Non-invasive and high-throughput electrophysiological analysis of apical and basolateral membranes of RPE cells

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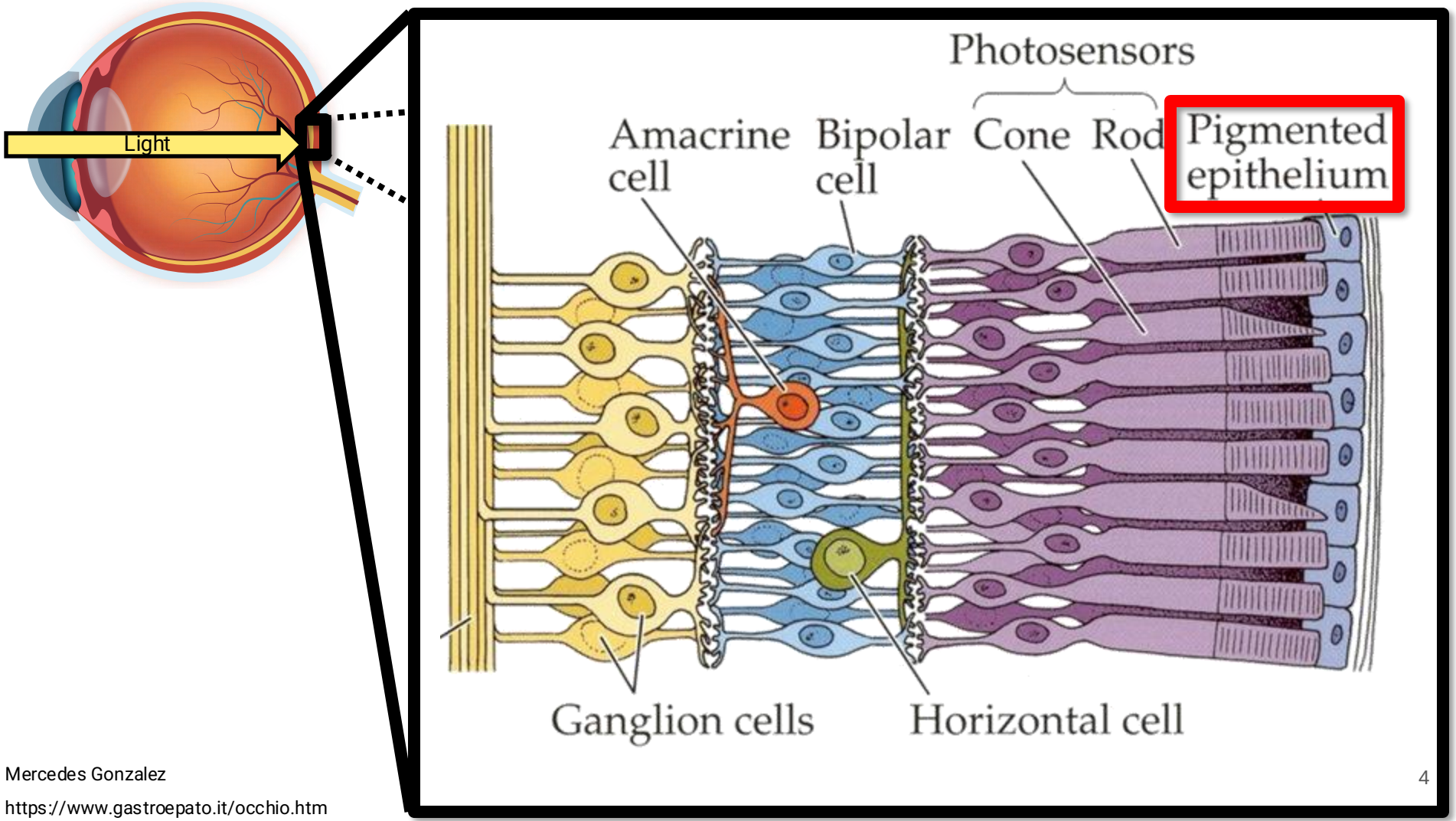
Outline

- Background of RPE electrophysiology
- Derivation of impedance model constraint
- Validation of impedance model constraint
- Testing using LCA16 iPSC-RPE line

Electrophysiology background

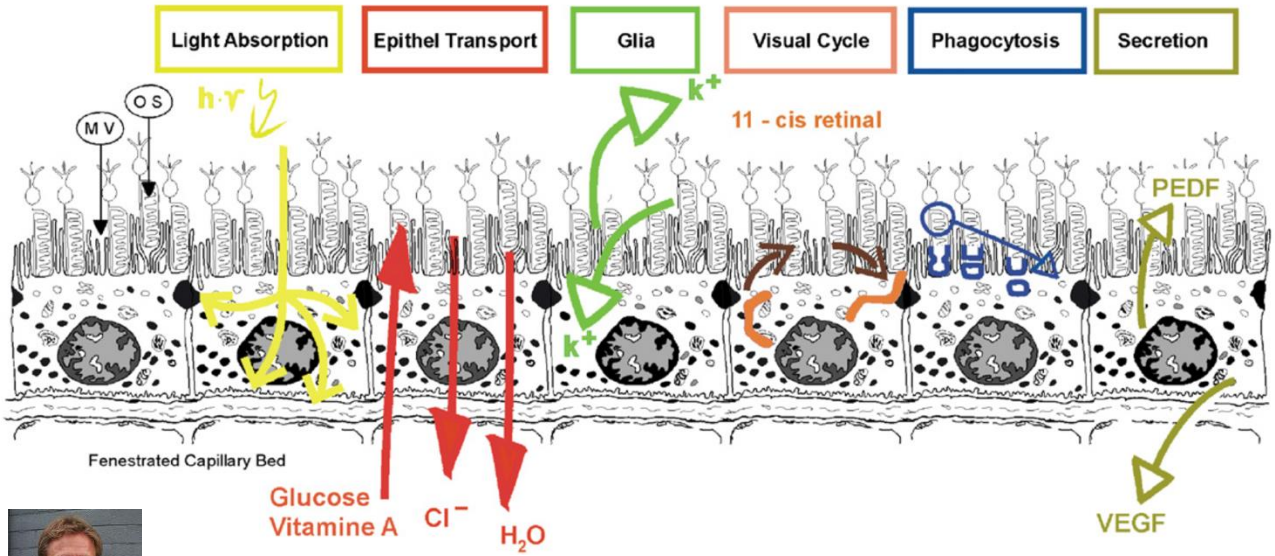


Mercedes Gonzalez

https://www.gastroepato.it/occhio.htm

Key Features of RPE

Functions

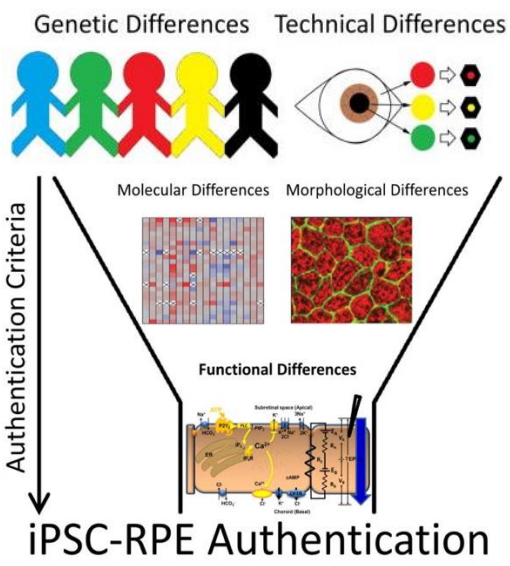


Characteristics

1. Polarized
2. Pigmented
3. Hexagonal shape
4. Tight junctions
5. Tightly adhered
6. Non-proliferative

Strauss (2005). *Physiological Reviews*

Validation of iPSC-Derived RPE



Sharma et al. (2019)
Science Translational Medicine

Characteristics Validation

- **Cell purity:** flow cytometry
- **Morphology:** quantitative shape metrics **Molecular markers:** RPE-specific gene expression
- **Membrane protein localization:** Polarized distribution of proteins (Na+/K+ ATPase, ezrin, ZO-1)

Functional Validation

- **Polarized secretion:** VEGF secretion assays
- **Phagocytosis & lysosomal function:** Uptake and degradation of photoreceptor outer segments
- **Barrier integrity:** Trans-epithelial resistance
- **Ion transport:** Calcium signaling, ion flux assays (e.g., patch-clamp)
- **Metabolism:** Mitochondrial activity profiling

Snapshot of cell quality and function

Validation of iPSC-Derived RPE

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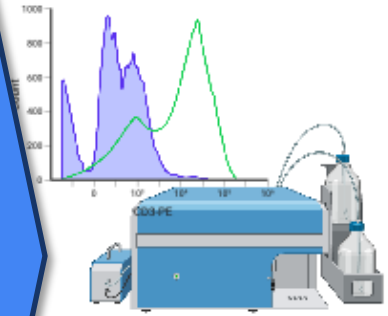
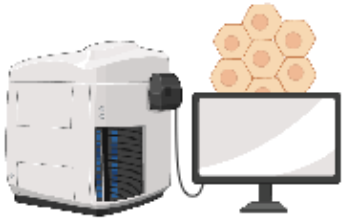
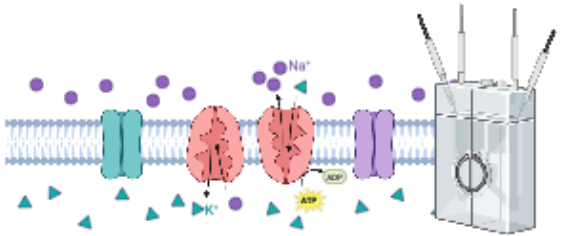




