

# **Example Thesis/Dissertation**

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## **THESIS/DISSERTATION TITLE**

by

Full Name

A dissertation submitted to the faculty of  
The University of Utah  
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of xx

The University of Utah

xx yyyy

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# The University of Utah Graduate School

## STATEMENT OF DISSERTATION APPROVAL

The dissertation of \_\_\_\_\_ **Full Name** \_\_\_\_\_

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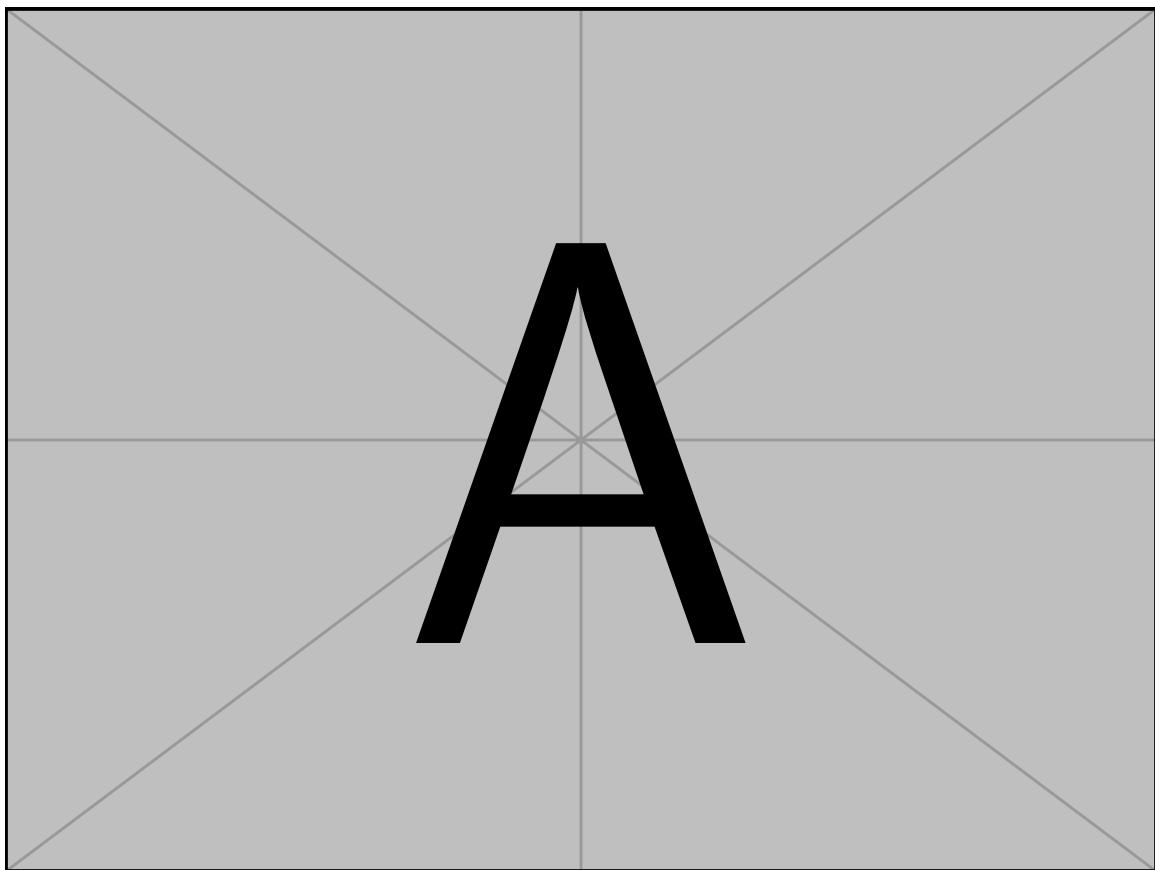
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## **ABSTRACT**

The usability of dissertation abstracts depends largely on their content. Many journals within the medical community have settled on a seven sentence structure, which is also gaining acceptance in the social sciences, education and business. In it, the purpose of the study and methodological choices are outlined succinctly, allowing the reader or researcher to quickly scan and evaluate a number of studies to easily choose ones that meet their particular demands. The structure contains variations on the following seven sentence stems: "The purpose of this study is...." "The scope of this study...." "The methodology...." "The Findings..." "Conclusions reached are ..." "Limitations of this study include...." "This study contributes...." Abstracts of dissertation proposals contain the same seven concepts, substituting data collection and analysis in place of findings and conclusions. Abstracts are limited in the United States by the UMI to 350 words.

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“Quote”

---

—Famous Individual

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2.5	Example table 5 ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula. . . . .	49
4.1	... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.	92
4.2	... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.	93
4.3	... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.	101

- 4.4 ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula. 102
- 4.5 ...Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. . . . . 109

## NOTATION AND SYMBOLS

---

x	<i>Var</i>	- the variable 'x'.
y	<i>y</i>	- the variable 'y'.
m	<i>Slope</i>	- The slope is one of the essential characteristics of a line and helps us measure the rate of change. The slope of a straight line is the ratio of the change in $y$ to the change in $x$ , also called the rise over run.
F	<i>Force</i>	- Force vector.
$\pi$	<i>Pi</i>	- The number $\pi$ is a mathematical constant. It is defined as the ratio of a circle's circumference to its diameter, and it also has various equivalent definitions. It appears in many formulas in all areas of mathematics and physics.

---

## **ACKNOWLEDGEMENTS**

The dissertation acknowledgements section is where you thank those who have helped and supported you during the research and writing process. This includes both professional and personal acknowledgements. ....

More acknowledgement info can be found here: <https://www.scribbr.com/dissertation/acknowledgements/>.



# **CHAPTER 1**

## **INTRODUCTION**

### **1.1 Overview**

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### **1.2 Background and Significance**

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Nulla malesuada porttitor diam. Donec felis erat, congue non, volutpat at, tincidunt tristique, libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum. Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim. Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus. Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus eu enim. Vestibulum pellentesque felis eu massa.

## 1.3 Hypothesis and Rationale

### 1.3.1 Aim 1

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### **1.3.2 Aim 2**

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### **1.3.3 Aim 3**

Nulla malesuada porttitor diam. Donec felis erat, congue non, volutpat at, tincidunt tristique, libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum. Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim. Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus. Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus eu enim. Vestibulum pellentesque felis eu massa.

## **1.4 Impact and Intellectual Contributions**

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semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

## **CHAPTER 2**

### **MULTILINE**

#### **TITLE**

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### **2.1 Abstract**

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## 2.2 Introduction

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Nulla malesuada porttitor diam. Donec felis erat, congue non, volutpat at, tincidunt tristique, libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum. Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim. Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus. Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus eu enim. Vestibulum pellentesque felis eu massa.

Quisque ullamcorper placerat ipsum. Cras nibh. Morbi vel justo vitae lacus tincidunt ultrices. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. In hac habitasse platea dictumst. Integer tempus convallis augue. Etiam facilisis. Nunc elementum fermentum

wisi. Aenean placerat. Ut imperdiet, enim sed gravida sollicitudin, felis odio placerat quam, ac pulvinar elit purus eget enim. Nunc vitae tortor. Proin tempus nibh sit amet nisl. Vivamus quis tortor vitae risus porta vehicula.

Fusce mauris. Vestibulum luctus nibh at lectus. Sed bibendum, nulla a faucibus semper, leo velit ultricies tellus, ac venenatis arcu wisi vel nisl. Vestibulum diam. Aliquam pellen-tesque, augue quis sagittis posuere, turpis lacus congue quam, in hendrerit risus eros eget felis. Maecenas eget erat in sapien mattis porttitor. Vestibulum porttitor. Nulla facilisi. Sed a turpis eu lacus commodo facilisis. Morbi fringilla, wisi in dignissim interdum, justo lectus sagittis dui, et vehicula libero dui cursus dui. Mauris tempor ligula sed lacus. Duis cursus enim ut augue. Cras ac magna. Cras nulla. Nulla egestas. Curabitur a leo. Quisque egestas wisi eget nunc. Nam feugiat lacus vel est. Curabitur consectetur.

## 2.3 Section

### 2.3.1 Subsection

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luctus mauris. **Figure 2.1** and **Table 2.1** Nunc sed pede. Praesent vitae lectus. Praesent neque justo, vehicula eget, interdum id, facilisis et, nibh. Phasellus at purus et libero lacinia dictum. Fusce aliquet. Nulla eu ante placerat leo semper dictum. Mauris metus. Curabitur lobortis. Curabitur sollicitudin hendrerit nunc. Donec ultrices lacus id ipsum. [1].

The L<sup>A</sup>T<sub>E</sub>X code used to create **Figure 2.1** is in **Script 1**.

</>    **Script 1:** ... L<sup>A</sup>T<sub>E</sub>X to display a figure with one three side-by-side images and no space.

```

1 %-----%
2 \afterpage{
3 \begin{figure}[p]
4   \centering
5   \begin{subfigure}[b]{0.33\textwidth}
6     \centering
7     \includegraphics[width=\linewidth]
8     {example-image-a} % File path to graphics
9     \subcaption{\label{fig:a}}
10 \end{subfigure}%' indicates no space between figures
11 \begin{subfigure}[b]{0.33\textwidth}
12   \centering
13   \includegraphics[width=\linewidth]
14   {example-image-b} % File path to graphics
15   \subcaption{\label{fig:b}}
16 \end{subfigure}%' indicates no space between figures
17 \begin{subfigure}[b]{0.33\textwidth}
18   \centering
19   \includegraphics[width=\linewidth]
20   {example-image-c} % File path to graphics
21   \subcaption{\label{fig:c}}
22 \end{subfigure}%' indicates no space between figures
23 \caption{Example figure with three side-by-side images with no space
24 between each figure. \subref{fig:a} \ldots \subref{fig:b} \ldots
25 \subref{fig:c} \ldots \lipsum[75]}
26 \label{fig:Figure_1}
27 \end{figure}
28 \clearpage

```

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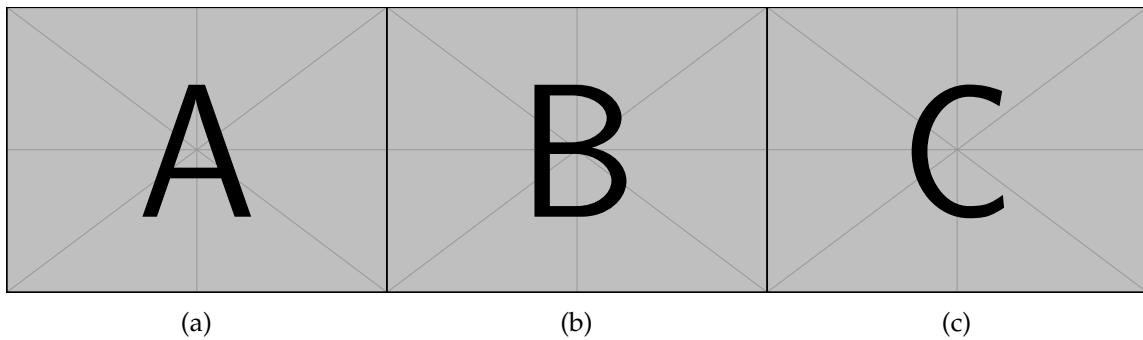


Figure 2.1: Example figure with three side-by-side images with no space between each figure. (a) ... (b) ... (c) ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

Table 2.1: Example table 1. ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

$\alpha$	$\beta$	$\gamma$
0	2	4
1	3	5

semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

The  $\text{\LaTeX}$  code used to create [Table 2.1](#) is in [Script 2](#).

```
</>      Script 2: ... \LaTeX to display a table with three columns and three rows. </>
1 %-----%
2 \afterpage{
3 \begin{table}[p]
4   \centering
5   \caption{Example table 1. \ldots \lipsum[75]}
6   \begin{tabularx}{0.5\textwidth}{XXX}
7     \toprule
8     $\alpha$ & $\beta$ & $\gamma$ \\
9     \midrule
10    0 & 2 & 4 \\
11    1 & 3 & 5 \\
12    \bottomrule
13 \end{tabularx}
14 \label{tab:Table_1}
15 \end{table}
16 \clearpage
17 %-----%
```

### 2.3.1.1 Cross Reference Label Definition

To cross reference anything in  $\text{\LaTeX}$  you need to define a **label**. For example, *Chapters*, *Sections*, *Subsections*, *SubSubSections*, *Paragraphs*, *Figures*, and *Tables* can be labeled in the following manner:

- A Chapter:

`'\chapter{Chapter}\label{chp:1}'`

- A Section:

`'\section{Section}\label{s:chp1_overview}'`

- A SubSection:

'\subsection{SubSection}\label{ss:Subsection1}'

- A SubSubSection:

'\subsubsection{SubSubSection}\label{sss:sss1}'

- A Paragraph:

'\paragraph{Paragraph}\label{p:paragraph1}'

- Inside a figure environment:

'\label{fig:figure\_1}'

- Inside a table environment:

'\label{tab:table\_1}'

### 2.3.1.2 Cross Referencing

**2.3.1.2.1 Document sections** To cross reference *Chapters*, *Sections*, *Subsections*, *SubSubSections*, *Paragraphs*, *Figures*, *Tables*, *References*, and *Codes* use the following L<sup>A</sup>T<sub>E</sub>X commands from the `cleveref` package:

- A Chapter:

'\cref{chp:1}' → Chapter 1.

- A Section:

'\cref{s:chp1\_overview}' → Section 1.1.

- A SubSection:

'\cref{ss:Subsection1}' → Section 2.3.1.

- A SubSubSection:

'\cref{sss:cross\_referencing}' → Section 2.3.1.2.

- A Paragraph:

'\cref{p:paragraph\_1}' → Paragraph 2.5.2.1.1.

- Multiple *Chapters*, *Sections*, *SubSections*, *SubSubSections*

'\cref{chp:1,s:chp1\_overview,ss:Subsection1,sss:cross\_referencing}' → Chapter 1 and Sections 1.1, 2.3.1 and 2.3.1.2.

*with no space between each item being referenced.*

**2.3.1.2.2 References/Citations/Bibliography** Use a citation manager to load your references and export a `bibliography.bib` file. Include this `bibliography.bib` file in your main dissertation file using the `\addbibresource{\subfix{Chapter2/bib_files/bibliography.bib}}` before the command: `\begin{document} ... \end{document}`. It is recommended to use [Mendeley](#) for their ease of adding references and exporting `bibliography.bib` files. [Mendeley Web Importer](#) also has a Google Chrome extension where you can add references via the web browser.

- Single references from a `bibliography.bib` file

`'\autocite{Feltgen_2014}' —> [1]`

- Multiple references (2) from a `bibliography.bib` file

`'\autocite{Gandorfer_2001, Feltgen_2014}' —> [1], [2]`

- Multiple references (3+) from a `bibliography.bib` file

`'\autocite{Fivgas_2001, Gandorfer_2001, Feltgen_2014}' —> [1]–[3]`

### 2.3.1.2.3 Figures

- A single figure:

`'\cref{fig:Figure_1}' —> Figure 2.1.`

- Multiple figures:

`'\cref{fig:Figure_1,fig:Figure_2}' —> Figures 2.1 and 2.2`

*with no space between each item being referenced.*

### 2.3.1.2.4 Tables

- A single Table:

`'\cref{tab:Table_1}' —> Table 2.1.`

- Multiple Tables:

`'\cref{tab:Table_1,tab:Table_2}' —> Tables 2.1 and 2.2`

*with no space between each item being referenced.*

### 2.3.1.2.5 Figures and Tables

- A Figure and a Table:

`'\cref{fig:Figure_1,tab:Table_1}'` → Figure 2.1 and Table 2.1.

*with no space between each item being referenced.*

### 2.3.1.2.6 Codes

- A single code:

`'\cref{code:Figure_1}'` → Script 1.

- Multiple codes:

`'\cref{code:Figure_1,code:Figure_2}'` → Scripts 1 and 3

*with no space between each item being referenced.*

## 2.4 Figure and Table Placement

Due to the strict requirement of figure/table placement, it is recommended to have figure/tables placed immediately after they are referenced on a separate page. We are using the L<sup>A</sup>T<sub>E</sub>X commands from the `afterpage` package to do this. Careful placement in the text needs to be considered to ensure that there are not additional pages of text before the figure/table is placed. In some rare instances the figure/table will need to be coded in a previous paragraph/section to have the display be on the subsequent page after the first mention. The L<sup>A</sup>T<sub>E</sub>X code used to create Figure 2.1 is in Script 1.

If the figure/tables are to instead be placed in the text at either the top or bottom of the page then the placement option `[tbp]` forces the figure/table to be either placed at the ‘top’, ‘bottom’, or ‘on a separate page centered vertically’. The location of the figure/table in the text will have to be shifted such that the figure shows up immediately after the figure/table is referenced in the text. L<sup>A</sup>T<sub>E</sub>X will try and find the best location to minimize white space. In doing so, sometimes the figure/tables will *float* in a less desirable location. Hence, why it is suggested to use the method described above using the `afterpage` package.

### 2.4.1 Table Creation

$\text{\LaTeX}$  can be a bit tricky when it comes to tables. Therefore, it is recommended to use the two following methods to easily create your table for publication.

1. [tablesgenerator](#)
2. [Excel2\LaTeX](#) package: Making tables in  $\text{\LaTeX}$  can be tedious, especially if some columns are calculated. This converter allows you to write a table in Excel instead, and export the current selection as  $\text{\LaTeX}$  markup which can be pasted into an existing  $\text{\LaTeX}$  document, or exported to a file and included via the `\input` command.

## 2.5 Section

  Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetuer id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

  Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

### 2.5.1 Subsection

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetuer id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris. **Figure 2.2**

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetuer id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet,

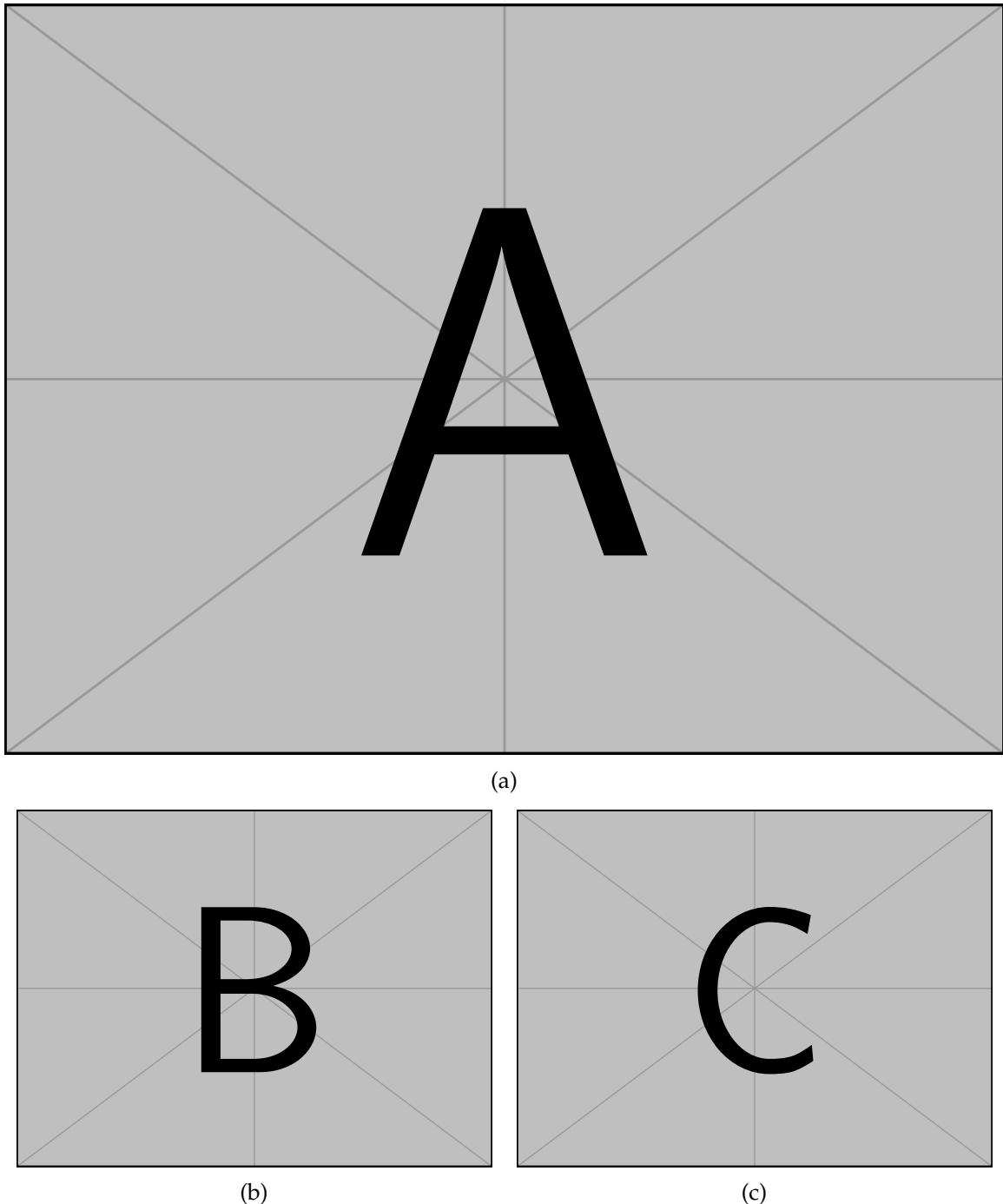


Figure 2.2: Three figures, one on the top and two side-by-side with gaps between figures.  
...**(a)** ...**(b)** ...**(c)** ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

Nulla malesuada porttitor diam. Donec felis erat, congue non, volutpat at, tincidunt tristique, libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum. Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim. Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus. Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus eu enim. Vestibulum pellentesque felis eu massa.

Quisque ullamcorper placerat ipsum. Cras nibh. Morbi vel justo vitae lacus tincidunt ultrices. Lorem ipsum dolor sit amet, consectetuer adipiscing elit. In hac habitasse platea dictumst. Integer tempus convallis augue. Etiam facilisis. Nunc elementum fermentum wisi. Aenean placerat. Ut imperdiet, enim sed gravida sollicitudin, felis odio placerat quam, ac pulvinar elit purus eget enim. Nunc vitae tortor. Proin tempus nibh sit amet nisl. Vivamus quis tortor vitae risus porta vehicula.

The  $\text{\LaTeX}$  code used to create [Figure 2.2](#) is in [Script 3](#).

</>    **Script 3:** ...  $\text{\LaTeX}$  to display a figure with one image above and two side-by-side images. </>

```

1 %-----%
2 \afterpage{
3 \begin{figure}[p]
4   \centering
5   \begin{subfigure}[b]{1.0\textwidth}
6     \centering
7     \includegraphics[width=\linewidth]
8     {example-image-a} % File path to graphics
9     \subcaption{\label{fig:d}}
10 \end{subfigure} \\[1ex]
11 \begin{subfigure}[b]{0.5\textwidth}
12   \centering

```

```

13   \includegraphics[width=0.95\linewidth]
14   {example-image-b} % File path to graphics
15   \subcaption{\label{fig:e}}
16 \end{subfigure} % '%' indicates no space between figures
17 \begin{subfigure}[b]{0.5\textwidth}
18   \centering
19   \includegraphics[width=0.95\linewidth]
20   {example-image-c} % File path to graphics
21   \subcaption{\label{fig:f}}
22 \end{subfigure}
23 \caption{Three figures, one on the top and two side-by-side with gaps
24 between figures. \ldots \subref{fig:d} \ldots \subref{fig:e} \ldots
25 \subref{fig:f} \ldots \lipsum[75]}
26 \label{fig:Figure_2}
27 \end{figure}
28 \clearpage
29 %-----%

```

## 2.5.2 SubSection

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### 2.5.2.1 SubSubSection

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Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

**2.5.2.1.1 Paragraph1** Suspendisse vel felis. Ut lorem lorem, interdum eu, tincidunt sit amet, laoreet vitae, arcu. Aenean faucibus pede eu ante. Praesent enim elit, rutrum at, molestie non, nonummy vel, nisl. Ut lectus eros, malesuada sit amet, fermentum eu, sodales cursus, magna. Donec eu purus. Quisque vehicula, urna sed ultricies auctor, pede lorem egestas dui, et convallis elit erat sed nulla. Donec luctus. Curabitur et nunc. Aliquam dolor odio, commodo pretium, ultricies non, pharetra in, velit. Integer arcu est, nonummy in, fermentum faucibus, egestas vel, odio.

Sed commodo posuere pede. Mauris ut est. Ut quis purus. Sed ac odio. Sed vehicula hendrerit sem. Duis non odio. Morbi ut dui. Sed accumsan risus eget odio. In hac habitasse platea dictumst. Pellentesque non elit. Fusce sed justo eu urna porta tincidunt. Mauris felis odio, sollicitudin sed, volutpat a, ornare ac, erat. Morbi quis dolor. Donec pellentesque, erat

ac sagittis semper, nunc dui lobortis purus, quis congue purus metus ultricies tellus. Proin et quam. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos hymenaeos. Praesent sapien turpis, fermentum vel, eleifend faucibus, vehicula eu, lacus.

### Figure 2.3

The L<sup>A</sup>T<sub>E</sub>X code used to create Figure 2.3 is in [Script 4](#).

```
</>          Script 4: ... LATEX to display a figure with two side-by-side images.          </>
1 %-----%
2 \afterpage{
3 \begin{figure}[p]
4   \centering
5   \begin{subfigure}[b]{0.5\textwidth}
6     \centering
7     \includegraphics[width=\linewidth]
8     {example-image-a}
9     \subcaption{\label{fig:g}}
10 \end{subfigure}%
11 \begin{subfigure}[b]{0.5\textwidth}
12   \centering
13   \includegraphics[width=\linewidth]
14   {example-image-b}
15   \subcaption{\label{fig:h}}
16 \end{subfigure}%
17 \caption{Two figures side-by-side. \ldots \subref{fig:g} \ldots
18 \subref{fig:h} \ldots \lipsum[75]}
19 \label{fig:Figure_3}
20 \end{figure}
21 \clearpage
22 %-----%
```

Suspendisse vel felis. Ut lorem lorem, interdum eu, tincidunt sit amet, laoreet vitae, arcu. Aenean faucibus pede eu ante. Praesent enim elit, rutrum at, molestie non, nonummy vel, nisl. Ut lectus eros, malesuada sit amet, fermentum eu, sodales cursus, magna. Donec eu purus. Quisque vehicula, urna sed ultricies auctor, pede lorem egestas dui, et convallis elit erat sed nulla. Donec luctus. Curabitur et nunc. Aliquam dolor odio, commodo pretium, ultricies non, pharetra in, velit. Integer arcu est, nonummy in, fermentum faucibus, egestas vel, odio.

Sed commodo posuere pede. Mauris ut est. Ut quis purus. Sed ac odio. Sed vehicula hendrerit sem. Duis non odio. Morbi ut dui. Sed accumsan risus eget odio. In hac habitasse platea dictumst. Pellentesque non elit. Fusce sed justo eu urna porta tincidunt. Mauris felis odio, sollicitudin sed, volutpat a, ornare ac, erat. Morbi quis dolor. Donec pellentesque, erat ac sagittis semper, nunc dui lobortis purus, quis congue purus metus ultricies tellus. Proin

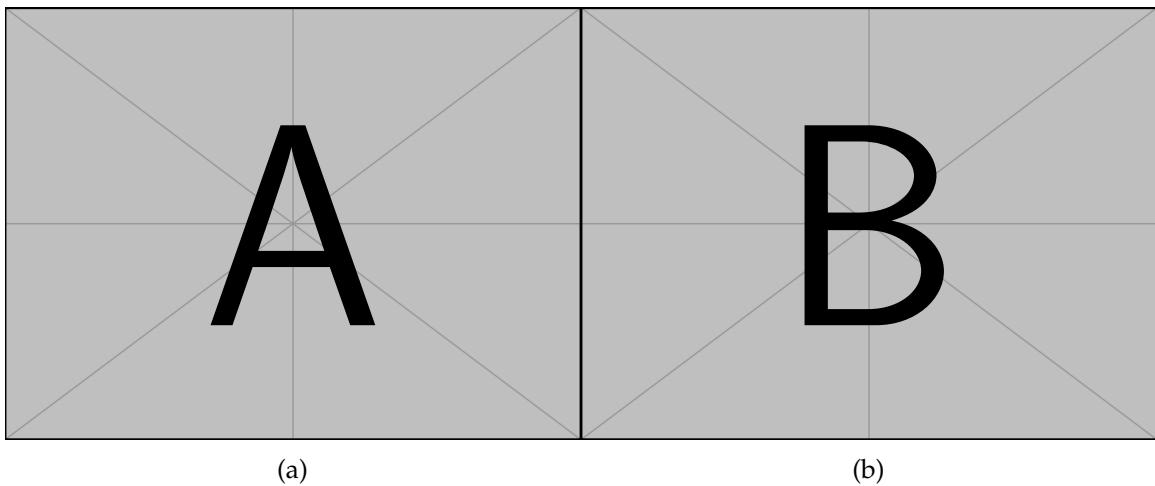


Figure 2.3: Two figures side-by-side. ...**(a)** ...**(b)** ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

et quam. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos hymenaeos. Praesent sapien turpis, fermentum vel, eleifend faucibus, vehicula eu, lacus.

**2.5.2.1.2 Paragraph2** Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Donec odio elit, dictum in, hendrerit sit amet, egestas sed, leo. Praesent feugiat sapien aliquet odio. Integer vitae justo. Aliquam vestibulum fringilla lorem. Sed neque lectus, consectetur at, consectetur sed, eleifend ac, lectus. Nulla facilisi. Pellentesque eget lectus. Proin eu metus. Sed porttitor. In hac habitasse platea dictumst. Suspendisse eu lectus. Ut mi mi, lacinia sit amet, placerat et, mollis vitae, dui. Sed ante tellus, tristique ut, iaculis eu, malesuada ac, dui. Mauris nibh leo, facilisis non, adipiscing quis, ultrices a, dui.

Morbi luctus, wisi viverra faucibus pretium, nibh est placerat odio, nec commodo wisi enim eget quam. Quisque libero justo, consectetur a, feugiat vitae, porttitor eu, libero. Suspendisse sed mauris vitae elit sollicitudin malesuada. Maecenas ultricies eros sit amet ante. Ut venenatis velit. Maecenas sed mi eget dui varius euismod. Phasellus aliquet volutpat odio. Vestibulum ante ipsum primis in faucibus orci luctus et ultrices posuere cubilia Curae; Pellentesque sit amet pede ac sem eleifend consectetur. Nullam elementum, urna vel imperdiet sodales, elit ipsum pharetra ligula, ac pretium ante justo a nulla. Curabitur tristique arcu eu metus. Vestibulum lectus. Proin mauris. Proin eu nunc eu urna hendrerit faucibus. Aliquam auctor, pede consequat laoreet varius, eros tellus scelerisque quam, pellentesque hendrerit ipsum dolor sed augue. Nulla nec lacus.

Suspendisse vitae elit. Aliquam arcu neque, ornare in, ullamcorper quis, commodo eu, libero. Fusce sagittis erat at erat tristique mollis. Maecenas sapien libero, molestie et, lobortis in, sodales eget, dui. Morbi ultrices rutrum lorem. Nam elementum ullamcorper leo. Morbi dui. Aliquam sagittis. Nunc placerat. Pellentesque tristique sodales est. Maecenas imperdiet lacinia velit. Cras non urna. Morbi eros pede, suscipit ac, varius vel, egestas non, eros. Praesent malesuada, diam id pretium elementum, eros sem dictum tortor, vel consectetur odio sem sed wisi. **Figure 2.4**

The L<sup>A</sup>T<sub>E</sub>X code used to create **Figure 2.4** is in **Script 5**.

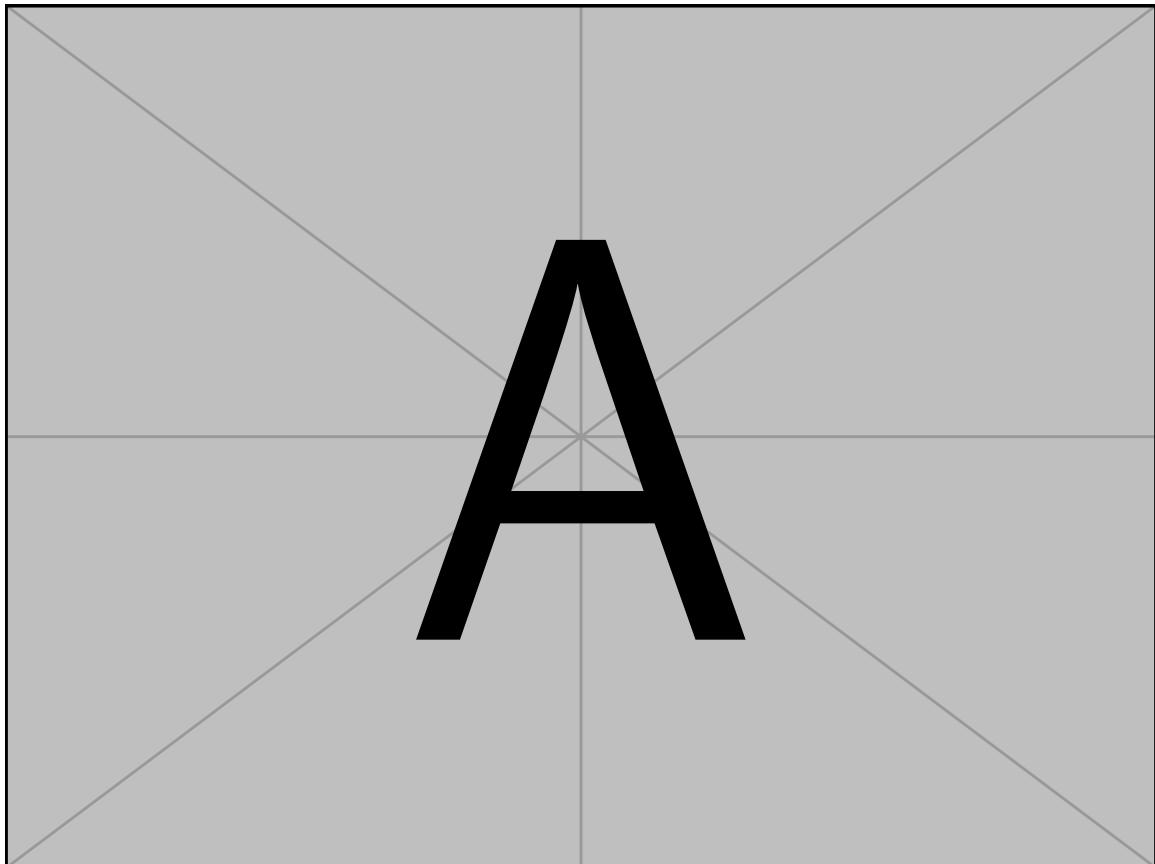


Figure 2.4: Single image ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

&lt;/&gt;

**Script 5:** ... *LATEX* to display a figure of a single image.

&lt;/&gt;

```

1 %-----%
2 \afterpage{
3 \begin{figure}[p]
4   \centering
5   \includegraphics[width=1.0\textwidth]
6   {example-image-a}
7   \caption{Single image \ldots \lipsum[75]}
8   \label{fig:Figure_4}
9 \end{figure}
10 \clearpage
11 %-----%

```

Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Donec odio elit, dictum in, hendrerit sit amet, egestas sed, leo. Praesent feugiat sapien aliquet odio. Integer vitae justo. Aliquam vestibulum fringilla lorem. Sed neque lectus, consectetur at, consectetur sed, eleifend ac, lectus. Nulla facilisi. Pellentesque eget lectus. Proin eu metus. Sed porttitor. In hac habitasse platea dictumst. Suspendisse eu lectus. Ut mi mi, lacinia sit amet, placerat et, mollis vitae, dui. Sed ante tellus, tristique ut, iaculis eu, malesuada ac, dui. Mauris nibh leo, facilisis non, adipiscing quis, ultrices a, dui.

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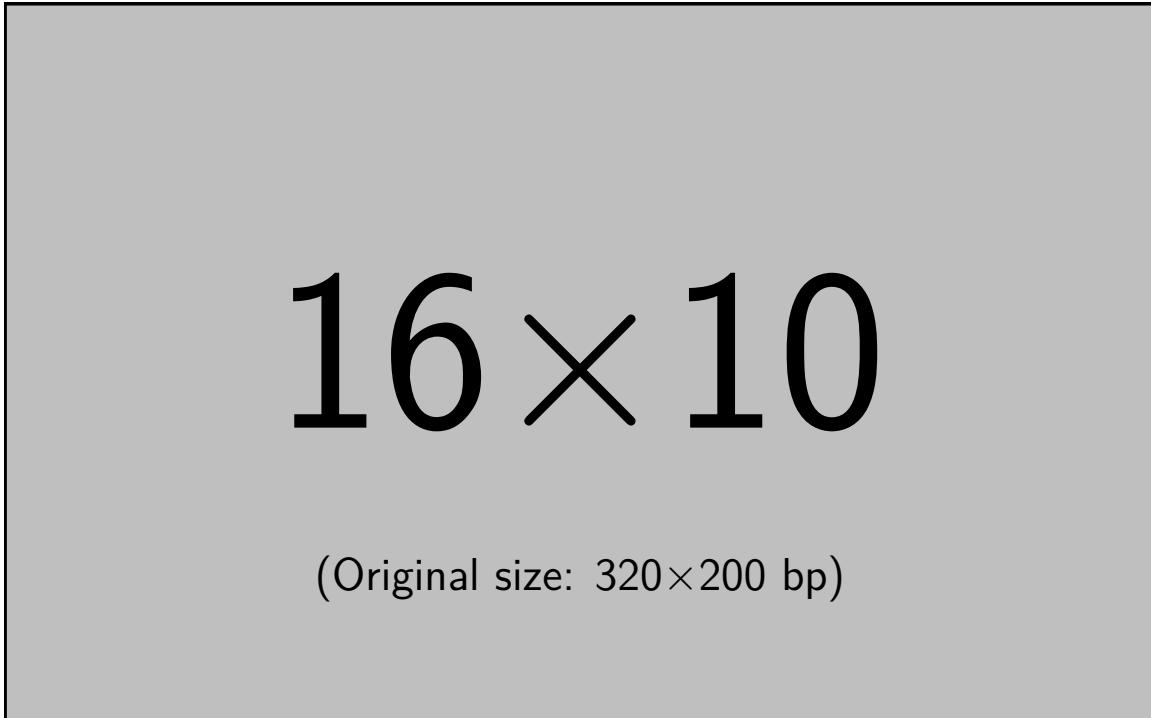
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### 2.5.3 SubSection

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16×10

(Original size: 320×200 bp)

Figure 2.5: Single image ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

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## 2.6 Results

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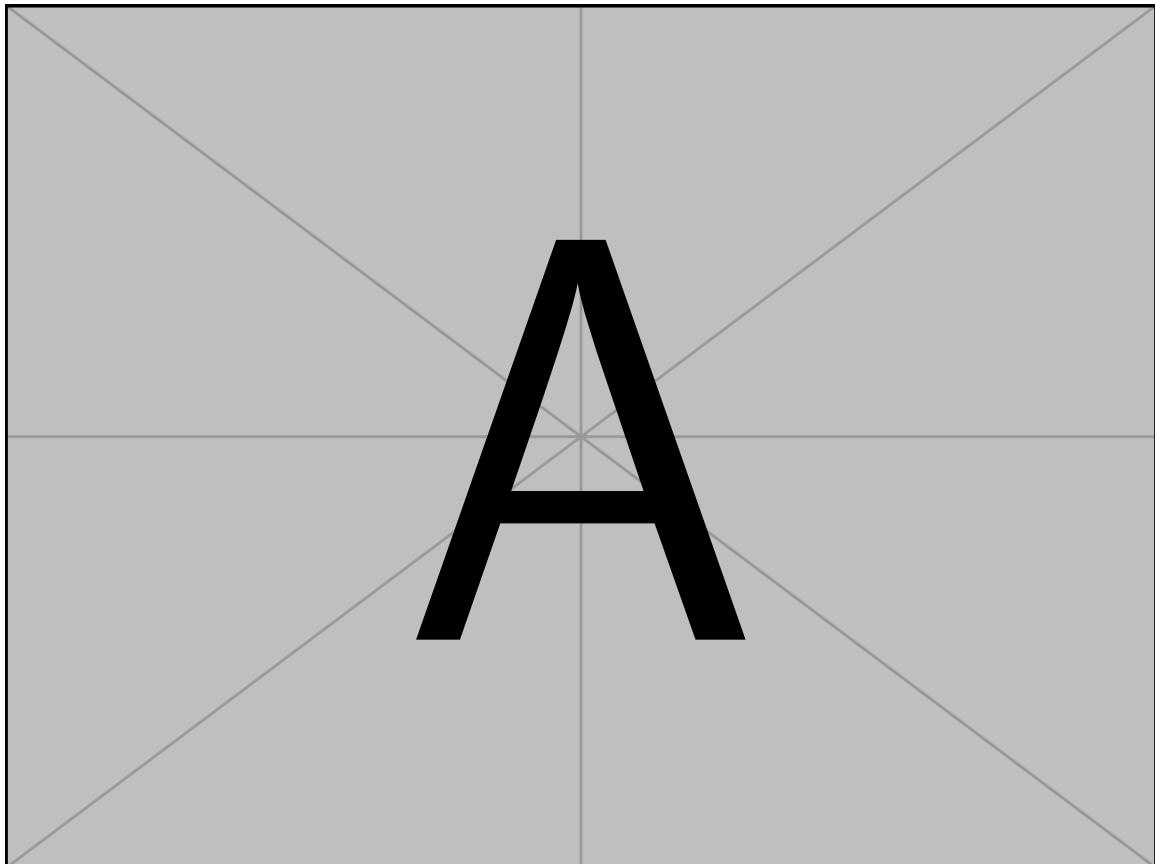


Figure 2.6: Example figure ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

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### 2.6.1 Animation

Using the  $\text{\LaTeX}$  package `animate` you can embed PDF gifs in your PDF. When printed, java-enabled macros will disable the play icon and will print the first figure in the sequence. An example animation is in [Figure 2.7](#).

#### 2.6.1.1 $\text{\LaTeX}$ Code for Animations

[Figure 2.7](#) was created using [Script 6](#).

```
</>          Script 6: ... \LaTeX script using the package \animate to embed gifs.      </>
1 %-----%
2 \afterpage{
3 \begin{figure}[tbp]
4   \centering
5   \animategraphics[loop, controls, width=1.0\textwidth]{20}
6   {pngs/anim-}{0}{24}
7   \caption{Example of an embedded animation in the PDF using the {\LaTeX} package \texttt{\textbackslash href{https://ctan.org/pkg/animate?lang=en}\{animate\}}.}
8   \label{fig:animation}
9 \end{figure}
10 \clearpage
11 %
12 %-----%
```

### 2.6.2 Subsection

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Figure 2.7: Example embedded animation in the PDF using the L<sup>A</sup>T<sub>E</sub>X package `animate`.

purus urna posuere velit, et commodo risus tellus quis tellus. Vivamus leo turpis, tempus sit amet, tristique vitae, laoreet quis, odio. Proin scelerisque bibendum ipsum. Etiam nisl. Praesent vel dolor. Pellentesque vel magna. Curabitur urna. Vivamus congue urna in velit. Etiam ullamcorper elementum dui. Praesent non urna. Sed placerat quam non mi. Pellentesque diam magna, ultricies eget, ultrices placerat, adipiscing rutrum, sem.

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Donec a nibh ut elit vestibulum tristique. Integer at pede. Cras volutpat varius magna. Phasellus eu wisi. Praesent risus justo, lobortis eget, scelerisque ac, aliquet in, dolor. Proin id leo. Nunc iaculis, mi vitae accumsan commodo, neque sem lacinia nulla, quis vestibulum justo sem in eros. Quisque sed massa. Morbi lectus ipsum, vulputate a, mollis ut, accumsan placerat, tellus. Nullam in wisi. Vivamus eu ligula a nunc accumsan congue. Suspendisse ac libero. Aliquam erat volutpat. Donec augue. Nunc venenatis fringilla nibh. Fusce accumsan pulvinar justo. Nullam semper, dui ut dignissim auctor, orci libero fringilla massa, blandit pulvinar pede tortor id magna. Nunc adipiscing justo sed velit tincidunt fermentum.

