Pilot Study for Examination of Injection Site Trauma and Injection Material Pathway
Imaging through a Human Analog System

A Thesis Submitted to the College of
Graduate and Postdoctoral Studies
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Division of Biomedical Engineering
University of Saskatchewan
Saskatoon

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Abstract
There are three main sections that are examined in this research. The first is whether or not a porcupine quill would be a suitable model for future needle designs in order to reduce the pain of pediatric vaccination. The second section delves into whether the Biomedical Imaging and Therapy Beamline at the Canadian Light Source Synchrotron can be used to monitor the path of an injection in a live animal through imaging. The third section involves engineering designs for the Biomedical Imaging and Therapy Beamline.

A literature review was completed which showed that porcupine quills enter human tissue with less force than is required for a typical needle. However, through in line phase contrast imaging, I was unable to conclusively show that there was less tissue damage caused by a porcupine quill than a conventional needle when it enters tissue. Based upon the literature review and the anecdotal evidence of the veterinarian performing the injections, I am able to conclude that it is possible that a metal needle modelled after a porcupine quill would damage tissue less than a standard hypodermic needle during insertion. This reduction in tissue damage could possibly reduce the pain during pediatric vaccination. A redesigned needle may also be suited for suture procedures, especially in ophthalmology, where the fine nerves around the eye are very sensitive to pain. Suture procedures do not require the needle to be pulled back against the grain and may therefore get the most benefit from the lower insertion force of the porcupine quill type needle.

Design and fabrication of such a needle would be the next step in possible research.

Examining the images obtained at the Canadian Light Source, it can be concluded that the absorption, distribution, metabolism and excretion of an injected contrast agent can be monitored through imaging on the Biomedical Imaging and Therapy Beamline. This was confirmed by comparing images obtained in a traditional computed tomography scanner with the images obtained at the Canadian Light Source.

The engineering designs created during this research were fabricated and put into use at the Biomedical Imaging and Therapy beamline at the Canadian Light Source Synchrotron.
Acknowledgements

I would like to acknowledge my two supervisors on this work: Dr. Dean Chapman and Dr. William McKay. Their experience, knowledge and patience have been very valuable to my growth as a student.

Thank you to Dr. Sheldon Wiebe and Dr. Michael Kelly for being on my thesis committee and offering advice that applies beyond the pages of this thesis.

I would like to thank Nazanin Samedi, Mercedes Martinson and Bassey Bassey for their help with the experimentation and data collection at the Canadian Light Source.

I would like to thank Melanie Van Der Loop and Gurpreet Aulakh for their assistance with mice used during experimentation.

Thank you, again, to Dr. Sheldon Wiebe for his assistance in obtaining time to use the Computed Tomography scanner at Royal University Hospital and his assistance in capturing data that day.

Thanks lastly, to Dr. Jeffrey Karp of Harvard University who gave our team access to the porcupine quills that his team uses in their research.
Dedication

To Jennifer Mason, for the love and support to help me finish this degree and succeed in my future endeavours and projects. I couldn’t ask for anyone better to be by my side on this journey.
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List of Abbreviations

BMIT: Biomedical Imaging and Therapy
CLS: Canadian Light Source
CT: Computed Tomography
DEI: Diffraction Enhanced Imaging
ILO: Industry Liaison Office
IM: Intra-Muscular
KES: K-edge Subtraction
RUH: Royal University Hospital
SC: Sub-Cutaneous
TM: Trademark
1 Scientific Framework

1.1 Context
The research for this thesis came from a project that I began working on in my final year of undergraduate mechanical engineering. Our capstone design project was to design an auto-injection system for anaphylaxis. The most well-known commercial device of that type is the Epipen™. The team I was involved with continued work on that project after we had graduated from the University of Saskatchewan for the next five years. In that time, we partnered with the University of Saskatchewan Industry Liaison Office (ILO) to obtain funding for prototypes, patenting costs and marketing. An image of that device from our patent application is shown in Figure 1.1:

![Figure 1.1: The Anadote](image)

The device that was created was a pocked sized auto-injection system that was capable of administering a dosage of epinephrine that was larger than the EpiPen™ in a delivery system that occupied 66% less spatial volume. The concept for this device came from Dr. William McKay, an anaesthesiologist at Royal University Hospital in Saskatoon, Saskatchewan, Canada. The goal was to create one device that would work on both adults and children as easily as possible. The device had to be small and portable so that it would stand out from its other competitors on the market. Through several iterative cycles, a design was selected. Part of this design selection
process was the selection of the type of needle that would be required. In researching needle
design and the benefits associated with each type, we discovered that there was a glaring lack of
research available on the tissue damage that is caused when a needle perforates the skin.

Concurrently to the work I was doing on the auto-injection device, which we had now named
The Anadote, I was enrolled in the Master of Engineering program in the College of Biomedical
Engineering at the University of Saskatchewan. As part of this program, a class I took
introduced me to the capabilities of the Biomedical Imaging and Therapy (BMIT) beamline at
the Canadian Light Source Synchrotron on the University of Saskatchewan campus. It occurred
to me and the two gentlemen that would become my supervisors for thesis, Dr. William McKay
and Dr. Dean Chapman, that there may be an opportunity to use the Canadian Light Source to
image needle trauma at a high resolution and therefore link the two projects that I had been
working on.

The research presented in this thesis occurred because of the desire of the Anadote team to select
a needle that would be as effective as possible while also causing as little pain as possible.
1.2 Objectives

1.2.1 Objective 1: Imaging of Tissue Damage

There were multiple sections to the research proposed for this master’s thesis. The first area of the research was to image needle injection trauma sites to examine the damage that occurs. There is surprisingly little research that has been conducted in the field of tissue damage caused by a needle. Hypodermic syringes have been in use since 1843 (Shephard, 2009) (Miller, 2000) however, there have been no studies conducted on the anatomical effects that these needles have.

The pain of syringe injection makes the vaccination process very painful and uncomfortable for children. For this reason, as a part of the pain reduction research being conducted by a group within the Saskatchewan Health Authority, we proposed to use the high quality imaging capabilities of the Canadian Light Source synchrotron to examine the trauma that occurs at the injection site during injection.

As part of this section of research, the difference in tissue damage between standard needles and porcupine quills was examined. It has been hypothesized that porcupine quills naturally evolved in such a way that they enter tissue with minimal damage and force so that they will embed themselves deeply. The damage that is caused by a porcupine quill occurs when it is removed, as the quill is made of thousands of microscopic rear facing barbs that grab the tissue as it is pulled back along the direction of entry (Cho, et al., 2012). Experimentation has shown that quills easily penetrate tissue; however, imaging at the quality proposed has not been done to examine the effects the quills have on the surrounding tissues.

The information gained from these images may be used as additional information for a project with a final goal of reducing the pain of injections in children by reducing the amount of damage caused to the tissue.
1.2.2 Objective 2: Exploration of the Ability of the BMIT Beamline to follow an Injected Substance in a Human Analog

The second part of the research was to follow an injected substance through the body of a mouse. Mice were imaged multiple times to follow injections through their body as they absorb, distribute, metabolize and were then excreted. The ability to follow the path of an injection through the body in real time may be an intriguing idea for another research facility at the University of Saskatchewan: the Vaccine and Infectious Disease – International Vaccine Center (VIDO-INTERVAC). Vaccines need to be studied before they can begin to move into large scale use. The ability to accurately map their pathway through the body at a research facility that is essentially next door could be very valuable. If a newly developed vaccine could be bonded with a contrast agent for imaging, a complete profile of its pharmacokinetics could be developed.

Imaging contrast agents over time is not a new concept; however, imaging iodine using spectral k-edge imaging at a synchrotron in a mouse is a novel aspect to this research.

1.2.3 Objective 3: Biomedical Engineering Design for the BMIT Beamline

The final portion of the research conducted was related to engineering design using 3D modelling technology to develop new devices for the Biomedical Imaging and Therapy Beamline at the Canadian Light Source synchrotron. As part of this Master of Science degree, I worked on creating the 3D models for three different devices for use in the beamline. Those devices were a frame bender, a cooling frame bender and a refraction test object. The frame bending design was built and used during the synchrotron imaging for this thesis.

The data obtained in this pilot study could be the first step along the path to creating a less painful injection needle. This has importance as it is recommended that children receive more than 20 vaccinations during infancy and childhood. Injections are painful and can result in needle phobia. If there is the possibility that the vaccination procedure could become less painful for children, then new needle design is a goal worth working towards.
1.3 Review of Literature

1.3.1 Literature on Imaging Techniques

1.3.1.1 Diffraction Enhanced Imaging
One possible method of imaging that could be used in this pilot study is diffraction enhanced imaging (DEI). DEI is a type of imaging that uses synchrotron light to obtain high contrast images of tissue due to the attenuation, refraction and diffraction properties of the sample (Chapman D., et al., 1997). As opposed to traditional x-ray imaging where the radiation is absorbed by an object to create a negative image, DEI uses the radiation that is diffracted by an object to create an image.

At the Canadian Light Source Synchrotron, high energy radiation is created by passing the white beam through a double crystal monochromator to select a narrow energy bandwidth. This filtered beam intersects the object being researched, passes through it and then is diffracted by an analyzer crystal. The beam that has not been attenuated or scattered is captured by an area detector where the energy is turned into a data sample that can be transformed into an image (see figure 1.2).

![Synchrotron Radiology Setup](image)

Figure 1.2: Synchrotron Setup for Diffraction Enhanced Imaging (Image courtesy of Dean Chapman)
Diffraction enhanced imaging is capable of providing two different types of images: absorption and refraction (Chapman D., et al., 1996). A spike in intensity on the detector occurs when the angle of the analyzer begins to match the angle of the monochromator. This spike in intensity is the “rocking curve” that DEI depends upon.

If images are taken with the analyzer angle such that the relative intensity is roughly 50% of the peak intensity, then both absorption and refraction information will be captured. This occurs because refraction in one direction moves the intensity up the rocking curve and creates a lighter spot on the image while refraction in the other direction moves the intensity down and creates a darker spot on the image. Orientating the analyzer crystal to the same reflectivity on the other side of the rocking curve will give an image that will have the same absorption information as the previous image but will have the opposite refraction information. This concept is shown in Figure 1.3.

![Figure 1.3: Example of the DEI Rocking Curve and Images Taken at the Same Relative Intensity (Image Courtesy of Dean Chapman)](image)

Computer analysis of these two images can result in absorption only images and refraction only images. The absorption images created from DEI are highly detailed because any of the
incoming radiation that was scattered by too great an angle is not passed onto the detector via the analyzer crystal.

Refraction images can be very highly detailed because they are dependent on the density of the materials the radiation traverses (Chapman D., et al., 1996) and not on the attenuation. Therefore, tissue densities and other subtle anatomical differences are able to be seen using diffraction enhanced imaging.

1.3.1.2 In-Line Phase Contrast Imaging

In phase contrast imaging, the intent is to determine the distributions of the scattering properties of an object (Bravin, Coan, & Suortti, 2013). In this methodology, images are formed because of the propagation of the x-ray beam after it interacts with an object (Nesterets, Wilkins, Gureyev, & Stevenson, 2005). After the beam interacts with the object, the waves that are refracted (phase shifted) interfere with the un-refracted waves. The angle of refraction is very small, so small that it is difficult to detect interference patterns close to the sample. If the distance from the sample to the detector is optimized, strong interference patterns in the intensity can be detected which gives great detail in the sample that was imaged (Arfelli, 2000). Figure 1.4 depicts the setup of phase contrast imaging used the synchrotron radiation facility in Trieste, Italy.