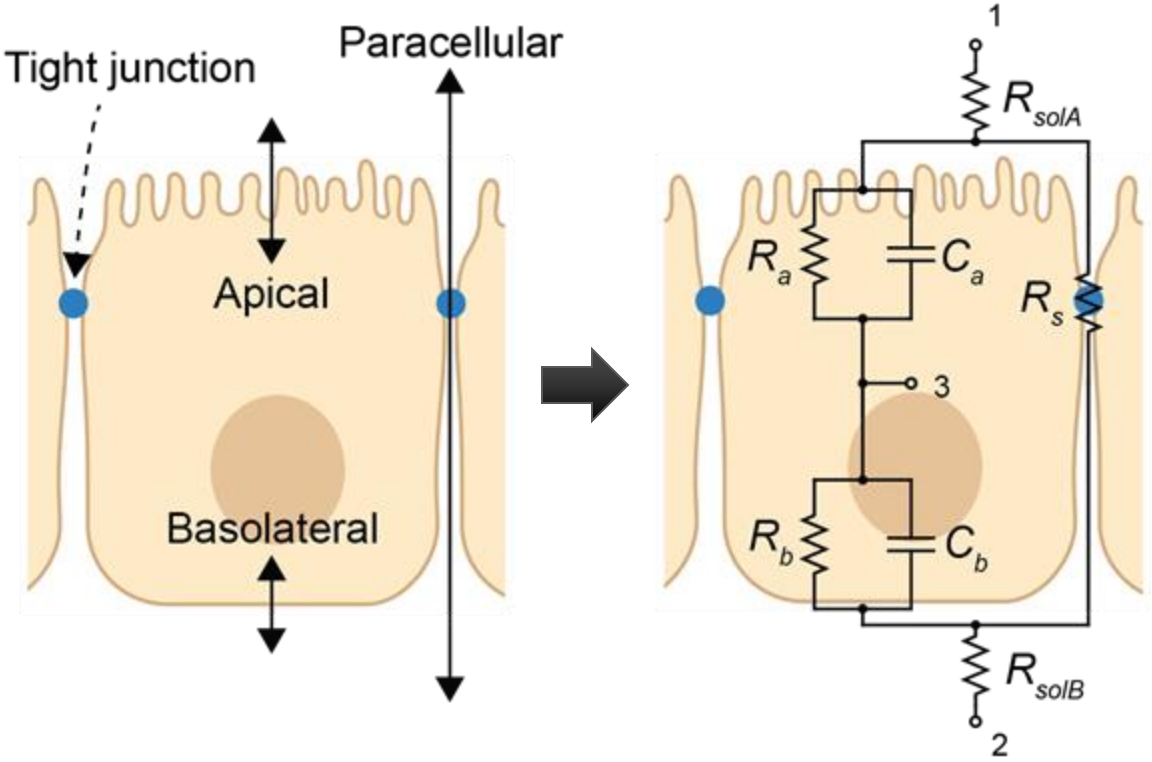
High throughput *assessment of these properties is* ***expensive*** *and* ***impractical*** *for most studies*



How can we **minimize time** and **maximize measured quality indicators** for high throughput analysis?

How can we increase the **sensitivity** of these measurements?

RPE polar properties represented by equivalent circuit



Example image of setup for measurements (historical)

$R_{a/b/s/sol}$ - apical/basal/shunt/solution **resistance**
$C_{a/b}$ - apical/ basal **capacitance**

ATP response of RPE

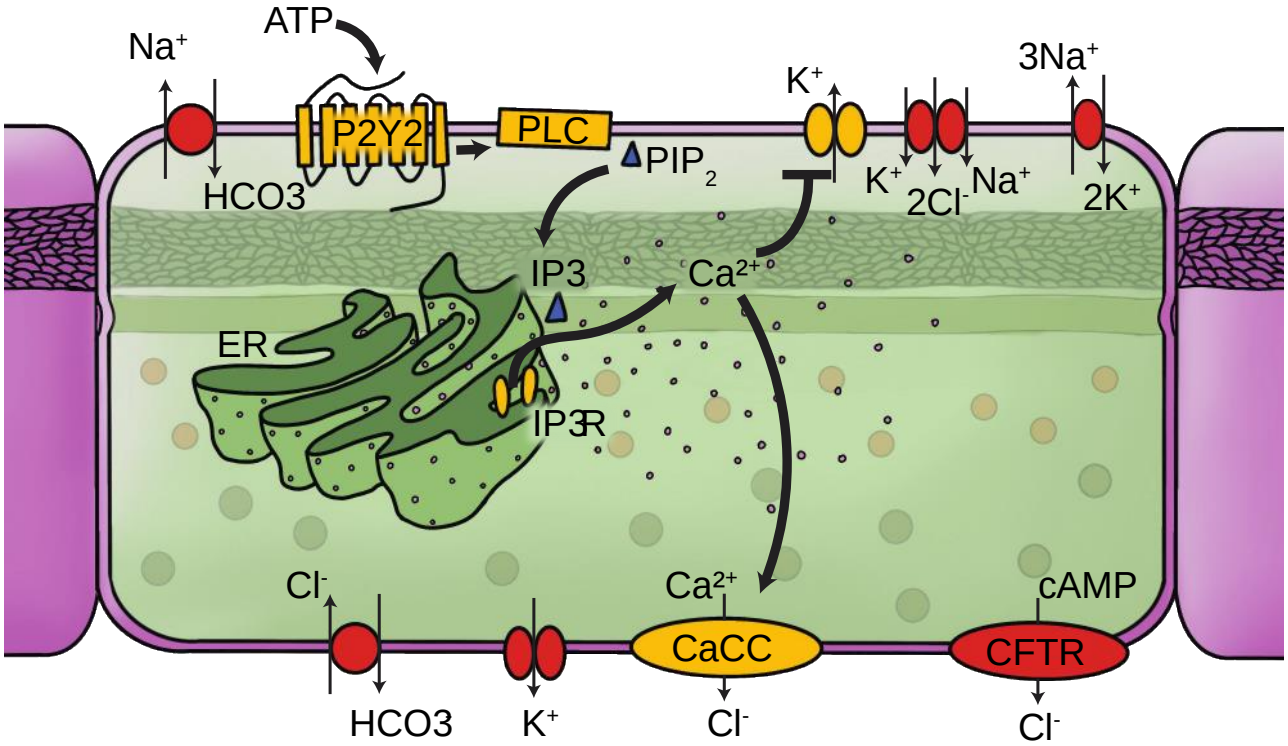
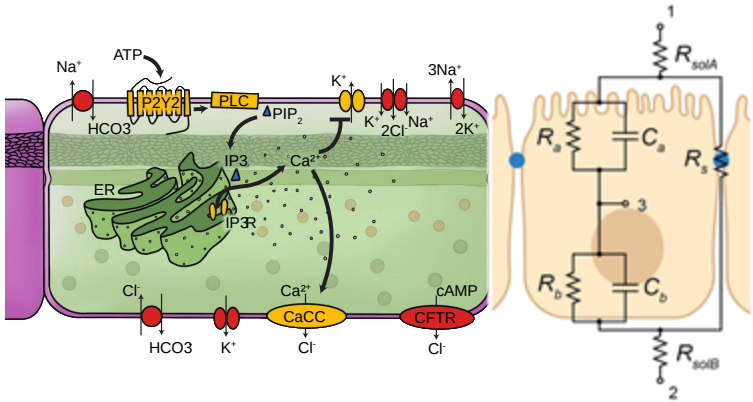
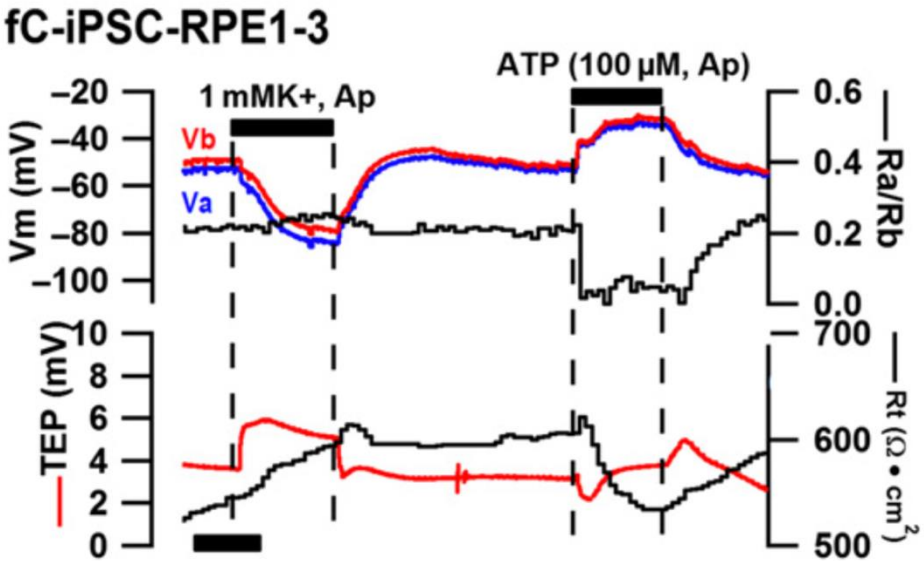