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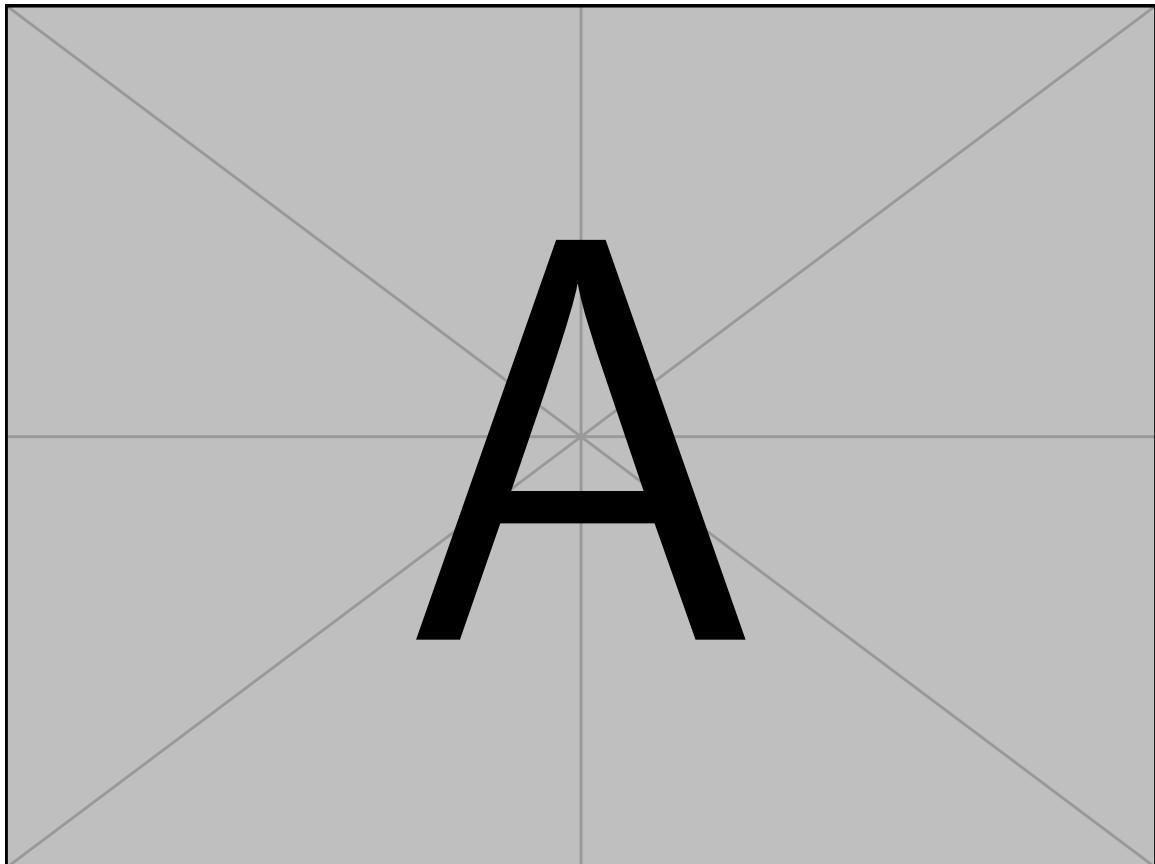


Figure 2.8: Example image ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

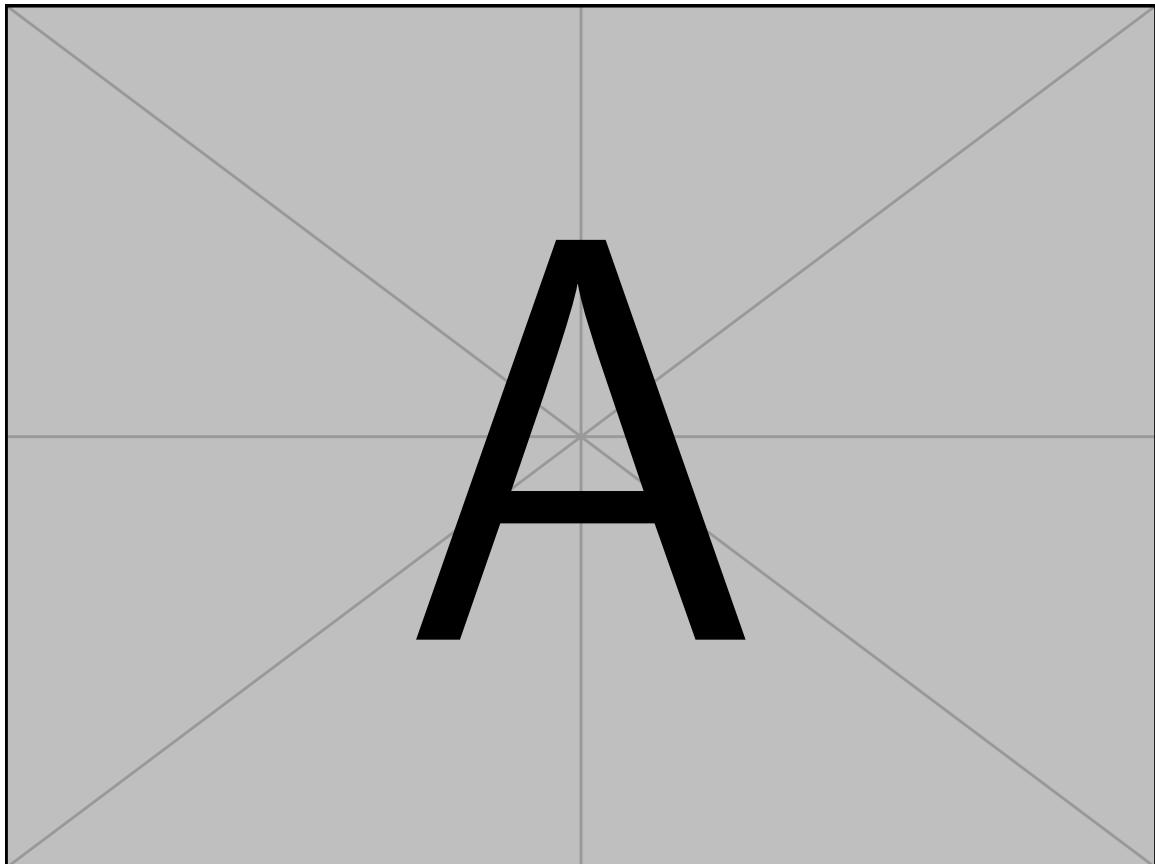


Figure 2.9: Example image ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

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### 2.6.3 Subsection

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

Etiam vel ipsum. Morbi facilisis vestibulum nisl. Praesent cursus laoreet felis. Integer adipiscing pretium orci. Nulla facilisi. Quisque posuere bibendum purus. Nulla quam mauris, cursus eget, convallis ac, molestie non, enim. Aliquam congue. Quisque sagittis

Table 2.2: Example table 2 ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

A	B	C	D (mN)	E (mN)	F (mN)
P	E	12	9.185	9.372	2.459
	P	11	8.566	9.721	3.959
Q	E	6	7.579	7.415	3.797
	P	9	5.463	4.550	2.311
R	E	10	6.712	6.290	2.102
	P	11	7.558	7.829	2.903
S	E	11	16.673	14.650	7.446
P	12	8.322	8.364	2.535	

Table 2.3: Example table 3 ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

A	B	C	D (mN)	E (mN)	F (mN)
P	E	12	9.185	9.372	2.459
	P	11	8.566	9.721	3.959
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	P	9	5.463	4.550	2.311
R	E	10	6.712	6.290	2.102
	P	11	7.558	7.829	2.903
S	E	11	16.673	14.650	7.446
P	12	8.322	8.364	2.535	

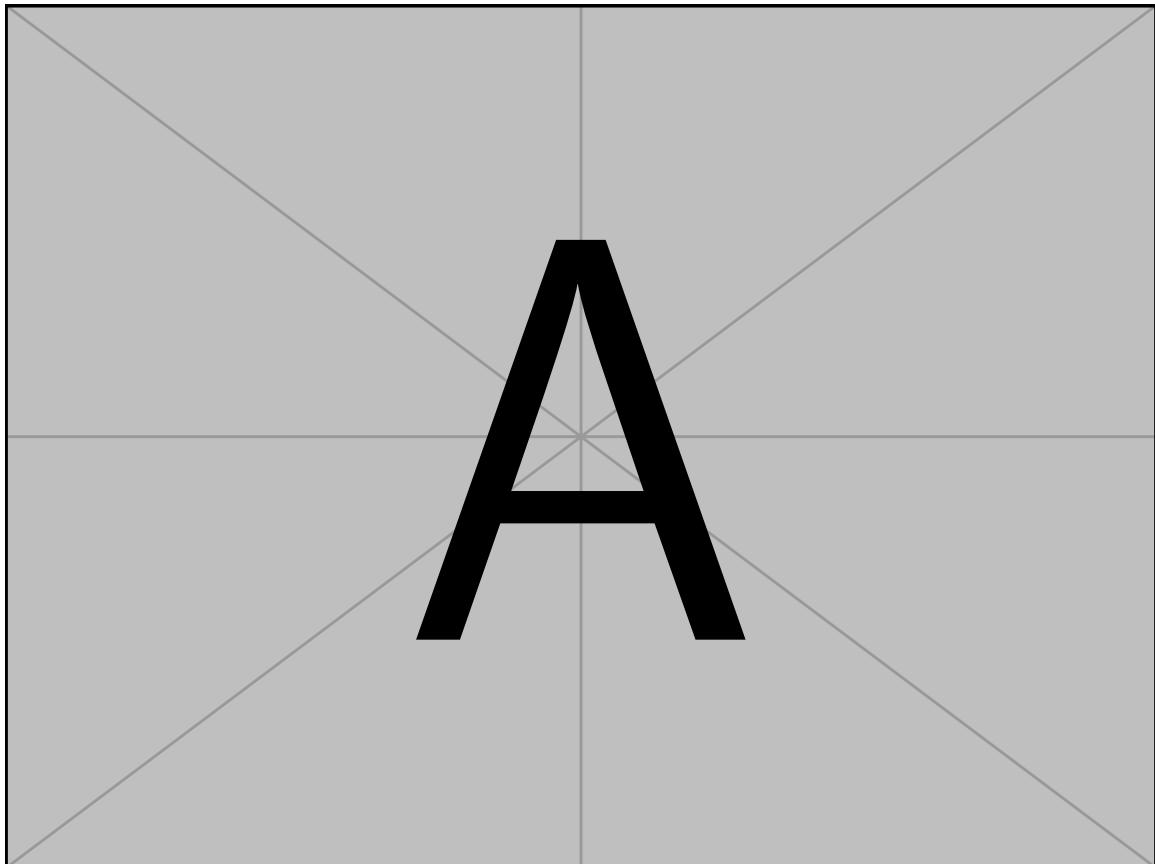


Figure 2.10: Example image ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

nonummy sapien. Proin molestie sem vitae urna. Maecenas lorem. Vivamus viverra consequat enim. **Figure 2.10**

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

Etiam vel ipsum. Morbi facilisis vestibulum nisl. Praesent cursus laoreet felis. Integer adipiscing pretium orci. Nulla facilisi. Quisque posuere bibendum purus. Nulla quam mauris, cursus eget, convallis ac, molestie non, enim. Aliquam congue. Quisque sagittis nonummy sapien. Proin molestie sem vitae urna. Maecenas lorem. Vivamus viverra consequat enim. **Figure 2.11**

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

Etiam vel ipsum. Morbi facilisis vestibulum nisl. Praesent cursus laoreet felis. Integer adipiscing pretium orci. Nulla facilisi. Quisque posuere bibendum purus. Nulla quam mauris, cursus eget, convallis ac, molestie non, enim. Aliquam congue. Quisque sagittis nonummy sapien. Proin molestie sem vitae urna. Maecenas lorem. Vivamus viverra consequat enim. **Figure 2.12**

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum.

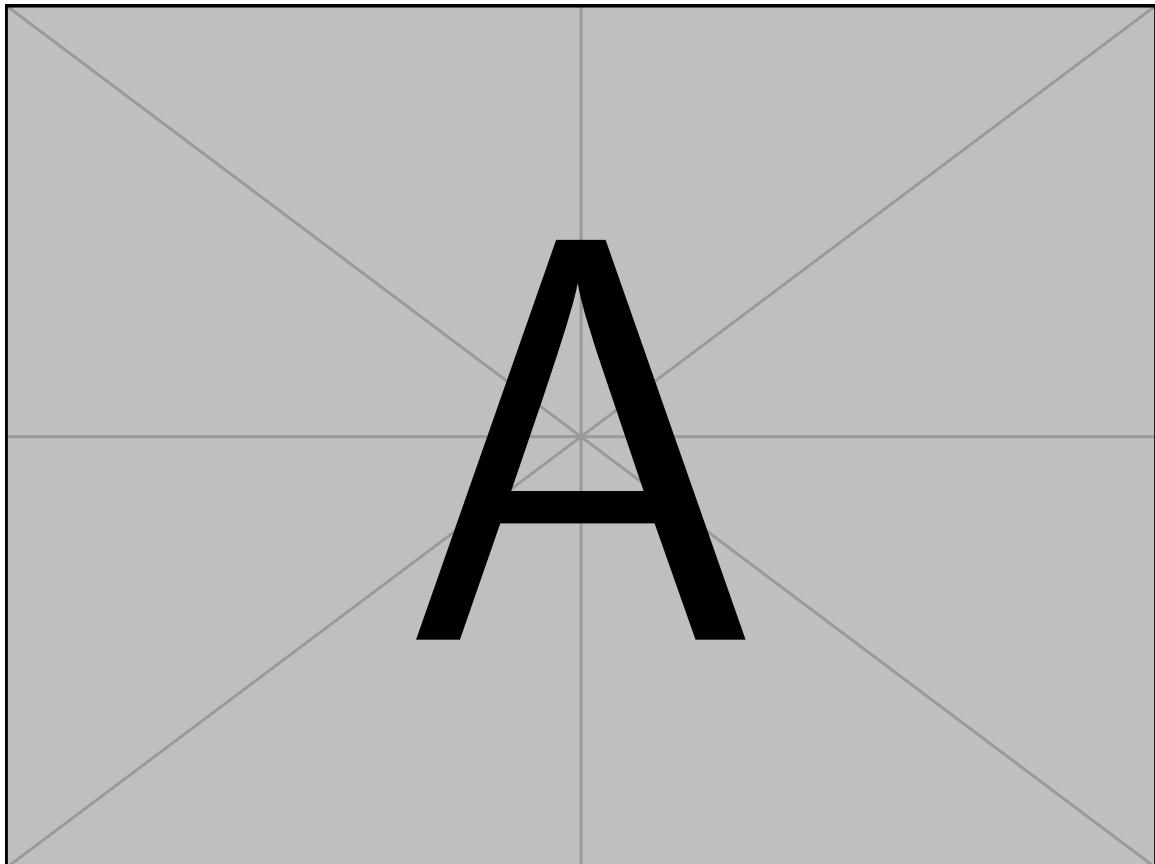


Figure 2.11: Example image ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

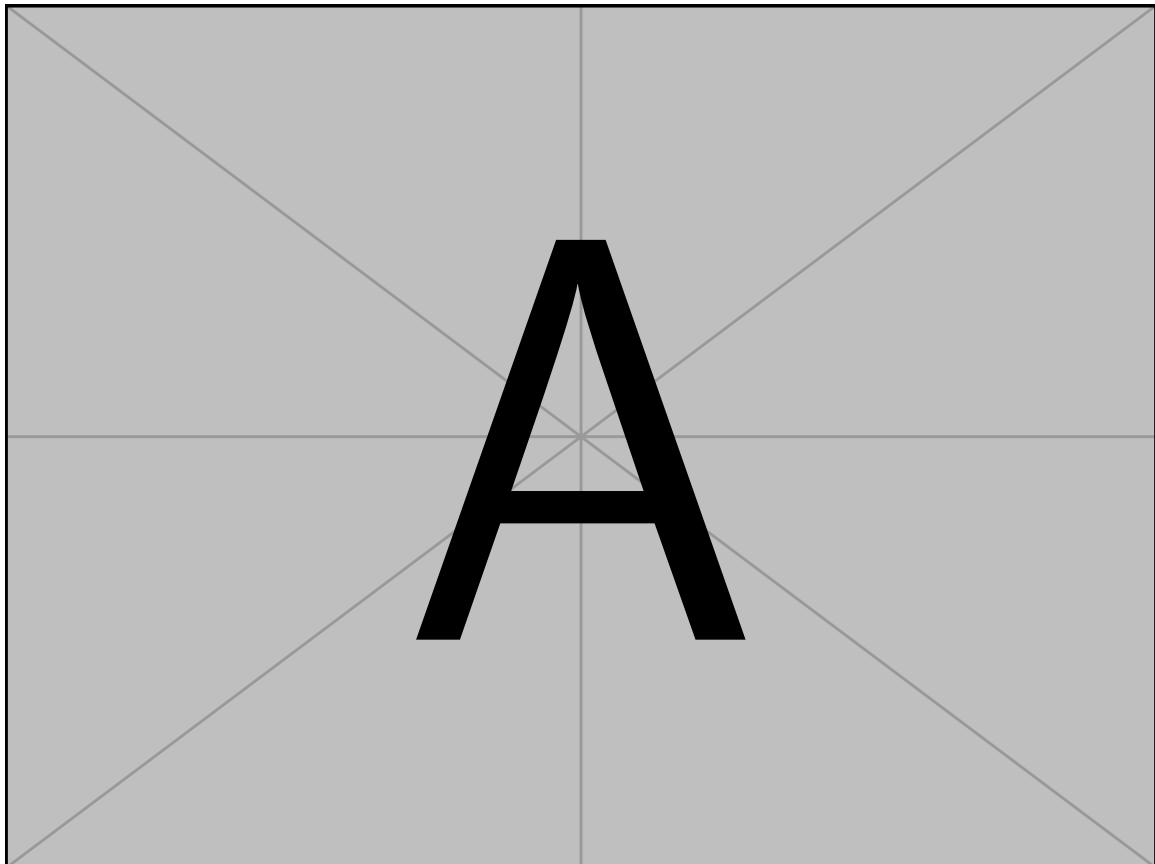


Figure 2.12: Example image ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

Etiam vel ipsum. Morbi facilisis vestibulum nisl. Praesent cursus laoreet felis. Integer adipiscing pretium orci. Nulla facilisi. Quisque posuere bibendum purus. Nulla quam mauris, cursus eget, convallis ac, molestie non, enim. Aliquam congue. Quisque sagittis nonummy sapien. Proin molestie sem vitae urna. Maecenas lorem. Vivamus viverra consequat enim. [Figure 2.13](#)

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

Etiam vel ipsum. Morbi facilisis vestibulum nisl. Praesent cursus laoreet felis. Integer adipiscing pretium orci. Nulla facilisi. Quisque posuere bibendum purus. Nulla quam mauris, cursus eget, convallis ac, molestie non, enim. Aliquam congue. Quisque sagittis nonummy sapien. Proin molestie sem vitae urna. Maecenas lorem. Vivamus viverra consequat enim. [Table 2.4](#)

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

Etiam vel ipsum. Morbi facilisis vestibulum nisl. Praesent cursus laoreet felis. Integer adipiscing pretium orci. Nulla facilisi. Quisque posuere bibendum purus. Nulla quam

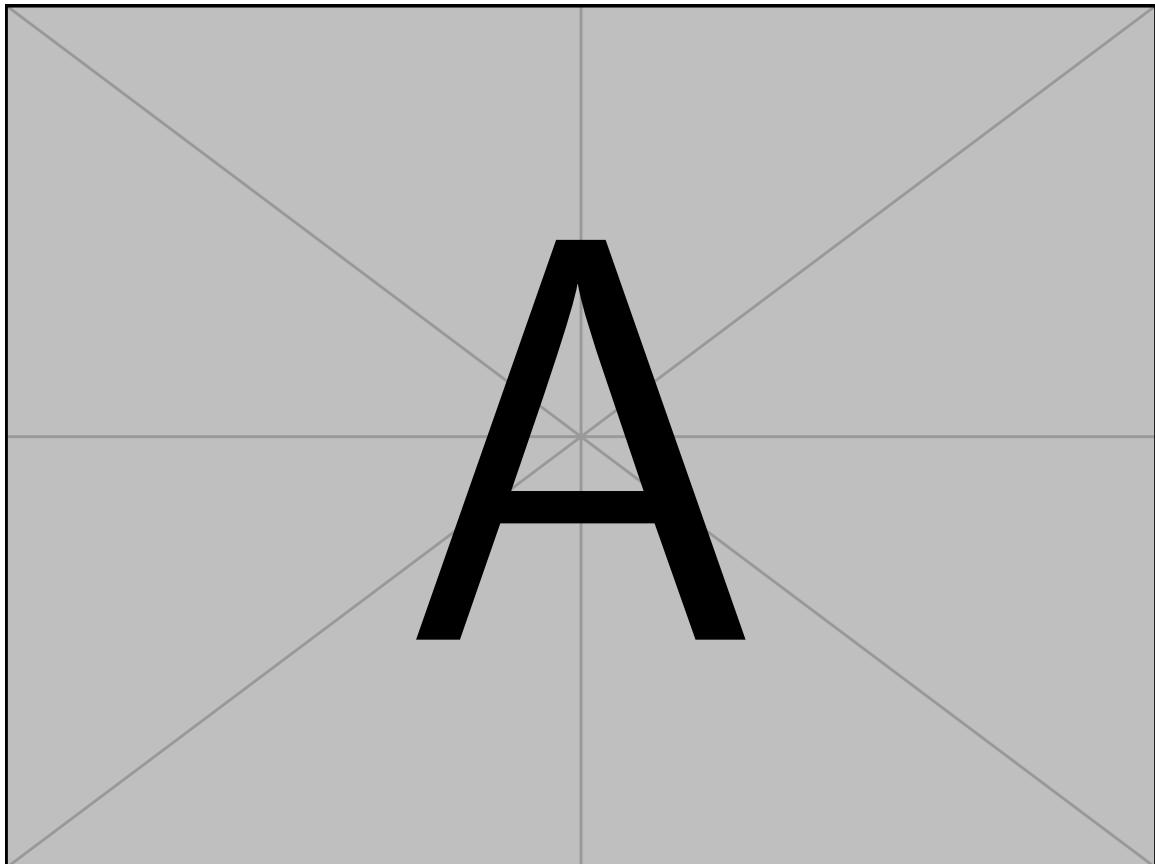


Figure 2.13: Example image ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

Table 2.4: Example table 4 ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

A	B	C	D (mN)	E (mN)	F (mN)
P	E	12	9.185	9.372	2.459
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	P	9	5.463	4.550	2.311
R	E	10	6.712	6.290	2.102
	P	11	7.558	7.829	2.903
S	E	11	16.673	14.650	7.446
P	12	8.322	8.364	2.535	

mauris, cursus eget, convallis ac, molestie non, enim. Aliquam congue. Quisque sagittis nonummy sapien. Proin molestie sem vitae urna. Maecenas lorem. Vivamus viverra consequat enim. [Table 2.5](#)

#### 2.6.4 SubSection

Aliquam tortor. Morbi ipsum massa, imperdiet non, consectetur vel, feugiat vel, lorem. Quisque eget lorem nec elit malesuada vestibulum. Quisque sollicitudin ipsum vel sem. Nulla enim. Proin nonummy felis vitae felis. Nullam pellentesque. Duis rutrum feugiat felis. Mauris vel pede sed libero tincidunt mollis. Phasellus sed urna rhoncus diam euismod bibendum. Phasellus sed nisl. Integer condimentum justo id orci iaculis varius. Quisque et lacus. Phasellus elementum, justo at dignissim auctor, wisi odio lobortis arcu, sed sollicitudin felis felis eu neque. Praesent at lacus.

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [Figures 2.14a to 2.14c](#) [Figures 2.14d to 2.14f](#).

Aliquam tortor. Morbi ipsum massa, imperdiet non, consectetur vel, feugiat vel, lorem. Quisque eget lorem nec elit malesuada vestibulum. Quisque sollicitudin ipsum vel sem. Nulla enim. Proin nonummy felis vitae felis. Nullam pellentesque. Duis rutrum feugiat felis. Mauris vel pede sed libero tincidunt mollis. Phasellus sed urna rhoncus diam euismod bibendum. Phasellus sed nisl. Integer condimentum justo id orci iaculis varius. Quisque et lacus. Phasellus elementum, justo at dignissim auctor, wisi odio lobortis arcu, sed sollicitudin felis felis eu neque. Praesent at lacus.

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum.

Table 2.5: Example table 5 ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

A	B	C	D (mN)	E (mN)	F (mN)
P	E	12	9.185	9.372	2.459
	P	11	8.566	9.721	3.959
Q	E	6	7.579	7.415	3.797
	P	9	5.463	4.550	2.311
R	E	10	6.712	6.290	2.102
	P	11	7.558	7.829	2.903
S	E	11	16.673	14.650	7.446
P	12	8.322	8.364	2.535	

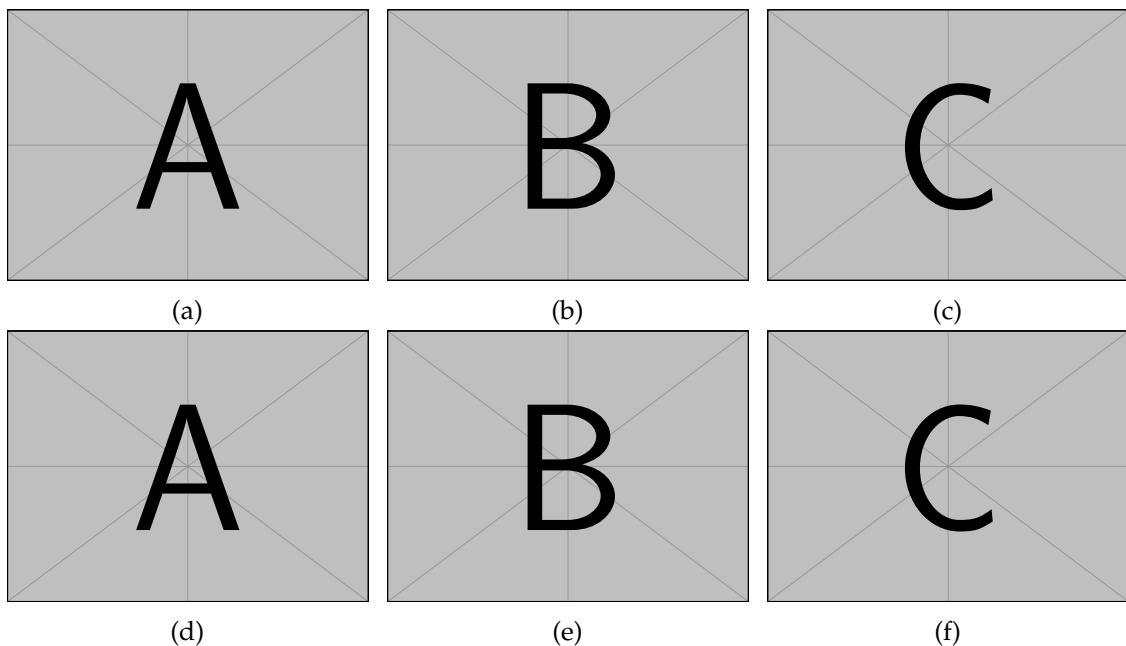


Figure 2.14: This image contains six subfigures. ...((a)-(c)) ... (a). ... (b), ... (c). .... ((d)-(f)), ... (d), ... (e). ... (f). Example image ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

Etiam vel ipsum. Morbi facilisis vestibulum nisl. Praesent cursus laoreet felis. Integer adipiscing pretium orci. Nulla facilisi. Quisque posuere bibendum purus. Nulla quam mauris, cursus eget, convallis ac, molestie non, enim. Aliquam congue. Quisque sagittis nonummy sapien. Proin molestie sem vitae urna. Maecenas lorem. Vivamus viverra consequat enim.

Nunc sed pede. Praesent vitae lectus. Praesent neque justo, vehicula eget, interdum id, facilisis et, nibh. Phasellus at purus et libero lacinia dictum. Fusce aliquet. Nulla eu ante placerat leo semper dictum. Mauris metus. Curabitur lobortis. Curabitur sollicitudin hendrerit nunc. Donec ultrices lacus id ipsum.

Donec a nibh ut elit vestibulum tristique. Integer at pede. Cras volutpat varius magna. Phasellus eu wisi. Praesent risus justo, lobortis eget, scelerisque ac, aliquet in, dolor. Proin id leo. Nunc iaculis, mi vitae accumsan commodo, neque sem lacinia nulla, quis vestibulum justo sem in eros. Quisque sed massa. Morbi lectus ipsum, vulputate a, mollis ut, accumsan placerat, tellus. Nullam in wisi. Vivamus eu ligula a nunc accumsan congue. Suspendisse ac libero. Aliquam erat volutpat. Donec augue. Nunc venenatis fringilla nibh. Fusce accumsan pulvinar justo. Nullam semper, dui ut dignissim auctor, orci libero fringilla massa, blandit pulvinar pede tortor id magna. Nunc adipiscing justo sed velit tincidunt fermentum.

The *L<sup>A</sup>T<sub>E</sub>X* code used to create [Figure 2.14](#) is in [Script 7](#).

```
</>          Script 7: ... LATEX to display a figure with two side-by-side images.          </>
1 %-----%
2 \afterpage{
3 \begin{figure}[p]
4   \centering
5   \begin{subfigure}[b]{0.33\textwidth}
6     % A
7     \centering
8     \includegraphics[width=.95\linewidth]
9     {example-image-a} % File path to graphics
10    \subcaption{\label{fig:A}}
11  \end{subfigure}%
```

```

12 \begin{subfigure}[b]{0.33\textwidth}
13   % B
14   \centering
15   \includegraphics[width=.95\linewidth]
16   {example-image-b} % File path to graphics
17   \subcaption{\label{fig:B}}
18 \end{subfigure}%
19 \begin{subfigure}[b]{0.33\textwidth}
20   % C
21   \centering
22   \includegraphics[width=.95\linewidth]
23   {example-image-c} % File path to graphics
24   \subcaption{\label{fig:C}}
25 \end{subfigure}%
26
27 \begin{subfigure}[b]{0.33\textwidth}
28   % D
29   \centering
30   \includegraphics[width=.95\linewidth]
31   {example-image-a} % File path to graphics
32   \subcaption{\label{fig:D}}
33 \end{subfigure}%
34 \begin{subfigure}[b]{0.33\textwidth}
35   % E
36   \centering
37   \includegraphics[width=.95\linewidth]
38   {example-image-b} % File path to graphics
39   \subcaption{\label{fig:E}}
40 \end{subfigure}%
41 \begin{subfigure}[b]{0.33\textwidth}
42   % F
43   \centering
44   \includegraphics[width=.95\linewidth]
45   {example-image-c} % File path to graphics
46   \subcaption{\label{fig:F}}
47 \end{subfigure}%
48 \caption{This image contains six subfigures. \ldots
49 (\subref{fig:A}-\subref{fig:C}) \ldots \subref{fig:A}. \ldots
50 \subref{fig:B}, \ldots \subref{fig:C}. \ldots \ldots
51 (\subref{fig:D}-\subref{fig:F}), \ldots \subref{fig:D}, \ldots
52 \subref{fig:E}. \ldots \subref{fig:F}. Example image \ldots
53 \lipsum[75]}
54 \label{fig:ABCDEF}
55 \end{figure}
56 \clearpage
57 %-----%

```

## 2.7 Discussion

Fusce mauris. Vestibulum luctus nibh at lectus. Sed bibendum, nulla a faucibus semper, leo velit ultricies tellus, ac venenatis arcu wisi vel nisl. Vestibulum diam. Aliquam pellen-  
tesque, augue quis sagittis posuere, turpis lacus congue quam, in hendrerit risus eros eget  
felis. Maecenas eget erat in sapien mattis porttitor. Vestibulum porttitor. Nulla facilisi. Sed

a turpis eu lacus commodo facilisis. Morbi fringilla, wisi in dignissim interdum, justo lectus sagittis dui, et vehicula libero dui cursus dui. Mauris tempor ligula sed lacus. Duis cursus enim ut augue. Cras ac magna. Cras nulla. Nulla egestas. Curabitur a leo. Quisque egestas wisi eget nunc. Nam feugiat lacus vel est. Curabitur consectetur.

Suspendisse vel felis. Ut lorem lorem, interdum eu, tincidunt sit amet, laoreet vitae, arcu. Aenean faucibus pede eu ante. Praesent enim elit, rutrum at, molestie non, nonummy vel, nisl. Ut lectus eros, malesuada sit amet, fermentum eu, sodales cursus, magna. Donec eu purus. Quisque vehicula, urna sed ultricies auctor, pede lorem egestas dui, et convallis elit erat sed nulla. Donec luctus. Curabitur et nunc. Aliquam dolor odio, commodo pretium, ultricies non, pharetra in, velit. Integer arcu est, nonummy in, fermentum faucibus, egestas vel, odio.

Sed commodo posuere pede. Mauris ut est. Ut quis purus. Sed ac odio. Sed vehicula hendrerit sem. Duis non odio. Morbi ut dui. Sed accumsan risus eget odio. In hac habitasse platea dictumst. Pellentesque non elit. Fusce sed justo eu urna porta tincidunt. Mauris felis odio, sollicitudin sed, volutpat a, ornare ac, erat. Morbi quis dolor. Donec pellentesque, erat ac sagittis semper, nunc dui lobortis purus, quis congue purus metus ultricies tellus. Proin et quam. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos hymenaeos. Praesent sapien turpis, fermentum vel, eleifend faucibus, vehicula eu, lacus.

Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Donec odio elit, dictum in, hendrerit sit amet, egestas sed, leo. Praesent feugiat sapien aliquet odio. Integer vitae justo. Aliquam vestibulum fringilla lorem. Sed neque lectus, consectetur at, consectetur sed, eleifend ac, lectus. Nulla facilisi. Pellentesque eget lectus. Proin eu metus. Sed porttitor. In hac habitasse platea dictumst. Suspendisse eu lectus. Ut mi mi, lacinia sit amet, placerat et, mollis vitae, dui. Sed ante tellus, tristique ut, iaculis eu, malesuada ac, dui. Mauris nibh leo, facilisis non, adipiscing quis, ultrices a, dui.

Morbi luctus, wisi viverra faucibus pretium, nibh est placerat odio, nec commodo wisi enim eget quam. Quisque libero justo, consectetur a, feugiat vitae, porttitor eu, libero. Suspendisse sed mauris vitae elit sollicitudin malesuada. Maecenas ultricies eros sit amet ante. Ut venenatis velit. Maecenas sed mi eget dui varius euismod. Phasellus aliquet volutpat odio. Vestibulum ante ipsum primis in faucibus orci luctus et ultrices

posuere cubilia Curae; Pellentesque sit amet pede ac sem eleifend consectetuer. Nullam elementum, urna vel imperdiet sodales, elit ipsum pharetra ligula, ac pretium ante justo a nulla. Curabitur tristique arcu eu metus. Vestibulum lectus. Proin mauris. Proin eu nunc eu urna hendrerit faucibus. Aliquam auctor, pede consequat laoreet varius, eros tellus scelerisque quam, pellentesque hendrerit ipsum dolor sed augue. Nulla nec lacus.

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## 2.8 Conclusion

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## 2.9 Acknowledgment

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## **CHAPTER 3**

### **PREVIOUSLY PUBLISHED ARTICLE**

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# Changes in Vitreoretinal Adhesion With Age and Region in Human and Sheep Eyes

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While several studies have qualitatively investigated age- and region-dependent adhesion between the vitreous and retina, no studies have directly measured the vitreoretinal strength of adhesion. In this study, we developed a rotational peel device and associated methodology to measure the maximum and steady-state peel forces between the vitreous and the retina. Vitreoretinal adhesion in the equator and posterior pole were measured in human eyes from donors ranging 30 to 79 years of age, and in sheep eyes from premature, neonatal, young lamb, and young adult sheep. In human eyes, maximum peel force in the equator ( $7.24 \pm 4.13$  mN) was greater than in the posterior pole ( $4.08 \pm 2.03$  mN). This trend was especially evident for younger eyes from donors 30 to 39 years of age. After 60 years of age, there was a significant decrease in the maximum equatorial ( $4.69 \pm 2.52$  mN,  $p = 0.016$ ) and posterior pole adhesion ( $2.95 \pm 1.25$  mN,  $p = 0.037$ ). In immature sheep eyes, maximum adhesion was  $7.60 \pm 3.06$  mN, and did not significantly differ between the equator and posterior pole until young adulthood. At this age, the maximum adhesion in the equator nearly doubled ( $16.67 \pm 7.45$  mN) that of the posterior pole, similar to the young adult human eyes. Light microscopy images suggest more disruption of the inner limiting membrane (ILM) in immature sheep eyes compared to adult sheep eyes. Interestingly, in human eyes, ILM disruption was significantly greater in the posterior pole ( $p < 0.05$ ) and in people over 60 years of age ( $p < 0.02$ ). These findings supplement the current discussion surrounding age-related posterior vitreous detachment, and the risk factors and physiological progressions associated with this condition. In addition, these data further our understanding of the biomechanical mechanisms of vitreoretinal adhesion, and can be used to develop age- appropriate computational models simulating retinal detachment, hemorrhaging, or retinal trauma.

**Keywords:** peel, vitreous, retina, inner limiting membrane, posterior vitreous detachment, retinal detachment

## INTRODUCTION

Any disruption to the layers of the retina, particularly the separation of the photosensitive cells from the retinal pigment epithelium, can result in blindness or severe visual impairment. The most common cause of disruption is retinal detachment. Retinal detachment occurs in one of every 10,000 people (Mitry et al., 2010) and does not discriminate between children and adults (Rosner et al., 1987; Fivgas and Capone, 2001). Ocular trauma and age-related vitreous degradation are

common causes of detachment, and the principal mechanism for each of these etiologies is retinal force mediated by adhesion to the vitreous.

Little is quantitatively known about adhesion at the vitreoretinal interface. Sebag (1991) manually peeled the posterior vitreous from the retina in 59 post-mortem eyes from donors with ages spanning 33 weeks gestation to 100 years old. They reported that peeling was "more difficult" in younger ages (20 years old or younger). Additionally, they report disruption of the Müller cells after peeling in 40% of the younger eyes, suggesting vitreoretinal adhesion in the younger group was stronger than adhesion between individual layers of the retina. This study provides evidence for changes in adhesion with age. It also correlates with theories that suggest vitreoretinal adhesion is a function of collagen density and structural integrity (Sebag, 1992; Fivgas and Capone, 2001; Gondorfer et al., 2001, 2002; Bishop et al., 2004; Ponsioen et al., 2008; Mitry et al., 2010). The amount of vitreal collagen present at birth does not change throughout life, so the relative density of collagen in the eye decreases as the eye grows (Balazs and Denlinger, 1982), and degrades with time (Bishop et al., 2004). If adhesion is dependent on collagen density and structure, the decrease and weakening of collagen with age would alter and diminish adhesion at the vitreoretinal interface.

Vitreoretinal adhesion has qualitatively been shown to vary with region. Gondorfer et al. (2001) injected plasmin into the vitreous of 24 porcine eyes and evaluated the vitreoretinal interface using scanning and transmission electron microscopy. Greater dosages and incubation times were required to eliminate and/or separate collagen fibrils on the inner limiting membrane (ILM) of the equator compared to the posterior pole. None of the dosages evaluated were able to completely separate the vitreous cortex from the ILM at the vitreous base. This suggests that vitreoretinal adhesion increases from the posterior pole to the equator to the vitreous base. The exact mechanism of adhesion is not well-established and may be different in the vitreous base compared to the equator or the posterior pole. It is possible the plasmin used in the Gondorfer study was more successful in detaching the vitreous from the retina in the equator and/or posterior pole because of regional adhesion mechanisms rather than adhesive strength.

No studies to date have directly measured adhesive forces at the vitreoretinal interface, but there are several studies that quantified retinal separation at the pigment epithelium (RPE). The earliest series of studies were by Zauberman and deGuillebon (Zauberman and Berman, 1969; DeGuillebon et al., 1971; DeGuillebon and Zauberman, 1972; Zauberman, 1972) in monkey, cat, and rabbit eyes. Both groups excised rectangular specimens from the eye containing retina, choroid, and sclera. The specimens were laid flat in a saline-filled petri dish. A metallic rod was attached to the ILM of the retina and pulled either manually (Zauberman and Berman, 1969), or with a computer-controlled linear actuator (Zauberman, 1972). The adhesive forces between the RPE and choroid were higher in the equator compared to the posterior region (Zauberman and Berman, 1969), and this regional dependence was reported to be greater in younger rabbits (1–2 months old) compared to adult rabbits (DeGuillebon et al., 1971). Additional experiments

report significantly increased RPE adhesion with peel rate (DeGuillebon et al., 1972) and significantly decreased adhesion with post-mortem time (Zauberman and DeGuillebon, 1972). A subsequent study by Endo et al. (1988), however, reported that refrigeration of enucleated eyes delayed deterioration up to at least 18 h.

The limitation of these peel studies is the potential disruption of the interface between the RPE and choroid prior to testing. Sandwich specimens were physically removed from the spherical eye and laid flat for testing. The excision likely damages structures at the cut interface, and straightening the sample likely causes shear forces between the layers which may compromise the interface. To overcome these limitations, Kita et al. (1990) used a bleb technique to estimate adhesion at the RPE. Briefly, a balanced salt solution was slowly injected into the subretinal space to generate retinal separation and a fluid-filled bleb. The choroidal retinal adhesive force was estimated from measured pressure differences between the vitreous and the fluid-filled subretinal space. The accuracy of these calculations is based on the assumption that the fluid-filled bleb is spherical. Previous bleb studies have reported that subretinal injections typically result in blebs with flattened rather than spherical tops (Marmor et al., 1980). The error this may cause in adhesion force estimations is unknown.

The objective of our study was to quantify vitreoretinal adhesive forces in sheep and human eyes, and evaluate how measurements change with age and region of the eye. To achieve this objective, an innovative testing device was created to overcome many of the limitations of the previous retinal adhesion methods. This device allows the retina to be peeled from the vitreous without altering the curvature of the specimen, or requiring dissection of the retina. Using this technique, quantitative vitreoretinal adhesive forces can be directly measured. These forces will be necessary to understand the biomechanics of vitreoretinal adhesion and create numerical tools for predicting retinal detachment.

## METHODS

### Materials

The adhesive strength between the vitreous and retina was measured in sheep ( $n = 43$ ) and human ( $n = 17$ ) eyes. Sheep eyes were used to investigate differences in adhesion between immature and mature eyes. Sheep eyes were selected because they have a well-defined retinal structure and holangiotic vasculature, and their vitreous composition is similar to human eyes (Balazs and Denlinger, 1982; Sebag, 1993; Ponsioen et al., 2010). Of the sheep eyes, four age groups were compared: adult ( $n = 15$ , 4–6 years old), young lamb ( $n = 10$ , 18 weeks old), neonatal ( $n = 5$ , 1–5 days old), and premature ( $n = 13$ , 128–136 days gestational age). There are no known human age equivalents for sheep based on ocular anatomy. The young adult sheep used in this study have a human age equivalence of 28–36 years old based on reproductive maturity and life span (Lévy et al., 2017). The immature groups (young lamb, neonatal, and premature) do not have well-established age equivalencies. Sheep brain development peaks *in utero* at ~85 days gestation (Dobbing, 1974). Based on the brain development patterns, our premature and neonatal

groups are estimated to have human equivalent ages of 6–12 and 14–20 months old, respectively. The young lamb group is estimated to have a human equivalent age of a 5–7 years of age. This lamb age equivalency was estimated based on extrapolation of the early brain maturation data and reproductive maturity timelines.

Sheep eyes were removed immediately upon sacrifice and refrigerated *en bloc* in phosphate-buffered saline (PBS) until testing. Adult human eyes (30–80 years old) were purchased from the Utah Lions Eye Bank in PBS and refrigerated until time of testing. Left and right eyes for all sheep and human subjects were collected and tested within 24 h of death. All studies were reviewed by the University of Utah IRB and IACUC compliance boards and determined to be exempt from regulation.

Testing was performed in the equatorial and posterior pole regions for each eye. The order in which samples were tested (right/left, equator/posterior pole) was randomly selected. Extraocular tissue and the optic nerve were removed from the globe prior to all dissections. For equatorial peels, a cut through the sclera, leaving the choroid, retina, and vitreous intact, was made anteriorly from the small opening at the optic nerve head to the equatorial region, ending ~15 mm posterior to the cornea (Cut 1 in **Figure 1A**). A second cut was made perpendicular to the initial cut, and along the equatorial region of the eye, ~25 mm in length (Eq. Cut in **Figure 1A**). Third and fourth cuts were made to create an 8 × 25 mm rectangular window of choroid (**Figure 1B**). Forceps were used to carefully pull away the choroid and expose the underlying retinal pigment epithelium.

For posterior peels, an initial cut was made similar to the equatorial peels (Cut 1 in **Figure 1A**). A circumferential cut was made using forceps and dissection scissors around the globe along the posterior cut line (Pos. Cut in **Figure 1A**), and the posterior sclera was removed entirely from the globe. This provided better visualization and access to the retina in the posterior pole. The choroid was removed using forceps and dissection scissors. The posterior retinal testing region was always oriented at an angle to avoid the fovea (**Figure 1C**).

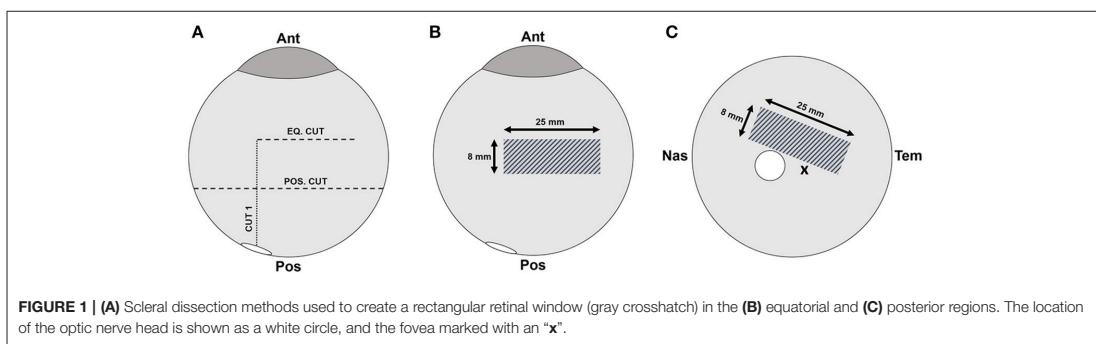
### Peel Test Setup and Protocol

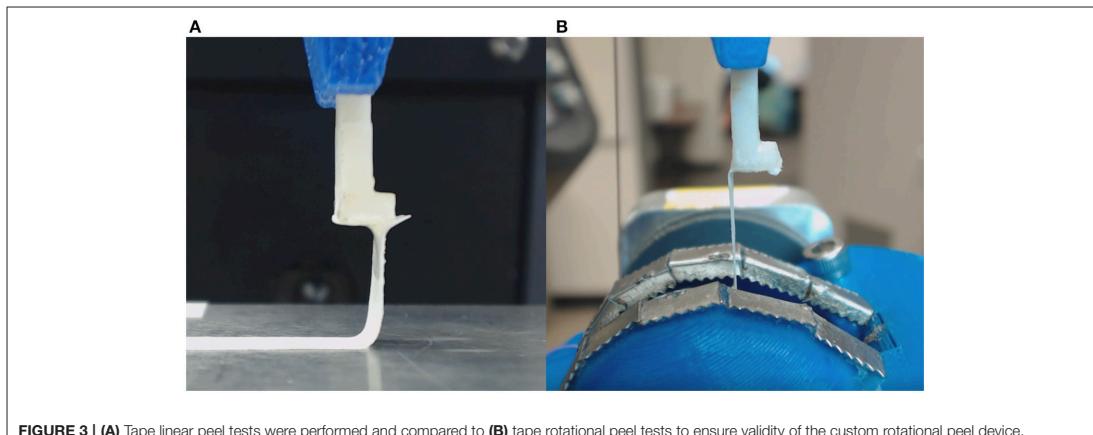
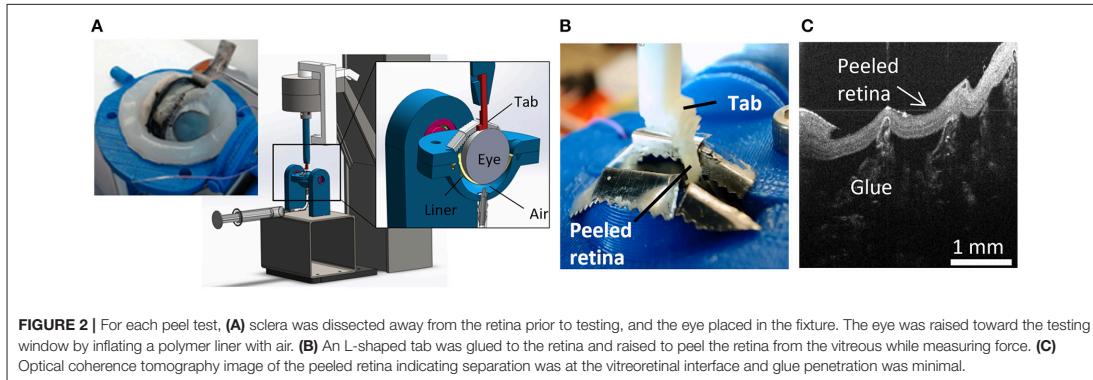
A peel test system was created to test vitreoretinal adhesion with minimal dissection and disruption to the vitreoretinal

interface. This was achieved by keeping the retina and vitreous in their natural configuration, and rotating the eye as the retina was peeled away from the vitreous. The prepared eye with a window of exposed retina was placed onto a flexible membrane (Dragon Skin, Smooth-On, Macungie, PA) molded to cup the eye (**Figure 2A**). The eye and membrane were loaded into a custom 3D-printed holder with a rectangular opening that lined up with the exposed retina. Air was pumped into the cavity beneath the flexible membrane to enforce a spherical shape at the vitreoretinal interface. Two different sizes of membranes and eye holders were designed. The large size (diameter = 30 mm) was used to hold adult and young lamb sheep eyes. The small size (diameter = 20 mm) was used for neonatal and preterm sheep and human eyes.