Figure 1.4: Conceptual Beam Layout for Phase Contrast Imaging (Arfelli, 2000)
Due to the high coherence of synchrotron light, the interference patterns caused by the object being imaged create sharp edge enhancement which improves the quality of the images. As stated in an article published in Physics in Medicine and Biology, “This is ideal for the weakly absorbing details like those often encountered in biology and medicine” (Bravin, Coan, & Suortti, 2013).

1.3.1.3  **K-Edge Subtraction Imaging**

K-edge subtraction is an imaging technique that involves taking two separate images of the object being analyzed; one below the K-edge of a certain element and one above the K-edge. It is also referred to as dual-energy subtraction imaging (Zhu, 2012). The K-edge energy of an element is the binding energy that is holding the electrons in place around an atom. At the K-edge, there is a large jump in the absorption coefficient ($\mu/\rho$) of the element (approximately 5.5 times for Iodine) (Zhu, 2012) where $\mu$ is the attenuation coefficient and $\rho$ is the density of the material. This means that when synchrotron radiation is used to take an image of an object below and then above the K-edge, the absorption of that element will be drastically different in each image. Imaging software, such as Image J (Java open source code https://imagej.nih.gov/ij/), can be used to subtract the images. For all intents and purposes, all of the other material in the images will disappear and all that will be left is the difference in absorption of the one element above and below its K-edge. An example of this is shown in the image on the next page. The vasculature of the heart is not clear in either the high or low energy image, however, when the two are subtracted, the bone and water disappears and all that remains is the iodine that has been absorbed.
1.3.1.4 **Spectral K-Edge Subtraction Imaging**

This type of imaging is similar to traditional K-Edge Subtraction imaging. In fact, spectral KES is a generalized version of traditional KES. It also has a very simple set up which is important when imaging live specimens. In this methodology, instead of imaging at energies below and above the K-absorption edge of an element, a continuous spectrum of hundreds of energies is used to capture information on the object for computer reconstruction (Zhu, et al., 2014). The body systems in living specimens are constantly moving (breathing, circulation, etc.) and as such, imaging can be difficult to obtain clear of artifacts. One large benefit of this type of imaging is that the simultaneous energies eliminate motion artifacts which allows for high quality imaging of living specimens.

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**Figure 1.5: K-Edge Imaging Example (Image courtesy of B Thomlinson & M Renier c/o Dean Chapman)**
1.3.1.5 Computed Tomography Imaging

Computed tomography imaging, more commonly referred to as a CT scan, is an imaging method that is used to generate three dimensional images of the inside of an object without needing to cut it open. CT scanning is based upon the difference in x-ray absorption between different tissues; the same as what happens in a conventional x-ray. However, CT scans are able to produce a cross sectional image, referred to as a slice (U.S. Food and Drug Administration, 2014). CT scans work by moving the object being imaged horizontally through the machine. As the object moves through the imaging system, the x-ray source rotates around the circular opening of the machine to capture a three hundred and sixty degree slice of the object undergoing imaging. Detectors on the opposite side of the source capture the x-rays that exit the object at the imaging position. These images are spliced together by a computer in order to reconstruct a three dimensional slice of the object being imaged. When these slices are added together, a complete three dimensional image of the object is obtained which enables viewing of the inside of the object. (U.S. Food and Drug Administration, 2014).

Shown on the next page, in Figure 1.6, is one of the computed tomography scanners at Royal University Hospital in Saskatoon, Saskatchewan, Canada. The object to be imaged (normally a patient) is placed on the horizontal bed and is slowly moved into the scanner in order to capture the imaging slices required for recreation of the object in its virtual format.
Figure 1.6: Computed Tomography Machine at Royal University Hospital Saskatoon (University of Saskatchewan, 2016)
1.3.2 Literature on Tissue Damage and Needle Design

1.3.2.1 Hypodermic Syringe Design

The modern hypodermic syringe that is one of the most commonly used instruments in medicine has remained relatively unchanged since its creation in the mid-19th century (Kravetz, 2005). Alexander Wood published an article entitled “A New method of Direct Application of Opiates and Painful Points” (Wood, 1855) at the same time that Charles Parvcaz documented using a similar device for the first time. These two men are credited with the creation of the hypodermic syringe. This initial design of Dr. Wood is shown in Figure 1.7.

![Figure 1.7: Original Hypodermic Syringe of Dr. Alexander Wood (Wellcome Library, 2015)](image)

This design bears a striking resemblance to the syringes that are currently used in medicine today. Since this initial design, there have been improvements to the design but no substantial changes. Mainly, hypodermic syringes can now be made stronger, thinner and cheaper. In the 1960s, disposable plastic syringes and needles were developed and continue to be used in the market today. While new methods of pharmaceutical administration continue to be developed, such as micro needles and patch based methods, the classic needle and syringe combination has not been updated in over 50 years (Kravetz, 2005).

1.3.2.2 Pain and Tissue Damage

According to the International Association for the study of Pain; “Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey & Bogduk, 1994). Pain, and what causes it, has been a question that
has been plaguing humans throughout recorded history. Several theories existed throughout the 1800 and 1900s on what caused pain. These theories included specificity theory, which saw pain as “a specific sensation, with its own sensory apparatus independent of touch and other senses” (Bonica, 1978). This means that pain was essentially thought of as a sixth sense of the body; an input that the brain uses to understand the current state.

What pain is and how it is registered is still not completely understood today. However, the modern theory that relates to vaccinations involves pain signals being caused by stimulation of sensory nerve fibres (nociceptors). Nociceptors are capable of responding to mechanical, thermal or chemical stimuli. This pain signal then travels from the periphery of the body to the spinal cord along A-delta or C-delta fibres. A–delta fibres are thicker and sheathed in myelin which enables the pain signal to travel faster through them than C-fibres (Beaulieu, Lussier, Porreca, & Dickenson, 2010). Pain felt by signals travelling on the A-delta fibres is a sharp pain and is felt quickly. C-fibres carry a signal that is felt as a duller pain (Skevington, 1995).

Therefore, when a vaccination is given, there is mechanical force that is penetrating the skin and crushing and tearing tissue which causes nociceptors to begin the process of signalling the brain of a potentially damaging stimulus (Purves & Augustine, 2001). The pain carried by the A-delta fibres would be related to the sharp pain felt from the puncture and the signals carried by the C-delta fibres would characterize the dull ache that can be felt after injection.

1.3.2.3 Porcupine Quills as a New Needle Design

The North American porcupine has approximately 30,000 quills that are released when the animal is contacted by what it deems to be a predatory force (Yong, 2012). Each quill has miniscule rear facing barbs located on its tip. According to Harvard Medical School professor Jeffrey Karp: “This [porcupine quills] is the only system with dual functionality, where a single
feature – the barbs – both reduces penetration force and increases pull-out force” (Yong, 2012).

A high resolution image of a porcupine quill is shown in the Figure 1.8.

![Figure 1.8: Electron Microscopic Image of a Porcupine Quill (Cho, et al., 2012)](image)

Research conducted by Dr. Karp and his team shows that the porcupine quill enters tissue using smaller forces than are required for a conventional 18 gauge needle. The results of their team are summarized in Table 1.1. The African porcupine quill is a naturally barbless quill (Yong, 2012).

Table 1.1: Summary of the Forces and Work Required for Penetration and Removal of Quills and Needles in Tissue (Cho, et al., 2012)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbed Quill</td>
<td>0.33 ± 0.08</td>
<td>0.44 ± 0.06</td>
<td>1.08 ± 0.37</td>
<td>1.73 ± 0.41</td>
</tr>
<tr>
<td>Barbless Quill</td>
<td>0.71 ± 0.09</td>
<td>0.11 ± 0.02</td>
<td>2.41 ± 0.28</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>18 Gauge Needle</td>
<td>0.58 ± 0.11</td>
<td>0.04 ± 0.005</td>
<td>2.76 ± 0.70</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>African Porcupine Quill</td>
<td>0.65 ± 0.03</td>
<td>0.06 ± 0.01</td>
<td>2.13 ± 0.04</td>
<td>0.22 ± 0.06</td>
</tr>
</tbody>
</table>
1.4 **Hypothesis**

As mentioned above, there are three distinct parts to this research. The first part of the study is to image needle injection trauma sites to determine the damage that occurs. By imaging the insertion sites of both conventional needles and porcupine quills, I believe this pilot study will show porcupine quills will cause less tissue damage than a conventional needle during insertion due to their natural shape. The results of this pilot could lead to the design a new needle based on the surface profile of a porcupine quill.

While it is clear that I will not be able to determine the amount of pain a person would feel during an injection by looking at an image of tissue, I anticipate that I will be able to accurately examine the stretching and deformation of the tissue structures and nerves within the tissue to make an accurate hypothesis about the relative amount of pain incurred. The quills will cause damage to the tissue during extraction, however, by imaging while they are still inserted I expect to be able to compare the deformation and damage caused compared to a standard needle because of the quality of in line phase contrast imaging.

For the second part of the research, I am confident that I will be able to accurately follow injections as they progress through the bodies of the mice because of the quality of the imaging methods being used. This will confirm the potential that the Biomedical Imaging and Therapy (BMIT) may have for research regarding the pharmacokinetics of their new vaccine formulations if they can be tagged with a contrast agent. This research will be able to show in great detail which organs and body systems that the injections travel to and will be able to compare this to the sites in which they are supposed to attach and affect the body.
The images in Figure 1.9 show the quality of detail I expect to obtain from the images taken in this research. The DEI image (comparable in quality to in-line phase contrast) is able to clearly show lung and muscle tissue. The KES image clearly shows the vasculature of the heart.

**DEI Image**

**KES Image**

Figure 1.9: DEI and KES Images (Courtesy of Dean Chapman)

I expect the engineering design portion of this research to contribute to progression within the field of biomedical synchrotron imaging.
2 Experimental Methods

2.1 Animal Use

2.1.1 Animal Ethics Approvals
In 2013, an application for research using animals was submitted to the University of Saskatchewan Animal Research Ethics Board and was approved on July 30th, 2013. The application can be found in Appendix A and the approval form is Appendix B.

2.1.2 Canadian Light Source
This study involved the use of 2 animals. The mice were of BALB/c breed and required no special treatment. The mice that were imaged were placed into an anaesthetic induced sleep using isoflurane inhalational anesthetic based upon a protocol developed by Gurpreet Aulakh using equipment that is available on the BMIT beam line. The mice then received an intra-muscular injection and a subcutaneous injection of Sodium Iodide (NaI, 0.5 mL dose, 320mg/mL concentration) in its hind leg. A porcupine needle was inserted into subcutaneous tissue and muscle of the hind leg of the mouse so that its effect on the tissue could be compared to the hollow needles. This was completed by a qualified animal handler.

The mouse model is an effective analog for the human body in this research because they are mammals with vascular and connective tissue as well as striated muscle that are very similar to that of humans. The data collected on the tissue damage and the ability to follow the clearance of the injection through the body was enough to draw conclusions that could have human impact.