Image credit: Mercedes Gonzalez

ATP response of RPE



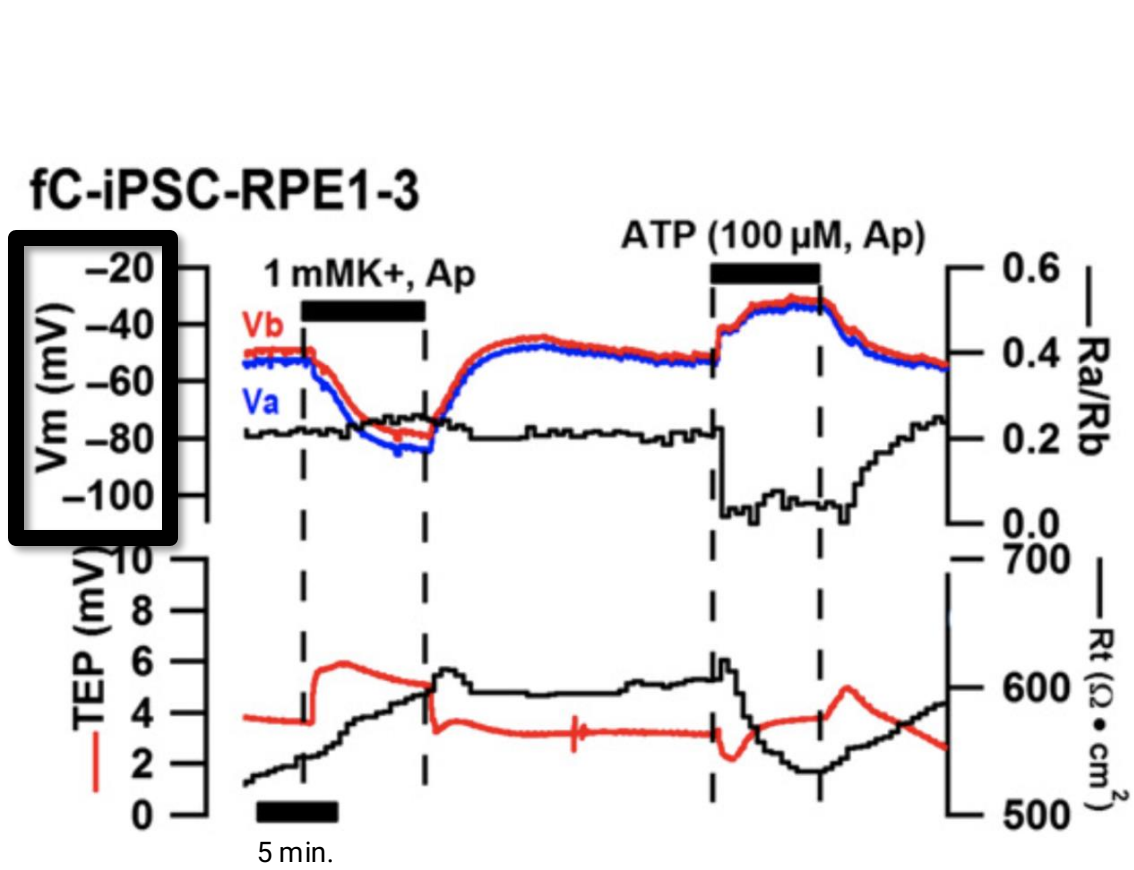


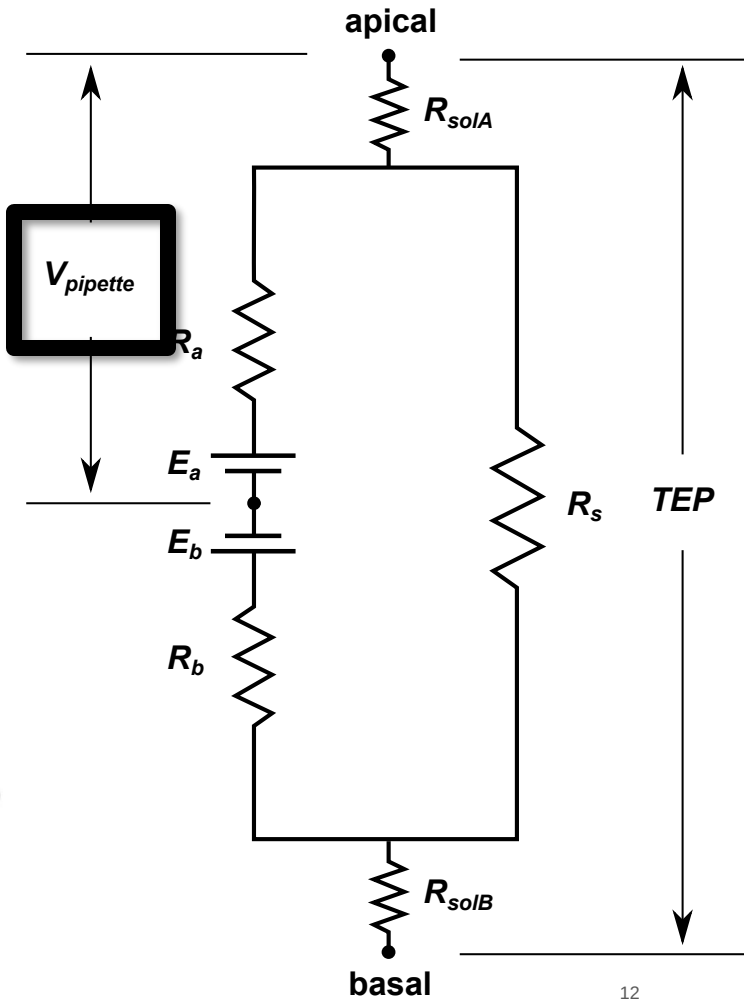
One assay reveals complex functional health of epithelia

- Epithelial polarization
- Tight junction formation

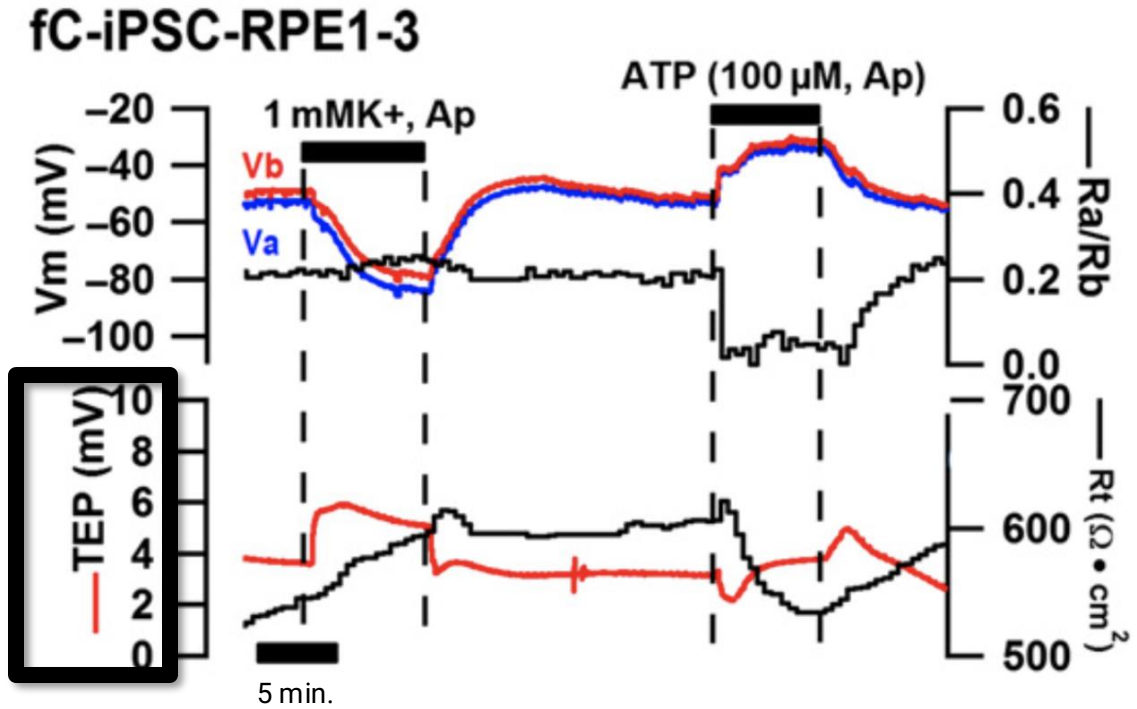
- Cell-to-cell homogeneity
- Intracellular ionic composition
- General molecular mechanisms

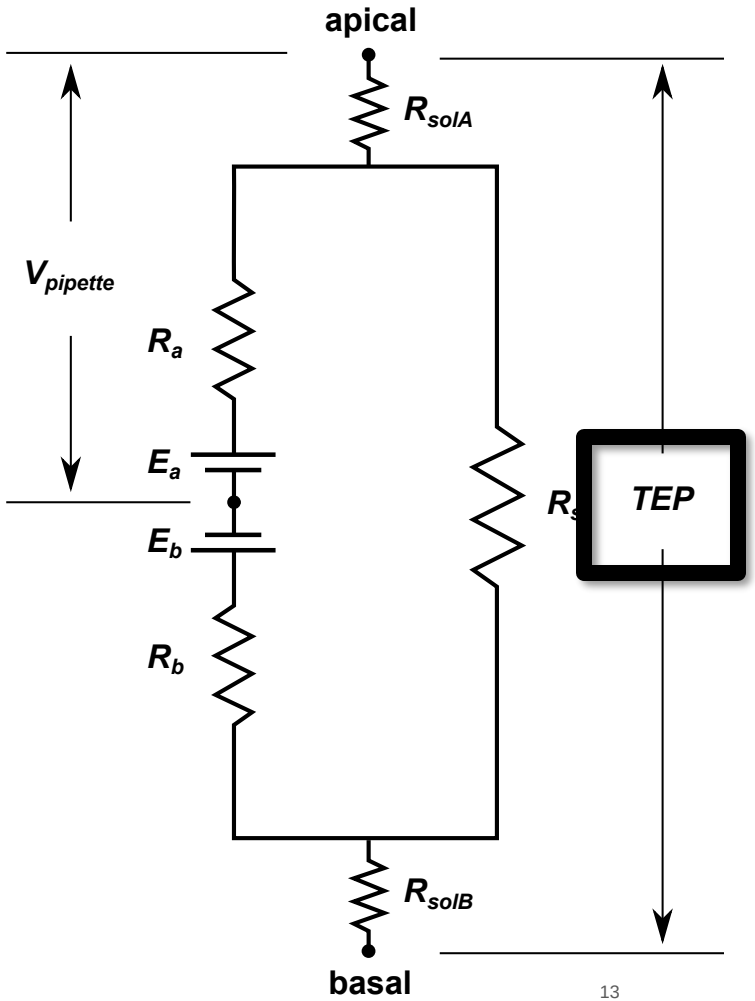
Miyagishima K. J.*, and Wan Q.*, et. al., 2016





