

# Scaling Up 3D Imaging, Analysis, and Culture of Complex Brain Models

Justin M. Swaney

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Supervised by:  
Professor Kwanghun Chung  
Department of Chemical Engineering

Massachusetts Institute of Technology  
Cambridge, MA  
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*I, Justin M. Swaney, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.*

# Abstract

The brain is the most complex human organ, containing components from the nanometer scale to the centimeter scale, such as synapses, neurons, and brain regions. However, many experimental techniques in imaging, analysis, and tissue culture have been optimized for smaller brain models. This thesis summarizes a body of work aimed at scaling up imaging, analysis, and tissue culture techniques for large-scale brain models. Fluorescence imaging of large-scale models is limited by diffusion of molecular probes and the resolution of long working distance objectives. We present a technique termed SWITCH that inhibits probe binding to allow for diffusion without the formation of a reaction front. To improve imaging resolution, we present a tissue expansion technique called MAP that physically magnifies tissue samples for super-resolution imaging. We demonstrate a 4-fold increase in effective resolution using long working distance objectives and show that the tissue distortion through expansion is comparable to the deformation that occurs during handling. Volumetric imaging of large-scale models generates petabyte scale data, for which we present horizontally scalable image processing pipelines for analysis of intact mouse brains and cerebral organoids. The mouse brain pipeline allows region-based statistical analysis of protein expression and cell counts. The cerebral organoid pipeline allows single-cell, cytoarchitectural, and morphological analyses to be combined into a hyper-dimensional statistical analysis. We use this pipeline to show phenotypic changes during development. In order to overcome limitations in multicolor imaging, we also present a 3D image coregistration pipeline capable of aligning multiple rounds of staining of the same whole-brain sample at single-cell resolution. We show that the average distance between corresponding nuclei after coregistration is less than 4 um. Finally, large-scale tissue cultures are

limited by nutrient transport since they lack a vascular system. To address this issue *in vitro*, we fabricated synthetic vasculature by two-photon photopolymerization of polyethylene glycol based resins. Printed micro-vessels were 100 um in outer diameter, durable yet flexible, and permeable to biomolecules in a tunable manner. Perfusion of vascularized cerebral organoids cultured for 30 days resulted in the expected neuronal differentiation as well as integration of the vascular network. Future studies can use and build on these technical advances to further our understanding of the brain through the use of large-scale brain models.

# Acknowledgements

I would like to acknowledge all of my colleagues at MIT for their continued support throughout various research projects. We accomplished a great deal and learned even more together. I have no doubt that those individuals that I have had the privilege to work with at MIT will continue to impact the world in positive ways.

I am grateful for my fiancé, Kassi, who has been with me side-by-side as an MIT PhD student since the beginning. We made it through grad school together, and I'm excited to share our hardships and victories in the future.

Of course, this work never would have been possible without my family. My brother, Jason, and his wife, Shanon, always gave me a place to stay whenever I went home to Wisconsin during my PhD. My sister, Amy, and neice, Lexi, also welcomed me home when I visited. My dad, Rich, has supported me throughout my entire life and academic career.

Lastly, I'd like to dedicate this thesis to my mother, Margaret Swaney, to whom I owe everything. She has been and will always be my guiding light.

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# Abbreviations

<b>API</b>	Application Programming Interface
<b>JSON</b>	JavaScript Object Notation

# Chapter 1

## Introduction, with a citation

### 1.1 Background

This is the introduction. Quisque finibus aliquet cursus. Integer in pellen-tesque tellus. Duis eu dignissim nulla, a porttitor enim. Quisque vehicula leo non ultrices finibus. Duis vehicula quis sem sit amet sollicitudin. Integer neque est, pharetra et auctor vel, iaculis interdum lectus.

To include a citation to the text, just add the citation key shown in the references.bib file. The style of the citation is determined by the ref\_format.csl file. For example, in The Living Sea you can find pictures of the Calypso (Cousteau Jacques & Dugan James 1963).

In neque mauris, maximus at sapien a, iaculis dignissim justo. Aliquam erat volutpat. Praesent varius risus auctor est ultricies, sit amet consequat nisi laoreet. Suspendisse non est et mauris pharetra sagittis non porta justo. Praesent malesuada metus ut sapien sodales ornare.

