Nanoscale mechanical analysis of biological cells in diabetic nephropathy

Project ID, Title: 19174, Nanomechanical analysis of tubular cell cytoskeleton
Student ID, Name: 27047440, Joseph Ashton
Supervisor Name, Email: Eleftherios Siamantouras, esiamantouras@lincoln.ac.uk
Module Leader Name, Email: Pouriya H Niknam, phniknam@lincoln.ac.uk

Assessment Name: Proposal
Module ID, Name: EGR3024, Individual Project
Institution: University of Lincoln

Project Proposal

Executive Summary

This document outlines a 3rd year individual project. In this project atomic force nanoindentation data of kidney tubular cells representing healthy and diabetic states will be processed and analysed. The key objective of this project is to quantify any difference in effective young's modulus between the healthy and diabetic samples.

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Background

Diabetic nephropathy is a serious complication of both type 1 and 2 diabetes resulting in kidney failure due to progressive damage to the nephrons, functional units responsible for filtering the blood.[1] One mechanism that contributes to this damage are mechanical alterations to the F-actin cytoskeleton that provide the structure of the renal tubules[2], a component of the nephron, these fine vessels line blood vessels and allow desirable molecules and ions to diffuse back into the blood.[1-1] The cytoskeleton is a semisolid gel layer within the cell comprised of bundles of actin proteins held together by binding proteins providing mechanical structure and support to the cell.[2-1] late stage diabetic nephropathy is associated with changes in the expression of actin binding proteins which in turn change the mechanical properties of the cytoskeleton of tubules. These changes can include reduced cell adhesion, cell coupling and cell-to-cell communication which have profound effects on overall integrity and function of the tubule.[3] As Diabetes is also associated with high blood pressure and inflammation these potentially weakened structures are under additional stress promoting kidney failure.

An improved understanding of the changes of the mechanical properties of tubular cell cytoskeleton can lead to novel understanding of the disease and development of single cell diagnostics and therapies related to nanomedicine.

The study of the physical properties of living cells is challenging, most techniques that would provide suitable resolution require manipulations like staining drying or freezing which compromise the validity of the results. Atomic force microscopy provides a non destructive means of observing single cells close to their native state.[4] Atomic force microscopes use the deflection of a very fine probe on the end of a cantilever to measure contact forces against a sample. In a process called nanoindentation a single cell can be advanced into the probe measuring the deflection of the cantilever against stage height to produce a force vs separation distance curve to gain insights into the physical properties of the cell.[5]

Project Summary

This project aims to quantify the change in tubular cell elasticity in healthy and diabetic states though analysis of nanoscale force-displacement data from AFM-single cell force spectroscopy (SCFS) measurements on individual cells. This project will require fine processing of nanoscale data, numerical analysis, development of data sets and statistical comparison.

Objectives

- Research
  - Identify the relevant Hertzian contact mechanics model and understand how the model applies to nano indentation force displacement curves
  - Conduct brief literature review on force displacement curves on single cell experiments
- Process raw data into force displacement curves
  - Identify errors and correct/reject curves as appropriate
  - Identify features such as:
    - contact/separation points
    - Linear region
    - features of the curve that correspond with the molecular structure.
- Statistical analysis
  - Calculation of Young's modulus
  - Graphs comparing healthy and diseased cells
  - If viable any parameters that may be effective for diagnosis along with Identifying Optimal indentation depth for tests

