Assessing Quality of Tissue Engineered Retinal Pigment Epithelia Using Absorbance Imaging & Artificial Intelligence

July 1, 2021

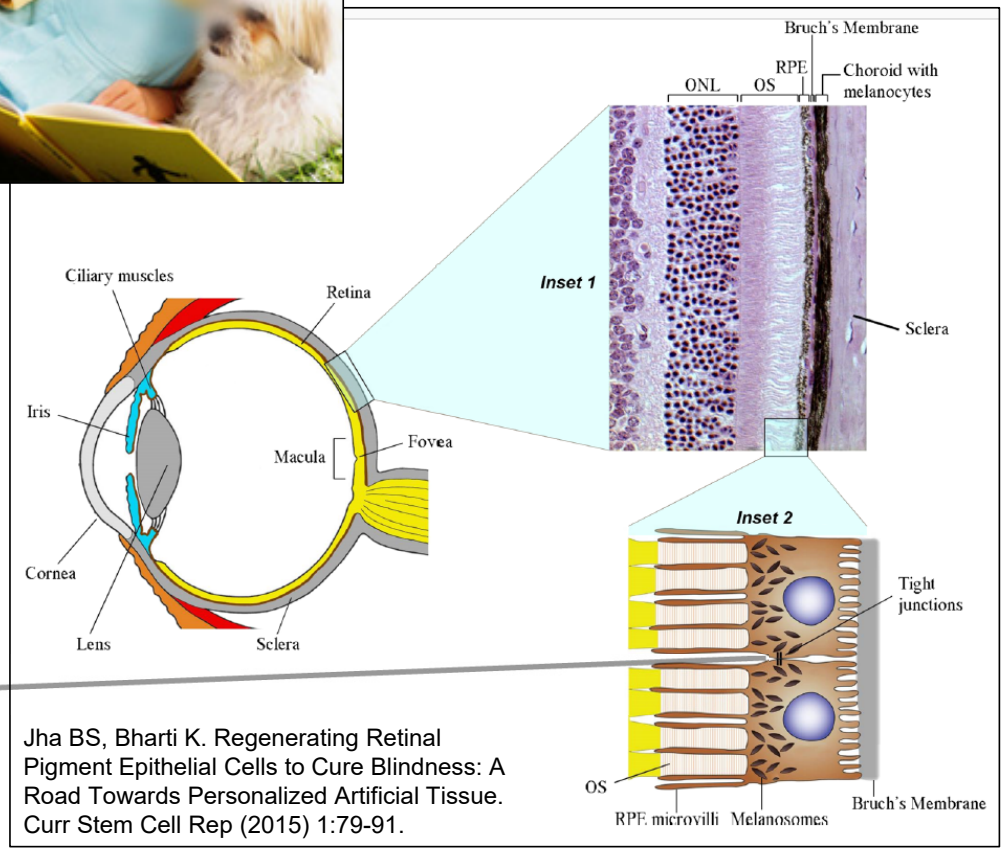
Carl Simon

Age-Related Macular Degeneration & Retinal Pigment Epithelial Cells (AMD & RPE)

https://www.cdc.gov/visionhealth/basics/ced/index.html

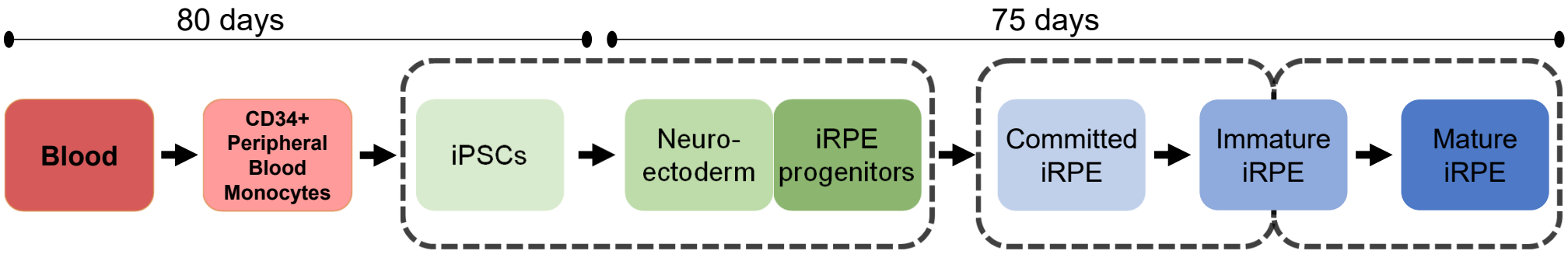
- RPE support rods & cones by delivering nutrients from the bloodstream & removing waste that the rods and cones generate
- In AMD, RPE stop performing their support functions & rods & cones die, resulting in loss of central vision
- AMD is a common cause of vision loss in developed countries, affecting 30 to 50 million people worldwide
  - No good treatment for 90% of AMD cases (dry AMD)
- **Treatment Goal:** Manufacture healthy RPE & deliver to eye to prevent rod and cone cell death

Formation of tight junctions is key for RPE function

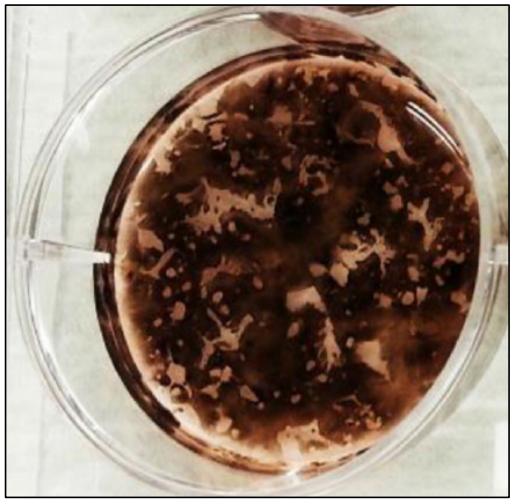


Jha BS, Bharti K. Regenerating Retinal Pigment Epithelial Cells to Cure Blindness: A Road Towards Personalized Artificial Tissue. Curr Stem Cell Rep (2015) 1:79-91.

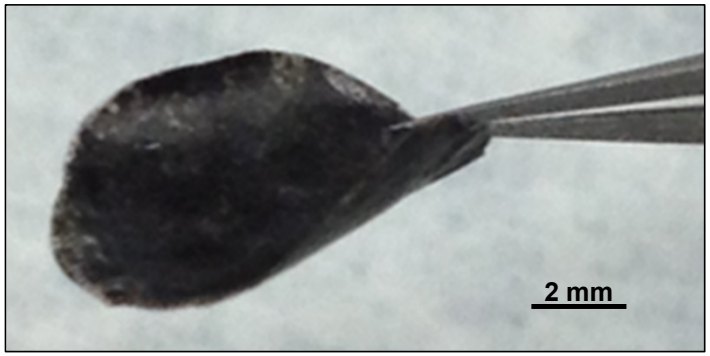
RPE Manufacturing in Bharti Lab at NIH: Takes 155 Days Per Patient



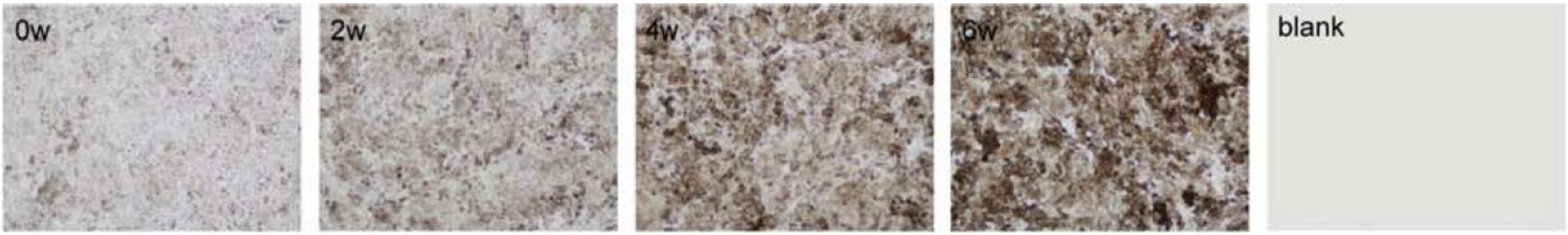
- Draw patient blood, centrifuge, retain PBMC fraction
- Isolate CD34+ monocytes using magnetic beads
- Generate bank of clinical grade induced-pluripotent stem cells (iPSCs) using 4 episomal factors



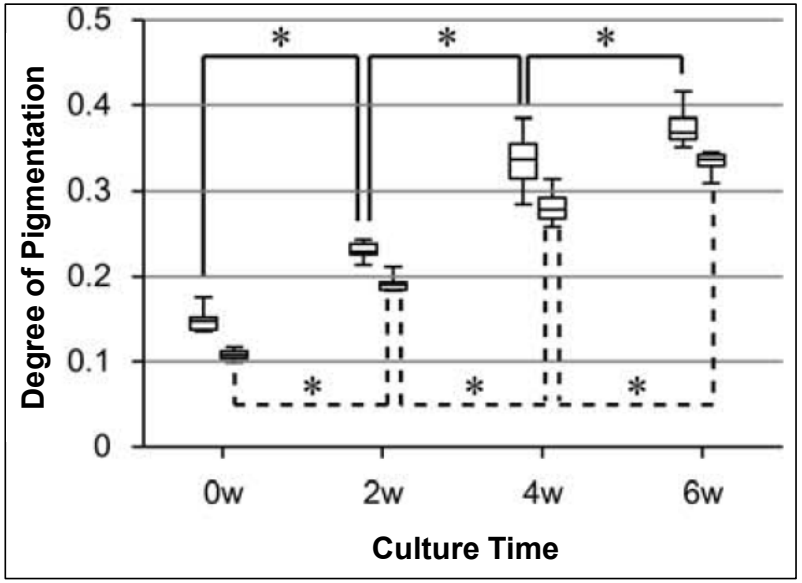
AMD Patient-Derived iPSC-derived RPE (iRPE)



Pigmentation Correlates with RPE Maturation



- RPE express melanin to absorb light
- Prevents light from entering the back of the eye
  - Reduce light scattering in the eye to improve vision
  - Protect tissue from exposure to light, reduce disease & cancerous lesions
- NOTE: Pigmentation is an indirect measure of function
  - *Pigmentation is not part of the mechanism of action for treating AMD*
  - MOA = support of the rods & cones

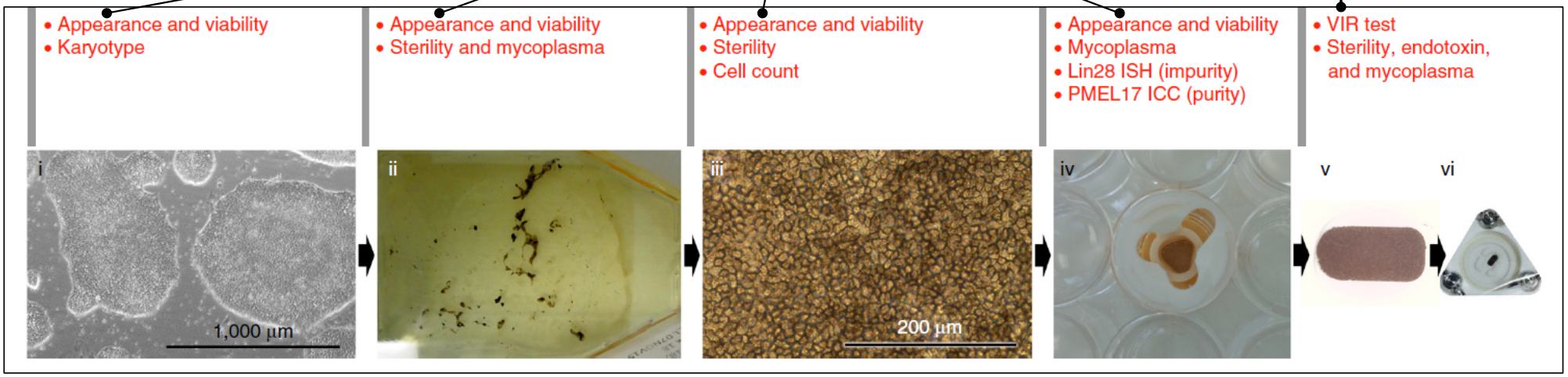


Kamao et al. Objective Evaluation of the Degree of Pigmentation in Human Induced Pluripotent Stem Cell–Derived RPE. Invest Ophthalmol Vis Sci. 2014;55:8309–8318.

