NANOMECHANICAL ANALYSIS OF TUBULAR CELL CYTOSKELETON

PROGRESS REPORT

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INTRODUCTION

- **Introduction**
- **Assessment Criteria**
- **Project Overview**
- Current Progress
- Reflection
- Future Plans

ASSESSMENT CRITERIA

- *Learning objectives stated in assessment brief, edited for brevity.*

- **(LO2) Propose solutions to unfamiliar problems**
- **(LO4) Investigate a problem using appropriate tools**
- **(LO5) Document the process**
- **(LO7) Communicate technical information**
- **(LO8) Time/Project management**

PROJECT OVERVIEW

Aims of the Project

- Determine the change in elasticity of tubular cells under the conditions of diabetic nephropathy
- Propose a method for quantifying progression of DN bases on single cell indentation data

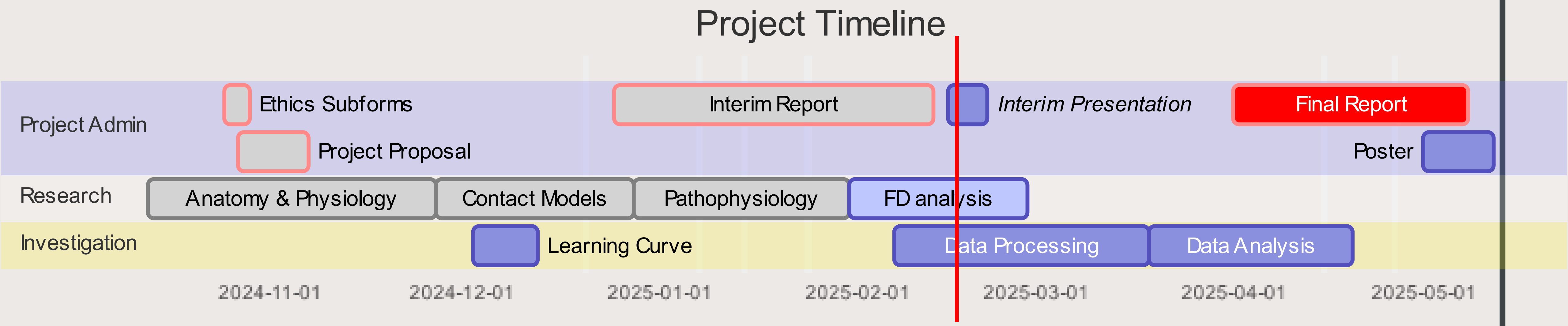
Why is it important?

- 30% of people with type 2 diabetes reach ESRD [1]
- Diabetic nephropathy increases mortality by 23% [2]

[1] G. L. Bakris, ‘Update on reducing the development of diabetic kidney disease and cardiovascular death in diabetes’, Kidney Int Suppl (2011), vol. 8, no. 1, p. 1, Jan. 2018, doi: 10.1016/j.kisu.2017.10.002.

[2] M. Afkarian *et al.*, ‘Kidney Disease and Increased Mortality Risk in Type 2 Diabetes’, *J Am Soc Nephrol*, vol. 24, no. 2, pp. 302–308, Jan. 2013, doi: 10.1681/ASN.2012070718.

PROJECT TIMELINE



CURRENT PROGRESS

Milestones

- Completed Literature review
- Produced first set of Young's modulus results

Roadblocks

- Have not had meeting time to discuss Learning Curve method
- Access to full experiment results dataset

