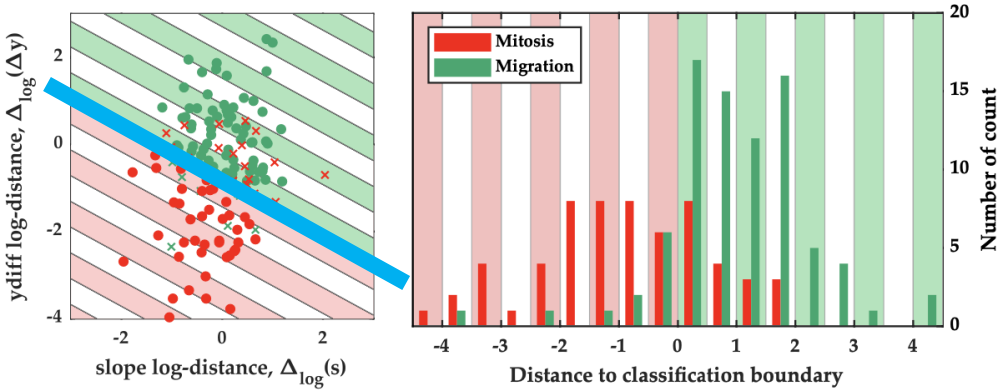
- P(mitosis) = Perfect-V similarity

	Perfect V	Else	
			
Δs	**Small**	Large	**Small**
$\Delta(\Delta y)$	**Small**	**Small**	Large
$P_{mitosis}$	**Large**	Low	Low

3-step Classification Algorithm

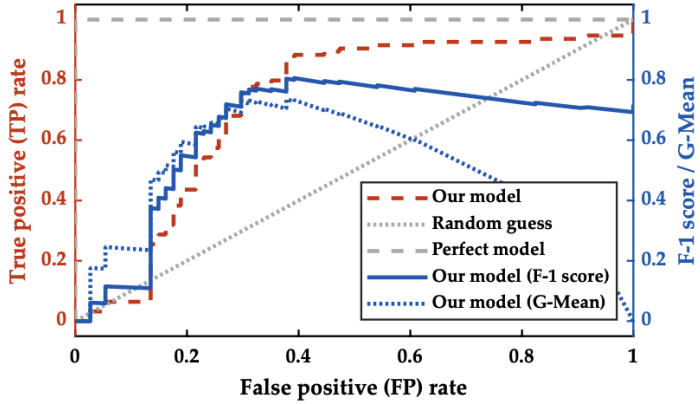
1. Represent in piecewise linear
2. Extract features
   - Derivative distance $\Delta_{log}(s)$
   - Amplitude distance $\Delta_{log}(\Delta y)$
3. Classify with SVM





Results – Comparison to another Feature Library

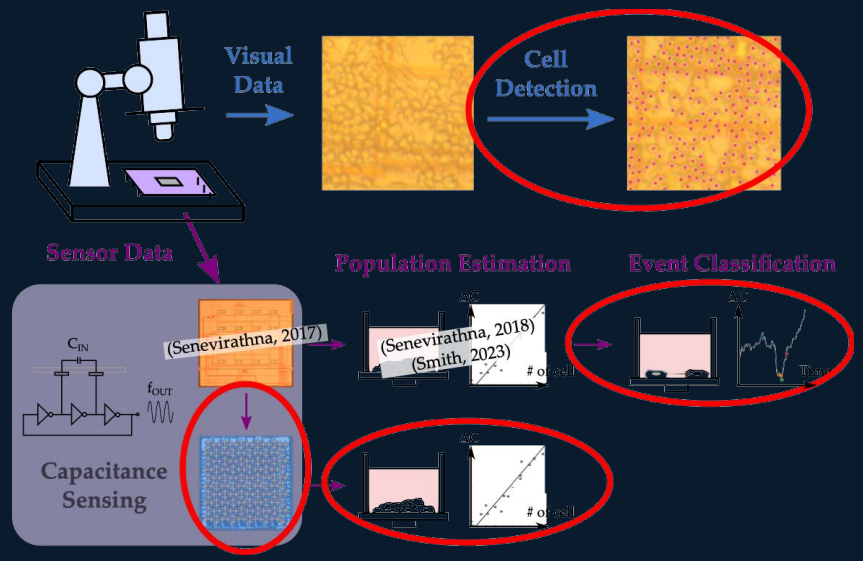
- Public dataset for signal and images (Senevirathna, 2019)
- Binary classification task
  - AUC = 0.719
- Compare to another feature library
  - TSFEL (~400 features)
  - Choose two best features
    - From DT, PCA, and LDA



	Pairs	Training Acc.	Validation Accuracy
DT	Median diff, $\Delta_{log}(\Delta y)$	**78.08**	**78.21 ± 4.50**
Manual	$\Delta_{log}(s)$, $\Delta_{log}(\Delta y)$	75.34	76.53 ± 5.20
DT*	Skewness, Spectral variation	73.97	72.85 ± 9.97
Manual*	$\Delta_{log}(s)$, $\Delta_{log}(\Delta y)$	73.97	71.00 ± 10.72
Manual*	Median diff, $\Delta_{log}(\Delta y)$	71.23	71.82 ± 10.45
LDA	ECDF_0, FFTMC_119	65.75	40.76 ± 10.72
DT	Centroid, FFTMC_22	64.38	66.36 ± 5.48
Manual*	Centroid, FFTMC_22	63.01	58.24 ± 14.64
PCA	FFTMC_185, FFTMC_216	58.90	50.87 ± 8.95
PCA*	FFTMC_185, FFTMC_166	58.90	46.72 ± 12.65
LDA*	Minimum peaks, FFTMC_88	42.47	43.25 ± 11.75

TSFEL = Time Series Feature Extraction Library
DT = Decision Tree
PCA = Principal Component Analysis
LDA = Linear Discriminative Analysis

Visual Signal Processing Algorithms



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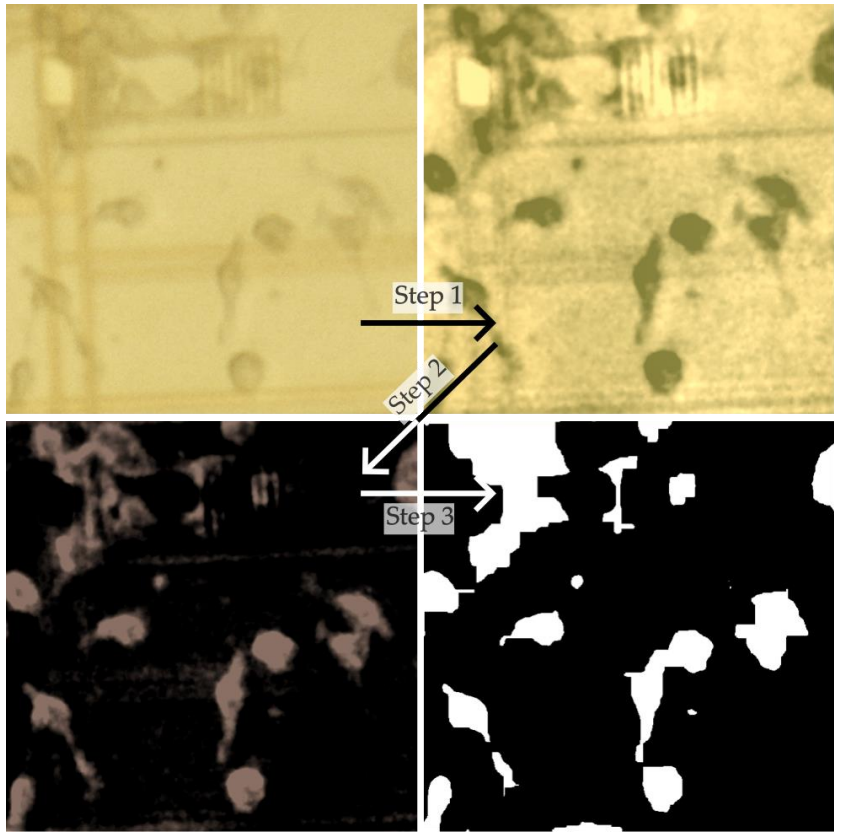
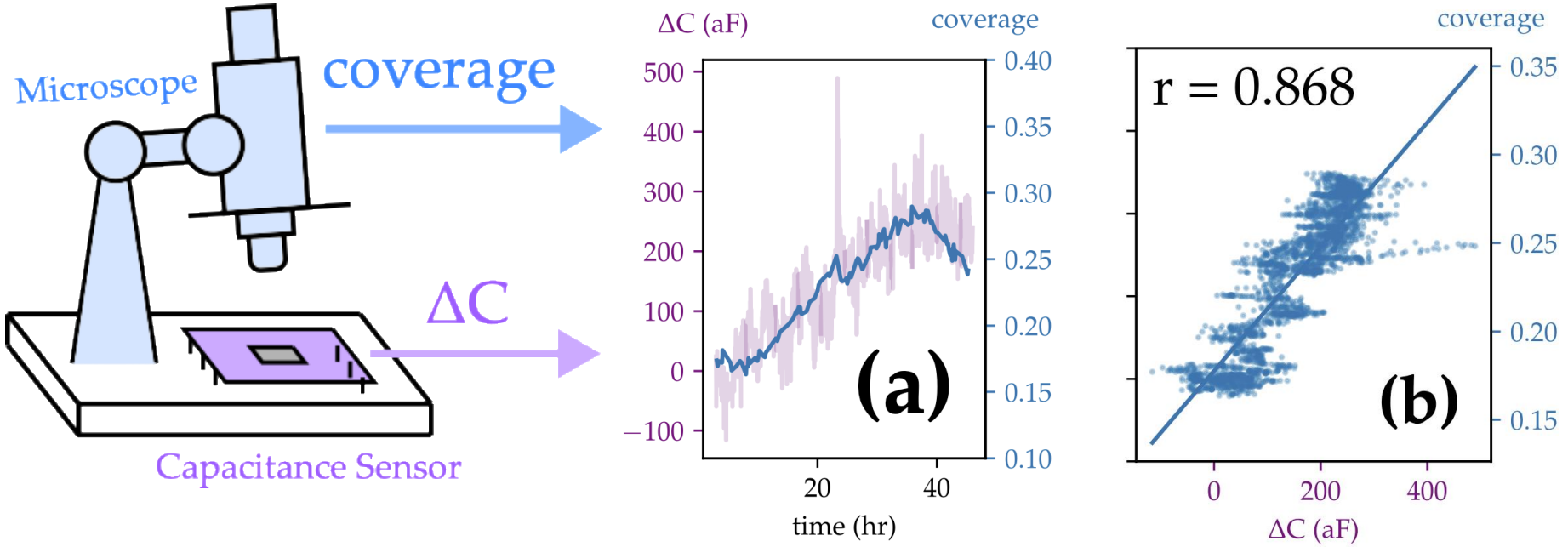
Visual Data Analysis – Coverage Estimation

- Cell images are
    1. Background removal
    2. Inverted + clipped
    3. Thresholding
- Cell coverage = Ratio after thresholding