The mice were anaesthetized using isoflurane and then injected with a contrast agent, iodine. These injections occurred in the hind thigh muscle of and scruff of the animal. Two mice were used in this pilot study and each animal received a different type of injection (either a subcutaneous injection or an intra muscular injection).
The injection site was imaged using in-line phase contrast imaging. This imaging modality provides good soft tissue contrast, especially in thick samples. Each mouse was then imaged with K-Edge Subtraction (KES) at the K-edge energy of the contrast agent (33.2 kilo electron volts for iodine) (Suortti & Thomlinson, 2003). Images were taken over time to follow the contrast agent as it moves underwent the absorption, distribution, metabolism, and elimination (ADME) process in the body. The anesthetized mouse went through the K-edge of the contrast agent at specific time intervals (see Table 2). This time elapse imaging method provided an accurate map of the injected agent as it progresses through the body. K-edge subtraction imaging is a very sensitive imaging method which allowed us to obtain accurate imaging projections of the contrast agent.

Following imaging, the mice were subjected to euthanasia anesthetic overdose. Tissue samples were then harvested from the mice where the injections occurred for further examination.

The mice also had a porcupine quill inserted into the muscle tissue prior to imaging. It has been hypothesized that the natural design of the quill causes minimal damage during insertion and only during extraction does significant damage occur. This hypothesis is based upon information from Dr. Jeffrey Karp of the Harvard School of Medicine (Cho, et al., 2012).

An advantage of this data collection plan was that all of the experimentation could take place at once and therefore the need for multiple animals is reduced.

Based upon our experimentation using a CT scanner at Royal University Hospital, the entire absorption, metabolism, distribution and excretion (ADME) process can imaged in less than an hour. Images were taken for one animal, examined and then the time between images was adjusted based upon the completeness of the last set of images. Once imaging was complete, the mouse was euthanized via anesthetic overdose, administered by Gurpreet Aulakh, and a computed tomography scan (CT scan) was performed.
Using the laboratory facilities at BMIT, tissue sections were taken from the location where the needles and porcupine quills were inserted. These tissue sections were then examined under a microscope. For the diffraction enhanced imaging technique to be used, images were taken on both sides of the rocking curve and then analyzed using the Image J computer software program. The use of diffraction enhanced imaging allows for extremely detailed images of the soft tissue near the injection that would not be visible from normal absorption imaging. The use of a third crystal in this technique allows the subtle differences in scattering angles produced by the tissue to be converted into intensity differences which can be picked up on the detector.

2.1.3 Royal University Hospital
During imaging at Royal University Hospital, an identical breed of mouse was used as was described above. The mouse underwent the same anaesthetic, injection and euthanasia procedures that were followed at the Canadian Light Source.
2.2 Synchrotron Experimental Pre-Imaging

In order to ensure the beamline was set up correctly for the experiment, initial imaging took place with a plasticine tube in the beam to ensure proper alignment and image quality. A second set of images was then taken with an iodine tube and a barium tube in order to tune the energy of the beam to correctly capture the K-edge of the materials being imaged. The second set of images obtained is shown in Figure 2.1.

![Figure 2.1: Test Object Water Equivalent Image](image)

Figure 2.1 shows the water equivalent image obtained during K-Edge Subtraction imaging. This highlights the relative densities of the objects in the image when compared to water (density of 1 gram/millilitre). The dense bar in the image is a bone analog made of hydroxyapatite. Based upon the density scale in the right hand side of the image, it is approximately 5 times the density of water. The iodine and barium tubes are only slightly denser than water because they are diluted in a water solution.
The KES iodine image clearly shows the location of the iodine tube in the image. The concentration of the iodine in the tube is 0.025 grams per millilitre.

2.3 Computed Tomography Injection Solution and Needle Size

The solution injected into the mouse at Royal University Hospital for imaging was Optiray 320™. This contrast agent contains 320 milligrams (mg) of Iodine per milliliter (mL) which enables the injection to be tracked through the body of the mouse. The needle used for injection was 22 gage for the computed tomography scan at Royal University Hospital. As outlined above in the animal use protocol, the injection sites were the scruff of the neck (sub cutaneous), the right hind leg (intramuscular) and the left rear leg (sub cutaneous).
2.4 **Canadian Light Source Synchrotron Injection Solution and Needle Size**

The solution injected into both mice was identical. The mixture was 0.25 millilitres of OptiRay240™ and 0.25 millilitres of Evans Blue. This 0.5 millilitre solution was injected into the mouse in two separate locations. 0.25 millilitres of solution was injected at each location. OptiRay240™ was used at the synchrotron and Optiray320™ at Royal University Hospital because of the availability of the material at the time of testing. The difference in concentration had no effect on the outcome of the imaging. A lower Iodine content was acceptable at the Canadian Light Source because of the increased sensitivity of the K-edge subtraction method compared to conventional imaging systems either in projection or computed tomography (Suortti & Thomlinson, 2003).

The Needles used were 21 and 25 gauge for the synchrotron imaging. Mouse 1 received a subcutaneous injection in its right hind leg with a 21 gage needle and a subcutaneous injection in the scruff of its neck with a 25 gage needle. Mouse 2 received an intramuscular injection in its right hind leg with a 21 gage needle and an intramuscular injection in its left hind leg with a 25 gage needle. The different needle sizes were selected to see if there was a difference in the tissue damage caused by each.

2.5 **Mouse Size**

For the synchrotron imaging, mouse 1 weighed 35.0 grams and mouse 2 weighed 33.3 grams. The mouse used at Royal University Hospital was 33.5 grams.

2.6 **Porcupine Quill Type and Location**

Mouse 1 had a barbed quill inserted into its right hind leg. Mouse 2 had a barbed quill inserted into its right hind leg and an un-barbed quill inserted into its left hind leg.
2.7 Synchrotron Imaging Schedule

The table below shows the time scheduled followed for imaging the mice. A larger gap was left after injection for the second mouse in order to have the contrast agent absorb more into the body of the mouse.

Table 2.1: Schedule of Synchrotron Imaging of the Mice

<table>
<thead>
<tr>
<th>Mouse 1</th>
<th>Mouse 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:00 Minutes</td>
<td>Injection</td>
</tr>
<tr>
<td>10:00 Minutes</td>
<td>Image 1</td>
</tr>
<tr>
<td>18:00 Minutes</td>
<td>Image 2</td>
</tr>
<tr>
<td>27:00 Minutes</td>
<td>Image 3</td>
</tr>
<tr>
<td>32:00 Minutes</td>
<td>Image 4</td>
</tr>
<tr>
<td>37:00 Minutes</td>
<td>Image 5</td>
</tr>
<tr>
<td>42:00 Minutes</td>
<td>Image 6</td>
</tr>
<tr>
<td>48:00 Minutes</td>
<td>Image 7</td>
</tr>
<tr>
<td>53:00 Minutes</td>
<td>Image 8</td>
</tr>
</tbody>
</table>
3 Results

3.1 Computed Tomography Imaging

The following series of images were created using Sante DICOM viewer to reconstruct the cross-sectional images obtained during the CT scan of a mouse. Figure 3.1, below, shows the mouse in its natural state. The internal organs are clearly visible and the brighter areas on the image are the bone structures and tail.

Figure 3.1: CT Image 1
Figure 3.2 shows a pre-injection view of the mouse that is taken at a more dorsal level than image 1. The bones of the skull and vertebrae are visible as are some of the internal organs.
The visuals shown below in Figure 3.3 were captured very quickly after injection occurred in the right hind leg of the mouse. The contrast agent is clearly visible as the bright area in the image on the left.

![Figure 3.3: CT Image 3: Anteroposterior and Lateral Views](image)

Figure 3.3: CT Image 3: Anteroposterior and Lateral Views
Image 4 was taken one minute after injection. The contrast agent is still concentrated in the right hind leg of the mouse.

Figure 3.4: CT Image 4: Anteroposterior View
The images below were taken very quickly after the second injection was given to the mouse. This injection was placed in the scruff of its neck. The bright area on the image is where the injection occurred.

Figure 3.5: CT Image 5: Anteroposterior and Lateral Views
The images below were taken 4 minutes after the first injection. The injection in the scruff has not disposed throughout the body to this point; however, the injection in the leg has started to be filtered into the bladder as shown on the image to the right.

Figure 3.6: CT Image 6: Anteroposterior and Lateral Views
The image below shows that the injections have started to be filtered by the kidneys and are entering the bladder.

Figure 3.7: CT Image 7: Anteroposterior and Lateral Views
The images below show that the injection has nearly dissipated completely from the hind leg and has filled the bladder, as shown by the very bright area on the image on the left. The injection in the scruff is still mainly located in its initial injection location.

Figure 3.8: CT Image 8: Anteroposterior and Lateral Views
The images below, taken at the same time as the set of images above, shows the migration of the injection to other parts of the body, such as the kidneys, lungs and other musculature surrounding the scruff.

Figure 3.9: CT Image 9: Anteroposterior and Lateral Views
3.1.1 Tissue Damage

It was difficult to analyze the tissue damage that occurred from the injections using computed tomography. The resolution of the images was not sufficient to draw any conclusions about the damage that would be done to the muscle in order to estimate the pain that would occur during injection.

No porcupine quills were inserted when imaging this mouse.

3.1.2 Injection Monitoring

Using the computed tomography imaging machine at Royal University Hospital in Saskatoon, it was very simple to follow the injected solution as it was distributed and metabolized in the body of the mouse. The images presented above clearly show the distribution of the injection throughout the body of the mouse over time. This was an expected result as CT images are very useful for differentiating contrast agents from other body tissues and structures.
3.2 Synchrotron Imaging

3.2.1 Examining Tissue Damage with In Line Phase Contrast Imaging
Over 1200 high resolution images (1000x1000 dpi) were taken of mouse one and mouse two using in line phase contrast imaging. Each mouse went through two imaging scans. Each imaging scan contained 4 spins (rotations of the mouse). Each spin contained 75 images (also known as slices). The representative image below (1 of the 1200) was analyzed using ImageJ to enhance the contrast of the raw data.

![Image](image.png)

**Figure 3.10: In Line Phase Contrast Image - Mouse 1**

In the image, the bright white structures are bone, the fatty layer of tissue underneath the skin and fur can be seen around the edge, and the internal organs are the lighter grey shapes in the middle. Each structure inside of the mouse can be seen in very high detail.

The image selected below shows the second mouse, which received sub cutaneous injections in the scruff of its neck and in the loose tissue of its right hind leg. The barbed porcupine quill was
inserted into its left posterior and an unsuccessful attempt to insert a plastic porcupine quill occurred on the right rear posterior of the mouse.

![Image of mouse with porcupine quill insertions highlighted]

**Figure 3.11: In Line Phase Contrast - Mouse 2, Scan 2, Spin 0, Slice 74**

In the image, the locations of the two porcupine quill insertions can be identified because of the deformation in tissue surrounding their insertion point. It appears that the location of the barbed porcupine quill filled with fat and/or other body fluids after the trauma of insertion. Unfortunately, the locations of the conventional needle insertion were not able to be identified in the individual imaging sets. This is likely due to the large gap in time between needle insertion and in-line phase contrast imaging.

To further explore how the insertion of porcupine quill was affecting the tissue, a three-dimensional model of the mouse was created. The model was created in the ImageJ 3D Viewer plugin using all 75 slices from scan 2, spin 0 of mouse 2. The model created is shown next in
multiple views. Each model is approximately 1.2 centimetres thick according to calculations within the ImageJ software.

Figure 3.12: 3D Image of Mouse Two – From Side

The image in Figure 3.12 above shows the power of in-line phase contrast imaging to distinguish the large bone structures and outer tissue layers of the mouse. To create this contrast, the transparency of the image had to be increased to 78% from 0%. However, despite the high resolution contrast, this angle sheds minimal light on tissue deformation.
The next image, Figure 3.13, shows a cephalad (from the bottom) view of the mouse. In this three dimensional model, it is possible to see the location of quill insertion, the skeletal features and the outer tissues of the mouse.