Schedule Implications

- Lack of progress on data analysis
- More work to do in March & April

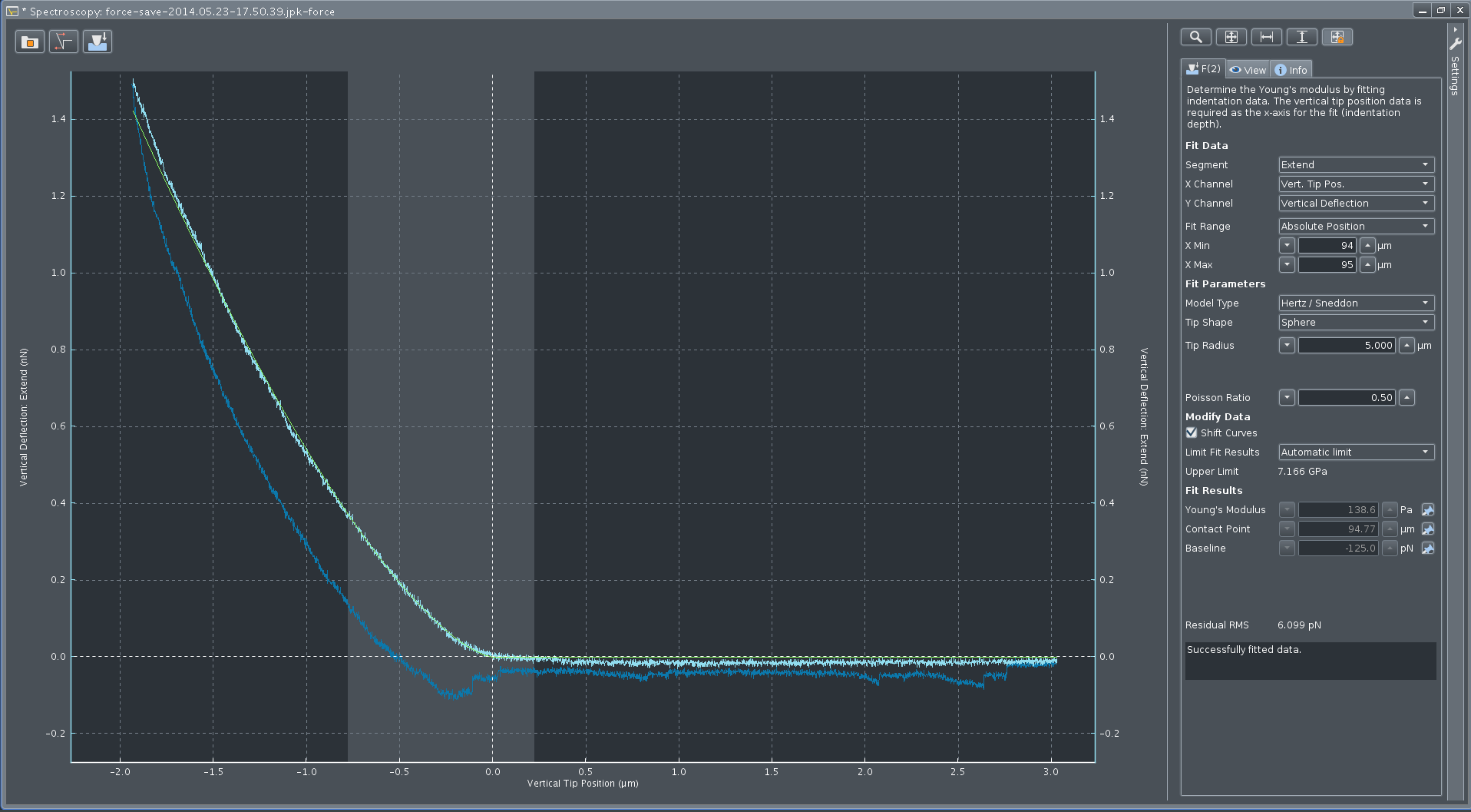
LITERATURE REVIEW

Paper	Cell Type	Young's Modulus
E. Siamantouras, C. E. Hills, P. E. Squires, and K.-K. Liu, 'Quantifying cellular mechanics and adhesion in renal tubular injury using single cell force spectroscopy', *Nanomedicine: Nanotechnology, Biology and Medicine*, vol. 12, no. 4, pp. 1013–1021, May 2016, doi: 10.1016/j.nano.2015.12.362.	**HK2** immortalised human kidney proximal tubule epithelial cell culture	**control:** 320Pa **TGF-β1:** 549 Pa
A. Jafari, A. Sadeghi, and M. Lafouti, 'Mechanical properties of human kidney cells and their effects on the atomic force microscope beam vibrations', *Microsc. Res. Tech.*, vol. 87, no. 8, pp. 1704–1717, 2024, doi: 10.1002/jemt.24543.	**HEK-293** immortalised human embryonic kidney cell culture	539.8 Pa
Y. Shimizu, T. Kihara, S. M. A. Haghparast, S. Yuba, and J. Miyake, 'Simple Display System of Mechanical Properties of Cells and Their Dispersion', *PLOS ONE*, vol. 7, no. 3, p. e34305, Mar. 2012, doi: 10.1371/journal.pone.0034305.	**HEK-293** immortalised human embryonic kidney cell culture	410 Pa

[3] E. Siamantouras, C. E. Hills, P. E. Squires, and K.-K. Liu, 'Quantifying cellular mechanics and adhesion in renal tubular injury using single cell force spectroscopy', Nanomedicine: Nanotechnology, Biology and Medicine, vol. 12, no. 4, pp. 1013–1021, May 2016, doi: 10.1016/j.nano.2015.12.362.