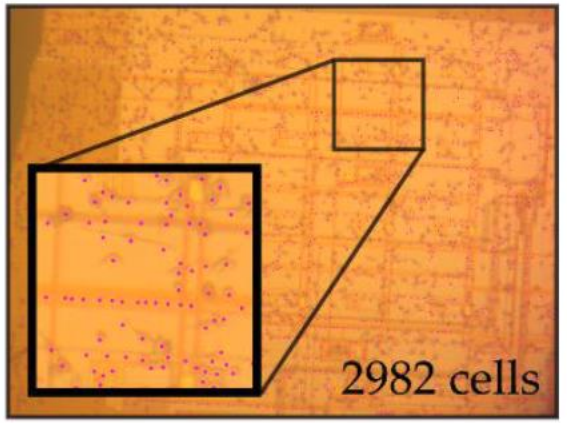
Preliminary Experiment (Lin, 2024)

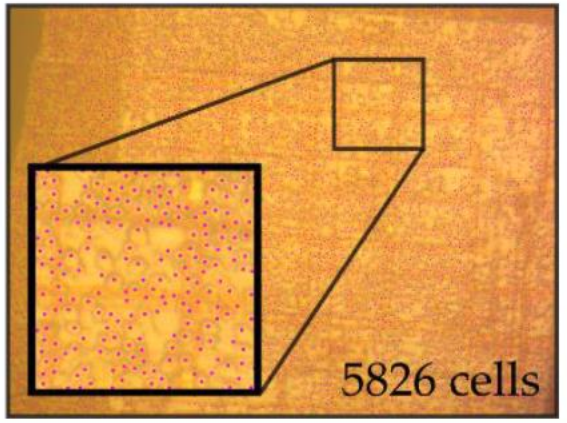
- First-generation chip + Breast cancer cell

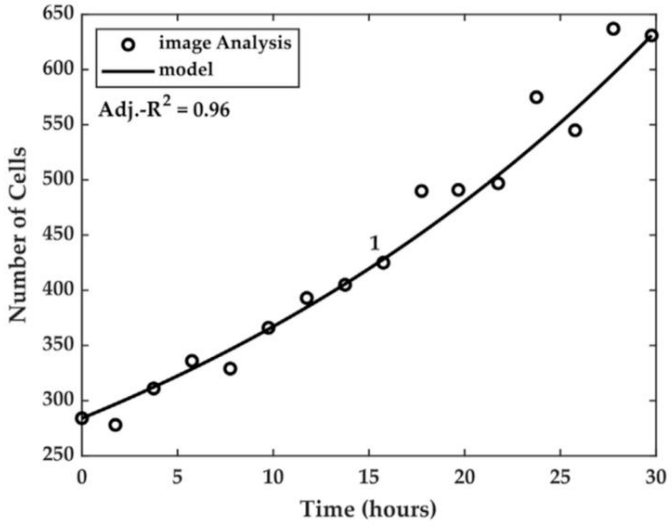


Visual Data Analysis – Cell Detection

- Characterize cell growth parameter (Smith, 2023)
- Validate treatment (Gilpin, 2022)

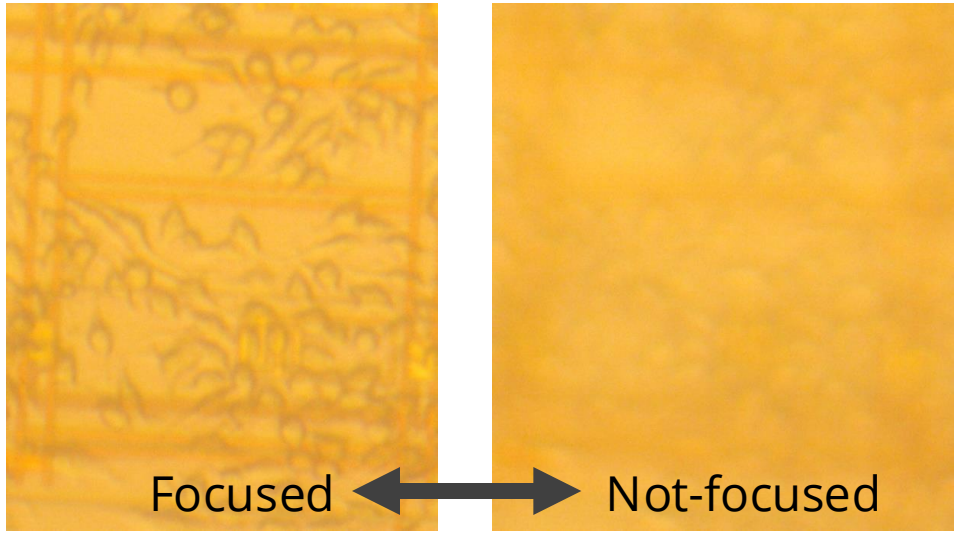


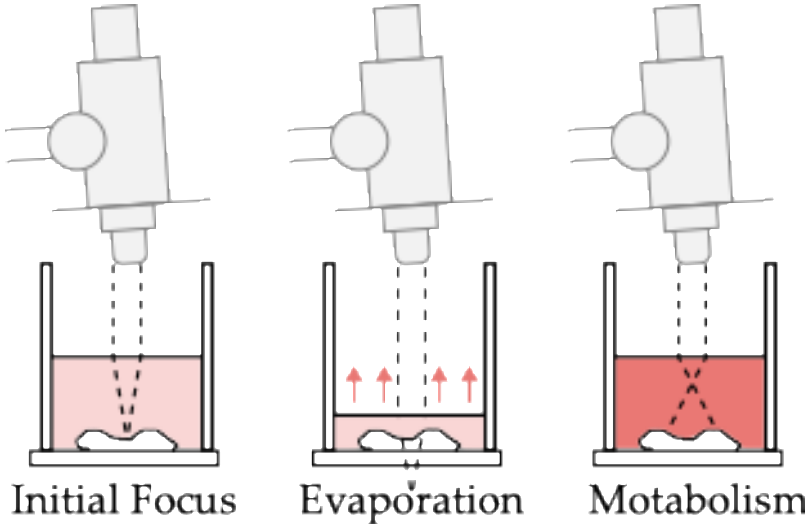




Challenges -- Focus Degradation

- Refractive index changes focal distance
  - Medium level from evaporation
  - Cell metabolism

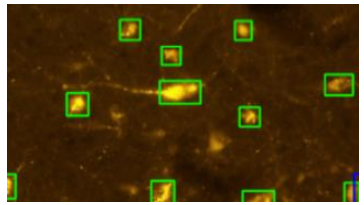




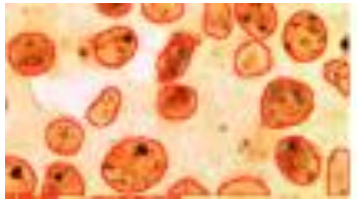
Visual Analysis Algorithms

- Our application has 1K~2K labeled cells

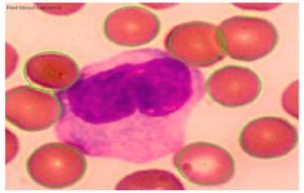
	Morphological	Template-based	Deep Learning
# of training data	Small	Small	**Large (>100k)**
Data-driven	**No**	Yes	Yes
Formulation	$\boldsymbol{a}cos\theta + \boldsymbol{b}sin\theta$	$\max(x \odot \boldsymbol{h}) > th$	$\mathcal{F}_{\boldsymbol{\theta}}(x) > th$
Parameters	1~5 ($\boldsymbol{a}, \boldsymbol{b}$)	64 x 64 ($\boldsymbol{h}$)	60M+ ($\boldsymbol{\theta}$)
	Edge (Choudhry, 2016) Hough (Mahmood, 2012)	Adaptive filter (Cibej, 2015)	Alexnet (Shahin, 2019) R-CNN (Kutlu, 2020)



(Morelli, 2021)

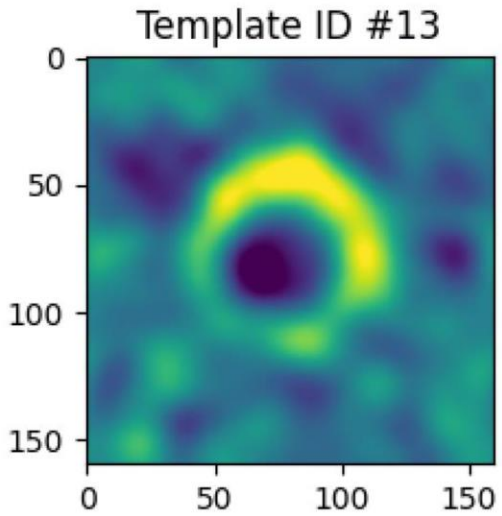


(Chen, 2013)



(Mahmood, 2012)

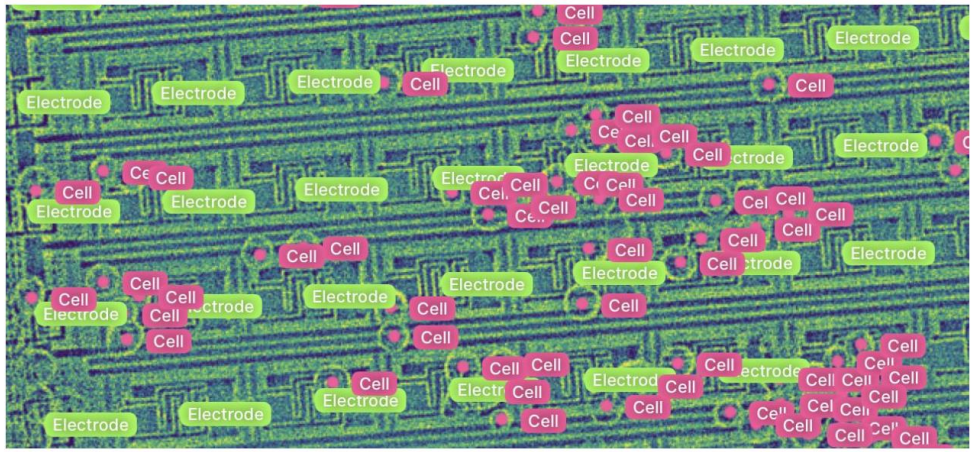
Template Matching-based Algorithr



- Formulation (linear kernel)

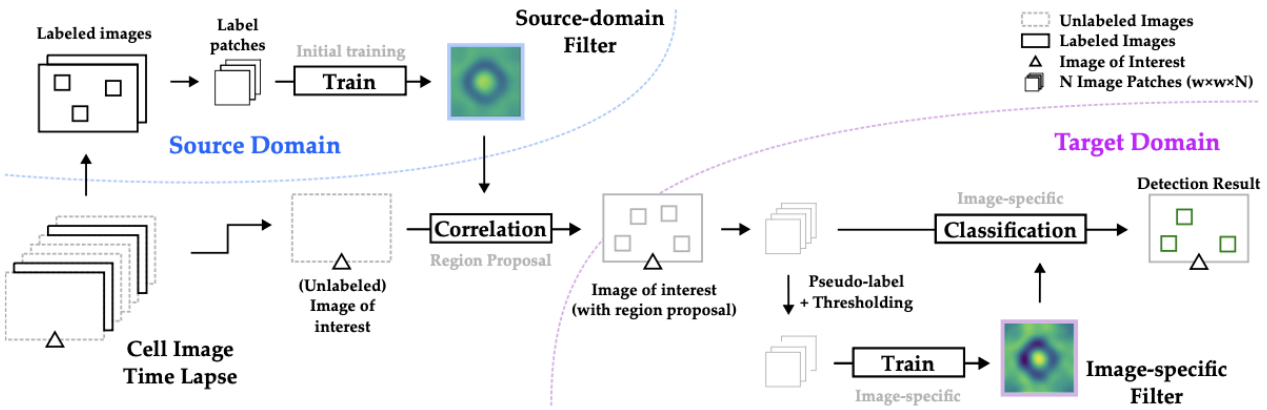
$$H = arg\min_{H} \Sigma |F_i \odot H^* - G_i|^2$$