Miyagishima K. J.*, and Wan Q.*, et. al., 2016





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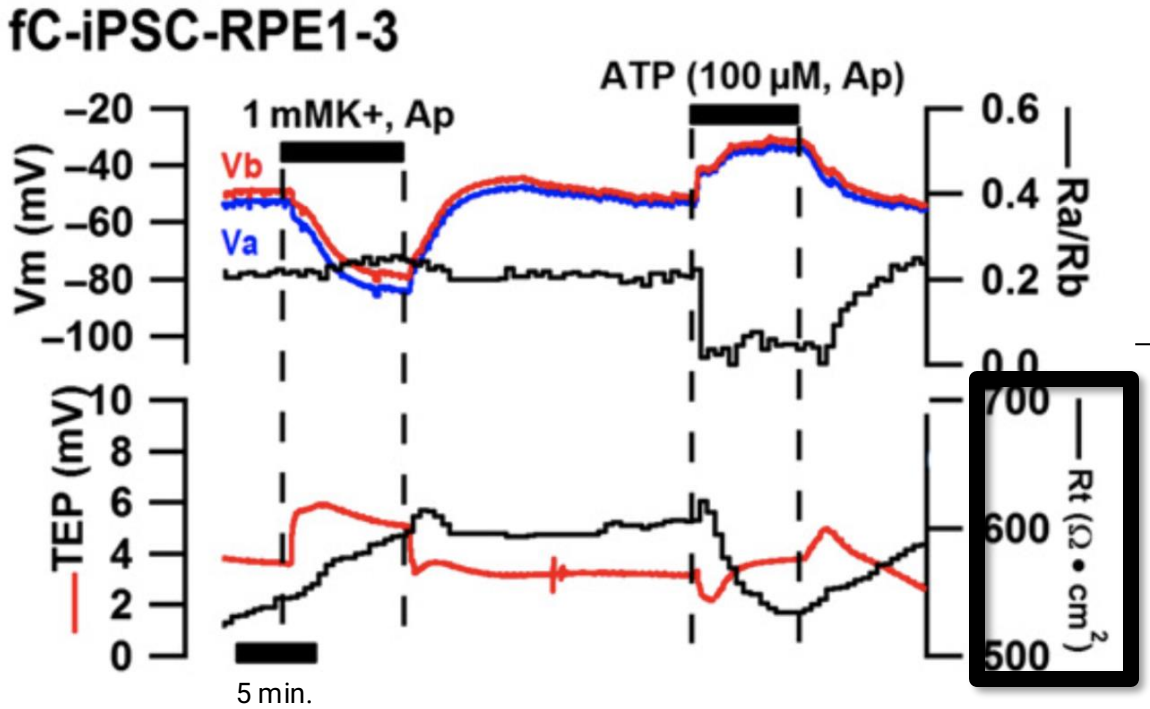
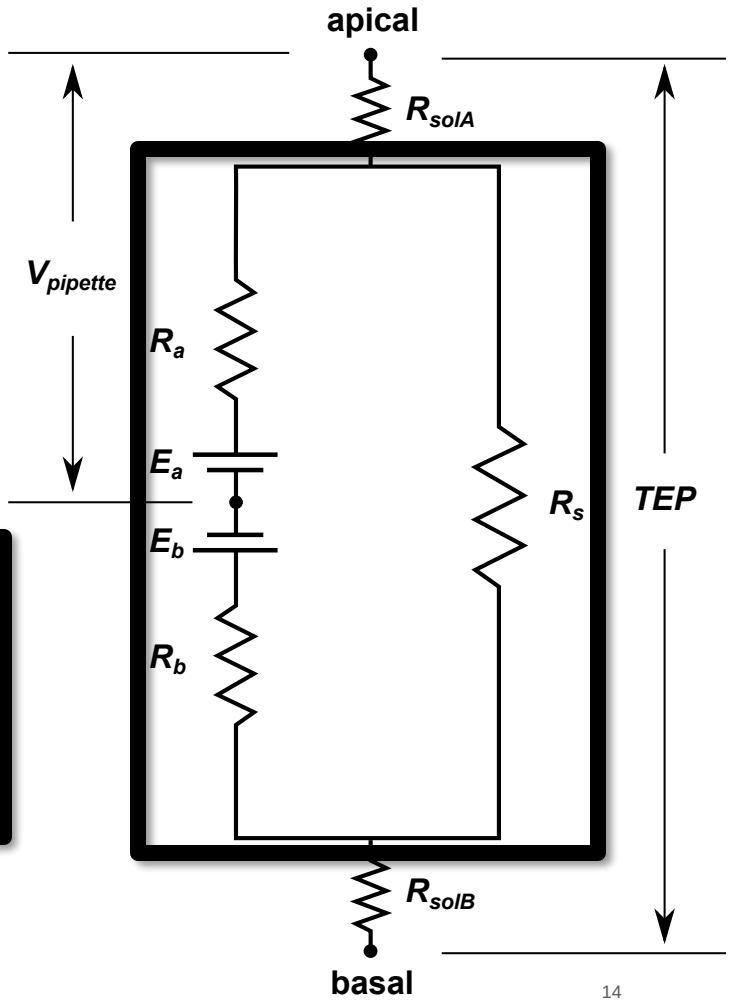
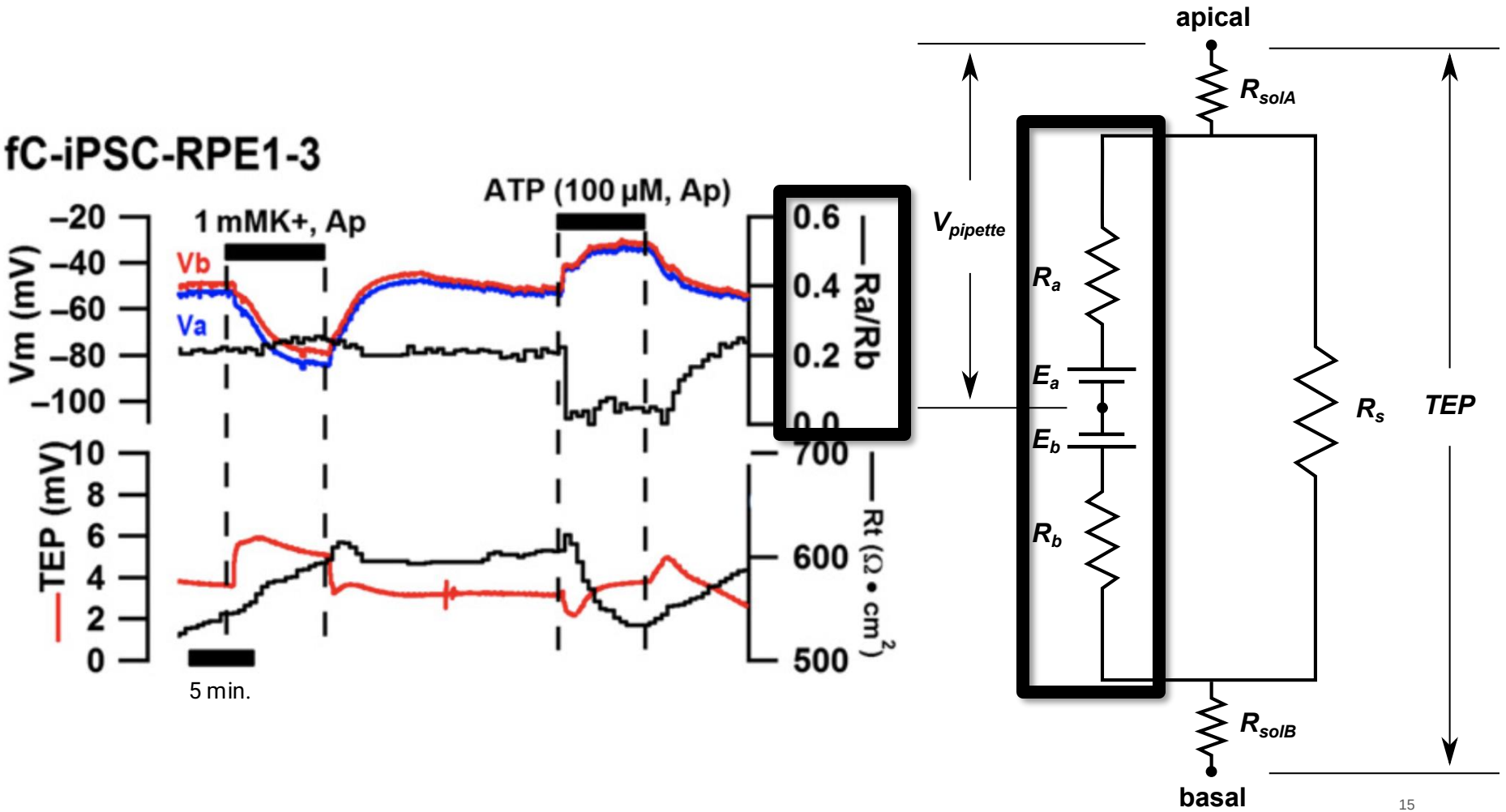