[4] A. Jafari, A. Sadeghi, and M. Lafouti, 'Mechanical properties of human kidney cells and their effects on the atomic force microscope beam vibrations', Microsc. Res. Tech., vol. 87, no. 8, pp. 1704–1717, 2024, doi: 10.1002/jemt.24543.

[5] Y. Shimizu, T. Kihara, S. M. A. Haghparast, S. Yuba, and J. Miyake, 'Simple Display System of Mechanical Properties of Cells and Their Dispersion', PLOS ONE, vol. 7, no. 3, p. e34305, Mar. 2012, doi: 10.1371/journal.pone.0034305.


* Spectroscopy: force-save-2014.05.23-17.50.39.jpk-force
Vertical Deflection: Extend (nN)
Vertical Tip Position (µm)
F(2)
View
Info
Settings
Determine the Young's modulus by fitting indentation data. The vertical tip position data is required as the x-axis for the fit (indentation depth).
Fit Data
Segment Extend
X Channel Vert. Tip Pos.
Y Channel Vertical Deflection
Fit Range Absolute Position
X Min 94 µm
X Max 95 µm
Fit Parameters
Model Type Hertz / Sneddon
Tip Shape Sphere
Tip Radius 5.000 µm
Poisson Ratio 0.50
Modify Data
Shift Curves
Limit Fit Results Automatic limit
Upper Limit 7.166 GPa
Fit Results
Young's Modulus 138.6 Pa
Contact Point 94.77 µm
Baseline -125.0 pN
Residual RMS 6.099 pN
Successfully fitted data.

CONTACT MODELS

Model	Force-Indentation relationship
Hertz [6, 7]	$$F = \frac{4}{3} E' \sqrt{R}\ \omega^{3/2}$$
DerjaguinMuller-Toporov (DMT) [6, 7]	$$F = F_{Hertz} - F_{det}$$ $$\delta = \frac{a}{2} \ln \frac{R_i + a}{R_i - a}$$
Fung [7, 8]	$$F = B\pi\left(\frac{a^5 - 15Ra^4 + 75R^2a^3}{5Ra^2 - 50R^2a + 125R^3}\right)\exp\left[b\left(\frac{a^3 - 15Ra^2}{25R^2a - 125R^3}\right)\right]$$

[6] H.-J. Butt, M. Jaschke, and W. Ducker, ‘Measuring surface forces in aqueous electrolyte solution with the atomic force microscope’, Bioelectrochemistry and Bioenergetics, vol. 38, no. 1, pp. 191–201, Aug. 1995, doi: 10.1016/0302-4598(95)01800-T.

[7] D. C. Lin, D. I. Shreiber, E. K. Dimitriadis, and F. Horkay, ‘Spherical indentation of soft matter beyond the Hertzian regime: numerical and experimental validation of hyperelastic models’, Biomech Model Mechanobiol, vol. 8, no. 5, pp. 345–358, Oct. 2009, doi: 10.1007/s10237-008-0139-9.

[8] Y. Fung, ‘Elasticity of soft tissues in simple elongation’, American Journal of Physiology-Legacy Content, vol. 213, no. 6, pp. 1532–1544, Dec. 1967, doi: 10.1152/ajplegacy.1967.213.6.1532.

REFLECTION ON PROGRESS

What Can Be Improved?

- Assessed work should be polished
- More results focused
- More oversight and discussion

Proposed Systematic Changes

- Work should be drafted, discussed and reviewed with supervisor before submission
- Bi-weekly check ins / Progress updates

FUTURE PLANS

Immediate Future

- Refine JPK process
- Create dataset from experimental data

Rest of Project

- Import data to Matlab
- Determine statistical significance
- Propose method for quantifying cells as diseased vs healthy

PURPOSE OF THE INTERIM ASSESSMENT

- Evaluate the student’s technical understanding, progress and future plans.
- Ensure the student’s approach to project planning and project management will allow them to complete their project.
- Uncover any problems within the project and provide feedback to help the student resolve these issues.
- Provide feedback from a member of academic staff other than the student’s supervisor.

- *Stated purpose taken from assessment brief, edited for brevity*

THANK YOU

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