

# Experimental Design Template

Date:

Experiment Title

**Using microfluidic dielectrophoresis to sense polarization differences between cell types**

**Brief Description**

Retinal stem cells are a rare cell type which exist in large populations of epithelial cells upon dissection from a primary cell sample from the eye. With limited information about surface-bound proteins which could be used as biomarkers to differentiate between retinal stem cells and other cell types, being able to detect differences in electrical properties of the cell surface using dielectrophoresis will provide a physical biomarker to use for cell sorting.

Hint:

Briefly explain what you hope to demonstrate with this experiment

**Rationale**

Retinal stem cells have been found to be quite small compared to the other cell types in primary cell samples. This suggests that a different dielectrophoretic signal could be measured for retinal stem cells compared to other cells, as dielectrophoretic force is a strong function of particle size.

Hint:

Findings from last experiment? What is the starting point?

**Background/ Helpful info**

It is important to consider that the dielectrophoretic signal is not only a function of size, but solution and cell permittivity, as well as applied electric field strength. With this, it is hard to decouple observed effects from just the contribution of size, so further extrapolation may be necessary.

Hint:

Are there additional things that will be helpful to remember when returning to this data?

**Hypothesis**

**Retinal stem cells can be uniquely identified in a primary cell sample from the ciliary epithelium by observing the dielectrophoretic force generated under an applied electric field in a cell sorting device.**

Hint:

If you have one, put it here... (If, then statement?) (Is the hypothesis falsifiable?)

**Predictions**

1)

Dielectrophoretic force will differ among cell types

2)

Heterogeneity within a single cell type will produce a range of dielectrophoretic force

3)

Cell clusters will interfere with results

4)

Sorting will be affected by inlet flow rate in the microfluidic device

5)

Hint:

This will help you decide what controls are necessary

**Methods**

Cell sample is produced by dissecting the ciliary epithelium of the mouse eye, digesting the tissue to produce a sample in serum which is in single-cell suspension. The cell sample is then put through a dielectrophoretic microfluidic cell sorting device with three sample outlets. The device will be driven using a flow-rate driven syringe pump. An inhomogeneous electric field is applied by applying an AC potential to interdigitated electrodes patterned on the device. The device will be placed under a bright field microscope to monitor the cell sorting, and outlet samples will be collected to perform a sphere-forming cell culture to determine how many retinal stem cells exist in each outlet.

Hint:

If you wish to include experimental details, include them here (instruments, methods of data collection)

<b>Metrics</b>	Spheres will be counted to determine which outlets contain retinal stem cells -- the larger the skew to one outlet populations, the better.
<b>Hint:</b>	What is the actual experimental readout? Flourescence intensity? Cell count? Colonies?

<b>Data Normalization</b>	The number of spheres (where each sphere is considered the product of one stem cell) will be divided by the total number of cells in the outlet population.
<b>Hint:</b>	Is the data manipulated post measurement? Normalized to what?

<b>Replicates and Stats</b>	<b>Biological n</b>	10	10 different mouse samples
	<b>Technical n</b>	30	3 devices per mouse sample
	<b>Error bar n if SEM</b>		
	<b>Error bars</b>		
	<b>Stats comparison?</b>		
<b>Hint:</b>	Enter and describe the experimental and technical replicates for the experiment		

DRAWING Space/ Extra Stats Description Space

<b>Controls</b>	<b>Positive</b>		
	<b>Negative</b>	No applied potential	To produce baseline for which outlet cells flow into
	<b>Other 1</b>	Known, homogeneous, cell line	To determine the efficiency of the device to sort homogeneous cells into a single outlet
	<b>Other 2</b>		
	<b>Other 3</b>		
<b>Additional Info/ Rationale for Controls</b>			

<b>Conclusions</b>	If dielectrophoretic signal differences can help differentiate between retinal stem cells and other ciliary epithelial cells, this cell sorting method can be used to isolate pure populations of retinal
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	stem cells to be used in differentiation experiments
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<b>Notes/ Reminders</b>	
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<b>File/ Data Location</b>	
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<b>Contact people</b>	
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## Experimental Design Template

Date:

**Experiment Title**

**Optimized versus existing automated external defibrillator locations**

**Brief Description**

Automated external defibrillators (AEDs) are commonly placed in areas of low out-of-hospital cardiac arrest (OHCA) risk and are often inaccessible during OHCA events. Mathematical optimization has been shown to be a promising approach to determining AED locations that improve accessibility but has yet to be compared to current placement strategies. If superior, optimization models should be integrated into current practice to improve outcomes while effectively using limited resources.

**Hint:** Briefly explain what you hope to demonstrate with this experiment

**Rationale**

**Optimization approaches has been shown to outperform random placements, population guided placement heuristics, and previous iterations of optimization models, but has never been benchmarked against current practice.**

**Hint:** Findings from last experiment? What is the starting point?

**Background/Helpful info**

The data used record bystander defibrillation rates as opposed to the commonly used bystander AED use rate. The two should be mistaken when interpreting results as defibrillation rates are expected to be lower than AED use alone.