Cruz et al. 2018: Phase 1 Clinical Study of an Embryonic Stem Cell-Derived RPE Patch in AMD

"Appearance" is a key quality attribute

VIR = Visual Inspection Release test



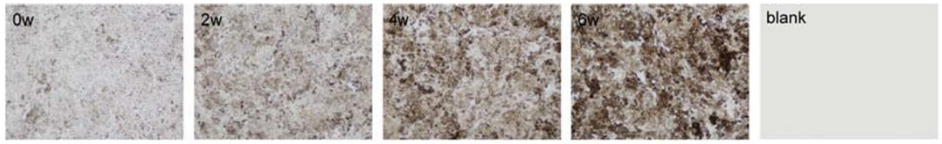
Cruz et al. Nature Biotechnology, 2018;36(4):328-337.

"RPE cells were assessed using a light microscope for pigmentation, cobblestone morphology, health and signs of contamination and processed further, only if they passed this visual check."

"On the day of surgery...The patch is assessed visually through the clear lid of the storage container for integrity, pigmented cell coverage and viability."

Measurement Issue

Pigmentation Correlates with RPE Maturation



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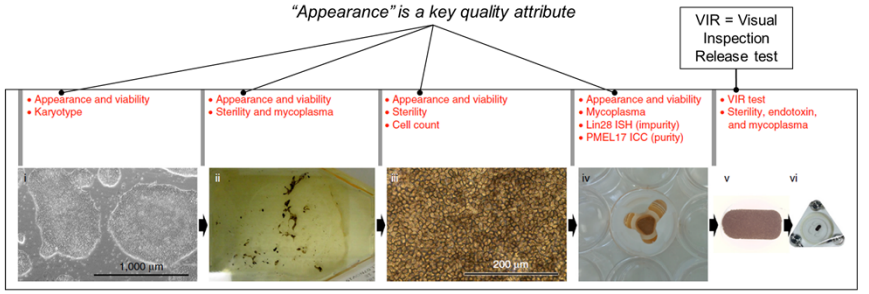

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How to replace "Appearance" qualitative visual inspection tests using image as a key quality attribute of iRPE patches with quantitative measurements?

Quantitative Bright-Field Absorbance Microscopy (QBAM)

- Use brightfield microscope as a spectrophotometer
- Each pixel in an image is a *quantitative* measure of pigmentation

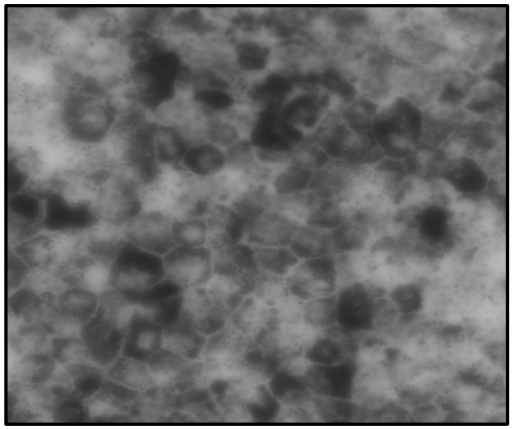
Calculate Absorbance Image

$$A = -\log_{10} \frac{I_{Cell} - I_{Min}}{I_{Max} - I_{Min}}$$

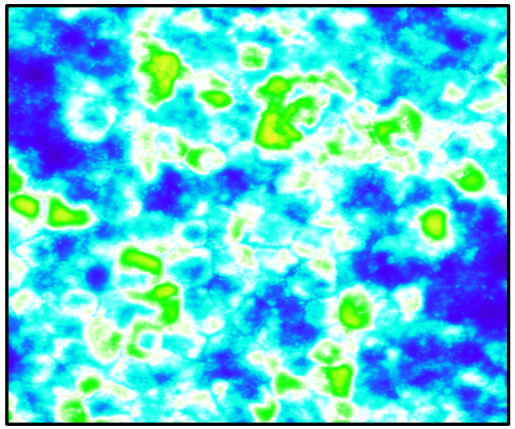
Shutter Closed (I_{Min})

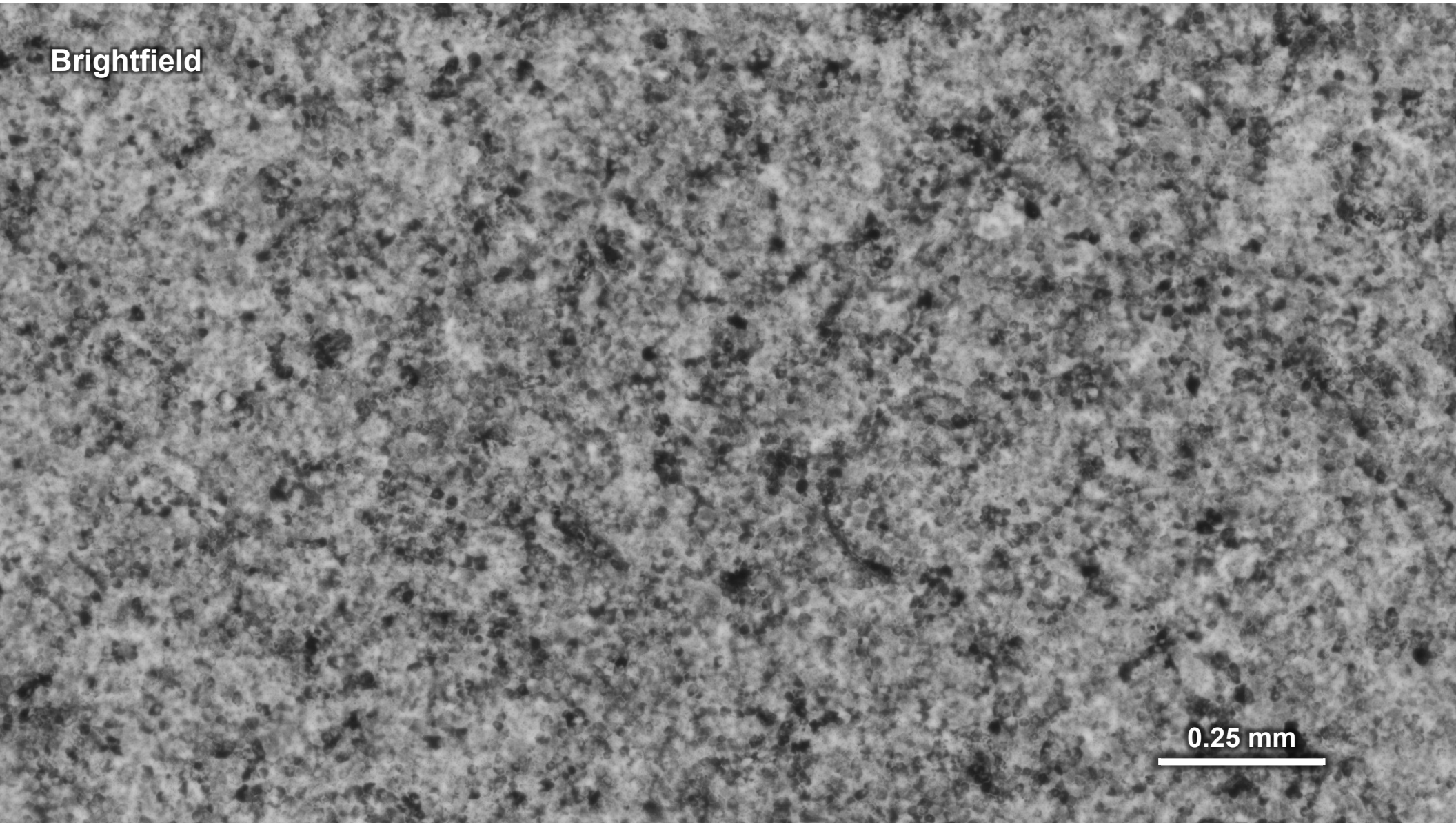
Shutter Open (I_{Max})

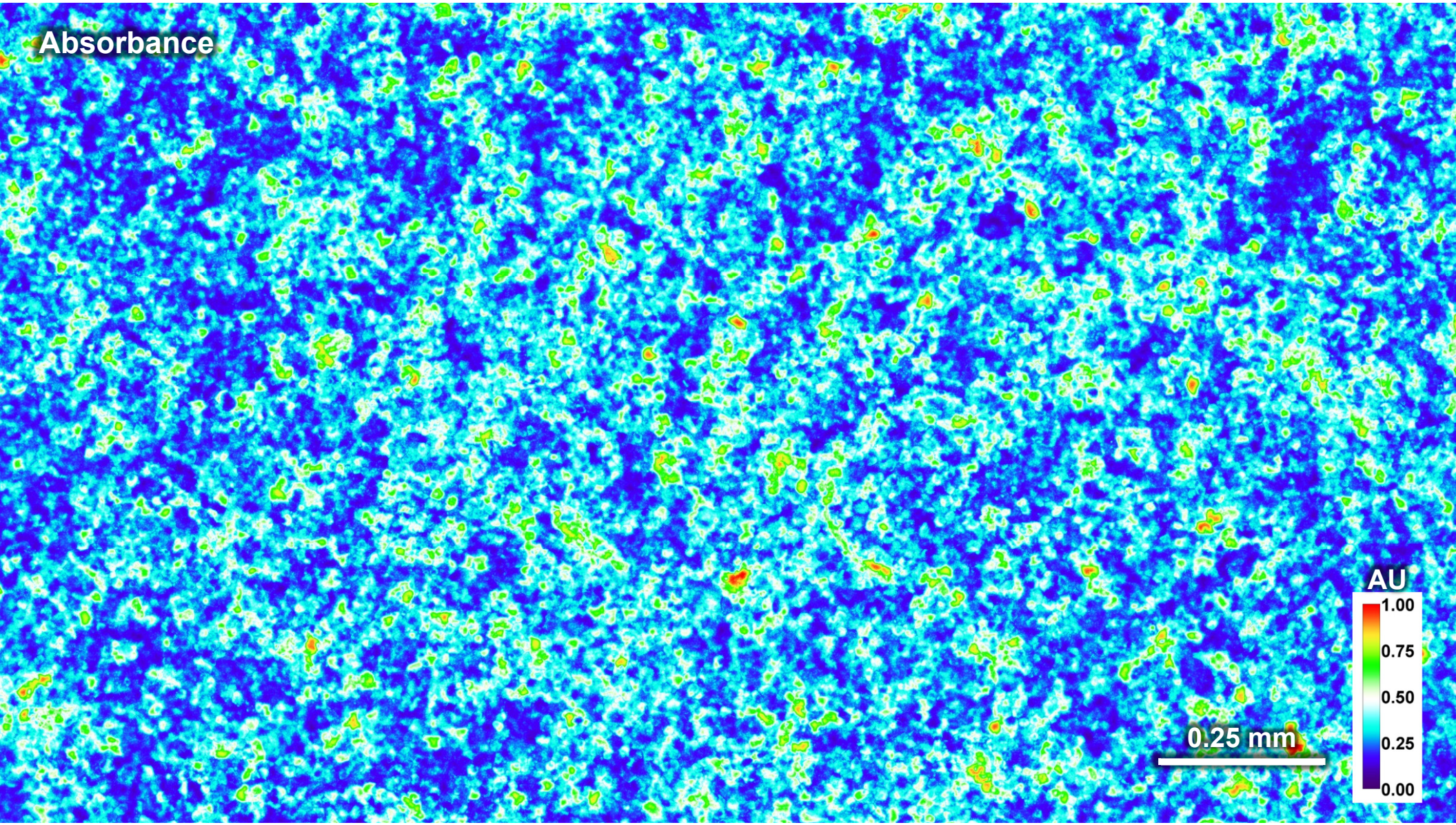
RPE (I_{Cell})