Deliverables

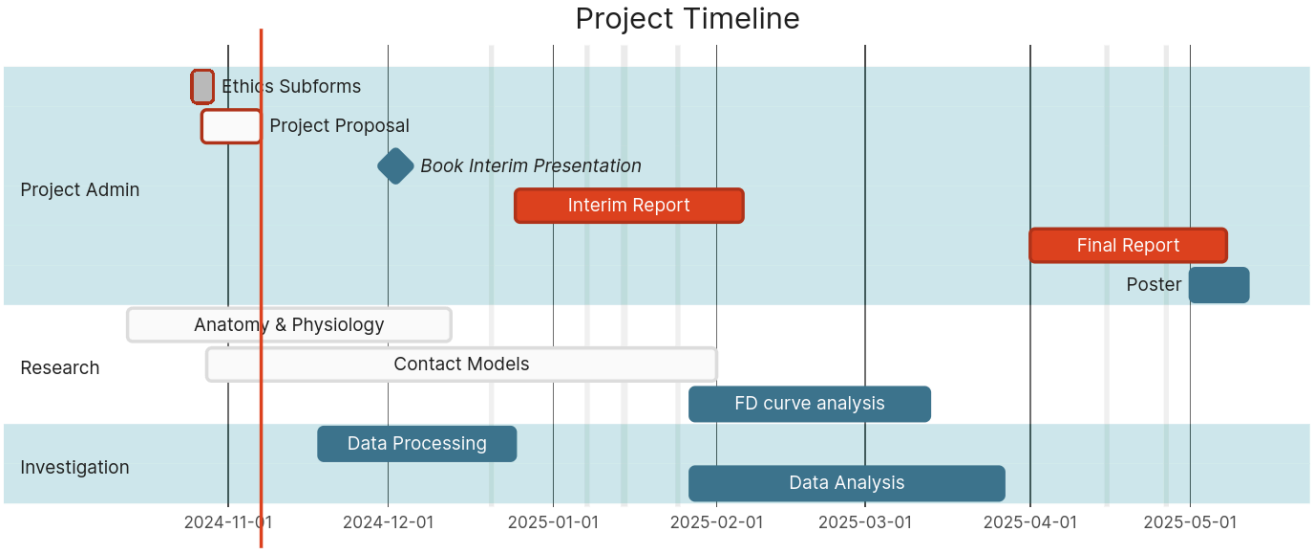
The first stage of this project that broadly lines up with the first semester will be spent on research and processing the data into force displacement curves and will conclude in an interim report and oral presentation.

The second stage of this project that takes place over the second semester will be focused on extracting insights from the force displacement curves with numerical

calculations for young's modulus and identification of features that may prove useful for distinguishing healthy from diseased samples. This will be presented in a dissertation like report along with an academic poster.

Project Outline

Gantt Chart



Research / Project Requirements

During the first month of the project some time will be dedicated to the broader background of the project, putting into context the data that will be analysed. This means a rudimentary understanding of atomic force microscopy, the means by which the data was produced, as well as a working understanding of the relevant physiology.

In order to process the data into a form conducive to this investigation I will need to build a working knowledge of the JPK signal processing software package along with developing my data processing skill set in python.

A suitable model for the interaction must be developed to interoperate the results. This will involve building a working knowledge of the various mathematical models of the behaviours at play and an appreciation for their respective strengths and weaknesses.

Data Processing

The raw data must be scrutinised for validity & viability, where systematic or recording errors are found the datasets will be corrected or rejected as appropriate.

The raw data is recorded in the from of photodiode voltage versus the scanner position, this must be converted to force displacement curves. This involves accounting for things like;

- calibration of the equipment,
- elasticity of the cantilever,
- relative displacement of the probe,
- point of contact with the cell membrane,

Once the curves can be effectively plotted their features must be identified and labelled such as;[6]

- the point of contact with the cell membrane,
- the region of linear elastic deformation,
- features that describe the molecular structure,
- any influential non-idealities.

Analysis

The the linear regions of the curves, produced in the Data Processing stage, are investigated using an appropriate contact model, as found in the Research stage. This will be to to determine young's modulus, first of the graphs, then the cells, then healthy vs diseased. When dealing with nano scale data this can require accounting for or overcoming complexities like;[5-1]

- dielectric attraction / repulsion,
- hydrophilic repulsion & surface tension,
- viscosity of the fluids in the cell,
- contamination of the probe tip,
- out of plane relative motion.

Once the curves are understood visualisations will be produced in order to compare the behaviours of healthy and diseased cells. This will be in the hopes of identifying parameters that could be useful in single cell diagnosis.