In both sets of images, even when zoomed in on the location of insertion, it is difficult to determine the effect that the porcupine quill is having on the tissue. There is a large deformity in the tissue surrounding the quill; however, this was not able to be compared to the deformity caused by the needle insertion. Another feature is visible in the three dimensional reconstruction may be the temperature probe as it corresponds to an area of high contrast in the individual images.
3.2.2 Injection Monitoring with K-Edge Subtraction Imaging

The following images are the results of the K-edge subtraction imaging; which was used to monitor the location of the Iodine injection through the body of the mouse over time. The images on the left are the K-Edge Subtraction Water Equivalent Images and the images on the right are the K-Edge Subtraction Iodine Images. The group of images is of Mouse 1. Image set 1 clearly shows the two injection sites for this mouse; the scruff of the neck and the right hind leg.

Figure 3.14: Mouse 1 – KES 1
Image set 2 shows that the injections have started to diffuse through the tissues near the injection sites. The largest concentration of the injection is still located at the injection site.

Figure 3.15: Mouse 1 – KES 2
Image set 3 shows further diffusion of the injection solution through the tissues surrounding the injection site. Faint amounts of the injection can start to be seen in the kidneys.

Figure 3.16: Mouse 1 – KES 3
Image set 4 continues to display the dispersion of the injection throughout the tissues of the mouse. The kidneys can be seen more clearly than the last image.

Figure 3.17: Mouse 1 – KES 4
The fifth set of images shows minimal change from the fourth as the diffusion process continues.

Figure 3.18: Mouse 1 – KES 5
In the image set below, the injection continues to move through the tissues surrounding the injection site. The concentration of the dosage is getting weaker at the site of injection.

Figure 3.19: Mouse 1 – KES 6
In image sets seven and eight below, the diffusion process continues over time as the injected dosage gets absorbed and metabolized by the mouse.

Figure 3.20: Mouse 1 – KES 7
Figure 3.21: Mouse 1 – KES 8
This next series of images shows the second mouse, Mouse 2, which received an intramuscular injection in its right hind leg and an intramuscular injection in its left hind leg. An un-barbed porcupine quill was inserted into the scruff of the neck at the end of this set of images.

Figure 3.22: Mouse 2 – KES 1
The imaging process on mouse 2 started later after initial injection than mouse 1. As such, enough time has passed that the injected solution has been absorbed and metabolized by the mouse. This time lapse results in the image below which shows the concentration of the contrast agent located in the bladder of the mouse.

![Figure 3.23: Mouse 2 – KES 2](image-url)
Images three, four and five show little change over time. More of the injected solution is processed into the bladder as would be expected.

Figure 3.24: Mouse 2 – KES 3
Figure 3.25: Mouse 2 – KES 4
Figure 3.26: Mouse 2 – KES 5
3.3 Engineering Design

As part of this Master of Science degree, I worked on three different small-scale engineering projects for the Biomedical Imaging and Therapy (BMIT) beamline at the Canadian Light Source Synchrotron in Saskatoon, Saskatchewan, Canada. These projects and the results are discussed in the following sections. One of the projects was used in the imaging for this thesis.

3.3.1 Frame Bender

The frame bending apparatus was designed to be able to hold a circular silicon wafer in a fixed position in the path of the beam. It was designed using the three-dimensional computer assisted drawing software program SolidWorks.

3.3.1.1 Design Specifications

Table 3.1: Design Specifications of the Frame Bending Apparatus

<table>
<thead>
<tr>
<th>Part</th>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main Plate</td>
<td>Height</td>
<td>200 mm</td>
</tr>
<tr>
<td></td>
<td>Width</td>
<td>200 mm</td>
</tr>
<tr>
<td></td>
<td>Thickness</td>
<td>6 mm</td>
</tr>
<tr>
<td>Support Arms</td>
<td>Height</td>
<td>250 mm</td>
</tr>
<tr>
<td></td>
<td>Width</td>
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</tr>
<tr>
<td></td>
<td>Thickness</td>
<td>8 mm</td>
</tr>
<tr>
<td>Base Plate</td>
<td>Height</td>
<td>8 mm</td>
</tr>
<tr>
<td></td>
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<td>300 mm</td>
</tr>
<tr>
<td></td>
<td>Thickness</td>
<td>125 mm</td>
</tr>
</tbody>
</table>
3.3.1.2 Additional Features

The face of the apparatus that the wafer is attached to has a radius of 3000 millimeters. This gives the wafer a slight curve which helps to focus the beam coming through it onto the detector. This radius is shown in the screenshot from SolidWorks below.

![Radius of the Frame Bending Apparatus](image)

Figure 3.27: Radius of the Frame Bending Apparatus
The apparatus also has a section of material removed from the non-wafer side so that less of the beam will be scattered as it passes through the material on the way to the detector. The scattering and absorption of x-rays increases with distance they have to traverse through an object (need citation here). The hollowed out section is 170mm x 37.5 mm; which allows a beam of the same size to pass through the object with minimal obstruction. This feature is shown in the image below.

Figure 3.28: Frame Bending Apparatus with Material Removed
3.3.1.3 Fabricated Version used in this Research

The fabricated version of the frame bending apparatus is shown in the image below (with a wafer taped to the face). The bolts on the side can be tightened with an Allan key to hold the plate at the desired angle. There are several holes in the base plate that can have a bolt placed through them to secure the device to the stage in the beamline. Having the device attach to the stage enables it to be positioned directly in the path of the beam.

During imaging, the frame bender was placed in the path of the beam to help focus the beam onto the detector. It worked as expected and can continue to be used at the BMIT beamline for future imaging projects.

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Figure 3.29: Final Version of the Wafer Apparatus
3.3.2 Frame Bending Cooling Plate

The purpose of the cooling plate is to cool a wafer that has been placed in the beamline. The high energy of the x-rays that hit the wafer transfer heat to it. The wafers can get very warm and therefore, designing a device that can cool them is of use at the synchrotron. The concept of the cooling plate was to have a solid that the wafer can be placed on that is also able to cool its temperature.

To achieve this, two plates were designed with recessed channels on their inside faces. These two plates would be bolted together to make one complete solid. The recessed channel would be attached to a water source so that a stream of water can be run through it. The water is used to transfer the heat away from the wafer so that it will maintain a reasonable temperature.

The three dimensional model of the inside of one of the plates is shown below. The channel runs around the area the wafer would contact and there is a large window in the middle. This window exists so that the synchrotron beam is only traversing the wafer and will not be affected by the material of the cooling plate. This allows for more of the beam to reach the detector because it will not be attenuated by the cooling plate.

![SolidWorks Representation of the Cooling Plate](image)

Figure 3.30: SolidWorks Representation of the Cooling Plate
### 3.3.2.1 Design Specifications

Table 3.2: Design Specifications of the Cooling Plate

<table>
<thead>
<tr>
<th>Part</th>
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<th>Value</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
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<td>Width</td>
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</tr>
<tr>
<td></td>
<td>Thickness</td>
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</tr>
<tr>
<td>Channel</td>
<td>Radius of Groove</td>
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</tr>
</tbody>
</table>
3.3.3 Refraction Test Object
The refraction test object was designed based upon a suggestion from University of Saskatchewan professor Dr. Dean Chapman. The theory behind the design is that when the object is placed in the path of the beam, the scattering effects will be different based upon which part of the object that the beam hits.

Figure 3.31: Oblique View of the Refraction Test Object
3.3.3.1 Design Specifications

Table 3.3: Design Specifications for the Refraction Test Object

<table>
<thead>
<tr>
<th>Plate Number</th>
<th>Slope</th>
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<th>Left Height (mm)</th>
<th>Right Height (mm)</th>
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<td>0.00</td>
<td>10.00</td>
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</tr>
<tr>
<td>3</td>
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<td>26.57</td>
<td>2.50</td>
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</tr>
<tr>
<td>4</td>
<td>0.25</td>
<td>14.04</td>
<td>3.75</td>
<td>6.25</td>
</tr>
<tr>
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<td>0.00</td>
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<td>5.00</td>
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<td>-45.00</td>
<td>10.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>
4 Discussion

4.1 Main Findings

There were three main purposes to this research:

1. To determine whether the insertion of a needle or a porcupine quill causes more damage to tissue at the location of insertion.
2. To determine whether the Biomedical Imaging and Therapy Beamline at the Canadian Light Source Synchrotron can image the absorption, distribution, metabolism and excretion pattern of an injected solution in a live animal.
3. To use engineering design to further the field of biomedical imaging at a synchrotron facility.

4.1.1 Tissue Damage

The types of imaging obtained in this research were not able to show if there was a significant difference in tissue damage between the insertion of a needle and the insertion of a porcupine quill.

The barbed porcupine quill was much easier to insert into the tissue than either the synthetic version that was created by Dr. Jeffrey Karp and his lab. There was difficulty in making the synthetic quill pierce the flesh. A large amount of force was required for insertion of the synthetic quills in our experiment and as such it can be inferred that the pain caused by that design may be larger than a regular needle or a natural barbed porcupine quill. The difficulty in insertion was caused by the deflection of the synthetic quills when they were pressed against the hard tissue. If the synthetic quill had been made from a harder material while still keeping the natural scale pattern of a barbed quill, it is possible that it would have inserted into the tissue as easily as the standard needle or natural quill.

Hypodermic needles must be both inserted and withdrawn from tissue, meaning a porcupine-type metal needle design will likely inflict less damage going in, but more damage pulling out than a smooth needle. However, most suturing is done with semicircular curved needles pulling a swaged-on suture. These needles go into one side of the wound and out the other side always in
the same forward direction. Therefore, barbed suturing needles that have been modelled after a porcupine quill might cause less tissue damage and hasten healing.

4.1.2 Injection Monitoring
As hypothesized, the location of the injected bolus in a live mouse was able to be monitored in both the computed tomography scan and the K-edge subtraction imaging. It was simple to determine the location of the tagged injection over time through both types of imaging. The resolution of the K-edge subtraction image was better than the computed tomography scan. Therefore, it was easier to distinguish what tissues and organs the injection had been absorbed into in order to be metabolized.

The Biomedical Imaging and Therapy Beamline proved that it is capable of monitoring an injected solution through the body of a living creature in order to study the paths it takes through the body. The hypothesis that vaccine injection (which has been bonded to a contrast agent) could be monitored in the body of a live animal shows feasibility. The novel data obtained in this research is that of injected iodine being monitored over time using spectral K-edge subtraction imaging in a mouse. This type of imaging/injection monitoring has not been tried before in other research in this field.

4.1.3 Engineering Design
The three designs that were created during this research were developed with the beamline staff to further the imaging capabilities of the BMIT facility at the Canadian Light Source. The designs were fabricated and performed as expected during imaging, allowing for improved use of the facility for future researchers.