Absorbance




Brightfield
0.25 mm


Absorbance
AU
1.00
0.75
0.50
0.25
0.00
0.25 mm

Experimental Overview

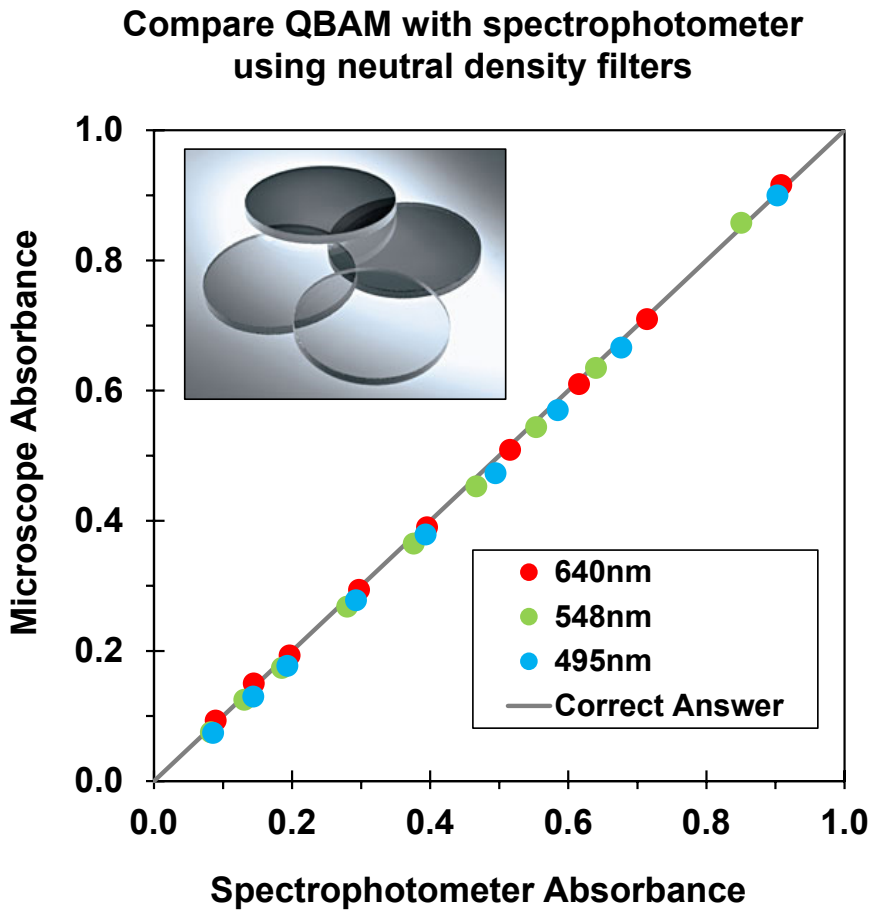
The information in the pixels is used to assess RPE quality by 2 approaches:

1. Deep neural network (DNN): uses image-level spatial patterning of pixel intensities
    - Avoids image processing/feature engineering
    - Creates a “Black box” model
    - Tissue-level & cell-level
2. Traditional Machine Learning (TML): uses single cell-level metrics: shape, intensity, texture
    - Requires extensive image processing/feature engineering
    - Provides some biological insights based on important cell metrics/features
    - Cell-level only

QBAM Quality Assurance

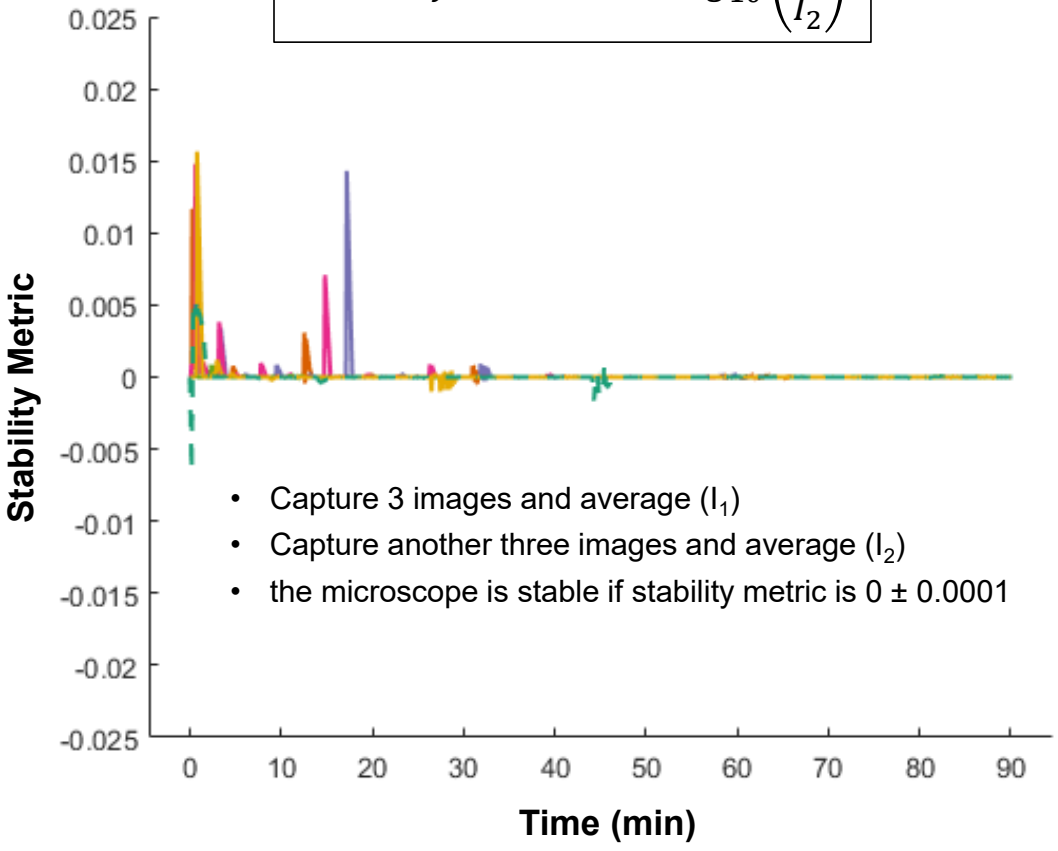
Data Collection Routines:

- Data collection automated using custom plugin for MicroManager
- Starts with “microscope stability” routine where it collects images at different exposure times until **95% confidence interval of each pixel is 0.01 absorbance units**
- The best exposure time for each pixel is used to generate an “optimized” image
- Takes images at 3 wavelengths by using three different filters (red, green, blue)
    - This helps to account for scattering, since scattering is less dependent on wavelength than is absorbance



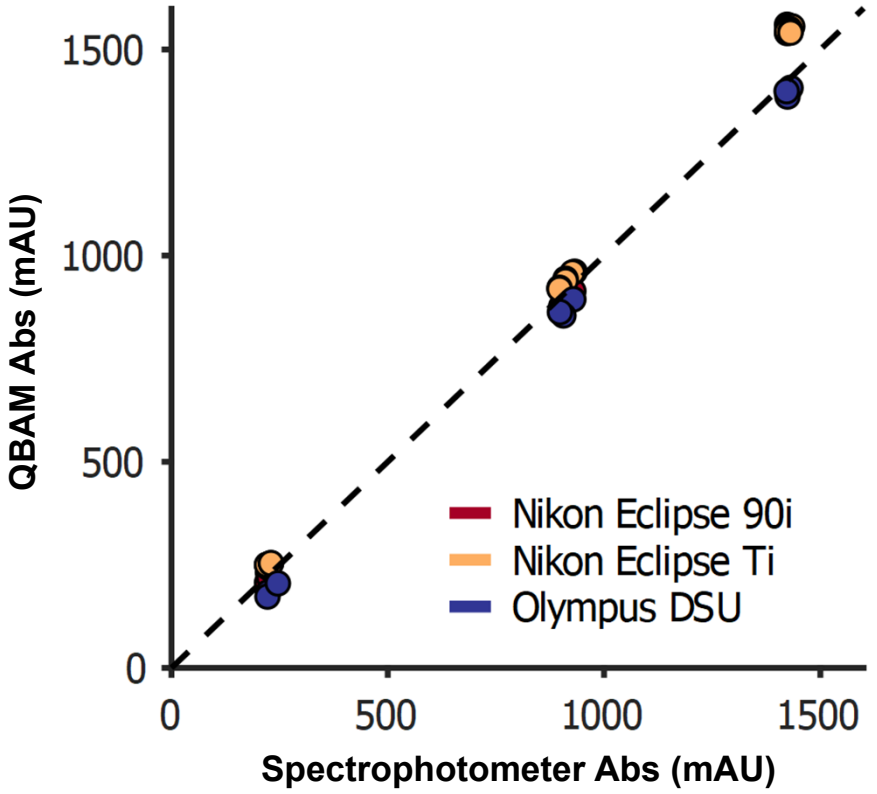
QBAM Quality Assurance

$$Stability\ metric = -Log_{10}\left(\frac{I_1}{I_2}\right)$$

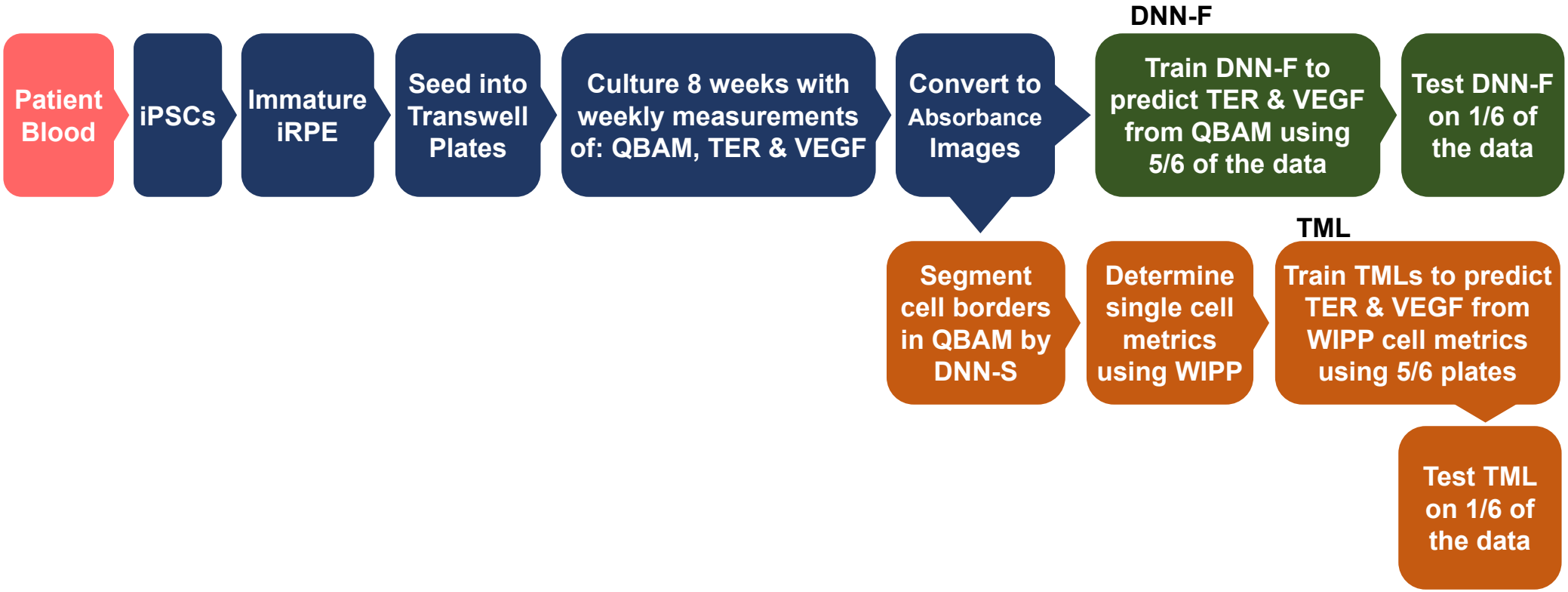


- Capture 3 images and average (I_1)
- Capture another three images and average (I_2)
- the microscope is stable if stability metric is 0 ± 0.0001