### 1.2 The middle bit

This is the middle bit. Phasellus quis ex in ipsum pellentesque lobortis tincidunt sed massa. Nullam euismod sem quis dictum condimentum. Sus-

pendisse risus metus, elementum eu congue quis, viverra ac metus. Donec non lectus at lectus euismod laoreet pharetra semper dui. Donec sed eleifend erat, vel ultrices nibh. Nam scelerisque turpis ac nunc mollis, et rutrum nisl luctus.

Duis faucibus vestibulum elit, sit amet lobortis libero. Class aptent taciti sociosqu ad litora torquent per conubia nostra, per inceptos himenaeos. Sed at cursus nibh. Sed accumsan imperdiet interdum. Proin id facilisis tortor. Proin posuere a neque nec iaculis. Suspendisse potenti. Nullam hendrerit ante mi, vitae iaculis dui laoreet eu.

Cras eleifend velit diam, eu viverra mi volutpat ut. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Donec finibus leo nec dui imperdiet, tincidunt ornare orci venenatis. Maecenas placerat efficitur est, eu blandit magna hendrerit eu.

### 1.2.1 SUBSECTION OF THE MIDDLE BIT

This is a subsection of the middle bit. Quisque sit amet tempus arcu, ac suscipit ante. Cras massa elit, pellentesque eget nisl ut, malesuada rutrum risus. Nunc in venenatis mi. Curabitur sit amet suscipit eros, non tincidunt nibh. Phasellus lorem lectus, iaculis non luctus eget, tempus non risus. Suspendisse ut felis mi.

## 1.3 Summary of chapters

This is a brief outline of what went into each chapter. **Chapter 1** gives a background on duis tempus justo quis arcu consectetur sollicitudin. **Chapter 2** discusses morbi sollicitudin gravida tellus in maximus. **Chapter 3** discusses vestibulum eleifend turpis id turpis sollicitudin aliquet. **Chapter 4** shows how phasellus gravida non ex id aliquet. Proin faucibus nibh sit amet augue blandit varius.

# Chapter 2

## Literature review, with maths

### 2.1 Introduction

This is the introduction. Duis in neque felis. In hac habitasse platea dictumst. Cras eget rutrum elit. Pellentesque tristique venenatis pellentesque. Cras eu dignissim quam, vel sodales felis. Vestibulum efficitur justo a nibh cursus eleifend. Integer ultrices lorem at nunc efficitur lobortis.

### 2.2 The middle

This is the literature review. Nullam quam odio, volutpat ac ornare quis, vestibulum nec nulla. Aenean nec dapibus in mL/min<sup>-1</sup>. Mathematical formula can be inserted using Latex:

$$(1) \quad f(x) = ax^3 + bx^2 + cx + d$$

Nunc eleifend, ex a luctus porttitor, felis ex suscipit tellus, ut sollicitudin sapien purus in libero. Nulla blandit eget urna vel tempus. Praesent fringilla dui sapien, sit amet egestas leo sollicitudin at.

Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Sed faucibus pulvinar volutpat. Ut semper fringilla erat

non dapibus. Nunc vitae felis eget purus placerat finibus laoreet ut nibh.

## 2.3 Conclusion

This is the conclusion. Donec pulvinar molestie urna eu faucibus. In tristique ut neque vel eleifend. Morbi ut massa vitae diam gravida iaculis. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas.

- first item in the list
- second item in the list
- third item in the list

# Chapter 3

## First research study, with code

### 3.1 Introduction

This is the introduction. Nam mollis congue tortor, sit amet convallis tortor mollis eget. Fusce viverra ut magna eu sagittis. Vestibulum at ultrices sapien, at elementum urna. Nam a blandit leo, non lobortis quam. Aliquam feugiat turpis vitae tincidunt ultricies. Mauris ullamcorper pellentesque nisl, vel molestie lorem viverra at.

### 3.2 Method

Suspendisse iaculis in lacus ut dignissim. Cras dignissim dictum eleifend. Suspendisse potenti. Suspendisse et nisi suscipit, vestibulum est at, maximus sapien. Sed ut diam tortor.

