Nanomechanical Analysis of Renal Tubular Cell Cytoskeleton to Measure Renal Disease

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TARGET 3·4

REDUCE MORTALITY FROM NON-COMMUNICABLE DISEASES AND PROMOTE MENTAL HEALTH

Abstract

This project investigates changes in mechanical properties of kidney cells when exposed to TGF-β1, which is known to induce renal disease [1]. The aim of this project is to provide insight on the progression of diabetic nephropathy from a mechanical perspective based on changes in mechanical properties observed in single cells using atomic force microscopy.

Equations

$$F(\delta) = \frac{4}{3} \cdot \frac{E}{1-\nu^2} \cdot \sqrt{R} \cdot \delta^{3/2} \qquad (1)$$

The Hertz/Sneddon spherical indentation model (Eq. 1) is matched to the force indentation curve to find the apparent elasticity of an experiment.

F::Force
E::Young's Modulus
ν::Poisson's Ratio
R::Indenter Radius
δ::Indentation depth

$$\hat{P}(G_2 \mid x) = \frac{P(x \mid G_2) \cdot P(G_2)}{P(x \mid G_1) \cdot P(G_1) + P(x \mid G_2) \cdot P(G_2)} \qquad (2)$$

The Bayesian classifier is a function based on Bayes theorem that finds a posterior probability (the probability of a precondition given the result).

$P(G \mid x)$::Posterior
$P(x \mid G)$::Likleyhood
$P(G)$::Prior
$P(x)$::Evidence

Where the likelihood of a given group is determined by fitting the observed occurrences to a distribution / Probability Density Function (PDF). 3 distribution models are tested: Gaussian (Eq. 3), Skewed Normal (Eq. 4), and Kernel Density Estimation (Eq. 5).

$$\hat{P}(x \mid G) = \frac{1}{\sigma_G \sqrt{2\pi}} e^{\frac{-1}{2}\left(\frac{x-\mu_G}{\sigma_G}\right)^2} \qquad (3)$$

$$\hat{P}(x \mid G) = \phi(x; \mu_G, \sigma_G) \cdot \Phi\left(\alpha_G \cdot \frac{x-\mu_G}{\sigma_G}\right) \qquad (4)$$

$$\hat{P}(x \mid G) = \frac{1}{nh} \sum_{i=1}^{n} K\left(\frac{x - x_{i_G}}{h}\right) \qquad (5)$$

$\hat{P}(x \mid G)$ :: Group Probability Density Function
x :: Observation (i.e. Young's Modulus)
σ_G :: Group Standard Deviation
μ_G :: Group Mean
$\phi(x; \mu, \sigma)$ :: Normal PDF evaluated at x
α_G :: Group Skew Parameter
$\Phi(z)$ :: Standard Normal CDF
x_{i_G} :: Observed Data Points from Group G
n :: Number of Observations
$K(\cdot)$ :: Kernel Function (i.e. Gaussian)
h :: Bandwidth (Smoothing Parameter)

Introduction

This project investigates the predictive power of renal tubular epithelial cell stiffness as a biomarker for the progression of Diabetic Nephropathy (DN). DN is a common and serious complication of diabetes resulting in kidney failure due to progressive damage to the nephrons, the functional units of the kidney responsible for filtering the blood [2]. This loss of function is due to physical changes at the cellular level induced by cytokine TGF-β 1 associated with an observable change in cytoskeleton stiffness [3].

A force against indentation curve of a cells can be observed using Atomic Force Microscopy (AFM) where the deflection of a very fine probe on a flexible cantilever is measured to detect contact forces. From the spring constant of the cantilever the indentation and force exerted can be determined as the assembly is advanced into the sample. This curve can then be fitted against an elastic deformation model to determine an apparent Young's Modulus (YM).

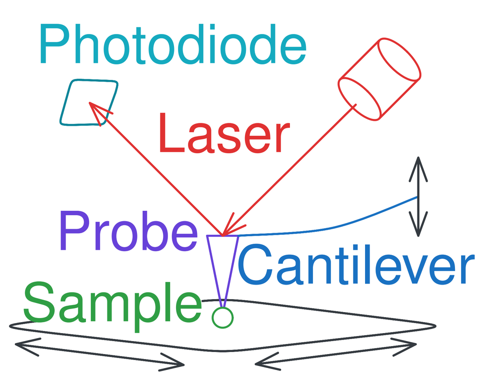

Fig. 1: AFM Diagram

The probability a given cell is healthy or diseased can be predicted from the observed distributions of YM of cells that have not been exposed to TGF-β (Control) and those that have (Treated) by a Bayesian classifier.

Methodology

Observe Cell Response → Elasticity Modeling → Determine Effect Strength → Construct Classifier

Observe Cell Response
- Single cell indentation tests via atomic force microscopy X5 per Cell
- Pre-processing raw data to force vs indentation depth curves

Elasticity Modeling
- Estimate YM via for each test by fitting observed response to an indentation model
- Estimate apparent YM for each cell and account for uncertainty and systemic error

Determine Effect Strength
- Estimate healthy vs diseased group characteristics, and uncertainty
- Quantify statistical significance and predictive power of the observed effect

Construct Classifier
- Determine suitable likelihood probability density functions
- Construct Bayesian classifiers and assess performance

Results

Discussion

Applying the methodology described on larger datasets would allow for a more robust estimate with more extensive testing and validation.

References

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