Assessing comparability using QBAM to measure absorbance of 3 different neutral density filters imaged on 3 different microscopes using different filters



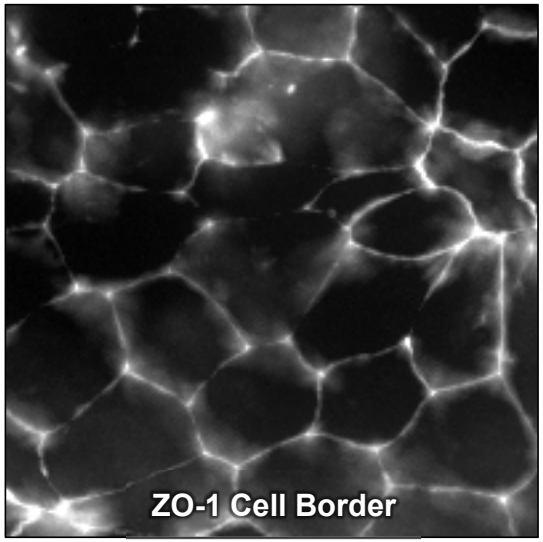
Experimental Overview

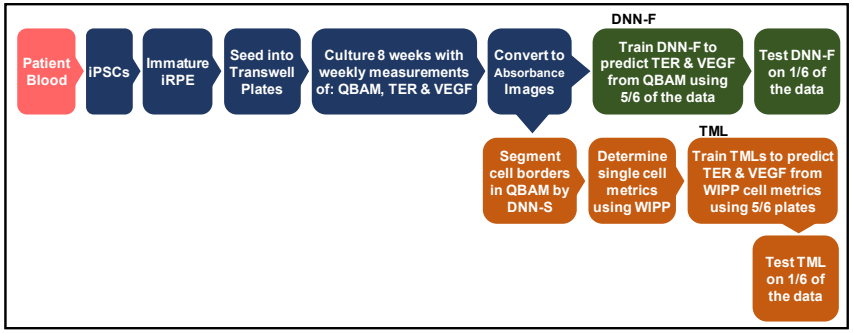


Experimental Overview

Goal for DNN-Z & DNN-S: Segment single cells in QBAM images

- By using QBAM images to predict where a human would identify a cell border in ZO-1 immunostained fluorescence image





DNN-Z

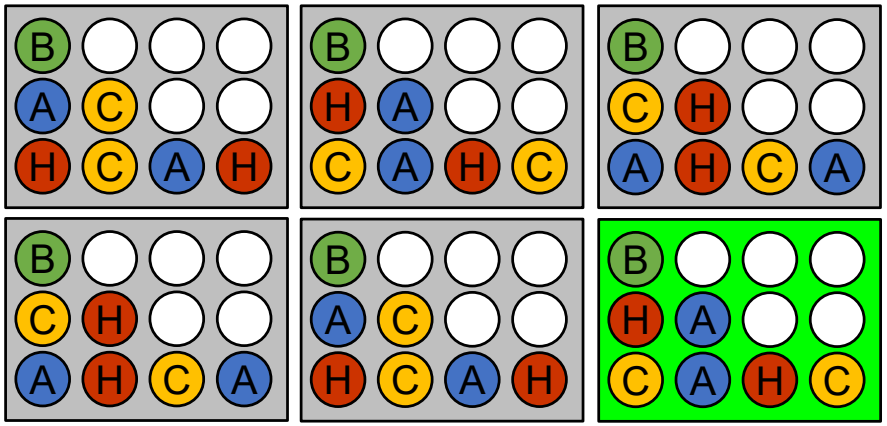
Wide variety of mature RPE with cell borders immunostained for ZO-1 → Image by fluor-escence → Segment fluorescence images for cell borders using Fogbank + hand-corrections → Train DNN-Z to predict ZO-1 segmentations from ZO-1 fluorescence images using Fogbank+hand-corrected segmentations as truth → Test DNN-Z on subset of fluorescence images

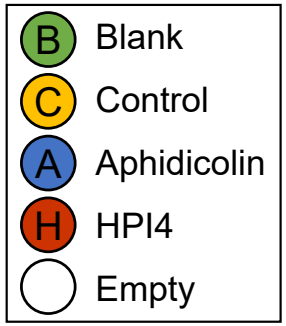
DNN-S

Mature iRPE with cell borders immunostained for ZO-1 → Image by QBAM & fluor-escence → Co-register QBAM & fluor-escence images so they perfectly overlay → Segment fluor-escence images for cell borders using DNN-Z + hand-corrections → Train DNN-S to predict cell borders in QBAM using DNN-Z+hand-corrected segmentations as truth → Test DNN-S on subset of QBAM

Experimental Design

Train on 5 plates…test on 1 plate





Cell seeding →
Start treatments →

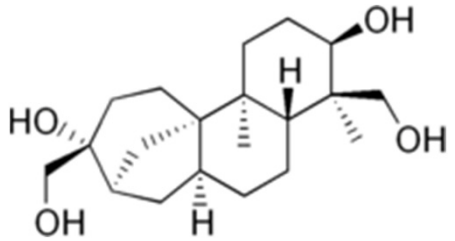
Week	QBAM	TER	VEGF Ratio
0	X		
1	X		
2	X		
3	X	X	
4	X	X	X
5	X	X	X
6	X	X	X
7	X	X	X
8	X	X	X

- 12-well transwell plates
- 6 plates x 6 wells/plate = 36 wells
- iPSC-derived RPE from healthy donor
- 3 treatments (control, aphidicolin, HPI4)
- QBAM
  - 12 overlapping fields of view per well (4 x 3) per time point
  - Use three different color filters (red, green, blue)
- TER measurement on all wells and plates 1X/week for 6 weeks
- VEGF measured on each well in 2 plates 1X/week

Generating Good & Bad RPE: Aphidicolin & HPI4

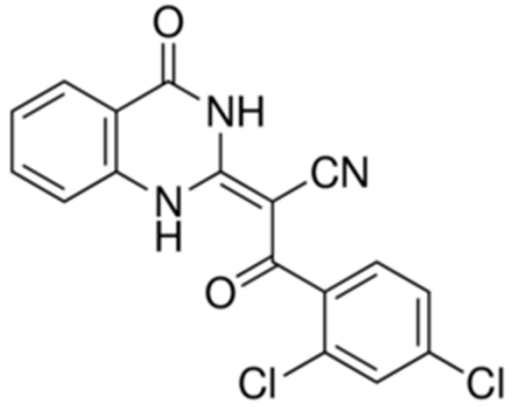
Aphidicolin

- **inducer** of RPE maturation
- antibiotic that inhibits eukaryotic nuclear DNA replication and blocks the cell cycle at early S phase



HPI4

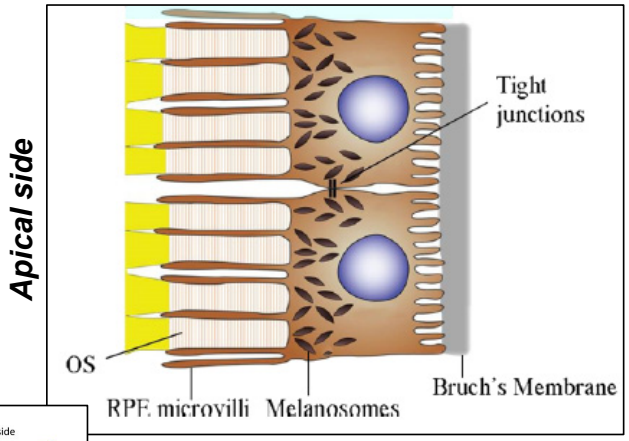
- **inhibitor** of RPE maturation
- hedgehog pathway inhibitor-4, HPI4
- Hedgehog signaling pathway transmits information to embryonic cells required for proper cell differentiation

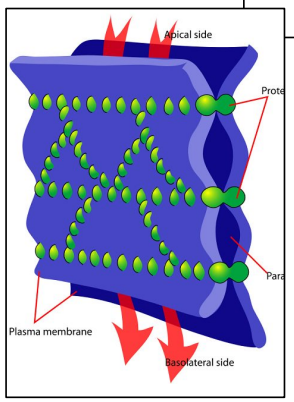


RPE Functional Attributes:

1) Trans-Epithelial Resistance (TER) &
2) Polarized VEGF Secretion (VEGF Ratio)

Tight junctions are key for RPE function

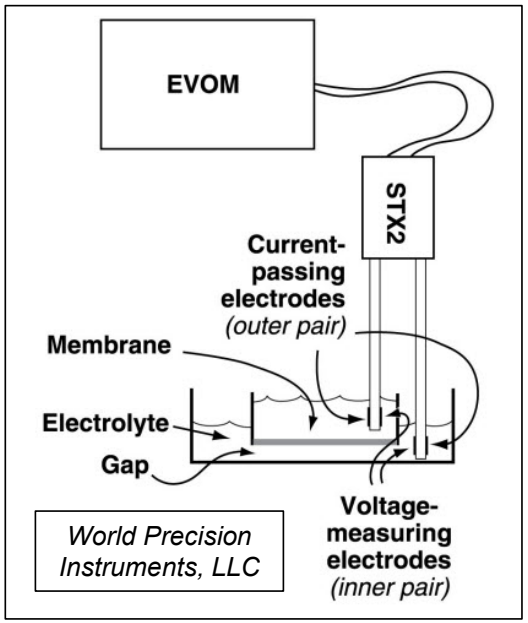




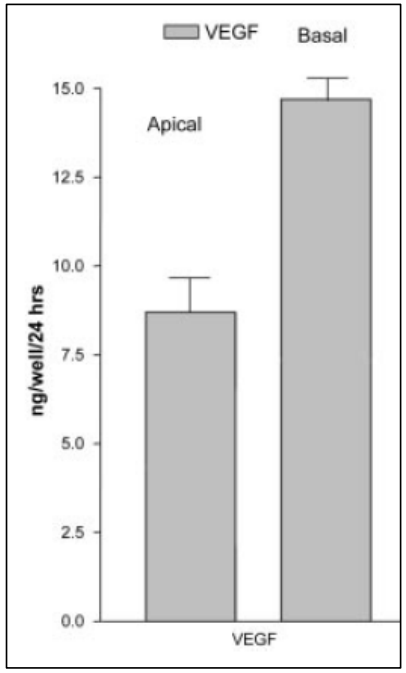
biologydictionary.net

Tight junctions: multiprotein junctional complexes common in epithelial cell layers that function to prevent passage of solutes & water between cells

TER



VEGF Ratio



Maminishkis et al. Confluent monolayers of cultured human fetal retinal pigment epithelium exhibit morphology and physiology of native tissue. Invest Ophthalmol Vis Sci. 2006;47(8):3612-24.