#### 3.2.1 SUBSECTION 1 WITH EXAMPLE CODE BLOCK

This is the first part of the methodology. Cras porta dui a dolor tincidunt placerat. Cras scelerisque sem et malesuada vestibulum. Vivamus faucibus ligula ac sodales consectetur. Aliquam vel tristique nisl. Aliquam erat volutpat. Pellentesque iaculis enim sit amet posuere facilisis. Integer egestas

quam sit amet nunc maximus, id bibendum ex blandit.

For syntax highlighting in code blocks, add three ““” characters before and after a code block:

```
mood = 'happy'  
if mood == 'happy':  
    print("I am a happy robot")
```

Alternatively, you can also use LaTeX to create a code block as shown in the Java example below:

Listing 3.1: Main.java

```
1 /**
2 * Hello, world – example in Java.
3 */
4 public class Main{
5     // says hello to the world
6     public static void main(String[] args) {
7         System.out.println("Hello, world!");
8     }
9 }
```

If you use `javaCodeStyle` as defined in the `preamble.tex`, it is best to keep the maximum line length in the source code at 80 characters.

### 3.2.2 SUBSECTION 2

This is the second part of the methodology. Proin tincidunt odio non sem mollis tristique. Fusce pharetra accumsan volutpat. In nec mauris vel orci rutrum dapibus nec ac nibh. Praesent malesuada sagittis nulla, eget commodo mauris ultricies eget. Suspendisse iaculis finibus ligula.

## 3.3 Results

These are the results. Ut accumsan tempus aliquam. Sed massa ex, egestas non libero id, imperdiet scelerisque augue. Duis rutrum ultrices arcu et

ultricies. Proin vel elit eu magna mattis vehicula. Sed ex erat, fringilla vel feugiat ut, fringilla non diam.

### 3.4 Discussion

This is the discussion. Duis ultrices tempor sem vitae convallis. Pellentesque lobortis risus ac nisi varius bibendum. Phasellus volutpat aliquam varius. Mauris vitae neque quis libero volutpat finibus. Nunc diam metus, imperdiet vitae leo sed, varius posuere orci.

### 3.5 Conclusion

This is the conclusion to the chapter. Praesent bibendum urna orci, a venenatis tellus venenatis at. Etiam ornare, est sed lacinia elementum, lectus diam tempor leo, sit amet elementum ex elit id ex. Ut ac viverra turpis. Quisque in nisl auctor, ornare dui ac, consequat tellus.

# Chapter 4

## Research containing a figure

### 4.1 Introduction

This is the introduction. Sed vulputate tortor at nisl blandit interdum. Cras sagittis massa ex, quis eleifend purus condimentum congue. Maecenas tristique, justo vitae efficitur mollis, mi nulla varius elit, in consequat ligula nulla ut augue. Phasellus diam sapien, placerat sit amet tempor non, lobortis tempus ante.

### 4.2 Method

Donec imperdiet, lectus vestibulum sagittis tempus, turpis dolor euismod justo, vel tempus neque libero sit amet tortor. Nam cursus commodo tincidunt.

#### 4.2.1 SUBSECTION 1

This is the first part of the methodology. Duis tempor sapien sed tellus ultrices blandit. Sed porta mauris tortor, eu vulputate arcu dapibus ac. Curabitur sodales at felis efficitur sollicitudin. Quisque at neque sollicitudin, mollis arcu vitae, faucibus tellus.

### 4.2.2 SUBSECTION 2

This is the second part of the methodology. Sed ut ipsum ultrices, interdum ipsum vel, lobortis diam. Curabitur sit amet massa quis tortor molestie dapibus a at libero. Mauris mollis magna quis ante vulputate consequat. Integer leo turpis, suscipit ac venenatis pellentesque, efficitur non sem. Pellentesque eget vulputate turpis. Etiam id nibh at elit fermentum interdum.

## 4.3 Results

These are the results. In vitae odio at libero elementum fermentum vel iaculis enim. Nullam finibus sapien in congue condimentum. Curabitur et ligula et ipsum mollis fringilla.