  - F_i: Image patches with cells
  - G_i: Target response (A 2-D Gaussian)
  - $F_i \odot H^*$: Detection response

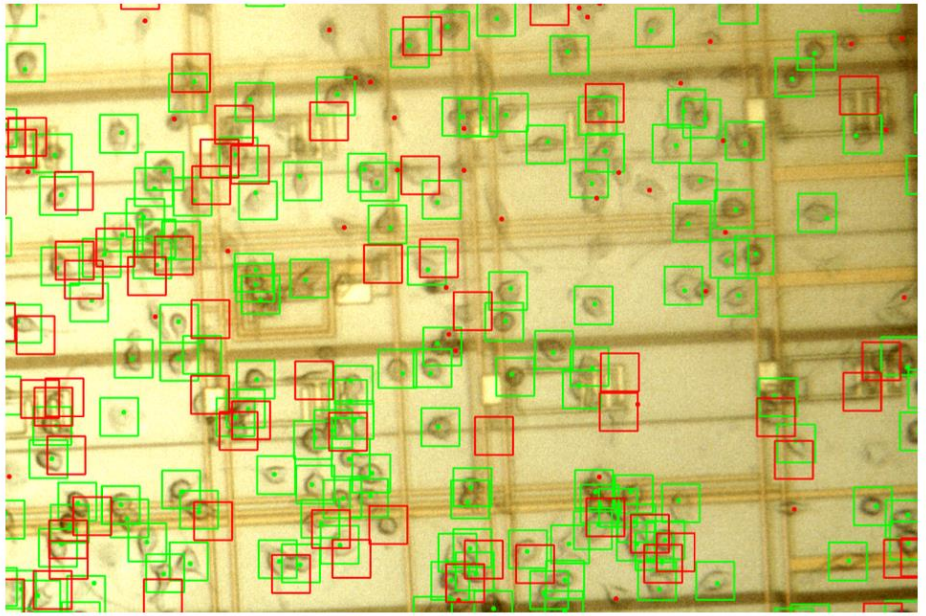


Transductive Cell Detection Overview

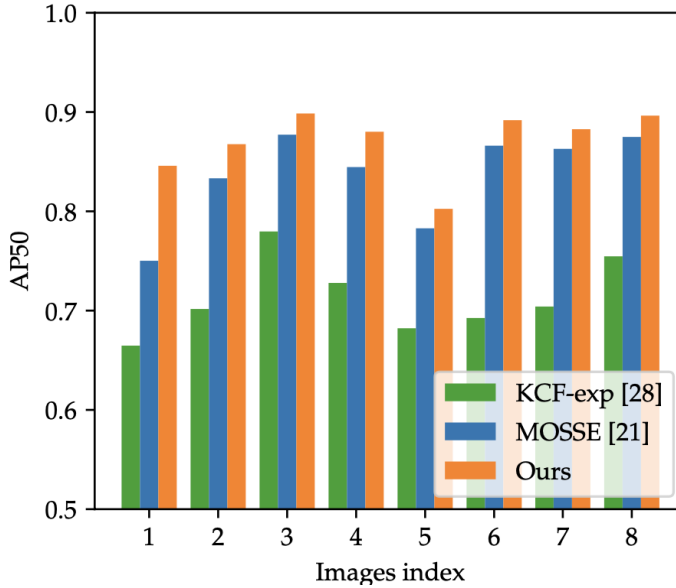
- Two rounds of training
  - Round 1 (Source domain): Large amount but include all focus
  - Round 2 (Target domain): One (test) image with exact focus



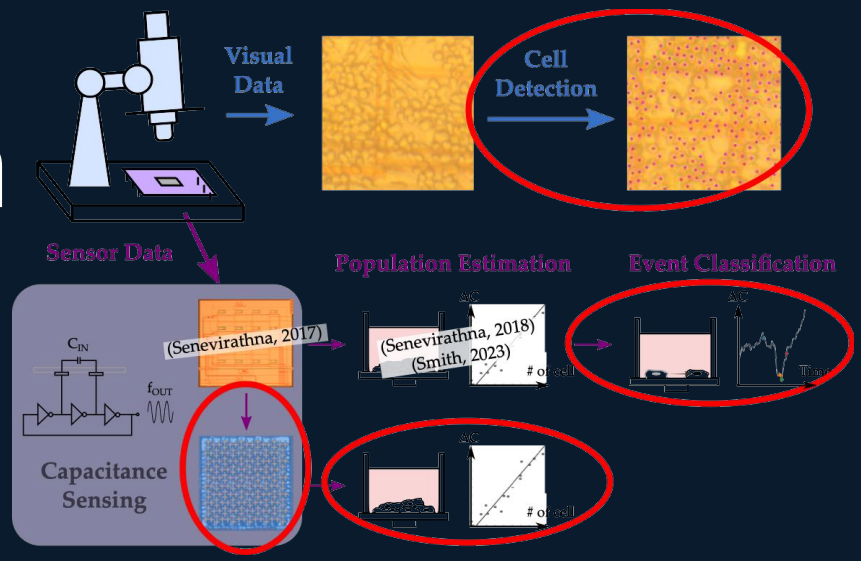
Results



- Classification results
  - Green / Red boxes: True / False detection
  - Green / Red dots: Detected / Undetected cells
- Compare two template-based works
  - MOSSE
  - KCF-exp
  - Ours



Next-generation Capacitance Sensor Design



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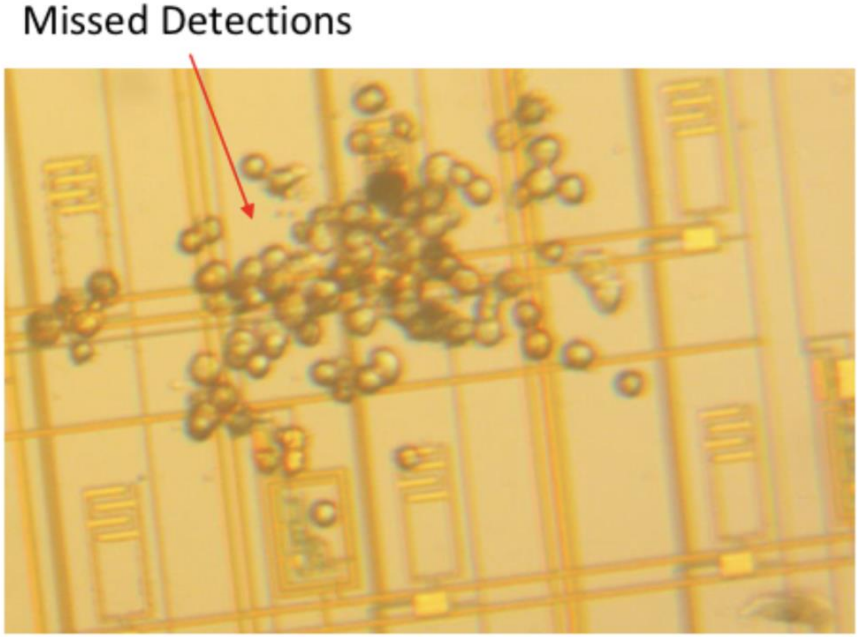
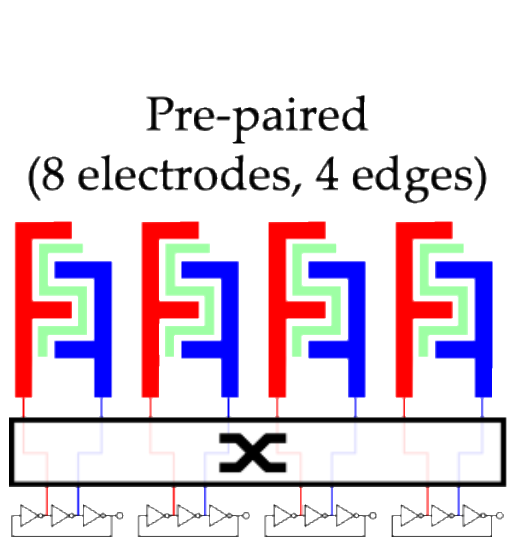
Recap – Miss Detection in Previous Sensor

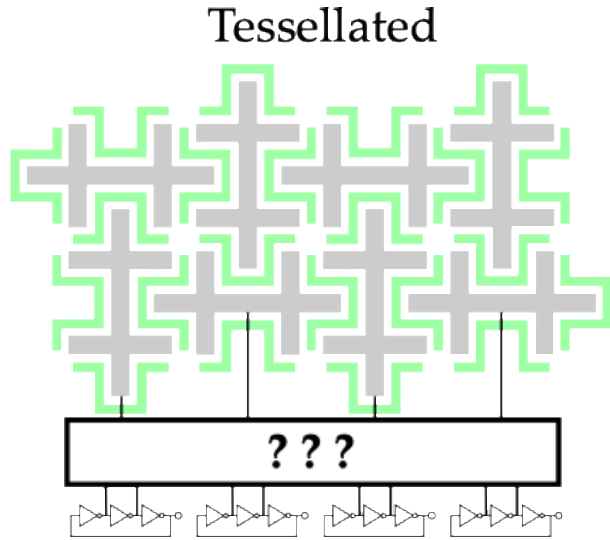
- Sensitive to detect population and single-cell event
- Low spatial resolution -> Few coverage
- Goal
  - High spatial resolution
  - Medium sensitivity



Motivation for High Spatial Resolution

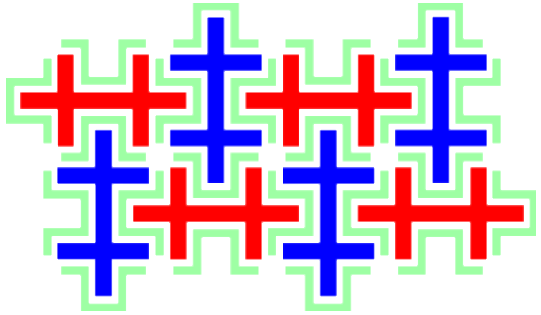
- Tessellated electrode array
  - Potentially more edges
  - Denser with large coverage
- Challenges
  - How to make every edge a sensing edge in C2F?
  - How to configure the array?



