SRTP Project Description
Summer 2015

Name of Supervisor: Martin Sandig and Timothy D. Wilson
Department: Anatomy and Cell Biology
Office Address: MSB472
Email: martin.sandig@schulich.uwo.ca
Phone: X86815
Supervisor’s homepage (if available): www.anatatorium.com

Project Title:
Three-dimensional visualization of stromal and immune cells within secondary lymphoid organs for research and teaching.

Project Description – include background, hypothesis, proposed methodology, and expected outcomes (one page maximum; you may attach a page to this form):

Please see attached page

Research Environment - Description of the number of research personnel, size of lab, etc.:

Dr. Sandig’s lab is located in the Department of Anatomy and Cell Biology, room M475. It contains all necessary reagents and equipment required for the project. Two Zeiss confocal microscopes and expertise for training is available within the department. One or two Clinical Anatomy graduate students will be working on similar projects in the lab over the two summers. The primary supervisor, Dr. Sandig, will spend his upcoming entire sabbatical year 2015-2016 in the lab working alongside the students to further develop technology in the field of 3D histology and tissue visualization. Dr. Wilson’s lab, the Corps for Research of Instructional and Perceptional Technologies (CRIPT), will provide ample computing and visualization equipment, and expertise. The SRTP student will thus be embedded in a rich learning environment supported by faculty and senior graduate students alike. Weekly lab meetings will monitor progress and will give opportunity for presentations and scientific exchange.

Expected Objectives/Accomplishments for Student for Year 1:
The student will be expected to become familiar with the techniques of optical clearing, immunocytochemistry and confocal microscopy.
1) The student will prepare and incubate mouse spleens and lymph nodes according to the CLARITY protocol.
2) The student will be staining tissues with antibodies to visualize specific cells within the lymphoid organs by double and triple immunocytochemistry.
3) The student will learn laser scanning confocal microscopy.
4) The student will image the 3D architecture of reticular cells and lymphocytes in the lymphoid follicles.
Due to the lengthy periods of times (up to 4 weeks) that tissues need to spend incubating in clearing solutions, the student will practice immunostaining and microscopy on other tissues, such as the kidney, that have been optically cleared previously by other students in the lab.

**Expected Objectives/Accomplishments for Student for Year 2:**
Depending on the progress and outcomes of the project in year one, the student will perfect and complete confocal imaging of optically cleared tissue and generate a digital 3D learning tool.
1) The student will import image stacks generated by confocal microscopy into Amira software to generate 3D visualizations of the imaged structures.
2) The student will design a digital learning tool using unity software that can be used to study the cellular organization of lymphoid follicles. This e-learning tool should be interactive and customizable for learners at various educational levels.

A future project will be using the digital e-learning tool to test its efficacy in different learning environments.

**If REB approval is required for this project, please provide REB Number: ____
OR provide the status of the application for REB approval:**

*Note: REB approval should be obtained prior to the start of Summer 2015.*

Please submit complete application form, together with an abbreviated CV (not more than 4 pages – please do not include your entire CV) to Stacey Bastien at srop-srtp@schulich.uwo.ca

**Deadline for submissions: Monday, December 8, 2014.**
Project Description

Background
Three-dimensional (3D) imaging and visualization of anatomical structure has become indispensable in research, education, and clinical practice. However, visualization of anatomical structure at the cellular and microscopic level still relies heavily on examination of 2D images from histological sections cut through complex multicellular tissues and organs. Particularly in organs where many different cell types interact in spatially well-defined but complex compartments, such as the kidney or secondary lymphatic organs, visualization of the 3D spatial arrangement of cells within the tissue is necessary for an understanding of normal and pathological structure/function relationships. For e-learning purposes we have recently used serial histological sections and Amira software to reconstruct the renal corpuscle in 3D. This proved to be a time-consuming and labor-intensive enterprise. To overcome these limitations we have recently imaged an entire renal glomerulus in 3D without the use of histological sectioning by employing a novel optical clearing method, called CLARITY. This method renders entire organs optically transparent by crosslinking proteins in situ within a hydrogel and removing lipids with detergents. This has been successfully used for 3D visualization of connections within the entire brain by confocal microscopy following immunofluorescence staining of particular antigens and cells.