4.2 Limitations
Due to the high demand for imaging time at both Royal University Hospital and the Canadian Light source, it was possible to only get time for one set of images. Drawing conclusions from one set of images can be a flawed methodology; however, because the results obtained match with the expected results based upon literature review, it is very likely that our results are strong enough to draw proper conclusions from.
4.3 Practical Implications and Future Research

There are two practical implications that come from this research. The first would be the possible new design of a needle that incorporates the scaled features of a natural porcupine quill. Based upon the research conducted by Dr. Jeffrey Karp and his team (Cho, et al., 2012); it is clear that the insertion force for a porcupine quill is lower than a conventional needle. The features of the porcupine quill that make it easier (and possibly less traumatic) to insert are best utilized in the design of a novel curved suture needle, where, the needle is passed into the skin and tissues on one side of an incision, then across the incision into the tissues on the other side, and then out without ever being pulled in a withdrawing “against-the-grain” direction. This may prove to be quite atraumatic. It may be possible to design an injecting (hollow) needle that is less traumatic than current smooth needles, but this is less intuitive and the anticipated cost of manufacturing would not be worth the possible small reduction in pain.

The second practical implication comes from the usage of the Biomedical Imaging and Therapy beamline. That research facility can be used to monitor injections in living animals so that their distribution patterns could be mapped. Having an organization (such as the Vaccine and Infectious Disease Organization -International Vaccine Centre (VIDO-INTERVAC) group) in close proximity to the Canadian Light Source, could lead to future partnerships in research and in evaluation of vaccine pharmacokinetics.
5 Conclusions

There are two conclusions to draw from this research:

1. A metal needle developed in the mould of a natural porcupine quill would have the potential to reduce tissue damage during insertion into tissue. This reduction in tissue damage may reduce the pain associated with pediatric vaccinations, which was the initial inquiry behind this research degree. A redesigned needle may be best suited for suture procedures, especially in ophthalmology, where the fine nerves around the eye are very sensitive to pain. Suture procedures do not require the needle to be pulled back against the grain and may therefore get the most benefit from the lower insertion force of the porcupine quill type needle.

2. The Canadian Light Source Synchrotron is a facility that is capable of monitoring a vaccination (that has been bonded with a contrast material) through its absorption, distribution, metabolism and excretion processes in the body of a living creature. The image quality is high enough that the injection can be monitored as it enters different organs in the body for metabolism and excretion.
Works Cited


EmittingProducts/RadiationEmittingProductsandProcedures/MedicalImaging/MedicalX-Rays/ucm115318.htm


Appendix A: Animal Use Protocol

University Committee on Animal Care and Supply (UCACS)
Animal Research Ethics Board (AREB)
Animal Use Protocol - RESEARCH Application Form

Use this Animal Use Protocol application form for use of animals in research, testing or production.
The UCACS AREB must approve the use of animals for research prior to the commencement of any project. This use must comply with the Canadian Council on Animal Care (CCAC) guidelines, the University of Saskatchewan Animal Care and Use Procedures, and the Tri-Council MOU – Schedule 3: Ethical Review of Research Involving Animals. Annual review and approval is required for ongoing studies. Every 4 years, a new animal use protocol application form must be submitted for review by the AREB.

### CONFIDENTIAL

<table>
<thead>
<tr>
<th>UCACS AUP#</th>
<th>Code</th>
<th>Date Received</th>
<th>Date Approved</th>
<th>Exempt</th>
<th>Required</th>
<th>Paid</th>
</tr>
</thead>
</table>

| UCACS AREB Chair Approval | Date |
| UCAS Veterinarian Approval | Date |
| UCAC Community Representative Approval | Date |

### Section 1 - Principal Investigator Information (PI is primary emergency contact)

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Department/Research Unit/College</th>
<th>Phone 1</th>
<th>Phone 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="mailto:deane.chapman@usask.ca">deane.chapman@usask.ca</a></td>
<td>3069664111</td>
<td>3062303469</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Email</th>
<th>Office Phone</th>
<th>Laboratory Phone</th>
<th>After Hours Phone</th>
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</thead>
<tbody>
<tr>
<td>Chapman</td>
<td>Dean</td>
<td>Anatomy and Cell Biology</td>
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</tr>
</tbody>
</table>

### Section 2 - General Protocol Information

Protocol Title:
Exploration of Imaging Techniques for Needle Injection Trauma Sites and Vaccination Pathways

Type of Project: [ ] Pilot Study  [ ] New Project

Proposed Start Date: ASAP  Expected Date of Completion: Apr 30, 2014  or Ongoing [ ]
1. Descriptive Summary (required for reporting to the CCAC):
In 40 words or less and in terms understandable to a non-scientist provide a descriptive summary of the nature of the procedures involved in the proposed project. For example: “Low levels of mercury exposure to fish may alter function of the thyroid gland. This study will expose fish to different levels of mercury and blood samples will be collected to assess thyroid function.”

Hypodermic syringes cause a large pain response when used in vaccination. This study will examine the extent of damage that occurs during injection with the hope of devising a method for reducing injection pain. It will also follow the pathway of the injected vaccine through the body.

2. Please indicate all sources of funding for this AUP:

<table>
<thead>
<tr>
<th>Add</th>
<th>Source /Agency</th>
<th>Funding Status</th>
<th>Scientific Peer Review Status</th>
<th>New or Existing Fund</th>
<th>UniHi Fund and Org Code</th>
</tr>
</thead>
<tbody>
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<td>Awarded</td>
<td>No Peer Review</td>
<td>Existing</td>
<td></td>
</tr>
<tr>
<td>X</td>
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<td>Awarded</td>
<td>No Peer Review</td>
<td>Existing</td>
<td></td>
</tr>
</tbody>
</table>

Section 3 - Personnel Involved in the Protocol

1. Please complete the table below for the Principal Investigator and each person associated with this AUP:

<table>
<thead>
<tr>
<th>Add</th>
<th>Last Name</th>
<th>First Name</th>
<th>Position</th>
<th>NSID</th>
<th>Email address</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Chapman</td>
<td>Dean</td>
<td>Co-PI</td>
<td></td>
<td><a href="mailto:dean.chapman@usask.ca">dean.chapman@usask.ca</a></td>
</tr>
<tr>
<td>X</td>
<td>McKay</td>
<td>William</td>
<td>Co-PI</td>
<td></td>
<td><a href="mailto:william.mckay@saskatoonhealthregion.ca">william.mckay@saskatoonhealthregion.ca</a></td>
</tr>
<tr>
<td>X</td>
<td>Campbell</td>
<td>Dougas</td>
<td>GS</td>
<td>109350 42</td>
<td><a href="mailto:dcs350@mail.usask.ca">dcs350@mail.usask.ca</a></td>
</tr>
<tr>
<td>X</td>
<td>Aulakh</td>
<td>Gurpreet</td>
<td>PDF</td>
<td>110594 99</td>
<td><a href="mailto:gka240@mail.usask.ca">gka240@mail.usask.ca</a></td>
</tr>
</tbody>
</table>

Note: Animal users must be listed as authorized workers on the biosafety permit(s) associated with this protocol.

2. For each animal user listed above, please provide the following information (does not apply for animal care staff employed at the Beef Cattle Research and Teaching Unit, Dairy Unit, LASU, Livestock Research Unit, Poultry Centre, PSCI, Sheep Unit, VIDO, WBDC, WCVM ACU, Goodale Research Farm or VMC):

<table>
<thead>
<tr>
<th>Add</th>
<th>Last Name</th>
<th>Description of animal user's education, training and experience</th>
<th>Procedures animal user will be responsible to perform under this AUP</th>
<th>UCACS Use Partial Additional Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Chapman</td>
<td>Ph.D. Physics</td>
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<td></td>
</tr>
<tr>
<td>X</td>
<td>McKay</td>
<td>MD FRCP (Anesthesia)</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>Campbell</td>
<td>B. Eng</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>Aulakh</td>
<td>M.Pharm (Pharmacology), Ph.D. (Vet. Biomedical Sciences), pharmacological and immunological techniques + in-vivo animal model development, intravitral microscopy</td>
<td>Anesthesia + of animals, animal monitoring, euthanasia, tissue sectioning</td>
<td></td>
</tr>
</tbody>
</table>

3. Will University animal facility staff provide any technical services beyond normal husbandry?

☐ Yes  ☒ No

4. Would you or other personnel involved in the AUP like to receive additional training for any of the procedures to be performed under this protocol?

☐ Yes  ☒ No
Section 4 - Purpose of Animal Use and Category of Invasiveness

1. Please choose the appropriate CCAC Purpose of Animal Use (PAU) for this protocol:
   - [X] 0
   - [ ] 1
   - [ ] 2
   - [ ] 3
   - [ ] 4

2. Please check all that apply:
   - [X] Research
     - [ ] Basic or Applied
     - [ ] Field or Wildlife Study
     - [ ] Diagnostic
     - [ ] Other, please specify
     - [ ] Testing
     - [ ] Production (breeding/ herd/ colony maintenance)

   This project does not involve any work on conscious vertebrate animals (e.g., animals are immediately anaesthetized without recovery, or immediately killed to obtain tissues/cells, etc)

3. Please choose the appropriate CCAC Category of Invasiveness (CoI) level for this research:

   **Animal Experiments**
   - [X] Level B
   - [ ] Level C
   - [ ] Level D
   - [ ] Level E

   **Wildlife Studies**
   - [ ] Level B
   - [ ] Level C
   - [ ] Level D
   - [ ] Level E

Section 5 - Lay Summary

1. Lay Summary:
   Provide an abstract of 250 words or less in simple language (Grade 8 reading level and understood by someone not familiar with scientific research). Outline objectives, experimental approach, and significance of the expected results to human and/or animal health. The UCACS AREB may need to release this abstract to the public in order to provide information about ongoing animal use at the University of Saskatchewan.

   The objective of this pilot study is to ensure that the information to be collected in a future study is attainable using the imaging capabilities of the Biomedical Imaging and Therapy beam line at the Canadian Light Source. We will be using k-edge subtraction imaging to follow the injection of a contrast agent as it proceeds through the body of a mouse. This will be used in a future experiment to track new vaccines from VIDO in the body to ensure they are acting as expected. We will also be using diffraction enhanced imaging to examine the site of injection. This will shed light on the tissue damage that occurs during injection with the hope of determining a way to reduce this damage and therefore reduce the pain of injection as well.

Section 6 - Source and Description of Animals

1. Please provide the following information for each species and strain requested. For multiple year studies, indicate the anticipated animal numbers for years 1 through 4. Justify these numbers in Section 8 of the protocol.

<table>
<thead>
<tr>
<th>Add</th>
<th>Species (common name)</th>
<th>Strain Identification (if applicable)</th>
<th>Stock # (if applicable)</th>
<th>Supplier/Source</th>
<th>Total Animal Numbers</th>
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<tbody>
<tr>
<td>X</td>
<td>Mouse</td>
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<td>Charles River</td>
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<td>Mouse</td>
<td>Balb/c</td>
<td></td>
<td>Other*</td>
<td>2</td>
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</tbody>
</table>
1(a). If applicable, provide additional information regarding the supplier/source:

We would like two deceased mice that are of no use to anyone else at the university to do a preliminary experiment to determine baselines for tissue damage caused by injection.

2. Will animals be bred specifically for this project?
   - [ ] Yes
   - [x] No

Section 7 - Housing of Animals and Location of Proposed Studies

1. Please identify the facility/location where animals will be housed and where procedures will be conducted.

<table>
<thead>
<tr>
<th>Add new - if multiple facilities are required</th>
<th>Animal Housing</th>
<th>Animal Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Lab Animal Services Unit (LASU) - Health Sci Bldg</td>
<td>Canadian Light Source (CLS)</td>
</tr>
</tbody>
</table>

1(a). If OTHER, please describe.

2. Is transport of animals to other buildings required?
   - [x] Yes
   - [ ] No

2(a). Please provide details regarding the transport.