References

1. A. Madrazo-Ibarra and P. Vaitla, “Histology, Nephron,” 01-Jan-2024. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK554411/. [Accessed: 31-Oct-2024]. ↩ ↩
2. G. M. Cooper, “Structure and Organization of Actin Filaments,” 01-Jan-2000. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK9908/. [Accessed: 31-Oct-2024]. ↩ ↩
3. C. E. Hills, E. Siamantouras, S. W. Smith, P. Cockwell, K.-K. Liu, and P. E. Squires, “TGFβ modulates cell-to-cell communication in early epithelial-to-mesenchymal transition,” 01-Mar-2012. [Online]. Available: https://link.springer.com/article/10.1007/s00125-011-2409-9#Sec1. [Accessed: 06-Nov-2024]. ↩
4. Y. F. Dufrêne, “Atomic Force Microscopy, a Powerful Tool in Microbiology,” 01-Oct-2002. [Online]. Available: https://journals.asm.org/doi/10.1128/jb.184.19.5205-5213.2002. [Accessed: 06-Nov-2024]. ↩
5. H.-J. Butt, M. Jaschke, and W. Ducker, “Measuring surface forces in aqueous electrolyte solution with the atomic force microscope,” 01-Aug-1995. [Online]. Available: https://www.sciencedirect.com/science/article/pii/030245989501800T?via%3Dihub. [Accessed: 06-Nov-2024]. ↩ ↩
6. Siamantouras E, et al, Quantifying cellular mechanics and adhesion in renal tubular injury using single cell force spectroscopy. Nanomedicine: NBM 2016;12:1013-1021, http://dx.doi.org/10.1016/j.nano.2015.12.362 ↩

Annexes

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Meeting Log

First Meeting with Eleftherios Siamantouras

2024-10-22 @ 11:00

Summarising project breif

there is some AFM-single cell force spectroscopy (SCFS) data, this is measuring:

- cell to cell adhesion (not feasible)
- Cell elasticity

contact mechanics of biological systems.

Cells, being fluid covered by cytoskeleton have complex mechanical properties.

At the molecular level not all proteins return to their original shape when deformed, this is largely due to the energy dissipated by the friction of the fluid.

This can be understood with the Hertzian contact mechanics model.

Project

1. Processing of force displacement curves
2. Analysis, calculation, of Young's modulus
3. Finite element analysis or Statistical analysis,
4. JPK Signal Processing
5. SPSS or Python

Next steps

Look at Loading and unloading Force displacement, especially elastic component of loading of the molecular structure that corresponds to force displacement indentation, F-actin cytoskeleton.

About Siamantouras

From Athens, Greece

Built device, had a kid,

World leading expert on single cell force microscopy

Mechanical Biology applications

soft Bio materials
Most commercial
Single cell diagnostics - 10 years ahead
Dr Siamantouras area of expertise
mechanotransduction - 30 years ahead
About merging biochemistry with bio-mechanics.

ToDo

Write up Background and future aspirations.

Look into
https://www.researchgate.net/publication/272825925_Contact_Mechanics
https://en.wikipedia.org/wiki/Contact_mechanics

JPK Data Processing 3.4

Meeting Reviewing Project Proposal

2024-11-05 @ 14:15

Misc Notes

In order to avoid viscoelastic drag all measurements where recorded with a velocity of less than 5 micrometers per second.

Single section for anatomy and pathophysiology.

Read: **claire hills diabetologia 2012** specifically:

- abstract
- atomic force spectroscopy
    - association between loss of structure (ie mechanics) and loss of function.

Indentor will have only moved through 10% of

Each cell has 3 force displacement curves, and will require 3 analysis.
Optimal indentation depth

Do analysis in JPK software, do notation in excel

Aims

Use mechanics to understand loss of function of the tubular part of the nephron by analysing atomic force microscopy nano indentation.

By taking a mechanical approach we avoid the prohibitively complicated biochemistry.

Key objectives

- Identify and Understand the relevant Hertzian contact mechanics model
- How the model applies to nano indentation force displacement curves
- Conduct brief literature review on force displacement curves on single cell experiments
- Process raw force displacement data,
    - Identify errors and correct/reject curves as appropriate
        - systematic
        - recording
    - Identify contact point
        - Identify contact with cell membrane

    - Identify Linear region
    - Identify molecular structure that correspond with features of the curve
    - Apply model
- Calculation of Young's modulus
    - Refine parameters
- Statistical analysis
    - Graphs between healthy and diseased cells
    - Which parameters are most effective for diagnosis
    - Identify Optimal indentation depth

Collaborative workflows

Whenever asking for edits/corrections we will use word with tracked changes.