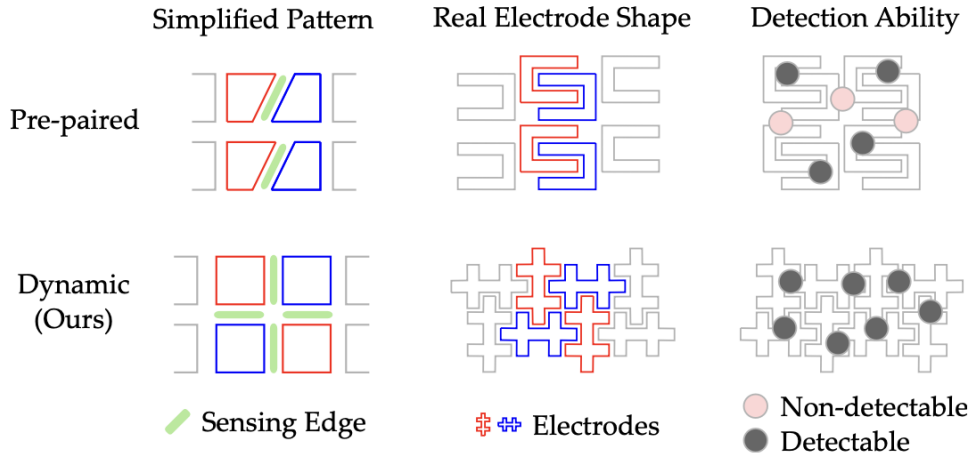


(Senevirathna, 2019)

Control Realization

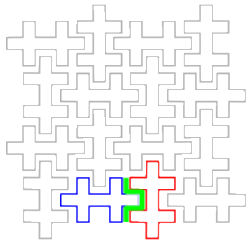


- Chess-coloring
  - Map black / white checkerboard to red / blue electrode
  - Every edge connects to two nodes
- How many edges per acquisition unit

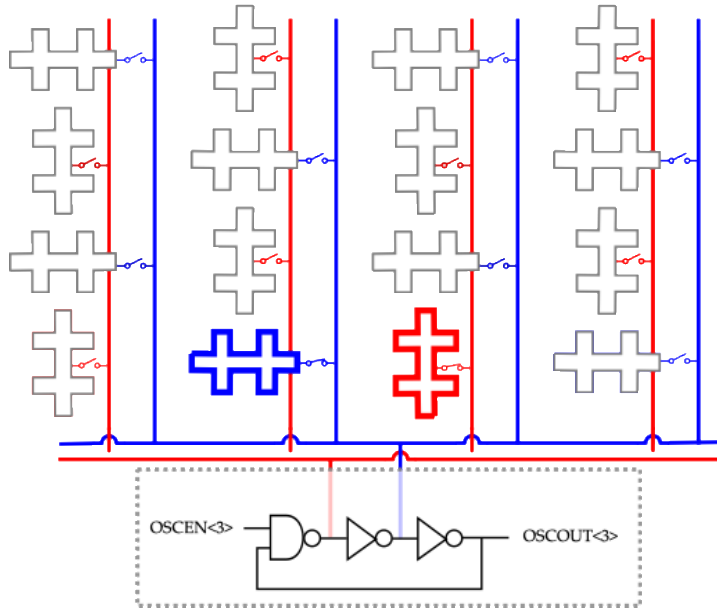


Data Acquisition and Oscillator Sharing

- 4x4 electrodes per C2F acquisition unit
- Inconsistent routing effort
  - Configuration mismatch
- Connection from transmission gate

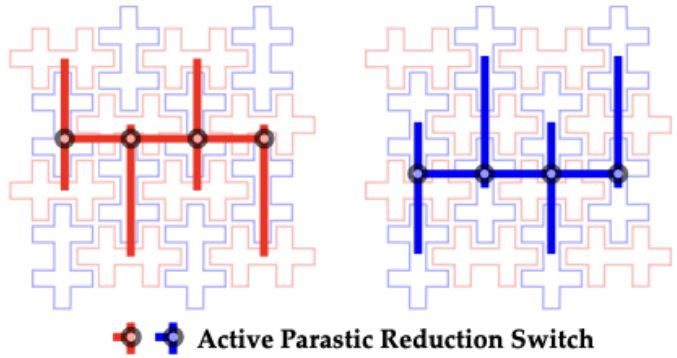


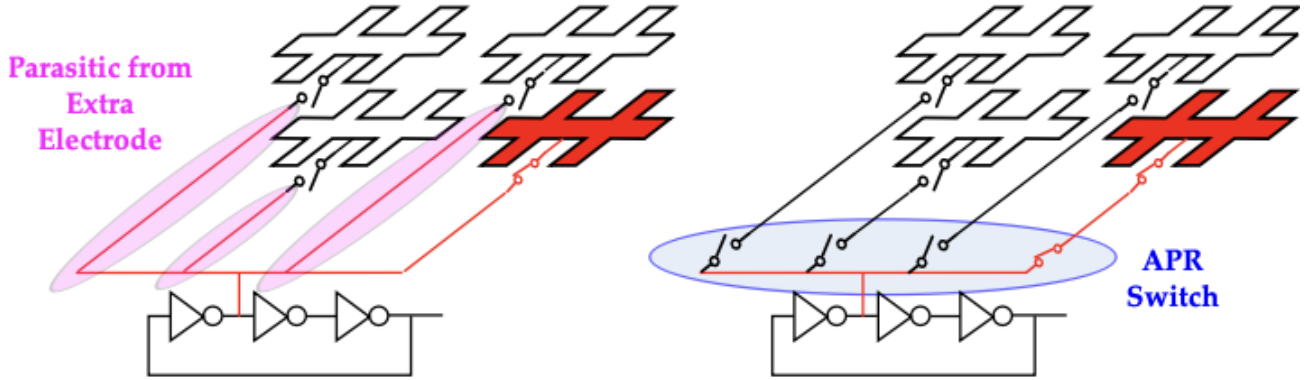
  - Activated electrodes are connected
  - Non-activated electrodes are not connected
    - But still contribute routing effect



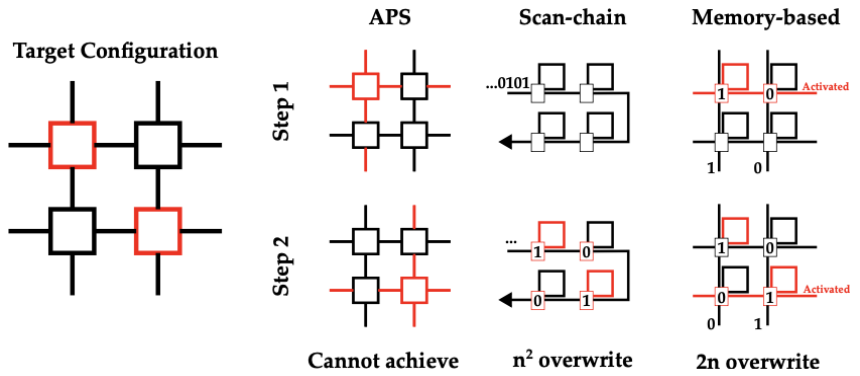
Active Parasitic Reduction (APR)

- Non-activated electrodes still contribute parasitic
- Disconnect unused connection (APR switch)
  - Pros: Less parasitic
  - Cons: Extra control (4 osc * 2 nodes * 4 cols = 32)





Configure Electrode Array

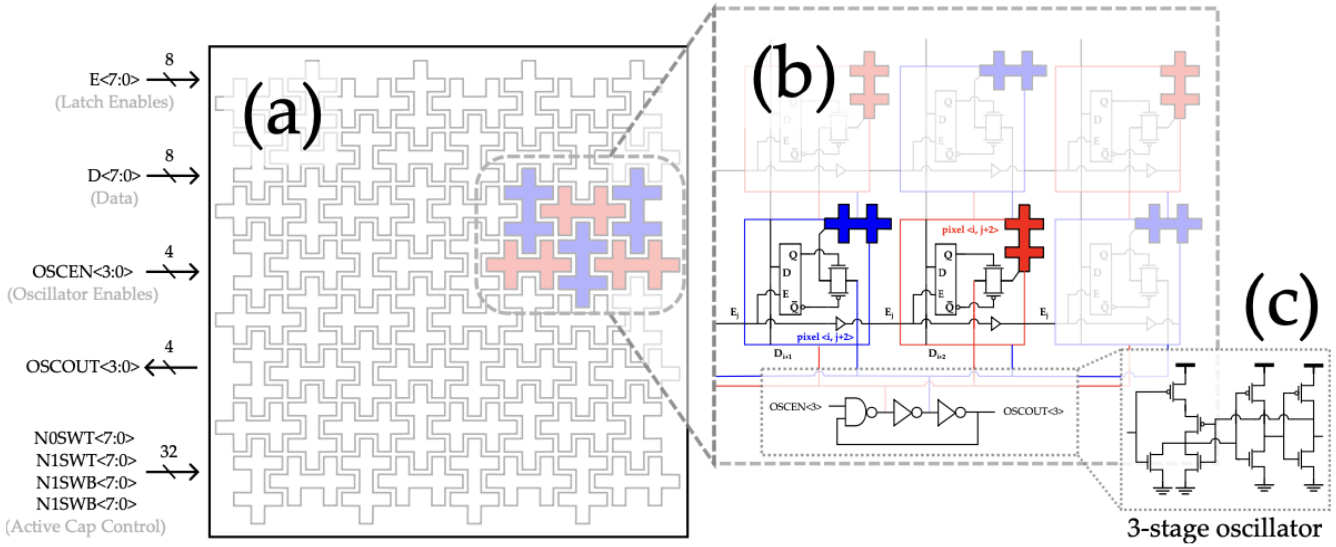


- Goal: Reconfiguration for any pattern
- Three potential solution (to activate 1 electrode)

	APS	Scan-chain	Memory-based
Memory Unit	X	V	V
Arbitrary pattern	**X**	V	V
# of IO	n + n	1	n + n
Time complexity	1	**n^2**	1

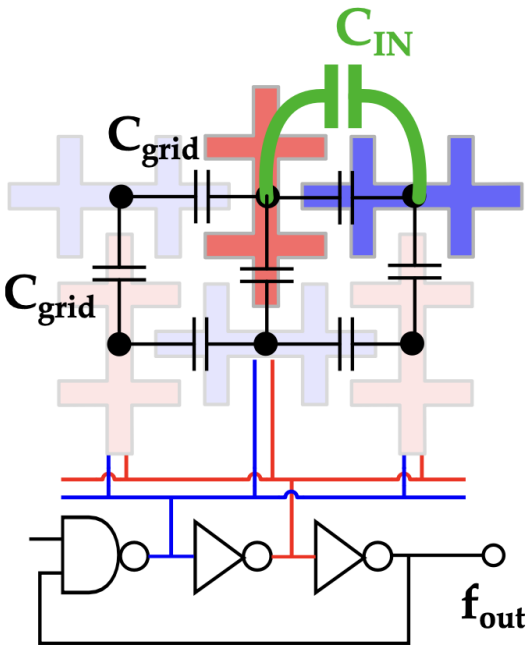
Sensor Array Schematic

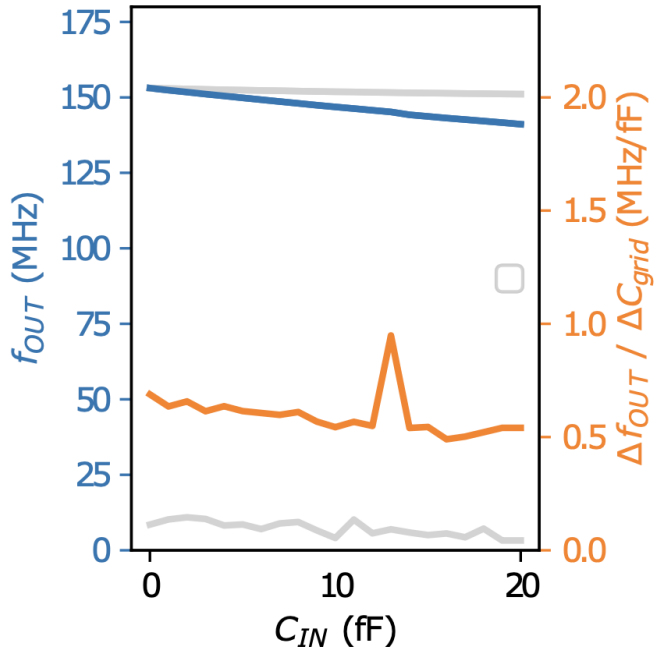
- Control Signals
  - 8-bit latch row enables + 8-bit data column
  - 4-bit oscillator enables + 4-bit oscillator outputs
  - 32-bit APR control
- Pixel implementation
  - Connection: T-gates
  - Memory: D-Latch