Miyagishima K. J.*, and Wan Q.*, et. al., 2016

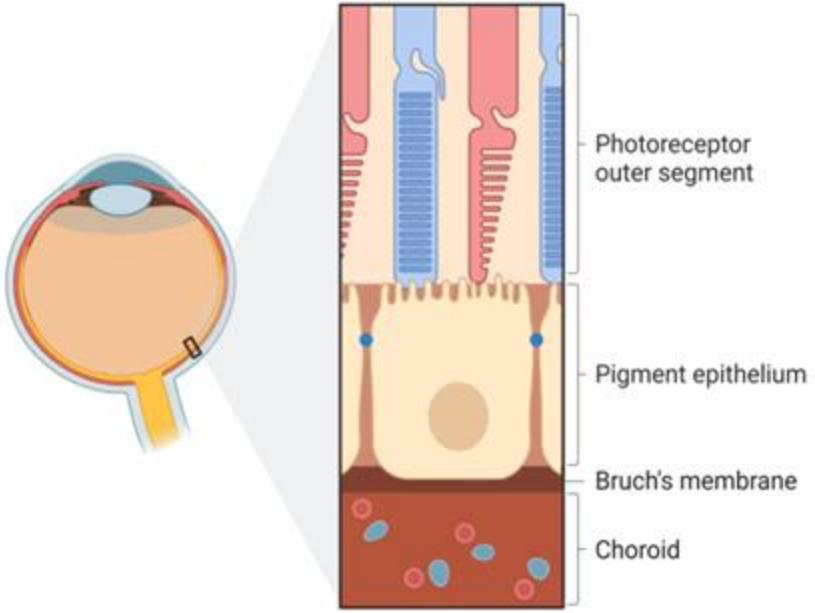


Miyagishima K. J.*, and Wan Q.*, et. al., 2016

Limitations

1. Difficult (requires training and precision)
2. Low throughput (1-3 measurements/day)
3. Expensive (>$100k)
4. Unreliable (can lose cells, hard to make pipettes, etc.)
5. Invasive (requires puncturing/touching the cells)

Epithelial polarity facilitates essential retinal function



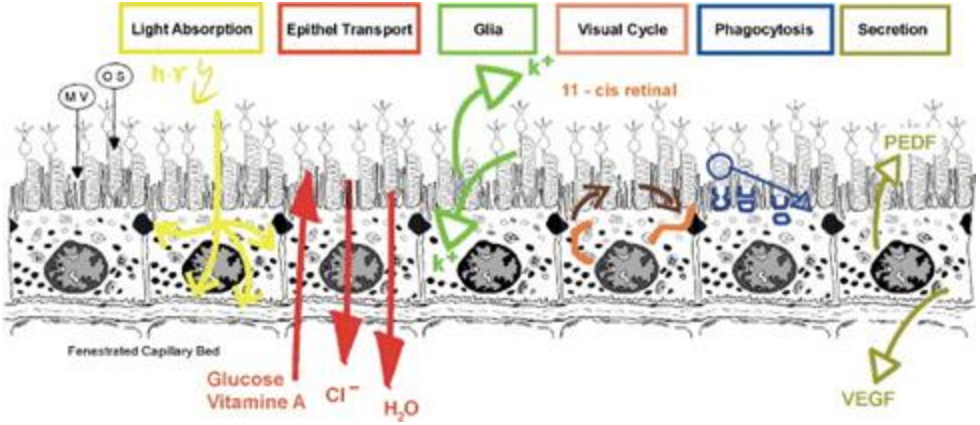
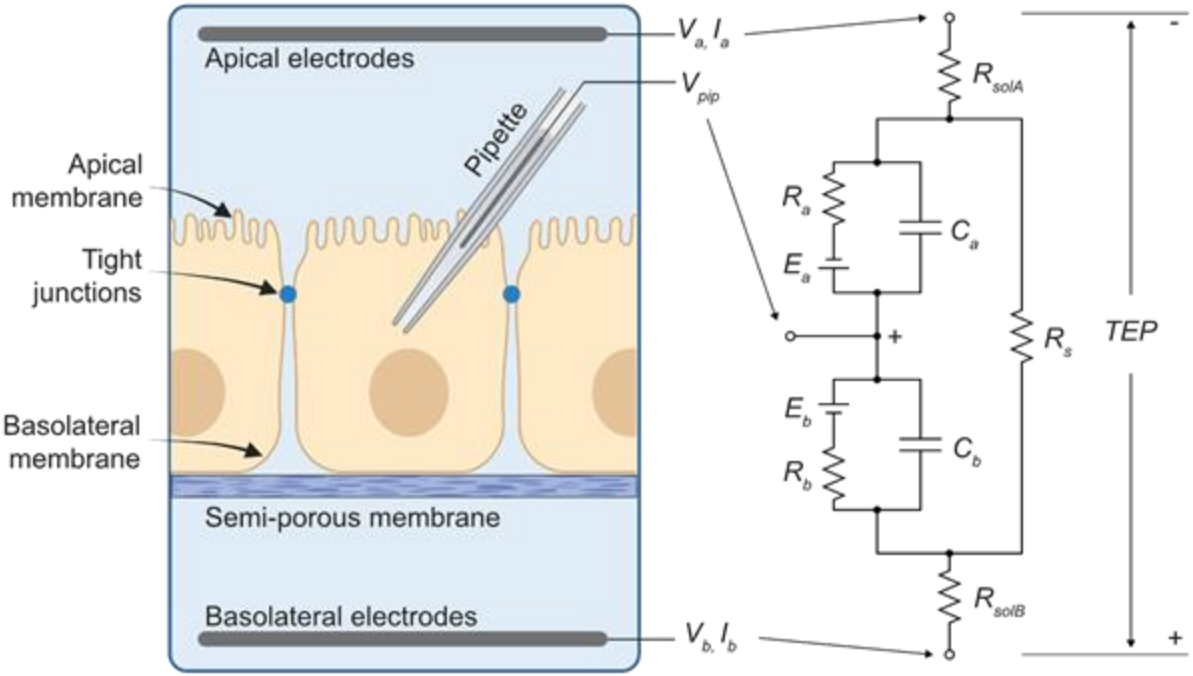


Image from biorender.com and Olaf Strauss

Current method to measure parameters of epithelial polarity



INVASIVE and **LOW-THROUGHPUT**

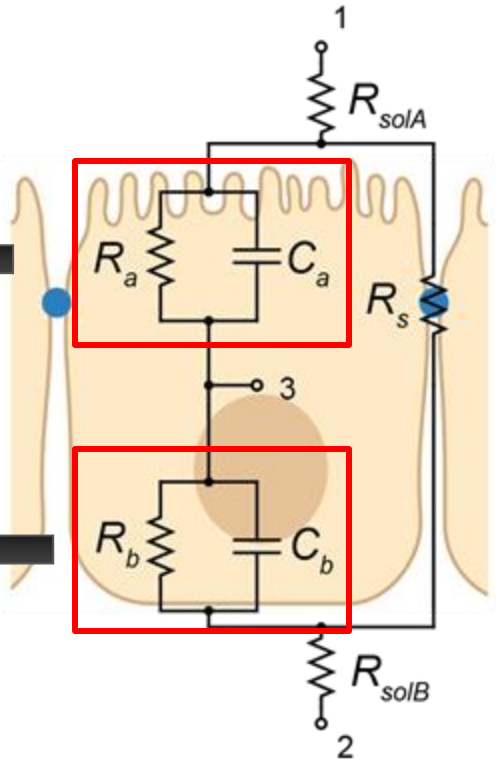
Goal: Develop a **non-invasive** and **high-throughput** method for the electrophysiological analysis of polarized epithelial cells using extracellular electrochemical impedance spectroscopy (EIS)

Derivation of impedance model

Derivation of equivalent impedance $Z_{eq}(w)$

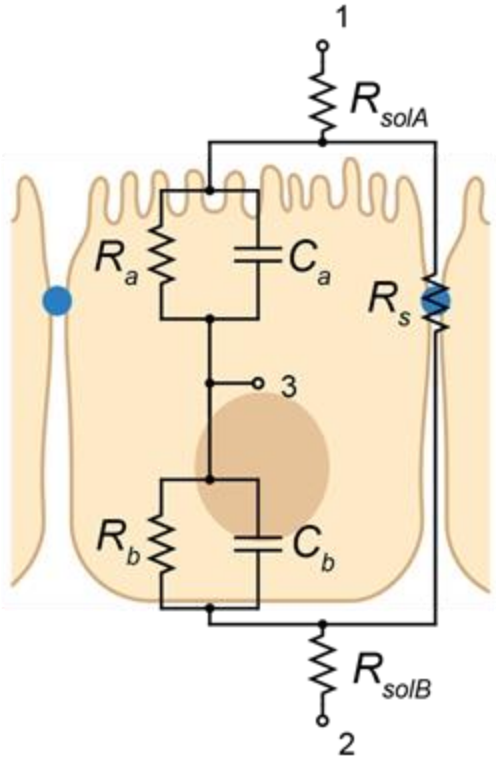
$$Z_a(\omega) = \frac{R_a}{1 + i\omega R_a C_a}$$

$$Z_b(\omega) = \frac{R_b}{1 + i\omega R_b C_b}$$



Imaginary number: $i = \sqrt{-1}$

Derivation of equivalent impedance model: $Z_{eq}(w)$



Combining the circuit elements

$$Z_{eq}(\omega) = R_{blank} + \frac{R_s\left(Z_a(\omega) + Z_b(\omega)\right)}{R_s + Z_a(\omega) + Z_b(\omega)}$$

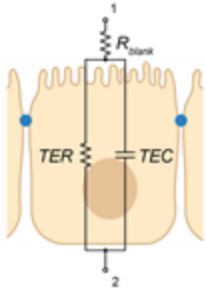
Epithelial resistance and capacity parameters can be extrapolated from measured impedance