Mice would be requested for transport by LASU staff to CLS on the day of experiment. Thereafter, mice would be humanely euthanized following imaging.

Section 8 - Description of Project, Experimental Design and Procedures

1. Rationale and Hypotheses:

   In 1-2 paragraphs briefly and clearly describe the rationale for this project, its primary objectives, hypotheses to be tested, and benefit to humans or animals.

   The pain of syringe injection makes the vaccination process very painful and uncomfortable for children. For this reason, as a part of the pain reduction research being conducted by a group within the Saskatoon Health Region, we are proposing to use the very high quality imaging capabilities of the Canadian Light Source to examine the injection sites for different types of injections. The information gained from these images will be used as baseline information for a project with a final goal of reducing the pain of injections in children by reducing the amount of damage caused to the tissue.

   To gain even more information from this pilot study, the injection that is given to the mice will be of a contrast agent which we will be able to follow through the body as it absorbs, distributes, metabolizes and is excreted. By showing the ability to follow the contrast agent in the body, we will be showing that the Canadian Light Source has the ability to follow a vaccine that has been tagged with a contrast agent though the body. This will enable future experiments to follow the paths of new vaccines through the body to ensure that they are behaving as expected and there are no unforeseen complications with their formulation and what organs and tissues they disperse into.

2. Experimental Design and Procedures:

   Clearly outline the study design, treatment groups, group size (example: 5 animals x 3 treatments x 2 replicates = 30 animals) and procedures you propose to conduct on the animals, and a description of what happens to the animals during the experiments. The design should contain sufficient animal numbers to ensure statistical significance and covers attrition rates. If multiple tests, procedures or surgeries are performed on the same animal(s), include a flow chart with timelines detailing animal use. Describe any food or water deprivation (duration, reason) and provide...
justification if the interval is >12hrs. Indicate if single housing or altered light cycles are required. If applicable, include results of pilot studies relating to this new protocol.

We would initially like to use two deceased mice to perform an intra-muscular and sub-cutaneous injection in the BMIT facility laboratory at the Canadian Light Source. This laboratory is included on biosafety permit R-ANA-06. We would then examine the tissue sites under a microscope to determine the amount of tissue damage that can be expected. This would give us a baseline to ensure that the results obtained using the synchrotron will be relevant.

The synchrotron portion of this study will involve the use of 8 animals. The mice will be of normal breed and will require no special treatment. The mouse to be imaged will be placed into an anaesthetic induced sleep using isoflurane inhalational anesthetic based upon a protocol developed by Ken Gagnon using equipment that is available on the BMIT beam line. The mouse will then receive an intra-muscular injection and a subcutaneous injection of contrast material in its hind leg. A porcine needle will also be inserted into the subcutaneous tissue and muscle of the hind leg of the mouse so that its effect on the tissue can be compared to the hollow needles. This will be completed by Gurpreet Aulakh, a qualified animal handler.

K-edge subtraction imaging is a very sensitive imaging method which will allow us to obtain incredibly accurate imaging projections of the contrast agent. This time elapsed imaging method will provide an accurate map of the injected agent as it progresses through the body.

The determination of the time between images will require standardization to ensure that the entire absorption, metabolism, distribution and excretion (ADME) process is imaged. Images would be taken for one animal, examined and then the time between images would be adjusted based upon the completeness of the last set of images.

Once imaging is complete, the mouse will be euthanized via anesthetic overdose. Tissue sections will then be taken from the location where the mouse was injected. These tissue sections will then be imaged using Diffraction Enhanced Imaging.

Our hypothesis is that we will be able to show that the Canadian Light Source is able to accurately track injectable contrast agents though the body of a mouse for use in future vaccination studies. We also will be able to image the tissue damage that occurs from injection to use this information for possible pain reducing methods of injection.

3. Standard Operating Procedures:
Please indicate the UCACS approved SOP numbers for all manipulations and techniques to be performed on the animals, including surgery, testing, substance administration, sample collection, etc. If no UCACS approved SOP is available, please append new SOPs, keeping in mind that not all members of the Animal REB have a scientific background.

3(a). UCACS approved SOP numbers:

3(b). Are you willing to share any new SOPs associated with this protocol in the UCACS SOP databank?
☐ Yes  ☐ No

4. Administration of Substances:
Provide a description of each administered substance (e.g. chemical, cells) for each species and procedure. Do not list sedatives, anaesthetics or analgesics associated with surgical procedures in this table.

<table>
<thead>
<tr>
<th>Add</th>
<th>Species (common name)</th>
<th>Agent Name</th>
<th>Concentration (e.g. mg/mL)</th>
<th>Dose (e.g. mg/kg)</th>
<th>Volume (e.g. mL)</th>
<th>Route (e.g. IV)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Balb/c</td>
<td>Sodium Iodide</td>
<td>0.15 mg/mL</td>
<td>0.05 mL</td>
<td></td>
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<tr>
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<td>Agent Name</td>
<td>Concentration (e.g. mg/mL)</td>
<td>Dose (e.g. mg/kg)</td>
<td>Volume (e.g. mL)</td>
<td>Route (e.g. IV)</td>
<td>Frequency</td>
</tr>
<tr>
<td>---------</td>
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<tr>
<td>X</td>
<td>Balb/c</td>
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<td>10-20 ml/kg</td>
<td>0.5 ml</td>
<td>SC</td>
<td>Once</td>
</tr>
</tbody>
</table>

5. Are you using any analgesics or sedatives for any non-surgical procedure?
   - Yes   ✗ No

5(a). Provide scientific justification for withholding anaesthesia or analgesia IF any procedure usually requires anaesthesia or analgesia OR indicate 'Not Applicable'.

   Not Applicable

6. Will you perform surgical procedures?
   - Yes   ✗ No

6(e). Provide details on the anaesthesia and analgesia specifically related to the surgical procedures:

<table>
<thead>
<tr>
<th>Add Row</th>
<th>Pre-medications(s)</th>
<th>Drug</th>
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<th>Dose (e.g. mg/kg)</th>
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<th>Route (e.g. IV)</th>
<th>Frequency</th>
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<tr>
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<td>Route (e.g. IV)</td>
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<tr>
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<td>Frequency</td>
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<tr>
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<td>Analgesia</td>
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<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

*** If you have any questions about drugs or dosages to be used in this protocol, please contact the [UCACS Animal Welfare Veterinarian](mailto:animal.welfare@ucacs.ac.uk).

6(f). Provide scientific justification for withholding anaesthesia or analgesia IF any procedure usually requires anaesthesia or analgesia OR indicate 'Not Applicable'.

7. Collection of Samples:
   List all samples (e.g. blood, other fluids, tissues, etc) collected from live animals. Do not list samples collected during terminal procedures.

<table>
<thead>
<tr>
<th>Add Row</th>
<th>Species (common name)</th>
<th>Sample</th>
<th>Site</th>
<th>Amount/Volume</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Does this protocol involve field studies using wild animals?
   - Yes   ✗ No
Section 9 - The Three Rs Principles

The CCAC requires that Principal Investigators provide information on the effective implementation of the three R's of animal care: Replacement, Reduction and Refinement. Refer to the [CCAC Three Rs Microsite](http://www.go3r.org/) for more information.

**Replacement:** use of an inanimate system as an alternative (e.g. a computer model or program); replacement of sentient animals (e.g. vertebrates) with less sentient animals (e.g. invertebrates, such as worms, bacteria, etc); cell or tissue cultures.

1. Justify the use of animals and choice of sentient species identified in the experimental design.

   Mice are used in this experiment because they will allow us to obtain results that can be directly translated to humans. The muscular and circulatory system of these mice will enable us to obtain accurate results about the damage done to tissue during injection while also following the injected substance through the body. The mice used must be alive for the drug to advance though the body, but will receive doses of radiation too large for humans.

2. Please access the CCAC Three Rs Search Guide or another search engine for alternatives (e.g. [http://www.go3r.org/](http://www.go3r.org/)). Did the search identify replacement alternatives that could meet the objectives of the study?

   - [ ] Yes
   - [x] No

2(a). Provide brief results from your search.

   The mice should be kept in comfortable quarters. This will help ensure that the results obtained from these mice is accurate because they are not under stress or suffering before experimentation (1). Isofluorane is an accepted and commonly used method for anesthesia in mice (2).


**Reduction:** a decrease in the number of animals used previously with no loss of useful information achieved by reducing the number of variables through data sharing, good experimental design (eg repeated measures), by using genetically homogeneous animals or by ensuring that the conditions of the experiment are rigorously controlled.

3. Briefly describe the considerations made to minimize the number of animals used?

   This is a pilot study, so 8 animals will enable design of a definitive study with hypothesis-testing. Fewer animals risk negating the value of the study if unforeseeable problems occur. Doing a small study with two already deceased mice will also show us if our research outcomes will be significant before we proceed with more mice.

**Refinement:** a change in some aspect of the experiment that results in a reduction or replacement of animals or in a reduction of any pain, stress or distress that animals may experience. The establishment of early endpoints for intervention in a study that has the potential to cause pain or distress is an example of refinement.

4. Are the procedures described in this protocol new to the Principal investigator?

   - [ ] Yes
   - [x] No

4(a). Please describe any refinements that have been made to procedures and describe any "lessons learned" that will improve animal welfare in this new protocol submission.

   None
Section 10 - Monitoring

The CGAC Guide to the Care and Use of Experimental Animals states that normal, healthy experimental animals should be observed at least once a day (CCAC, 1993). However, more frequent observation is necessary during critical periods with respect to impairment. The increased frequency of observations depends on the potential for increasing pain and/or distress. See CCAC Guidelines on: Choosing an Appropriate Endpoint in Experiments using Animals for Research, Teaching and Testing, 1998. All animals undergoing surgery require documentation of surgical/anesthetic and postoperative monitoring.

1. Frequency of animal monitoring:
How often will the animals’ condition be monitored: (a) during the course of the study; and (b) during critical times?

Mice would be continuously monitored during imaging. Once anaesthesia is administered, confirmation through pedal reflexes would be done and the mouse is in the imaging hutch, there would be real time visual camera aided monitoring from the control room.

2. Documentation of animal monitoring:
Has a monitoring schedule been developed for any procedure in this protocol?

☐ Yes ☐ No

3. Who is responsible for monitoring and record keeping? What training has (have) this individual(s) received?

Because the experiments are acute and terminal in nature, it does not require extensive monitoring. Gurpreet would be taking note of the mice during the experiments.

4. What is the chain of command for reporting monitoring results when animals are reaching the endpoint (including authority to euthanize)? Provide authorization within the Humane Intervention Point (HIP) checklist.

Gurpreet, Dean Chapman, Melanie van der Loop

Section 11 - Humane Intervention Point (HIP) Checklist (Endpoints)

When experimental procedures produce ongoing pain/distress a Humane Intervention Point (HIP) checklist is required to ensure that an animal’s discomfort, pain and/or distress is terminated, minimized or reduced. Please refer to the CCAC Guidelines on: Choosing an Appropriate Endpoint in Experiments using Animals for Research, Teaching and Testing, 1998.

1. In terms of behavioural changes and physiological signs, and signs of toxicity (if applicable), list the criteria that will be used to trigger the decision to remove an animal from the experiment or to terminate the experiment.

Mice would checked for deep anesthesia, appropriate temperature ambience and breathing rate before proceeding it to the imaging hutch. Thereafter, video surveillance will make sure of the signs of anesthesia. If there are signs of any distress, the mouse would be immediately brought out and humanely euthanized.