Capacitance Sensitivity

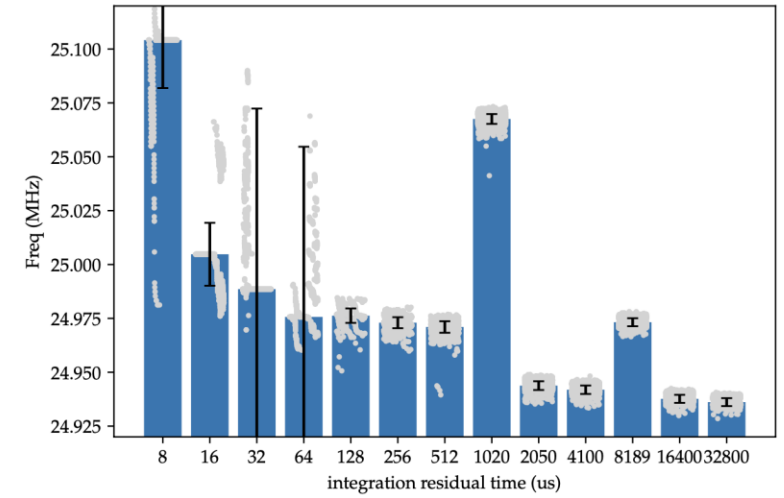
- (The last) Post-layout simulation
  - C_{grid} for media bias
  - C_{IN} for analyte change
- Sensitivity = 0.688 (MHz/fF)
  - Bias @ C_{grid} = 11fF

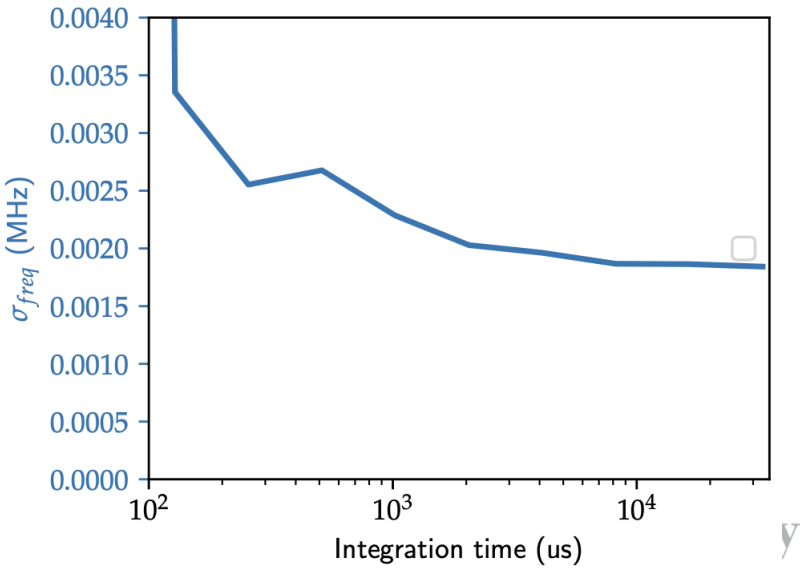




Integration Time vs Noise

- Larger integration time T_{INT}
  - Reduces σ_{freq}
    - Stable after T_{INT} > 2ms
  - Reduces sampling rate
- We choose T_{INT} = 20ms
  - LoD = $\mathbf{3.3} \times \frac{\sigma_{freq}}{S_{freq}} = 9.59$ (aF)

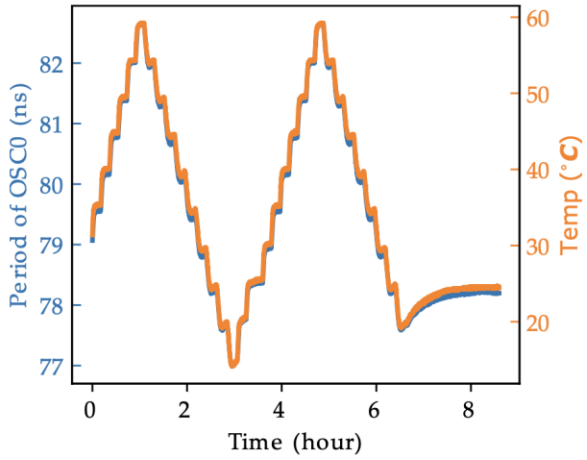


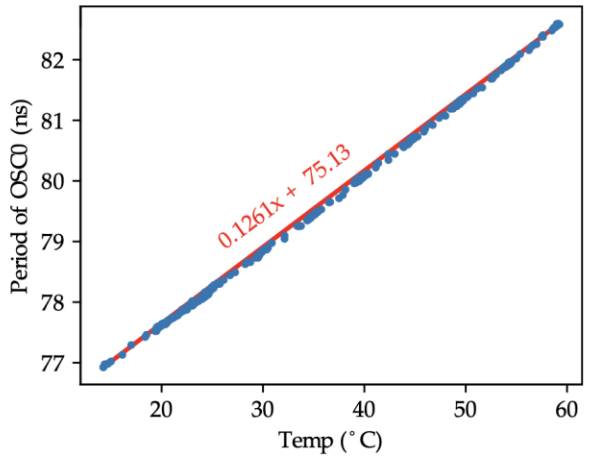


LoD = Limit of detection

Temperature Sensitivity

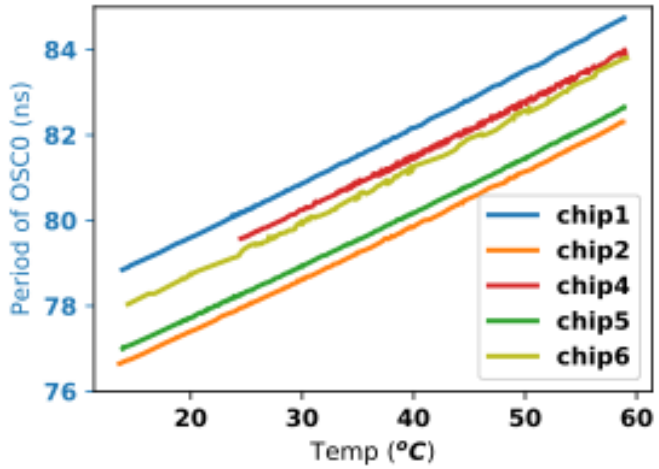
- Sweep in a programmable temperature chamber
- Temperature sensitivity
  - 0.1261 (ns/°C)
  - -0.020 (MHz/°C)
  - 29.07 (aF/°C)
    - $\Delta C_{cell} \approx 100$ (aF)

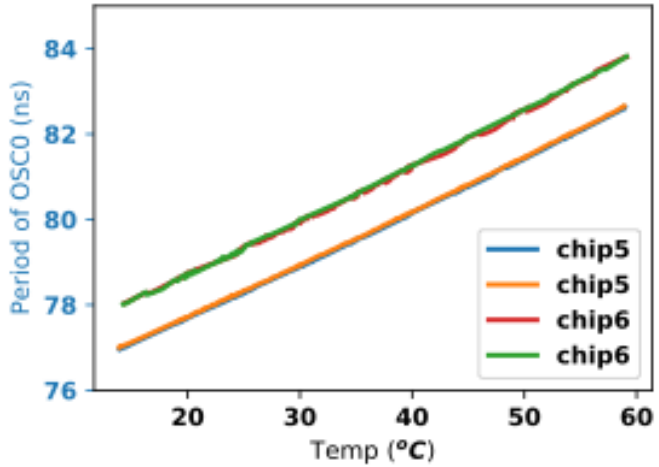




Die-to-die Mismatch

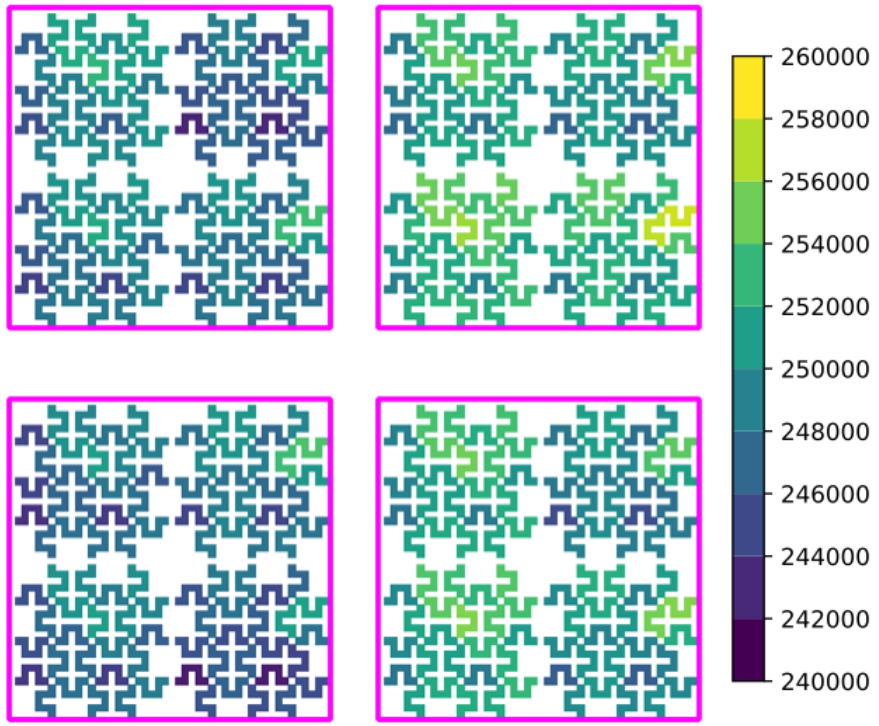
- Two experiments
  - Temperature sweep with five dies
  - Two-round sweep with two dies
- Oscillator has a per-die mismatch