In the secondary lymphatic organs stromal reticular cells provide a physical and molecular framework that is essential in establishing biochemical niches for lymphocyte interactions and activation by antigen. The 3D architecture of these cellular interactions is largely unknown and cannot easily be determined using reconstruction by serial sections and histological staining. We are therefore proposing to examine in 3D at high resolution the extensive meshwork of reticular cells and their relationships with lymphocytes in lymphoid nodules within lymph nodes. We hypothesize that optical clearing with CLARITY and immunostaining of entire lymph nodes will allow us to better visualize the spatial relationships of follicular reticular cells and different subsets of lymphocytes within lymphoid follicles.

Methodology
Mice will be transcardially perfused with cold PBS followed by hydrogel solution (HS) containing acrylamide and paraformaldehyde. Spleen and inguinal lymph nodes will be excised and incubated for 3 days at 4°C to allow for diffusion of the hydrogel solution into the tissue. Hydrogel polymerization will occur at 37°C in a solution that contains a mix of acrylamide, bis-acrylamide, paraformaldehyde and thermal initiator. Optical clearing will take place over a period of 4 weeks in a solution that contains detergents. Spleen and lymph nodes will be incubated with primary antibodies against specific surface antigens expressed by reticular cells, as well as B- and T-cells, and labeled with fluorescent secondary antibodies. Whole organs will be imaged by laser scanning confocal microscopy, and image stacks will be imported into Amira and processed for 3D reconstruction. Three-dimensional visualizations of the lymph node and spleen will then form the basis of generating an interactive e-learning tool using Unity software. This learning tool will be designed for students to study the cellular organization of the lymphoid organs in addition to using histological sections.

Expected Outcomes
We predict that optical clearing of lymphoid tissue in combination with confocal imaging of select cellular markers will:
1. Generate 3D visualizations of cellular constituents in unprecedented detail and resolution.
2. Provide a more comprehensive view of cellular interactions in a 3D spatial context.
3. Help us generate novel e-learning tools to better teach the histology of the lymphoid system.
Outcomes of this study will provide novel understanding into the cell physiological and pathological structure/function relationships within the lymphoid system.
CURRICULUM VITAE

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T : (519) 661-2111 ext. 86815, F: (519) 661-3936, Martin.Sandig@schulich.uwo.ca

EDUCATION

Degrees and Universities Attended:
Ph.D. 1990 University of Toronto, Department of Anatomy and Cell Biology
Thesis title: The organization and development of the zonulae adhaerentes and the associate circumferential microfilament bundles in retinal pigment epithelial cells.
Supervisor: Dr. Vitauts Kalnins

M.Sc. 1982 Freie Universität Berlin, Department of Zoology
Thesis title: The cleavage pattern in the gloissiphoniid leech Theromyzon tessulatum.
Supervisor: Dr. Wolfgang Dohle

B.Sc. 1978 Freie Universität Berlin, Department of Biology

Postdoctoral Training:
1992-96 University of Toronto, Department of Biomedical Research, Charles H. Best Institute
Topic: The role of the neural cell adhesion molecule (NCAM) in neurite outgrowth
Supervisor: Dr. Chi-Hung Siu

1990-92 McGill University, Bloomfield Centre for Research in Aging
Topic: The role of statin in liver regeneration and growth control.
Supervisor: Dr. Eugenia Wang

ACADEMIC APPOINTMENTS

2001-present Associate Professor with tenure
University of Western Ontario, Department of Anatomy and Cell Biology

1996-01 Assistant Professor
University of Western Ontario, Department of Anatomy and Cell Biology

AWARDS

1996-01 Heart and Stroke Foundation of Canada Research Scholarship
1992 McMurrich Award for Excellence in Publication
1990-92 Lady Davis Postdoctoral Fellowship
1988-89 Ontario Graduate Scholarship
1987-88 University of Toronto Open Fellowship
1985-86 Ontario Graduate Scholarship
1984-85 University of Toronto Open Fellowship
RESEARCH FUNDING

Student Salary Awards:
2011    J. Roth    Instructional Innovation and development Fund, SSMD
2010    E. Roszell  Undergraduate NSERC URSA

Educational Research Grants Held:
2011    Instructional Innovation and development Fund, Schulich $4,251
         Martin Sandig (PI)

Operating Grants Held:
2006-11 NSERC, Discovery Grant $140,000/5 years
         Tumor cell transendothelial migration
         Martin Sandig (PI)
2008-10 Heart and Stroke Foundation of Ontario, Grant in Aid $159,600/2 years
         Vascular smooth muscle cell phenotype switching and elastin synthesis in 3D tissue
         engineered coronary artery substitutes
         Kibret Mequanint (PI), Martin Sandig (Co-PI)
2007-09 Heart and Stroke Foundation of Ontario, Grant in Aid $167,062/2 years
         Integrin signaling in monocyte transendothelial migration
         Martin Sandig (PI)

PEER REVIEWED PUBLICATIONS


Xia Y., Bhattacharyya A., Roszell E., Sandig M., Mequanint K. (2012). The role of endothelial cell-bound


and extracellular matrix production of human coronary artery smooth muscle cells in 3D scaffolds.