A plastic L-shaped tab was connected to an Instron Universal Testing Instrument (Model No. 5943, Instron, Norwood, MA) where load measurements were made with a 5 N uniaxial load cell (Model No. 2530-5N, Instron, Norwood, MA). A thin layer of cyanoacrylate adhesive was applied to the bottom surface of the tab (5.0 × 4.5 mm) and lowered until it was in contact with the retina (**Figure 2B**). The retina was scored on both sides of the tab as well as one end of the peel region to ensure a clean rectangular peel shape throughout the duration of the test. The tab was raised at a quasistatic rate of 0.02 mm/s in accordance with other previous peel test literature (DeGuillebon and Zauberman, 1972; Zauberman and DeGuillebon, 1972; ASTM, 2016). The eye holder rotated simultaneously using a pulley system connected to the Instron crosshead. This rotation was critical for maintaining a constant perpendicular angle of peel during testing. Tests lasted anywhere from 5 to 12 min depending on the length of the steady-state peel. A Logitech web cam (Logitech C920 HD Pro, Logitech, Newark, CA) recorded video of each peel test. Optical coherence tomography (Envisu R2200, Leica Microsystems, Wetzlar, Germany) imaging was used to verify the glue between the tab and the retina did not penetrate deep into the retina and affect adhesion measurements (**Figure 2C**).

Following rotational peel tests, 2 mm square sections of peeled retina not directly attached to the tab were removed to characterize damage to the vitreoretinal interface. Specimens were placed in a 1% buffered formaldehyde and 1.25%





glutaraldehyde solution for 24 h. For processing, samples were placed in a 0.1 M sodium cacodylate buffer rinse twice for 5 min while being agitated. Samples were then placed in a 1:1 mixture of diluted osmium tetroxide  $\text{OsO}_4$  (4% in  $d\text{H}_2\text{O}$ ) and 0.2 M sodium cacodylate buffer and agitated for 1 h. The samples were rinsed with DI water to remove excess osmium, and then placed in a 4% uranyl acetate solution, and agitated for 1 h. The samples were then dehydrated in increasing percentages of ethanol alcohol (50, 70, 95, and 100%) and then placed in acetone. Processed specimens were infiltrated using unpolymerized resin plastic and cured at an elevated temperature in an oven overnight. Specimens were cut to 0.5  $\mu\text{m}$  thick slices, stained with toluidine blue, and imaged on an upright microscope (Olympus CX41, Olympus, Center Valley, PA).

### Peel Test Validation

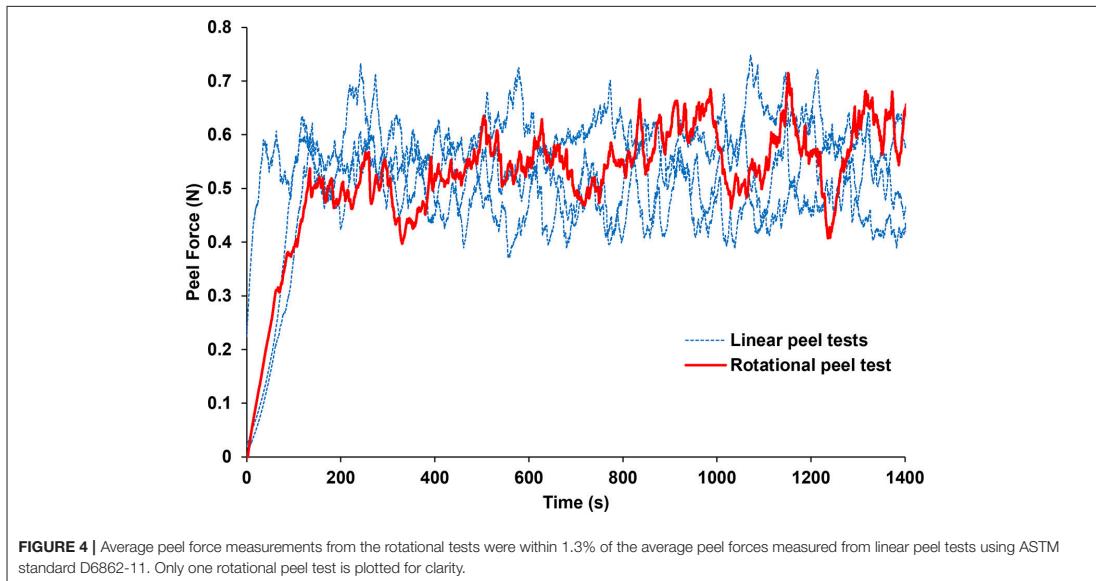
The novel rotational peel system was validated by measuring adhesion between metal and tape (Daigger, Vernon Hills, IL) and comparing measurements in the rotational system to measurements from linear peel testing using ASTM standard

D6862-11 (Figure 3A; ASTM, 2016). For the rotational test, tape was adhered to a aluminum ball bearing and placed inside the eye holder (Figure 3B). The tape was glued to the tab similar to the retinal experiments and followed the protocol described in section Peel Test Setup and Protocol. The linear peel test setup was identical to the rotational test, however it differed by adhering the tape to a flat aluminum surface (Figure 3A). After gluing the tab to the tape, the aluminum plate was horizontally actuated as the tab moved upward to maintain a 90° peel angle. The adhesive force over time for the rotational ( $n = 6$ ) and linear ( $n = 3$ ) peel tests were compared.

### Data Analysis

In any peel test, a maximum force is required to initiate a peel, and a steady-state peel force maintains the peel. In this study, these attributes were extracted through careful examination of videos of each peel test in conjunction with the force-time data.

The maximum peel force was defined as the maximum force measured prior to separation between the retina and the vitreous. The period of steady-state peel was defined as the time period



of relatively constant force after the vitreous separated from the retina, leaving vitreous in contact at the base of the peel. A video showing a typical peel test is provided as **Supplemental Data**. The steady-state peel force was calculated as the average force across the steady-state peel period. Retinal samples that detached from the L-shaped tab or tore before steady-state peeling were excluded from analysis.

A two-way ANOVA with repeated measures was performed to evaluate the effect of region and age on the maximum and steady-state peel force in the sheep eyes and human eyes. Repeated measures were used because regional data was collected from the same eyes. To perform this test in humans, donor ages were binned into the 4, 5, 6, 7, or 8th decade of life. Tukey-Kramer *post-hoc* tests were selected to evaluate pairwise comparisons within each effect. A linear regression was also performed in the human eyes to see if the maximum and steady-state peel force significantly decreased with age. The linear regression analysis was executed separately for each region (equator, posterior pole).

To identify significant differences in mechanisms of failure, the light microscopy images of the peeled retina were rated according to the following criteria: 0-ILM cleanly separated with no disruption or evidence of traction; 1-ILM cleanly separated, but ILM is undulated or there is evidence of traction on the ILM; 2-ILM cleanly separated, with the exception of 1–2 small localized disruptions (typically around vessels); 3-ILM torn and disrupted. A chi-square test was performed on these scores to identify significant differences between region and age. A logistic regression was also used to determine if the maximum and steady-state peel forces were predictive of failure type (i.e., 0, 1, 2, or 3).

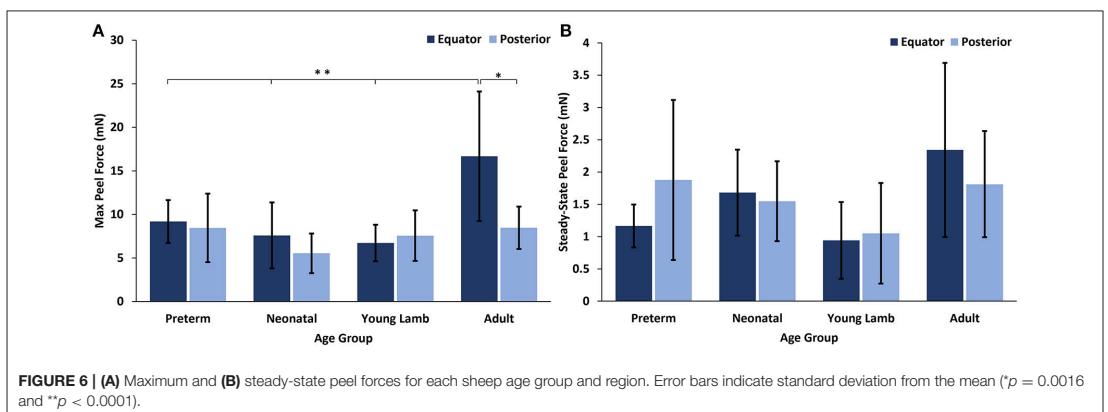
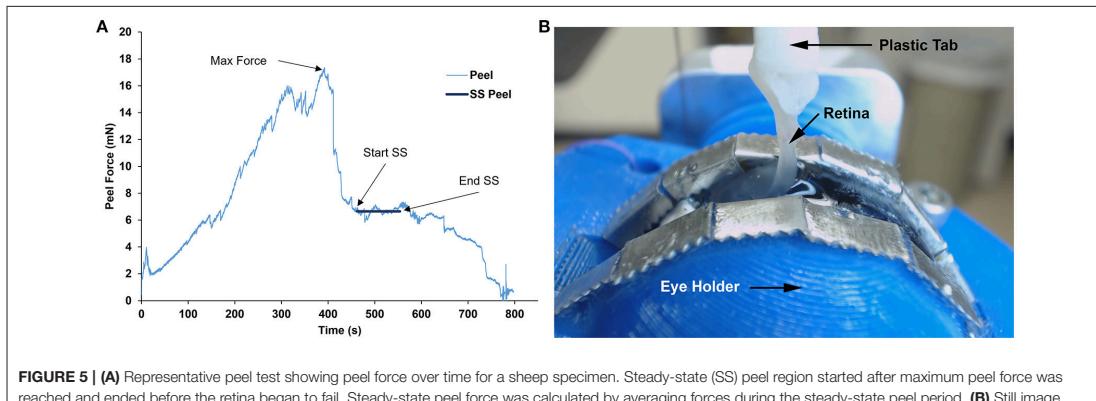
For all statistical tests, *p*-values < 0.05 were considered significant.

## RESULTS

The forces measured from the linear and rotational tape peel tests did not exhibit a definitive peak force (**Figure 4**), so steady-state peel force was calculated as the average of the data after the initial ramp up period. The resulting linear and rotational steady-state peel forces were  $0.534 \pm 0.062$  and  $0.527 \pm 0.075$  N, respectively, validating the device and methodology. All human and sheep peel tests exhibited a distinct maximum peel force (**Figure 5A**) followed by a steady-state peel region (**Figure 5B**). Decreases in force after the steady-state period were due to defects in the retina causing it to prematurely tear, retinal thinning or stretching during particularly long peel tests, or manual cutting by the test observer to end the test.

### Effect of Age and Region in Sheep Eyes

No significant differences with region were seen for either the max or steady-state peel force except for the adults which had significantly higher max peel force in the equatorial region ( $16.67 \pm 8.46$  mN) compared to the posterior region ( $8.46 \pm 2.43$  mN, *p* = 0.0016, **Figure 6**). Maximum peel force in the equator was also significantly higher in the adult compared to the younger ages ( $7.96 \pm 2.81$  mN, *p* < 0.0001). In most premature eyes, the steady-state adhesion in the posterior pole ( $1.88 \pm 1.24$  mN) was greater than the equator ( $1.12 \pm 0.33$  mN), but this finding was not statistically significant.



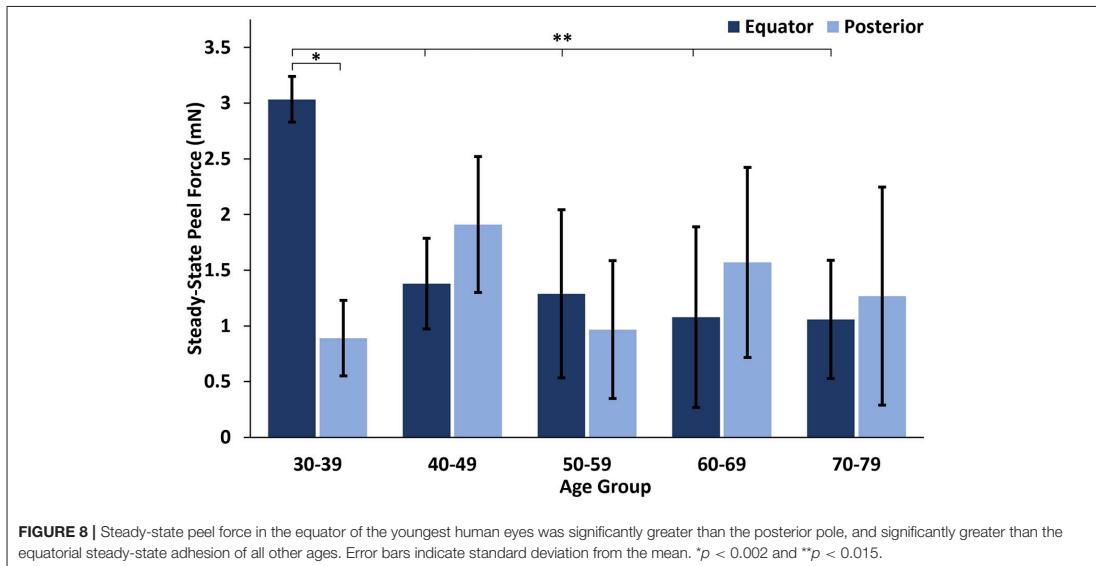
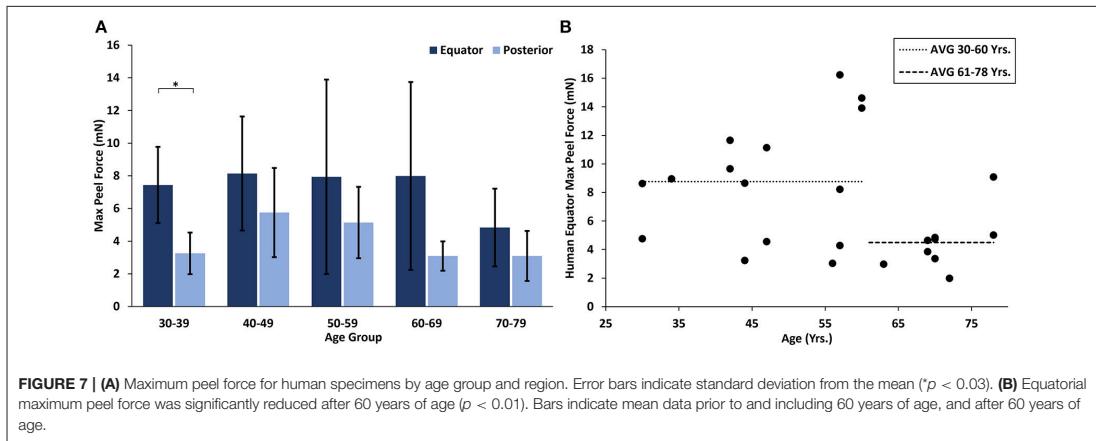
### Effect of Age and Region in Human Eyes

At all ages, the maximum peel forces in the equator ( $7.16 \pm 4.08$  mN) were greater than those in the posterior pole ( $4.08 \pm 2.03$  mN, Figure 7A), but this regional difference was only significant in the youngest adult ages ( $p < 0.03$ ). With age groups defined by decade of life, there was no significant difference in maximum adhesion in the equator or posterior pole with age. This finding was confirmed by the linear regression with age. However, when maximum peel force was plotted against age, there was a noticeable decrease in equatorial vitreoretinal adhesion after 60 years of age from  $8.76 \pm 4.22$  to  $4.50 \pm 2.00$  mN (Figure 7B). This decrease was found to be significant when evaluated with a Student's *t*-test ( $p < 0.01$ ). Steady-state peel force was significantly affected by age ( $p < 0.006$ ), region ( $p < 0.0001$ ), and their interaction ( $p < 0.001$ ). Specifically, vitreoretinal adhesion in the equator of the youngest adults ( $7.44 \pm 2.34$  mN, 30–39 years old) was significantly greater than the posterior pole in that age group ( $3.25 \pm 1.27$  mN,  $p < 0.002$ ) and significantly greater than

the equatorial adhesion in the other age groups ( $p < 0.015$ , Figure 8).

### Locations of Failure

Inspection of light microscopy images in the sheep revealed generally clean separation at the vitreoretinal interface (Figure 9A). Occasional disruption or failure of the ILM was observed (Figure 9B), especially in the presence of blood vessels (Figure 9C) and in immature eyes (i.e., premature, neonatal, and young lamb). Retinal stretching without ILM disruption was often seen in the nerve fiber layer, ganglion cell layer, or the outer plexiform layer. These observations were not different between the equator and posterior pole. In the human eyes, large disruption of the ILM occurred in the posterior pole (Figure 9D), and was significantly different than the failure location in the equator ( $p < 0.05$ ) which exhibited a clean separation (Figure 9E), oftentimes with indication of tractional pulling (Figure 9F). ILM disruption in the posterior pole was significantly greater in eyes from donors  $> 60$  years of



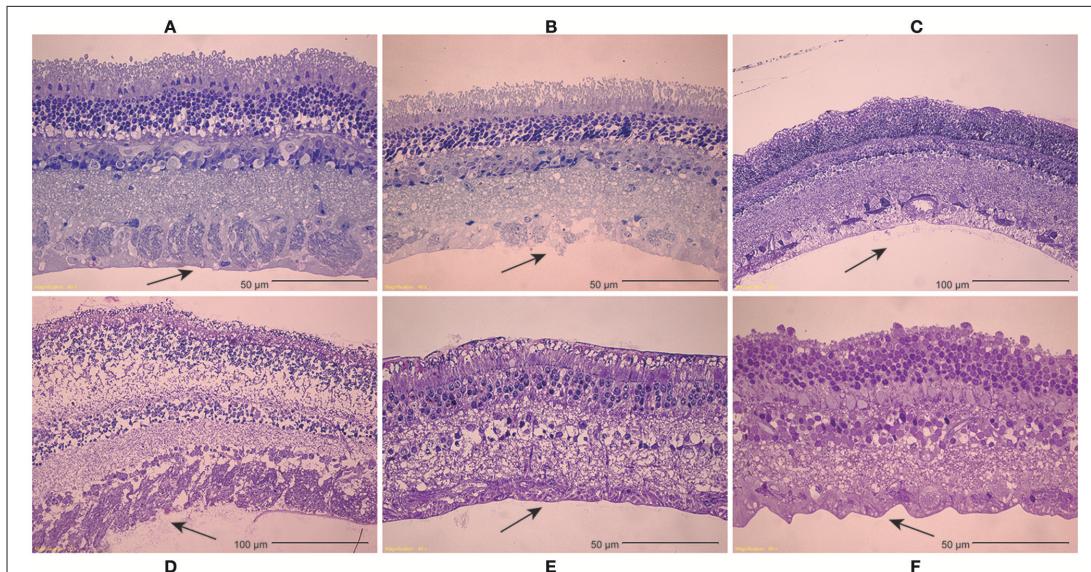
age ( $p < 0.02$ ). No significant differences in failure location were found with gender, and ILM disruption was not predicted by the maximum or steady-state forces.

## DISCUSSION

In this study, we developed a novel rotational peel test system to measure the strength of adhesion at the vitreoretinal interface. We used the device to quantify age- and region-dependent vitreoretinal adhesion in human and sheep eyes. These data are critical to improving biomechanical understanding of the vitreoretinal interface. They can be implemented

into computational models to investigate posterior vitreous detachment, or to simulate traumatic ocular injury. Further, the peel test system and associated data can be used to evaluate the effectiveness of chemical compounds to assist in surgical separation of the retina and vitreous.

In both sheep and human eyes, there was an interesting distinction between the maximum force of vitreoretinal adhesion in the equator and posterior pole that was highly dependent on the maturity of the animal. In premature, neonatal, and young lamb eyes, there were no regional differences. By the time the sheep developed into young adults (~28–36 years human age equivalent), the equatorial vitreoretinal adhesion significantly increased while adhesion in the posterior pole



**FIGURE 9 | (A–C)** Light microscopy images of peeled sheep retinas indicate **(A)** failure primarily occurred at the ILM. **(B)** In some images, focal ILM disruption was noted, **(C)** particularly in the presence of blood vessels. There were no regional differences in failure location, but immature sheep eyes tended to have more ILM disruption than young adult sheep eyes. **(D–F)** For human eyes, **(D)** peeling in the posterior pole typically disrupted the ILM, **(E)** while peeling in the equator had a clean separation between the vitreous and retina. **(F)** This clean separation was sometimes accompanied by traction-like undulations on the retinal surface.

remained unchanged from the immature sheep eyes. This may be due to posterior extension of the vitreous base and ILM thickening with age. Wang et al. investigated the anteroposterior length of the vitreous base in 58 pairs of human eyes from subjects spanning ages of 8–96 years old (Wang et al., 2003). They report a continual posterior extension of the length of the vitreous base until ~80 years old, at which time the length decreases. A similar study has not been performed in sheep, but a posterior extension of the vitreous base with age would explain the increased equatorial adhesion in the adult sheep eyes compared to immature sheep eyes.

Similar to the sheep eyes, the maximum peel force in the equator of the human eyes was greater than in the posterior pole. This was only significant in the youngest age group (30–39 years old), but all binned age groups younger than 70 years of age had higher average adhesion in the equator than in the posterior pole. One interesting finding from our dataset was a significant decrease in equatorial maximum peel force in eyes from human subjects > 60 years of age (Figure 7B). This suggests the existence of age-related changes that reduce equatorial adhesion, and overshadow any increased adhesion due to vitreous base extension. The distinct drop-off in equatorial max peel force around 60 years of age corresponds with the timeline of vitreous liquefaction (Foos, 1972; Balazs and Denlinger, 1982; Sebag, 1987, 1989; Uchino et al., 2001) and may be protective to the retina by facilitating posterior vitreous detachment (PVD) before retinal tearing or retinal detachment occurs. In PVD,

vitreoretinal separation is typically thought to begin near the macula and progress anteriorly (Kakehashi et al., 1997, 2014). As liquefaction occurs, increased traction is placed on the retina at locations of strong adhesion. The decreased equatorial adhesion after 60 years of age, a time when PVD is most prevalent, reduces the risk of retinal tearing or detachment in the equator. Further to this point, the steady-state peel force in our study was similar in both the posterior pole and the equator for all ages except the youngest age group (30–39 years old). This suggests that for most adults, PVD can gradually develop anteriorly into the equatorial region with minimal resistance and risk for retinal tearing. However, in young ages, there will be an increased risk for retinal tearing or detachment. This may explain why severe myopes, who have an increased disposition to liquefaction at young ages, have an increased risk for retinal detachment (Akiba, 1993).

Our study is the first quantitative measurement of vitreoretinal adhesion, but other groups have qualitatively evaluated adhesion at the vitreoretinal interface (Sebag, 1992; Marmor et al., 1994; Gandorfer et al., 2001). Their observations of changes in adhesion with age or region correlate with our findings. Sebag (1992) microscopically examined vitreoretinal adhesion in the posterior pole in human eyes from donors aged from 33 weeks of gestation to 94 years. He reported disruption to Müller's cells during peeling in 6 of the 15 eyes from donors < 20 years old, suggesting young adults and children have stronger posterior pole adhesion than older adults. In sheep eyes,

we found no significant difference in posterior pole adhesion between immature and adult sheep. The young adult sheep in our study correspond to 26–38 years old for humans. We visually observed minimal to no vitreous liquefaction during dissection of the eyes, similar to the visually observed levels of liquefaction in our youngest group of human eyes (30–39 years old). If we had used older adult sheep, we may have seen distinctions in the posterior pole adhesion similar to Sebag.

Microscopic examination of peeled sheep retinas suggested generally clean separation at the ILM, regardless of age or region. Occasionally, the ILM was torn in immature eyes, regardless of region. In adult sheep, the ILM was never torn, but the nerve fiber layer was often stretched and occasionally disrupted locally around blood vessels. There was a single premature specimen with failure occurring in the ganglion cell layer, which is similar to Sebag. We obtained light microscopy specimens from 37% of our sheep peel tests. A more extensive microscopic analysis might correlate more closely with observations by Sebag.

In human adult peeled retinas, there was significant distinction between failure in the equator and posterior pole. The peeled retina in the equator was generally smooth with some undulation or traction-like peaks on the surface of the ILM, while many of the posterior peeled retinas exhibited complete disruption of the ILM. This was surprising given that adhesion in the posterior pole is significantly lower than the equator. We hypothesize that numerous collagen penetrations in the posterior pole create large disruptions of the ILM when pulled, and that there is less collagen penetration in the equator where adhesion is thought to be dominated by adhesive proteins acting as an extracellular glue. Failure of this “glue” would result in cleaner separation. The traction-like characteristics may be caused by sparse penetration of collagen fibrils. Interestingly, ILM disruption in the posterior pole was significantly greater in eyes from donors older than 60 years of age. Because the adhesive strength is weak at this age, we hypothesize that the disruption of the ILM is an indication of decreased retinal structural integrity. Regardless, these data show adhesive strength cannot be inferred solely from locations of damage. Mechanism of adhesion and changes in retinal structural integrity with age likely contribute to patterns of failure.

Gandorfer et al. evaluated the effect of plasmin in different regions of porcine eyes (Gandorfer et al., 2001). After the same dosage and incubation time (2 U/0.1 mL plasmin, 60 min), they report a bare ILM in the posterior pole, sparse collagen fibrils in the equator, and a dense network of collagen fibrils at the vitreous base. This may suggest that adhesion is greater anteriorly to posteriorly, but different mechanisms of adhesion may occur in different regions of the eye, and may be affected by plasmin differently. Plasmin is known to digest fibrin and laminin, which are more prominent in the posterior pole compared to the equator (Mitry et al., 2010). There is some indirect digestion of collagen, but the extent is unknown. Future clarification is still needed to elucidate the role of glycoproteins in adhesion of the vitreoretinal interface at the vitreous base, equator, and posterior pole.

Several studies have quantitatively measured adhesion between the neurosensory layer and retina pigment epithelium (RPE) (Zauberman and Berman, 1969; Lincoff et al., 1970; DeGuillebon et al., 1971, 1972; DeGuillebon and Zauberman, 1972; Zauberman, 1972; Zauberman and DeGuillebon, 1972; Zauberman et al., 1972; Owczarek et al., 1975; Kain, 1984). Similar to our results, these studies report significantly greater adhesion in the equator compared to the posterior pole. Zauberman and Berman (1969) reported 1.18–2.45 mN in the equator compared to 0.59–0.88 mN in the posterior pole in cats. DeGuillebon et al. (1971) reported 0.77–1.41 mN in the equator compared to 0.86–1.206 mN in the posterior pole in rabbits. One exception to this is a study by DeGuillebon et al. (1972) that found increasing peel rates increased RPE adhesion in the posterior pole compared to the equator. The rates used in the present study are quasistatic, and suitable for PVD investigations. Additional vitreoretinal adhesion studies at higher rates will need to be performed to understand region and age-related differences associated with ocular trauma.

Retinal detachment or tearing can occur due to vitreoretinal traction, so adhesion at the vitreoretinal interface is likely greater than adhesion at the RPE in healthy young adults. No studies have measured RPE adhesion in sheep or in humans, making comparison of our measurements with the literature challenging. Kita and Marmor (1992) used subretinal injections to calculate posterior RPE adhesion in young adult primates (3.8–7.9 kg). They report average adhesive forces of  $140 \pm 3$  dynes/cm using a subretinal bleb technique. Without a reported bleb circumference, it is impossible to convert their measurements to mN and compare to our study. However, they state primate adhesive forces were 140% greater than that of rabbits, and an earlier study by the same group (Kita et al., 1990) reported  $1.979 \pm 0.22$  mN (converted using reported bleb circumferences). This results in an approximate primate RPE adhesion of  $2.771 \pm 0.31$  mN, and is 1.7 times lower than the average adhesion human eyes from donors 30–60 years of age in our study. Of note, the RPE adhesion measured in primates was only 10% lower than the maximum posterior peel force in our oldest age group (70–79 years old). None of the eyes we tested had PVD, so this oldest age group likely had greater vitreoretinal adhesion than those at risk for PVD.

In standard peel tests, a thin membrane is typically peeled from a solid surface. In these studies, we peeled a thin membrane (retina) from a gel (vitreous). This resulted in deformation of both materials prior to and during peeling. The maximum peel forces measured in this study were defined as the maximum force before clear separation of the vitreous from the retina. With this definition, the maximum peel force may incorporate some retinal stretching or separation from the scored retinal edges in addition to the peeling force. During steady-state peeling, the retina also experienced some deformation, however, careful examination of video in conjunction with the peel force data provided confidence that peeling, and not retinal deformation, was the primary contributor to the steady-state measurements. A computational simulation of the peel tests is planned for a future study to separate tissue adhesion and vitreous deformation in the maximum peel force measurements.

The data collected in this study was on the low end of the load cell limit. Because of this, we had the load cell carefully calibrated at its reported lower limit of 9 mN. The uncertainty measurement at this limit was 0.002 gf, or 0.0196 mN, and decreased with decreasing load. The maximum peel force data in our study ranged from 4 to 15 mN, which is near the lower limit of the load cell. The steady state peel forces were lower than the maximum peel forces (1–3 mN), but were still on the same order of magnitude as the calibrated limit, and still two orders of magnitude larger than the uncertainty measurements. It is possible the load cell limits contributed to the variability in the steady-state measurements, but we have strong confidence in the conclusions and trends of the study.

Vitreoretinal adhesion to large blood vessels is thought to be greater than adhesion in regions without blood vessels. Our observations of ILM tearing surrounding blood vessels support this notion. Further, we observed steady-state peel forces drop after passing a blood vessel. For this study, we extracted steady-state values from regions without the blood vessels in order to maintain a consistent comparison across all ages and regions. A comprehensive and focused assessment of the effect of blood vessels on adhesion will be performed in a future study.

## CONCLUSION

We developed a novel device to quantify vitreoretinal adhesion in the equator and posterior pole of human and sheep eyes. Maximum vitreoretinal adhesion in adult human eyes (30–79 years old) was greater in the equator than in the posterior pole, especially at young ages (30–39 years old). After 60 years of age, there was a significant drop in equatorial adhesion that may be protective to the retina by facilitating vitreous detachment during liquefaction. In immature (premature, neonatal, and young lamb) and mature (young adult) sheep eyes, there was no significant difference in posterior vitreoretinal adhesion, but maximum equatorial adhesion in mature eyes was 2 times greater than immature eyes. This may be caused by the extension of the vitreous base during development. These data are the first quantitative measurements of vitreoretinal adhesion, and

will be useful in the development of computational models for simulating posterior vitreous detachment or ocular trauma. The methods and technology developed for this study can be used to evaluate mechanisms of adhesion, and assess the efficacy of enzymes to remove or reduce vitreoretinal adhesion for surgical interventions.

## DATA AVAILABILITY

All data used in this manuscript is available via doi: 10.7278/S5BK19H3 located within the University of Utah Research Data Repository (<https://hive.utah.edu>).

## AUTHOR CONTRIBUTIONS

CC performed testing, sectioning, preliminary analysis and manuscript writing. JC assisted with all aspects of the data analysis and manuscript writing. BC was responsible for the study conception and design, data interpretation, and manuscript editing.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fbioe.2018.00153/full#supplementary-material>

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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### 3.7 PDF Import

Using the L<sup>A</sup>T<sub>E</sub>X package `pdfpages`, you can import PDFs into your PDF. An example of this is used to import a previously published journal article in [Script 8](#). There are function keywords that allow you to add sections to the table of contents, list of figures, and the list of tables.

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List of Figures, and List of Tables.

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5 % list of tables
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16   4,subsection,1,Peel Test Validation,chp3M_3,
17   4,subsection,1,Data Analysis,chp3M_4,
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20   6,subsection,1,Effect of Age and Region in Human Eyes,chp3R_2,
21   6,subsection,1,Locations of Failure,chp3R_3,
22   7,section,1,Discussion,chp3D,
23   10,section,1,Conclusion,chp3C},
24 addtolist={
25   3,figure,{(A) Scleral dissection methods used to create a rectangular
26     retinal window (gray crosshatch) in the (B) equatorial and (C)
27     posterior regions. The location of the optic nerve head is shown as a
28     white circle, and the fovea marked with an ``x''.},3fig1,
29   4,figure,{For each peel test, (A) sclera was dissected away from the
30     retina prior to testing, and the eye placed in the fixture. The eye was
31     raised toward the testing window by inflating a polymer liner with air.
32     (B) An L-shaped tab was glued to the retina and raised to peel the
33     retina from the vitreous while measuring force. (C) Optical coherence
34     tomography image of the peeled retina indicating separation was at the
35     vitreoretinal interface and glue penetration was minimal.},3fig2,
36   4,figure,{(A) Tape linear peel tests were performed and compared to (B)
37     tape rotational peel tests to ensure validity of the custom rotational
38     peel device.},3fig3,
39   5,figure,{Average peel force measurements from the rotational tests were
40     within 1.3\% of the average peel forces measured from linear peel tests}
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41     using ASTM standard D6862-11. Only one rotational peel test is plotted
42     for clarity.},3fig4,
43 ,figure,{Representative peel test showing peel force over time for a
44     sheep specimen. Steady-state (SS) peel region started after maximum
45     peel force was reached and ended before the retina began to fail.
46     Steady-state peel force was calculated by averaging forces during the
47     steady-state peel period. (B) Still image taken from the webcam video
48     of a sheep retina peeling away from the vitreous at 90$^\circ$ during
49     the steady-state peel region.},3fig5,
50 ,figure,{(A) Maximum and (B) steady-state peel forces for each sheep age
51     group and region. Error bars indicate standard deviation from the mean
52     ($^*p = 0.0016$ and $^{**}p < 0.0001$).},3fig6,
53 ,figure,{(A) Maximum peel force for human specimens by age group and
54     region. Error bars indicate standard deviation from the mean ($^*p <
55     0.03$). (B) Equatorial maximum peel force was significantly reduced
56     after 60 years of age ($p < 0.01$). Bars indicate mean data prior to
57     and including 60 years of age, and after 60 years of age.},3fig7,
58 ,figure,{Steady-state peel force in the equator of the youngest human
59     eyes was significantly greater than the posterior pole, and
60     significantly greater than the equatorial steady-state adhesion of all
61     other ages. Error bars indicate standard deviation from the mean. $^*p
62     < 0.002$ and $^{**}p < 0.015$.},3fig8,
63 ,figure,{(A-C) Light microscopy images of peeled sheep retinas indicate
64     (A) failure primarily occurred at the ILM. (B) In some images, focal
65     ILM disruption was noted, (C) particularly in the presence of blood
66     vessels. There were no regional differences in failure location, but
67     immature sheep eyes tended to have more ILM disruption than young adult
68     sheep eyes. (D-F) For human eyes, (D) peeling in the posterior pole
69     typically disrupted the ILM, (E) while peeling in the equator had a
70     clean separation between the vitreous and retina. (F) This clean
71     separation was sometimes accompanied by traction-like undulations on
72     the retinal surface.},3fig9},
73 scale=0.8]
74 {./Chapter3/media/fbioe-06-00153}
75
76 \end{document}

```

## CHAPTER 4

# THIS IS ANOTHER MULTI-LINE TITLE THAT NEEDS TO HAVE AN INVERTED PYRAMID SHAPE WHERE EACH LINE IS NOT WIDER THAN 4.6 IN.

### 4.1 Abstract

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### 4.2 Introduction

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nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [3], [14].

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neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [18], [19]. Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

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wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [20]–[24] Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [20]

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## 4.3 Methods

### 4.3.1 Subsection

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scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [19], [28], [29].

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### 4.3.2 Subsection

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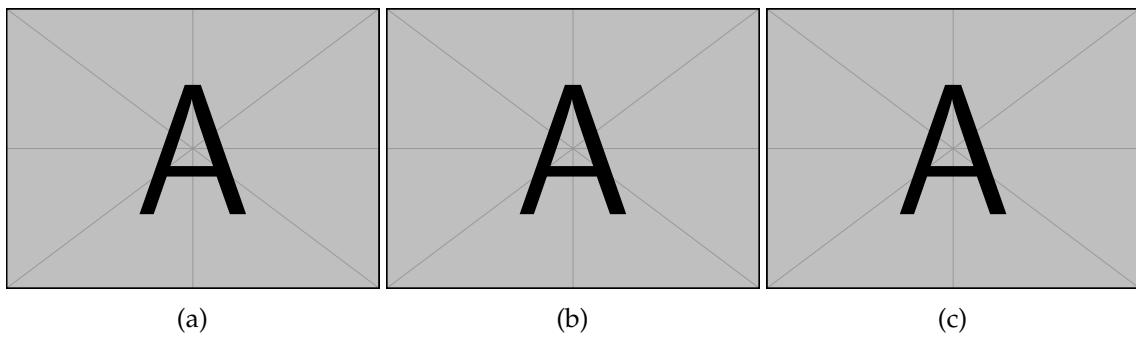


Figure 4.1: Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. ... (a) ... (b) ... (c) ...

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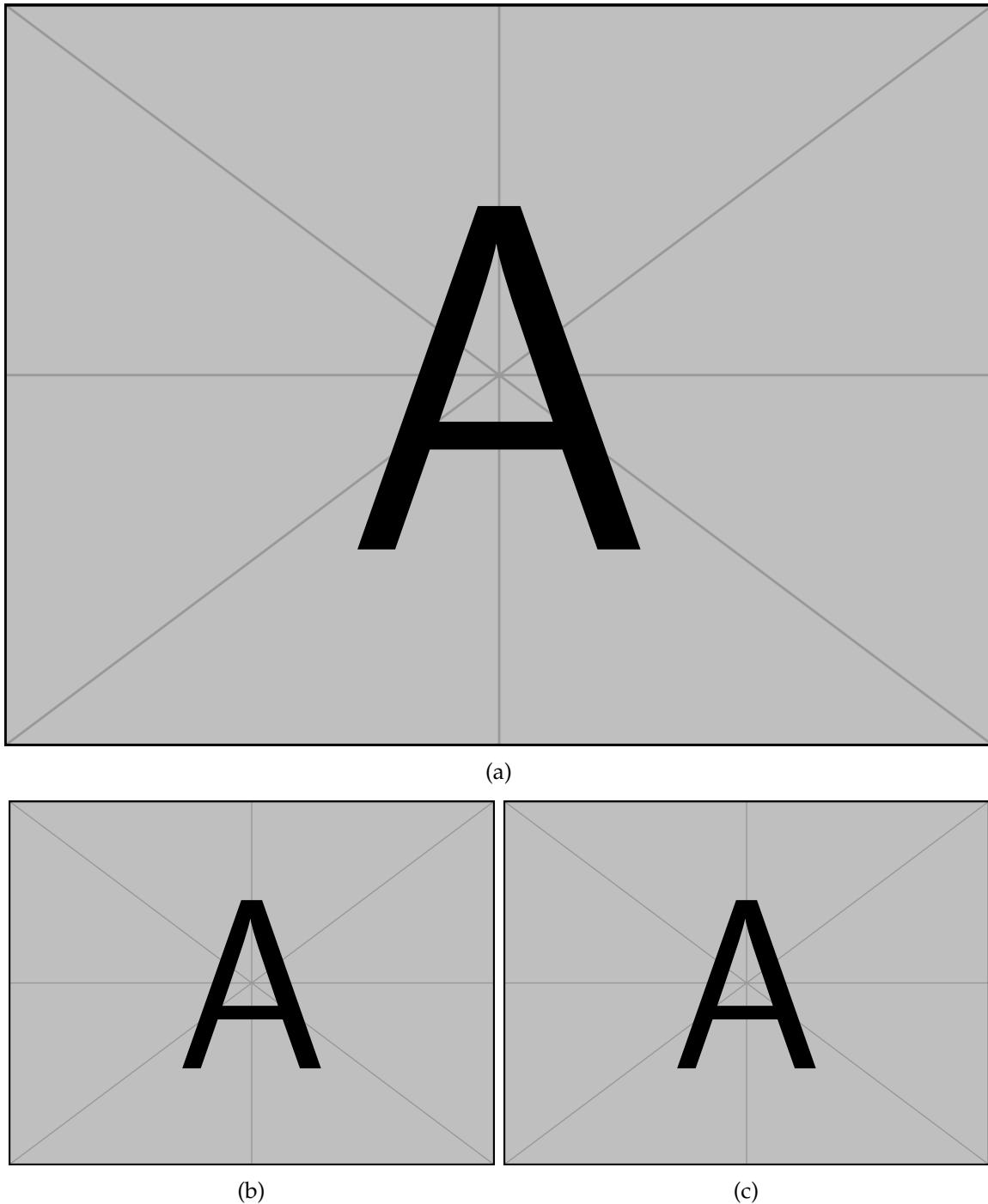


Figure 4.2: Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. (a) ... (b) ... (c) ...

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#### 4.3.2.1 Chemistry Formulations

For chemistry formulas use the following L<sup>A</sup>T<sub>E</sub>X commands from the `chemfig` package:

An example is seen here:

```
\chemfig{OsO_4} (4\% in \emph{d}\chemfig{H_2O}) \longrightarrow OsO4 (4% in dH2O)
```

#### 4.3.3 Subsection

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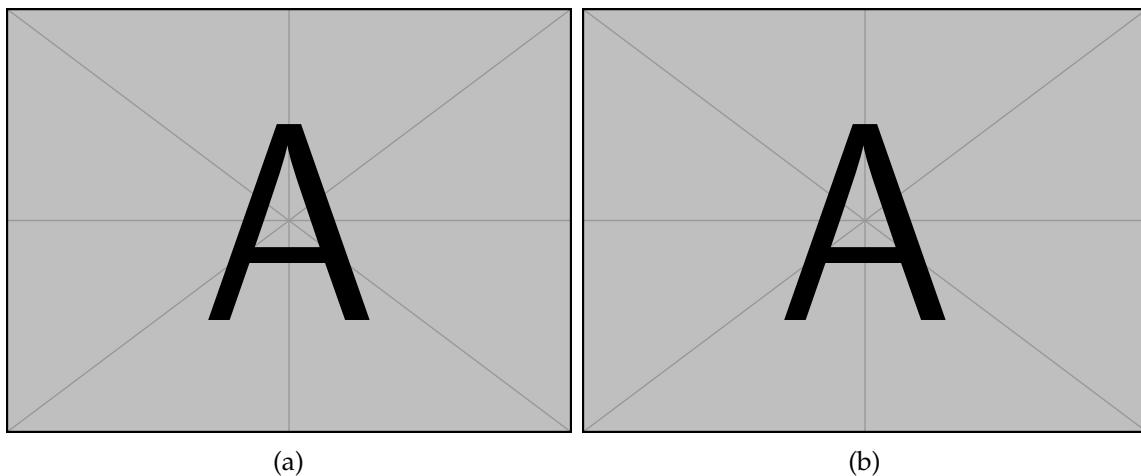


Figure 4.3: Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. (a) ... (b)

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#### **4.3.4 Subsection**

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## 4.4 Results

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tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. ([Figures 4.4 to 4.6](#))

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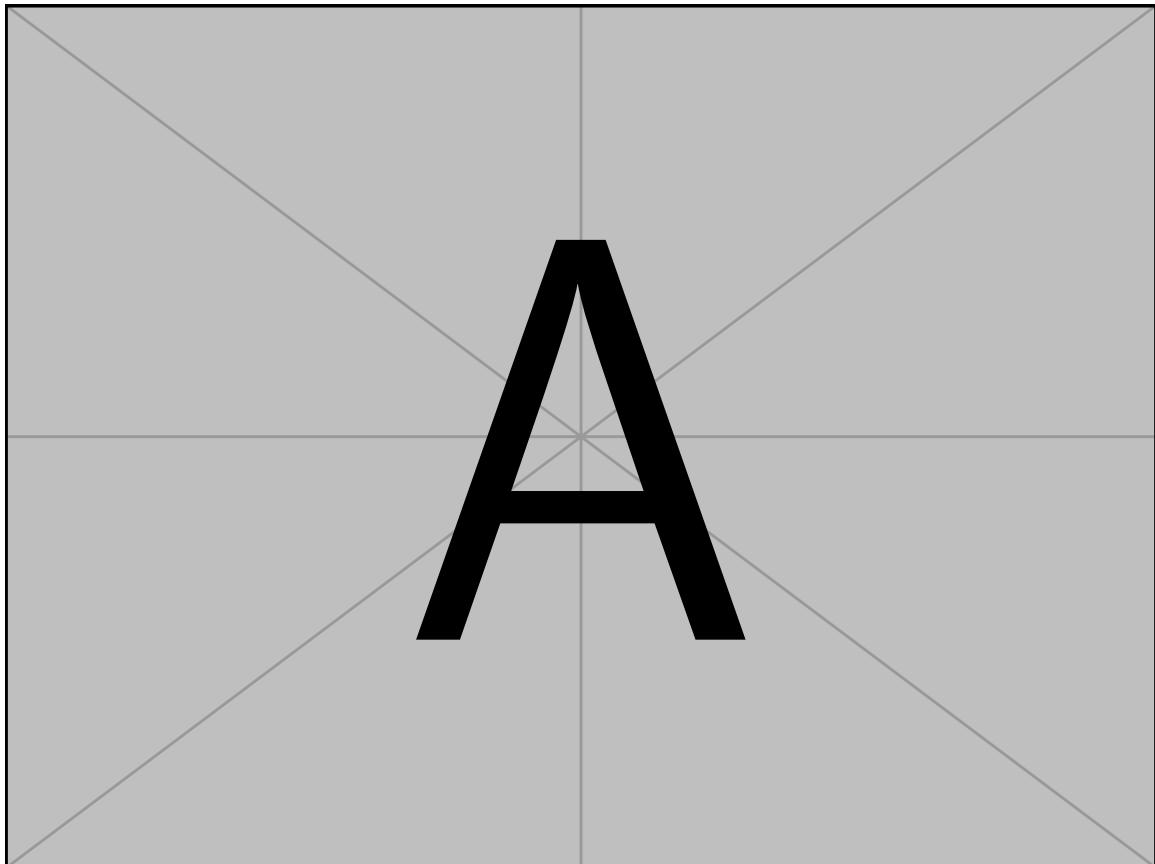


Figure 4.4: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

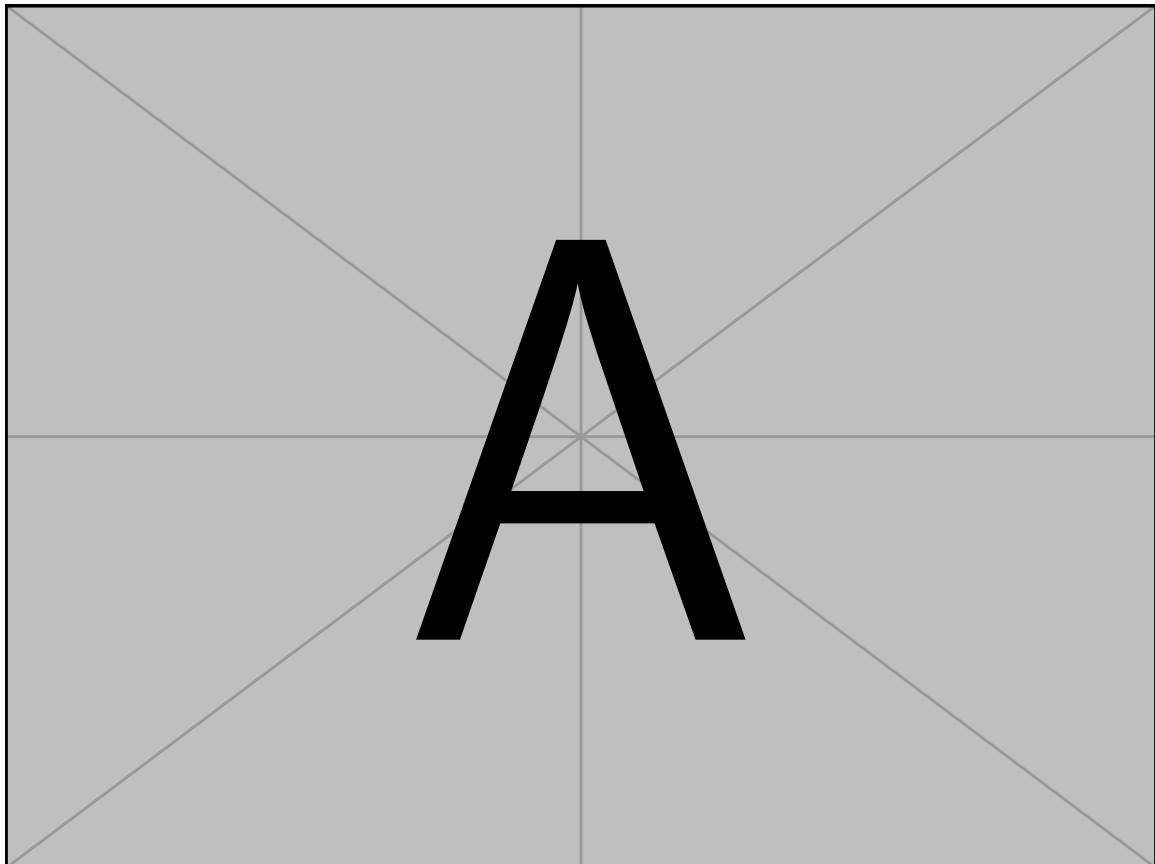


Figure 4.5: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

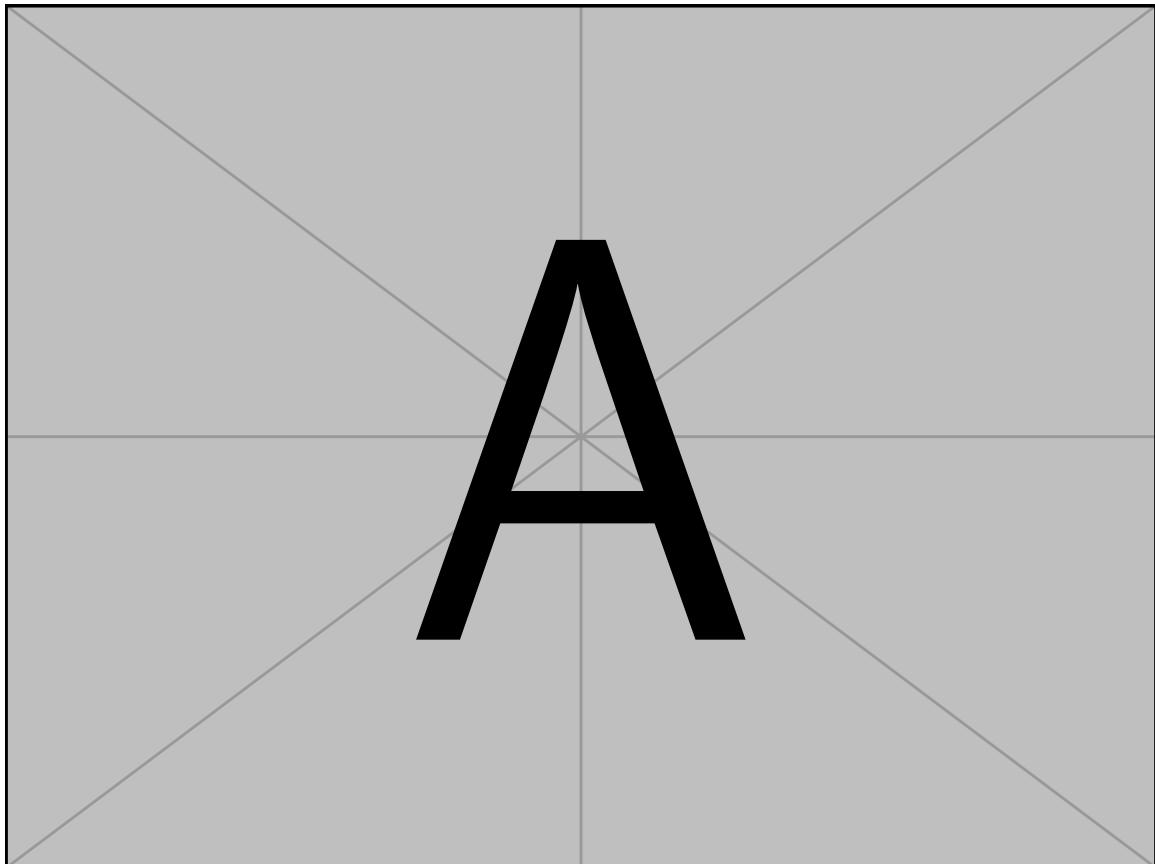


Figure 4.6: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

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#### 4.4.1 Subsection

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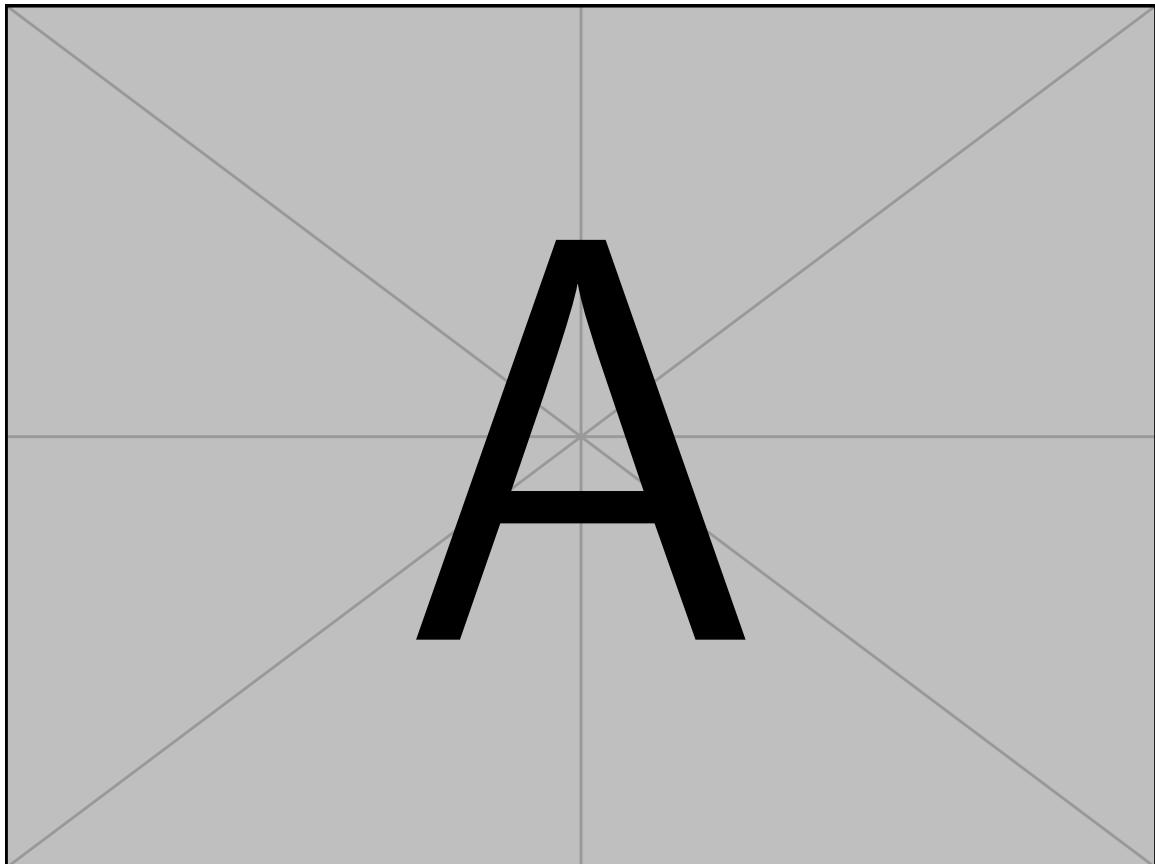


Figure 4.7: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

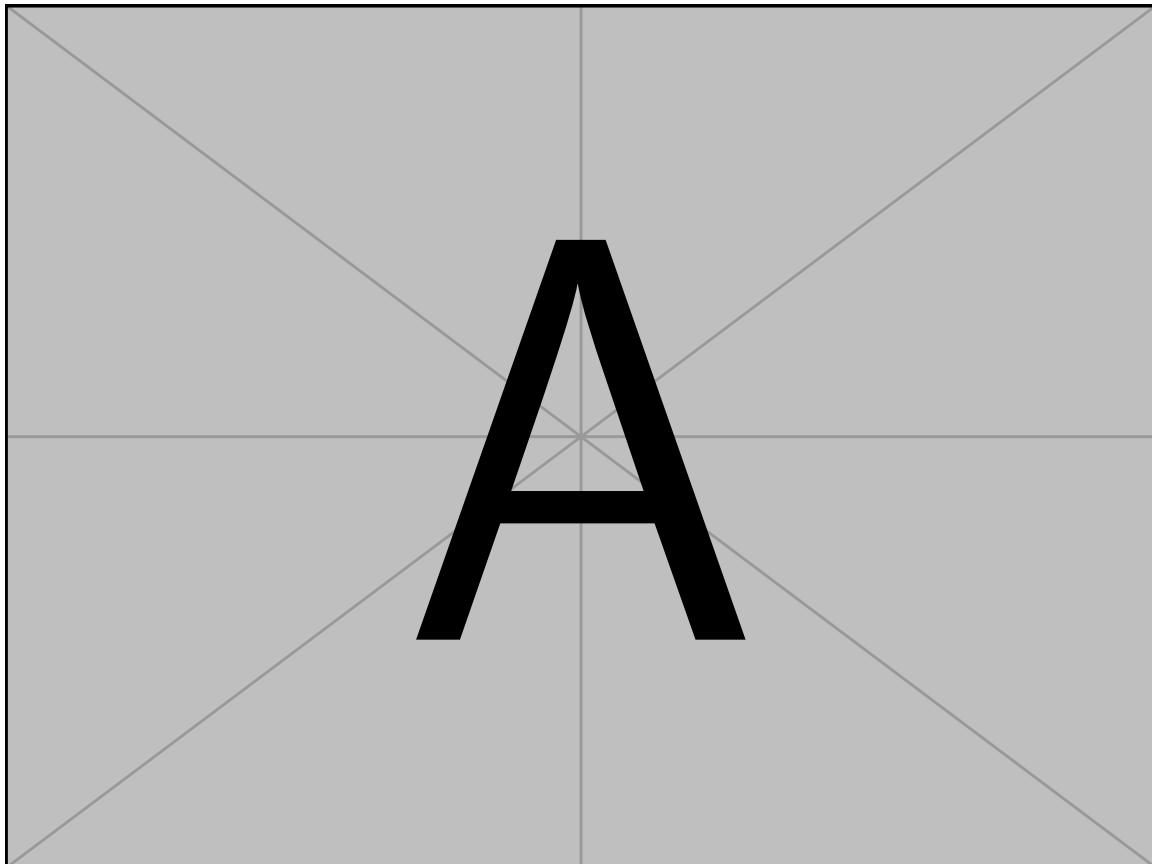


Figure 4.8: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

Table 4.1: ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

xx	xx	xx	xx (mN)	xx (mN)	xx (mN)
xx	xx	12	9.185	9.372	2.459
	xx	11	8.566	9.721	3.959
xx	xx	6	7.579	7.415	3.797
	xx	9	5.463	4.550	2.311
xx	xx	10	6.712	6.290	2.102
	xx	11	7.558	7.829	2.903
xx	xx	11	16.673	14.650	7.446
	xx	12	8.322	8.364	2.535

Table 4.2: ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

xx	xx	xx	xx	xx	xx
			(mN)	(mN)	(mN)
xx	xx	8	1.166	1.166	0.333
	xx	10	1.878	1.730	1.241
xx	xx	6	1.682	1.478	0.664
	xx	9	1.548	1.507	0.618
xx	xx	9	0.941	0.994	0.596
	xx	10	1.052	0.900	0.780
xx	xx	10	2.285	2.162	1.324
	xx	8	1.812	1.755	0.822

nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

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#### 4.4.2 Subsection

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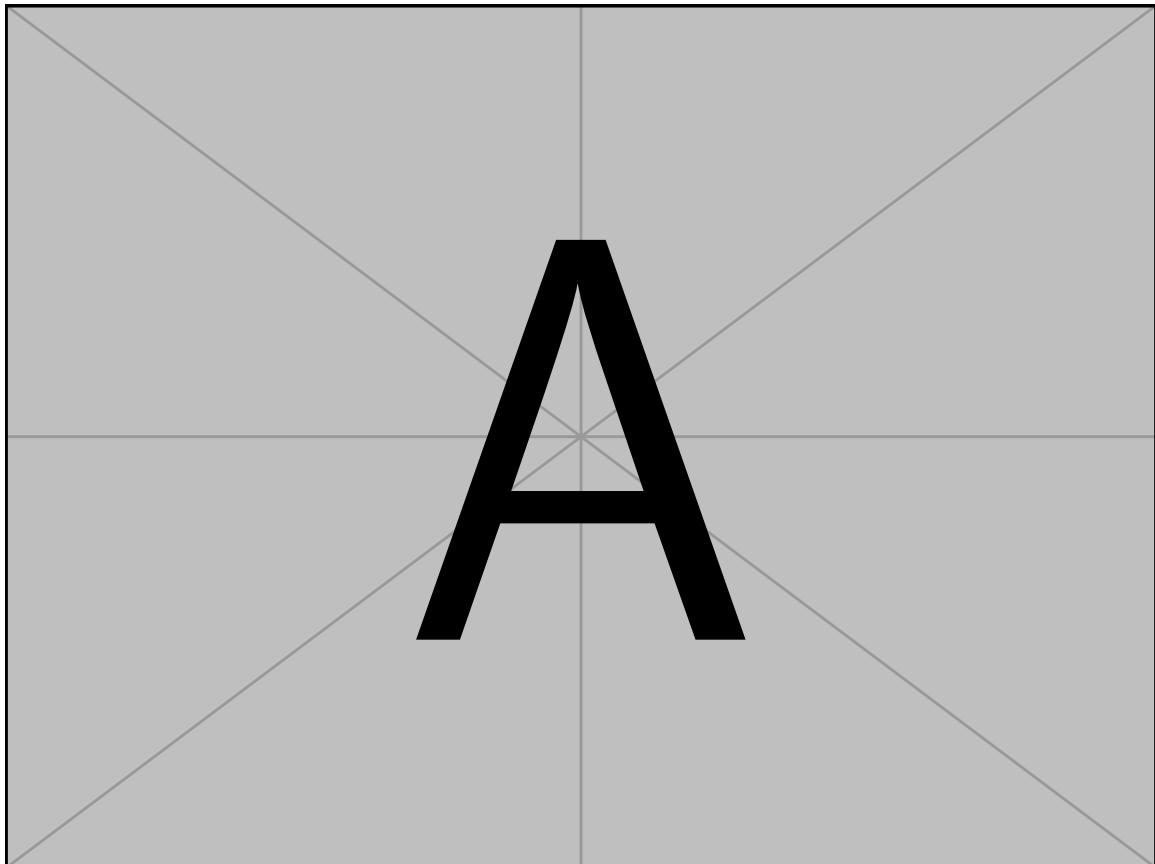


Figure 4.9: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

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#### Figure 4.10)

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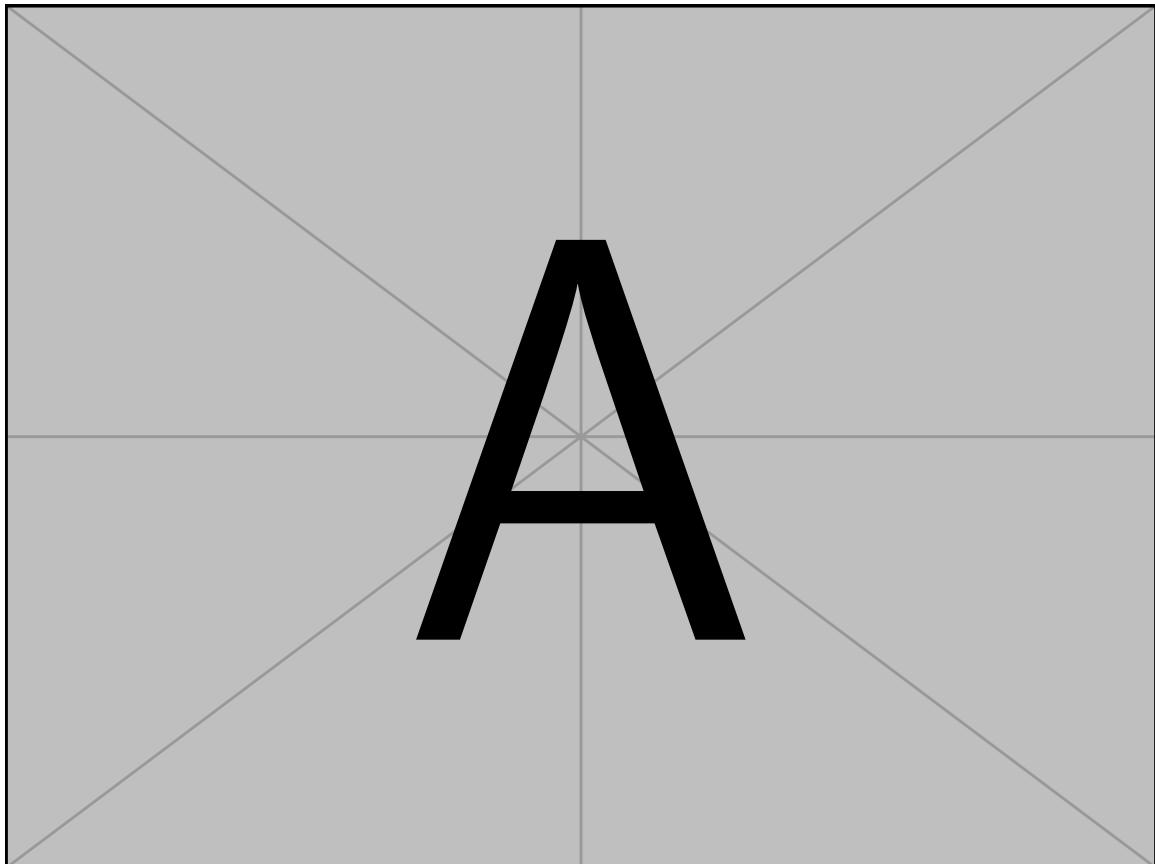


Figure 4.10: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

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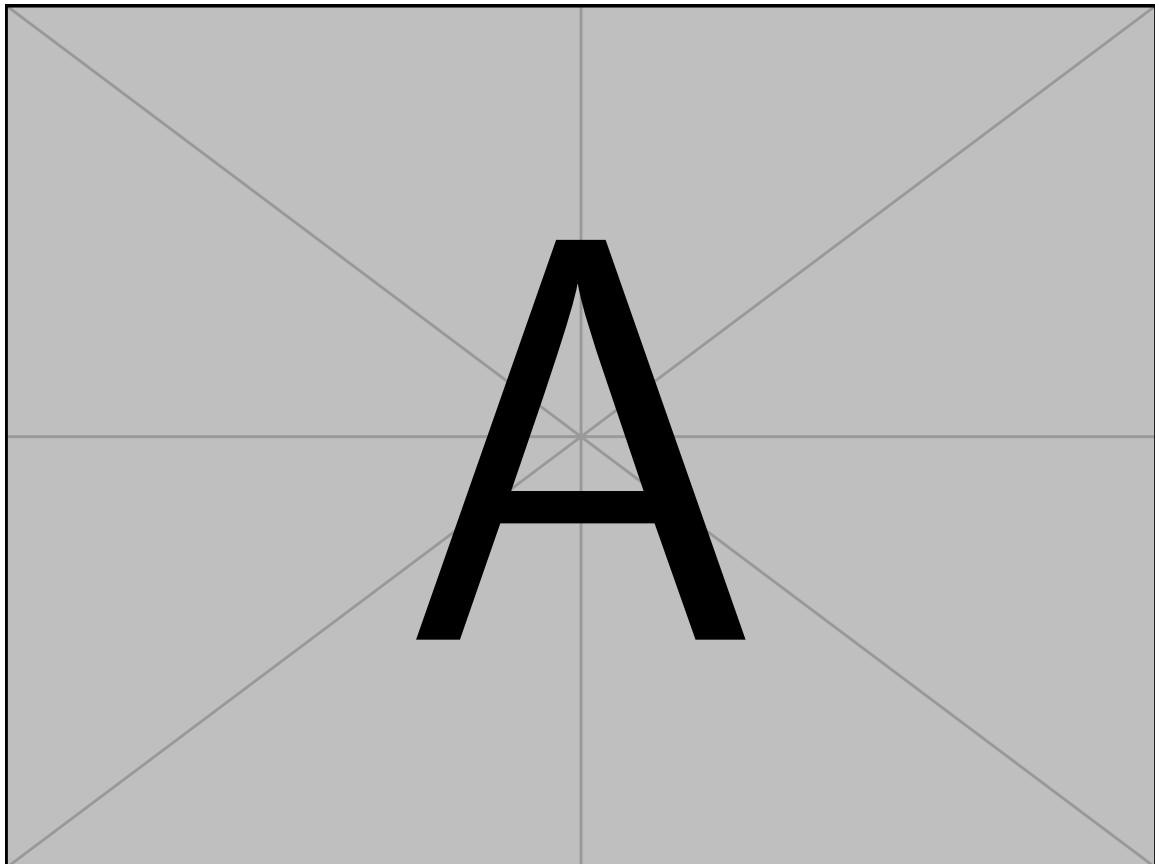


Figure 4.11: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

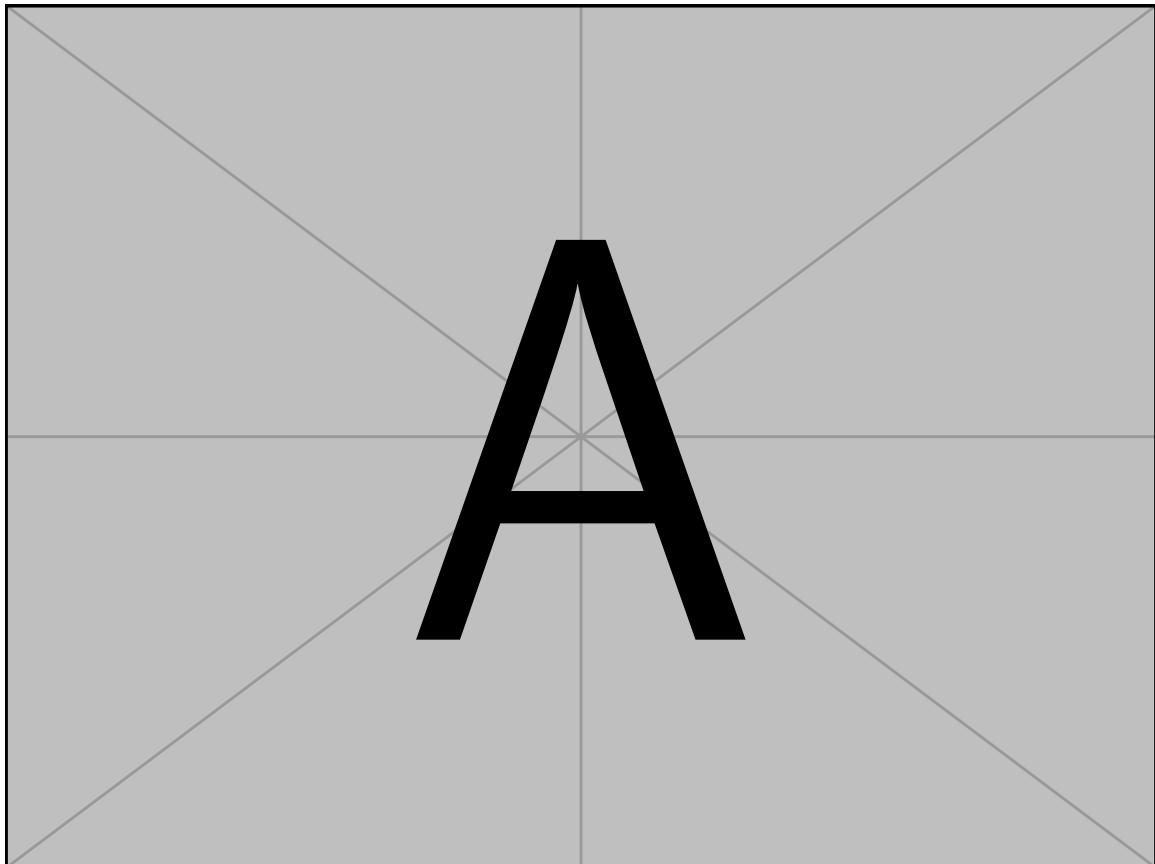


Figure 4.12: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

Table 4.3: ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

	xx (yr.)	xx	xx	mean (mN)	median (mN)	std (mN)
30-39	xx	3	7.440	8.620	2.335	
	xx	4	3.249	3.287	1.271	
40-49	xx	6	8.143	9.149	3.492	
	xx	4	5.753	4.722	2.732	
50-59	xx	4	7.939	6.248	5.954	
	xx	7	5.139	5.549	2.183	
60-69	xx	5	7.991	4.637	5.752	
	xx	5	3.087	2.908	0.894	
70-79	xx	6	4.835	4.789	2.382	
	xx	6	3.092	2.716	1.534	

Table 4.4: ... Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

	xx (yr.)	xx	xx	mean (mN)	median (mN)	std (mN)
30-39	xx	2	3.034	3.034	0.205	
	xx	4	0.891	0.845	0.339	
40-49	xx	5	1.380	1.424	0.407	
	xx	4	1.910	1.740	0.610	
50-59	xx	4	1.288	1.231	0.754	
	xx	6	0.967	0.996	0.619	
60-69	xx	4	1.079	0.865	0.811	
	xx	4	1.570	1.177	0.853	
70-79	xx	6	1.059	1.144	0.530	
	xx	5	1.269	0.863	0.978	

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Fusce mauris. Vestibulum luctus nibh at lectus. Sed bibendum, nulla a faucibus semper, leo velit ultricies tellus, ac venenatis arcu wisi vel nisl. Vestibulum diam. Aliquam pellentesque, augue quis sagittis posuere, turpis lacus congue quam, in hendrerit risus eros eget felis. Maecenas eget erat in sapien mattis porttitor. Vestibulum porttitor. Nulla facilisi. Sed a turpis eu lacus commodo facilisis. Morbi fringilla, wisi in dignissim interdum, justo lectus sagittis dui, et vehicula libero dui cursus dui. Mauris tempor ligula sed lacus. Duis cursus enim ut augue. Cras ac magna. Cras nulla. Nulla egestas. Curabitur a leo. Quisque egestas wisi eget nunc. Nam feugiat lacus vel est. Curabitur consectetur.

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Sed commodo posuere pede. Mauris ut est. Ut quis purus. Sed ac odio. Sed vehicula hendrerit sem. Duis non odio. Morbi ut dui. Sed accumsan risus eget odio. In hac habitasse platea dictumst. Pellentesque non elit. Fusce sed justo eu urna porta tincidunt. Mauris felis odio, sollicitudin sed, volutpat a, ornare ac, erat. Morbi quis dolor. Donec pellentesque, erat ac sagittis semper, nunc dui lobortis purus, quis congue purus metus ultricies tellus. Proin et quam. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos hymenaeos. Praesent sapien turpis, fermentum vel, eleifend faucibus, vehicula eu, lacus.

Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Donec odio elit, dictum in, hendrerit sit amet, egestas sed, leo. Praesent feugiat sapien aliquet odio. Integer vitae justo. Aliquam vestibulum fringilla lorem. Sed neque lectus, consectetur at, consectetur sed, eleifend ac, lectus. Nulla facilisi. Pellentesque eget lectus. Proin eu metus. Sed porttitor. In hac habitasse platea dictumst. Suspendisse eu lectus. Ut mi mi, lacinia sit amet, placerat et, mollis vitae, dui. Sed ante tellus, tristique ut, iaculis eu, malesuada ac, dui. Mauris nibh leo, facilisis non, adipiscing quis, ultrices a, dui.

#### 4.4.3 Subsection

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Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus.

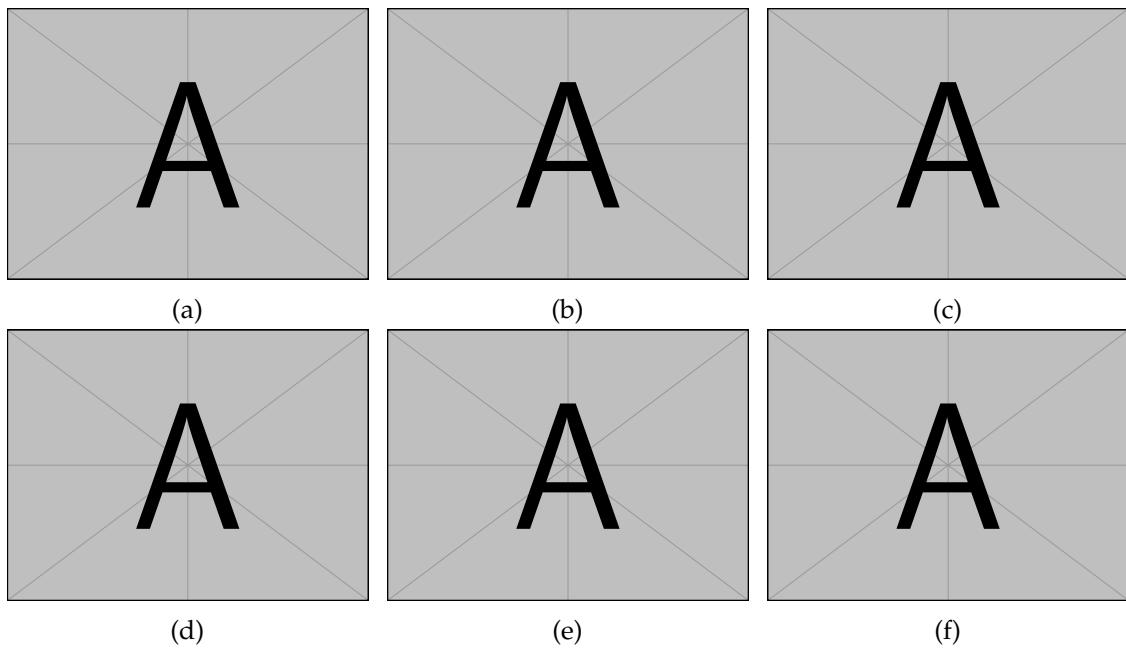


Figure 4.13: ... Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. ((a)-(c)) ... (a)...(b) ... (c) ... ((d)-(f)) ... (d) ... (e) ... (f).

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Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. ( $p < 0.05$ , [Figure 4.13d](#)) Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. ( $p < 0.05$ , [Figure 4.13e](#)) Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique

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## 4.5 Discussion

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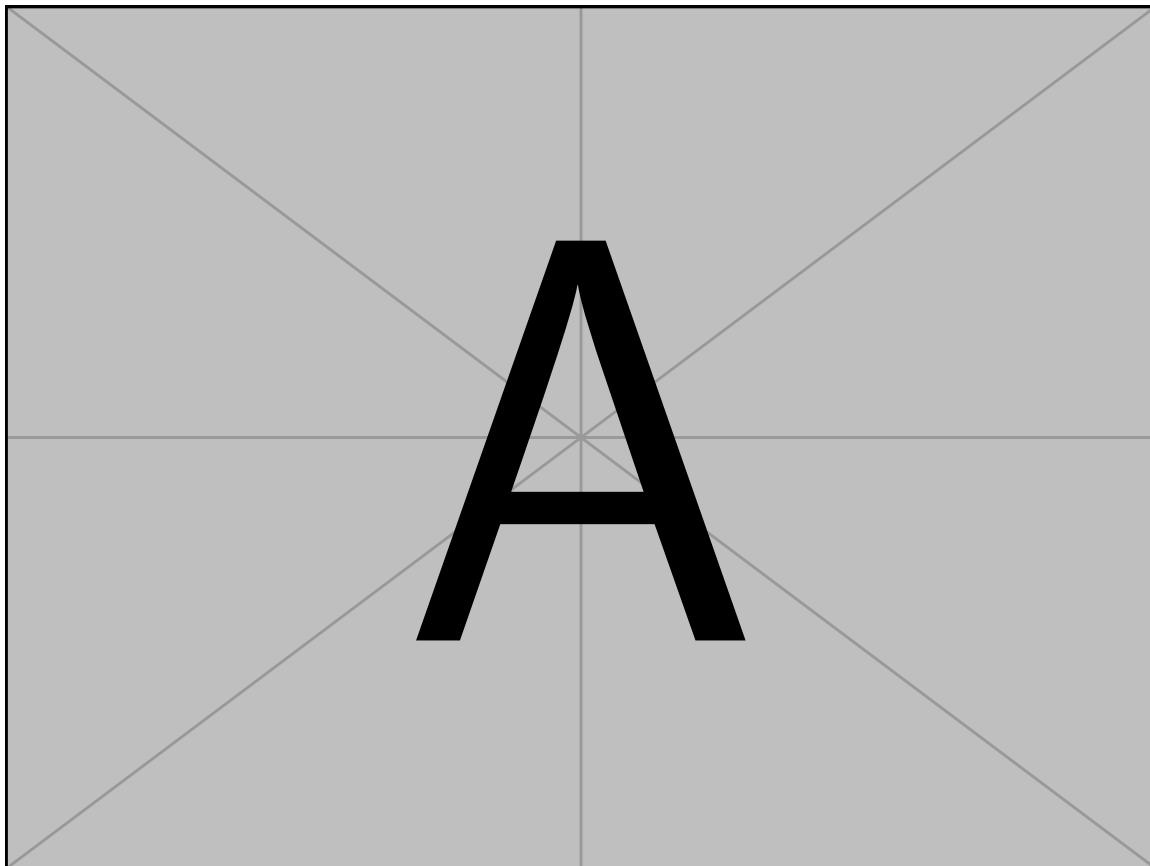


Figure 4.14: ... Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

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	xx	xx	xx
0	xx	6	
	xx	5	
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	xx	7	
2	xx	4	
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3	xx	1	
	xx	8	

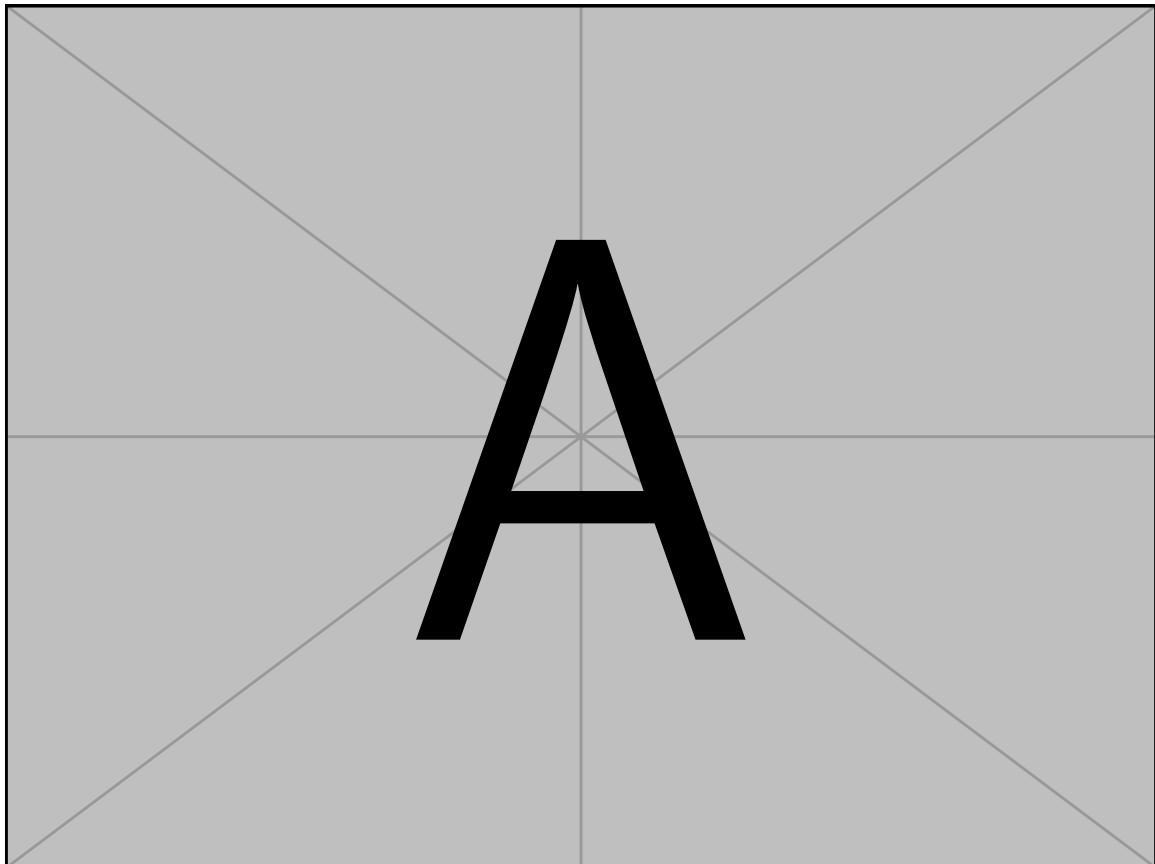


Figure 4.15: ...Pellentesque interdum sapien sed nulla. Proin tincidunt. Aliquam volutpat est vel massa. Sed dolor lacus, imperdiet non, ornare non, commodo eu, neque. Integer pretium semper justo. Proin risus. Nullam id quam. Nam neque. Duis vitae wisi ullamcorper diam congue ultricies. Quisque ligula. Mauris vehicula.

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Nunc sed pede. Praesent vitae lectus. Praesent neque justo, vehicula eget, interdum id, facilisis et, nibh. Phasellus at purus et libero lacinia dictum. Fusce aliquet. Nulla eu ante placerat leo semper dictum. Mauris metus. Curabitur lobortis. Curabitur sollicitudin hendrerit nunc. Donec ultrices lacus id ipsum. [19], [31]–[34]

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [35], [36].

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [37].

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Nunc sed pede. Praesent vitae lectus. Praesent neque justo, vehicula eget, interdum id, facilisis et, nibh. Phasellus at purus et libero lacinia dictum. Fusce aliquet. Nulla eu ante placerat leo semper dictum. Mauris metus. Curabitur lobortis. Curabitur sollicitudin hendrerit nunc. Donec ultrices lacus id ipsum.

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Mauris tempus eros at nulla. Sed quis dui dignissim mauris pretium tincidunt. Mauris ac purus. Phasellus ac libero. Etiam dapibus iaculis nunc. In lectus wisi, elementum eu, sollicitudin nec, imperdiet quis, dui. Nulla viverra neque ac libero. Mauris urna leo, adipiscing eu, ultrices non, blandit eu, dui. Maecenas dui neque, suscipit sit amet, rutrum a,

laoreet in, eros. Ut eu nibh. Fusce nec erat tempus urna fringilla tempus. Curabitur id enim. Sed ante. Cras sodales enim sit amet wisi. Nunc fermentum consequat quam. [2], [17], [38].

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

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Donec a nibh ut elit vestibulum tristique. Integer at pede. Cras volutpat varius magna. Phasellus eu wisi. Praesent risus justo, lobortis eget, scelerisque ac, aliquet in, dolor. Proin id leo. Nunc iaculis, mi vitae accumsan commodo, neque sem lacinia nulla, quis vestibulum justo sem in eros. Quisque sed massa. Morbi lectus ipsum, vulputate a, mollis ut, accumsan placerat, tellus. Nullam in wisi. Vivamus eu ligula a nunc accumsan congue. Suspendisse ac libero. Aliquam erat volutpat. Donec augue. Nunc venenatis fringilla nibh. Fusce accumsan pulvinar justo. Nullam semper, dui ut dignissim auctor, orci libero fringilla massa, blandit pulvinar pede tortor id magna. Nunc adipiscing justo sed velit tincidunt fermentum.

Integer placerat. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Sed in massa. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos hymenaeos. Phasellus tempus aliquam risus. Aliquam rutrum

purus at metus. Donec posuere odio at erat. Nam non nibh. Phasellus ligula. Quisque venenatis lectus in augue. Sed vestibulum dapibus neque.

Mauris tempus eros at nulla. Sed quis dui dignissim mauris pretium tincidunt. Mauris ac purus. Phasellus ac libero. Etiam dapibus iaculis nunc. In lectus wisi, elementum eu, sollicitudin nec, imperdiet quis, dui. Nulla viverra neque ac libero. Mauris urna leo, adipiscing eu, ultrices non, blandit eu, dui. Maecenas dui neque, suscipit sit amet, rutrum a, laoreet in, eros. Ut eu nibh. Fusce nec erat tempus urna fringilla tempus. Curabitur id enim. Sed ante. Cras sodales enim sit amet wisi. Nunc fermentum consequat quam.

## 4.6 Conclusion

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci.

## 4.7 Acknowledgment

Vivamus sit amet pede. Duis interdum, nunc eget rutrum dignissim, nisl diam luctus leo, et tincidunt velit nisl id tellus. In lorem tellus, aliquet vitae, porta in, aliquet sed, lectus. Phasellus sodales. Ut varius scelerisque erat. In vel nibh eu eros imperdiet rutrum. Donec ac odio nec neque vulputate suscipit. Nam nec magna. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nullam porta, odio et sagittis iaculis, wisi neque fringilla sapien, vel commodo lorem lorem id elit. Ut sem lectus, scelerisque eget, placerat et, tincidunt scelerisque, ligula. Pellentesque non orci. [39]–[43]

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# CHAPTER 5

## CONCLUSIONS AND FUTURE WORK

### 5.1 Summary of Key Findings

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## 5.2 Conclusion

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### 5.3 Future Work

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**APPENDIX A**

**CODE SCRIPT EXAMPLES**

## A.1 Example Cross product codes

Find cross products using [Scripts 9 to 12](#).

### A.1.1 c

</>	<b>Script 9: ... c to solve cross products.</b>	</>
-----	---	-----