Fun Facts

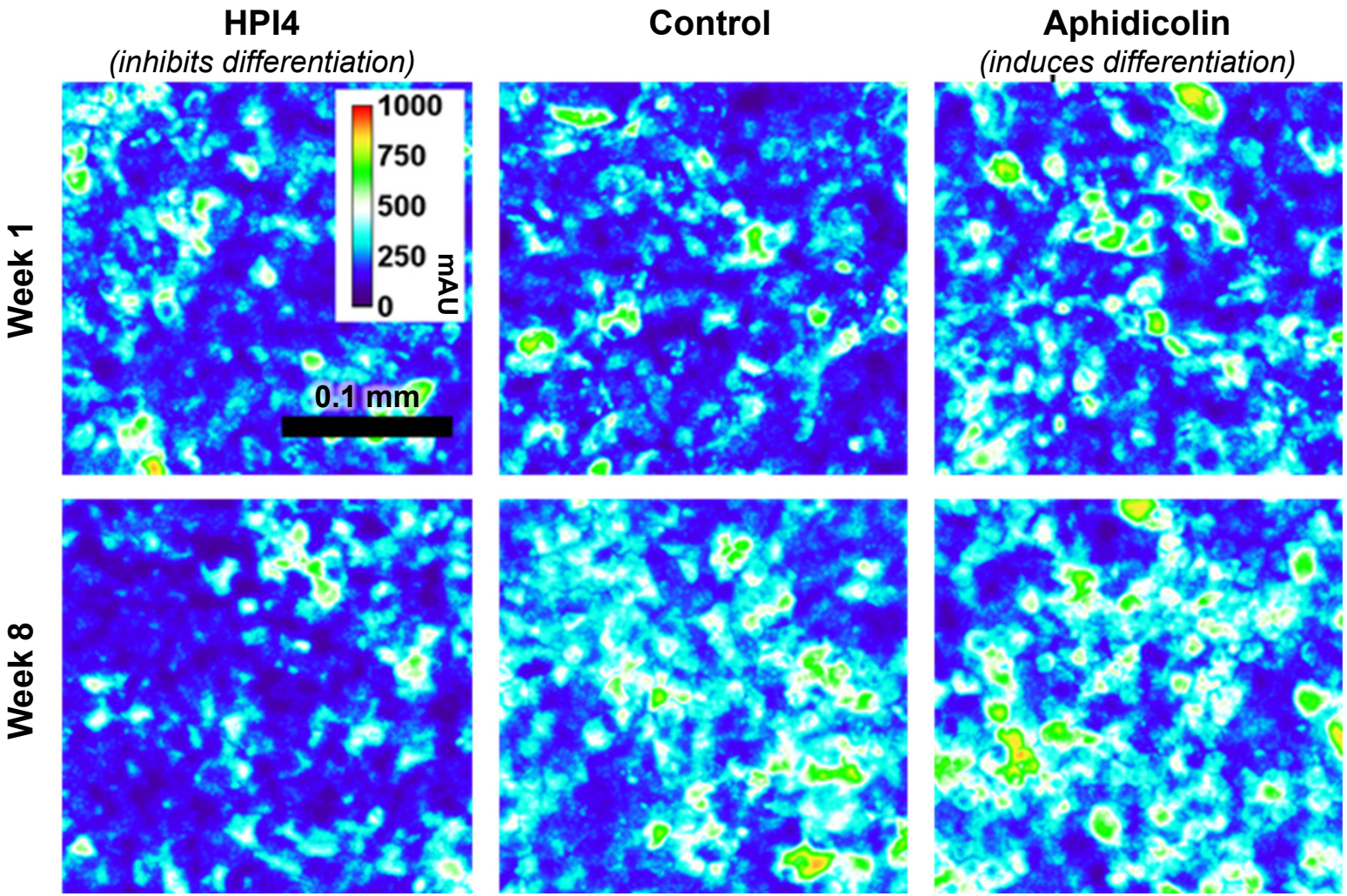
Scope

- iRPE Manufacturing takes 155 days
    - Bharti lab has 2 dedicated staff that only manufacture RPE for use by the rest of the lab
    - $10K in growth factors required to make a batch of RPE
- Cost per patient is unknown (~$1M per patient)
- iPSC technology is new, discovered in 2006, only 6 patients have rec'd them
    - 6 in Japan (1 iRPE patch & 5 iRPE suspensions)
    - 0 in USA
- Data from iRPE from 10 donors
- Implemented 53 DNN & ML AI routines to
- Data Dissemination (600 GB): https://isg.nist.gov/deepzoomweb/data/RPEimplants

Challenges

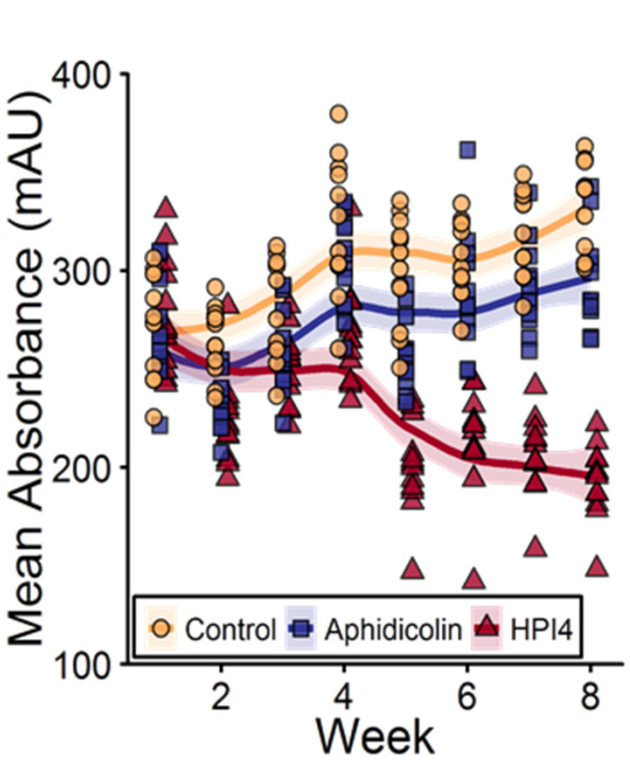
- Reliability: microscope stability, optimal exposure time, background, blank, 3 colors, different wells
- Speed: Optimizing speed to minimize time that cultures are out of the incubator
- Big data: 200K images, 1TB, 12M single cells
    - 4 channels of data: QBAM RGB + ZO-1 fluorescence
    - organizing, moving, annotating
- Processing: background subtraction, generating absorbance images from brightfield images
- Stitching: 4x3 tiling from each well
- 7 summer students participated in hand segmentations
- Build prototype at NIST & convince NIH to install it
- GLP: NIH gave us access to GLP facility (risky for them)

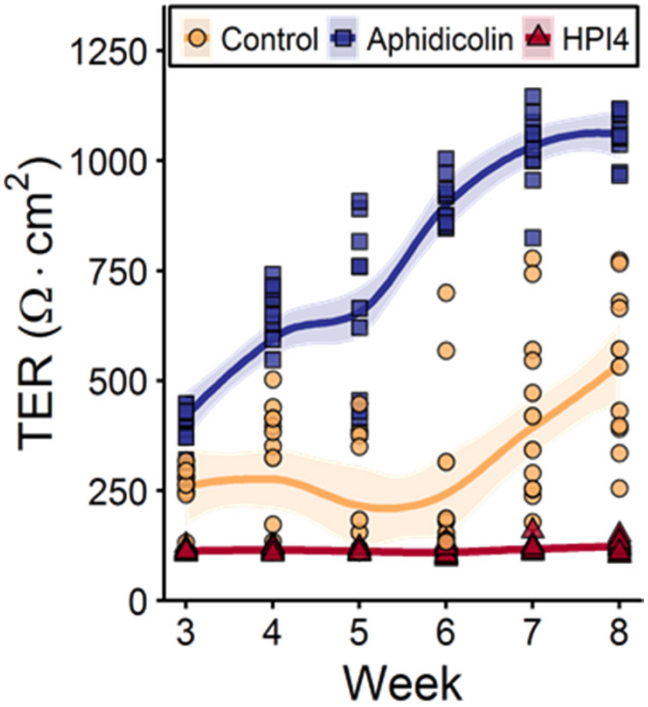
Quantitative Brightfield Absorbance Microscopy (QBAM)