ABSTRACTS

Conciatori, T., De Santis-Smith, A., Rogers, K., and Sandig M. (2014). 3D Visualization of the glomerulus within kidney tissue made transparent through passive optical clearing. AAA annual meeting, March 2014, Boston, MA.


Awards and Honours

2013-14 Nominee for the American Association of Anatomy Basmajian Award for Young Scientist and Educator
• award recognizes health science faculty who are in the formative stages of their career (within 10 years of their highest earned degree at time of nomination), teach human or veterinary gross anatomy, can document excellence in their contribution to the teaching of gross anatomy, and have outstanding accomplishments in biomedical research or scholarship in education.

2012 The Marilyn Robinson Teaching Award of Excellence
• award for excellence in teaching was established at Western based on evidence of outstanding contributions in the area of classroom, laboratory, or clinical instruction
• have seven years or less of full-time university teaching experience at the time of their nomination

I. Research and Scientific Contributions
* indicates student supervision

Peer Reviewed Publications in 2014


   • graduate student supervision, managed project, developed hypotheses, and contributed to writing of the manuscript.

   • interdisciplinary team effort to understand challenges and rewards, challenges, and limitations of interdisciplinary graduate supervision.

   • graduate student co-supervision

   • graduate student supervision

**II. Teaching**

**Lifetime Effectiveness Rating: 6.34 /7 ± 0.416
90.5 % on course as a learning experience**

<table>
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<th>Course Title / Number</th>
<th>Class Size</th>
<th>Mandatory (M), Elective (E), Graduate (G), Undergraduate (UG), Professional (P)</th>
<th>Contact Hrs. Lecture/Lab (# teaching Assistants)</th>
<th>Overall Effectiveness Rating (7 point scale / % effectiveness)</th>
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<td>2013-14 ACB 2221 (Kin)</td>
<td>70</td>
<td>UGM</td>
<td>28/42 (4TA, 3 volunteers)</td>
<td>6.7±0.6 / 96%</td>
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<td>GM</td>
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<td>5.9±1.0 / 84%</td>
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<td>OT 9528</td>
<td>55</td>
<td>GM</td>
<td>28</td>
<td>5.4±1.1 / 77%</td>
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updated 2014-12-03
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<td>PM</td>
<td>7/5 (3 TA)</td>
<td>6.7±0.7 / 96%</td>
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<td>Systemic Anatomy</td>
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<tr>
<td>Dentistry 5160</td>
<td>2013-14</td>
<td>PM</td>
<td>11/5 (3 TA)</td>
<td>6.6±0.8 / 94%</td>
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<td>Systemic Anatomy</td>
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<tr>
<td>ACB 9566/9666 Professionalism</td>
<td>2013-14</td>
<td>GM</td>
<td>50</td>
<td>6.0±1.8 / 85%</td>
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### Trainee Mentorship and Supervision

#### Schulich Student Supervision (3)

**Schulich Research Training Programme (1) 2014-15**
- Mark Dawidek - Developing virtual visual training exercises for individuals of lower spatial ability: A view to better laparoscopic training.

**Schulich Research Training Programme (1) 2011-12**
- Manisha Mistry - Impact of Stereoscopy on Endoscopic Skills Translation in Novices
  - see publications

**Schulich Research Opportunities Programme (1) 2009-10**
- Jeffery Yeung - Development and testing of Cranial Nerve Simulation Trainer
  - see publications

### Graduate Supervision and Co-supervision

#### PhD (5 lifetime)

**In Progress (2)**

- **09/13 - ongoing** Danielle Brewer - The Effects of Concussion on Student Cognition. The Varsity Football Effect.
- **09/11 - ongoing** Victoria Roach - Visuospatial Perception and Surgical Training

#### MSc (24 lifetime)

### Undergraduate 4th Year Honours Projects (20 lifetime)

updated 2014-12-03