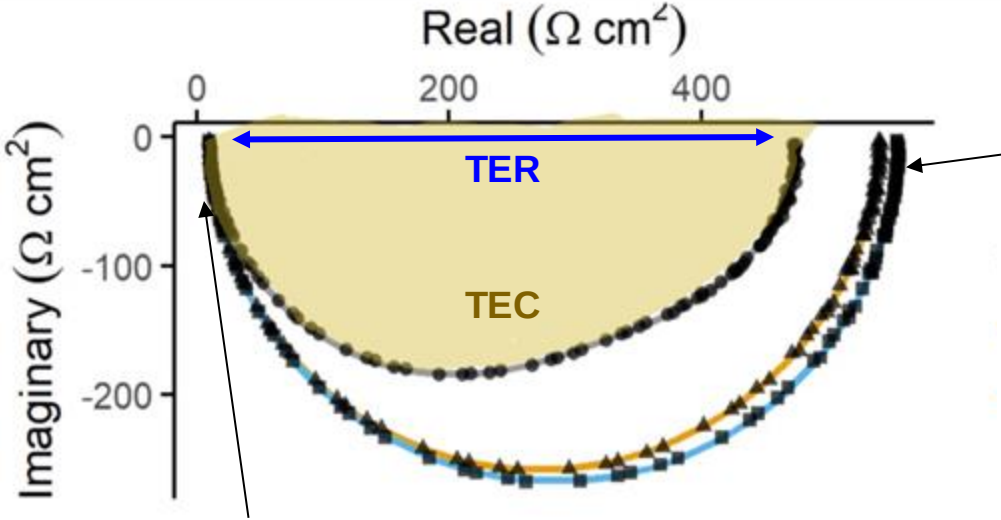
$$Z_{eq}(\omega) = R_{blank} + \frac{R_s\left(Z_a(\omega) + Z_b(\omega)\right)}{R_s + Z_a(\omega) + Z_b(\omega)}$$

$$R_{blank} = Z_{eq}(\infty)$$

$$TER = Z_{eq}(0) = R_{blank} + \frac{R_s(R_a + R_b)}{R_s + R_a + R_b}$$

$$\frac{1}{TEC} = \frac{2}{\pi}\int_0^{\infty} \Re\left(Z_{eq}(\omega) - R_{blank}\right) d\omega$$





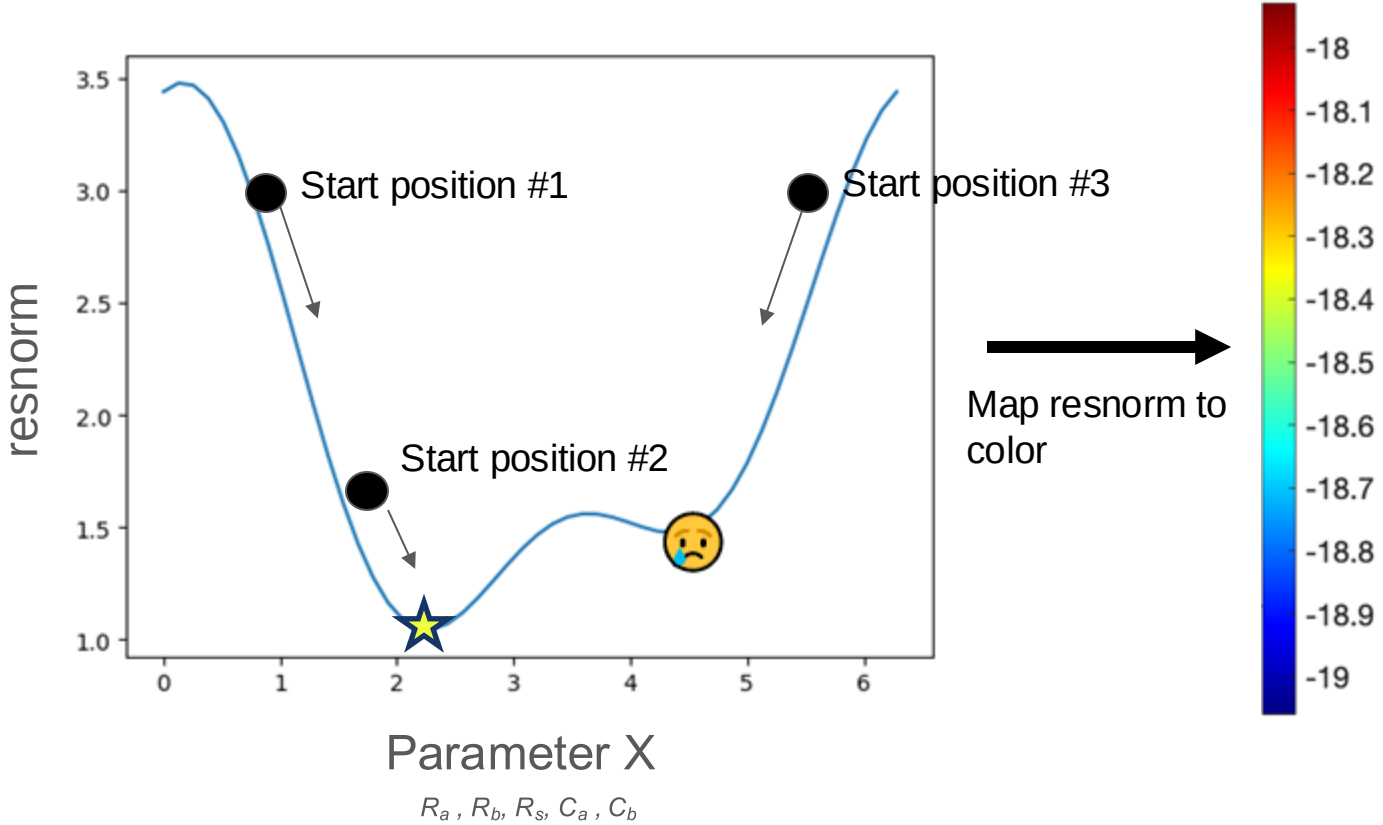
Low frequency

High frequency

NOT membrane specific

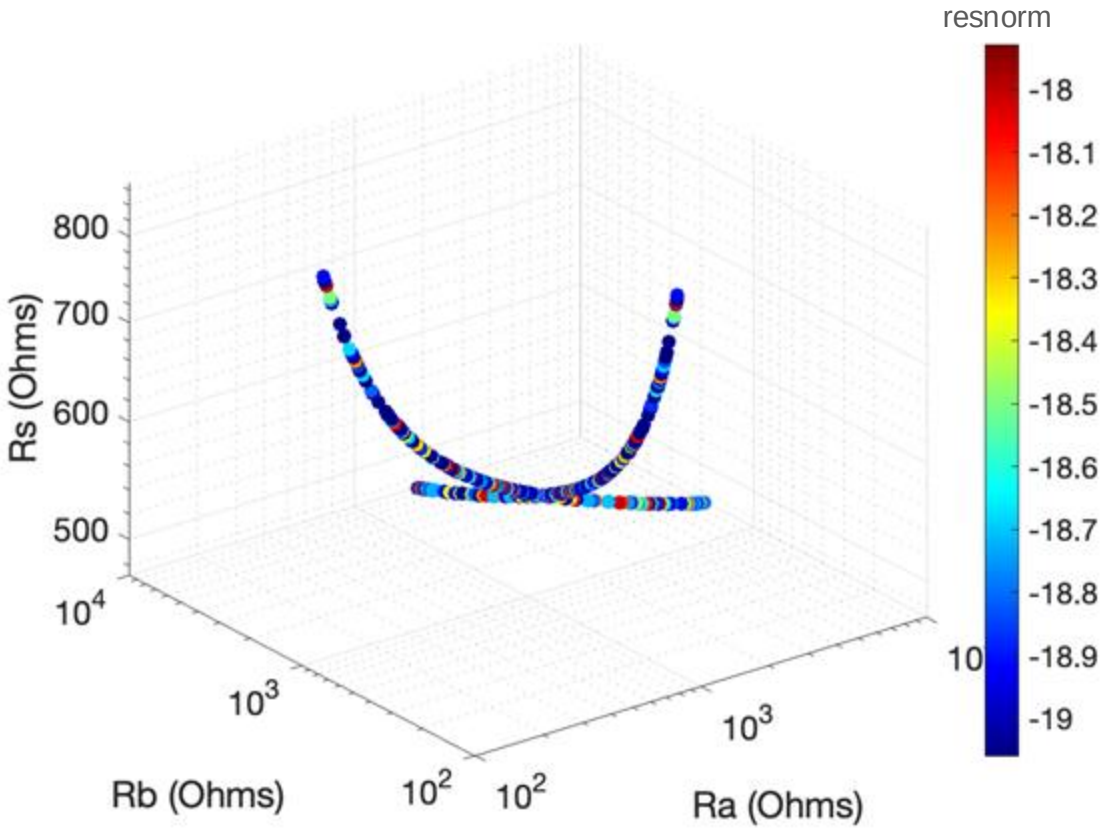
Resolving underdetermined fit

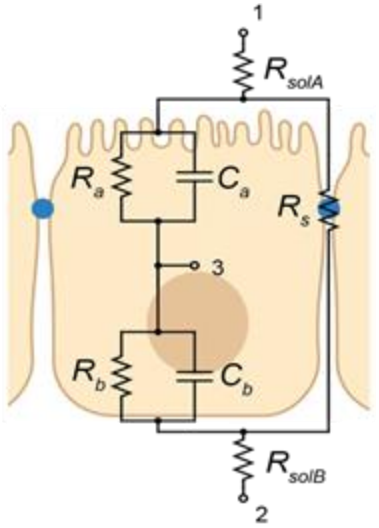
Principle of fitting



Location of best fit

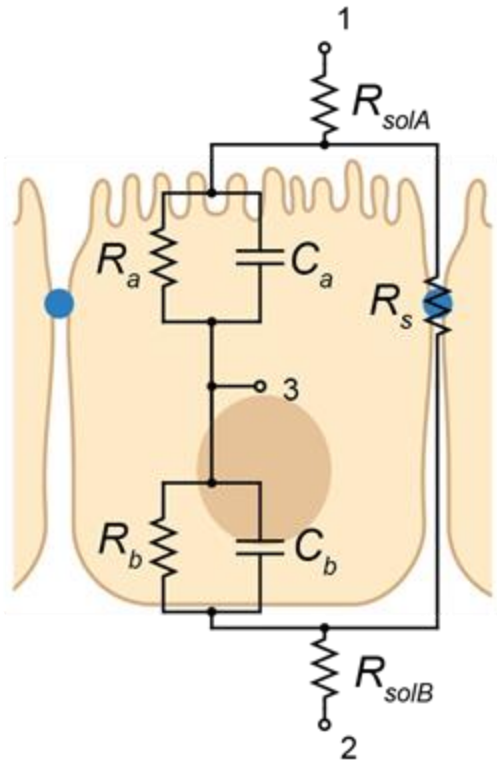
No clear minimum when fitting Zeq(w)