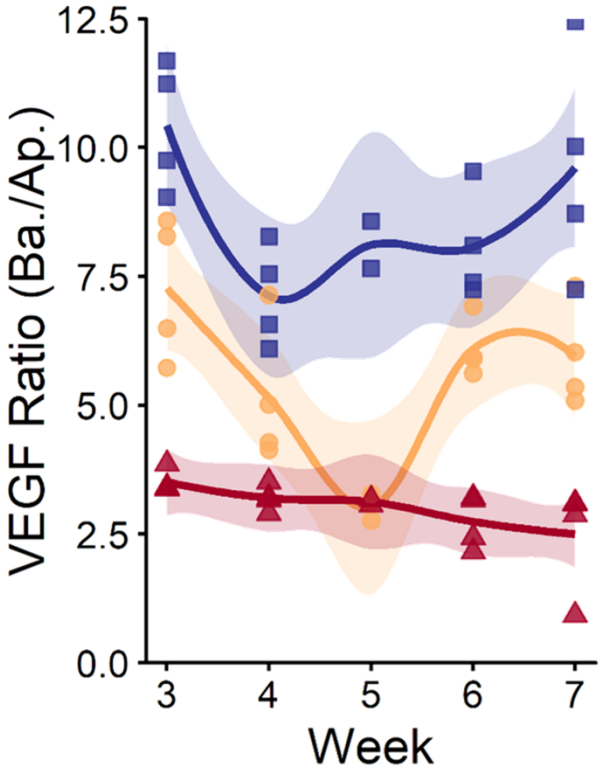


RPE Maturation: Mean Absorbance, TER & VEGF Ratio

Each data point is a well

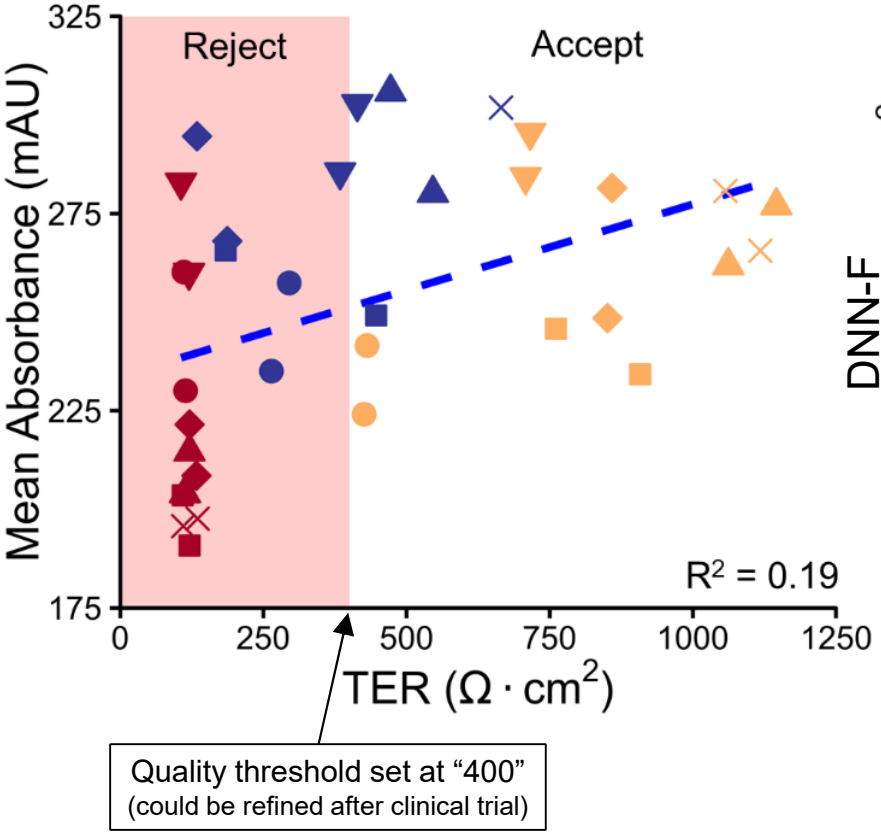


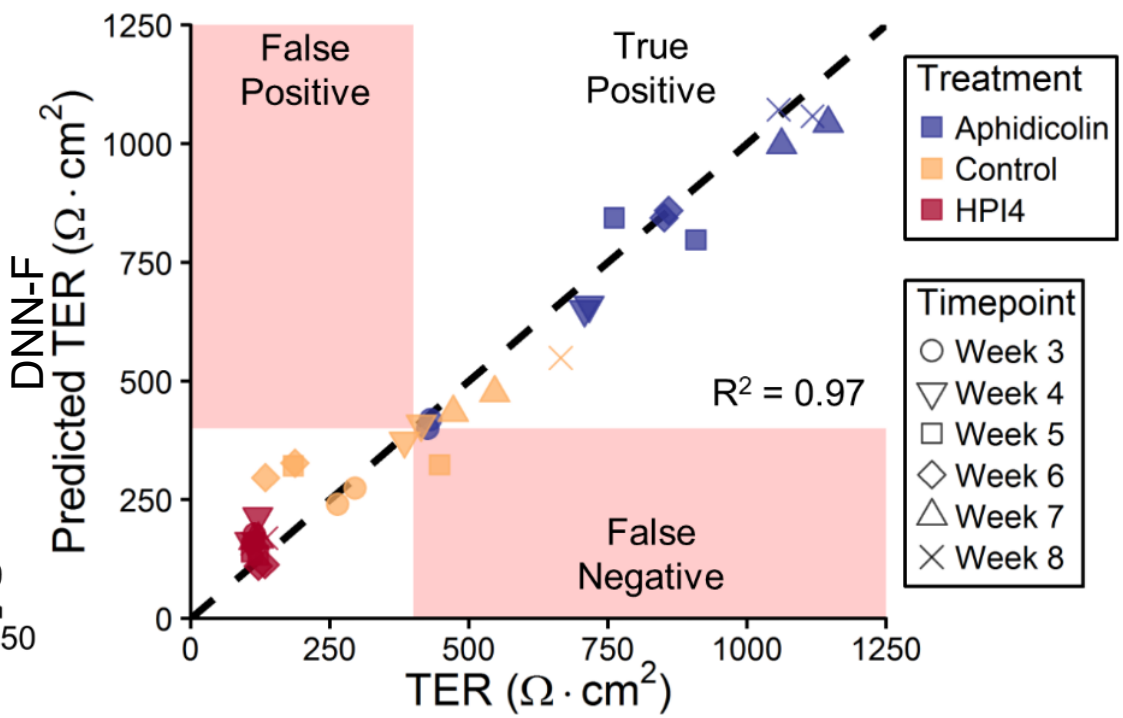




Weekly QBAM imaging did not impact iRPE maturation

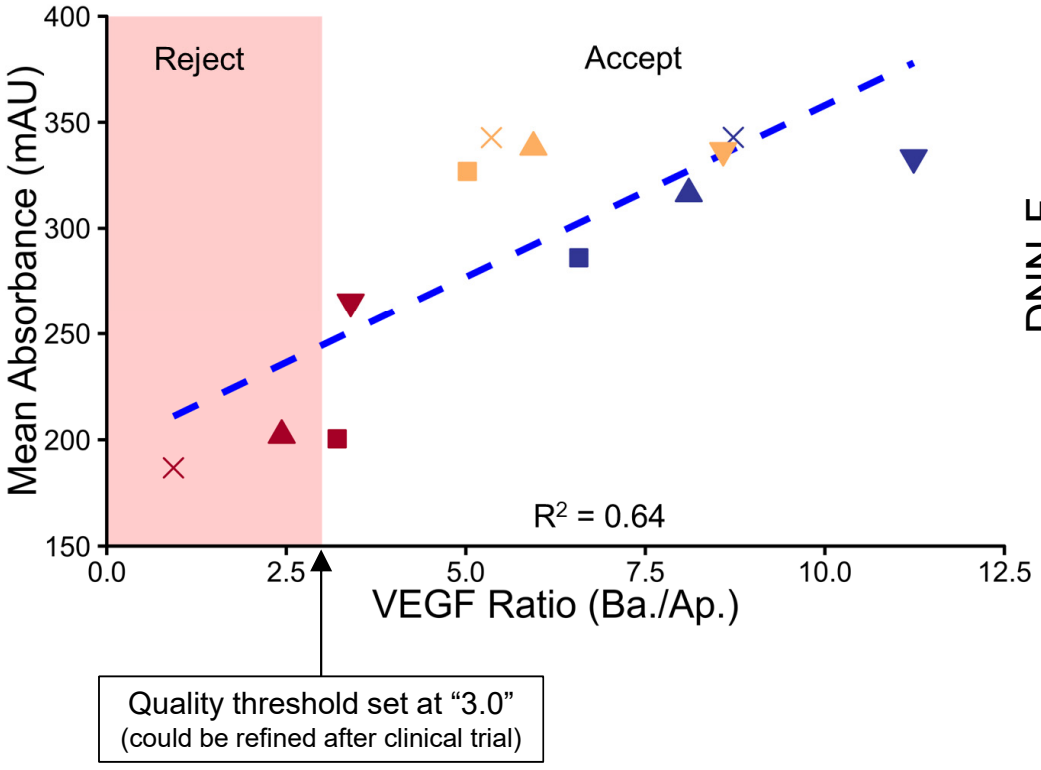
Mean Absorbance vs. AI: Trans-Epithelial Resistance (TER)



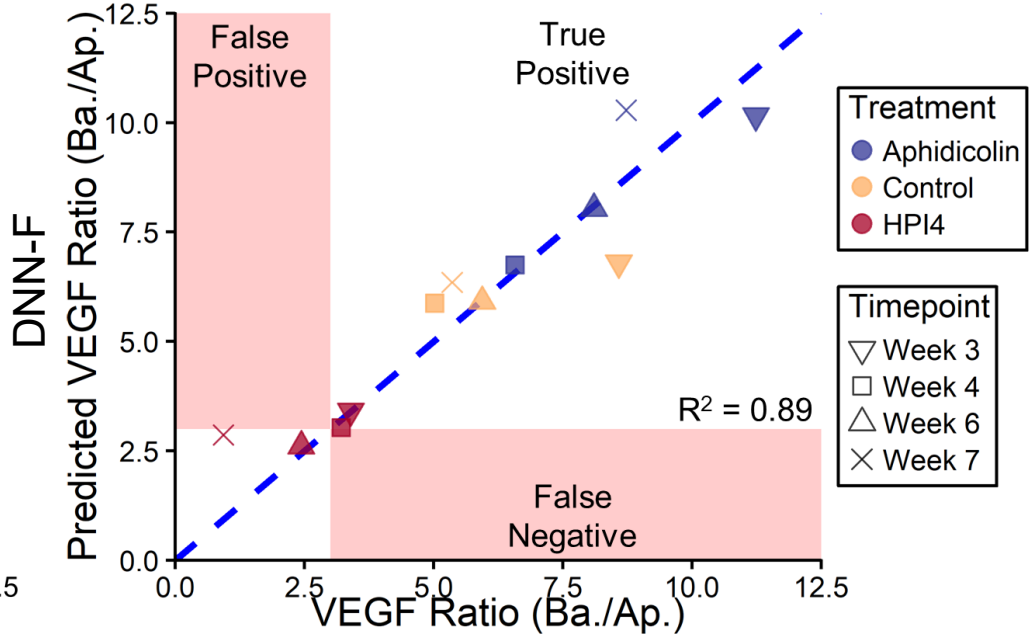


Root Mean Squared Error (RMSE): 70.6 Ω·cm²
Accuracy: 94%, Sensitivity: 1.0, Specificity: 0.9

Mean Absorbance vs. AI: VEGF Ratio

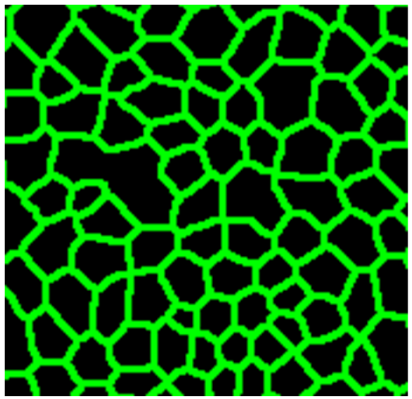


Quality threshold set at “3.0”
(could be refined after clinical trial)

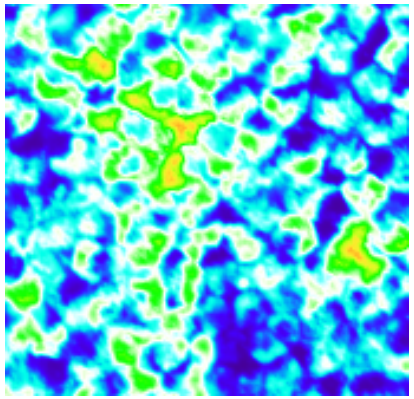


Root Mean Squared Error (RMSE): 1.1
Accuracy: 100%, Sensitivity: 1.0, Specificity: 1.0

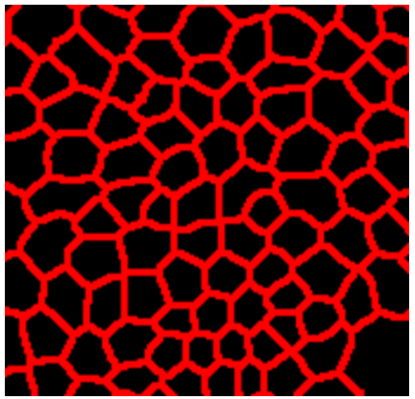
Deep Neural Network Prediction of RPE Image Segmentation



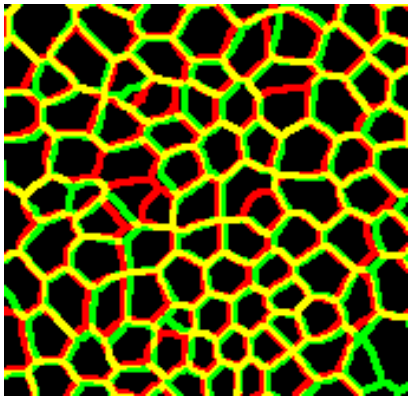
Truth: DNN-Z segmentation of ZO-1 fluorescence Images