ToDo

Make edits to Diabetic nephropathy Individual Project Proposal and send to Eleftherios Siamantouras for final comments.

Project Brief

Breif

Diabetic nephropathy is a serious complication of diabetes resulting in end-stage kidney failure, a life-threatening condition that requires dialysis or kidney transplantation. Progression of the disease is associated with complex defects at the cellular and molecular level of the kidney's filtering unit, the nephron. The condition ultimately leads to dysfunction of the tubular structure of the nephron that is responsible for filtration, with devastating effects for the organ.

This project focuses on the investigation of mechanical alterations of tubular cells, which are responsible for the structural integrity of the nephron. Nanomechanical analysis at single cell level in healthy and diseased conditions, contains valuable information about the dynamic behaviour of the cellular network, called the cytoskeleton, which maintains the physiological structure of cells. Analysis of the mechanical changes of the cytoskeleton in diabetic nephropathy can lead to novel understanding of the disease and development of single cell diagnostics and therapies related to nanomedicine.

However, due to the intricate nature of the cell at the molecular level, mechanical data are often complex, requiring fine processing, iterative modelling and sound statistical analysis. The aim of this project is to quantify mechanical properties of biological cells in healthy and diseased states by developing analytical methods to process nanoscale force-displacement measurements and calculate the mechanical properties of cells using mathematical models. The experimental data are available in a raw form and tasks include fine processing of nanoscale data, numerical analysis, development of data sets and statistical comparison between two or more variables. The results of the statistical analysis will be associated with the underlying molecular changes of the cytoskeleton during diabetic nephropathy and the potential for mechanical diagnostic information at the cellular level will be addressed.

Understanding the Project brief

Diabetic Nephropathy

Summary

Nephropathy means kidney disease.