```

1 #include <stdio.h>
2 #include <math.h>
3
4 typedef struct{
5     float i,j,k;
6 }Vector;
7
8 Vector vecAdd(Vector a, Vector b)
9 {
10     Vector c = {a.i + b.i, a.j + b.j, a.k + b.k};
11     return c;
12 }
13
14 Vector vecSubtract(Vector a, Vector b)
15 {
16     Vector c = {a.i - b.i, a.j - b.j, a.k - b.k};
17     return c;
18 }
19
20 float dotProduct(Vector a, Vector b)
21 {
22     return a.i*b.i + a.j*b.j + a.k*b.k;
23 }
24
25 float vecMag(Vector a)
26 {
27     return pow(a.i*a.i + a.j*a.j + a.k*a.k, 0.5);
28 }
29
30 Vector crossProduct(Vector a,Vector b)
31 {
32     Vector c = {a.j*b.k - a.k*b.j,
33                 a.k*b.i - a.i*b.k,
34                 a.i*b.j - a.j*b.i};
35     return c;
36 }
37
38 void printVector(Vector a)
39 {
40     printf("( %f, %f, %f)\n", a.i, a.j, a.k);
41 }
42
43 int main(){
44     double F, t, r, m_x;
45
46     // Part-a using scalar
47     F = 100;
48     t = 60*M_PI/180;

```

```

49     r = 250;
50
51     m_x = -F*sin(t)*r;
52
53     // Part-a vector approach
54     Vector o = {0, 0, 0};
55     Vector R = {0, 250, 0};
56     Vector f = {0, F*cos(t), -F*sin(t)};
57
58     // Unit vector
59     Vector x = {1, 0, 0};
60
61     // Position vector
62     Vector d = vecSubtract(R, o);
63
64     // Calculate moment
65     Vector M = crossProduct(R, f);
66
67     // Vector magnitude
68     float m;
69     m = vecMag(M);
70
71     printf("\n Part a (Scalar approach)\n %f (N-mm)\n", m_x);
72     printf("\n Part b (Vector approach)\n r x F = "); printVector(M);
73     printf("\n Magnitude = %f (N-mm)\n ", m);
74     printf("\n Magnitude of M about x-axis = %f (N-mm)\n", dotProduct(M, x));
75
76 }
```

## A.1.2 Fortran

</>                    Script 10: ...Fortran to solve cross products.                    </>

```

1      module cross_product
2      implicit none
3      contains
4      FUNCTION cross(a, b)
5          REAL, DIMENSION(3) :: cross
6          REAL, DIMENSION(3), INTENT(IN) :: a, b
7          cross(1) = a(2) * b(3) - a(3) * b(2)
8          cross(2) = a(3) * b(1) - a(1) * b(3)
9          cross(3) = a(1) * b(2) - a(2) * b(1)
10     END FUNCTION cross
11     end module cross_product
12
13     PROGRAM HW3_Prob_2
14         use cross_product
15         IMPLICIT NONE
16         REAL, DIMENSION(3) :: r, f
17         REAL, DIMENSION(3) :: m
18         ! Part a
19         r = [2.0, 5.0, 11.0] ! ft
20         f = [0.0, 44.0, -60.0] ! lbs
21         m = cross(r, f) ! Cross product
22         print*, "Part a ---- r x F = "
23         write (*, *) m, "lb-ft"
```

```

24
25      ! Part b
26      r = [1.4, 3.0, 5.7] ! m
27      f = [13.0, 25.0, -8.0] ! N
28      m = cross(r, f) ! Cross product
29      print*, "Part b ---- r x F = "
30      write (*, *) m, "N-m"
31
32      ! Part c
33      r = [-10.0, 15.0, -5.0] ! m
34      f = [500.0, -250.0, -300.0] ! N
35      m = cross(r, f) ! Cross product
36      print*, "Part c ---- r x F = "
37      write (*, *) m, "N-m"
38 END PROGRAM HW3_Prob_2

```

### A.1.3 Matlab

</> Script 11: ... Matlab to solve cross products. </>

```

1 clear all
2 clc
3
4 % Part A
5 r_a = [2, 5, 11]; % ft
6 F_a = [0, 44, -60]; % lbs
7
8 r_x_F_a = cross(r_a, F_a);
9
10 fmt = ['r X F: [', repmat('%g, ', 1, numel(r_x_F_a)-1), '%g] lb-ft\n'];
11 fprintf(fmt, r_x_F_a)
12
13 % Part B
14 r_b = [1.4, 3, 5.7]; % m
15 F_b = [13, 25, -8]; % kN
16
17 r_x_F_b = cross(r_b, F_b);
18
19 fmt = ['r X F: [', repmat('%g, ', 1, numel(r_x_F_b)-1), '%g] kN-m\n'];
20 fprintf(fmt, r_x_F_b)
21
22 % Part C
23 r_c = [-10, 15, -5]; % m
24 F_c = [500, -250, -300]; % N
25
26 r_x_F_c = cross(r_c, F_c);
27
28 fmt = ['r X F: [', repmat('%g, ', 1, numel(r_x_F_c)-1), '%g] N-m\n'];
29 fprintf(fmt, r_x_F_c)

```

### A.1.4 Python

```
</>                               Script 12: ... Python to solve cross products. </>
1 import numpy as np
2
3 # Part A
4 r_a = np.array([2, 5, 11]) # ft
5 F_a = np.array([0, 44, -60]) # lbs
6
7 r_x_F_a = np.cross(r_a, F_a)
8
9 print('r X F:', r_x_F_a, 'lb-ft')
10
11 # Part B
12 r_b = np.array([1.4, 3, 5.7]) # m
13 F_b = np.array([13, 25, -8]) # kN
14
15 r_x_F_b = np.cross(r_b, F_b)
16
17 print('r X F:', r_x_F_b, 'kN-m')
18
19 # Part C
20 r_c = np.array([-10, 15, -5]) # m
21 F_c = np.array([500, -250, -300]) # N
22
23 r_x_F_c = np.cross(r_c, F_c)
24
25 print('r X F:', r_x_F_c, 'N-m')
```

## A.2 Additional Example codes

### A.2.1 Matlab

```
</>                               Script 13: ... Matlab to solve a problem. </>
1 clear all
2 clc
3
4 % Part A
5 r_a = 2; % ft
6 F_a = 8; % lbs
7
8 M_a = -r_a*F_a;
9
10 fprintf('M: %f lb-ft\n', M_a)
11
12 % Part B
13 r_b = 1.5; % ft
14 F_b = 20; % lbs
15
16 M_b = -r_b*F_b;
17
18 fprintf('M: %f lb-ft\n', M_b)
19
```

```

20 % Part C
21 r_c_1 = 4; % m
22 F_c_1 = 75; % N
23
24 r_c_2 = 1.5; % m
25 F_c_2 = 50; % N
26
27 M_c = -r_c_1*F_c_1 + -r_c_2*F_c_2;
28
29 fprintf('M: %f N-m\n', M_c)

```

## A.3 Code Highlighting Using `minted`

### A.3.1 Highlighting Function

To use the code highlighting function that renders scripts for publication with line numbers/language specification/file path and more, please see the following function:

```
% The "\codeFromFile" function is used in the following manner:
\codeFromFile
  {language}      % Programming language
  {\subfix{path}} % File path
  {Header}        % Script heading info
  {label}         % LaTeX label for cross referencing
  {fontsize}      % Fontsize
  {backgroundcolor} % Text background color
  {mintedStyle}    % Minted text style (default)
```

### A.3.2 Code Highlighting Example

Here is the text to display the code used to highlight  $\text{\LaTeX}$  code in [Script 14](#).

```
\section{Scripting language to call scripts}
\subsection{\texttt{\hologo{LaTeX}}}
  \codeFromFile{LaTeX}
  {\subfix{code/LaTeX/exampleCode.tex}}
  {\ldots \hologo{LaTeX} to call other scripts.}
  {exampleCode}
  {\footnotesize}
  {latexcodebg}
  {default}
```

- Lines 1-8 in [Script 14](#) refer to [Script 9](#)
- Lines 10-17 in [Script 14](#) refer to [Script 10](#)
- Lines 19-26 in [Script 14](#) refer to [Script 11](#)

- Lines 28-35 in [Script 14](#) refer to [Script 12](#)

### A.3.3 L<sup>A</sup>T<sub>E</sub>X Script

</>      **Script 14:** ... L<sup>A</sup>T<sub>E</sub>X to call other scripts using the *codeFromFile* function.      </>

```

1 \subsection{\texttt{c}}
2   \codeFromFile{c}
3   {\subfix{code/c/HW3-4_58.c}}
4   {\ldots \texttt{c} to solve cross products.}
5   {ex1}
6   {\footnotesize}
7   {ccodebg}
8   {default}
9
10 \subsection{\texttt{Fortran}}
11   \codeFromFile{fortran}
12   {\subfix{code/fortran/HW3-Problem_2.f}}
13   {\ldots \texttt{Fortran} to solve cross products.}
14   {ex2}
15   {\footnotesize}
16   {fortrancodebg}
17   {default}
18
19 \subsection{\texttt{Matlab}}
20   \codeFromFile{matlab}
21   {\subfix{code/matlab/HW3-2_cross_product.m}}
22   {\ldots \texttt{Matlab} to solve cross products.}
23   {ex3}
24   {\footnotesize}
25   {matlabcodebg}
26   {default}
27
28 \subsection{\texttt{Python}}
29   \codeFromFile{LaTeX}
30   {\subfix{code/python/HW3-2_cross_Product.py}}
31   {\ldots \texttt{Python} to solve cross products.}
32   {ex4}
33   {\footnotesize}
34   {pythoncodebg}
35   {default}
```

**APPENDIX B**

**EQUATIONS**

## B.1 Supporting relationships

The mass-energy equivalence is described by the famous equation

$$E = mc^2 \quad (\text{B.1})$$

discovered in 1905 by Albert Einstein. In natural units ( $c = 1$ ), the formula expresses the identity

$$E = m \quad (\text{B.2})$$

**APPENDIX C**

**TISSUE PROCESSING**

## C.1 Electron Microscopy Preparation

### C.1.1 Tissue Processing

## Tissue Processing Dehydration to Plastic

Christopher Creveling, Graduate Student \*

June 6th, 2018

#### **Abstract**

Tissue preparation in the electron microscopy lab to use TEM to look at the vitreo-retinal interface in eyes.

### §1 Introduction

This document is intended to be used to process tissue from formalin to embedded plastic to be used on the transmission electron microscope (TEM) to identify the orientation of collagen fibers.

#### §1.1 Sorting

Begin first by sorting the tissue in two piles of tissue that was peeled and tissue that was adjacent to the peeled region. Then write down the identification ID # on the paper to keep the proper vial straight during the tissue process.

##### §1.1.1 Identification ID #

Sheep #, L/R, E/P, P/A

For example, *UL-15A-B Left Equator Peel* can be reduced to *UL15LEP*

### §2 Dehydration

First place samples in glass vials. Use forceps if it is required to remove excess waste from the container. Properly label the samples from before section 1.1.1 and place the label on the vial. Before adhering the label to the vial, write down the number of specimens in the vial to ensure that the specimens don't get lost during the process. Use tape to ensure that the label will not be removed from the vial during the process.

---

\*N. Chandler was with the Electron Microscopy Facility, University of Utah, Salt Lake City, UT, 84112 USA e-mail: (see <http://www.bioscience.utah.edu/molecular-biology/core-facilities.php>).

**§2.1 Buffer Rinse**

Remove the fixative from the existing vial using the micropipette. Be sure not to suck out the tissue. Then fill the vial with buffer - 0.1M Sodium Cacodylate buffer.

**§2.1.1 Agitation**

Put the sample vials in the rotating agitator for 5 minutes.

**§2.2 Buffer Rinse**

Remove the buffer from section 2.1 and replace with new buffer - 0.1M Sodium Cacodylate buffer.

**§2.2.1 Agitation**

Put the sample vials in the rotating agitator again for 5 minutes.

**§2.3 Osmium dilution**

During the previous agitation step in section 2.2.1 dilute the osmium tetroxide  $OsO_4$  (4% in  $dH_2O$ ) with 0.2 M Sodium Cacodylate buffer in a 1:1 mixture. Be sure to filter the Osmium tetroxide with a millipore filter to remove any excess particulate that would otherwise result in artifacts inside the tissue.

**§2.4 Osmium rinse**

Remove the 0.1M Sodium cacodylate buffer from the vials and replace with the diluted Osmium from section 2.3. Use just enough diluted Osmium to cover the tissue.

**§2.4.1 Agitation**

Put the sample vials back in the rotating agitator again for one hour.

**§2.5 DI water rinse**

Remove the diluted Osmium tetroxide from the vials and rinse with DI water. The DI water will be filtered <sup>1</sup>. This step is done to remove excess osmium.

**§2.5.1 Agitation**

Put the sample vials back in the rotating agitator again for 5 minutes.

**§2.6 Uranyl Acetate rinse**

Remove the DI water from the vials and replace with Saturated 4% Aqueous Uranyl Acetate. The Uranyl Acetate also needs to be filtered using a millipore filter<sup>1</sup> on a 10 ml syringe.

**§2.6.1 Agitation**

Put the sample vials back in the rotating agitator again for one hour.

<sup>1</sup>The millipore filter is used to remove any excess particulate that would otherwise result in artifacts inside the tissue.

### §3 Final acetone dehydration step

The final step of the dehydration process is to replace all of the moisture in the tissue from  $H_2O$  to pure acetone. This is done with a series of rinses in various percentages of alcohol with the last set of rinses in acetone. \*\*Note - if there is not enough alcohol mixtures in the hood then you will need to make more. When making the dilutions, use the graduated cylinder that is in the sink and mix the highest concentrations first to ensure that the percentages of alcohol is correct. Start with 95 then 70 then 50 etc. Also be sure that the ethanol containers are covered to prevent evaporation during each step of the dehydration process.

#### §3.1 50% Ethanol Alcohol

Remove the urinal acetate from the vial in section 2.6 to the appropriate container. Next use the micropipette and fill the vial with 50% Ethanol Alcohol; ensure that the tissue specimen is well covered.

##### §3.1.1 Agitation

Put the sample vials in the rotating agitator again for 10 minutes.

#### §3.2 70% Ethanol Alcohol

Remove the 50% Ethanol Alcohol from the vial. Next use the micropipette and fill the vial with 70% Ethanol Alcohol; ensure that the tissue specimen is well covered.

##### §3.2.1 Agitation

Put the sample vials in the rotating agitator again for 10 minutes.

#### §3.3 95% Ethanol Alcohol

Remove the 70% Ethanol Alcohol from the vial. Next use the micropipette and fill the vial with 95% Ethanol Alcohol; ensure that the tissue specimen is well covered.

##### §3.3.1 Agitation

Put the sample vials in the rotating agitator again for 10 minutes.

#### §3.4 95% Ethanol Alcohol

Remove the 95% Ethanol Alcohol from the vial. Next use the micropipette and fill the vial with 95% Ethanol Alcohol; ensure that the tissue specimen is well covered.

##### §3.4.1 Agitation

Put the sample vials in the rotating agitator again for 10 minutes.

#### §3.5 100% Ethanol Alcohol

Remove the 95% Ethanol Alcohol from the vial. Next use the micropipette and fill the vial with 100% Ethanol Alcohol; ensure that the tissue specimen is well covered.

**§3.5.1 Agitation**

Put the sample vials in the rotating agitator again for 10 minutes.

**§3.6 100% Ethanol Alcohol**

Remove the 100% Ethanol Alcohol from the vial. Next use the micropipette and fill the vial with 100% Ethanol Alcohol; ensure that the tissue specimen is well covered.

**§3.6.1 Agitation**

Put the sample vials in the rotating agitator again for 10 minutes.

**§3.7 100% Ethanol Alcohol**

Remove the 100% Ethanol Alcohol from the vial. Next use the micropipette and fill the vial with 100% Ethanol Alcohol; ensure that the tissue specimen is well covered.

**§3.7.1 Agitation**

Put the sample vials in the rotating agitator again for 10 minutes.

**§3.8 100% Ethanol Alcohol**

Remove the 100% Ethanol Alcohol from the vial. Next use the micropipette and fill the vial with 100% Ethanol Alcohol; ensure that the tissue specimen is well covered.

**§3.8.1 Agitation**

Put the sample vials in the rotating agitator again for 10 minutes.

**§3.9 Acetone**

Remove the 100% Ethanol Alcohol from the vial. Next use the micropipette and fill the vial with acetone; ensure that the tissue specimen is well covered.

**§3.9.1 Agitation**

Put the sample vials in the rotating agitator again for 10 minutes.

**§3.10 Acetone**

Remove the acetone from the vial. Next use the micropipette and fill the vial with acetone; ensure that the tissue specimen is well covered.

**§3.10.1 Agitation**

Put the sample vials in the rotating agitator again for 10 minutes.

**§3.11 Acetone**

Remove the acetone from the vial. Next use the micropipette and fill the vial with acetone; ensure that the tissue specimen is well covered.

**§3.11.1 Agitation**

Put the sample vials in the rotating agitator again for 10 minutes.

**§3.12 Acetone**

Remove the acetone from the vial. Next use the micropipette and fill the vial with acetone; ensure that the tissue specimen is well covered.

**§3.12.1 Agitation**

Put the sample vials in the rotating agitator again for 10 minutes.

**§4 Infiltration**

Once the tissue samples have been completely dehydrated and all moisture in the sample has been replaced with acetone, the next step is to infiltrate with plastic. This will allow the tissue to be embedded and then cut using the Ultramicrotomes. This will also take a few steps that still incorporate various mixtures of acetone and plastic.

**§4.1 Acetone & Plastic**

The first step is to remove the acetone from the vial using a micropipette and replacing it with a 1:1 mixture of acetone and plastic. Again, as mentioned before, the vial does not need to be filled up to the brim, just enough to thoroughly allow plastic to infiltrate the tissue.

**§4.1.1 Agitation**

Put the sample vials in the rotating agitator again for one hour.

**§4.2 Acetone & Plastic Overnight Option\*\***

If you are to finish the process for the day and return the next, then perform the following option, if not skip to section 4.3. First remove the 1:1 mixture from section 4.1 and replace with a 3:1 mixture of plastic to acetone and let it sit overnight.

**§4.3 Acetone & Plastic**

If you are to finish the process the same day then skip section 4.2. First remove the 1:1 mixture from section 4.1 and replace with a 3:1 mixture of plastic to acetone.

**§4.3.1 Agitation**

Put the sample vials in the rotating agitator again for one hour.

#### §4.4 Pure Plastic

First remove the 3:1 mixture from either section 4.2 or 4.3 and replace with pure plastic.

##### §4.4.1 Agitation

Put the sample vials in the rotating agitator again for one hour.

##### §4.4.2 Vacuum

Place all of the vials with the lids removed inside the vacuum chamber. Turn the pump on to remove air from the chamber. This will remove all air from the samples that has been embedded inside the tissue and will allow the infiltration of plastic to fully take affect. Let the samples sit inside the vacuum chamber for one hour.

#### §4.5 Pure Plastic

Remove the pure plastic from section 4.4 and replace with pure plastic again.

##### §4.5.1 Agitation

Put the sample vials in the rotating agitator again for one hour.

##### §4.5.2 Vacuum

Place all of the vials with the lids removed inside the vacuum chamber. Turn the pump on to remove air from the chamber. This will remove all air from the samples that has been embedded inside the tissue and will allow the infiltration of plastic to fully take affect. Let the samples sit inside the vacuum chamber for one hour.

### §5 Embedding

The next step is to embed the plasticized tissue into the mold. Before forgoing with this process, a list of all of the specimens will need to be created on Excel to print and cut out. For example if there are five specimens in the same vial, make a list of sample names with the specimen ID (A), specimen ID (B), ... specimen ID (E). Next, grab a razor blade and a wooden stir stick. Simply use the razor blade to shave away wood from the stir stick to make a flat surface. The flat surface will be used to transfer specimens from the vials to the mold. Place the printed out label inside the mold and set the mold inside the oven to let it bake the specimens to cure the plastic.

### §6 Cutting

After the plastic has cured, remove the specimen to be cut and use the microtome to shave away thin layers to be used for TEM.

### §7 Grid Staining

Once thin sections have been placed on grids from section 6 the grids will need to be stained to increase the contrast for TEM. Two chemicals will be Uranyl Acetate and Lead Citrate.

### **§7.1 Preparation**

Using the square petri-dish and wax from the cupboard cut the wax to fit the inside the petri-dish. Clean the wax with alcohol and DI water to remove any impurities on the wax that would alter the grid samples. This will also prevent the drops from coagulating together on the wax. Simply rinse the wax to clean it off.

### **§7.2 Chemical Prep**

After the wax has been cleaned and cut remove the saturated Uranyl Acetate and Reynold's Lead Citrate from the refrigerator. Grab two small 1 ml syringes from the drawer and fill up each syringe with either UA or Lead Citrate. Then place one small filter on the end of the syringe filled with UA and two filters on the syringe filled with Lead Citrate.

### **§7.3 UA Stain**

Using the 1 ml syringe with a single filter place a droplet of UA for each grid that you need to stain evenly spaced on the wax pad. Use the forceps and remove the grids from the grid holder and place on top of the UA droplet. Be sure to place the grid shiny side down to allow the UA to stain the specimen.

### **§7.4 Timer - 18 minutes**

Set the timer for 18 minutes. During this time fill up enough 30 ml syringes with DI water for rinsing both UA and Lead Citrate. You will need approximately 10 ml per sample per rinse. Place a large filter on the end of the syringe.

### **§7.5 Staging Area**

Grab a small round petri dish and insert two filter papers to absorb the water following the rinse. Use a pen or pencil to mark the paper to help organize the order of specimens to prevent a mix up.

### **§7.6 First Rinse**

After 18 minutes, pick up the grid with forceps and rinse with 10 ml of DI water. Hold the forceps at a 60° angle from the horizontal and drip the water down the curved section of the forceps. After the rinse, place the specimens inside the round petri dish to remove excess DI water. Once all of the specimens have been placed on the filter paper, a few sodium hydroxide crystals will need to be placed inside the square petri dish. The NaOH will help prevent any sort of moisture from interfering with the grid during the staining process. Next, use the other 1 ml syringe with Lead Citrate and place drops on the wax pad following the same procedure mentioned before in section 7.3.

### **§7.7 Lead Citrate Rinse**

Using the forceps, grip the grid and place it on top of the Lead Citrate droplet with the shiny side down which allows the grid to be stained. Set the timer for eight minutes.

### **§7.8 Second Rinse**

After eight minutes have passed, repeat the same step as in 7.6. Once the grids have completely dried, place them back in the grid holder and they are ready for the TEM.

**§7.9 Cleanup**

Dispose of the petri dish in the unwanted UA container.

**§8 Transmission Electron Microscopy**

Transmission electron microscopy (TEM) is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through it.

**§8.1 TEM**

Head over to the TEM and begin imaging!

#	Step	Instruction	Time	<input checked="" type="checkbox"/>							
1	Dehydration	0.1 M Sodium Cacodylate buffer	A* 5 minutes								
2	Dehydration	0.1 M Sodium Cacodylate buffer	A* 5 minutes								
3	Fix	4% <i>OsO<sub>4</sub></i> with 0.2 M Sodium Cacodylate buffer (1:1 filtered)	A* 60 minutes								
4	Rinse	DI water rinse	A* 5 minutes								
5	Stain	Saturated 4% Aqueous Uranyl Acetate (filtered)	A* 60 minutes								
6	Dehydration	50% Ethanol	A* 10 minutes								
7	Dehydration	70% Ethanol	A* 10 minutes								
8	Dehydration	95% Ethanol	A* 10 minutes								
9	Dehydration	95% Ethanol	A* 10 minutes								
10	Dehydration	100% Ethanol	A* 10 minutes								
11	Dehydration	100% Ethanol	A* 10 minutes								
12	Dehydration	100% Ethanol	A* 10 minutes								
13	Dehydration	100% Ethanol	A* 10 minutes								
14	Dehydration	Acetone	A* 10 minutes								
15	Dehydration	Acetone	A* 10 minutes								
16	Dehydration	Acetone	A* 10 minutes								
17	Dehydration	Acetone	A* 10 minutes								
18	Infiltration	1:1 Plastic to Acetone	A* 60 minutes								
19	Infiltration	3:1 Plastic to Acetone	A* 60 minutes								
20	Infiltration	Pure Plastic	A* 60 minutes								
21	Vacuum	Vacuum	V* 60 minutes								
22	Infiltration	Pure Plastic	A* 60 minutes								
23	Vacuum	Vacuum	V* 60 minutes								
24	Embedding	Embedding	Limitless								

Table 1: Simplified instructions to check off the steps during the tissue processing by hand. A\* indicates Agitation, and V\* indicates Vacuum.

Station #	Step	Instruction	Time	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
1	-	-	-	-	-	-	-
2	Dehydration	0.1 M Sodium Cacodylate buffer	A* 10 minutes				
3	Fix	4% <i>OsO<sub>4</sub></i> with 0.2 M Sodium Cacodylate buffer (1:1 filtered)	A* 60 minutes				
4	Rinse	DI water rinse	A* 10 minutes				
5	Stain	Saturated 4% Aqueous Uranyl Acetate (filtered)	A* 60 minutes				
6	Dehydration	50% Ethanol	A* 10 minutes				
7	Dehydration	70% Ethanol	A* 10 minutes				
8	Dehydration	95% Ethanol	A* 10 minutes				
9	Dehydration	95% Ethanol	A* 10 minutes				
10	Dehydration	100% Ethanol	A* 10 minutes				
11	Dehydration	100% Ethanol	A* 10 minutes				
12	Dehydration	100% Ethanol	A* 10 minutes				
13	Dehydration	100% Ethanol	A* 10 minutes				
14	Dehydration	Acetone	A* 10 minutes				
15	Dehydration	Acetone	A* 10 minutes				
16	Dehydration	Acetone	A* 10 minutes				
17	Dehydration	Acetone	A* 10 minutes				
18	Infiltration	1:1 Plastic to Acetone	A* 60 minutes				
19	Infiltration	3:1 Plastic to Acetone	A* 60 minutes				
20	Infiltration	Pure Plastic	A* & V* 60 minutes				
21	Infiltration	Pure Plastic	A* & V* 60 minutes				
22	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-

Table 2: Simplified instructions to check and make sure the automatic tissue processor is set up at the correct stations. Each vial should be filled with 20 ml when processing. Be sure to check the program on the automatic tissue processor; it should be marked by program #2. A\* indicates Agitation, and V\* indicates Vacuum.

### C.1.2 Tissue Processing

## Tissue Processing Dehydration to Plastic

Christopher Creveling, Graduate Student \*

June 8th, 2018

### Abstract

Tissue preparation in the electron microscopy lab to prepare the EMbed 812 for tissue processing.

### §1 Introduction

This document is intended to be used to process tissue from formalin to embedded plastic to be used on the transmission electron microscope (TEM) to identify the orientation of collagen fibers.

### §2 Embed 812

#### §2.1 Personal Protective Equipment

Begin first by grabbing a lab coat and then use paper towels and acetone to clean off the scale used for measuring out the mass of various resin mixtures.

#### §2.2 Recipe for EMbed 812

Remove the four chemicals for the EMbed 812 resin from the cabinet by using the WPE-147. Where W.P.E. is the Weight per Epoxide Equivalent).

Ingredient	Unit
EMbed 812 Resin	51.80 g
DDSA	26.68 g
NMA	21.67 g
BDMA	2.5 ml

Table 1: Simplified instructions to check and make sure the automatic tissue processor is set up at the correct stations. Each vial should be filled with 20 ml when processing. Be sure to check the program on the automatic tissue processor; it should be marked by program #2.

---

\*N. Chandler was with the Electron Microscopy Facility, University of Utah, Salt Lake City, UT, 84112 USA e-mail: (see <http://www.bioscience.utah.edu/molecular-biology/core-facilities.php>).

### §3 Lab Equipment

Grab one (400 ml) Tripore container along with four clean pipettes.

### §4 EMbed 812

Balance the scale with the Tripore container and pour in 51.80 g of EMbed 812 Resin. Clean the bottle and cap with Kimwipes and throw out the pipette. Grab a strip of Parafilm to stretch over the cap to ensure an air-tight seal.

### §5 DDSA

Balance the scale after 51.80 g of EMbed 812 Resin has been added to the Tripore container. Add 26.68 g of DDSA to the container by first underpouring and then using a pipette to add the rest of the DDSA. Clean the bottle and cap with Kimwipes and throw out the pipette. Grab a strip of Parafilm to stretch over the cap to ensure an air-tight seal.

### §6 NMA

Balance the scale after 26.68 g of DDSA has been added to the Tripore container. Add 21.67 g of NMA to the container by first underpouring and then using a pipette to add the rest of the NMA. Clean the bottle and cap with Kimwipes and throw out the pipette. Grab a strip of Parafilm to stretch over the cap to ensure an air-tight seal.

### §7 Stir

Move the Tripore container inside the hood. Add a stirbar to the Tripore container containing EMbed 812 Resin, DDSA, and NMA to stir the mixture for 10 minutes in the hood.

### §8 BDMA

In the hood, while the Tripore container is being stirred, use a graduated micropipette and obtain 2.5 ml of BDMA. The BDMA is used as the accelerator for polymerization.

### §9 Stir

Stir the Tripore container containing EMbed 812 Resin, DDSA, NMA, and BDMA mixture for 10 minutes in the hood. The mixture should turn orange after the BDMA has been added.

### §10 Parafilm

Using Parafilm wrap, ensure that each chemical lid has been wrapped to keep an air-tight seal.

**§11 Syringe**

Grab eight syringes and caps from the cupboards and prepare them for filling up with the resin. Place the newly filled resin syringes in the freezer.

**§12 Clean-up**

Clean the magnetic stirbars with acetone. Be sure to put vinyl liners inside the gloves.

**§13 Embed**

Embed the tissue samples!

## C.2 Ridge Detection Input Parameters

</> Script 15: Matlab script that determine ridge detection parameters using TEM images.

```

1 % Sigma selection parameter
2 % Christopher Creveling
3
4 close all
5 clear
6 clc
7
8 [file_name_root, dirname] = uigetfile('*.tif');
9 info = imfinfo(file_name_root);
10 % Gathers the resolution from the image data
11 resolution = info.XResolution;
12
13 line_width = 0.026; % Micron length
14 U = 204; % Image upper intensity value (background)
15 P = 160; % Pixel intensity for the contrast value
16
17 % line_width = input('Max of four line width measurements (Microns)\n');
18 fprintf('Resolution %f (pixels/micron)\n', resolution);
19
20 fprintf('Line width %f (microns)\n', line_width);
21 % resolution = 623.1429; % conversion between length and pixels
22
23 L = line_width*resolution; %Line width in pixels
24 fprintf('Line width %f (pixels)\n', L);
25 w = L/2; % width of a line in pixels
26 sigma = w/sqrt(3) + 0.4; % calculated sigma value
27 % sigma = 3.1
28 fprintf('Sigma = %f\n', sigma)
29
30 % sigma = 3.8; % approximate value
31
32 fprintf('U --- %d\n', U);
33 fprintf('P --- %d\n', P);
34 % Contrast (difference between upper and selected pixel intensity values)
35 h = U - P;
36 %h = 42;
37 fprintf('h = %d\n', h)
38
39 % First derivative of the gaussian kernel [Equation 4] - 1D
40 g_p1Dx = @(x, sigma)-x/(sqrt(2*pi)*sigma^3)*exp(-(x^2)/(2*sigma^2));
41 % Second directional derivative approximation [Equation 8]
42 rb_pp1Dx = @(x) h*(g_p1Dx(x + w, sigma) - g_p1Dx(x - w, sigma));
43 % Evaluate the second order approximation at zero to find out the upper
44 % threshold value 1D
45 fprintf('1D upper threshold approximation is %f\n', abs(rb_pp1Dx(0)))
46
47 % First derivative of the 2D gaussian kernel [Equation 4]
48 % g_p2Dx = @(x, y, sigma)-x/(2*pi*sigma^4)*exp(-(x^2 + y^2)/(2*sigma^2));
49 % First derivative of the 2D gaussian kernel [Equation 4]
50 % g_p2Dy = @(x, y, sigma)-y/(2*pi*sigma^4)*exp(-(x^2 + y^2)/(2*sigma^2));
51 % Second directional derivative approximation [Equation 8]
52 % rb_pp2D = @(x, y) h*(g_p2Dx(x + w, y, sigma) - ...

```

```

53 %      g_p2Dx(x - w, y, sigma) + g_p2Dy(x, y + w, sigma) - ...
54 %      g_p2Dy(x, y - w, sigma));
55 % Evaluate the second order approximation at zero to find out the upper
56 % threshold value 1D
57 % fprintf('2D upper threshold approximation is %f\n', abs(rb_pp2D(0, 0)))
58 % s = 0.006:0.001:0.03; % Range of sigma values
59 %
60 % for i = 1:length(s)
61 %     H(i) = abs(h*(g_p1Dx(0 + w, s(i)) - g_p1Dx(0 - w, s(i))));
62 % end
63 % H';

```

### C.3 Analyze Ridge Detection Output

</> Script 16: Matlab script that analyzes ridge detection output from TEM images. </>

```

1 % Name: Christopher Creveling
2 % Date: 11/13/18
3 % Title: Image analysis Ridge Detection Interpretation
4
5 % Description: After running a non-local means filter and further running
6 % a Ridge-Detection algorithm through Fiji I am trying to learn to extract
7 % what the output is giving me
8
9 %{
10 %Output from Ridge-Detection
11 /** This class holds one extracted line. The field num contains the number of
12 points in the line. The coordinates of the line points are given in the
13 arrays row and col. The array angle contains the direction of the normal
14 to each line point, as measured from the row-axis. Some people like to
15 call the col-axis the x-axis and the row-axis the y-axis, and measure the
16 angle from the x-axis. To convert the angle into this convention, subtract
17 PI/2 from the angle and normalize it to be in the interval [0, 2*PI). The
18 array response contains the response of the operator, i.e., the second
19 directional derivative in the direction of angle, at each line point. The
20 arrays width_l and width_r contain the width information for each line point
21 if the algorithm was requested to extract it; otherwise they are NULL. If
22 the line position and width correction was applied the contents of width_l
23 and width_r will be identical. The arrays asymmetry and contrast contain
24 the true asymmetry and contrast of each line point if the algorithm was
25 instructed to apply the width and position correction. Otherwise, they are
26 set to NULL. If the asymmetry, i.e., the weaker gradient, is on the right
27 side of the line, the asymmetry is set to a positive value, while if it is
28 on the left side it is set to a negative value. */
29 %}
30
31 clear all;
32 close all force; % Force the message boxes to close
33 clear;
34 clc;
35
36 cd 'Z:\students\Yousef\TEM\Ridge detection\Fiji Output'
37
38 %%%%%%%%%%%%%%
39 % Real TEM Image Data

```

```

40 % #####
41 % Import the data from the CSV file
42 synthetic = false;
43
44 % file root name _crop
45 file_name_root = 'H160993LPA-3_12_L4';
46 % File name extension _h85_H191_L06_S4
47 file_name_extension = '_C41_U351_L02_S220_W0010';
48 % file_name_extension = '';
49
50 % Ridge Detection Results
51 table_1 = readtable(strcat(file_name_root, file_name_extension, '_RD.csv'));
52 % Ridge Detection Junction Results
53 % table_2 = readtable(strcat(file_name_root, file_name_extension, ...
54 % '_RD_J.csv'));
55 % Ridge Detection Summary Results
56 table_3 = readtable(strcat(file_name_root, file_name_extension, '_RD_S.csv'));
57 % Extract information from the original image
58 img = imread(strcat(file_name_root, '.tif'));
59 info = imfinfo(strcat(file_name_root, '.tif'));
60 x_scale = info.XResolution;
61 y_scale = info.YResolution;
62 val = 1;%input(prompt);
63
64 fiber_color_num = 11; % the number of fiber divisions for the visual output
65
66 height = size(img, 2);
67 width = size(img, 1);
68
69 % Set up the file for outputting data
70 fileID = fopen(strcat(file_name_root, file_name_extension, '.txt'), 'w');
71
72 %%
73 ##### Identify how to properly shift the TEM image
74 % Identify how to properly shift the TEM image
75 #####
76 prompt = ['Are the collagen fibers on the top (1), right (2), ' ...
77 'bottom (3), or left (4)? \n'];
78 % val = 2;%input(prompt);
79 if (val == 1)
80     % No need to shift pixels
81     shift_x = 0;
82     shift_y = 0;
83 elseif (val == 2)
84     % shift pixels to the right
85     shift_x = max(width) - max(table_1.X*x_scale);
86     shift_y = 0;
87 elseif (val == 3)
88     % shift pixels down
89     shift_x = 0;
90     shift_y = max(width) - max(table_1.X*y_scale);
91 elseif (val == 4)
92     % No need to shift pixels
93     shift_x = 0;
94     shift_y = 0;
95 else
96     err = 'Invalid input';
97     error(err);

```

```

98 end
99
100
101 %%
102 %%%%%%
103 % Ask for collagen
104 %%%%%%
105 figure
106 imshow(img);
107
108
109 answer = questdlg('Do Collagen fibers exist?');
110 switch answer
111 case 'Yes'
112     close
113
114 %%
115 % Plot the RD classification color for all of the fiber segments detected
116 % by the algorithm
117 %%%%%%
118 %figure
119 %imshow(img);
120 %hold on
121 %RD_classification = unique(table_1.Class);
122 %RD_class_vals = []; % Empty array
123 %C = hsv(length(RD_classification));
124 % for i = 1:length(RD_classification)
125 %     Ridge_Detection_Class{i} = RD_classification(i);
126 %     RD_class_vals(i).XY = [table_1.X(categorical(table_1.Class) ==
127 %         RD_classification{i}), ...
128 %             table_1.Y(categorical(table_1.Class) ==
129 %                 RD_classification{i})*x_scale;
130 %             plot(RD_class_vals(i).XY(:, 1) + shift_x, RD_class_vals(i).XY(:, 2) +
131 %                 shift_y, '.', 'Color', C(i, :), 'markersize', 5, 'linewidth', 3);
132 %             % Legend(i) = num2str(RD_classification{i));
133 %         end
134 % legend(RD_classification, 'location', 'best');
135
136
137 %%
138 % Ridge-Detection results
139
140 figure;
141 imshow(img);
142 title('\bf Original Image')
143
144 msgStr = ['Select two points that define the ILM (Right to Left' ...
145             ' if Collagen Fibrils are above, Left to Right if' ...
146             ' Collagen Fibrils are below'];
147 % Indicate the ILM used for angle calculations
148 f = msgbox(msgStr, 'ILM');
149 pause(3);
150 [ILM.x, ILM.y] = ginput(2);
151 % delete(f); % Delete the message box
152 hold on

```

```

153 plot(ILM.x, ILM.y, 'bo', 'linewidth', 2);
154 % Sorts rows of the input to maintain correct order (ascending)
155 % ILM.x = sortrows(ILM.x);
156 ILM_slope = [];
157 ILM_angle = [];
158 for i = 1:length(ILM.x)-1
159     numerator = (ILM.y(i+1) - ILM.y(i));
160     denominator = (ILM.x(i+1) - ILM.x(i));
161     % slope of the line
162     ILM_slope(i) = numerator/denominator;
163     % Angle of the ILM relative to the x-axis
164     ILM_angle(i) = -atan(numerator/denominator)*180/pi;
165 end
166 fprintf('ILM slope = %f\n', ILM_slope);

167
168 slope = mean(ILM_slope); % Mean slope between the points
169 y_int = ILM.y(1) - slope*ILM.x(1); % Solve for the y-intercept
170
171
172
173 %% Create Rectangle
174
175 x1 = linspace(ILM.x(1), ILM.x(2));
176 y1 = linspace(ILM.y(1), ILM.y(2));
177 d = 1 * x_scale;      %distance in microns
178
179 height = size(img, 2);
180 width = size(img, 1);
181 aLine = [-ILM_slope, 1, -y_int];
182
183 fcn = @(x)ILM_slope*x + y_int; % Function handle
184 fplot(fcn, [0, width], 'r');
185
186 start_ = [ILM.x(1) ILM.y(1)];
187 goal_ = [ILM.x(2) ILM.y(2)];
188
189 n = 2;
190 t = linspace(0, 1, n);
191 v = goal_ - start_;
192 x3 = start_(1) + t*v(1);
193 y3 = start_(2) + t*v(2);
194 v = d* v / norm(v);
195
196 for i=1:n
197     line([x3(i) - v(2)], [y3(i) + v(1)]);
198     plot([x3(i) - v(2)], [y3(i) + v(1)], 'ro', 'linewidth', 2);
199 end
200
201 x3f = x3 - v(2);
202 y3f = y3 + v(1);
203
204 % Coordinates of the region of interest within the 1 micron rectangle
205 xv = [ILM.x(1), x3f(1), x3f(2), ILM.x(2)];
206 yv = [ILM.y(1), y3f(1), y3f(2), ILM.y(2)];
207
208 % Plots the 1 micron rectangle
209 plot(xv, yv, 'r--', 'LineWidth', 1.5)
210

```

```

211 Answer = questdlg('Is this correct?');
212
213 switch Answer
214   case 'Yes'
215     In = inpolygon(table_1.X*x_scale, table_1.Y*y_scale, xv, yv);
216
217     table_1.X = In .* table_1.X;
218     table_1.Y = In .* table_1.Y;
219
220     table_1(~table_1.X, :) = [];
221
222   case 'No'
223     fprintf('Please run code again')
224     msgbox('Please run code again');
225
226   return
227 end
228
229
230 %%%%%% End of create Rectangle
231
232 %%
233 % Define the input parameters for the line to border points (Ax+By+C=0)
234 % A = slope
235 % B = integer in front of y
236 % C = y-intercept
237 aLine = [-slope, 1, -y_int];
238
239
240 % extrapolate the ILM line on the image as well as calculate the distance
241 ILM_x_pts = linspace(0, width, 100);
242 for i = 1:length(ILM_x_pts)
243   ILM_line(i) = slope*ILM_x_pts(i) + y_int; % + ILM.x(end)
244 end
245 ILM_length = sqrt((ILM.x(2)-ILM.x(1))^2 + (ILM.y(2) - ILM.y(1))^2);
246 ILM_length = ILM_length/x_scale;
247 fprintf('ILM length = %f microns\n', ILM_length);
248 ILM_angle = (mean(ILM_angle));
249 fprintf(['ILM angle is %f degrees relative to the x-axis ' ...
250 '(Unit circle)\n'], ILM_angle);
251
252 fiber_min_length = 0.044962164;
253
254 % Indicate the five points on the ILM used for thickness measurements
255 figure
256 imshow(img)
257 for i = 1:5
258   f = msgbox(['Select the first two points that define the ' ...
259   'ILM thickness'], 'ILM');
260   % pause(1);
261   [ILM_thick(i).x, ILM_thick(i).y] = ginput(2);
262   hold on
263   plot(ILM_thick(i).x, ILM_thick(i).y, 'g-o', 'linewidth', 1);
264   % Pythagorean theorem
265   ILM_thick(i).measurement = sqrt((ILM_thick(i).x(1) - ILM_thick(i).x(2))^2
266   ↵ + ...
267   (ILM_thick(i).y(1) - ILM_thick(i).y(2))^2);
268   delete(f); % Delete the message box

```

```

268     end
269     for i = 1:5
270         ILM_measurement(i) = ILM_thick(i).measurement;
271     end
272     L{4} = 'ILM thickness measurements';
273     %legend(L, 'location', 'best');
274     axis image;
275
276     ILM_thickness = mean(ILM_measurement)/x_scale*1000;
277     fprintf('Average ILM thickness is %f nanometers \n', ILM_thickness);
278
279
280
281
282
283     %%
284     % Loop over all of the unique Contour ID's and identify the length of each
285     % one
286     ID_num = unique(Contour_ID);
287     for i = 1:length(ID_num)
288         unique_ID_lengths(i) = mean(table_1.Length(table_1.ContourID ==
289             ID_num(i)));
290         unique_ID_widths(i) = mean(table_1.LineWidth(table_1.ContourID ==
291             ID_num(i)));
292         unique_ID_ang_of_norm(i) = mean(table_1.AngleOfNormal(table_1.ContourID
293             == ID_num(i)));
294     end
295
296
297     %%
298     % figure;
299     % imshow(img);
300     % hold on
301
302     % fiber_color_num = 12; % the number of fiber divisions for the visual
303     % output (chosen from up above)
304
305     % Properly match the associated ContourID with the unique_ID number and the
306     % specified fiber length
307
308     fiber_length = linspace(min(Length_segment), ...
309         max(Length_segment)*0.8, fiber_color_num); %
310     C = hsv(length(fiber_length)); % Splits up the colormap into 11 unique values
311     m_size = 5;
312
313     % Loop over the unique fiber segment lengths to break them apart by lengths
314     for i = 1:length(fiber_length)
315         % if the length of the fibers is longer than the specified bin put them
316         % here
317         if i == length(fiber_length)
318             % extract X & Y coordinates of each point based on the criteria
319             fiber(i).x = table_1.X(table_1.Length > fiber_length(i));
320             % extract X & Y coordinates of each point based on the criteria
321             fiber(i).y = table_1.Y(table_1.Length > fiber_length(i));
322             % Calculate fiber area (LineLength *LineWidth)
323             % fiber(i).area = datatbl.Length(datatbl.Length >
324             % fiber_length(i)).*datatbl.LineWidth(datatbl.Length >
325             fiber_length(i));
326             fiber(i).len = table_1.Length(table_1.Length > fiber_length(i));

```