2. Has an Humane Intervention Point (HIP) checklist been established? Contact the UCACs for examples of HIP checklists for different species.

☐ Yes ☐ Not Applicable

2(a). Please append the HIP checklist that uses the criteria identified above and clearly describes the point at which animals will be terminated (humanely euthanized) due to pain and/or distress or discomfort, or given treatment to relieve pain and/or distress or discomfort regardless of study endpoints or completion of the study.

Section 12 - Method of Disposition of Animals

1. Check the method of disposition of the animals and provide further details if required.

☐ Kept as stock/colony animals

☐ Sold

☐ Donated
Returned to Owner
☒ Humane Euthanized

Confirm individual(s) who will perform this procedure:

☐ Other

1(a). Indicate the method of euthanasia:
☒ Anaesthetic Overdose
Please specify the agent:

☐ Isoflurane
☐ Carbon Dioxide - Note: CO₂ should not be used where other methods are practical for the experiment and the species.
☐ Anaesthesia and exsanguination
☐ Decapitation (under anaesthesia)
☐ Cervical Dislocation
☐ Decapitation without Anaesthesia
☐ Captive Bolt
☐ Other

Section 13 - Emergency Veterinary Care

1. Dealing with unexpected complications:
What provisions will be made to deal with any unexpected problems or with animals showing severe pain or distress?

Mice would be euthanized immediately

Reminder - Submit a UCACS MMI Report Form for any incidents that occur under this protocol as per the UCACS Procedures on Submission of a Morbidity Mortality Animal Welfare Incident Report Form.

2. Please confirm the facility veterinarian who is responsible to provide oversight for this protocol. Note: If you are the principal investigator of this AUP and are also a designated UCACS Facility Veterinarian, then please select the Animal Welfare Veterinarian, Dr. Melanie van der Loop.

<table>
<thead>
<tr>
<th>Add Row</th>
<th>Facility Veterinarian</th>
</tr>
</thead>
<tbody>
<tr>
<td>☒</td>
<td>Dr. Melanie van der Loop (LASU, ACU, CLS, Fish Facilities, Natural Sciences Museum)</td>
</tr>
</tbody>
</table>

2(a). If OTHER, please describe. Note: Other is chosen only when work is conducted at a facility not affiliated with the University of Saskatchewan.

The UCACS Animal Welfare Veterinarian can be contacted if you require veterinary assistance for your animals. Please note that the Animal Welfare Veterinarian, University Veterinarian or designate is obligated to treat or euthanize animals in distress. If you cannot be contacted after a reasonable attempt, the decision of the UCACS Veterinarian is final. Ensure that arrangements are in place to permit consultation on a 24-hour a day, 7-day a week basis.
Section 14 - Occupational Health and Safety (Biosafety, Chemical, Radiation)

According to CCAC Guidelines on protocol review, use of biohazardous, infectious, biological, chemical or radioactive agents in living animals must be clearly identified, and institutional approval of this use provided. BIOSAFETY, RADIATION SAFETY OR CHEMICAL SAFETY REQUIREMENTS, AS APPROPRIATE TO THIS PROTOCOL, MUST BE IN PLACE BEFORE APPROVAL CAN BE GRANTED.

1. Indicate all that are applicable:

- [X] Non-infectious biological material (Risk Group Level 1)
- [ ] Infectious biological material (Risk Group Level 2 or Level 3)
  - [ ] Bacteria
  - [ ] Virus
  - [ ] Fungi
  - [ ] Parasites
  - [ ] Toxins
  - [ ] Other
- [ ] Chemical
- [ ] Radiisotopes
- [ ] Carcinogens
- [ ] Genetically Modified (GM) Biological Material
  - [ ] Recombinant DNA/RNA
  - [ ] GM Microorganisms
  - [ ] Transgenic Animals

2. Indicate the Biosafety Permit number(s) that cover this protocol. Also, state the Radiation Safety Permit number (if applicable).

RANA05

3. List all biological agents, both infectious and non-infectious, and/or toxins to be used:

None

4. Please identify where animals will be housed/procedures conducted and state the biosafety level of containment that is required.

<table>
<thead>
<tr>
<th>Add row - if multiple facilities are required</th>
<th>Location (Building and Room #)</th>
<th>Level of Containment (1, 2 or 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Canadian Light Source (CLS)</td>
<td>Biosafety Level 1</td>
</tr>
</tbody>
</table>

4(a). If OTHER, please describe.

5. State where any samples, tissues or other material will be analyzed or processed.

CLS

6. Will any potentially hazardous materials (chemical, radiisotope, or biological) be used in this AUP for which occupational exposures to animal care staff in animal facilities may occur? If there are no real risks, please state so.

- [ ] Yes
- [X] No

Section 15 - Declaration and Submission of AUP

The Principal Investigator’s signature below indicates that:

1. All animals used in this research project/course will be cared for and used in accordance with the Guidelines of the Canadian Council on Animal Care, and the Regulations of the University Committee on Animal Care and Supply;
2. The UCACs Veterinarian has the authority on behalf of the UCACs to order treatment or euthanasia for any animal in emergency situations, if in the veterinarian’s professional judgment such action is urgently required. You acknowledge that it is your responsibility to inform the veterinarian of contraindicated treatments or medications.
3. You have searched the literature and the proposed animal use does not unnecessarily duplicate other animal use;
4. You have considered alternative procedures that do not involve the use of living animals and that an alternative to the proposed animal use is not feasible;
5. All manipulations which have the potential to cause pain and discomfort, wherever possible, have been refined in technique and reduced in numbers to achieve the desired results with a minimum degree of discomfort to the animal.
6. All animal manipulations will be carried out by experienced, trained and competent personnel using recognized techniques. You accept personal responsibility as principal investigator for all animal manipulations in this project.
7. You will provide all personnel involved in the project with the most current version of the approved AUP.
8. You accept responsibility for keeping the AUP information current, especially with respect to methodology.
9. You will notify the AREB of any revisions to this protocol and submit a protocol modification request.
10. You will keep copies of all approved AUPs, AUP summaries, revisions and amendments in an accessible file.

<table>
<thead>
<tr>
<th>Dean Chairman</th>
<th>Mar 7, 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal Investigator (Typed Name)</td>
<td>Principal Investigator (Signature)</td>
</tr>
<tr>
<td>Ben Rosser</td>
<td>Mar 7, 2013</td>
</tr>
<tr>
<td>Department Head / Dean / Director (Typed Name)</td>
<td>Department Head / Dean / Director (Signature)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Add row - if multiple signatures are required</th>
<th>Animal Facility Manager * (Typed Name)</th>
<th>Animal Facility Manager (Signature)</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Michele Moroz</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Add row - if multiple signatures are required</th>
<th>Other Authorizing Signature ** (Typed Name)</th>
<th>Other Authorizing Signature (Signature)</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Animal Facility Manager approval/signature is required for use of animals in ALL research, teaching, testing or production in UofS animal facilities. Only field or wildlife studies, or animal use conducted off-campus in non-UofS facilities does not require Facility Manager approval.

** Authorizing Signatures include for example: VMC Director, WSEP Biosafety Manager, WSEP Chemical Safety Manager, WSEP Radiation Safety Manager, WCVM representatives responsible for oversight of the resident animal teaching herds.

Submit by Email to UofS Research Ethics Office

Submit 1 signed original (hard copy) plus 1 electronic copy (email to ucacs.office@usask.ca) of this protocol application to the UCACS AREB
c/o Research Ethics Office, Box 5000 RPO University, Saskatoon, SK S7N 4J8
Physical location: 1607 - 110 Gymnasium Place, NRC-PBI Building

Applications received by the 1st Friday of the month will be reviewed by the AREB the same month.

IMPORTANT! If you do not receive a reply email confirming acknowledgement of receipt of this form within three business days after its submission, please contact the Research Ethics Office at 966-4126 or ucacs.office@usask.ca.
Appendix B: Animal Research Ethics Board Certificate of Approval

UNIVERSITY OF SASKATCHEWAN

Animal Research Ethics Board
Certificate of Approval

PRINCIPAL INVESTIGATOR
Dr. L. Dean Chapman

DEPARTMENT/ORGANIZATION
Anatomy & Cell Biology

ANIMAL USE PROTOCOL #
20130041

TITLE
Exploration of imaging techniques for needle injection trauma sites and vaccination pathways

SPONSORING AGENCIES
Canadian Institutes of Health Research (CIHR)

BIOSAFETY NUMBER
RANA-06

UNIFI FUND #

APPROVAL DATE:
July 20, 2013

APPROVAL OF:
Now Animal Use Protocol

EXPIRY DATE:
July 31, 2014

Full Board Meeting ☐ AREB Subcommittee ☐ AREB Chair and University Veterinarian ☐

CERTIFICATION
The University of Saskatchewan Animal Research Ethics Board reviewed the above-named research project. The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to the conditions outlined in the original protocol submitted for ethics review. This Certificate of Approval is valid for the above time period.

PROTOCOL MODIFICATIONS
Any modifications to this protocol must be approved by the UCACS AREB Chair prior to implementation, using the AUP Modification Form.

ONGOING REVIEW REQUIREMENTS
Research programs that extend beyond one year must receive annual review. For the annual renewal, an annual review form (and progress report) must be submitted to the AREB within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the Research Ethics Office website for further instructions.

Michael Corcoran, Chair
Animal Research Ethics Board
University of Saskatchewan

cc: Doug Campbell, Anatomy & Cell Biology
    Gurpoor Aulakh, Anatomy & Cell Biology

July 30, 2013
Date Issued

Please send all correspondence to:
Research Ethics Office
University of Saskatchewan
Box 0000 RPO University, 1007-110 Gymnasium Place
Saskatoon SK, S7N 4X8
Telephone: (306) 966-7028 Fax: (306) 966-2080 Email: ucasr.office@usask.ca

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Appendix C: CLSI Beam Time Proposal

**CLSI PROPOSAL SUBMISSION**

**GENERAL USER**

<table>
<thead>
<tr>
<th>Title of Proposal:</th>
<th>Biomedical Imaging of Needle Injection Trauma Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Proposal:</td>
<td>General User</td>
</tr>
<tr>
<td>Proposal Duration:</td>
<td>2 cycles (12 months)</td>
</tr>
<tr>
<td>Subject of Research</td>
<td>Life Sciences</td>
</tr>
<tr>
<td>Industrial Partners Involved?</td>
<td>No</td>
</tr>
<tr>
<td>Five Key Words</td>
<td>Injection, Trauma, Contrast, Vaccines, Medical</td>
</tr>
<tr>
<td>Funding Sources</td>
<td>NSERC, CIHR</td>
</tr>
</tbody>
</table>

### RESEARCH TEAM

**First Name** | **Last Name** | **Email** | **Institute/Affil.** | **Department** | **Classification** | **Status** |
--- | --- | --- | --- | --- | --- | --- |
Douglas | Campbell | dac390@mail.usask.ca | University of Saskatchewan | Anatomy and Cell Biology | Student | Coming to CLS |
Dean | Chapman | dean.chapman@usask.ca | University of Saskatchewan | Anatomy and Cell Biology | Faculty | Coming to CLS |
William | McKay | bill.mckay@usask.ca | University of Saskatchewan | Anesthesia | Faculty | Coming to CLS |
Gurpreet | Aulakh | gurpreet.aulakh@usask.ca | University of Saskatchewan, College of Medicine | Anatomy & Cell Biology | Post Doctoral Fellow | Coming to CLS |
Jeff | Karp | jeffkarp@mit.edu | Brigham and Women's Hospital (Harvard Medical School) | Medicine | Faculty | Remote |

### INVOICING DETAILS

| Name | Address | City | State / Province | Postal / Zip Code | Country |
--- | --- | --- | --- | --- | --- |
Douglas Campbell | 314 Jan Crescent | Saskatoon | Saskatchewan | S7N 5T7 | Canada |

**BRIEF DESCRIPTION**

Summarizes: (1) the importance and goal of the research project; (2) what experiments will be carried out during the requested beam time and (3) how the acquired data will contribute towards achieving the goal explained in (1).