Background

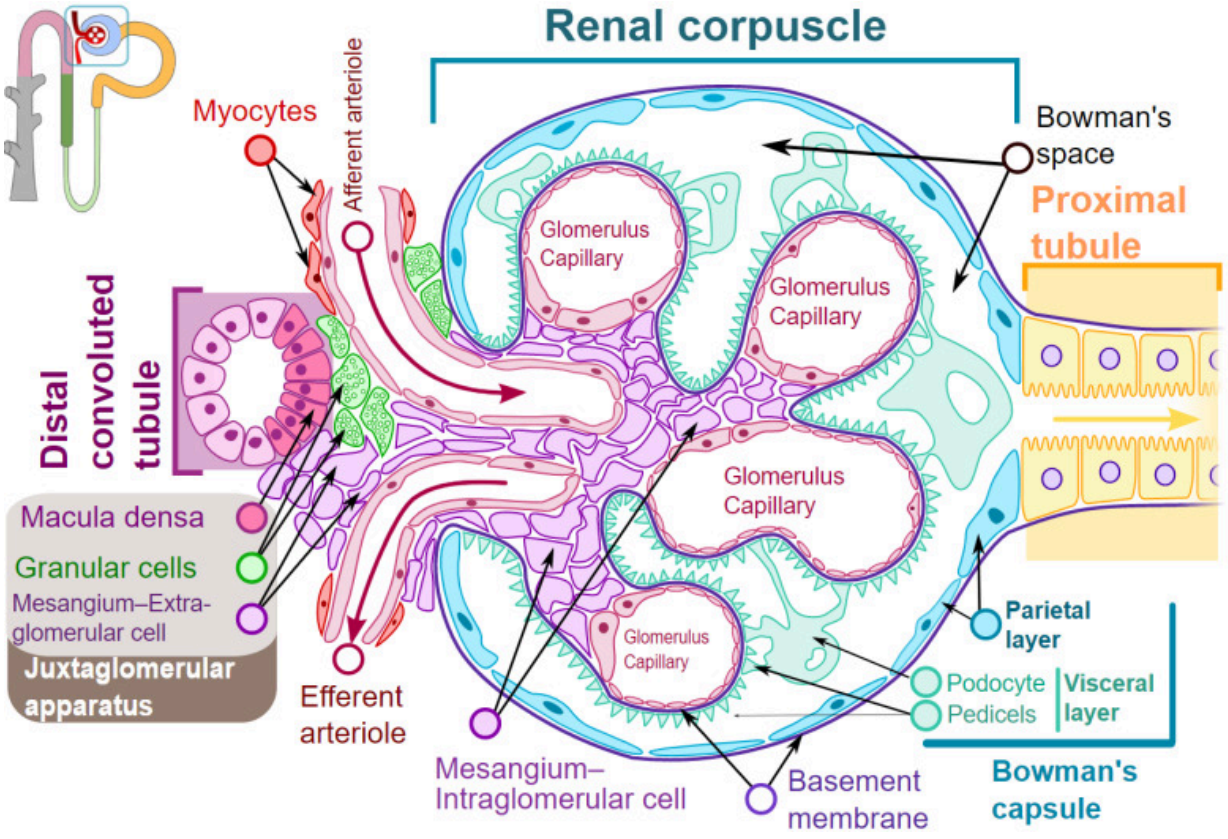
Anatomy

Kidney

Nephron

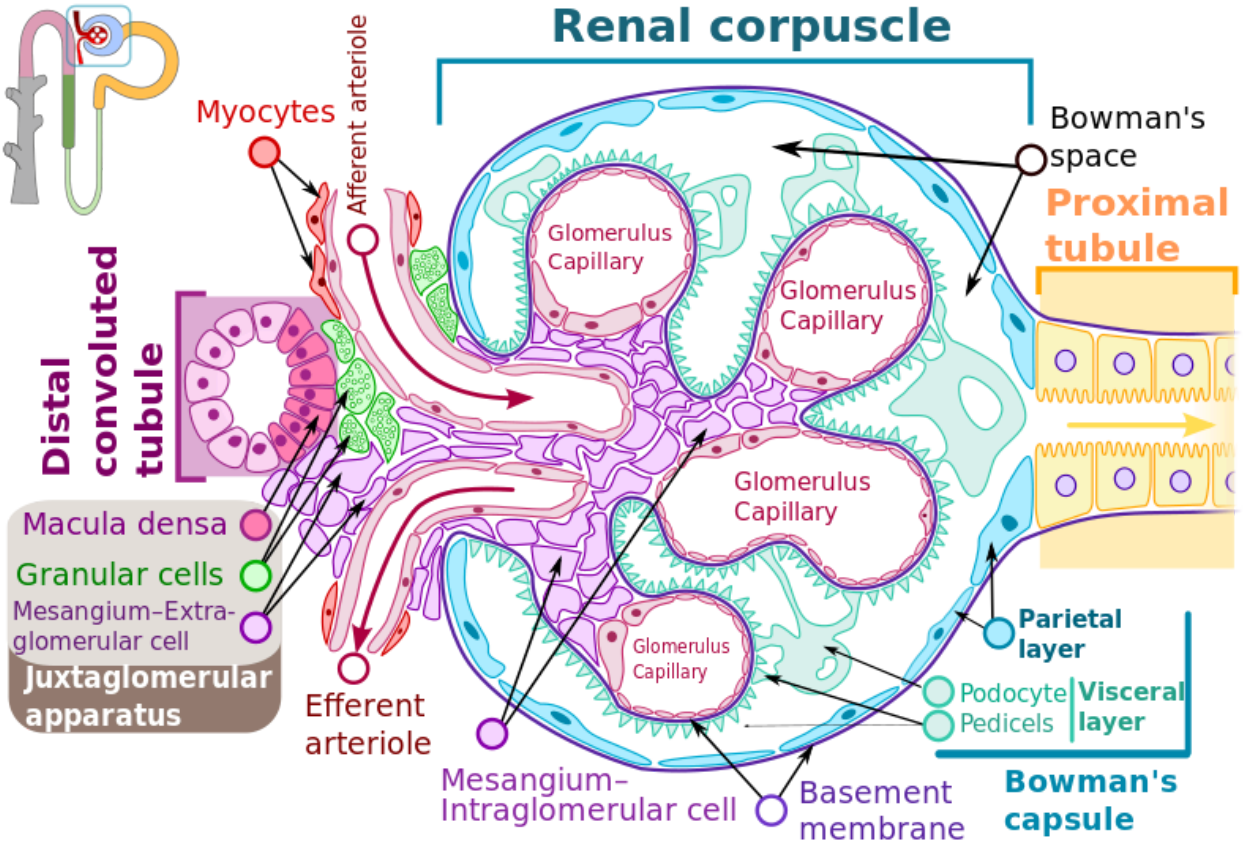
Summary

The Nephron are the filtration units of the kidney.



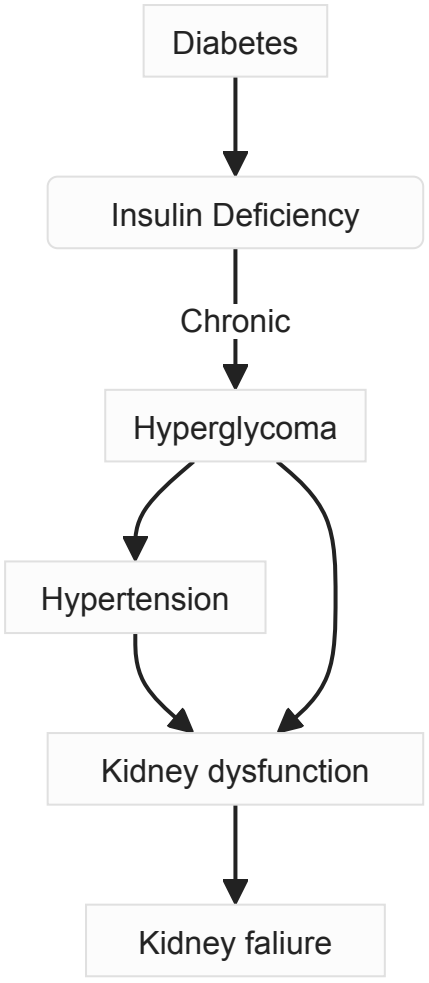
Glomerulus

A network of capillaries at the begining of a nephron in the kidney



Mechanism

Mechanism Overview



https://www.youtube.com/watch?v=sGt-Wxde_6Q

Contact Mechanics

Standard cases

Cylindrical contact

Spherical contact

Elastic

$$F = \frac{4}{3} E' \sqrt{R}\, \omega^{3/2}$$

$$A = \pi R \omega = \pi a^2$$

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Point of Yielding

The following 3 equations give the critical values of deformation, contact area, and contact force at the point where the stress is sufficient to cause the material to yield:

$$\omega_c = \left(\frac{\pi \cdot S_y \cdot C}{2E'} \right)$$

$$A_c = \pi^3 \left(\frac{S_y C R}{2E'} \right)^2$$

$$P_c = \frac{4}{3} \left(\frac{R}{E'} \right)^2 \left(\pi \cdot S_y \cdot \frac{C}{2} \right)^2$$

Where:

- ω_c : is the deformation at the onset of plastic deformation
- A_c : is the contact area at the onset of plastic deformation
- P_c : is the contact force at the onset of plastic deformation
- S_y : is the yield strength
- C : is the critical yield stress coefficient, in this case approximated as: $C = 1.295e^{0.736\nu}$
  where:
  - ν : is Poisson's ratio
- R : is the equivalent radius, given by the superposition of the radii of the contacting surfaces:
  $\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2}$
- E' : is the effective Young's modulus, given by:
  $\frac{1}{E'} = \frac{1-\nu_1^2}{E_1} + \frac{1-\nu_2^2}{E_2}$
  where:
  - E : is Young's modulus
  - ν : is Poisson's ratio
  - $_1$ & $_2$: indicate the different surfaces