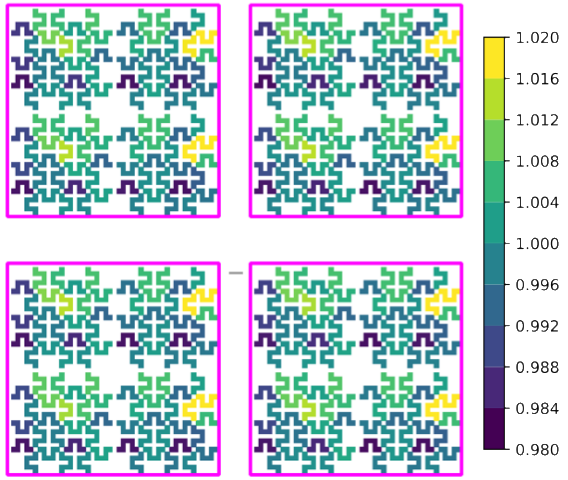


Raw Configuration Mismatch

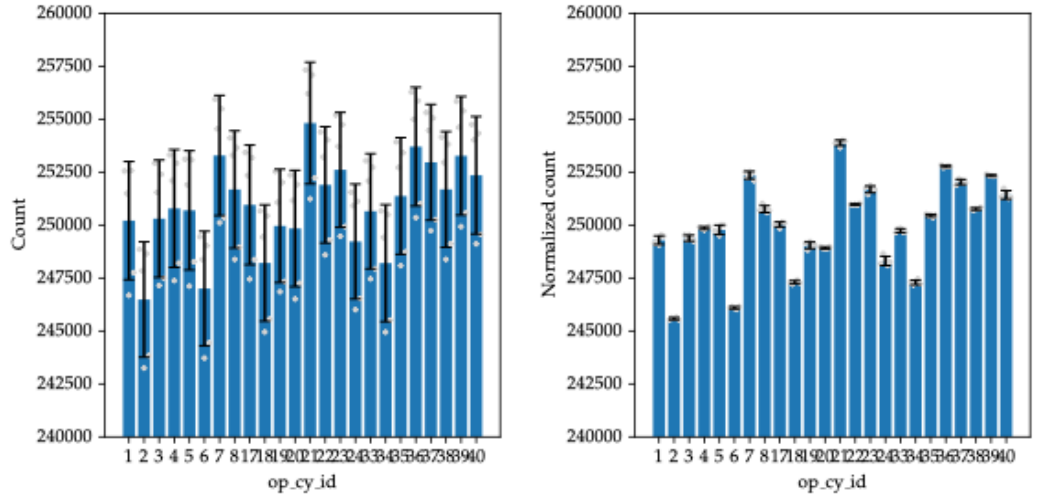
- From inconsistent routing effort
- Raw measurement result
  - 4 different dies
  - Counter count in 20ms
- Observation
  - Same trend
  - Different baseline



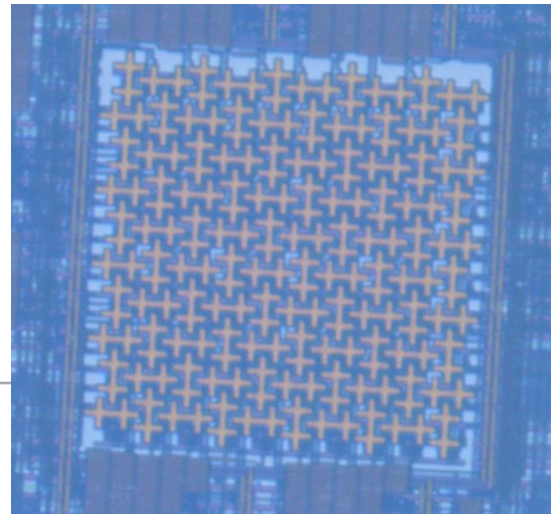
Normalized Configuration Mismatch



- Calculate relative count γ_{cfg} for a configuration cfg
  - $\gamma_{cfg} = \frac{T_{cfg}}{\frac{1}{|S|}\sum_{i \in S} T_i} = \frac{count_{cfg}}{\overline{count}}$
  - Reference to average count
  - Scope: S (Same-oscillator edges)
- From die-to-die
  - Consistent mismatch γ_{cfg}
  - Different oscillator mismatch k_{osc}



Comparison Table



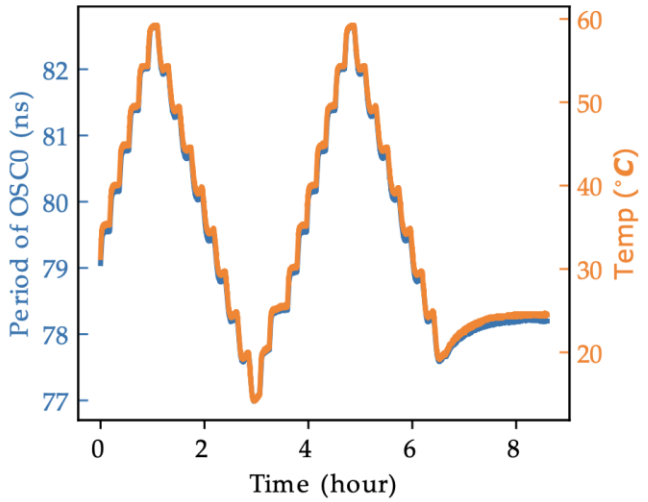
- Highest resolution in 350nm
- Highest normalized resolution (resolution / process)

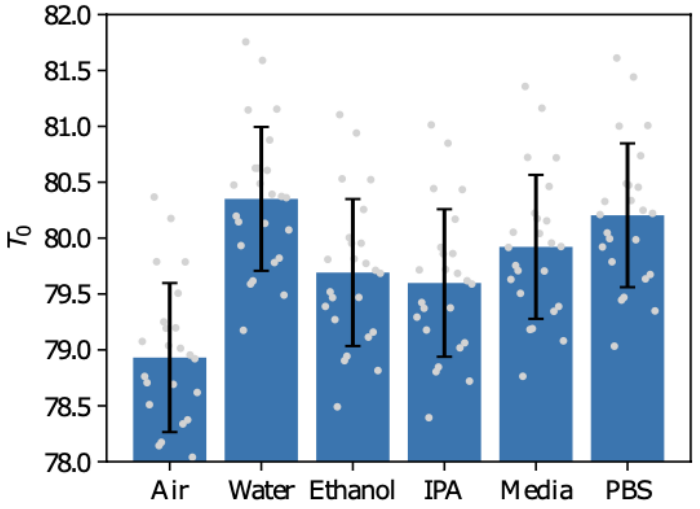
	Nabovati 2016	Senevirathna 2017	Tabrizi 2022	Hu 2022	This work
Process Node	350	350	350	180	350
Sensing	CBCM	C2F	CCO	ECIS	C2F
Detection Limit (aF)	10	17.5	416	**0.13**	9.59
Sensitivity	350 (mV/fF)	593 (aF/kHz)	-	-	688 (fF/kHz)
Sample Rate	**450 (kHz)**	1 (Hz)	-	1 (Hz)	50 (Hz)
Power (mW)	-	8	-	58.8	11.05
Pixel Size (um)	50 x 50	30 x 30	228 x 54	**10 x 10**	22 x 22
Effective resolution (um)	50	180	111	**10**	18
Normalized resolution (λ)	142.9	514.3	317.1	55.6	**51.4**
Array size	8x8	4x4	1x2	512x256	8x8
Target Biomarker	Cells	Cancer Cells	Oral Cells	Bacterial Biofilms	Cancer Cells

Capacitance Signal Processing For the New Sensor

Non-ideality from Temperature and Configuration

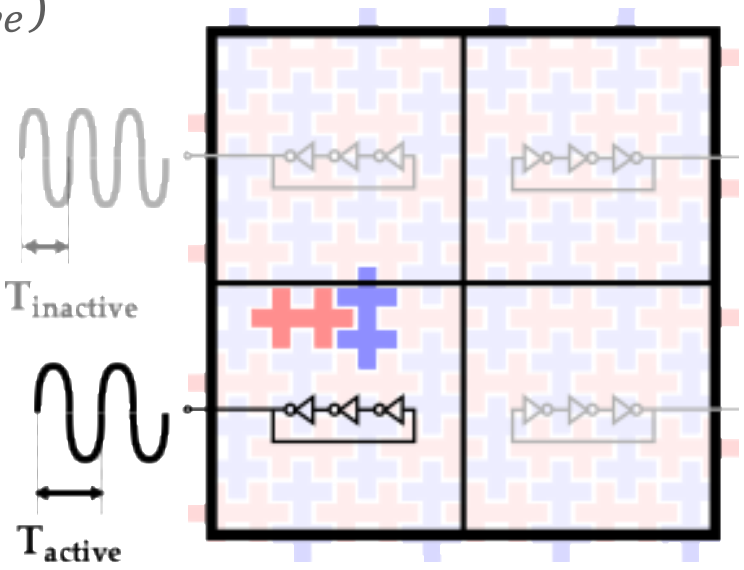
- Temperature effect from temperature sweep
- Configuration mismatch from material sweep





Period Modeling

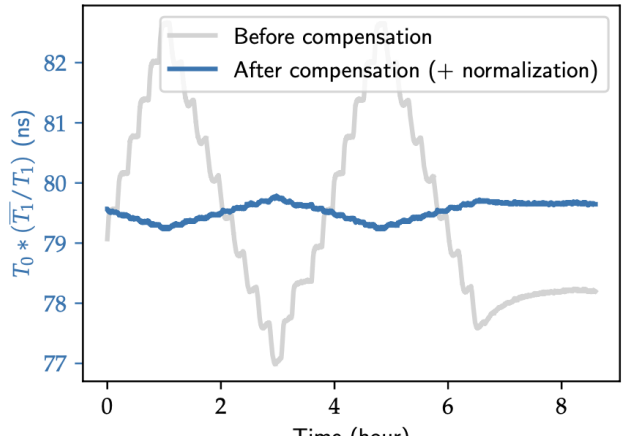
- We model period of active and inactive oscillator
  - $$\begin{cases} T_{active} = T(\epsilon) \times k_{temp} \times \gamma_{cfg} \times k(osc_{active}) \\ T_{inactive} = T_{base} \times k_{temp} \times k(osc_{inactive}) \end{cases}$$
- Normalization
  - $R_{normalized} = \frac{T_{active}}{T_{inactive}} \times \frac{1}{\gamma_{cfg}} \propto \frac{T(\epsilon)}{T_{base}}$
    - Temperature k_{temp} is canceled
    - Mismatch γ_{cfg} can be estimated

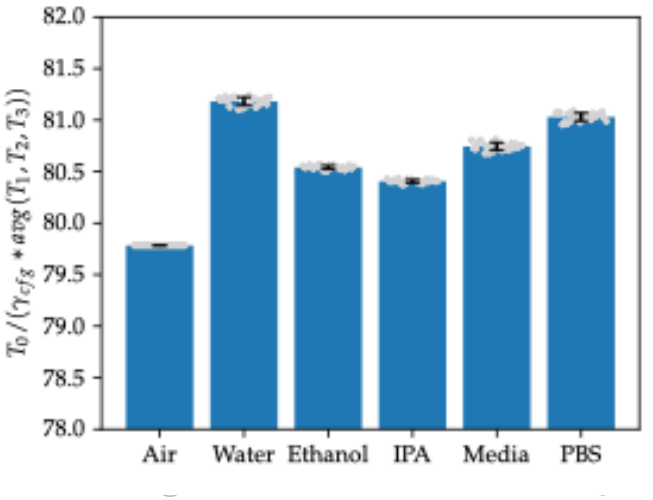


$$R_{normalized} = \frac{T_{active}}{T_{inactive}} \times \frac{1}{\gamma_{cfg}} \propto \frac{T(\epsilon)}{T_{base}}$$

Preliminary -- Temperature / Material Sweep

- Temperature sweep
  - Scale back from $R_{normalized} \times \bar{T}$
- Material sweep experiment: Air + 5 materials
  - Estimates mismatch $\gamma_{cfg} = \frac{T_{cfg}}{\frac{1}{|S|}\sum_{i \in S} T_i} = \frac{T_{cfg}}{\bar{T}}$
    - From air experiment
  - Normalization: $R_{normalized} = (\frac{T_{active}}{T_{inactive}})_{cfg} \times \frac{\overline{T_{air}}}{T_{air,cfg}}$





Reality – Mismatch is ϵ-dependent

- One-order mismatch: $\gamma_{cfg}(\epsilon) = \alpha_{cfg}\epsilon + \beta_{cfg}$
- New model

$$T_{active,cell} = T(\hat{\epsilon}_{media} + \hat{\epsilon}_{cell}) \times k_{temp} \times (\boldsymbol{\hat{\gamma}_{air,cfg}} \times \hat{\epsilon}_{media} + \boldsymbol{\hat{\beta}_{media,cfg}}) \times k(osc_{active})$$