```

321     fiber(i).wid = table_1.LineWidth(table_1.Length > fiber_length(i));
322     % Fiber area = length * width (pixels)
323     fiber(i).area = fiber(i).len.*fiber(i).wid;
324     % Calculates the angle of the fiber
325     % fiber(i).angle = atan2(max(fiber(i).y) - min(fiber(i).y),
326     % max(fiber(i).x) - min(fiber(i).x))*180/pi;
327     else
328         % extract X & Y coordinates of each point based on the criteria
329         fiber(i).x = table_1.X(table_1.Length > fiber_length(i) & ...
330             table_1.Length <= fiber_length(i+1));
331         % extract X & Y coordinates of each point based on the criteria
332         fiber(i).y = table_1.Y(table_1.Length > fiber_length(i) & ...
333             table_1.Length <= fiber_length(i+1));
334         % Calculate fiber area (LineLength *LineWidth)
335         % fiber(i).area = datatbl.Length(datatbl.Length > fiber_length(i) &
336         % ...
337         % datatbl.Length <=
338         % fiber_length(i+1)).*datatbl.LineWidth(datatbl.Length > fiber_length(i) & ...
339         % datatbl.Length <= fiber_length(i+1));
340         fiber(i).len = table_1.Length(table_1.Length > fiber_length(i) & ...
341             table_1.Length <= fiber_length(i+1));
342         fiber(i).wid = table_1.LineWidth(table_1.Length > fiber_length(i) &
343             ...
344             table_1.Length <= fiber_length(i+1));
345         fiber(i).area = fiber(i).len.*fiber(i).wid; % Fiber area = length *
346         % width (pixels)
347         % Calculates the angle of the fiber
348         % fiber(i).angle = atan2(max(fiber(i).y) - min(fiber(i).y), ...
349         % max(fiber(i).x) - min(fiber(i).x))*180/pi;
350     end
351     tot_fiber_area(i) = sum(fiber(i).area); % sum up fiber area
352 end
353
354 % fiber_area = sum(tot_fiber_area); % fiber area
355 % fprintf('Area of fiber segments [pixels]) %f\n', fiber_area);
356 % fprintf(['Collagen fiber segment density (Area of fibers ' ...
357 % '[pixels]/ILM length (nanometers)) %f\n'], ...
358 % fiber_area/ILM_length);
359
360 % Plot the fibers
361 % for i = 1:length(fiber_length)
362 % plot(fiber(i).x*x_scale + shift_x, fiber(i).y*y_scale + shift_y, '.', ...
363 % 'color', C(i, :), 'markersize', m_size);
364 % end
365 %
366 % title('\bf Scatter Plot of Collagen fiber segments with corresponding
367 % lengths');
368 %
369 % % Create the legend based upon the length in the fiber array
370 % for i = 1:length(fiber_length)
371 % if i == length(fiber_length)
372 % Legend{i} = strcat('L \geq', num2str(fiber_length(i)), '\mu', 'm');
373 % else
374 % Legend{i} = strcat(num2str(fiber_length(i)), ...
375 % '< L \leq', num2str(fiber_length(i+1)), '\mu', 'm');
376 % end
377 % end
378 %

```

```

372 % [h, ~] = legend(Legend);
373 % // children of legend of type line
374 % ch = findobj(get(h, 'children'), 'type', 'line');
375 % set(ch, 'Markersize', 24); // set value as desired
376 % set(h, 'Interpreter', 'latex', 'location', 'best');
377 % axis image;
378 % set(gca, 'DataAspectRatio', [1 1 1]) % Adjust the aspect ratio for printing
379
380 %
381 %
382 %
383 %
384 % % Data from the Ridge Detection Junction Results CSV file
385 % %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
386 % %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
387 % T2_x = table_2.X;
388 % T2_y = table_2.Y;
389 % T2_ID1 = table_2.ContourID1;
390 % T2_ID2 = table_2.ContourID2;
391 %
392 % figure
393 % imshow(img);
394 % hold on;
395 % C = hsv(length(T2_x));
396 % for i = 1:length(T2_x)
397 %     % plot(All_Fibers(i).XYRes(:, 1), All_Fibers(i).XYRes(:, 2), '.', ...
398 %     % 'color', C(i, :), 'linewidth', 2);
399 %     % plot(T2_x(i)*x_scale + shift_x, T2_y(i)*y_scale + shift_y, 'o',
400 %     % 'linewidth', 3, 'markersize', 8, 'color', C(i, :));
401 %     % % hold on;
402 % end
403 % axis image
404
405 %
406 % % Data from the Ridge Detection Summary Results CSV file
407 % %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
408 % Length_T3 = table_3.Length;
409 % Width_T3 = table_3.MeanLineWidth;
410 % ContourID_T3 = table_3.ContourID;
411 %
412 % figure
413 % subplot(1, 2, 1);
414 % hist(Length_T3, fiber_color_num); % histogram of the lengths from the
415 % summary results file
416 % xlabel('\bf Lengths from summary results file');
417 %
418 % subplot(1, 2, 2);
419 % hist(Width_T3, fiber_color_num); % histogram of the lengths from the
420 % summary results file
421 % xlabel('\bf Mean line width from summary results file');
422
423 % % Plots all of the junction points from the Fiji Output
424 % figure;
425 % imshow(img);
426 % hold on

```

```

425      % plot(c_X + shift_x, c_Y + shift_y, '.', bj_X + shift_x, bj_Y + shift_y, '.', 
426      ej_X + shift_x, ej_Y + shift_y, '.', sj_X + shift_x, sj_Y + shift_y, '.', nj_X + 
427      shift_x, nj_Y + shift_y, '.', 'markersize', 5)
428      % title('bf Ridge-Detection Results')
429      % Legend_1 = legend({'Closed Points', 'Both Junction', 'End Junction', 'Start 
430      Junction', 'No Junction'}, 'location', 'best');
431      % axis image
432      % [h, ~] = legend(Legend_1);
433      % ch = findobj(get(h, 'children'), 'type', 'line'); %// children of legend of 
434      type line
435      % set(ch, 'Markersize', 24); %// set value as desired
436      % set(h, 'Interpreter', 'latex', 'location', 'best');
437      % axis image;
438      % set(gca, 'DataAspectRatio', [1 1 1]) % Adjust the aspect ratio for printing
439      %%
440
441      % Identify the fiber segments that are greater than the threshold and
442      % identify whether or not they overlap and combine them into a single fiber
443      % if they do
444
445      % close all force;
446      % clc;
447
448      % extract the ContourID & Length in an array
449      ID_Length = unique([table_1.ContourID, table_1.Length], 'rows');
450      % Identify fiber segments that are greater than the minimum length
451      segments = ID_Length(ID_Length(:, 2) >= fiber_min_length);
452      % Identify fiber segments that are less than the minimum length
453      short_segments = ID_Length(ID_Length(:, 2) < fiber_min_length);
454
455      % Loop over all of the unique segments to identify which ones are contained
456      % in the longer fibers by looking at all combinations. i.e. if two fiber
457      % segments have matching coordinates/slope they would be combined into a
458      % single fiber and the list of potential fibers would decrease
459
460      % Go over the matching fibers and further eliminate duplicates
461
462      % Initialize the arrays
463      tic
464      atol = 0.02; % relative tolerance
465      rtol = 0.01; % absolute tolerance
466
467      c1 = 1; % while loop 1 counter
468      c3 = 1; % fiber match counter
469      count = 1; % iteration counter
470      Lib = [];
471      fiber_union = []; % Fiber unions
472      fiber_segment = []; % initialize the array to be zero
473      condition_segment = []; % Initialize the array to be zero
474      lone_fibers = [];
475      check_1 = false; % Initialize the while loop statements
476
477
478

```

```

479 while (check_1 == false)
480     check_2 = false; % Initialize the while loop statements
481     c2 = 2; % while loop 2 counter
482     while (check_2 == false)
483
484         % X-coordinates
485         A1 = table_1.X(table_1.ContourID == segments(c1));
486         % Y-coordinates
487         A2 = table_1.Y(table_1.ContourID == segments(c1));
488
489         % X-coordinates
490         B1 = table_1.X(table_1.ContourID == segments(c2));
491         % Y-coordinates
492         B2 = table_1.Y(table_1.ContourID == segments(c2));
493
494         A = [A1, A2]; % [X, Y] coordinates from contour ID (i)
495         B = [B1, B2]; % [X, Y] coordinates from contour ID (j)
496
497         % Find the number of matches between array A and B and store them
498         % every iteration
499         % compares the two arrays to find matches (:, 1:2)(:, 1:2)
500         Lib.logical = double(ismember(A, B, 'rows'));
501         % finds the mean value of the comparison array
502         Lib.mean = mean(Lib.logical);
503         % finds the mode value of the comparison array
504         Lib.mode = mode(Lib.logical);
505         % Sums the zeros
506         Lib.num_zero = sum(Lib.logical == 0);
507         % sums the ones
508         Lib.num_one = sum(Lib.logical == 1);
509         % Identifies the combination of contour IDs
510         Lib.IDs = [segments(c1), segments(c2)];
511
512         % Consider looking at the slope of each line segment
513         % Pass in an array of coordinates to find the slope & y-intercept [
514             ← a_0 + a_1*x]
515             MA = Least_Squares(A);
516             % Pass in an array of coordinates to find the slope & y-intercept [
517             ← a_0 + a_1*x]
518             MB = Least_Squares(B);
519
520             Ax = A(:, 1);
521             Ay = A(:, 2);
522             Bx = B(:, 1);
523             By = B(:, 2);
524
525             % Find the distance between the segments
526             C_A = [mean(Ax), mean(Ay)]; % Center of mass for A
527             C_B = [mean(Bx), mean(By)]; % Center of mass for B
528
529             % Distance between fiber centers
530             D_AB = sqrt((C_A(2) - C_B(2))^2 + (C_A(1) - C_B(1))^2);
531
532             % Local extrema of each fiber segment
533             A_E(1) = min(Ax);
534             A_E(2) = max(Ax);
535             A_E(3) = min(Ay);
536             A_E(4) = max(Ay);

```

```

535     B_E(1) = min(Bx);
536     B_E(2) = max(Bx);
537     B_E(3) = min(By);
538     B_E(4) = max(By);
539
540     % Distance between local extrema for each fiber segment assuming
541     % they are linear
542     % Distance between fiber centers
543     L_A = sqrt((A_E(2) - A_E(1))^2 + (A_E(4) - A_E(3))^2);
544     % Distance between fiber centers
545     L_B = sqrt((B_E(2) - B_E(1))^2 + (B_E(4) - B_E(3))^2);
546
547     % Three conditions need to be satisfied
548     % Looks at the mode of the overlap values if there are any
549     condition_1 = (Lib.mode == 1);
550     % Compares how close the two slopes of similar segments are
551     condition_2 = (all(abs(MA(2) - MB(2)) <= atol + rtol*abs(MB(2)))) ;
552     % Compares how close the two y-intercepts are
553     condition_3 = (all(abs(MA(1) - MB(1)) <= atol + rtol*abs(MB(1)))) ;
554     % Is the distance between the fiber centers less than the length of
      ← the fiber segment
555     condition_4 = ((D_AB < L_A) || (D_AB < L_B));
556     condition_5 = (c1 ~= c2); % checks to see if A & B are duplicates
557
558     % Used for debugging
559     [condition_1, condition_2, condition_3, condition_4, ...
560      condition_5, segments(c1), segments(c2), count, ...
561      (max(table_1.ContourID) + 1)];
562
563     % Five conditions need to be satisfied
564     if [condition_1 && condition_5 || condition_2 && ...
565          condition_3 && condition_4 && condition_5]
566
567         A3 = table_1.Length(table_1.ContourID == segments(c1));
568         A4 = table_1.Contrast(table_1.ContourID == segments(c1));
569         A5 = table_1.Asymmetry(table_1.ContourID == segments(c1));
570         A6 = table_1.LineWidth(table_1.ContourID == segments(c1));
571         A7 = table_1.AngleOfNormal(table_1.ContourID == segments(c1));
572
573         B3 = table_1.Length(table_1.ContourID == segments(c2));
574         B4 = table_1.Contrast(table_1.ContourID == segments(c2));
575         B5 = table_1.Asymmetry(table_1.ContourID == segments(c2));
576         B6 = table_1.LineWidth(table_1.ContourID == segments(c2));
577         B7 = table_1.AngleOfNormal(table_1.ContourID == segments(c2));
578
579         A = [A, A3, A4, A5, A6, A7]; % Combine A with A3:A7
580         B = [B, B3, B4, B5, B6, B7]; % Combine B with B3:B7
581
582         fiber_pair = [segments(c1), segments(c2)];
583
584         % Update the vertical array of matching fiber segment overlaps
585         fiber_segment = vertcat(fiber_segment, fiber_pair);
586
587         % write down which conditions were satisfied per segment
588         condition_quad = [condition_1, condition_2, ...
589                          condition_3, condition_4, condition_5];
590         condition_segment = vertcat(condition_segment, ...
591                               condition_quad);

```

```

592
593          % merge the two contourID's (XY) coordinates together without
594          % duplicating points
595          fiber_union(c3).XY = [union(A, B, 'rows', 'stable')];
596          f_len = length(fiber_union(c3).XY); % length of the matched fiber
597          % segment
598
599          % Length of the new segments is going to be a mixture of the
600          % two fiber segments
601          L_A = unique(table_1.Length(table_1.ContourID == segments(c1)));
602          L_B = unique(table_1.Length(table_1.ContourID == segments(c2)));
603
604          % Fiber A contains all of fiber B
605          case_1 = (Lib.mode == 1) && (Lib.num_zero == 0);
606          % Fiber A contains the majority of fiber B
607          case_2 = (Lib.mode == 1) && (condition_2 == 1) && ...
608              (condition_3 == 1) && (condition_4 == 1);
609          % Fiber A contains the minority of fiber B
610          case_3 = (Lib.mode == 0) && (condition_2 == 1) && ...
611              (condition_3 == 1) && (condition_4 == 1);
612          % Fiber A does not contain fiber B
613          case_4 = (Lib.num_one == 0) && (condition_2 == 1) && ...
614              (condition_3 == 1) && (condition_4 == 1);
615
616          if case_1 == 1
617              % Max of the two fiber segments length
618              new_fiber_len = max([A3;B3]);
619          elseif case_2 == 1
620              overlap = Lib.num_one;
621              % If the majority of the points overlap, find the percentage
622              new_fiber_len = (L_A + L_B - ...
623                  (overlap/length(A)*L_A + ...
624                      overlap/length(B)*L_B)/2);
625          elseif case_3 == 1
626              overlap = Lib.num_one;
627              % If the majority of the points overlap, find the percentage
628              new_fiber_len = L_A + L_B - ...
629                  (overlap/length(A)*L_A + ...
630                      overlap/length(B)*L_B)/2;
631          elseif case_4 == 1
632              % if the two fibers don't overlap
633              new_fiber_len = L_A + L_B;
634          else
635              % Average the two lengths
636              new_fiber_len = 0.5*(L_A + L_B);
637          end
638
639          % store the matching contourID with the coordinates
640          fiber_union(c3).segment_match = fiber_pair;
641          % adds a new ContourID number (max(ContourID) + 1)
642          fiber_union(c3).New_ContourID = ones(f_len, 1) *
643          (max(table_1.ContourID) + 1);
644          if strcmp(table_1.Properties.VariableNames{1}, 'Var1')
645              % update the number Var1 number. Some of the outputs have
646              % this. If not, comment out
647              fiber_union(c3).Var1 = ones(f_len,
648              1).*table_1.Var1(end):table_1.Var1(end) + f_len - 1;
649          end

```

```

645     fiber_union(c3).Frame = ones(f_len, 1);
646     fiber_union(c3).Pos_ = 1:f_len;
647     fiber_union(c3).X = fiber_union(c3).XY(:, 1);
648     fiber_union(c3).Y = fiber_union(c3).XY(:, 2);
649     %; % Update new fiber length
650     fiber_union(c3).Length = ones(f_len, 1)*new_fiber_len;
651     fiber_union(c3).Contrast = fiber_union(c3).XY(:, 4);
652     fiber_union(c3).Asymmetry = fiber_union(c3).XY(:, 5);
653     fiber_union(c3).LineWidth = fiber_union(c3).XY(:, 6);
654     fiber_union(c3).AngleOfNormal = fiber_union(c3).XY(:, 7);
655     fiber_union(c3).Class(1:f_len) = {'new_fiber'};
656     fiber_union(c3).Class = fiber_union(c3).Class(1:f_len)';
657
658     % create a shortcut for the list
659     fu = fiber_union(c3);
660     % transpose the position
661     fu.Pos_ = fu.Pos_;
662     % If the attribute is in the CSV file add the info
663     if strcmp(table_1.Properties.VariableNames{1}, 'Var1')
664         fu.Var1 = fu.Var1'; % Transpose the column
665         % new matching segment info
666         table_1_new_fiber_segment = table(fu.Var1, ...
667             fu.Frame, fu.NewContourID, fu.Pos_, fu.X, ...
668             fu.Y, fu.Length, fu.Contrast, fu.Asymmetry, ...
669             fu.LineWidth, fu.AngleOfNormal, fu.Class);
670     else
671         % If the attribute is not in the CSV file, move on without it
672         % new matching segment info
673         table_1_new_fiber_segment = table(fu.Frame, ...
674             fu.NewContourID, fu.Pos_, fu.X, fu.Y, ...
675             fu.Length, fu.Contrast, fu.Asymmetry, ...
676             fu.LineWidth, fu.AngleOfNormal, fu.Class);
677     end
678     % stores the variable names to the new table for merging
679     table_1_new_fiber_segment.Properties.VariableNames =
680     → table_1.Properties.VariableNames;
681     % append new matching segment info to table1
682     table_1 = [table_1;table_1_new_fiber_segment];
683
684     % % Plot both segments that are being eliminated
685     % figure;
686     % imshow(img);
687     % hold on
688     % plot(Ax*x_scale + shift_x, Ay*y_scale + shift_y, 'r.',
689     → 'markersize', 5);
690     % plot(Bx*x_scale + shift_x, By*y_scale + shift_y, 'bo',
691     → 'markersize', 5);
692     % txt = {'\leftarrow A -s#', num2str(segments(c1)), '\leftarrow B
693     → -s#', num2str(segments(c2))};
694     % text(mean(Ax*x_scale) + shift_x, mean(Ay*x_scale) + shift_y,
695     → strcat(txt{1}, txt{2}));
696     % text(mean(Bx*x_scale) + shift_x, mean(By*x_scale) + shift_y,
697     → strcat(txt{3}, txt{4}));
698     %
699     % % Used for debugging
700     % fprintf('A ----- %f, B ----- %f, New Fiber #%d ----- %f\n', ...
701     % L_A, L_B, unique(fiber_union(c3).NewContourID), ...
702     % new_fiber_len);

```

```

697
698
699      % If the length of segment_A is longer than segment_B get rid
700      % of the smaller segment (segment_B)
701      if(length(A) > length(B))
702          % Update the table with the new ContourID #
703          segments(c1) = max(table_1.ContourID);
704          % Delete the ID number from list 'B'
705          segments(c2) = [];
706          % start from the top of the list
707          % c2 = 1;
708
709      % If the two segments are identical
710      elseif (segments(c1) ~= segments(c2))
711          % Update the table with the new ContourID #
712          segments(c2) = max(table_1.ContourID);
713          % Delete the ID number from list 'A'
714          segments(c1) = [];
715          % start from the top of the list
716          % c1 = 1;
717      end
718      % restart from the top of the list
719      c1 = 1;
720      % c2 = 2;
721      % Update the matched pairs counter
722      c3 = c3 + 1;
723  end
724
725      % If the length of segments is 1 or 0, or the last iteration of the
726      → loop
727      if (length(segments) <= 1) || (length(segments) == c2)
728          % If there are no more matches after the end of looping through
729          → the
730          % it is considered a 'lone fiber'
731          lone_fibers = [lone_fibers;segments(c1)];
732          % Delete the ID number from list 'A'
733          segments(c1) = [];
734          % restart from the top of the list
735          c1 = 1;
736          fprintf(['Segment # %.0f removed from the list ' ...
737                  'of potential segments (%d)\n'], ...
738                  lone_fibers(end), length(segments));
739          % If there are no more combinations that can be ...
740          % checked then all unique fibers have been ...
741          % identified and concatenated
742          check_2 = true;
743          if (length(segments) == 0) || (length(segments) == 1) % (c1 ==
744          → length(segments)) || (c1 > length(segments))
745              % If there are no more combinations that can
746              % be checked then all unique fibers have been
747              % identified and concatenated
748              check_1 = true;
749  end
750
751      if (condition_1 && condition_5 || ...
752          condition_2 && condition_3 && ...
753          condition_4 && condition_5)

```

```

752         % restart from the top of the list if a segment was removed
753         c2 = 2;
754     else
755         % Update the iteration for while loop #2
756         c2 = c2 + 1;
757     end
758     count = count + 1; % Update the number of iterations
759 end
760 % c1 = c1 + 1; % Update the iteration for while loop #1 % We don't need
761 % to update this because we are eliminating the c1 point if there are
762 % not matches after each c2 iteration through all of the segments. We
763 % should probably eliminate the first while loop because it is
764 % unnecessary to increment now in this 2.0 version of the code by
765 % eliminating the c1 point.
766 end
767 toc
768
769 %%
770 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
771 % Plot the fiber segments that matched from the previous step
772 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
773
774 % sort the fibers from the previous loop to color code by length
775 combined_and_lone_fibers = [segments;lone_fibers];
776 fiber_len_array = []; % zero array
777 for i = 1:length(combined_and_lone_fibers)
778     fiber_len = [combined_and_lone_fibers(i), ...
779                 mean(table_1.Length(table_1.ContourID == ...
780                         combined_and_lone_fibers(i)))];
781     fiber_len_array = vertcat(fiber_len_array, fiber_len);
782 end
783
784 % Sort the fibers based on their length
785 combined_and_lone_fibers = sortrows(fiber_len_array, 2);
786 C = parula(length(combined_and_lone_fibers));
787 % Overlay of the fibers and the original image
788 h = figure;
789 imshow(img);
790 hold on
791 for i = 1:length(combined_and_lone_fibers)
792     % figure;
793     % imshow(img);
794     % hold on
795     x1 = table_1.X(table_1.ContourID == combined_and_lone_fibers(i));
796     y1 = table_1.Y(table_1.ContourID == combined_and_lone_fibers(i));
797     % Plot dots instead of connected lines
798     plot(x1*x_scale + shift_x, y1*y_scale + shift_y, '.', 'markersize', 5,
    ← 'color', C(i, :));
799     % i % Plot the ID # i
800     % txt = '|<arrow #', num2str(combined_and_lone_fibers(i));
801     % text(mean(x1*x_scale) + shift_x, mean(y1*y_scale) + shift_y,
    ← strcat(txt{1}, txt{2}));
802     title('\bf True Fibers');
803 end
804 plot(xv, yv, 'r--', 'LineWidth', 1.5)
805 plot(ILM.x, ILM.y, 'r--', 'LineWidth', 1.5)
806 title('\bf True Fibers!');
807 % Saves the figure as a Tif

```

```

808     saveas(h, strcat(file_name_root, file_name_extension, '.tif'));
809
810     % % Look at the matching fibers that were used to construct the complete
811     % % fiber
812     % for i = 1:length(fiber_segment)
813     %     figure;
814     %     imshow(img);
815     %     hold on
816     %     x1 = table_1.X(ContourID == fiber_segment(i, 1));
817     %     y1 = table_1.Y(ContourID == fiber_segment(i, 1));
818     %     x2 = table_1.X(ContourID == fiber_segment(i, 2));
819     %     y2 = table_1.Y(ContourID == fiber_segment(i, 2));
820     %     plot(x1*x_scale, y1*y_scale, 'r.', 'markersize', 5);
821     %     plot(x2*x_scale, y2*y_scale, 'bo', 'markersize', 10);
822     % end
823
824     % filtered out contour ID's that were too small
825     % ID_eliminated = unique(table_1.ContourID(table_1.Length <
826     % length_threshold));
827     % filtered out contour ID's that were too small
828     % ID_eliminated = unique(table_1.ContourID((table_1.Length <
829     % fiber_min_length)));
830
831     %%%
832     filtered_fibers = length(short_segments);
833     fprintf('Filtered out %d fiber segments\n', filtered_fibers);
834     fprintf('Remaining eligible fibers = %d fibers\n', ...
835             length(segments));
836
837
838
839     % Loop over all the current IDs that satisfy the criteria
840     for i = 1:length(combined_and_lone_fibers)
841         cur_x = table_1.X(find(table_1.ContourID == ...
842             combined_and_lone_fibers(i)));
843         cur_y = table_1.Y(find(table_1.ContourID == ...
844             combined_and_lone_fibers(i)));
845         %     cur_xRes = cur_x*x_scale + shift_x;
846         %     cur_yRes = cur_y*y_scale + shift_y;
847         Filt_Fibers_XY = [cur_x, cur_y];
848         %     Filt_Fibers_XYRes = [cur_xRes, cur_yRes];
849         Filt_Fibers(i).Length = unique(table_1.Length(table_1.ContourID == ...
850             combined_and_lone_fibers(i)));
851         Filt_Fibers(i).Width = table_1.LineWidth(table_1.ContourID == ...
852             combined_and_lone_fibers(i));
853         Filt_Fibers(i).ID = combined_and_lone_fibers(i);
854         Filt_Fibers(i).Area = Filt_Fibers(i).Length.*Filt_Fibers(i).Width; % Area
855     % of fibers
856     %     sort_cur_x = sort(table_1.X(combined_and_lone_fibers(i)));
857     %     sort_cur_y = sort(table_1.Y(combined_and_lone_fibers(i)));
858     %     angle = []; % clears the array during each loop
859     %     slope = []; % array of slopes
860     %     for j = 1:length(cur_x)-1
861     %         % Consider using the polyfit
862     %         numerator = (cur_y(j+1) - cur_y(j));

```

```

863 %
864 %
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889
890 % Calculate slope & y-intercept from linear fit
891 [F] = Least_Squares(Filt_Fibers_XY);
892 %Slope
893 Filt_Fibers(i).slope = F(2);
894 % inverse tangent of the slope
895 Filt_Fibers(i).Angle = -atan(F(2))*180/pi;
896 % Clear the dataset from the array for the next iteration
897 Filt_Fibers_XY = [];
898
899 % % average the slope for each individual contour ID
900 % Filt_Fibers(i).slope = mean(slope);
901 % %ILM_angle - ...
902 % Filt_Fibers(i).Angle = angle;
903 % % Mean angle of each countour ID
904 % Filt_Fibers(i).mean_Angle = mean(angle);
905 end
906
907 for i = 1:length(combined_and_lone_fibers)
908 % Puts each mean angle into an array
909 filt_ang(i) = Filt_Fibers(i).Angle;
910 % Calculates mean fiber length
911 filt_len(i) = Filt_Fibers(i).Length;
912 % Average width of the fiber and puts it into an array
913 filt_wid(i) = mean(Filt_Fibers(i).Width);
914 % Calculates the average fiber area (length*width of pixels)
915 filt_area(i) = mean(Filt_Fibers(i).Area);
916 % Number of points in each contour ID# and puts it into an array
917 filt_num(i) = length(Filt_Fibers(i).Width);
918 % Average slope of each contour ID#
919 filt_slo(i) = Filt_Fibers(i).slope;
920 end

```

```

921
922 for i = 1:length(short_segments)
923     cur_x = table_1.X(find(table_1.ContourID == ...
924         short_segments(i)));
925     cur_y = table_1.Y(find(table_1.ContourID == ...
926         short_segments(i)));
927     %     cur_xRes = cur_x*x_scale + shift_x;
928     %     cur_yRes = cur_y*y_scale + shift_y;
929     No_Filt_Fibers_XY = [cur_x, cur_y];
930     %     No_Filt_Fibers(i).XYRes = [cur_xRes, cur_yRes];
931     No_Filt_Fibers(i).Length = unique(table_1.Length(table_1.ContourID == ...
932         short_segments(i)));
933     No_Filt_Fibers(i).Width = table_1.LineWidth(table_1.ContourID == ...
934         short_segments(i));
935     No_Filt_Fibers(i).ID = short_segments(i);
936
937     % Calculate slope & y-intercept from linear fit
938     [F] = Least_Squares(No_Filt_Fibers_XY);
939     %Slope
940     No_Filt_Fibers(i).slope = F(2);
941     % inverse tangent of the slope
942     No_Filt_Fibers(i).Angle = atan(F(2))*180/pi;
943     % Clear the dataset from the array for the next iteration
944     No_Filt_Fibers_XY = [];
945
946     % % average the slope for each individual contour ID
947     % No_Filt_Fibers(i).slope = mean(slope);
948     % %ILM_angle - ...
949     % No_Filt_Fibers(i).Angle = angle;
950     % % Mean angle of each countour ID
951     % % No_Filt_Fibers(i).mean_Angle = mean(angle);
952 end
953
954 for i = 1:length(short_segments)
955     % Puts each mean angle into an array
956     No_filt_ang(i) = No_Filt_Fibers(i).Angle;
957     % Puts each fiber length into an array
958     No_filt_len(i) = No_Filt_Fibers(i).Length;
959     % Average width of the fiber and puts it into an array
960     No_filt_wid(i) = mean(No_Filt_Fibers(i).Width);
961     % Number of points in each contour ID# and puts it into an array
962     No_filt_num(i) = length(No_Filt_Fibers(i).Width);
963     % Average slope of each contour ID#
964     No_filt_slo(i) = No_Filt_Fibers(i).slope;
965 end
966
967 % %
968 % % Plot individual fibers on a single sheet
969 % %
970 % % Do not run this on a real image
971 % %
972 % C = hsv(length(segments)); % Color array for the fibers
973 % for i = 1:length(segments)
974 %     figure
975 %     imshow(img);
976 %     hold on
977 %     plot(Filt_Fibers(i).XYRes(:, 1), Filt_Fibers(i).XYRes(:, 2), '.', 
978 % →      'Color', C(i, :));

```

```

978 % end
979 % title('bf Filterd image', 'fontsize', 18);
980 %
981 % [~, index] = sortrows([Filt_Fibers.Length].');
982 % Filt_Fibers = Filt_Fibers(index);
983 % clear index; % Sort the Filt_Fibers by Length
984 %
985 %
986 % % Plot individual fibers on the same sheet just pausing for half a second
987 % C = hsv(length(segments)); % Color array for the fibers
988 % figure
989 % imshow(img);
990 % hold on
991 % for i = 1:length(segments)
992 %     waitbar(i/length(segments));
993 %     plot(Filt_Fibers(i).XYRes(:, 1), Filt_Fibers(i).XYRes(:, 2), '.', 
994 % → 'Color', C(i, :));
995 %         % pause(0.01)
996 % end
997 % title('bf Filterd image', 'fontsize', 18);
998 %
999 %
1000 % [~, index] = sortrows([No_Filt_Fibers.Length].');
1001 % No_Filt_Fibers = No_Filt_Fibers(index);
1002 % clear index; % Sort the Filt_Fibers by Length
1003 %
1004 %
1005 % % Plot individual fibers on the same sheet just pausing for half a second
1006 % C = hsv(length(short_segments)); % Color array for the fibers
1007 % figure
1008 % imshow(img);
1009 % hold on
1010 % for i = 1:length(short_segments)
1011 %     waitbar(i/length(short_segments));
1012 %     plot(No_Filt_Fibers(i).XYRes(:, 1), No_Filt_Fibers(i).XYRes(:, 2), '.', 
1013 % → 'Color', C(i, :));
1014 %         % pause(0.01)
1015 % end
1016 % title('bf Non-Filterd image', 'fontsize', 18);

1017 %
1018 % The combined_and_lone_fibers list needs to be sorted by fiber length
1019 % before calculating attributes such as slope, and angle
1020 for i = 1:length(combined_and_lone_fibers)
1021 % unique length of the connected fibers *1000 for nanometers
1022 len = unique(table_1.Length(table_1.ContourID == 
1023 → combined_and_lone_fibers(i)));
1024 % converted average angle from y-axis to the x-axis -pi/2
1025 angle = (mean(table_1.AngleOfNormal(table_1.ContourID == ...
1026 combined_and_lone_fibers(i)))-pi)*180/pi;
1027 % angle from calculating the inverse tangent of the slope
1028 calc_ang = filt_ang(i);
1029 %difference in angle
1030 difference = angle - calc_ang;
1031 % density of collagen fibers / ilm length
1032 density(i) = filt_area(i)/ILM_length;
1033 fprintf(['Fiber # %d -- length = %.4f nanometers, -- ' ...
1034 'avg. angle RD = %.2f degrees, -- angle Calc = ' ...

```

```

1033      '%.2f degrees, -- angle diff %.2f\n'], ...
1034      combined_and_lone_fibers(i), len, angle, calc_ang, ...
1035      difference);
1036
1037 % density of collagen fibers / ilm length
1038 fprintf('Collagen fiber density = %f microns\n', sum(density));
1039
1040 % Plots the histogram of the calculated angles
1041 % figure
1042 % hist(filt_ang);
1043 % title('\bf Calculation of fiber angles');
1044 % fprintf(['Collagen fiber angle is %f \n ' ...
1045 % '(relative to the x-axis)\n'], mean(filt_ang));
1046
1047 % Plots the angle vs. fiber segment length
1048 %figure
1049 %plot(filt_ang, filt_len, '.');
1050 %set(gca, 'XDir', 'reverse');
1051 % xlabel('\bf Fiber Angle');
1052 % ylabel('\bf Fiber Length');
1053 %title('\bf Fiber Angle vs. Length');
1054
1055 % Plots the angle vs. fiber segment length on a polar grid
1056 %figure
1057 % plot(ang, len, '.');
1058 % pax = gca; % 2018a
1059 % pax.ThetaAxisUnits = 'radians'; % 2018a
1060 %polarplot(filt_ang*pi/180, filt_len, '.')
1061 % xlabel('\bf Fiber Angle'); % 2018a
1062 % ylabel('\bf Fiber Length'); % 2018a
1063 % axis([min(ang), max(ang), min(len), max(len)]);
1064 %title('\bf Fiber Angle vs. Length');
1065
1066 % Plot each unique fiber with a different color
1067 % figure
1068 % imshow(img);
1069 % hold on
1070 % C = hsv(length(unique(combined_and_lone_fibers)));
1071 % for i = 1:length(combined_and_lone_fibers)
1072 %     plot(All_Fibers(i).XYRes(:, 1), All_Fibers(i).XYRes(:, 2), '.', 'color',
→   C(i, :), 'linewidth', 2);
1073 %     hold on;
1074 % end
1075 % axis image;
1076 % title('\bf Unique ContourID fiber identification');
1077
1078 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
1079 % Plot the histogram of the image
1080 % figure
1081 % if synthetic == true
1082 %     img2 = rgb2gray(img); % Converts the RGB image to grayscale
1083 %     [counts, grayLevels] = imhist(img, 256);
1084 %     imhist(img2); % Looks at the histogram of pixel intensities
1085 % else
1086 %     [counts, grayLevels] = imhist(img, 256);
1087 %     imhist(img); % Looks at the histogram of pixel intensities
1088 % end
1089 % title('\bf Histogram of TEM image pixel intensities');

```

```

1090
1091 % Plot the contour map for the image overlayed with the detected fibers
1092 % h = figure;
1093 % image(img)
1094 % hold on
1095 % contourf(img, 10)
1096 % axis image
1097 % colormap gray
1098 % for i = 1:length(combined_and_lone_fibers)
1099 %     % figure;
1100 %     % imshow(img);
1101 %     hold on
1102 %     x1 = table_1.X(table_1.ContourID == combined_and_lone_fibers(i));
1103 %     y1 = table_1.Y(table_1.ContourID == combined_and_lone_fibers(i));
1104 %     plot(x1*x_scale + shift_x, y1*y_scale + shift_y, '.', 'markersize', 5,
1105 %           'color', C(i, :)); % Plot dots instead of connected lines
1106 % % txt = t'\leftarrow #', num2str(combined_and_lone_fibers(i)); % i %
1107 % Plot the ID # i
1108 % % text(mean(x1*x_scale) + shift_x, mean(y1*x_scale) + shift_y,
1109 % strcat(txt{1}, txt{2})); % % title('\bf True Fibers');
1110 % end
1111 %title('\bf True Fibers overlayed on a contour filled plot!');
1112 %saveas(h, strcat(file_name_root, file_name_extension, '_contour.tif')); % Saves the figure as a Tif
1113
1114 fprintf(fileID, 'Total unique fibers = %d fibers\n', ...
1115     length(combined_and_lone_fibers));
1116 fprintf(fileID, ...
1117     'Width of the rectangle ILM measurement = %d microns\n', ...
1118     ILM_length);
1119 fprintf(fileID, ...
1120     'ILM angle is %f degrees \n (relative to the x-axis)\n', ...
1121     ILM_angle);
1122 fprintf(fileID, ...
1123     'Average ILM thickness is %f nanometers \n', ...
1124     ILM_thickness);
1125 fprintf(fileID, ...
1126     ['Abs Mean Collagen fiber angle is %f \n ' ...
1127     '(relative to the x-axis)\n'], ...
1128     nanmean(abs(filt_ang)));
1129 fprintf(fileID, ...
1130     ['Abs Median Collagen fiber angle is %f \n ' ...
1131     '(relative to the x-axis)\n'], ...
1132     nanmedian(abs(filt_ang)));
1133 fprintf(fileID, ...
1134     ['Abs Mean Collagen fiber angle is %f \n ' ...
1135     '(relative to the ILM)\n'], ...
1136     nanmean(abs(filt_ang-ILM_angle)));
1137 fprintf(fileID, ...
1138     ['Abs Median Collagen fiber angle is %f \n ' ...
1139     '(relative to the ILM)\n'], ...
1140     nanmedian(abs(filt_ang-ILM_angle)));
1141
1142 %fprintf(fileID, 'ILM slope = %f\n', ILM_slope);
1143 %fprintf(fileID, 'ILM length = %f microns\n', ILM_length);

```

```

1144
1145     %fprintf(fileID, 'Mimimum fiber length is %f microns\n', fiber_min_length);
1146
1147     %fprintf(fileID, 'Filtered out %d fiber segments\n', filtered_fibers);
1148     %fprintf(fileID, 'Remaining eligible fibers = %d fibers\n',
1149     ↪ length(segments));
1150
1151         % for i = 1:length(combined_and_lone_fibers)
1152             %     fprintf(fileID, 'Fiber # %d -- length = %.4f nanometers, -- avg. angle
1153             ↪ RD = %.2f degrees, -- angle Calc = %.2f degrees, -- angle diff %.2f\n',
1154             ↪ combined_and_lone_fibers(i), len, angle, calc_ang, difference);
1155             % end
1156             %fprintf(fileID, 'Collagen fiber density = %f microns\n', sum(density)); %
1157             ↪ density of collagen fibers / ilm length
1158
1159             fprintf(fileID, 'Average collagen fiber length = %f microns\n', ...
1160                     mean(filt_len));
1161             fclose(fileID); % close the txt file for the output information
1162
1163
1164 case 'No'
1165     %Calculate only ILM thickness if no collagen
1166     figure
1167     imshow(img);
1168     % Indicate the five points on the ILM used for thickness measurements
1169     for i = 1:5
1170         f = msgbox(['Select the first two points that define ' ...
1171                     'the ILM thickness'], 'ILM');
1172         %     pause(1);
1173         [ILM_thick(i).x, ILM_thick(i).y] = ginput(2);
1174         hold on
1175         plot(ILM_thick(i).x, ILM_thick(i).y, 'g-o', 'LineWidth', 1);
1176         % Pythagorean theorem
1177         ILM_thick(i).measurement = sqrt((ILM_thick(i).x(1) - ILM_thick(i).x(2))^2
1178         ↪ + ...
1179                     (ILM_thick(i).y(1) - ILM_thick(i).y(2))^2);
1180         delete(f); % Delete the message box
1181     end
1182     for i = 1:5
1183         ILM_measurement(i) = ILM_thick(i).measurement;
1184     end
1185     L{4} = 'ILM thickness measurements';
1186     axis image;
1187     ILM_thickness = mean(ILM_measurement)/x_scale*1000;
1188     fprintf('Average ILM thickness is %f nanometers \n', ILM_thickness);
1189 end

```

## C.4 Human Data Analysis

</>    **Script 17:** *Python script analyzes human data, performs statistics, and creates figures.* </>

```

1 # -*- coding: utf-8 -*-
2 """
3 Created on Mon Nov 23 21:48:15 2020
4
5 @author: Kiffer Creveling
6 """
7
8 import pandas as pd
9 import os
10 import numpy as np
11 import seaborn as sns
12 from statannot import add_stat_annotation
13 import matplotlib.pyplot as plt
14 from matplotlib.patches import PathPatch
15 plt.rcParams['figure.figsize'] = [16, 10]
16 from scipy import stats
17 import pdb
18
19 # In[Functions]
20
21 # fcn for plotting
22 def yfit(x):
23     return slope*x + intercept
24
25 # In[Read values from Database]
26 """ Read from the database """
27
28 df = pd.read_csv('JMP_Data.csv') # Data from JMP
29 df = pd.read_excel('Human Data Paper 2 TEM only (Updated Jul 10 2020).xlsx',
30                     engine='openpyxl')
31 df = pd.read_excel('Human Data Paper 2 TEM only (Updated April 17 2021).xlsx',
32                     engine='openpyxl')
33
34 """ Simplification of code """
35 SF = 'StatisticsFigures' # Figure directory
36 TMD = 'TEM Mean Density'
37 TMA = 'TEM Mean Angle'
38 TAA = 'TEM Angle ABS'
39 ILM = 'ILM Thickness (nm)'
40 FL = 'Fiber Length (um)'
41 MPF = 'Maximum peel force (mN)'
42 SSPF = 'Steady-state peel force (mN)'
43 mpf_mN = 'Max peel force (mN)'
44 R = 'Region'
45 Eq = 'Equator'
46 Po = 'Posterior'
47 AG = 'AgeGroup'
48 A60 = 'Age60'
49 Aleq60 = r'Age $\leq$ 60'
50 Ag60 = 'Age $>$ 60'
51 A = 'Age'
52 MN = 'Max [N]'
```

```

53 MmN = 'Max [mN]'
54 SSN = 'SS [N]'
55 SSmN = 'SS [mN]'
56
57 # Plot attributes (labels, etc)
58 A_yrs = 'Age (yr.)'
59 A_G = 'Age Group (yr.)'
60 DensityUnit = (r'Collagen Fibril Density
61 → $\left(\frac{\#\text{ of fibrils}}{\text{ILM length (nm)}}\right)$')
62 FibrilLengthUnit = r'Collagen Fibril length ($\mu\text{m}$)'
63 OrientationUnit = r'Collagen Fibril Angle Relative to the ILM $(^\circ)$'
64
65 # convert from N to mN
66 df[mpf_mN] = df[SSN]*1000
67 df[SSmN] = df[SSN]*1000
68
69 # Exclude the cells that have duplicates or have been excluded due to
70 # video analysis
71 df = df[df['Excluded'] != 'yes']
72
73 # In[Create AgeGroup bins]
74 bins = [30, 40, 50, 60, 70, 80, 90]
75 labels = ['30-39', '40-49', '50-59', '60-69', '70-79', '80-89']
76 # Create binned AgeGroups
77 df[AG] = pd.cut(df[A], bins, labels=labels, right=False)
78
79 bins = [0, 60, 90]
80 labels = [Aeq60, Ag60]
81 # Create binned AgeGroups
82 df[A60] = pd.cut(df[A], bins, labels=labels, right=True)
83
84 # In[Pivot Table]
85 # Simplify pivot table output
86
87 pvtOut = {'count', np.median, np.mean, np.std} # pivot table outputs
88
89 # In[Plots]
90
91 standardError = 68 # Used for confidence intervals
92
93 sns.set_theme(context='paper', style='darkgrid', palette="Paired",
94                 font_scale=2)
95 custom_style = {'axes.facecolor': 'white',
96                  'axes.edgecolor': 'black',
97                  'axes.grid': False,
98                  'axes.axisbelow': True,
99                  'axes.labelcolor': 'black',
100                 'figure.facecolor': 'white',
101                 'grid.color': '.8',
102                 'grid.linestyle': '-',
103                 'text.color': 'black',
104                 'xtick.color': 'black',
105                 'ytick.color': 'black',
106                 'xtick.direction': 'out',
107                 'ytick.direction': 'out',
108                 'lines.solid_capstyle': 'round',
109                 'patch.edgecolor': 'w',

```

```

110     'patch.force_edgecolor': True,
111     'image.cmap': 'rocket',
112     'font.family': ['sans-serif'],
113     'font.sans-serif': ['Arial', 'DejaVu Sans', 'Liberation Sans',
114                         'Bitstream Vera Sans', 'sans-serif'],
115     'xtick.bottom': True,
116     'xtick.top': False,
117     'ytick.left': True,
118     'ytick.right': False,
119     'axes.spines.left': True,
120     'axes.spines.bottom': True,
121     'axes.spines.right': False,
122     'axes.spines.top': False}
123 # White background with ticks and black border lines, Turns grid off
124 ax = sns.set_style(rc=custom_style)
125
126 def boxPlotBlackBorder(ax):
127     # iterate over boxes in the plot to make each line black
128     for i,box in enumerate(ax.artists):
129         box.set_edgecolor('black')
130         # box.set_facecolor('white')
131
132     # iterate over whiskers and median lines
133     for j in range(6*i, 6*(i+1)):
134         ax.lines[j].set_color('black')
135
136 def smartPlot(data=None, x=None, y=None, hue=None, hue_order=None,
137               addBoxPair=None, ci=None, errcolor=None, capsize=None,
138               plot=None, test=None, sigLoc=None, text_format=None,
139               line_offset=None, line_offset_to_box=None, line_height=None,
140               fontsize=None, legLoc=None, verbose=None, xlabel=None,
141               ylabel=None, legendTitle=None, figName=None, folderName=None,
142               dataPoints=None):
143
144     # barplot
145     f, ax = plt.subplots()
146
147     if plot == 'barplot':
148         ax = sns.barplot(data=data, x=x, y=y, hue=hue, hue_order=hue_order,
149                           ci=ci, errcolor=errcolor, capsize=capsize)
150
151     elif plot == 'boxplot':
152         ax = sns.boxplot(data=data, x=x, y=y, hue=hue, hue_order=hue_order)
153
154     # Statistical test for differences
155     x_grps = list(data[x].unique()) # List of groups
156     if hue != None:
157         # Create combinations to compare
158         box_pairs_1 = [((x_grps_i, hue_order[0]),
159                         (x_grps_i, hue_order[1]))
160                         for x_grps_i in x_grps]
161     box_pairs = box_pairs_1
162
163     if addBoxPair != None:
164         # Additional box pairs
165         box_pairs = box_pairs_1 + addBoxPair
166
167     elif hue_order != None:

```