## 4.4 Discussion

Figure 4.1 shows how to add a figure. Donec ut lacinia nibh. Nam tincidunt augue et tristique cursus. Vestibulum sagittis odio nisl, a malesuada turpis blandit quis. Cras ultrices metus tempor laoreet sodales. Nam molestie ipsum ac imperdiet laoreet. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas.

## 4.5 Conclusion

This is the conclusion to the chapter. Quisque nec purus a quam consectetur volutpat. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. In lorem justo, convallis quis lacinia eget, laoreet eu metus. Fusce blandit tellus tellus. Curabitur nec cursus odio. Quisque tristique eros nulla, vitae finibus lorem aliquam quis. Interdum et malesuada fames ac ante ipsum primis in faucibus.



Figure 4.1: RV Calypso is a former British Royal Navy minesweeper converted into a research vessel for the oceanographic researcher Jacques-Yves Cousteau. It was equipped with a mobile laboratory for underwater field research.

# Chapter 5

## Research containing a table

### 5.1 Introduction

This is the introduction. Phasellus non purus id mauris aliquam rutrum vitae quis tellus. Maecenas rhoncus ligula nulla, fringilla placerat mi consectetur eu. Aenean nec metus ac est ornare posuere. Nunc ipsum lacus, gravida commodo turpis quis, rutrum eleifend erat. Pellentesque id lorem eget ante porta tincidunt nec nec tellus.

### 5.2 Method

Vivamus consectetur, velit in congue lobortis, massa massa lacinia urna, sollicitudin semper ipsum augue quis tortor. Donec quis nisl at arcu volutpat ultrices. Maecenas ex nibh, consequat ac blandit sit amet, molestie in odio. Morbi finibus libero et nisl dignissim, at ultricies ligula pulvinar.

#### 5.2.1 SUBSECTION 1

This is the first part of the methodology. Integer leo erat, commodo in lacus vel, egestas varius elit. Nulla eget magna quam. Nullam sollicitudin dolor ut ipsum varius tincidunt. Duis dignissim massa in ipsum accumsan imperdiet.

Maecenas suscipit sapien sed dui pharetra blandit. Morbi fermentum est vel quam pretium maximus.

### 5.2.2 SUBSECTION 2

This is the second part of the methodology. Nullam accumsan condimentum eros eu volutpat. Maecenas quis ligula tempor, interdum ante sit amet, aliquet sem. Fusce tellus massa, blandit id tempus at, cursus in tortor. Nunc nec volutpat ante. Phasellus dignissim ut lectus quis porta. Lorem ipsum dolor sit amet, consectetur adipiscing elit.

## 5.3 Results

Table 5.1 shows us how to add a table. Integer tincidunt sed nisl eget pellentesque. Mauris eleifend, nisl non lobortis fringilla, sapien eros aliquet orci, vitae pretium massa neque eu turpis. Pellentesque tincidunt aliquet volutpat. Ut ornare dui id ex sodales laoreet.

Table 5.1: This is the table caption. Suspendisse blandit dolor sed tellus venenatis, venenatis fringilla turpis pretium.

Column 1	Column 2	Column 3
Row 1	0.1	0.2
Row 2	0.3	0.3
Row 3	0.4	0.4
Row 4	0.5	0.6

## 5.4 Discussion

This is the discussion. Etiam sit amet mi eros. Donec vel nisi sed purus gravida fermentum at quis odio. Vestibulum quis nisl sit amet justo maximus molestie. Maecenas vitae arcu erat. Nulla facilisi. Nam pretium mauris eu enim porttitor, a mattis velit dictum. Nulla sit amet ligula non mauris volutpat fermentum quis vitae sapien.

## 5.5 Conclusion

This is the conclusion to the chapter. Nullam porta tortor id vehicula interdum. Quisque pharetra, neque ut accumsan suscipit, orci orci commodo tortor, ac finibus est turpis eget justo. Cras sodales nibh nec mauris laoreet iaculis. Morbi volutpat orci felis, id condimentum nulla suscipit eu. Fusce in turpis quis ligula tempus scelerisque eget quis odio. Vestibulum et dolor id erat lobortis ullamcorper quis at sem.