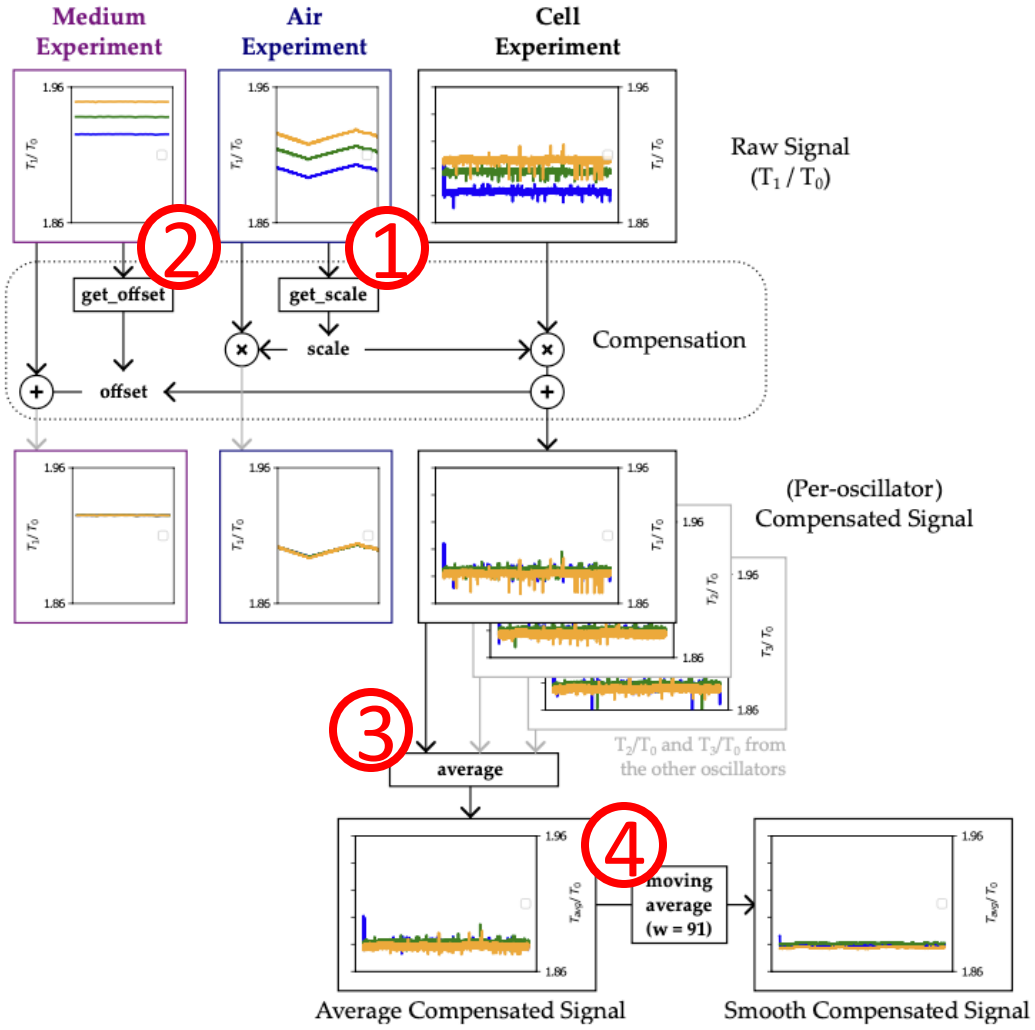
$$T_{inactive,cell} = T(\hat{\epsilon}_{base}) \times k_{temp} \times k(osc_{inactive})$$

  - New mismatch term $\boldsymbol{\hat{\gamma}_{air,cfg}} \times \hat{\epsilon}_{media} + \boldsymbol{\hat{\beta}_{media,cfg}}$
  - Two parameters to be estimated: $\boldsymbol{\hat{\gamma}_{air,cfg}}$ and $\boldsymbol{\hat{\beta}_{media,cfg}}$

 $\hat{\gamma}_{air,cfg} = \gamma_{cfg}(\epsilon_{air}) = \alpha_{cfg}\epsilon_{air} + \beta_{cfg}$, $\hat{\epsilon}_{media} = \frac{\epsilon_{media}}{\epsilon_{air}}$, $\hat{\beta}_{media,cfg} = \beta_{cfg} \times (1 - \hat{\epsilon}_{media})$

Compensation Algorithm

1. Scale $\hat{\gamma}_{air,cfg}$ temperature sweep
2. Offset $\hat{\beta}_{media,cfg}$ from material sweep
3. Average with inactive oscillators
4. Smooth with moving average filter



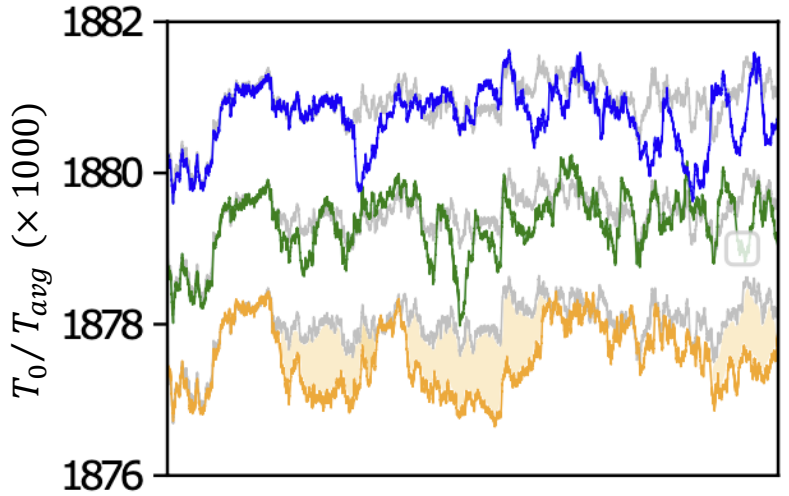
Biological Experiments

Estimate Cell Contribution

- Observation from cell signal
  - Negative spikes
  - Consistent trend with an offset
- Goal: Get negative residual from the signal

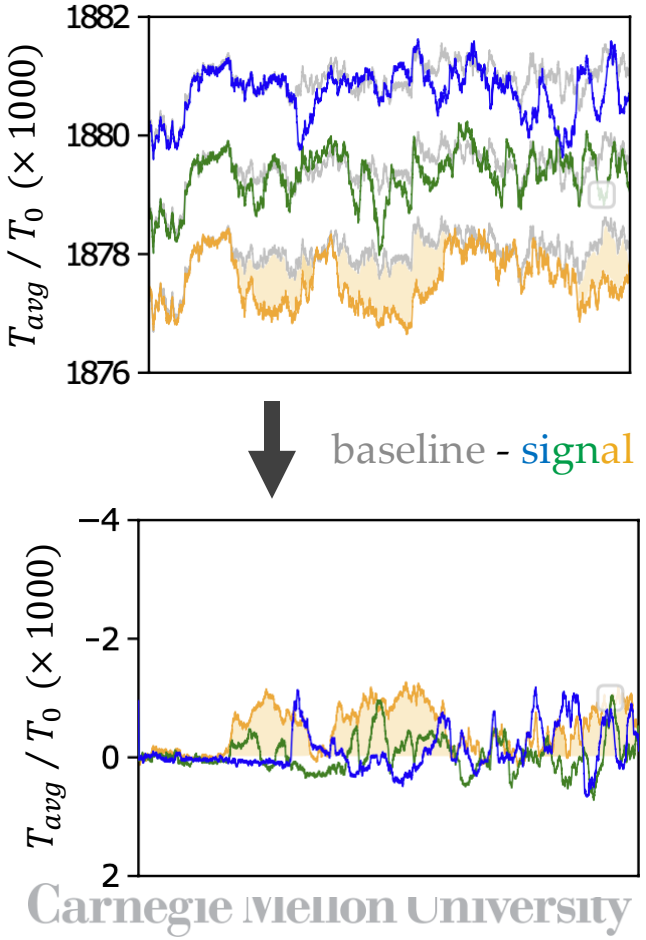
$$T_i(t) = CELL_i(t) + b(t) + c_i$$

  - $CELL_i(t)$: Per-pixel cell contribution
  - $b(t)$: A global trend
  - c_i : Per-pixel constant offset



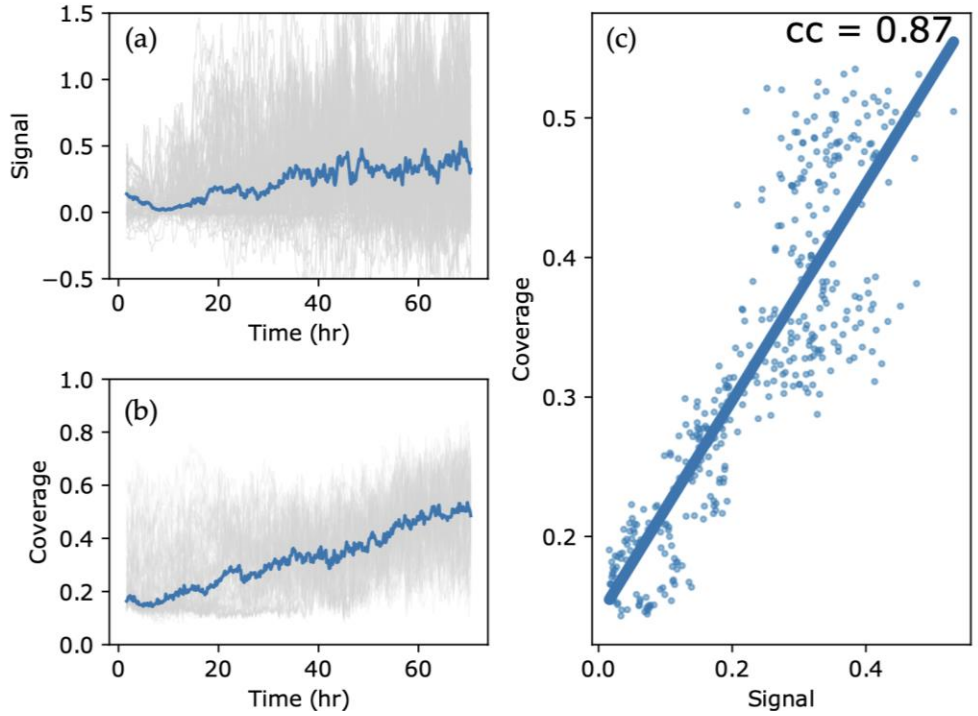
Solve $CELL_i(t)$ by Estimating $b(t)$ and c_i

- Cell contribution $CELL_i(t) = T_i(t) - b(t) - c_i$
- Two-step iterative solution
  - Fixed c_i, get $b(t)$: $b(t) = R_{80}(\{T_i(t) - c_i | i \in S\}$
  - Fixed $b(t)$, get c_i : One-sided weighted regression
    - $\begin{cases} w = 1, & T_i(t) - b(t) > 0 \\ w < 1, & T_i(t) - b(t) < 0 \end{cases}$