```

168     box_pairs = [(hue_order[0], hue_order[1])]
169
170     #Stats results and significant differences (SR)
171     SR = add_stat_annotation(ax, plot=plot, data=data, x=x, y=y, hue=hue,
172                             hue_order=hue_order, box_pairs=box_pairs,
173                             test=test, loc=sigLoc, text_format=text_format,
174                             verbose=verbose, comparisons_correction=None,
175                             line_offset=line_offset,
176                             line_offset_to_box=line_offset_to_box,
177                             line_height=line_height,
178                             fontsize=fontsize) # 'bonferroni'
179
180     if plot == 'boxplot':
181         boxPlotBlackBorder(ax) # Make borders black
182
183
184     if dataPoints == True:
185         # Add data points to the box plot
186         sns.stripplot(data=data, x=x, y=y, hue=hue, hue_order=hue_order,
187                     color='.5', size=5, linewidth=1, dodge=True)
188
189         # gather plot attributes for legends
190         handles, labels = ax.get_legend_handles_labels()
191
192         if hue != None:
193             l = plt.legend(handles[0:2], labels[0:2], title=legendTitle)
194
195     else:
196         if hue != None:
197             ax.legend(loc=legLoc).set_title(legendTitle)
198
199         ax.set_xlabel(xlabel)
200         ax.set_ylabel(ylabel)
201         ax = sns.despine() # takes the lines off on the right and top of the graph
202
203     if folderName != None:
204         # If a new folder name is given, put the files there
205
206         # New file path
207         NP = os.path.join(SF, folderName)
208
209         # Create folder if it doesn't exist
210         os.makedirs(NP, exist_ok=True)
211
212     else:
213         # Put the file in the same folder
214         NP = SF
215
216     f.savefig(os.path.join(NP, '{}.pdf'.format(figName)),
217               bbox_inches='tight')
218     plt.close()
219
220     # Special spacing
221
222     def adjust_box_widths(g, fac):
223         """
224             Adjust the widths of a seaborn-generated boxplot.
225         """

```

```

226
227     # iterating through Axes instances
228     for ax in g.axes:
229
230         # iterating through axes artists:
231         for c in ax.get_children():
232
233             # searching for PathPatches
234             if isinstance(c, PathPatch):
235                 # getting current width of box:
236                 p = c.get_path()
237                 verts = p.vertices
238                 verts_sub = verts[:-1]
239                 xmin = np.min(verts_sub[:, 0])
240                 xmax = np.max(verts_sub[:, 0])
241                 xmid = 0.5*(xmin + xmax)
242                 xhalf = 0.5*(xmax - xmin)
243
244                 # setting new width of box
245                 xmin_new = xmid - fac*xhalf
246                 xmax_new = xmid + fac*xhalf
247                 verts_sub[verts_sub[:, 0] == xmin, 0] = xmin_new
248                 verts_sub[verts_sub[:, 0] == xmax, 0] = xmax_new
249
250             # setting new width of median line
251             for l in ax.lines:
252                 if np.all(l.get_xdata() == [xmin, xmax]):
253                     l.set_xdata([xmin_new, xmax_new])
254
255 # In[TEM mean density by age +/- 60 and region]
256
257 """ TEM mean density by age +/- 60 and region """
258
259 pivotTEM_MeanDensityAgeGroup60 = pd.pivot_table(df, values=TMD,
260                                                 index=[A60, R],
261                                                 aggfunc=pvtOut)
262
263 print('pivotTEM_MeanDensityAgeGroup60')
264 print(pivotTEM_MeanDensityAgeGroup60)
265 # Add the index groups and convert NaN's to "-"
266 print(pivotTEM_MeanDensityAgeGroup60.to_latex(index=True, na_rep='-', escape=False,
267                                               float_format=".format"))
268
269 Folder = 'Density_Age60Region'
270
271 # Barplot
272 smartPlot(data=df, x=A60, y=TMD, hue=R, hue_order=[Eq, Po], ci='sd',
273             errcolor='black', caps=.2, plot='barplot', test='t-test_ind',
274             sigLoc='outside', text_format='star', line_offset=0.0,
275             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
276             legLoc='best', verbose=2,
277             xlabel=A_G, ylabel=DensityUnit, legendTitle=R,
278             figName='BarPlot', folderName=Folder)
279
280 # Boxplot
281 smartPlot(data=df, x=A60, y=TMD, hue=R, hue_order=[Eq, Po], plot='boxplot',
282             test='t-test_ind', text_format='star', sigLoc='outside',
283

```

```

284     line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
285     fontsize='small', legLoc='best', verbose=2,
286     xlabel=A_G, ylabel=DensityUnit,
287     legendTitle=R, figName='BoxPlot', folderName=Folder)
288
289 # Boxplot with data
290 smartPlot(data=df, x=A60, y=TMD, hue=R, hue_order=[Eq, Po], plot='boxplot',
291             test='t-test_ind', sigLoc='outside', text_format='star',
292             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
293             fontsize='small', legLoc='best', verbose=2,
294             xlabel=A_G, ylabel=DensityUnit,
295             legendTitle=R, figName='BoxPlotWithData', folderName=Folder,
296             dataPoints=True)
297
298
299 # In[TEM mean density grouped by region]
300
301 """ TEM mean density """
302
303 pivotTEM_MeanDensityRegion = pd.pivot_table(df, values=TMD, index=[R],
304                                              aggfunc=pvtOut)
305
306 print('pivotTEM_MeanDensityRegion')
307 print(pivotTEM_MeanDensityRegion)
308 # Add the index groups and convert NaN's to "----"
309 print(pivotTEM_MeanDensityRegion.to_latex(index=True, na_rep='--',
310                                              escape=False,
311                                              float_format="{:0.3f}".format))
312
313 Folder = 'Density_Region'
314
315 # Barplot
316 smartPlot(data=df, x=R, y=TMD, hue=None, hue_order=[Eq, Po], ci='sd',
317             errcolor='black', caps=.2, plot='barplot', test='t-test_ind',
318             sigLoc='outside', text_format='star', line_offset=0.0,
319             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
320             legLoc='best', verbose=2,
321             xlabel=R, ylabel=DensityUnit, legendTitle=R,
322             figName='BarPlot', folderName=Folder)
323
324 # Boxplot
325 smartPlot(data=df, x=R, y=TMD, hue=None, hue_order=[Eq, Po], plot='boxplot',
326             test='t-test_ind', sigLoc='outside', text_format='star',
327             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
328             fontsize='small', legLoc='best', verbose=2,
329             xlabel=R, ylabel=DensityUnit,
330             legendTitle=R, figName='BoxPlot', folderName=Folder)
331
332 # Boxplot with data
333 smartPlot(data=df, x=R, y=TMD, hue=None, hue_order=[Eq, Po], plot='boxplot',
334             test='t-test_ind', sigLoc='outside', text_format='star',
335             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
336             fontsize='small', legLoc='best', verbose=2,
337             xlabel=R, ylabel=DensityUnit,
338             legendTitle=R, figName='BoxPlotWithData', folderName=Folder,
339             dataPoints=True)
340
341 # matched_pairs student's t-test

```

```

342 dfTMD = df[df[TMD].notna()]
343
344 dfMP = dfTMD[dfTMD.duplicated(['MatchingID'], keep=False)]
345 f, p = stats.ttest_rel(dfMP[TMD][dfMP[R] == Eq],
346                         dfMP[TMD][dfMP[R] == Po])
347
348 print(f, p, "Matched Pairs Student's t-test")
349
350 f, p = stats.ttest_ind(dfTMD[TMD][dfTMD[R] == Eq],
351                         dfTMD[TMD][dfTMD[R] == Po])
352
353 print(f, p, "Student's t-test")
354
355 # In[TEM mean density grouped by age group decade and region]
356
357 pivotTEM_MeanDensity = pd.pivot_table(df, values=TMD, index=[R, AG],
358                                         aggfunc=pvtOut)
359
360 print('pivotTEM_MeanDensity')
361 print(pivotTEM_MeanDensity)
362 # Add the index groups and convert NaN's to "-"'
363 print(pivotTEM_MeanDensity.to_latex(index=True, na_rep='-', escape=False,
364                                         float_format="{:0.3f}".format))
365
366 Folder = 'Density_AgeDecadeRegion'
367
368 # Barplot
369 smartPlot(data=df, x=AG, y=TMD, hue=R, hue_order=[Eq, Po], ci='sd',
370             errcolor='black', capszie=.2, plot='barplot', test='t-test_ind',
371             sigLoc='outside', text_format='star', line_offset=0.0,
372             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
373             legLoc='best', verbose=2,
374             xlabel=A_G, ylabel=DensityUnit, legendTitle=R,
375             figName='BarPlot', folderName=Folder)
376
377 # Boxplot
378 smartPlot(data=df, x=AG, y=TMD, hue=R, hue_order=[Eq, Po], plot='boxplot',
379             test='t-test_ind', sigLoc='outside', text_format='star',
380             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
381             fontsize='small', legLoc='best', verbose=2,
382             xlabel=A_G, ylabel=DensityUnit,
383             legendTitle=R, figName='BoxPlot', folderName=Folder)
384
385 # Boxplot with data
386 smartPlot(data=df, x=AG, y=TMD, hue=R, hue_order=[Eq, Po], plot='boxplot',
387             test='t-test_ind', sigLoc='outside', text_format='star',
388             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
389             fontsize='small', legLoc='best', verbose=2,
390             xlabel=A_G, ylabel=DensityUnit,
391             legendTitle=R,
392             figName='BoxPlotWithData', folderName=Folder,
393             dataPoints=True)
394
395 # In[ILM thickness vs region age +/- 60]
396
397 """ TEM ILM thickness vs region age +/- 60 """
398
399 pivotTEM_ILM_ThicknessAge60 = pd.pivot_table(df, values=ILM, index=[A60, R],

```

```

400                                     aggfunc=pvtOut)
401
402 print('pivotTEM_ILM_ThicknessAge60')
403 print(pivotTEM_ILM_ThicknessAge60)
404 # Add the index groups and convert NaN's to "-"'
405 print(pivotTEM_ILM_ThicknessAge60.to_latex(index=True, na_rep='-' ,
406                                              escape=False,
407                                              float_format="{:0.3f}".format))
408
409 Folder = 'ILM_Age60Region'
410
411 # Barplot
412 smartPlot(data=df, x=A60, y=ILM, hue=R, hue_order=[Eq, Po], ci='sd',
413             errcolor='black', capsized=.2, plot='barplot', test='t-test_ind',
414             sigLoc='outside', text_format='star', line_offset=0.0,
415             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
416             legLoc='best', verbose=2,
417             xlabel=A_G, ylabel=ILM, legendTitle=R,
418             figName='BarPlot', folderName=Folder)
419
420 # Boxplot
421 smartPlot(data=df, x=A60, y=ILM, hue=R, hue_order=[Eq, Po], plot='boxplot',
422             test='t-test_ind', sigLoc='outside', text_format='star',
423             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
424             fontsize='small', legLoc='best', verbose=2,
425             xlabel=A_G, ylabel=ILM,
426             legendTitle=R, figName='BoxPlot', folderName=Folder)
427
428 # Boxplot with data
429 smartPlot(data=df, x=A60, y=ILM, hue=R, hue_order=[Eq, Po], plot='boxplot',
430             test='t-test_ind', sigLoc='outside', text_format='star',
431             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
432             fontsize='small', legLoc='best', verbose=2,
433             xlabel=A_G, ylabel=ILM,
434             legendTitle=R, figName='BoxPlotWithData', folderName=Folder,
435             dataPoints=True)
436
437
438 # In[ILM thickness vs region age group]
439
440 """ ILM thickness vs region and age group """
441
442 pivotTEM_ILM_Thickness = pd.pivot_table(df, values=ILM, index=[AG, R],
443                                         aggfunc=pvtOut)
444
445 print('pivotTEM_ILM_Thickness')
446 print(pivotTEM_ILM_Thickness)
447 # Add the index groups and convert NaN's to "-"'
448 print(pivotTEM_ILM_Thickness.to_latex(index=True, na_rep='-' ,
449                                              escape=False,
450                                              float_format="{:0.3f}".format))
451
452 Folder = 'ILM_Region'
453
454 # Barplot
455 smartPlot(data=df, x=AG, y=ILM, hue=R, hue_order=[Eq, Po], ci=68,
456             errcolor='black', capsized=.2, plot='barplot', test='t-test_ind',
457             sigLoc='outside', text_format='star', line_offset=0.0,

```

```

458     line_offset_to_box=0.0, line_height=0.015, fontsize='small',
459     legLoc='best', verbose=2,
460     xlabel=A_G, ylabel=ILM, legendTitle=R,
461     figName='BarPlot', folderName=Folder)
462
463 # Boxplot
464 smartPlot(data=df, x=AG, y=ILM, hue=R, hue_order=[Eq, Po], plot='boxplot',
465             test='t-test_ind', sigLoc='outside', text_format='star',
466             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
467             fontsize='small', legLoc='best', verbose=2,
468             xlabel=A_G, ylabel=ILM,
469             legendTitle=R, figName='BoxPlot', folderName=Folder)
470
471 # Boxplot with data
472 smartPlot(data=df, x=AG, y=ILM, hue=R, hue_order=[Eq, Po], plot='boxplot',
473             test='t-test_ind', sigLoc='outside', text_format='star',
474             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
475             fontsize='small', legLoc='best', verbose=2,
476             xlabel=A_G, ylabel=ILM,
477             legendTitle=R,
478             figName='BoxPlotWithData', folderName=Folder,
479             dataPoints=True)
480
481
482 # In[ILM fiber length vs region age group decade]
483
484 """ TEM ILM fiber length """
485
486 pivotTEM_FiberLength = pd.pivot_table(df, values=FL, index=[AG, R],
487                                         aggfunc=pvtOut)
488
489 print('pivotTEM_FiberLength')
490 print(pivotTEM_FiberLength)
491 # Add the index groups and convert NaN's to "-"
492 print(pivotTEM_FiberLength.to_latex(index=True, na_rep='-' ,
493                                         escape=False,
494                                         float_format=".format"))
495
496 Folder = 'FibrillLength_AgeDecadeRegion'
497
498 # Barplot
499 smartPlot(data=df, x=AG, y=FL, hue=R, hue_order=[Eq, Po], ci=68,
500             errcolor='black', caps=.2, plot='barplot', test='t-test_ind',
501             sigLoc='outside', text_format='star', line_offset=0.0,
502             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
503             legLoc='best', verbose=2,
504             xlabel=A_G, ylabel=FibrillLengthUnit, legendTitle=R,
505             figName='BarPlot', folderName=Folder)
506
507 # Boxplot
508 smartPlot(data=df, x=AG, y=FL, hue=R, hue_order=[Eq, Po], plot='boxplot',
509             test='t-test_ind', sigLoc='outside', text_format='star',
510             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
511             fontsize='small', legLoc='best', verbose=2,
512             xlabel=A_G, ylabel=FibrillLengthUnit,
513             legendTitle=R, figName='BoxPlot', folderName=Folder)
514
515 # Boxplot with data

```

```

516 smartPlot(data=df, x=A_G, y=FL, hue=R, hue_order=[Eq, Po], plot='boxplot',
517             test='t-test_ind', sigLoc='outside', text_format='star',
518             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
519             fontsize='small', legLoc='best', verbose=2,
520             xlabel=A_G, ylabel=FibrillLengthUnit,
521             legendTitle=R,
522             figName='BoxPlotWithData', folderName=Folder,
523             dataPoints=True)
524
525
526 # In[ILM fiber length vs region age group +/- 60]
527
528 """ TEM ILM fiber length """
529
530 pivotTEM_FiberLengthAge60 = pd.pivot_table(df, values=FL, index=[A60, R],
531                                              aggfunc=pvt0ut)
532
533 print('pivotTEM_FiberLengthAge60')
534 print(pivotTEM_FiberLengthAge60)
535 # Add the index groups and convert NaN's to "--"
536 print(pivotTEM_FiberLengthAge60.to_latex(index=True, na_rep='--',
537                                             escape=False,
538                                             float_format="{:0.3f}".format))
539
540 Folder = 'FibrillLength_Age60Region'
541
542 # Barplot
543 smartPlot(data=df, x=A60, y=FL, hue=R, hue_order=[Eq, Po], ci=68,
544             errcolor='black', capszie=.2, plot='barplot', test='t-test_ind',
545             sigLoc='outside', text_format='star', line_offset=0.0,
546             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
547             legLoc='best', verbose=2,
548             xlabel=A_G, ylabel=FibrillLengthUnit, legendTitle=R,
549             figName='BarPlot', folderName=Folder)
550
551 # Boxplot
552 smartPlot(data=df, x=A60, y=FL, hue=R, hue_order=[Eq, Po], plot='boxplot',
553             test='t-test_ind', sigLoc='outside', text_format='star',
554             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
555             fontsize='small', legLoc='best', verbose=2,
556             xlabel=A_G, ylabel=FibrillLengthUnit,
557             legendTitle=R, figName='BoxPlot', folderName=Folder)
558
559 # Boxplot with data
560 smartPlot(data=df, x=A60, y=FL, hue=R, hue_order=[Eq, Po], plot='boxplot',
561             test='t-test_ind', sigLoc='outside', text_format='star',
562             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
563             fontsize='small', legLoc='best', verbose=2,
564             xlabel=A_G, ylabel=FibrillLengthUnit,
565             legendTitle=R,
566             figName='BoxPlotWithData', folderName=Folder,
567             dataPoints=True)
568
569 # In[TEM Absolute Angle by age +/- 60 and region]
570
571 """ TEM Absolute Angle """
572
573 pivotTEM_MeanAngleABSAgeGroup60 = pd.pivot_table(df, values=TAA,

```

```

574                                         index=[A60, R],
575                                         aggfunc=pvt0ut)
576
577 print('pivotTEM_MeanAngleABSAgeGroup60')
578 print(pivotTEM_MeanAngleABSAgeGroup60)
579 # Add the index groups and convert NaN's to "-"
580 print(pivotTEM_MeanAngleABSAgeGroup60.to_latex(index=True, na_rep='-' ,
581                                         escape=False,
582                                         float_format="{:0.3f}".format()))
583
584 Folder = 'ABSAngle_Age60Region'
585
586 # Barplot
587 smartPlot(data=df, x=A60, y=TAA, hue=R, hue_order=[Eq, Po], ci=68,
588             errcolor='black', capszie=.2, plot='barplot', test='t-test_ind',
589             sigLoc='outside', text_format='star', line_offset=0.0,
590             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
591             legLoc='best', verbose=2,
592             xlabel=A_G, ylabel=OrientationUnit, legendTitle=R,
593             figName='BarPlot', folderName=Folder)
594
595 # Boxplot
596 smartPlot(data=df, x=A60, y=TAA, hue=R, hue_order=[Eq, Po], plot='boxplot',
597             test='t-test_ind', sigLoc='outside', text_format='star',
598             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
599             fontsize='small', legLoc='best', verbose=2,
600             xlabel=A_G, ylabel=OrientationUnit,
601             legendTitle=R, figName='BoxPlot', folderName=Folder)
602
603 # Boxplot with data
604 smartPlot(data=df, x=A60, y=TAA, hue=R, hue_order=[Eq, Po], plot='boxplot',
605             test='t-test_ind', sigLoc='outside', text_format='star',
606             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
607             fontsize='small', legLoc='best', verbose=2,
608             xlabel=A_G, ylabel=OrientationUnit,
609             legendTitle=R,
610             figName='BoxPlotWithData', folderName=Folder,
611             dataPoints=True)
612
613
614 # In[TEM angle]
615
616 pivotTEM_MeanAngle = pd.pivot_table(df, values=TMA, index=[R, AG],
617                                         aggfunc=pvt0ut)
618
619 print('pivotTEM_MeanAngle')
620 print(pivotTEM_MeanAngle)
621 # Add the index groups and convert NaN's to "-"
622 print(pivotTEM_MeanAngle.to_latex(index=True, na_rep='-' ,
623                                         escape=False,
624                                         float_format="{:0.3f}".format()))
625
626 OrientationUnitNoAbs = r'ILM angle $(^{\circ})$'
627 Folder = 'Angle_AgeRegion'
628
629 # Barplot
630 smartPlot(data=df, x=AG, y=TMA, hue=R, hue_order=[Eq, Po], ci=68,
631             errcolor='black', capszie=.2, plot='barplot', test='t-test_ind',

```

```

632     sigLoc='outside', text_format='star', line_offset=0.0,
633     line_offset_to_box=0.0, line_height=0.015, fontsize='small',
634     legLoc='best', verbose=2,
635     xlabel=A_G, ylabel=OrientationUnitNoAbs, legendTitle=R,
636     figName='BarPlot', folderName=Folder)
637
638 # Boxplot
639 smartPlot(data=df, x=AG, y=TMA, hue=R, hue_order=[Eq, Po], plot='boxplot',
640             test='t-test_ind', sigLoc='outside', text_format='star',
641             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
642             fontsize='small', legLoc='best', verbose=2,
643             xlabel=A_G, ylabel=OrientationUnitNoAbs,
644             legendTitle=R, figName='BoxPlot', folderName=Folder)
645
646 # Boxplot with data
647 smartPlot(data=df, x=AG, y=TMA, hue=R, hue_order=[Eq, Po], plot='boxplot',
648             test='t-test_ind', sigLoc='outside', text_format='star',
649             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
650             fontsize='small', legLoc='best', verbose=2,
651             xlabel=A_G, ylabel=OrientationUnitNoAbs,
652             legendTitle=R,
653             figName='BoxPlotWithData', folderName=Folder,
654             dataPoints=True)
655
656 # In[TEM ABS angle by age decade group and region]
657
658 pivotTEM_MeanAngleABS = pd.pivot_table(df, values=TAA, index=[R, AG],
659                                         aggfunc=pvtOut)
660 print('pivotTEM_MeanAngleABS')
661 print(pivotTEM_MeanAngleABS)
662 # Add the index groups and convert NaN's to "-"'
663 print(pivotTEM_MeanAngleABS.to_latex(index=True, na_rep='-' ,
664                                         escape=False,
665                                         float_format="{:0.3f}".format))
666
667 Folder = 'ABSAngle_AgeDecadeRegion'
668
669 # Barplot
670 smartPlot(data=df, x=AG, y=TAA, hue=R, hue_order=[Eq, Po], ci=68,
671             errcolor='black', capsize=.2, plot='barplot', test='t-test_ind',
672             sigLoc='outside', text_format='star', line_offset=0.0,
673             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
674             legLoc='best', verbose=2,
675             xlabel=A_G, ylabel=OrientationUnit, legendTitle=R,
676             figName='BarPlot', folderName=Folder)
677
678 # Boxplot
679 smartPlot(data=df, x=AG, y=TAA, hue=R, hue_order=[Eq, Po], plot='boxplot',
680             test='t-test_ind', sigLoc='outside', text_format='star',
681             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
682             fontsize='small', legLoc='best', verbose=2,
683             xlabel=A_G, ylabel=OrientationUnit,
684             legendTitle=R, figName='BoxPlot', folderName=Folder)
685
686 # Boxplot with data
687 smartPlot(data=df, x=AG, y=TAA, hue=R, hue_order=[Eq, Po], plot='boxplot',
688             test='t-test_ind', sigLoc='outside', text_format='star',
689             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,

```

```

690     fontsize='small', legLoc='best', verbose=2,
691     xlabel=A_G, ylabel=OrientationUnit,
692     legendTitle=R,
693     figName='BoxPlotWithData', folderName=Folder,
694     dataPoints=True)
695
696 # In[TEM absolute angle by region]
697
698 pivotTEM_MeanAngleABSRegion = pd.pivot_table(df, values=TAA, index=[R],
699                                              aggfunc=pvtOut)
700
701 print('pivotTEM_MeanAngleABSRegion')
702 print(pivotTEM_MeanAngleABSRegion)
703 # Add the index groups and convert NaN's to "-"'
704 print(pivotTEM_MeanAngleABSRegion.to_latex(index=True, na_rep='-' ,
705                                               escape=False,
706                                               float_format='{:.0f}'.format))
707
708 Folder = 'ABSAngle_Region'
709
710 # Barplot
711 smartPlot(data=df, x=R, y=TAA, hue=None, hue_order=[Eq, Po], ci=68,
712             errcolor='black', caps=.2, plot='barplot', test='t-test_ind',
713             sigLoc='outside', text_format='star', line_offset=0.0,
714             line_offset_to_box=0.0, line_height=0.015, fontsize='small',
715             legLoc='best', verbose=2,
716             xlabel=R, ylabel=OrientationUnit, legendTitle=R,
717             figName='BarPlot', folderName=Folder)
718
719 # Boxplot
720 smartPlot(data=df, x=R, y=TAA, hue=None, hue_order=[Eq, Po], plot='boxplot',
721             test='t-test_ind', sigLoc='outside', text_format='star',
722             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
723             fontsize='small', legLoc='best', verbose=2,
724             xlabel=R, ylabel=OrientationUnit,
725             legendTitle=R, figName='BoxPlot', folderName=Folder)
726
727 # Boxplot with data
728 smartPlot(data=df, x=R, y=TAA, hue=None, hue_order=[Eq, Po], plot='boxplot',
729             test='t-test_ind', sigLoc='outside', text_format='star',
730             line_offset=0.0, line_offset_to_box=0.0, line_height=0.015,
731             fontsize='small', legLoc='best', verbose=2,
732             xlabel=R, ylabel=OrientationUnit,
733             legendTitle=R,
734             figName='BoxPlotWithData', folderName=Folder,
735             dataPoints=True)
736
737 # In[ILM thickness vs age regression]
738
739 # Linear regression
740 f, ax = plt.subplots()
741 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
742                           "axes.labelsize":12})
743 # dict(Equator="r", Posterior="b") , 'color':'black', 'color':'blue'
744 ax = sns.lmplot(x=A, y=ILM, hue=R, markers=["o", "x"], data=df,
745                  legend_out=False, fit_reg=True, height=5, aspect=1.6,
746                  palette="Set1", truncate=False, ci=95, line_kws={'lw':0})
747 ax.set(ylabel=ILM, xlabel=A_yrs)

```

```

748
749 # Remove all NaN's from the data for regressions
750
751 # remove nans from ILM thickness
752 df_no_Nan = df.dropna(subset=[ILM])
753
754 # linear regressions for fitting
755 x = df_no_Nan[A][df_no_Nan[R] == Eq]
756 y = df_no_Nan[ILM][df_no_Nan[R] == Eq]
757
758 x_plot = np.linspace(min(x), max(x), 100)
759
760 slope, intercept, r_value1, p_value, std_err = stats.linregress(x, y)
761 plt.plot(x_plot, yfit(x_plot), '-', color='r', linewidth=1, label='line')
762 plt.text(80, yfit(80) + 20, r'$r={:.4f}$'.format(r_value1), color='r',
763         horizontalalignment='left', fontsize=8, weight='semibold') # r value
764
765 # linear regressions for fitting
766 x = df_no_Nan[A][df_no_Nan[R] == Po]
767 y = df_no_Nan[ILM][df_no_Nan[R] == Po]
768
769 x_plot = np.linspace(min(x), max(x), 100)
770 slope, intercept, r_value2, p_value, std_err = stats.linregress(x, y)
771 plt.plot(x_plot, yfit(x_plot), '-', color='b', linewidth=1, label='line')
772 plt.text(75, yfit(75) + 20, r'$r={:.4f}$'.format(r_value2), color='b',
773         horizontalalignment='left', fontsize=8, weight='semibold') # r value
774
775 # Axis limits
776 ax.set(ylim=(0, None))
777 ax.set(xlim=(None, None))
778
779 # New path
780 NP = os.path.join(SF, 'ILM_vs_Age')
781
782 # Create folder if it doesn't exist
783 os.makedirs(NP, exist_ok=True)
784
785 ax.savefig(os.path.join(NP, 'Regression.pdf'), bbox_inches='tight')
786 plt.close()
787
788 # In[Max peel force vs ILM thickness]
789
790 # Linear regression
791 f, ax = plt.subplots()
792 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
793                             "axes.labelsize":12})
794 ax = sns.lmplot(x=ILM, y=mpf_mN, hue=R, markers=["o", "x"], data=df,
795                  legend_out=False, fit_reg=True, height=5, aspect=1.6,
796                  palette="Set1", truncate=True, ci=95, line_kws={'lw':0})
797 ax.set(xlabel=ILM, ylabel=MPF)
798
799 # Remove all NaN's from the data for regressions
800 # remove nans from ILM thickness & Max
801 df_no_Nan = df.dropna(subset=[ILM, mpf_mN])
802
803 # linear regressions for fitting
804 x = df_no_Nan[ILM][df_no_Nan[R] == Eq]
805 # Convert to N

```

```

806 y = df_no_Nan[mpf_mN][df_no_Nan[R] == Eq]
807
808 x_plot = np.linspace(min(x), max(x), 100)
809
810 slope, intercept, r_value1, p_value, std_err = stats.linregress(x, y)
811 plt.plot(x_plot, yfit(x_plot), '-', color='r', linewidth=1, label='line')
812 plt.text(500, yfit(500) + 4, r'$r={:.4f}$'.format(r_value1), color='r',
813         horizontalalignment='left', fontsize=8, weight='semibold') # r value
814
815 print('Values for correlation between ' +
816       'ILM thickness and Max Force in the Equator\n',
817       'P={:.4f}'.format(p_value), 'r={:.4f}'.format(r_value1))
818
819 # linear regressions for fitting
820 x = df_no_Nan[ILM][df_no_Nan[R] == Po]
821 y = df_no_Nan[mpf_mN][df_no_Nan[R] == Po]
822
823
824 x_plot = np.linspace(min(x), max(x), 100)
825 slope, intercept, r_value2, p_value, std_err = stats.linregress(x, y)
826 plt.plot(x_plot, yfit(x_plot), '-', color='b', linewidth=1, label='line')
827 plt.text(1500, yfit(1500) + 1, r'$r={:.4f}$'.format(r_value2), color='b',
828         horizontalalignment='left', fontsize=8, weight='semibold') # r value
829
830 print('Values for correlation between ' +
831       'ILM thickness and Max Force in the Posterior\n',
832       f'P={p_value:.4f}', f'r={r_value2:.4f}')
833
834 # Axis limits
835 ax.set(ylim=(0, 18))
836 ax.set(xlim=(0, max(x)*1.1))
837
838 # New path
839 NP = os.path.join(SF, 'ILM_vs_MaxPeel')
840
841 # Create folder if it doesn't exist
842 os.makedirs(NP, exist_ok=True)
843
844 ax.savefig(os.path.join(NP, 'Regression.pdf'), bbox_inches='tight')
845 plt.close()
846
847 # In[Max peel force vs ILM thickness by age group]
848
849 # Linear regression
850 f, ax = plt.subplots()
851 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
852                           "axes.labelsize":12})
853 ax = sns.lmplot(x=ILM, y=mpf_mN, hue=A60, markers=["o", "x"], data=df,
854                  legend_out=False, fit_reg=True, height=5, aspect=1.6,
855                  palette="Set1", truncate=True, ci=95, line_kws={'lw':0})
856 ax.set(xlabel=ILM, ylabel=MPF)
857
858 # Remove all NaN's from the data for regressions
859 # remove nans from ILM thickness & Max
860 df_no_Nan = df.dropna(subset=[ILM, mpf_mN])
861
862 # linear regressions for fitting
863 x = df_no_Nan[ILM][df_no_Nan[A60] == A6eq60]

```

```

864 y = df_no_Nan[mpf_mN][df_no_Nan[A60] == Aleq60] # MmN
865
866 x_plot = np.linspace(min(x), max(x), 100)
867
868 # linear regression
869 slope, intercept, r_value1, p_value, std_err = stats.linregress(x, y)
870
871 # Linear regression line
872 plt.plot(x_plot, yfit(x_plot), '-', color='r', linewidth=1)
873 plt.text(1250, yfit(1250) + 0.75, r'$r={:.4f}$'.format(r_value1), color='r',
874         horizontalalignment='left', fontsize=8, weight='semibold') # r value
875
876 # linear regressions for fitting
877 x = df_no_Nan[ILM][df_no_Nan[A60] == Ag60]
878 y = df_no_Nan[mpf_mN][df_no_Nan[A60] == Ag60] # MmN
879
880 x_plot = np.linspace(min(x), max(x), 100)
881 # linear regression
882 slope, intercept, r_value2, p_value, std_err = stats.linregress(x, y)
883
884 plt.plot(x_plot, yfit(x_plot), '-', color='b', linewidth=1) # linear regression
885 plt.text(1000, yfit(1000) + 1, r'$r={:.4f}$'.format(r_value2), color='b',
886         horizontalalignment='left', fontsize=8, weight='semibold') # r value
887
888 # Legend
889 plt.legend(loc='best').set_title(A_G) # legend
890
891 # axis limits
892 ax.set(ylim=(0, 18))
893 ax.set(xlim=(0, 2200))
894
895 # New path
896 NP = os.path.join(SF, 'ILM_vs_MaxPeel_Age60')
897
898 # Create folder if it doesn't exist
899 os.makedirs(NP, exist_ok=True)
900
901 ax.savefig(os.path.join(NP, 'Regression.pdf'), bbox_inches='tight')
902 plt.close()
903
904
905 # In[Max peel force vs ILM thickness in the Equator]
906
907 # Linear regression
908 f, ax = plt.subplots()
909 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
910                  "axes.labelsize":12})
911 ax = sns.lmplot(x=ILM, y=mpf_mN, hue=A60, markers=["o", "x"],
912                  data=df[df[R] == Eq], legend_out=False, fit_reg=True, height=5,
913                  aspect=1.6, palette="Set1", truncate=False, ci=95,
914                  line_kws={'lw':0})
915 ax.set(xlabel=ILM, ylabel=MPF)
916
917 # Remove all NaN's from the data for regressions
918 # remove nans from ILM thickness & Max
919 df_no_Nan = df.dropna(subset=[ILM, mpf_mN])
920
921 # linear regressions for fitting

```

```

922 x = df_no_Nan[ILM][(df_no_Nan[A60] == Aleq60) & (df[R] == Eq)]
923 y = df_no_Nan[mpf_mN][(df_no_Nan[A60] == Aleq60) & (df[R] == Eq)] # MmN
924
925 x_plot = np.linspace(min(x), max(x), 100)
926
927 # linear regression
928 slope, intercept, r_value1, p_value, std_err = stats.linregress(x, y)
929
930 # Linear regression line
931 plt.plot(x_plot, yfit(x_plot), '-', color='r', linewidth=1)
932 plt.text(500, yfit(500) + 1, r'$r={:.4f}$'.format(r_value1), color='r',
933         horizontalalignment='left', fontsize=8, weight='semibold') # r value
934
935 # linear regressions for fitting
936 x = df_no_Nan[ILM][(df_no_Nan[A60] == Ag60) & (df[R] == Eq)]
937 y = df_no_Nan[mpf_mN][(df_no_Nan[A60] == Ag60) & (df[R] == Eq)] # MmN
938
939 x_plot = np.linspace(min(x), max(x), 100)
940 # linear regression
941 slope, intercept, r_value2, p_value, std_err = stats.linregress(x, y)
942
943 plt.plot(x_plot, yfit(x_plot), '-', color='b', linewidth=1) # linear regression
944 plt.text(500, yfit(500) - 1, r'$r={:.4f}$'.format(r_value2), color='b',
945         horizontalalignment='left', fontsize=8, weight='semibold') # r value
946
947 # Legend
948 plt.legend(loc='best').set_title("Equator Age group (yr.)") # legend
949
950 # axis limits
951 ax.set(ylim=(0, 20))
952 # ax.set(xlim=(0, None))
953
954 # New path
955 NP = os.path.join(SF, 'ILM_vs_MaxPeel_Age60_Equator')
956
957 # Create folder if it doesn't exist
958 os.makedirs(NP, exist_ok=True)
959
960 ax.savefig(os.path.join(NP, 'Regression.pdf'), bbox_inches='tight')
961 plt.close()
962
963 # In[Steady state peel force vs ILM density]
964
965 # Linear regression
966 f, ax = plt.subplots()
967 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
968                     "axes.labelsize":12})
969 ax = sns.lmplot(x=TMD, y=SSmN, hue=R, markers=["o", "x"], data=df,
970                 legend_out=False, fit_reg=True, height=5, aspect=1.6,
971                 palette="Set1", truncate=True, ci=95, line_kws={'lw':0})
972 ax.set(xlabel=DensityUnit, ylabel=SSPF)
973
974 # Remove all NaN's from the data for regressions
975 # remove nans from ILM thickness & Max
976 df_no_Nan = df.dropna(subset=[TMD, SSmN])
977 # figure out why zero's aren't being eliminated
978
979 # linear regressions for fitting

```

```

980 x = df_no_Nan[TMD][df_no_Nan[R] == Eq]
981 y = df_no_Nan[SSmN][df_no_Nan[R] == Eq]
982
983 x_plot = np.linspace(min(x), max(x), 100)
984
985 slope, intercept, r_value1, p_value, std_err = stats.linregress(x, y)
986 plt.plot(x_plot, yfit(x_plot), '-', color='r', linewidth=1, label='line')
987 plt.text(85, yfit(85) + 0.2, r'$r={:.4f}$'.format(r_value1), color='r',
988         horizontalalignment='left', fontsize=8, weight='semibold') # r value
989
990 print('Values for correlation between Steady-state and Equator\n',
991       f'P={p_value:.4f}', f'r={r_value1:.4f}')
992
993 # linear regressions for fitting
994 x = df_no_Nan[TMD][df_no_Nan[R] == Po]
995 y = df_no_Nan[SSmN][df_no_Nan[R] == Po]
996
997 x_plot = np.linspace(min(x), max(x), 100)
998 slope, intercept, r_value2, p_value, std_err = stats.linregress(x, y)
999 plt.plot(x_plot, yfit(x_plot), '-', color='b', linewidth=1, label='line')
1000 plt.text(70, yfit(70) + 0.3, r'$r={:.4f}$'.format(r_value2), color='b',
1001         horizontalalignment='left', fontsize=8, weight='semibold') # r value
1002
1003 print('Values for correlation between Steady-state and Posterior\n',
1004       f'P={p_value:.4f}', f'r={r_value2:.4f}')
1005
1006 # axis limits
1007 ax.set(ylim=(0, None))
1008 ax.set(xlim=(0, max(df_no_Nan[TMD])*1.05))
1009
1010 # New path
1011 NP = os.path.join(SF, 'Density_vs_SteadyStatePeel_Region')
1012
1013 # Create folder if it doesn't exist
1014 os.makedirs(NP, exist_ok=True)
1015
1016 ax.savefig(os.path.join(NP, 'Regression.pdf'), bbox_inches='tight')
1017 plt.close()
1018
1019
1020 # In[Maximum peel force vs ILM density]
1021
1022 # Linear regression
1023 f, ax = plt.subplots()
1024 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
1025                   "axes.labelsize":12})
1026 ax = sns.lmplot(x=TMD, y=mpf_mN, hue=R, markers=["o", "x"], data=df,
1027                   legend_out=False, fit_reg=True, height=5, aspect=1.6,
1028                   palette="Set1", truncate=False, ci=95, line_kws={'lw':0})
1029 ax.set(xlabel=DensityUnit, ylabel='Maximum peel force (mN)')
1030
1031 # Remove all NaN's from the data for regressions
1032 # remove nans from ILM thickness & Max
1033 df_no_Nan = df.dropna(subset=[TMD, mpf_mN])
1034 # figure out why zero's aren't being eliminated
1035
1036 # linear regressions for fitting
1037 x = df_no_Nan[TMD][df_no_Nan[R] == Eq]

```

```

1038 y = df_no_Nan[mpf_mN][df_no_Nan[R] == Eq]
1039
1040 x_plot = np.linspace(min(x), max(x), 100)
1041
1042 slope, intercept, r_value1, p_value, std_err = stats.linregress(x, y)
1043 plt.plot(x_plot, yfit(x_plot), '-', color='r', linewidth=1, label='line')
1044 plt.text(85, yfit(85) + 0.1, r'$r={:.4f}$'.format(r_value1), color='r',
1045         horizontalalignment='left', fontsize=8, weight='semibold') # r value
1046
1047 # linear regressions for fitting
1048 x = df_no_Nan[TMD][df_no_Nan[R] == Po]
1049 y = df_no_Nan[mpf_mN][df_no_Nan[R] == Po]
1050
1051 x_plot = np.linspace(min(x), max(x), 100)
1052 slope, intercept, r_value2, p_value, std_err = stats.linregress(x, y)
1053 plt.plot(x_plot, yfit(x_plot), '-', color='b', linewidth=1, label='line')
1054 plt.text(70, yfit(70) + 0.1, r'$r={:.4f}$'.format(r_value2), color='b',
1055         horizontalalignment='left', fontsize=8, weight='semibold') # r value
1056
1057 # axis limits
1058 ax.set(ylim=(0, None))
1059 # ax.set(xlim=(0, None))
1060
1061 # New path
1062 NP = os.path.join(SF, 'Density_vs_MaxPeel_Region')
1063
1064 # Create folder if it doesn't exist
1065 os.makedirs(NP, exist_ok=True)
1066
1067 ax.savefig(os.path.join(NP, 'Regression.pdf'), bbox_inches='tight')
1068 plt.close()
1069
1070 # In[Collagen fibril density vs age correlation (regression)]
1071
1072 # Linear regression
1073 f, ax = plt.subplots()
1074 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
1075                     "axes.labelsize":12})
1076 # dict(Equator='r', Posterior='b') , 'color':'black', 'color':'blue'
1077 ax = sns.lmplot(x=A, y=TMD, hue=R, markers=["o", "x"], data=df,
1078                  legend_out=False, fit_reg=True, height=5, aspect=1.6,
1079                  palette="Set1", truncate=False, ci=95, line_kws={'lw':0})
1080 ax.set(ylabel=DensityUnit, xlabel=A_yrs)
1081
1082 # Remove all NaN's from the data for regressions
1083
1084 # remove nans from ILM thickness
1085 df_no_Nan = df.dropna(subset=[TMD])
1086
1087 # linear regressions for fitting
1088 x = df_no_Nan[A][df_no_Nan[R] == Eq]
1089 y = df_no_Nan[TMD][df_no_Nan[R] == Eq]
1090
1091 x_plot = np.linspace(min(x), max(x), 100)
1092
1093 slope, intercept, r_value1, p_value, std_err = stats.linregress(x, y)
1094 plt.plot(x_plot, yfit(x_plot), '-', color='r', linewidth=1, label='line')
1095 plt.text(80, yfit(80) + 5, r'$r={:.4f}$'.format(r_value1), color='r',

```

```

1096         horizontalalignment='left', fontsize=8, weight='semibold') # r value
1097
1098 # linear regressions for fitting
1099 x = df_no_Nan[A][df_no_Nan[R] == Po]
1100 y = df_no_Nan[TMD][df_no_Nan[R] == Po]
1101
1102 x_plot = np.linspace(min(x), max(x), 100)
1103 slope, intercept, r_value2, p_value, std_err = stats.linregress(x, y)
1104 plt.plot(x_plot, yfit(x_plot), '-', color='b', linewidth=1, label='line')
1105 plt.text(75, yfit(75) + 5, r'$r={:.4f}$'.format(r_value2), color='b',
1106           horizontalalignment='left', fontsize=8, weight='semibold') # r value
1107
1108 # Axis limits
1109 ax.set(ylim=(0, None))
1110 ax.set(xlim=(None, None))
1111
1112 # New path
1113 NP = os.path.join(SF, 'Density_vs_Age')
1114
1115 # Create folder if it doesn't exist
1116 os.makedirs(NP, exist_ok=True)
1117
1118 ax.savefig(os.path.join(NP, 'Regression.pdf'), bbox_inches='tight')
1119 plt.close()
1120
1121
1122 # In[Collagen fibril Orientation vs age correlation (regression)]
1123
1124 # Linear regression
1125 f, ax = plt.subplots()
1126 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
1127                   "axes.labelsize":12})
1128 # dict(Equator="r", Posterior="b"), 'color':'black', 'color':'blue'
1129 ax = sns.lmplot(x=A, y=TAA, hue=R, markers=["o", "x"], data=df,
1130                   legend_out=False, fit_reg=True, height=5, aspect=1.6,
1131                   palette="Set1", truncate=False, ci=95, line_kws={'lw':0})
1132 ax.set(ylabel=OrientationUnit, xlabel=A_yrs)
1133
1134 # Remove all NaN's from the data for regressions
1135
1136 # remove nans from ILM thickness
1137 df_no_Nan = df.dropna(subset=[TAA])
1138
1139 # linear regressions for fitting
1140 x = df_no_Nan[A][df_no_Nan[R] == Eq]
1141 y = df_no_Nan[TAA][df_no_Nan[R] == Eq]
1142
1143 x_plot = np.linspace(min(x), max(x), 100)
1144
1145 slope, intercept, r_value1, p_value, std_err = stats.linregress(x, y)
1146 plt.plot(x_plot, yfit(x_plot), '-', color='r', linewidth=1, label='line')
1147 plt.text(80, yfit(80) + 2, r'$r={:.4f}$'.format(r_value1), color='r',
1148           horizontalalignment='left', fontsize=8, weight='semibold') # r value
1149
1150 print('Collagen fibril Equator orientation\n',
1151       f'P={p_value:.4f}', f'r={r_value1:.4f}')
1152
1153 # linear regressions for fitting

```