Set target Cb during fitting to get distinct solution

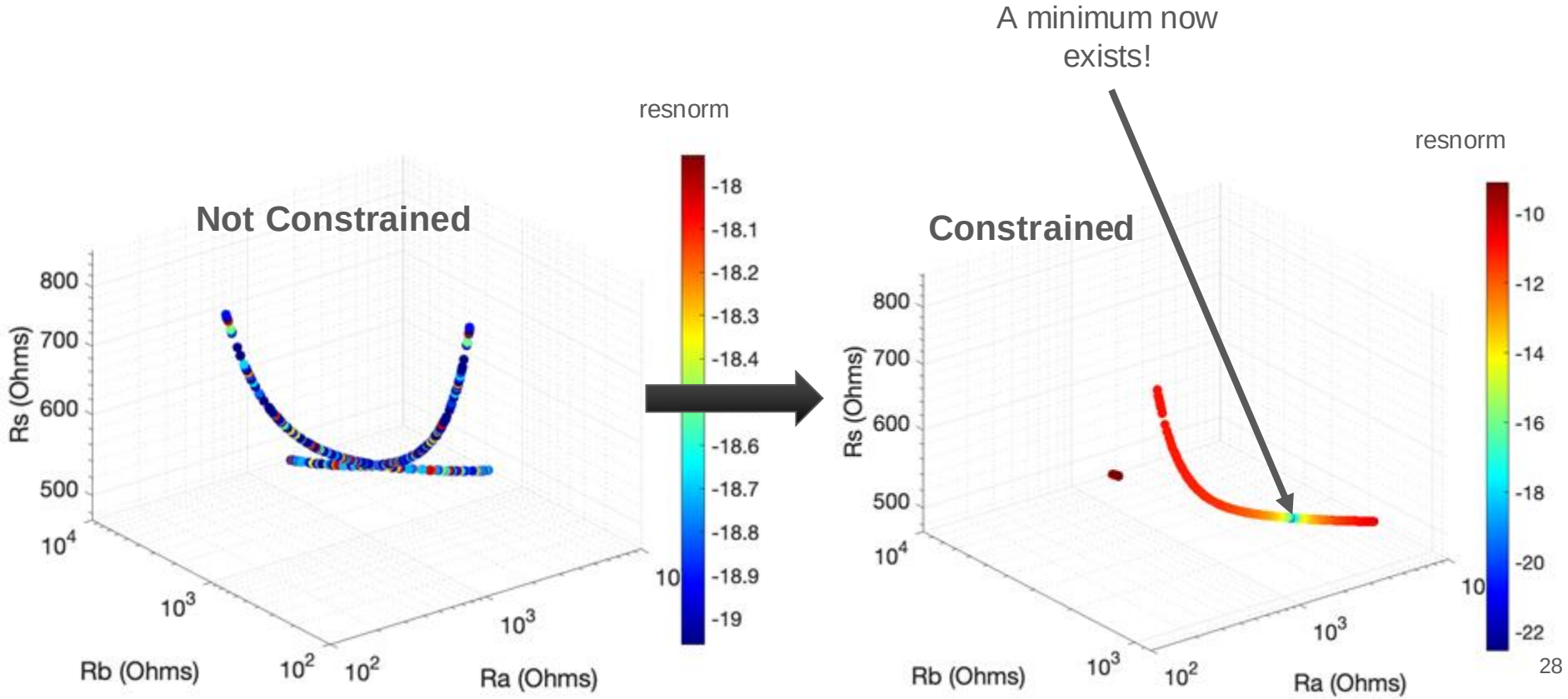
- Cb should not change much between cell lines.
- Primarily growth substrate dependent.



Arvydas.png

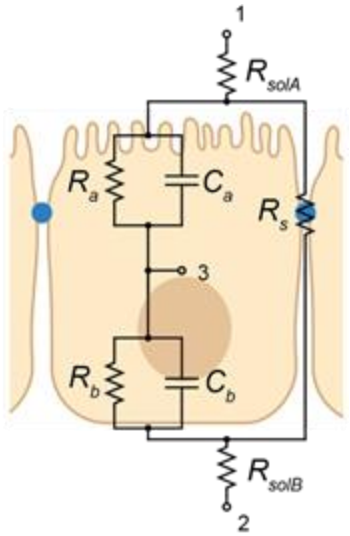
Arvydas Maminishkis,
MD, PhD

Simple constraint converges to distinct solution

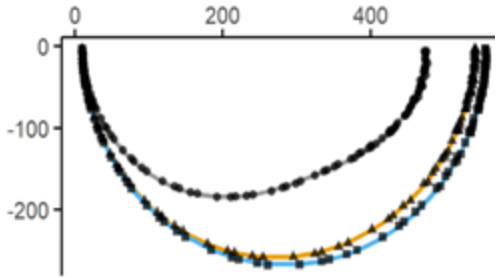


Validation

Validate constraint accuracy with **SIMULATED** data



simulate



fit

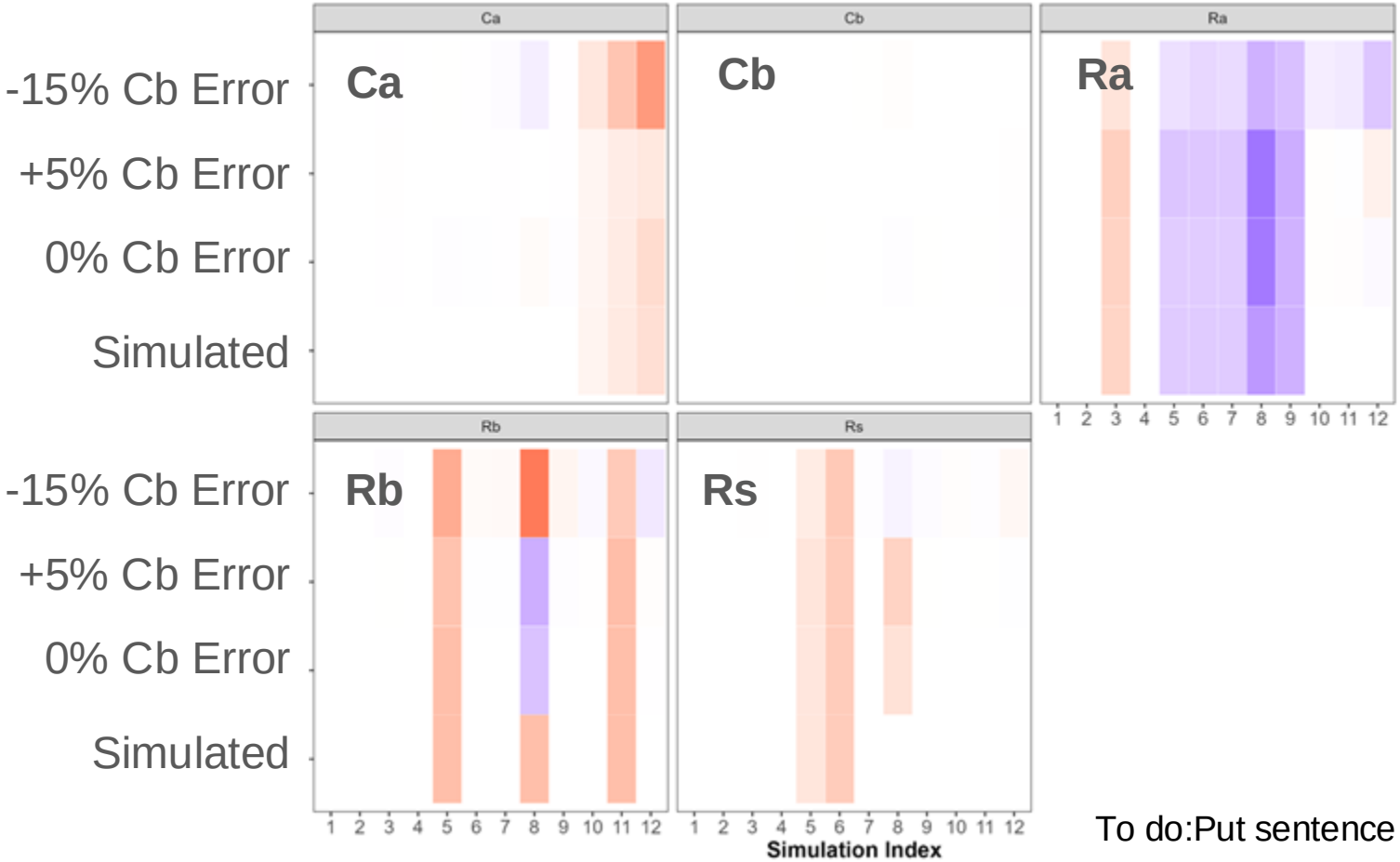
validate

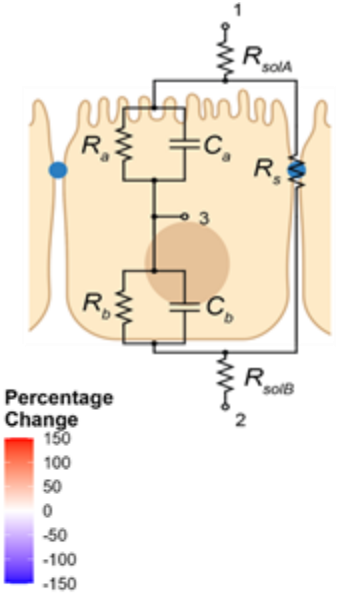
$$Z_{eq}(\omega) = R_{blank} + \frac{R_s\left(Z_a(\omega) + Z_b(\omega)\right)}{R_s + Z_a(\omega) + Z_b(\omega)}$$