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References

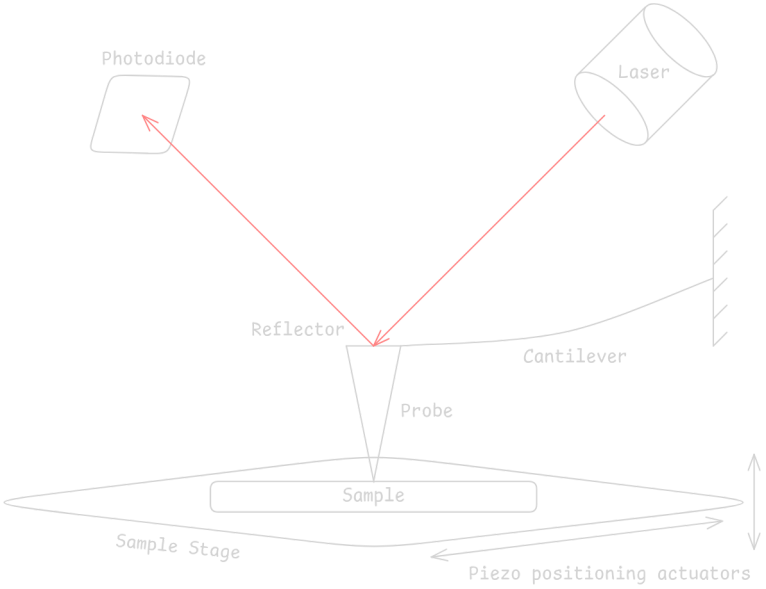
1 R. L. Jackson, H. Ghaednia, H. Lee, A. Rostami, X. Wang, P. L. Menezes, M. Nosonovsky, S. P. Ingole, S. V. Kailas, and M. R. Lovell, “Contact Mechanics,” 01-Jan-2013. Online. Available: https://link.springer.com/chapter/10.1007/978-1-4614-1945-7_3. [Accessed: 28-Oct-2024].

Atomic Force Microscopy

Summary

Mechanism

Atomic force microscopes use the deflection of a very fine probe on a flexible cantilever to detect atomic contact forces.



The sample stage can be moved precisely in all directions by piezoelectric actuators.
When the probe is acted on by contact forces from the sample will it will be deflected this in turn deflects the path of the reflected laser beam. Deviations of the beam are recorded by the photodiode. [1]

Modes

Contact

When probe tip is pushed into the sample to be deflected primarily by the short range repulsive forces.

Non Contact

This uses dynamic control to maintain constant distance from the sample.

Tapping

The probe is oscillated close to the natural frequency of the cantilever and interaction forces are identified by deviations from the induced oscillation.

Single cell

Force displacement curves

The sample cell can be advanced into the probe at a fixed x&y measuring the deflection of the cantilever against sample stage z (height). This can be converted to a force vs separation distance curve to gain insights into the physical properties of the cell.3

3 Y. F. Dufrêne, “Atomic Force Microscopy, a Powerful Tool in Microbiology,” 01-Oct-2002. [Online]. Available: https://journals.asm.org/doi/10.1128/jb.184.19.5205-5213.2002. [Accessed: 06-Nov-2024].