```

1154 x = df_no_Nan[A][df_no_Nan[R] == Po]
1155 y = df_no_Nan[TAA][df_no_Nan[R] == Po]
1156
1157 x_plot = np.linspace(min(x), max(x), 100)
1158 slope, intercept, r_value2, p_value, std_err = stats.linregress(x, y)
1159 plt.plot(x_plot, yfit(x_plot), '-', color='b', linewidth=1, label='line')
1160 plt.text(75, yfit(75) + 2, r'$r={:.4f}$'.format(r_value2), color='b',
1161           horizontalalignment='left', fontsize=8, weight='semibold') # r value
1162
1163 print('Collagen fibril Posterior orientation\n',
1164       f'P={p_value:.4f}', f'r={r_value2:.4f}')
1165
1166 # Axis limits
1167 ax.set(ylim=(0, None))
1168 ax.set(xlim=(None, None))
1169
1170 # New path
1171 NP = os.path.join(SF, 'Angle_vs_Age')
1172
1173 # Create folder if it doesn't exist
1174 os.makedirs(NP, exist_ok=True)
1175
1176 ax.savefig(os.path.join(NP, 'Regression.pdf'), bbox_inches='tight')
1177 plt.close()
1178
1179 # In[Collagen fibril orientation distributions]
1180
1181 # remove nans from ILM thickness
1182 df_no_Nan = df.dropna(subset=[TAA])
1183
1184 # Normal distribution plots
1185 f, ax = plt.subplots(figsize=(9.6, 6))
1186 sns.set_context("paper", rc={"font.size":12, "axes.titlesize":8,
1187                      "axes.labelsize":12})
1188
1189 ax = sns.kdeplot(data=df_no_Nan, x=TAA, hue=R, hue_order=[Eq, Po], fill=True,
1190                    legend=False, palette='Paired', multiple='layer',
1191                    cut=0, bw_adjust=0.7, alpha=0.3)
1192
1193 ax.set(xlabel=OrientationUnit, ylabel='Kernel Density Estimation')
1194
1195 # Legend
1196 plt.legend(labels=[Eq, Po], loc='best').set_title(R)
1197
1198 # Axis limits
1199 # ax.set(ylim=(0, None))
1200 # ax.set(xlim=(0, 90))
1201
1202 # New path
1203 NP = os.path.join(SF, 'Angle')
1204
1205 # Create folder if it doesn't exist
1206 os.makedirs(NP, exist_ok=True)
1207
1208 plt.savefig(os.path.join(NP, 'Distribution.pdf'), bbox_inches='tight')

```

## C.5 Additional Scripts

Additional scripts used for analysis: [https://gitlab.com/SkiEngineer/phd\\_dissertation\\_code/-/tree/master/AppendixC](https://gitlab.com/SkiEngineer/phd_dissertation_code/-/tree/master/AppendixC).

## **Part I**

# **Organization of Thesis/Dissertation**

(Not included in actual document)

**APPENDIX D**  
**THESIS/DISSERTATION**  
**ORGANIZATION**

To setup your Thesis/Dissertation, take advantage of the L<sup>A</sup>T<sub>E</sub>X package `subfiles` which allows you to break apart your document into various sections and compile them individually for editing purposes:

```
</>           Script 18: ... LATEX script used to setup the document.           </>
1 % The class file specifying the document structure
2 \documentclass{./LaTeX/tex/latex/MastersDoctoralThesis}
3
4 \input{StudentInfo.tex}
5
6 \input{./LaTeX/tex/latex/codeHighlighting.tex} % Code highlighting
7
8 %-----%
9 %             References
10 %-----%
11
12 \addbibresource{\subfix{Chapter1/bib_files/bibliography.bib}}
13 \addbibresource{\subfix{Chapter2/bib_files/bibliography.bib}}
14 \addbibresource{\subfix{Chapter3/bib_files/bibliography.bib}}
15 \addbibresource{\subfix{Chapter4/bib_files/bibliography.bib}}
16 \addbibresource{\subfix{Chapter5/bib_files/bibliography.bib}}
17
18 \raggedbottom
19 \begin{document}
20
21     \frontMatter
22
23 %-----%
24 %             Thesis Title, Copyright, Approval
25 %-----%
26
27 \begin{center}
28     \thesisTitle
29     \copyrightpage
30     \dissertationapproval
31 \end{center}
32
33 %-----%
34 %             Abstract
35 %-----%
36
37 \abstractPage
38
39 %-----%
40 %             Dedication
41 %-----%
42
43 \dedicationPage
44
45 %-----%
46 %             Frontispiece image (Image of importance relevance)
47 %-----%
48
49 \frontispiecePage
```

```

50
51 %-----%
52 %          Epigraph page (Inspirational quote) %
53 %-----%
54
55 \epigraphPage
56
57 %-----%
58 %          LIST OF CONTENTS/FIGURES/TABLES PAGES %
59 %-----%
60
61 \tableOfContents
62
63 %-----%
64 %          Notation and Symbols %
65 %-----%
66
67 \NotationSymbolsPage
68
69 %-----%
70 %          Acknowledge %
71 %-----%
72
73 \AcknowledgementPage
74
75 %-----%
76 %          Thesis/Dissertation Content (Chapters etc.) %
77 %-----%
78
79 \mainMatter
80
81 \subfile{\subfix{Chapter1/Chapter1}}
82 \subfile{\subfix{Chapter2/Chapter2}}
83 \subfile{\subfix{Chapter3/Chapter3}}
84 \subfile{\subfix{Chapter4/Chapter4}}
85 \subfile{\subfix{Chapter5/Chapter5}}
86
87 %-----%
88 %          List of items to work on (Editing phase) %
89 %-----%
90
91 \%listoftodos
92
93 %-----%
94 %          APPENDICES %
95 %-----%
96
97 \backMatter % Que to tell LaTeX that the following "chapters" are Appendices
98
99 % Include the appendices of the thesis as separate files from the
100 % Appendices folder
101 % Uncomment the lines as you write the Appendices
102
103 \subfile{\subfix{AppendixA/AppendixA}}
104 \subfile{\subfix{AppendixB/AppendixB}}
105 \subfile{\subfix{AppendixC/AppendixC}}
106
107 %-----%

```

```

108 %                               BIBLIOGRAPHY
109 %-----%
110
111 % Comment out because each chapter displays references/
112 % or comment out the individual references and keep the final bibliography
113 % \finalBibliography
114
115 \end{document}

```

## D.1 Student Info Setup

Script 19 is used to specify student-specific information.

</>                    **Script 19:** ...*LATEX script used for Student-Specific Info.*                    </>

```

1 \author      {Full Name}
2 \title       {Thesis/Dissertation Title}
3 \thesistype   {dissertation} % thesis/dissertation - variable to switch between
→ 3 and 5 committee members
4 \degreeAchieved {Doctor of Philosophy}
5 \department   {Department of xx}
6 \departmentLink {}
7 \submitdate   {xx yyyy}
8 \copyrightyear {\the\year{}}

9
10 \abstractString {The usability of dissertation abstracts depends largely on
11 their content. Many journals within the medical community have settled on a
12 seven sentence structure, which is also gaining acceptance in the social
13 sciences, education and business. In it, the purpose of the study and
14 methodological choices are outlined succinctly, allowing the reader or
15 researcher to quickly scan and evaluate a number of studies to easily choose
16 ones that meet their particular demands. The structure contains variations on
17 the following seven sentence stems: "The purpose of this study is...." "The
18 scope of this study...." "The methodology...." "The Findings..." "Conclusions
19 reached are ..." "Limitations of this study include...." "This study
20 contributes...." Abstracts of dissertation proposals contain the same seven
21 concepts, substituting data collection and analysis in place of findings and
22 conclusions. Abstracts are limited in the United States by the UMI to 350
23 words.\\\indent More info here.}

24
25 \dedication {Most books at the library will have a dedication page.
26 Normally, this page includes quotes like "For my mother" or "For Lucy who never
27 gave up on me." A dissertation dedication is the same concept. In this part of
28 the dissertation, the student must use a sentence or a paragraph to dedicate
29 their text. They may want to use the dedication to recognize an individual who
30 inspired them to go to college or someone who helped with the dissertation.
31 Dedicating the dissertation to someone is a way to honor them. After putting so
32 much work into this paper, it is a chance for the student to recognize the
33 people who influenced the process.}

34
35 \frontispiece {example-image-a} %

36
37 \epigraphQuote {``Quote''}
38 \epigraphAuthor {Famous Individual}
39
40 \Acknowledgement{The dissertation acknowledgements section is where you thank

```

```

41 those who have helped and supported you during the research and writing
42 process. This includes both professional and personal acknowledgements. \ldots
43 \\\\indent More acknowledgement info can be found here:
44 \url{https://www.scribbr.com/dissertation/acknowledgements/.}

45
46 \NotationAndSymbols{
47 \begin{tabularx}{\textwidth}{lX}
48   \toprule
49   x & \emph{Var} &- the variable `x'. \\
50   y & \emph{y} &- the variable `y'. \\
51   m & \emph{Slope} &- The slope is one of the essential characteristics of a
      → line and helps us measure the rate of change. The slope of a straight line is
      → the ratio of the change in  $y$  to the change in  $x$ , also called the rise
      → over run. \\
52   $\mathbf{F}$ & \emph{Force} &- Force vector. \\
53   $\pi$ & \emph{Pi} &- The number  $\pi$  is a mathematical constant. It is
      → defined as the ratio of a circle's circumference to its diameter, and it also
      → has various equivalent definitions. It appears in many formulas in all areas
      → of mathematics and physics. \\
54   \bottomrule
55 \end{tabularx}%
56 }
57
58 \approvaldepartment {Dept.}

59
60 \graduateDean {Graduate School Dean}
61 \departmentChair {Department Chair}
62 \departmentChairTitle {Chair}
63 \departmentCollegeSchool {Department}

64
65 \committeeChair {Graduate Advisor} % Graduate Advisor
66 \advisorTitle {Associate Professor, Associate Chair}

67
68 \committeeMemberII {Committee member 2}
69 \committeeMemberIII {Committee member 3}
70 \committeeMemberIIII {Committee member 4}
71 \committeeMemberIIIIII {Committee member 5}

72
73 \chairDateApproved {mm/dd/\the\year{}}
74 \committeeMemberIIDateApproved {mm/dd/\the\year{}}
75 \committeeMemberIIIDateApproved {mm/dd/\the\year{}}
76 \committeeMemberIIIIDateApproved {mm/dd/\the\year{}}
77 \committeeMemberIIIIIDateApproved {mm/dd/\the\year{}}

```

## D.2 Individual Chapter 1 Setup

</>                    Script 20: ... *L<sup>A</sup>T<sub>E</sub>X* script used for chapter 1.                    </>

```

1 %%% -*- LaTeX-*-
2 \documentclass[../Dissertation]{subfiles}
3
4 \doublespacing
5 \graphicspath{{Chapter1/Media/}{Media/}} % Graphics path for images
6
7 \begin{document}

```

```

8   % Needs to be capitalized (University Rules) and title width is no longer
9   % than 4.5 inches. Specify custom line breaks using "|protect||" for both
10  % the Chapter title page and the table of contents page
11  \chapter{\uppercase{Introduction}}\label{chp:1}
12
13
14  % If there is no text between the chapter and the first section, then we
15  % need to reduce the spacing with a negative skip. Otherwise, comment out
16  % the following line
17  \vspace{-2ex}
18
19  \subfile{\subfix{Chapter1/0_Overview}}
20  \subfile{\subfix{Chapter1/1_Background}}
21  \subfile{\subfix{Chapter1/2_Aims}}
22  \subfile{\subfix{Chapter1/3_Impact}}
23
24  % bibliography
25  \printbibliography[segment=\therefsegment,heading=references]
26
27 \end{document}

```

### D.3 Individual Chapter 2 Setup

</>                    **Script 21:** ... *LATEX script used to import a chapter.*                    </>

```

1  %% --LaTeX--
2  \documentclass[../Dissertation]{subfiles}
3
4  \doublespacing
5  \%graphicspath{{Chapter2/Media/}{Media/}} % Graphics path for images
6
7  \begin{document}
8
9  % Needs to be capitalized (University Rules) and title width is no longer
10  % than 4.5 inches. Specify custom line breaks using "|protect||" for both
11  % the Chapter title page and the table of contents page
12  \chapter[\uppercase{Multiline Title}]{\uppercase{Multiline
13  \protect\\ Title}}\label{chp:2}
14
15  \lipsum[1] % random text after the chapter title.
16
17  % If there is no text between the chapter and the first section, then we
18  % need to reduce the spacing with a negative skip. Otherwise, comment out
19  % the following line
20  % \vspace{-2ex}
21
22  \subfile{\subfix{Chapter2/0_Abstract}}
23  \subfile{\subfix{Chapter2/1_Introduction}}
24  \subfile{\subfix{Chapter2/2_Methods}}
25  \subfile{\subfix{Chapter2/3_Results}}
26  \subfile{\subfix{Chapter2/4_Discussion}}
27  \subfile{\subfix{Chapter2/5_Conclusion}}
28  \subfile{\subfix{Chapter2/6_Acknowledgment}}
29
30  % bibliography

```

```

31   \printbibliography[segment=\therefsegment,heading=references]
32
33 \end{document}

```

## D.4 Individual Chapter 3 Setup

</> Script 22: ... *LATEX* script used to import a chapter. </>

```

1  %% -*-LaTeX-*
2  \documentclass[../Dissertation]{subfiles}
3
4  \doublespacing
% \graphicspath{{Chapter3/Media/}{Media/}} % Graphics path for images
5
6
7  \begin{document}
8
9  % Needs to be capitalized (University Rules) and title width is no longer
10 % than 4.5 inches. Specify custom line breaks using "\protect\\\" for both
11 % the Chapter title page and the table of contents page
12 \chapter[\uppercase{Previously published article}]{\uppercase{Previously
13 published article}}\label{chp:3}
14
15 % If there is no text between the chapter and the first section, then we
16 % need to reduce the spacing with a negative skip. Otherwise, comment out
17 % the following line
18 %\vspace{-0.5\UofUDoubleSpace}
19
20 Research published: C. J. Creveling, J. Colter, and B. Coats, ``Changes in
21 Vitreoretinal Adhesion With Age and Region in Human and Sheep Eyes," Front.
22 Bioeng. Biotechnol., vol. 6, no. 153, pp. 1-11, Oct. 2018.
23
24 % \subfile{\subfix{Chapter3/0_Abstract}}
25 % \subfile{\subfix{Chapter3/1_Introduction}}
26 % \subfile{\subfix{Chapter3/2_Methods}}
27 % \subfile{\subfix{Chapter3/3_Results}}
28 % \subfile{\subfix{Chapter3/4_Discussion}}
29 % \subfile{\subfix{Chapter3/5_Conclusion}}
30 % \subfile{\subfix{Chapter3/6_Acknowledgment}}
31
32 \subfile{./code/PDFdocument}\%Chapter3/
33
34 \section{PDF Import}
35   Using the \holo{LaTeX} package
36   \url{https://ctan.org/pkg/pdfpages?lang=en}\{\texttt{pdfpages}\}, you
37   can import PDFs into your PDF. An example of this is used to import a
38   previously published journal article in \citet{code:PDF}. There are
39   function keywords that allow you to add sections to the table of
40   contents, list of figures, and the list of tables.
41
42   \mintinline{LaTeX}{addtotoc={<page
43   \hookrightarrow number>,<section>,<level>,<heading>,<label>}}
44
45   \mintinline{LaTeX}{addtolist={<page number>,<type>,<heading>,<label>}}
46
\codeFromFile{LaTeX}

```

```

47 {./Chapter3/code/PDFdocument.tex} %
48 {\ldots \holo{LaTeX} to import a PDF and add contents to the Table of
49 Contents, List of Figures, and List of Tables.}
50 {PDF}
51 {\footnotesize}
52 {\latextcodebg}
53 {default}

54 % bibliography
55 \printbibliography[segment=\therefsegment,heading=references]
56
57
58 \end{document}

```

## D.5 Individual Chapter 4 Setup

</>                    Script 23: ... *L<sup>A</sup>T<sub>E</sub>X* script used to import a chapter.                    </>

```

1 %%% -*- LaTeX -*-
2 \documentclass[../Dissertation]{subfiles}
3
4 \doublespacing
5 \graphicspath{{Chapter4/Media/}{Media/}} % Graphics path for images
6
7 \begin{document}
8
9 % Needs to be capitalized (University Rules) and title width is no longer
10 % than 4.5 inches. Specify custom line breaks using "\protect\\|\" for both
11 % the Chapter title page and the table of contents page
12 \chapter[\uppercase{This Is Another Multi-Line Title That Needs To Have An
13 \protect\\Inverted Pyramid Shape Where Each Line Is Not Wider Than 4.6
14 in.}]{\uppercase{This Is Another Multi-Line Title That \protect\\Needs To
15 Have An Inverted \protect\\Pyramid Shape Where Each \protect\\Line Is Not
16 Wider \protect\\Than 4.6 in.}}\label{chp:4}
17
18
19 % If there is no text between the chapter and the first section, then we
20 % need to reduce the spacing with a negative skip. Otherwise, comment out
21 % the following line
22 \vspace{-2ex}
23
24 \subfile{\subfix{Chapter4/0_Abstract}}
25 \subfile{\subfix{Chapter4/1_Introduction}}
26 \subfile{\subfix{Chapter4/2_Methods}}
27 \subfile{\subfix{Chapter4/3_Results}}
28 \subfile{\subfix{Chapter4/4_Discussion}}
29 \subfile{\subfix{Chapter4/5_Conclusion}}
30 \subfile{\subfix{Chapter4/6_Acknowledgment}}
31
32 % bibliography
33 \printbibliography[segment=\therefsegment,heading=references]
34
35 \end{document}

```

## D.6 Individual Chapter 5 Setup

```
</>           Script 24: ... LATEX script used to import a chapter.           </>
1  %%> -*-LaTeX-*-
2  \documentclass[../Dissertation]{subfiles}
3
4  \doublespacing
5  \graphicspath{{Chapter5/Media/}{Media/}} % Graphics path for images
6
7  \begin{document}
8
9  % Needs to be capitalized (University Rules) and title width is no longer
10 % than 4.5 inches. Specify custom line breaks using "\protect\\\" for both
11 % the Chapter title page and the table of contents page
12 \chapter{\uppercase{Conclusions and future work}}\label{chp:5}
13
14 % If there is no text between the chapter and the first section, then we
15 % need to reduce the spacing with a negative skip. Otherwise, comment out
16 % the following line
17 \vspace{-2ex}
18
19 \subfile{\subfix{Chapter5/0_Summary_of_key_findings}}
20 \subfile{\subfix{Chapter5/1_Chapter2_summary}}
21 \subfile{\subfix{Chapter5/2_Chapter3_summary}}
22 \subfile{\subfix{Chapter5/3_Chapter4_summary}}
23 \subfile{\subfix{Chapter5/4_Conclusion}}
24 \subfile{\subfix{Chapter5/5_Future_Work}}
25
26 % bibliography
27 \printbibliography[segment=\therefsegment,heading=references]
28
29 \end{document}
```

## D.7 Individual Appendix A Setup

```
</>           Script 25: ... LATEX script used to import an appendix.           </>
1  %%> -*-LaTeX-*-
2  \documentclass[../Dissertation]{subfiles}
3
4  \doublespacing
5
6  \begin{document}
7
8  \chapter{Code Script Examples}\label{AppendixA}
9
10 % If there is no text between the chapter and the first section, then we
11 % need to reduce the spacing with a negative skip. Otherwise, comment out
12 % the following line
13 \vspace{-0.5\UofUDoubleSpace}
14
15 \section{Example Cross product codes}
16   Find cross products using
17   \cref{code:ex1,code:ex2,code:ex3,code:ex4}.
```

```

18 \subsection{\texttt{c}}
19   \codeFromFile{c}
20   {\subfix{code/c/HW3-4_58.c}}
21   {\ldots \texttt{c} to solve cross products.}
22   {ex1}
23   {\footnotesize}
24   {ccodebg}
25   {default}

26 \subsection{\texttt{Fortran}}
27   \codeFromFile{fortran}
28   {\subfix{code/fortran/HW3-Problem_2.f}}
29   {\ldots \texttt{Fortran} to solve cross products.}
30   {ex2}
31   {\footnotesize}
32   {fortrancodebg}
33   {default}

34 \subsection{\texttt{Matlab}}
35   \codeFromFile{matlab}
36   {\subfix{code/matlab/HW3-2_cross_product.m}}
37   {\ldots \texttt{Matlab} to solve cross products.}
38   {ex3}
39   {\footnotesize}
40   {matlabcodebg}
41   {default}

42 \subsection{\texttt{Python}}
43   \codeFromFile{python}
44   {\subfix{code/python/HW3-2_cross_Product.py}}
45   {\ldots \texttt{Python} to solve cross products.}
46   {ex4}
47   {\footnotesize}
48   {pythancodebg}
49   {default}

50 \section{Additional Example codes}

51 \subsection{\texttt{Matlab}}
52   \codeFromFile{matlab}
53   {\subfix{code/matlab/HW3-problem_1.m}}
54   {\ldots \texttt{Matlab} to solve a problem.}
55   {HW3-problem_1}
56   {\footnotesize}
57   {matlabcodebg}
58   {default}

59 \section{Code Highlighting Using}
60 \href{https://ctan.org/pkg/minted?lang=en}{\texttt{minted}}
61 \subsection{Highlighting Function}
62 To use the code highlighting function that renders scripts for publication
63 with line numbers/language specification/file path and more, please see the
64 following function:
65
66 \begin{minted}[language=LaTeX]{}
67   \begin{spacing}{}
68     \begin{minted}[language=LaTeX]{}
69       % The "\codeFromFile" function is used in the following manner:
70     \end{minted}
71   \end{spacing}
72 \end{minted}

```

```

76 \codeFromFile
77   {language}      % Programming language
78   {\subfix{path}} % File path
79   {Header}        % Script heading info
80   {label}         % LaTeX label for cross referencing
81   {fontsize}      % Fontsize
82   {backgroundcolor} % Text background color
83   {mintedStyle}    % Minted text style (default)
84 \end{minted}
85 }

86
87 \subsection{Code Highlighting Example}
88 Here is the text to display the code used to highlight \hologo{LaTeX} code in
89 \cref{code:exampleCode}.
90
91 {\singespacing
92 \begin{minted}{LaTeX}
93 \section{Scripting language to call scripts}
94 \subsection{\texttt{\hologo{LaTeX}}}
95   \codeFromFile{LaTeX}
96   {\subfix{code/LaTeX/exampleCode.tex}}
97   {\ldots \hologo{LaTeX} to call other scripts.}
98   {exampleCode}
99   {\footnotesize}
100  {\latexcodebg}
101  {\default}
102 \end{minted}
103 }
104
105 \begin{itemize}
106   \item Lines \texttt{[1-8]} in \cref{code:exampleCode} refer to
107     \cref{code:ex1}
108   \item Lines \texttt{[10-17]} in \cref{code:exampleCode} refer to
109     \cref{code:ex2}
110   \item Lines \texttt{[19-26]} in \cref{code:exampleCode} refer to
111     \cref{code:ex3}
112   \item Lines \texttt{[28-35]} in \cref{code:exampleCode} refer to
113     \cref{code:ex4}
114 \end{itemize}
115
116
117 \subsection{\texttt{\hologo{LaTeX}}} Script
118 \codeFromFile{LaTeX}
119 {\subfix{code/LaTeX/exampleCode.tex}}
120 {\ldots \hologo{LaTeX} to call other scripts using the
121 \texttt{codeFromFile} function.} % \mintinline{LaTeX}{\codeFromFile}
122 {exampleCode}
123 {\footnotesize}
124 {\latexcodebg}
125 {\default}
126
127 \end{document}

```

## D.8 Individual Appendix B Setup

```
</>           Script 26: ... LATEX script used to import an appendix. </>
1  %%% -*-LaTeX-*
2  \documentclass[../Dissertation]{subfiles}
3
4  \doublespacing
5
6  \begin{document}
7
8  \chapter{Equations}\label{AppendixB}
9
10 \section{Supporting relationships}
11 The mass-energy equivalence is described by the famous equation
12
13 \begin{equation}
14     E=mc^2
15 \end{equation}
16
17 \noindent discovered in 1905 by Albert Einstein.
18 In natural units ( $c = 1$ ), the formula expresses the identity
19
20 \begin{equation}
21     E=m
22 \end{equation}
23
24 \end{document}
```

## D.9 Individual Appendix C Setup

```
</>           Script 27: ... LATEX script used to import an appendix. </>
1  %%% -*-LaTeX-*
2  \documentclass[../Dissertation]{subfiles}
3
4  \doublespacing
5
6  \begin{document}
7
8  \chapter{Tissue Processing}\label{AppendixC}
9
10 % Space used to make space between chapter title and section header the
11 % correct spacing
12 \vspace{-0.5\baselineskip}
13
14 \includepdf[
15   pages={1},
16   pagecommand={\section{Electron Microscopy Preparation}\subsection{Tissue
17   Processing}},
18   scale=0.8]
19   {\subfix{procedures/TissueProcessing/Release/TissueProcessing}}
20
21 % Because the page created new subsections for each page
22 \includepdf[pages={2-},
```

```

22 pagecommand={} ,
23 scale=0.8]
{\subfix{procedures/TissueProcessing/Release/TissueProcessing} }

25
26 \includepdf[pages={1},
27 pagecommand={\subsection{Tissue Processing}},
28 scale=0.8]
{\subfix{procedures/ResinPrep/Release/ResinPreparation} }

29
30 % Because the page created new subsections for each page
31 \includepdf[
32 pages={2-},
33 pagecommand={} ,
34 scale=0.8]
{\subfix{procedures/ResinPrep/Release/ResinPreparation} }

35
36 % \section{MatLab Least Squares}
37 % \codeFromFile{matlab}
38 % {\subfix{Matlab/Least_Squares.m}}
39 % {Matlab script that performs a least squares regression calculation.}
40 % {leastSquares}{\footnotesize}{matlabcodebg}{default}

41
42 \section{Ridge Detection Input Parameters}
43 \codeFromFile{matlab}
44 {\subfix{Matlab/Sigma_Selector.m}}
45 {Matlab script that determine ridge detection parameters using TEM images.}
46 {ridgeDetectionParams}{\footnotesize}{matlabcodebg}{default}

47
48 \section{Analyze Ridge Detection Output}
49 \codeFromFile{matlab}
50 {\subfix{Matlab/RidgeDetectionAnalysis.m}}
51 {Matlab script that analyzes ridge detection output from TEM images.}
52 {ridgeDetectionAnalysis}{\footnotesize}{matlabcodebg}{default}

53
54 \section{Human Data Analysis}
55 \codeFromFile{python}
56 {\subfix{Python/HumanDataStatisticsPaper2.py}}
57 {Python script analyzes human data, performs statistics, and creates
58 figures.}
59 {humanTEMDataAnalysis}{\footnotesize}{pythoncodebg}{default}

60
61 \section{Additional Scripts}
62 Additional scripts used for analysis:
63 \href{http://gitlab.com/SkiEngineer/phd_dissertation_code/-/tree/master/AppendixB}{}
64 {\texttt{\texttt{https://gitlab.com/SkiEngineer/\linebreak
65 phd\_dissertation\_code/-/tree/master/AppendixC}}}.
66
67
68
69 \end{document}

```

## D.10 How To Use This Thesis/Dissertation L<sup>A</sup>T<sub>E</sub>X Template?

In order to take advantage of the whole functionality of L<sup>A</sup>T<sub>E</sub>X to compile your Thesis/Dissertation, it is recommended to use a Linux system. This can be done on Microsoft Windows using the Linux subsystem [Ubuntu](#). The alternative is to use [Overleaf](#) which

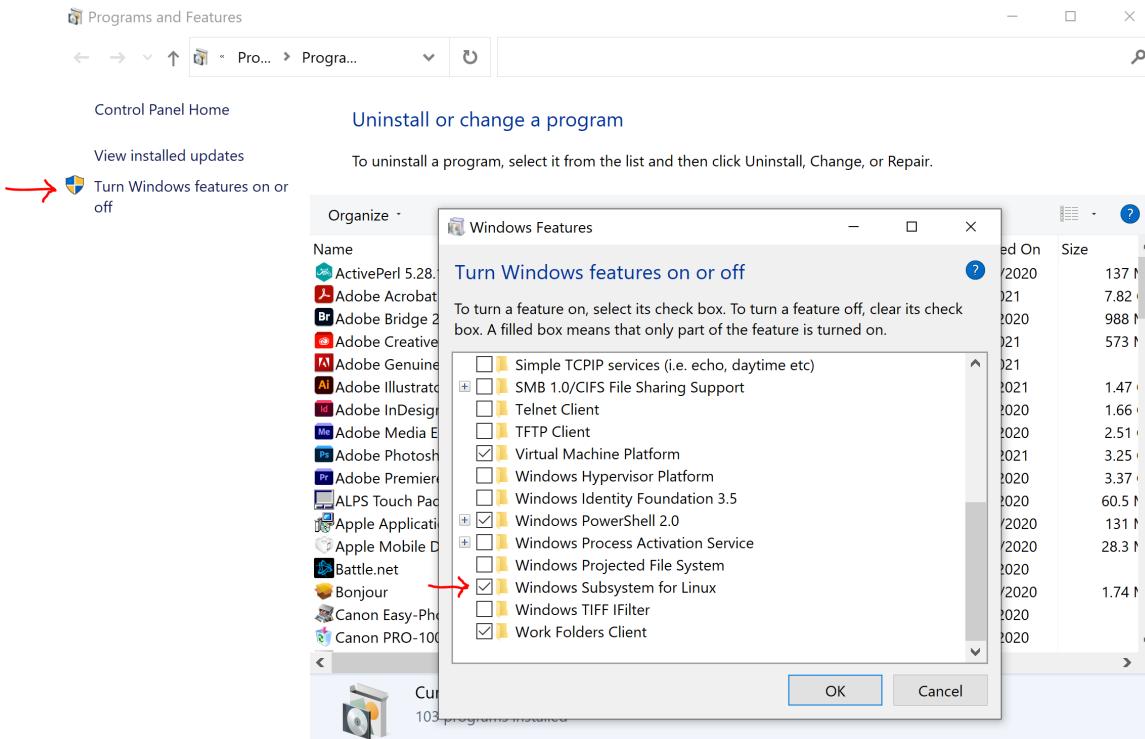


Figure D.1: Enable Windows Subsystem for Linux

takes care of all of the package installation requirements. The difficulty using Overleaf is that when you switch between files, it will compile each document. This takes a lot of time to wait for it to update. When the files are on your local machine, you can open files as much as you'd like and you only compile/update when you specify.

### D.10.1 Steps to Get Writing

Below are the steps you will need to take to get your system working:

1. Enable the Linux subsystem for Windows ([Figure D.1](#))
2. Download the Ubuntu application from the Microsoft App store ([Figure D.2](#))
3. Create a [GitLab](#) account to pull files. (You will learn how to do version control once you get your Thesis/Dissertation setup). There is a fabulous link to learn the basics of Git: <https://docs.gitlab.com/ee/gitlab-basics/start-using-git.html>
4. Initialize Ubuntu on your local machine and establish the connection to your own repository

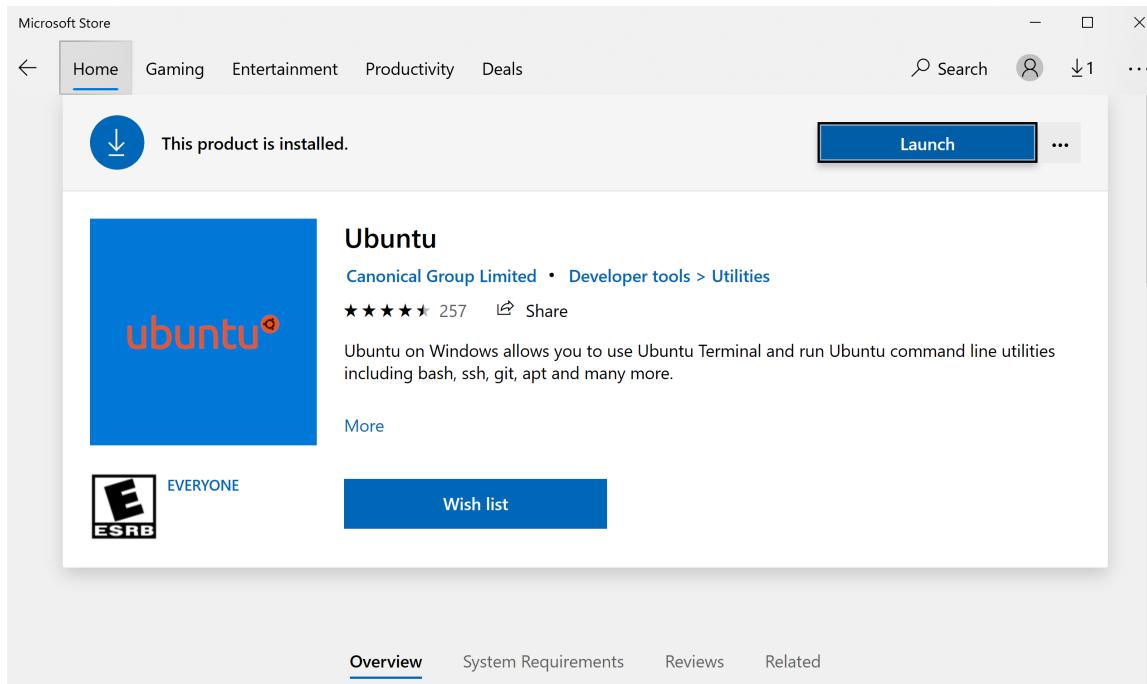


Figure D.2: Download Ubuntu

5. Clone the Thesis/Dissertation template to your local machine

```
git clone https://gitlab.com/SkiEngineer/uofuthesistemplate.git
```

6. Copy the files to your own Thesis/Dissertation folder on your local machine in the subsystem directory.
7. Add the new folder containing your Thesis/Dissertation to your own repository on GitLab

- (a) `git add New_Folder_Directory`
- (b) `git commit -m "Initial Commit"`
- (c) `git push`

#### **D.10.2 Required Ubuntu installations. Use a bash terminal to execute the following commands**

1. `sudo apt install texlive-latex-extra` → **LATEX** - is a document writing system
2. `sudo apt-get install build-essential` → The Linux make command is used to build and maintain groups of programs and files from the source code. In Linux, it is

```
kiffer@LAPTOP-9P769330: ~/uofuthesistemplate/thesis
kiffer@LAPTOP-9P769330:~/uofuthesistemplate/thesis$ make
```

Figure D.3: Screen snip of `Makefile` command to compile the Thesis/Dissertation.

one of the most frequently used commands by the developers. It assists developers to install and compile many utilities from the terminal.

3. `sudo apt-get install -y latexmk` —→ *Latexmk* completely automates the process of compiling a  $\text{\LaTeX}$  document. Essentially, it is like a specialized relative of the general make utility, but one which determines dependencies automatically and has some other very useful features. In its basic mode of operation latexmk is given the name of the primary source file for a document, and it issues the appropriate sequence of commands to generate a .dvi, .ps, .pdf and/or hardcopy version of the document.

### D.10.3 Editing Methods For Your Thesis/Dissertation

There are two ways you can edit the text of your document

1. Use [Notepad++](#)
2. Use `vi` via the command line interface (*Increased efficiency* - Your fingers will never leave the keyboard)
3. Add all images/figures to the `media` folder within each chapter

### D.10.4 How To Make Changes

Individual `makefiles` have been created to execute the necessary commands to compile your Thesis/Dissertation.

The basic commands inside of the `Ubuntu` terminal are:

- “`make`” —→ compiles the document. The associated screen snip in the Bash terminal is seen in [Figure D.3](#) to compile the Thesis/Dissertation.
- “`make release`” —→ cleans up the unnecessary files and moves the final PDF into the folder titled “Release”. To move the final PDF into the “Release” folder, simply

---

```

● kiffer@LAPTOP-9P769330: ~/uofuthesistemplate/thesis
kiffer@LAPTOP-9P769330:~/uofuthesistemplate/thesis$ make release

```

Figure D.4: Screen snip of Makefile command to clean up the file directory and move the PDF to the “Release” folder.

type “`make release`” and the PDF will be moved and the following L<sup>A</sup>T<sub>E</sub>X compilation documents will be deleted from the folder. This is seen in [Figure D.4](#).

- “`make clean`”
- “`make clear`”

#### D.10.4.1 Sample Makefiles

The following Makefile is used to compile the Thesis/Dissertation:

*</>      Script 28: ... L<sup>A</sup>T<sub>E</sub>X script used to compile the Thesis/Dissertation.      </>*

```

1 # Linux Makefile to create a final pdf of the project
2
3 # Variables
4 FILE = Dissertation
5
6 # PDF LaTeX specific
7 TEX = "pdflatex -interaction=nonstopmode -synctex=1 --shell-escape"
8
9 # You want latexmk to *always* run, because make does not have all the info.
10 # Also, include non-file targets in .PHONY so they are run regardless of any
11 # file of the given name existing.
12 .PHONY: ${FILE}.pdf all clean
13
14 # The first rule in a Makefile is the one executed by default ("make"). It
15 # should always be the "all" rule, so that "make" and "make all" are identical.
16 all: ${FILE}.pdf
17
18 # CUSTOM BUILD RULES
19
20 # In case you didn't know, '$@' is a variable holding the name of the target,
21 # and '$<' is a variable holding the (first) dependency of a rule.
22 # "raw2tex" and "dat2tex" are just placeholders for whatever custom steps
23 # you might have.
24
25 %.tex: %.raw
26     ./raw2tex $< > $@
27
28 %.tex: %.dat
29     ./dat2tex $< > $@
30
31 # MAIN LATEXMK RULE
32

```

```

33 # -pdf tells latexmk to generate PDF directly (instead of DVI).
34 # -pdflatex="" tells latexmk to call a specific backend with specific options.
35 # -use-make tells latexmk to call make for generating missing files.
36
37 # -interaction=nonstopmode keeps the pdflatex backend from stopping at a
38 # missing file reference and interactively asking you for an alternative.
39
40 # --shell-escape allows for *minted to run code highlighting
41
42 # -f forces latexmk to run until compiling has been complete regardless of
43 # cross referencing (i.e. it continues to run until references are in the
44 # correct location)
45
46 ${FILE}.pdf: ${FILE}.tex
47     latexmk -pdf -pdflatex=${TEX} -f -use-make ${FILE}.tex
48
49 # Clean up unnecessary files
50 clean:
51     latexmk -C
52     # specific to latexmk
53
54 clear:
55     rm -rf auto *_minted-* *.log *.aux *.synctex.gz *.out *.toc *.run *.bcf *.lof
56     ↳ *.lot *.tdo *.run.xml *.pdf *.bbl *.blk *.swp
57
58 release:
59     rm -rf Release # Once the folder is created, no need to create a new one
60     mkdir Release # Once the folder is created, no need to create a new one
61     cp *.pdf Release
62     make clear
63     make clean

```

The following Makefile is used to compile Chapter 1 of the Thesis/Dissertation:

**Script 29:** ... *LAT<sub>E</sub>X script used to compile Chapter1.*

```
1 # Linux Makefile to create a final pdf of the project
2
3 # Variables
4 CHAPTER = Chapter1
5 OVERVIEW = 0_Overview
6 BACKGROUND = 1_Background
7 AIMS = 2_Aims
8 IMPACT = 3_Impact
9
10 # PDF LaTeX specific
11 TEX = "pdflatex -interaction=nonstopmode -synctex=1 --shell-escape"
12
13 # You want latexm to *always* run, because make does not have all the info.
14 # Also, include non-file targets in .PHONY so they are run regardless of any
15 # file of the given name existing.
16 .PHONY: ${CHAPTER}.pdf all clean
17
18 # The first rule in a Makefile is the one executed by default ("make"). It
19 # should always be the "all" rule, so that "make" and "make all" are identical.
20 all: ${CHAPTER}.pdf
21
```

```

22 # CUSTOM BUILD RULES
23
24 # In case you didn't know, '$@' is a variable holding the name of the target,
25 # and '$<' is a variable holding the (first) dependency of a rule.
26 # "raw2tex" and "dat2tex" are just placeholders for whatever custom steps
27 # you might have.
28
29 %.tex: %.raw
30     ./raw2tex $< > $@
31
32 %.tex: %.dat
33     ./dat2tex $< > $@
34
35 chapter: ${CHAPTER}.pdf
36 overview: ${OVERVIEW}.pdf
37 background: ${BACKGROUND}.pdf
38 aims: ${AIMS}.pdf
39 impact: ${IMPACT}.pdf
40
41 # MAIN LATEXMK RULE
42
43 # -pdf tells latexmk to generate PDF directly (instead of DVI).
44 # -pdflatex="" tells latexmk to call a specific backend with specific options.
45 # -use-make tells latexmk to call make for generating missing files.
46
47 # -interaction=nonstopmode keeps the pdflatex backend from stopping at a
48 # missing file reference and interactively asking you for an alternative.
49
50 # --shell-escape allows for *minted to run code highlighting
51
52 # -f forces latexmk to run until compiling has been complete regardless of
53 # cross referencing (i.e. it continues to run until references are in the
54 # correct location)
55
56 ${CHAPTER}.pdf: ${CHAPTER}.tex
57     latexmk -pdf -pdflatex=${TEX} -f -use-make ${CHAPTER}.tex
58
59 ${OVERVIEW}.pdf: ${OVERVIEW}.tex
60     latexmk -pdf -pdflatex=${TEX} -f -use-make ${OVERVIEW}.tex
61
62 ${BACKGROUND}.pdf: ${BACKGROUND}.tex
63     latexmk -pdf -pdflatex=${TEX} -f -use-make ${BACKGROUND}.tex
64
65 ${AIMS}.pdf: ${AIMS}.tex
66     latexmk -pdf -pdflatex=${TEX} -f -use-make ${AIMS}.tex
67
68 ${IMPACT}.pdf: ${IMPACT}.tex
69     latexmk -pdf -pdflatex=${TEX} -f -use-make ${IMPACT}.tex
70
71
72 # Clean up unnecessary files
73 clean:
74     latexmk -C
75
76 clear:
77     rm -rf auto *_minted-* *.log *.aux *.synctex.gz *.out *.toc *.run *.bcf *.lof
    ↳ *.lot *.tdo *.run.xml *.pdf *.bbl *.blg *.swp
78

```

```

79 release:
80         # rm -rf Release # Once the folder is created, no need to create a new one
81         # mkdir Release # Once the folder is created, no need to create a new one
82         cp *.pdf Release
83         make clear
84         make clean

```

The following Makefile is used to compile Chapter 2 of the Thesis/Dissertation:

Script 30: ...*LATEX* script used to compile Chapter2.

```

</>                               Script 30: ...LATEX script used to compile Chapter2.                               </>
1 # Linux Makefile to create a final pdf of the project
2
3 # Variables
4 CHAPTER = Chapter2
5 ABSTRACT = 0_Abstract
6 INTRODUCTION = 1_Introduction
7 METHODS = 2_Methods
8 RESULTS = 3_Results
9 DISCUSSION = 4_Discussion
10 CONCLUSION = 5_Conclusion
11 ACKNOWLEDGE = 6_Acknowledgment
12
13 # PDF LaTeX specific
14 TEX = "pdflatex -interaction=nonstopmode -synctex=1 --shell-escape"
15
16 # You want latexmk to *always* run, because make does not have all the info.
17 # Also, include non-file targets in .PHONY so they are run regardless of any
18 # file of the given name existing.
19 .PHONY: ${CHAPTER}.pdf all clean
20
21 # The first rule in a Makefile is the one executed by default ("make"). It
22 # should always be the "all" rule, so that "make" and "make all" are identical.
23 all: ${CHAPTER}.pdf
24
25 # CUSTOM BUILD RULES
26
27 # In case you didn't know, '$@' is a variable holding the name of the target,
28 # and '$<' is a variable holding the (first) dependency of a rule.
29 # "raw2tex" and "dat2tex" are just placeholders for whatever custom steps
30 # you might have.
31
32 %.tex: %.raw
33     ./raw2tex $< > $@
34
35 %.tex: %.dat
36     ./dat2tex $< > $@
37
38 chapter: ${CHAPTER}.pdf
39 abstract: ${ABSTRACT}.pdf
40 introduction: ${INTRODUCTION}.pdf
41 methods: ${METHODS}.pdf
42 results: ${RESULTS}.pdf
43 discussion: ${DISCUSSION}.pdf
44 conclusion: ${CONCLUSION}.pdf
45 acknowledge: ${ACKNOWLEDGE}.pdf
46

```

```

47 # MAIN LATEXMK RULE
48
49 # -pdf tells latexmk to generate PDF directly (instead of DVI).
50 # -pdflatex="" tells latexmk to call a specific backend with specific options.
51 # -use-make tells latexmk to call make for generating missing files.
52
53 # -interaction=nonstopmode keeps the pdflatex backend from stopping at a
54 # missing file reference and interactively asking you for an alternative.
55
56 # --shell-escape allows for *minted to run code highlighting
57
58 # -f forces latexmk to run until compiling has been complete regardless of
59 # cross referencing (i.e. it continues to run until references are in the
60 # correct location)
61
62 ${CHAPTER}.pdf: ${CHAPTER}.tex
63     latexmk -pdf -pdflatex=${TEX} -f -use-make ${CHAPTER}.tex
64
65 ${ABSTRACT}.pdf: ${ABSTRACT}.tex
66     latexmk -pdf -pdflatex=${TEX} -f -use-make ${ABSTRACT}.tex
67
68 ${INTRODUCTION}.pdf: ${INTRODUCTION}.tex
69     latexmk -pdf -pdflatex=${TEX} -f -use-make ${INTRODUCTION}.tex
70
71 ${METHODS}.pdf: ${METHODS}.tex
72     latexmk -pdf -pdflatex=${TEX} -f -use-make ${METHODS}.tex
73
74 ${RESULTS}.pdf: ${RESULTS}.tex
75     latexmk -pdf -pdflatex=${TEX} -f -use-make ${RESULTS}.tex
76
77 ${DISCUSSION}.pdf: ${DISCUSSION}.tex
78     latexmk -pdf -pdflatex=${TEX} -f -use-make ${DISCUSSION}.tex
79
80 ${CONCLUSION}.pdf: ${CONCLUSION}.tex
81     latexmk -pdf -pdflatex=${TEX} -f -use-make ${CONCLUSION}.tex
82
83 ${ACKNOWLEDGE}.pdf: ${ACKNOWLEDGE}.tex
84     latexmk -pdf -pdflatex=${TEX} -f -use-make ${ACKNOWLEDGE}.tex
85
86 # Clean up unnecessary files
87 clean:
88     latexmk -C
89
90 clear:
91     rm -rf auto *_minted-* *.log *.aux *.synctex.gz *.out *.toc *.run *.bcf *.lof
92     ↳ *.lot *.tdo *.run.xml *.pdf *.bb1 *.blg *.swp
93 release:
94     # rm -rf Release # Once the folder is created, no need to create a new one
95     # mkdir Release # Once the folder is created, no need to create a new one
96     cp *.pdf Release
97     make clear
98     make clean

```

The variables in each Makefile are the L<sup>A</sup>T<sub>E</sub>X file names and you can simply type “`make methods`” to compile the methods section of a particular chapter. The same is true

for “`make abstract`”, “`make results`”, and “`make discussion`” which will compile the abstract, results, and discussion section, respectively. This is useful for editing sections of the document where you do not necessarily need to compile the entire document.