& Cb = constant

Keep the circuit diagram in all pertinent slides

Accurate prediction of membrane parameters is not impacted by accuracy of constrained basal capacitance value





To do:Put sentence explaining the impact o

Biology validation: polarized mutation

Test model with biological data (RPE)

KCNJ13 variants cause LCA16, disrupting Kir7.1 and apical RPE K+ conductance

Insert schematic of iPSC->RPE

David Gamm, MD, PhD

Bikash Pattnaik, PhD

Omar Memon

Jair Montford

SAM.png

Samuel Ramirez

Casey Cargill

[1]https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4594557/

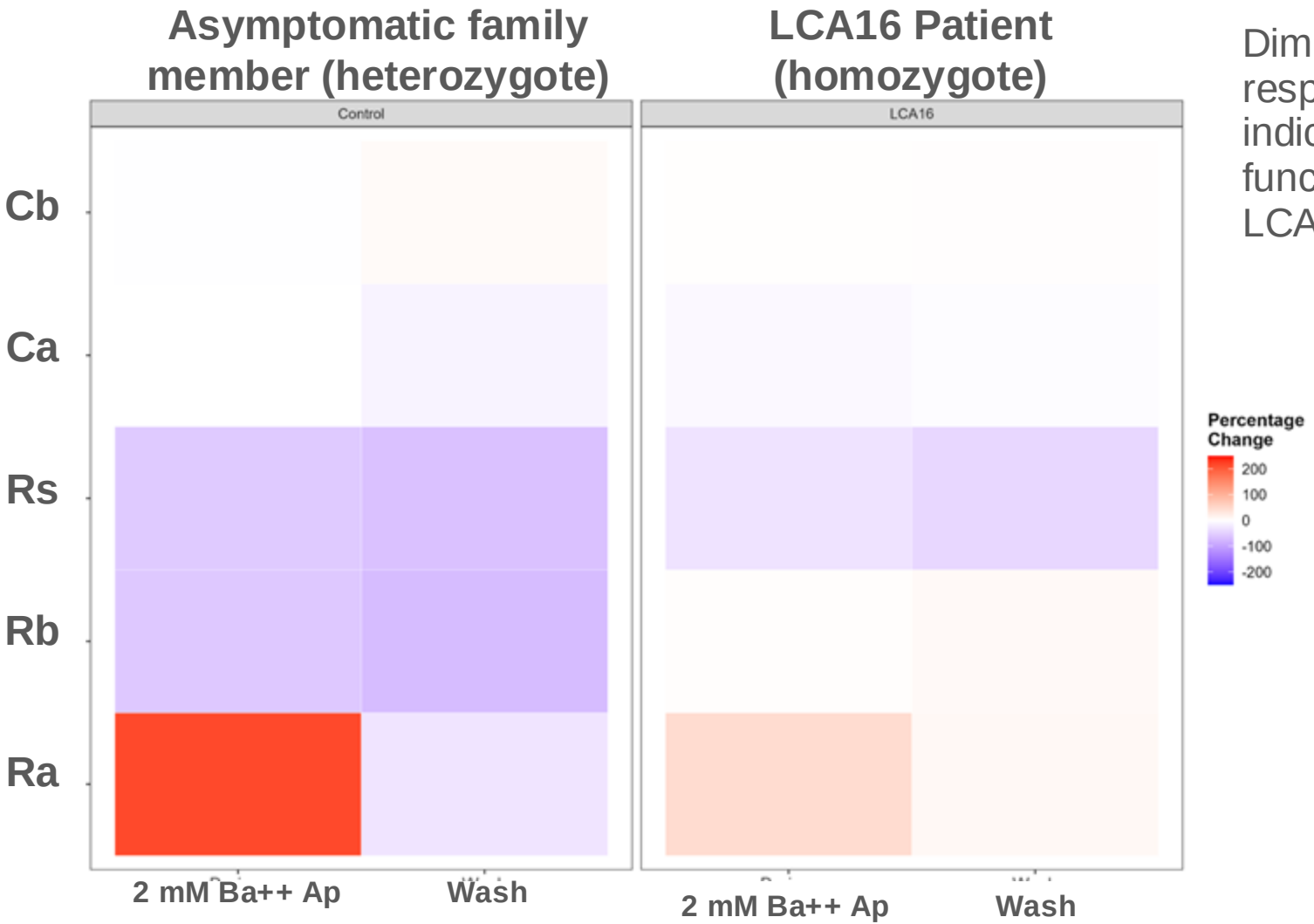
Kir maturation into RPE

Schematic depicting weekly measurements

Casey Cargill

SAM.png

Samuel Ramirez



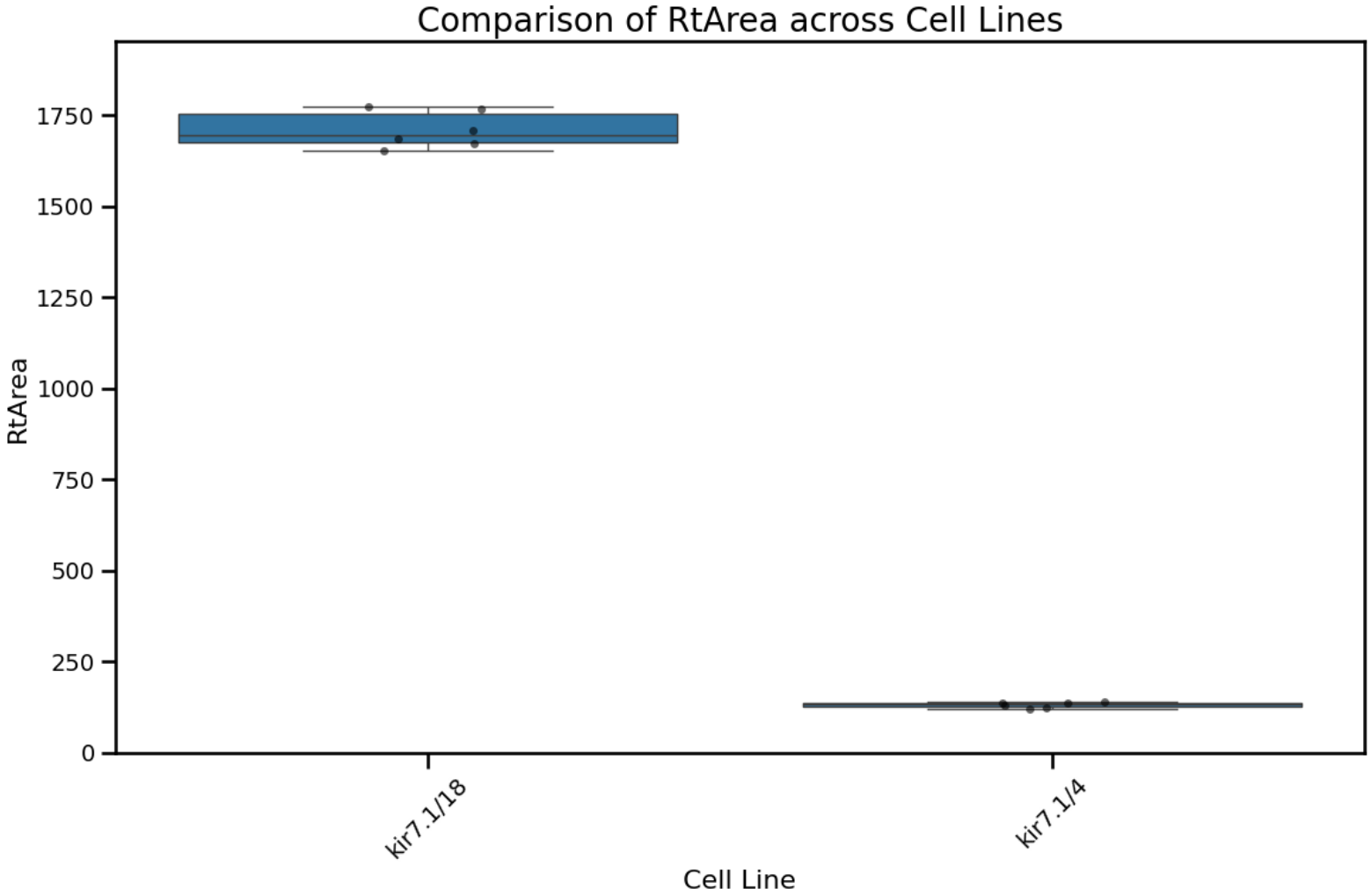
Diminished Ra response to Ba++ indicates lack of functional Kir7.1 in LCA16 patient line.

Casey Cargill

Conclusion: We generated a **non-invasive** alternative to intracellular electrophysiological assays for **high-throughput** analysis of polarized epithelial cells

Acknowledgements!

To do: add lab/collaborator names


Comparison of RtArea across Cell Lines
RtArea
1750
1500
1250
1000
750
500
250
0
kir7.1/18
kir7.1/4
Cell Line

Questions