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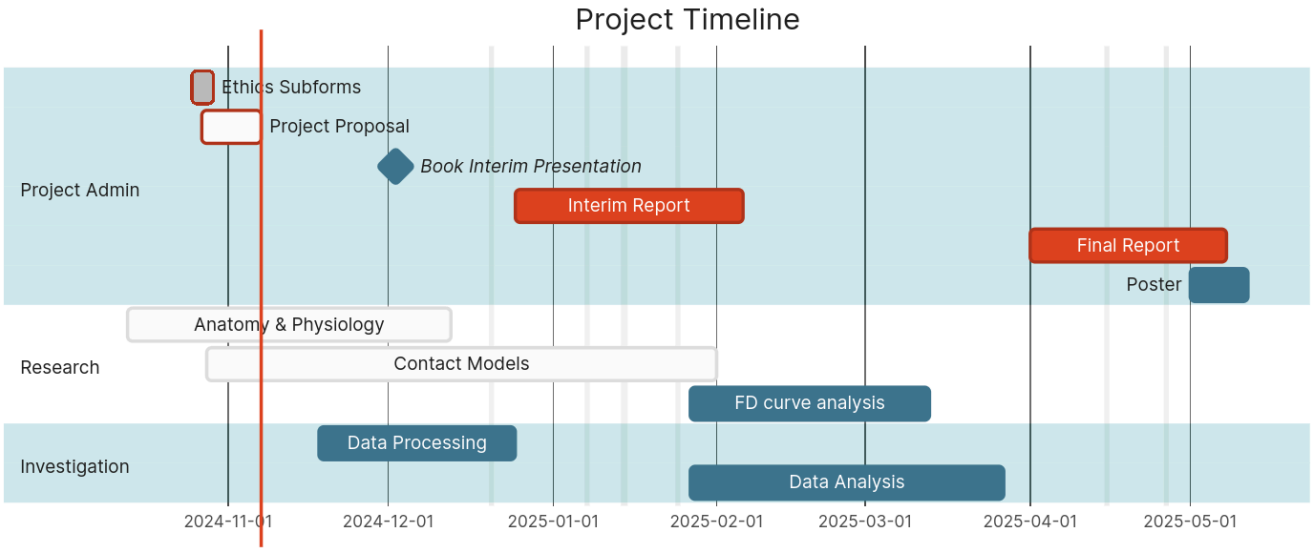
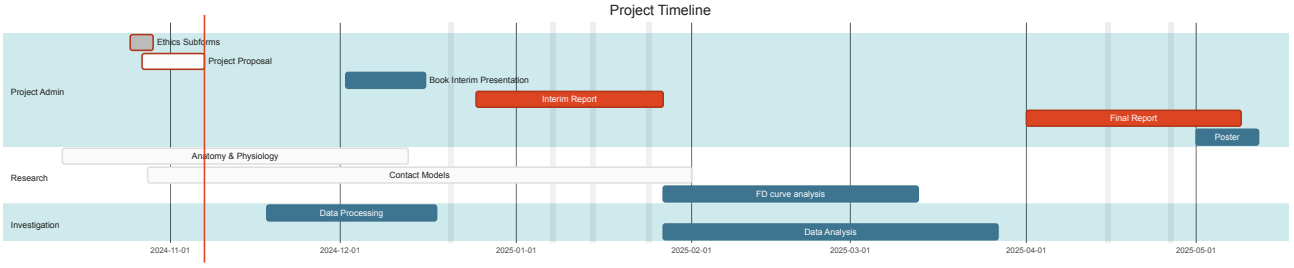
1. Nanoscience.de, “Scanning Probe Methods Group.” [Online]. Available: http://www.nanoscience.de/HTML/methods/afm.html. [Accessed: 06-Nov-2024]. ↩

Gantt chart





Mermaid

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section Project Admin
        Ethics Subforms :crit, done, 2024-10-25, 4d
        Project Proposal :crit, active, 2024-10-27, 2024-11-07
        Book Interim Presentation : 2024-12-02, 2w
        Interim Report :crit, 2024-12-25, 2025-01-27
        Final Report :crit, 2025-04-01, 2025-05-09
        Poster : 2025-05-01, 2025-05-12

section Research
        Anatomy & Physiology :active, 2024-10-13, 2M
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        Contact Models :active, 2024-10-28, 3M
        FD curve analysis: 2025-01-27, 45d

section Investigation
        Data Processing :2024-11-18 , 1M
        Data Analysis :2025-01-27, 2M

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Current Reading list

Contact mechanics

https://pmc.ncbi.nlm.nih.gov/articles/PMC3615644/

Pathophysiology of Diabetic Nephropathy

https://www.youtube.com/watch?v=sGt-Wxde_6Q

Anatomy and Physiology

https://www.ncbi.nlm.nih.gov/books/NBK9908/

https://www.britannica.com/science/kidney

https://www.sciencedirect.com/science/article/pii/B9780323086912000028

https://www.ncbi.nlm.nih.gov/books/NBK554411/

Single cell force spectroscopy

https://www.youtube.com/playlist?list=PL3592A61EEF52B29A

https://www.youtube.com/playlist?list=PLtkeUZItwHK748B1OpjtnlPLULyIUnQm6
https://www.youtube.com/playlist?list=PLtkeUZItwHK78uGpH76_hhsehADDWnKQB