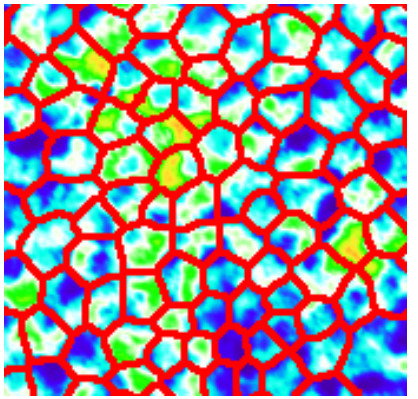
QBAM image



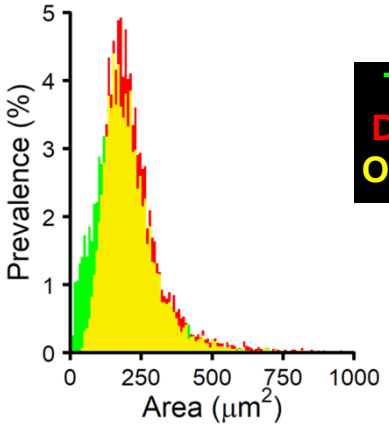
DNN-S segmentation



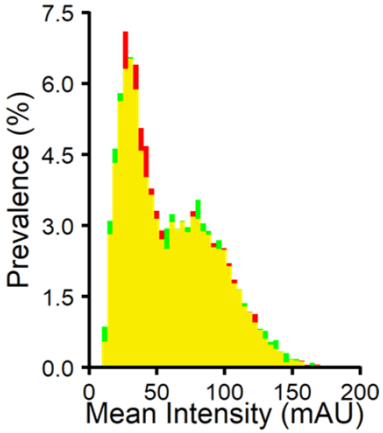
DNN-S overlaid on truth



DNN-S overlaid on QBAM

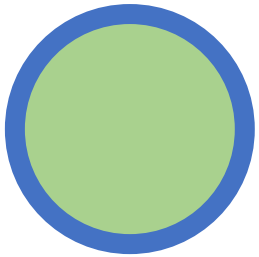


Truth
DNN-S
Overlap

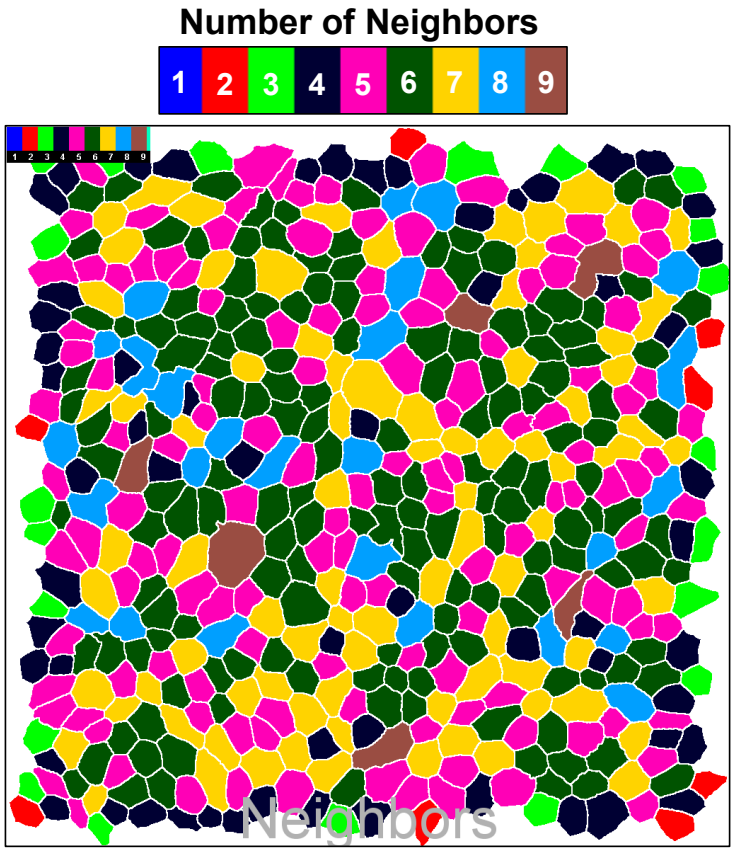


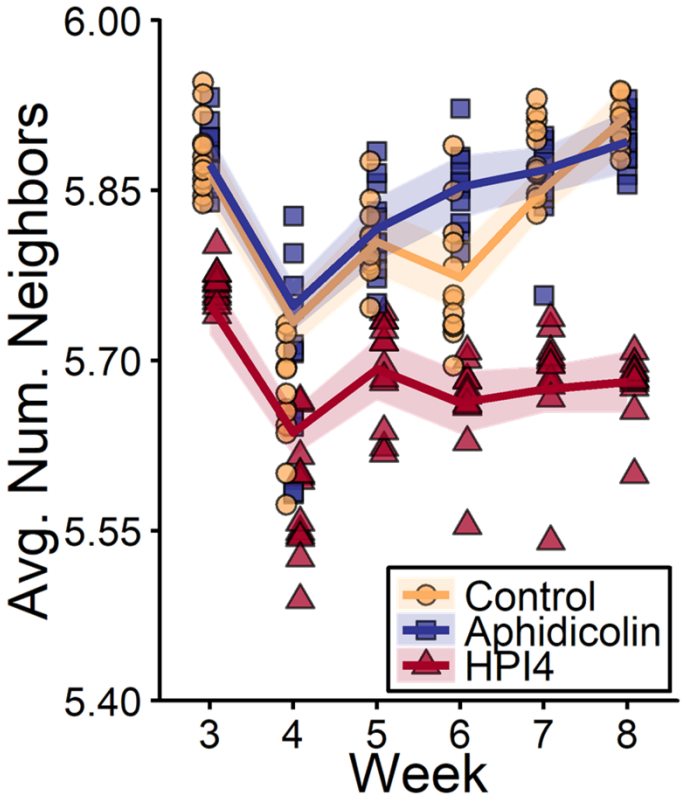
Comparative histograms of 44 cell image features of RPE segmented by hand or by DNN-S:

- F-2 = 0.71

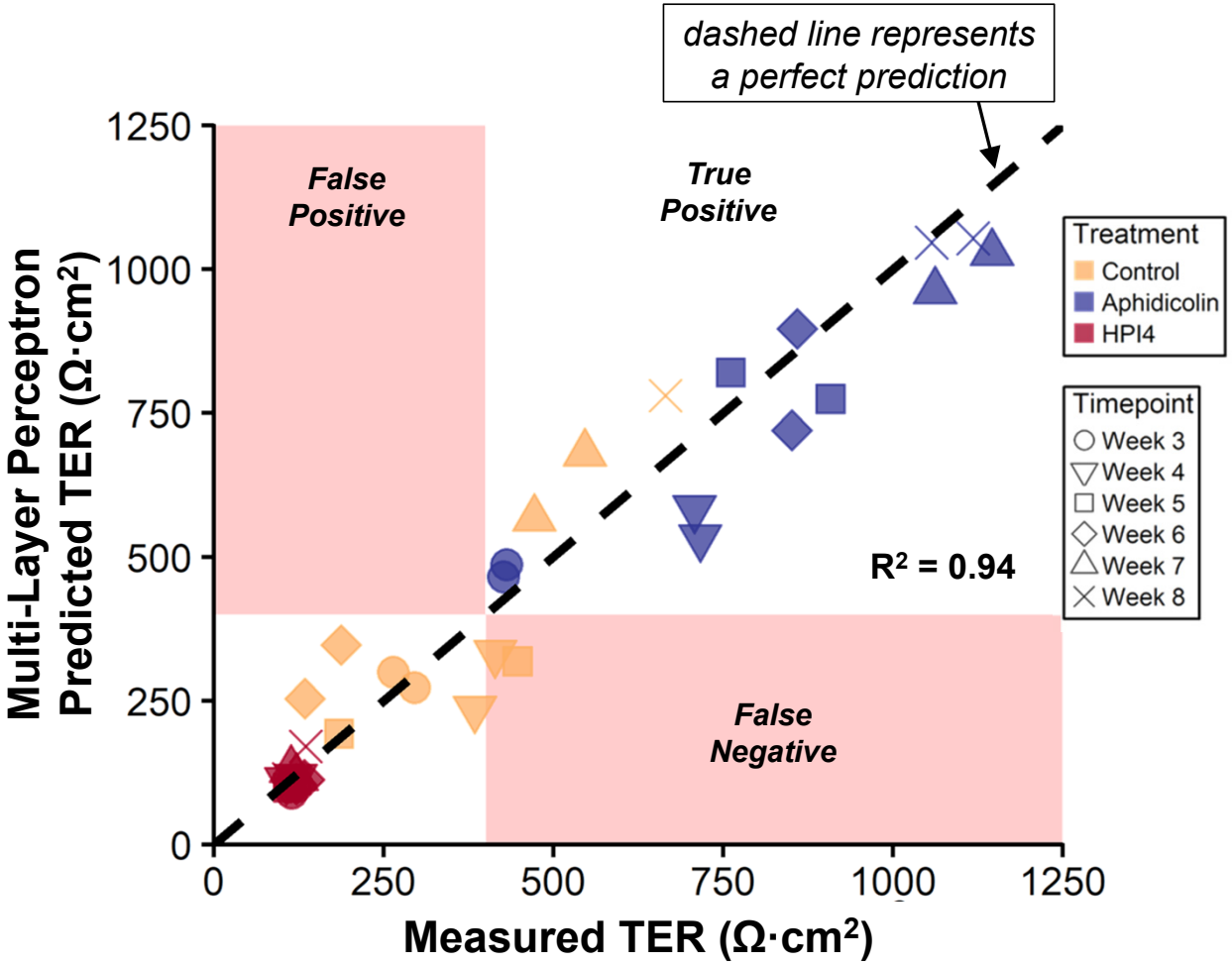


“Cobblestone Network”





Machine Learning Prediction of TER from WIPP Single Cell Features

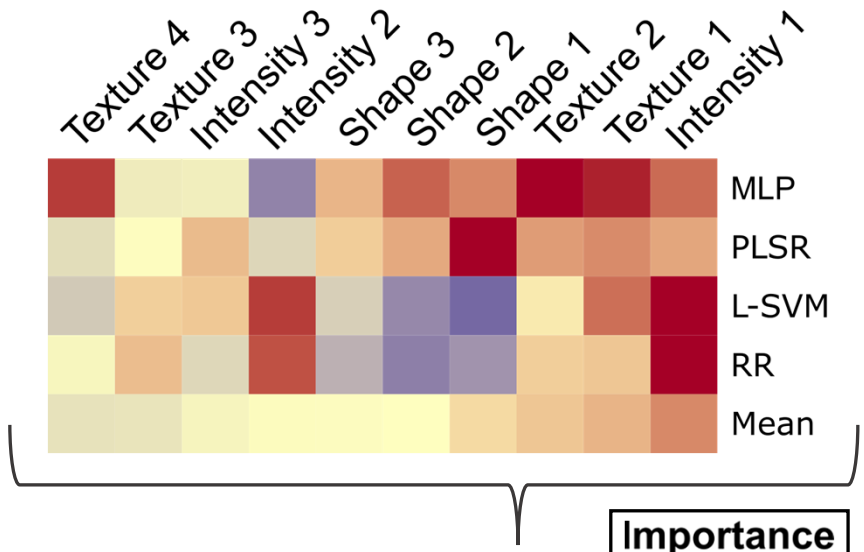


Summary of Algorithm Regression Errors	
Algorithm	**TER Root Mean Squared Error (Ω·cm2)**
DNN-F: Deep Learning Neural Network	70.6
MLP: Multi-Layer Perceptron	84.7
PLSR: Principle Least-squares Regression	100.1
L-SVM: Linear Support Vector Machine	102.7
RR: Ridge Regression	109.6
RF: Random Forest	116.4

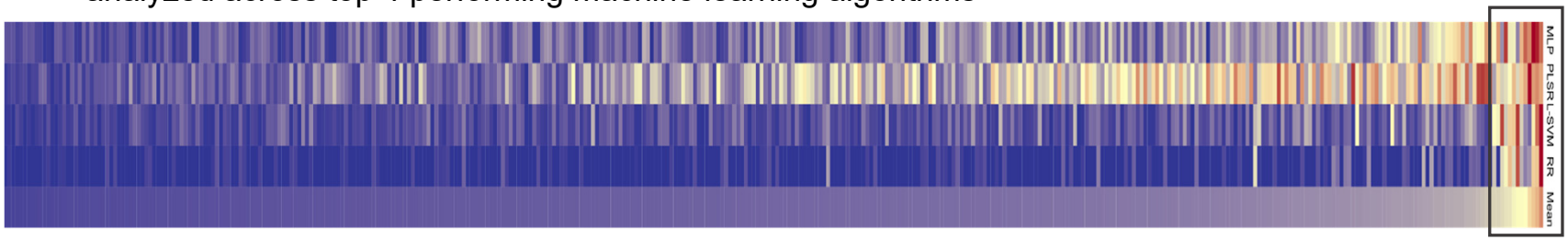
Different TML Models Key in on a Similar Set of Cell Features

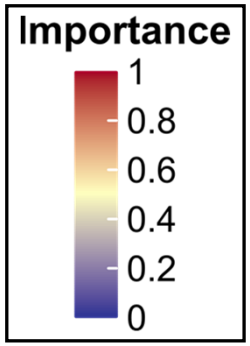
- Intensity 1 = average minimum intensity per cell
- Texture 1 = 3rd inverse difference moment at 45°
- Texture 2 = 3rd inverse difference moment at 135°
- Shape 1 = Zernike n4- l0 polynomial

What is the biological significance of these metrics?