**Hint:** Are there additional things that will be helpful to remember when returning to this data?

**Hypothesis**

**Optimized AED locations improve coverage of out-of-hospital cardiac arrests (OHCAs) compared to real AED locations**

**Hint:** If you have one, put it here... (If, then statement?) (Is the hypothesis falsifiable?)

**Predictions**

- |    |                                                                          |
|----|--------------------------------------------------------------------------|
| 1) | Optimization improves OHCA coverage compared to real placements          |
| 2) | OHCA coverage is directly related to clinical outcomes, such as survival |
| 3) | Optimization can improve OHCA outcomes to real placements                |
| 4) |                                                                          |
| 5) |                                                                          |

**Hint:** This will help you decide what controls are necessary

**Experimental Methods**

Retrospective comparison between two strategies. Develop 2 stage in-silico (simulated clinical trial) and predictive model framework to estimate impact of optimized AEDs placements. Specifically, retrospectively calculate OHCA coverage, then develop a predictive model to translate OHCA coverage to clinical outcomes. Compare these estimates to historical outcomes of the real AED placements. Differences calculated using pair-wise tests.

**Hint:** If you wish to include experimental details, include them here (instruments, methods of data collection)

- |    |                     |
|----|---------------------|
| 1) | Copenhagen EMS OHCA |
| 2) | Danish AED network  |

<b>Data Sources</b>	3)	Census Data
	4)	Building and land use information
	5)	City of Copenhagen GIS road network
<b>Hint:</b>	Identify organizations supplying data or open access databases	

<b>Study population and size</b>	<b>EMS treated, non-traumatic public OHCA; n=673</b>
<b>Hint:</b>	What population should be examined? Who will be impacted by proposed interventions?

<b>Performance Metrics/Study outcomes</b>	<b>OHCA coverage, 30-day survival, bystander defibrillation</b>
<b>Hint:</b>	What is the actual experimental readout? Fluorescence intensity? Cell count? Colonies?

<b>Feature and outcome engineering</b>	<b>Classification</b>	Yes	[Binary outcomes]
	<b>Regression</b>		
	<b>Supervised/Unsupervised</b>	Supervised	
	<b>Multicollinearity</b>	Possible	[Measure between covariates]
	<b>Normalization</b>	Test both	
	<b>Regularization</b>	No	
<b>Additional Notes (e.g. Is the data manipulated post measurement? Normalized to what?)</b>			
Metrics compared against historical outcomes. Report both absolute and relative gains.			

<b>Controls/Benchmarks</b>	1	Existing AED placements	Control/Current State
	2	Spatial-only optimization approaches	Existing Optimization Model
	3		
	4		
	5		
<b>Additional Info/ Rationale for Controls</b>			

<b>Conclusions</b>	Should optimization improve outcomes following OHCA, the results would support integrating optimization models into AED network design
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<b>Contact people</b>	
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## Model Design Template

Date:

**Model Title**

**Brief Description**

**Hint:** Briefly explain what you hope to demonstrate with this model

**Rationale**

**Hint:** Findings from last model? What is the starting point?

**Background/ Helpful info**

**Hint:** Are there additional things that will be helpful to remember when returning to this data?

**Hypothesis**

**There is an inverse relationship between home size and commuting distance**

**Hint:** If you have one, put it here... (If, then statement?) (Is the hypothesis falsifiable?)

**Predictions**

1)	estimated coefficient for the size of current home variable has a negative
2)	
3)	
4)	
5)	

**Hint:** This will help you decide what controls are necessary

**Methods**

Cobb-Douglas model of household commuting distance with 6 variables (Household income, Size of current home, Owns current home, Angle, Difference between longest and shortest commute distances (km), Distance between workplace1 and workplace2 (km)).  
Platform: "lm" package in the statistical software "R".

**Hint:** If you wish to include modelling details, include them here (mathematical framework, data analysis method, modelling platform)

**Metrics**

**Parameter estimate and t-stats**

**Hint:** What is the actual model readout? Name all model outputs (e.g., t-statistics value, parameter estimates)

<b>Data inputs and processing</b>	Data from a stated-adaptation survey entitled Car and Home Ownership decisions in the face of Increasing Commuting Expenses (CHOICE). Processing: Some respondents reported that their households had more than two workers. In these cases, the second worker in the respondent's household is defined as the individual who makes the highest income from the remaining workers (excluding the respondent).
<b>Hint:</b>	Where do the data come from? Is the data manipulated post modeling? Include all processing methods

<b>Modelling steps</b>	<p>1) Definition of the variables</p> <p>2) Cobb-Douglas log-linear model in which the natural logarithm of the dependent variable is expressed as a function of the natural logarithms of independent variables.</p> <p>3) Check that variables are statistically significant (Critical t-stats value of 1.64)</p>
<b>Hint:</b>	Enter and describe the modellin steps

<b>Model outputs</b>	1		Parameter estimate for "Size of current home": -0.227, t-stats = -2.379
	2		
	3		
	4		
	5		
<b>Additional Info/ Rationale for model outputs</b>			

<b>Conclusions</b>	<p>The estimated coefficient for this variable has a negative value, suggesting an inverse relationship between home size and commuting distance. A potential explanation for this finding is that households with larger homes dedicate a larger proportion of their household budget to home costs and therefore are sensitive to increases to commuting costs as presented in the survey.</p> <p>These household are likely to relocate in order to reduce their commuting distance and subsequently lowering household commuting costs.</p>
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