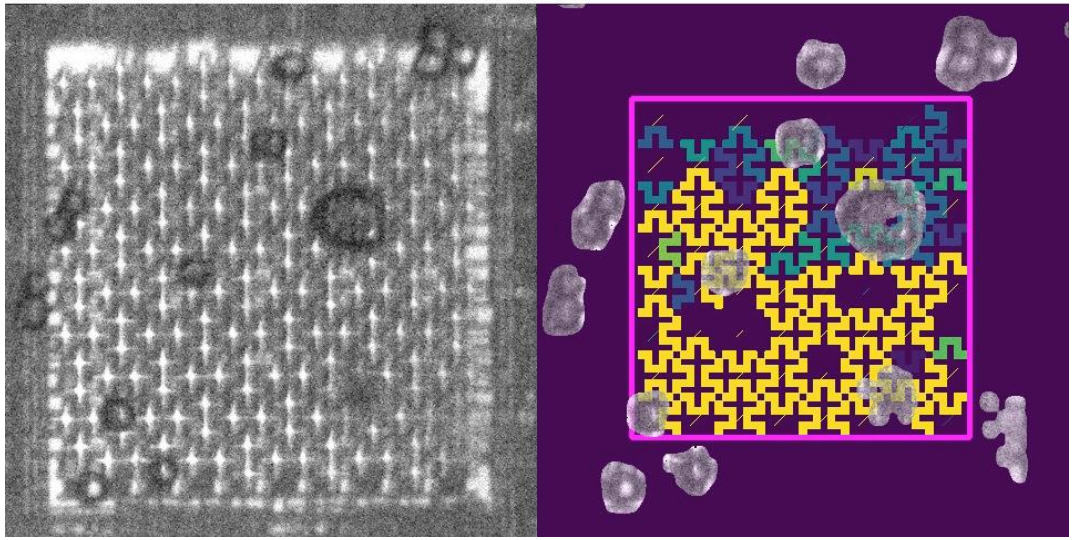
Population-based Evaluation

- Compare two series
  - Post-processed capacitance signal
  - Visual coverage estimated
    - Gray for each edge
    - Blue for spatial average



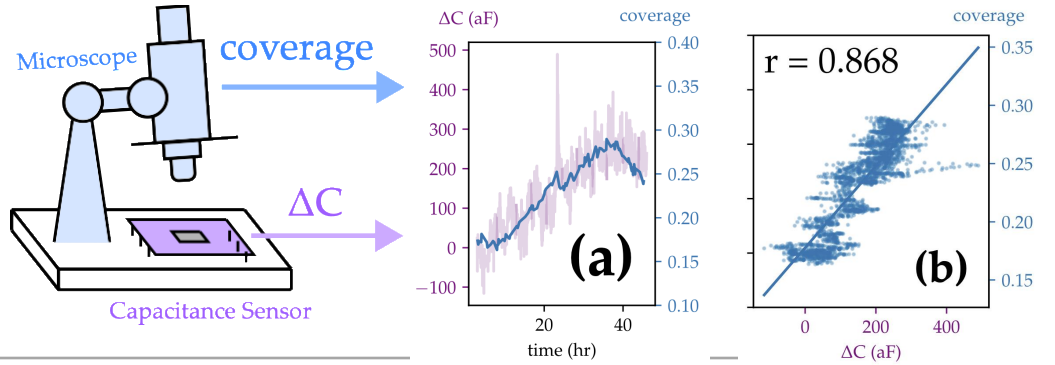
Visualization in a Video Format

- Two modalities overlayed in one frame
  - Left: Cell images
  - Right
    - Electrode-free images
    - Sensor signal visualization

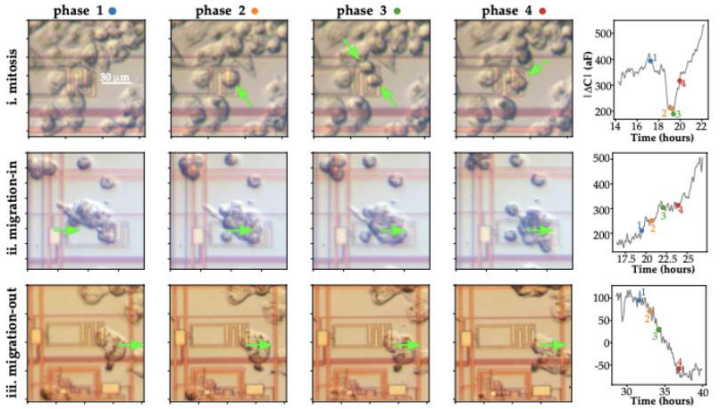


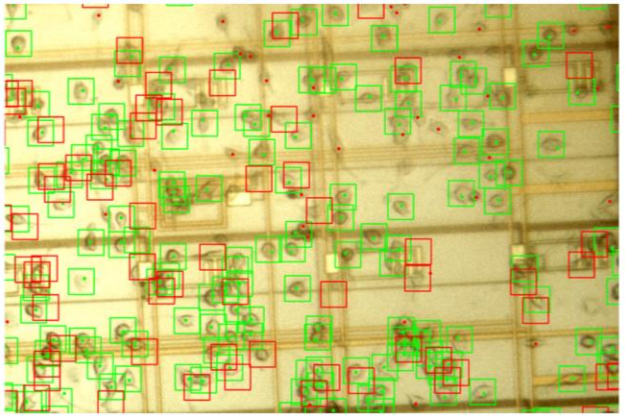
Video Archive
- https://drive.google.com/file/d/1N-E0Kpszv4prEKcCfEna41Fa5kSY39Ho/view?usp=sharing
- https://drive.google.com/file/d/11JE15zj_K8kuy5_PqyG1q58pO8t-tRVR/view?usp=sharing
- https://drive.google.com/file/d/1ycU4uadT6xkOrfuYGSrifCKmTjP0j-Rr/view?usp=sharing

Conclusions (1/2)

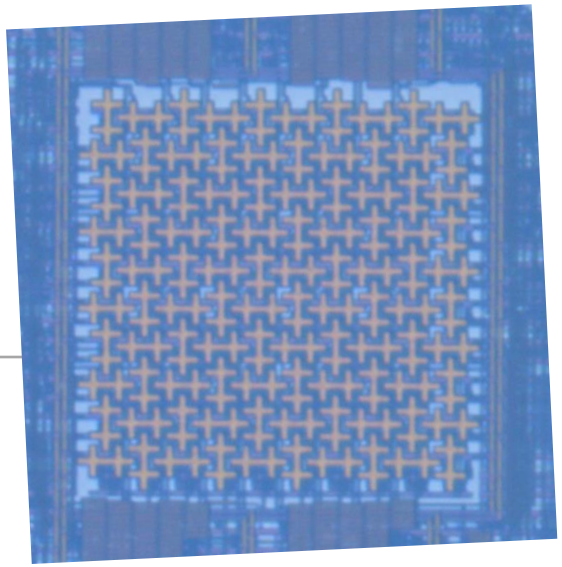


- Sensor signal processing for single-cell event
  - Event classification from existing C2F sensor
- Visual signal processing algorithm
  - Ground-truth for capacitance sensor
  - Match sensor signal with previous sensor
  - Provide an insight for biological researcher

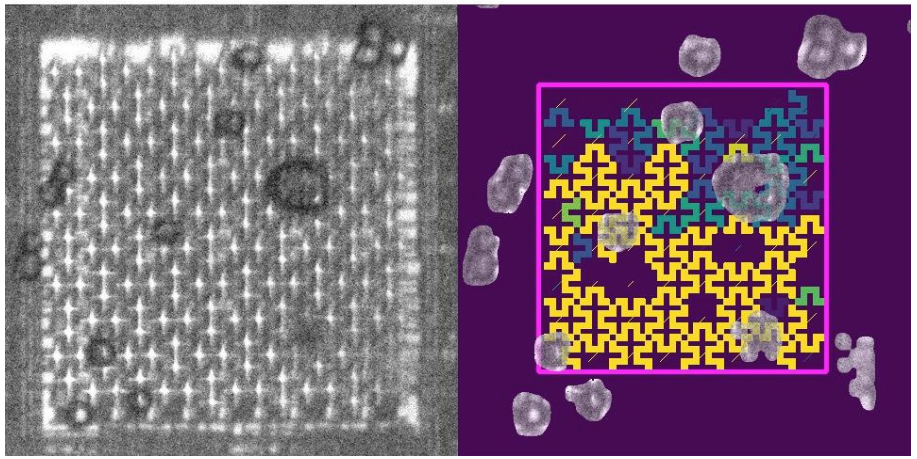




Conclusions (2/2)



- Sensor signal processing for single-cell event
- Visual processing considering focus degradation
- Enhanced sensing platform
  - Hardware with higher spatial resolution
  - Signal processing for sensor mismatch
  - Biological validation in a video format



Video Archive
- https://drive.google.com/file/d/1N-E0Kpszv4prEKcCfEna41Fa5kSY39Ho/view?usp=sharing
- https://drive.google.com/file/d/11JE15zj_K8kuy5_PqyG1q58pO8t-tRVR/view?usp=sharing
- https://drive.google.com/file/d/1ycU4uadT6xkOrfuYGSrifCKmTjP0j-Rr/view?usp=sharing

Publication Record

- **Ching-Yi Lin** and Marc Dandin. ”Machine Learning Identification and Classification of Cancer Cell Behaviors in a Lab-on-CMOS Capacitance Sensing Platform.” IEEE Journal of Biomedical and Health Informatics, doi: 10.1109/JBHI.2024.3486251
- **Ching-Yi Lin**, Yann Gilpin, Zixin Chen, Elizabeth Wayne and Marc Dandin, ”Towards a Lab-on-a- Chip as a Service (LoCaaS) Framework for in Vitro Cancer Cell Assays,” 2024 22nd IEEE Interregional NEWCAS Conference (NEWCAS), Sherbrooke, QC, Canada, 2024, pp. 268-272, doi: 10.1109/NewCAS58973.2024.10666355.
- Kyle Smith, **Ching-Yi Lin**, Yann Gilpin, Elizabeth Wayne, and Marc Dandin. ”Measuring and modeling macrophage proliferation in a lab-on-CMOS capacitance sensing microsystem.” Frontiers in Bioengineering and Biotechnology 11 (2023): 1159004.
- Yann Gilpin, **Ching-Yi Lin**, Mats Forssell, Siyang Zheng, Pulkit Grover, and Marc Dandin. ”Tracking the effects of tumor treating fields on human breast cancer cells in vitro using a capacitance sensing lab-on-CMOS microsystem.” In 2022 29th IEEE International Conference on Electronics, Circuits and Systems (ICECS), pp. 1-4. IEEE, 2022.
- **Ching-Yi Lin**, Md Sakibur Sajal, Yann Gilpin, Fahimeh Dehghandehnavi, Anna Batueva, Kai-Chun Lin, Nicole McFarlane, and Marc Dandin. ”CMOS bioelectronics: Current and future trends.” Bioelectronics (2022): 93-107.

Anticipated Publications

- Dissertation Chapter 4
  - Ching-Yi Lin and Marc Dandin, “Transductive Correlation-based Cell Detection with Small Data”, in BHI, 2025
- Dissertation Chapter 5
  - Ching-Yi Lin, Yann Gilpin, and Marc Dandin, “A High-spatial CMOS Biosensor for Cell Culture Assay”, in IEEE BioCAS, 2025
- Dissertation Chapter 6
  - Ching-Yi Lin, Yann Gilpin, and Marc Dandin, “Capacitance Sensor Signal Modeling and Compensation”, in MDPI Sensors, 2025

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Dr. Kai-Chun Lin
Dr. Daniel Bankman
Dr. Radu Marculescu

Thank You!

Any Questions?