# Chapter 6

## Final research study

### 6.1 Introduction

This is the introduction. Nunc lorem odio, laoreet eu turpis at, condimentum sagittis diam. Phasellus metus ligula, auctor ac nunc vel, molestie mattis libero. Praesent id posuere ex, vel efficitur nibh. Quisque vestibulum accumsan lacus vitae mattis.

### 6.2 Method

In tincidunt viverra dolor, ac pharetra tellus faucibus eget. Pellentesque tempor a enim nec venenatis. Morbi blandit magna imperdiet posuere auctor. Maecenas in maximus est.

#### 6.2.1 SUBSECTION 1

This is the first part of the methodology. Praesent mollis sem diam, sit amet tristique lacus vulputate quis. Vivamus rhoncus est rhoncus tellus lacinia, a interdum sem egestas. Curabitur quis urna vel quam blandit semper vitae a leo. Nam vel lectus lectus.

### 6.2.2 SUBSECTION 2

This is the second part of the methodology. Aenean vel pretium tortor. Aliquam erat volutpat. Quisque quis lobortis mi. Nulla turpis leo, ultrices nec nulla non, ullamcorper laoreet risus.

## 6.3 Results

These are the results. Curabitur vulputate nisl non ante tincidunt tempor. Aenean porta nisi quam, sed ornare urna congue sed. Curabitur in sapien justo. Quisque pulvinar ullamcorper metus, eu varius mauris pellentesque et. In hac habitasse platea dictumst. Pellentesque nec porttitor libero. Duis et magna a massa lacinia cursus.

## 6.4 Discussion

This is the discussion. Curabitur gravida nisl id gravida congue. Duis est nisi, sagittis eget accumsan ullamcorper, semper quis turpis. Mauris ultricies diam metus, sollicitudin ultricies turpis lobortis vitae. Ut egestas vehicula enim, porta molestie neque consectetur placerat. Integer iaculis sapien dolor, non porta nibh condimentum ut.

## 6.5 Conclusion

This is the conclusion to the chapter. Nulla sed condimentum lectus. Duis sed tempor erat, at cursus lacus. Nam vitae tempus arcu, id vestibulum sapien. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus.

# Chapter 7

## Conclusion

### 7.1 Thesis summary

In summary, pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Nunc eleifend, ex a luctus porttitor, felis ex suscipit tellus, ut sollicitudin sapien purus in libero. Nulla blandit eget urna vel tempus. Praesent fringilla dui sapien, sit amet egestas leo sollicitudin at.

### 7.2 Future work

There are several potential directions for extending this thesis. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Aliquam gravida ipsum at tempor tincidunt. Aliquam ligula nisl, blandit et dui eu, eleifend tempus nibh. Nullam eleifend sapien eget ante hendrerit commodo. Pellentesque pharetra erat sit amet dapibus scelerisque.

Vestibulum suscipit tellus risus, faucibus vulputate orci lobortis eget. Nunc varius sem nisi. Nunc tempor magna sapien, euismod blandit elit pharetra sed. In dapibus magna convallis lectus sodales, a consequat sem euismod. Curabitur in interdum purus. Integer ultrices laoreet aliquet. Nulla vel dapibus urna. Nunc efficitur erat ac nisi auctor sodales.

## Appendix 1: Some extra stuff

Add appendix 1 here. Vivamus hendrerit rhoncus interdum. Sed ullamcorper et augue at porta. Suspendisse facilisis imperdiet urna, eu pellentesque purus suscipit in. Integer dignissim mattis ex aliquam blandit. Curabitur lobortis quam varius turpis ultrices egestas.

## **Appendix 2: Some more extra stuff**

Add appendix 2 here. Aliquam rhoncus mauris ac neque imperdiet, in mattis eros aliquam. Etiam sed massa et risus posuere rutrum vel et mauris. Integer id mauris sed arcu venenatis finibus. Etiam nec hendrerit purus, sed cursus nunc. Pellentesque ac luctus magna. Aenean non posuere enim, nec hendrerit lacus. Etiam lacinia facilisis tempor. Aenean dictum nunc id felis rhoncus aliquam.

# References

Cousteau Jacques & Dugan James, 1963. *The Living Sea: by Jacques-Yves Cousteau*, London: Hamish Hamilton.