Heatmap of the cell image feature importance of all features analyzed across top 4 performing machine learning algorithms





Different TML Models Key in on a Similar Set of Cell Features

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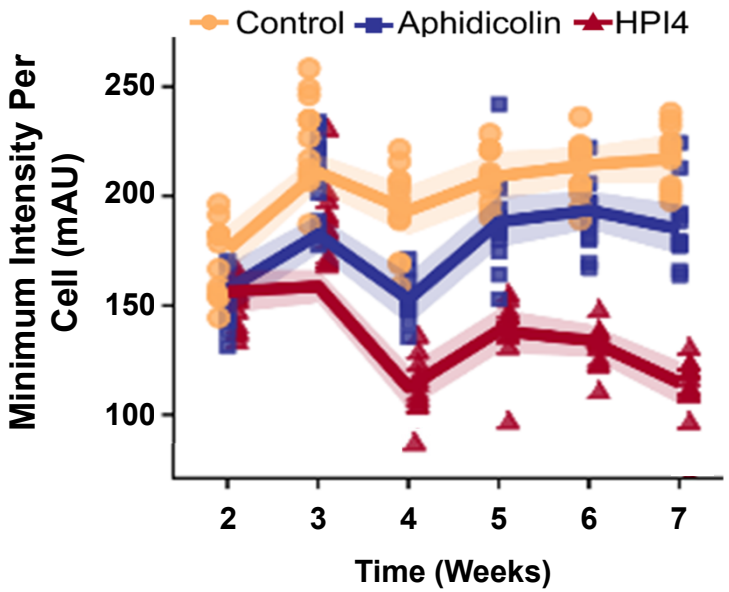
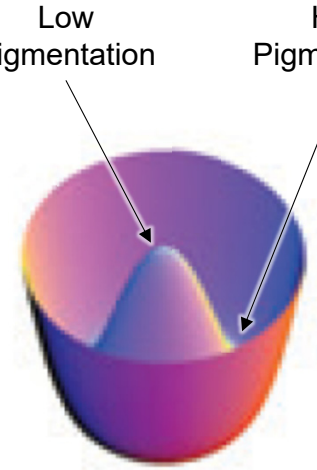
Local heterogeneity is non-homogenous

What is the biological significance of these metrics?

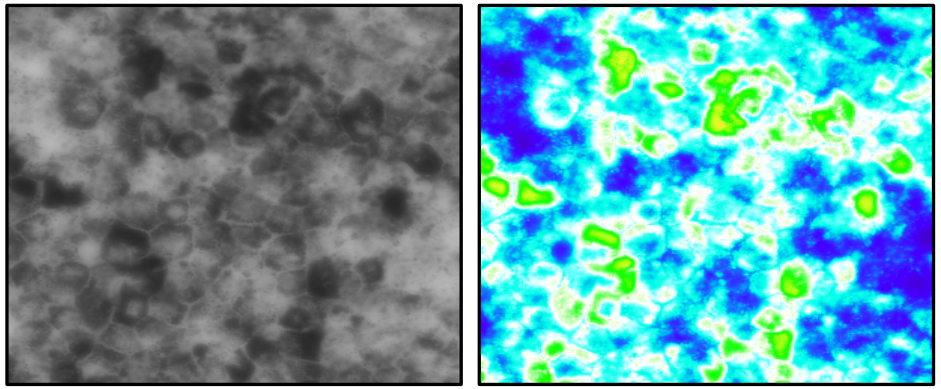


Zernike n4- l0 polynomial

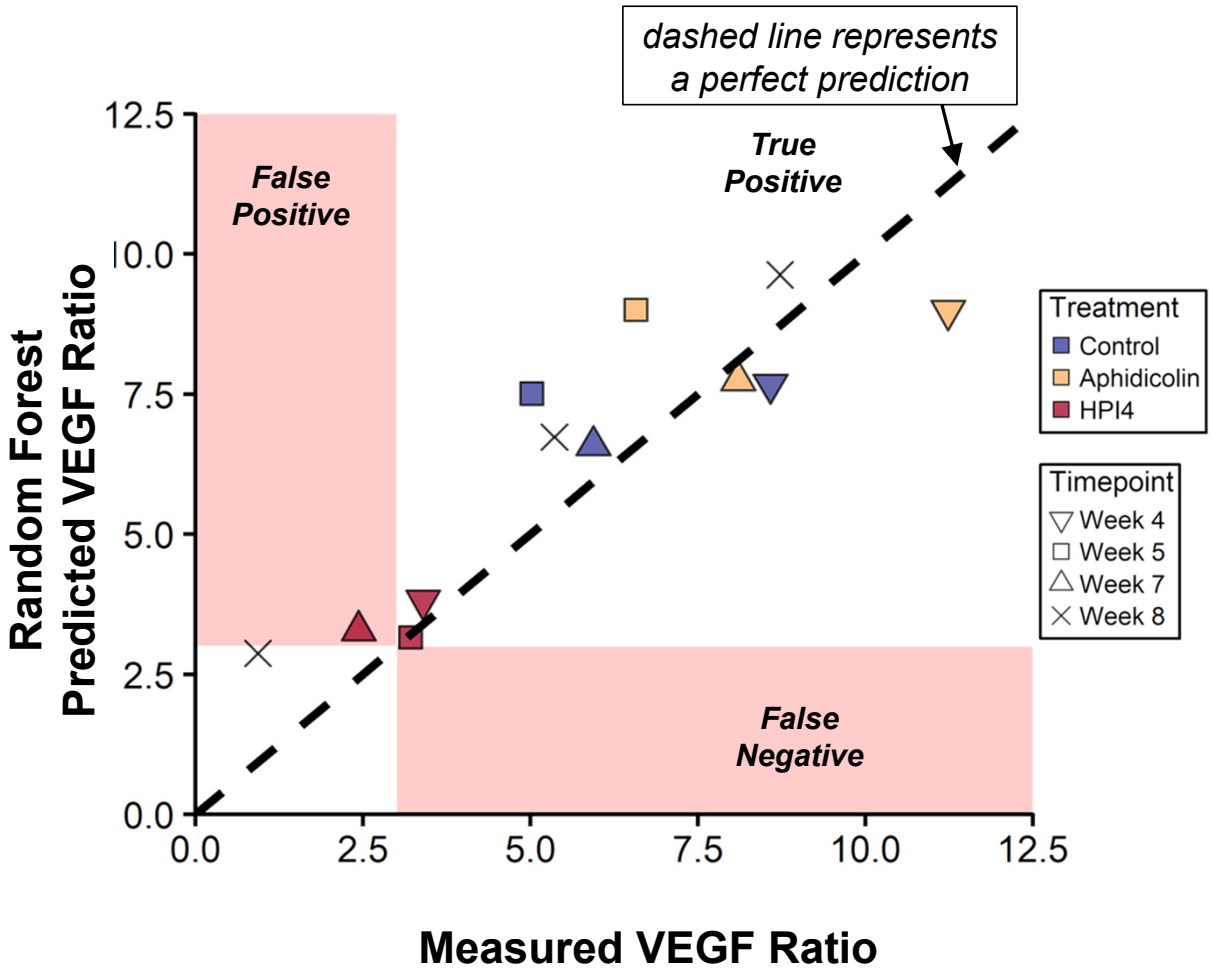
Z_4^0



Lakshminarayanan & Fleck. Zernike polynomials: a guide. J. of Modern Optics, 58, 545-561, 2011



Machine Learning Prediction of VEGF Ratio from WIPP Single Cell Features



Summary of Algorithm Regression Errors	
Algorithm	**VEGF Ratio Root Mean Squared Error**
DNN-F: Deep Learning Neural Network	1.01
RF: Random Forest	1.45
MLP: Multi-Layer Perceptron	1.47
L-SVM: Linear Support Vector Machine	1.59
PLSR: Principle Least-squares Regression	1.65
RR: Ridge Regression	1.84

Results not discussed

- Results confirmed in additional donors…
  - iRPE from 5 albino patients: verify QBAM measurements on iRPE with diagnosable differences in pigmentation
  - iRPE from 2 healthy donors: to verify that QBAM did not impact iRPE maturation
  - Clinical-grade iRPE from 3 AMD donors (and 2 or 3 clones from each of 3 donors): to verify AI predictions from QBAM worked for iRPE from AMD patients
- Using AI to predicting donor identity from QBAM images
  - FDA requires STR phenotyping of for manufactured autologous cell therapies

Conclusions

- Weekly QBAM imaging did not impact iRPE maturation (non-invasive)
- Unprocessed QBAM could predict iRPE function: TER & VEGF ratio
- DNN of unprocessed QBAM images more accurate than segmentation-TML
- Very important that training set have good & bad samples
    - Time: early timepoints less mature, later timepoints more mature
    - Treatments: HPI4 inhibits RPE maturation, aphidicolin promotes
    - BALANCE: If you only feed the AI great samples, then it will predict that the test samples are great

Dissemination

- Schaub NJ, Hotaling NA, Manescu P, Padi S, Wan Q, Sharma R, George A, Chalfoun J, Simon M, Ouladi M, Simon Jr CG, Bajcsy P, Bharti K (2020) Deep learning predicts function of live retinal pigment epithelium from quantitative microscopy. Journal of Clinical Investigation 130, 1010-1023.
  - https://doi.org/10.1172/JCI131187

- QBAM:
  - SQuIRE: Micromanager plugin to collect images
    - Github: https://github.com/Nicholas-Schaub/SQuIRE
  - CARPE: ImageJ plugin fto convert bright-field microscope images into absorbance images
    - Github: https://github.com/Nicholas-Schaub/CARPE

- Data: https://isg.nist.gov/deepzoomweb/data/RPEimplants
  - iRPE from healthy donors
    - Healthy1, live RPE, broadband (232 GB)
    - Healthy2, live RPE, narrowband (291 GB)
  - iRPE from 3 AMD patients (4 GB): 2 or 3 clones per donor, cells were fixed
  - iRPE from 5 albino patients (36 GB): Cells were fixed
  - QBAM and ZO-1 fluorescence images from segmenting routines (4 GB)

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