The pain of syringe injection makes the vaccination process very painful and uncomfortable for children. For this reason, as a part of the pain reduction research being conducted by a group within the Saskatoon Health Region, we are proposing to use the high quality imaging capabilities of the Canadian Light Source to examine the trauma that occurs at the injection site for different types of injections. The information gained from these images will be used as baseline information for a project with a final goal of reducing the pain of injections in children by reducing the amount of damage caused to the tissue.

The experiments will involve giving anesthetized mice both sub-cutaneous and intra-muscular injections with a standard 27 gauge needle. A porcine quill will also be inserted into the mice both sub-cutaneously and intra-muscularly. Tissue samples will be taken from the locations of trauma and these samples will be imaged using diffraction enhanced imaging. Tissue damaged as a result of the injection trauma will be visible in these images based upon findings in our preliminary lab testing. We intend on using the images obtained to observe at a very local level the deformation that is caused to the tissue structures. Our hypothesis is that due to the unique structure of the porcine quill it will cause less disruption in the tissue than the standard needle and as a result would
be the starting point for a possible new needle design.

As a bonus to the primary goal of this research, the injection that is given to the mice will be of a contrast agent which we will be able to follow through the body as it absorbs, distributes, metabolizes and is excreted. By following the contrast agent in the body, we will be showing that the Canadian Light Source has the ability to follow the path of new vaccines. This will lead to a partnership between the Biomedical Imaging and Therapy Beamline (BMIT) and the Vaccine and Infectious Disease Organization (VIDO). The images will be obtained using K-edge subtraction imaging.

**SCIENTIFIC MERIT** describes: (1) what is to be studied and the importance of it; (2) what hypothesis would be tested; (3) how the results will impact the field and (4) what is the likelihood of success? **Evaluation Criteria**

There are two distinct parts to this project. The first part of the project is to image needle injection trauma sites to determine the damage that occurs. There is surprising little research that has been conducted in this aspect of pain reduction. Hypodermic syringes and needles have been in use since 1853, however, the local anatomical effects of these needles has never been studied. This research will be used as an initial exploratory step in a program with the goal of reducing the pain of pediatric vaccination. This is an initiative in the anesthesiology department of Royal University Hospital, part of the Saskatoon Health Region.

We are proposing to inject the mice sub-cutaneously and intra-muscularly with both hypodermic needles and porcupine quills. The trauma that these instruments create will be examined as a baseline for research on creating a better needle for vaccine delivery for children. We are working with Dr. Jeffrey Karp, an associate professor of medicine at Harvard School of Medicine. He has brought to our attention that porcupine quills cause very little damage during insertion because of the way that they are naturally shaped. While it is clear that we will not be able to determine the amount of pain sustained from an image, we will be able to accurately examine the stretching and deformation of the tissue structures and nerves within them to make an accurate hypothesis about the relative amount of pain incurred. The quills will cause damage to the tissue during extraction, however, by imaging while they are still inserted we will be able to compare the deformation and damage caused compared to a standard needle because of the quality of diffraction enhanced imaging.

We feel that the data obtained in this portion of the experiment could be the first step along the path to creating a less painful injection needle. This is of great importance as it is recommended that children receive more than 30 vaccinations during infancy and childhood. These injections are somewhat painful and can result in needle phobia. If there is the possibility that the vaccination procedure could become less painful for children, then that is a goal worth working towards.

The second portion of the project will be to follow the path of vaccines that have been injected into the bodies of mice. The mice will be time lapse imaged using k-edge subtraction to follow the vaccines through the body as they absorb, distribute, metabolize and are then excreted. This is of great importance to the Vaccine and Infectious Disease International Vaccine Center (VIDO-Intervac) at the University of Saskatchewan as they regularly creating new vaccines. These vaccines need to be studied before they can begin to move into large scale use. This research will determine if the new vaccines tend to congregate in specific organs or the blood cells. This research will also determine how the administration of the drug affects its pharmacokinetics.

We have done this type of imaging in a Computed Tomography (CT) scanner at Royal University in Saskatoon. We were able to obtain accurate representations of the pharmacokinetics of the injections but were unable to have the resolution required to examine the fine details of muscular deformation that can be obtained from diffraction enhanced imaging.

We expect to obtain several novel results in this experiment. Our first hypothesis is that we will be able to shed light on the trauma that occurs at vaccination injection sites at an extremely local level. This will enable us to make accurate predictions about the relative amounts of pain caused by needles and porcupine quills as they enter the body. The use of diffraction enhanced imaging will show great detail in the tissues affected by these injections due to its very high contrast and will give us a great likelihood of obtaining high resolution images compared to what is available on a standard clinical CT image.

Based upon the quality of the imaging methods of K-edge subtraction, we hypothesize confidently that we will be able to accurately follow vaccines as they progress through the bodies of the mice. This will enable the BMIT team to slow the researchers at VIDO.Intervac that they will be able to obtain valuable information about the pharmacokinetics of their new vaccine formulations. We will also be able to show in great detail which organs and body systems that the vaccinations are attracted to and will be able to compare this to the sites in which they are supposed to attach and affect the body.

**EXPERIMENT PROCEDURE** describes sample procedures and explains the basis for the estimate of the amount of beam time needed as well as who will perform the measurements and how the data will be analyzed.

This study will involve the use of 2 animals. The mice will be of BALB/c breed and will require no special treatment. The mouse to be imaged will be placed into an anesthetic induced sleep using isoflurane inhalational anesthetic based upon a protocol developed by Gurpeet Aulakh using equipment that is available on the BMIT beam line. The mouse will then receive an intra-muscular injection and a subcutaneous injection of Sodium Iodide in its hind leg. A porcupine needle will also be inserted into the
subcutaneous tissue and muscle of the hind leg of the mouse so that its effect on the tissue can be compared to the hollow needles. This will be completed by Gurpreet Aulakh, a qualified animal handler.

The anesthetized mouse will be imaged through the k-edge of the contrast agent at specific time intervals. This time elapse imaging method will provide an accurate map of the injected agent as it progresses through the body. K-edge subtraction imaging is a very sensitive imaging method which will allow us to obtain accurate imaging projections of the contrast agent.

Based upon our experimentation using a CT scanner at Royal University Hospital, the entire absorption, metabolism, distribution and excretion (ADME) process can imaged in less than an hour. Images would be taken for one animal, examined and then the time between images would be adjusted based upon the completeness of the last set of images. Once imaging is complete, the mouse will be euthanized via anesthetic overdose, administered by Gurpreet, and a computed tomography scan (CT scan) will be performed.

Using the laboratory facilities at BMIT, tissue sections will then be taken from the location where the needles and porcupine quills were inserted. These tissue sections will then be imaged using Diffraction Enhanced Imaging. For the diffraction enhanced imaging technique to be used, images will be taken on both sides of the rocking curve and then analyzed using the ImageJ computer software program. The use of diffraction enhanced imaging will allow for extremely detailed images of the soft tissue near the injection that would not be visible from normal absorption imaging. The use of a third crystal in this technique allows the subtle differences in scattering angles produced by the tissue to be converted into intensity differences which can be picked up on the detector.

Our hypothesis is that we will be able to accurately image the local tissue damage and deformation that occurs from injection and draw conclusions about which type of injection was the least harmful to the animal. We will also be able to show that the Canadian Light Source is able to accurately track injectable contrast agents though the body of a mouse for use in future vaccination studies.

**SUITABILITY**

identifies (1) why a third generation synchrotron such as the CLS and the particular beamline/endstation is more suitable than others, (2) comments on whether complementary measurements will be made and (3) justifies the amount of beamtime requested in terms of the required development and number of samples. Evaluation Criteria.

The CLS is the optimal synchrotron for this experiment because it houses the BMIT-BM beamline. The BMIT facility is currently set up for use with biological samples as well as having wet and dry labs available for biological sample preparation. The BMIT beamline also enables us to complete both k-edge and diffraction enhanced imaging in the same location which is a very important aspect of our experiments.

The beam that comes out of the BM beamline is wide enough that it will enable us to scan down the sample vertically without having to move horizontally which will reduce imaging time. This is a very important consideration due to the dynamic nature of the experiment. The reduced scan time required per image is important because the contrast agent will be progressing through the specimen.

Large beam size will also reduce the time required for data acquisition which will in turn reduce the number of shifts required to complete the experiments. The experiment, once set up correctly should be able to be completed within six shifts, three for each cycle.

**PAST PRODUCTIVITY - Evaluation Criteria**

a) Briefly discuss results from previous shifts received at CLS. The table below identifies shifts received in the past two years if you were the spokesperson.
b) New User to CLS - comment on your research team’s recent synchrotron experience and evidence of capability. If you have no synchrotron experience, give evidence of productivity in other fields.

<table>
<thead>
<tr>
<th>Access Mechanism</th>
<th>Cycle 15</th>
<th>Cycle 16</th>
<th>Cycle 17</th>
<th>Cycle 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Shifts</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a) N/A
b) I have no previous experience using a synchrotron for data collection; however, I was a summer student for the SylMANN Beamline at the CLS for the summers of 2007 and 2008. In this position, I was part of the team readying the beamline for commissioning. It was my responsibility to write user manuals for several different pieces of equipment that are now located in the beamline cleanroom and hutch. I created a method for creating a flat surface on a material so that it can then be placed in beam to make micro and nano devices that is being used at this beamline currently.

I am currently a Fellow in the CIHR THRUST program at the University of Saskatchewan and am completing a Master of Engineering Degree through the College of Biomedical Engineering.

To aid me in my first synchrotron data collection endeavor, I have assembled a team of people that has logged several hours of research on the proposed beamline for the experiment, the BMIT-BM. Dean Chapman has an extensive background in data acquisition methods using BMIT and will likely be able to troubleshoot any issues that may arise. Please find attached a list of Dr.
Chapman’s most recent publications

Gurpreet Atulak is a skilled pharmacologist with a substantial amount of experience at the CLS. Her ability to monitor the mice in the experiment will be of the utmost importance. George Belev will be assisting with the operation of the BMIT beamline during data collection.

Dr. William McKay is an anesthesiologist conducting an extensive research program to study injection pain. Jeff Karp is an Associate Professor at Harvard Medical School and is Co-Director of the Center for Regenerative Therapeutics at the Brigham and Women's Hospital. He is Principal Faculty at the Harvard Stem Cell Institute and an Affiliate Faculty at MIT through the FST program. His interest in medical innovation and research with porcupine quills is perfect for this study.

<table>
<thead>
<tr>
<th>Beamline Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beamline:</strong></td>
</tr>
<tr>
<td><strong>Preferred Beamline:</strong></td>
</tr>
<tr>
<td><strong>CLS Staff Contacted:</strong></td>
</tr>
<tr>
<td><strong>Endstation:</strong></td>
</tr>
</tbody>
</table>
| **Technique:** | Computed Tomography (CT)  
Diffraction Enhanced Imaging (DEI)  
K-edge Subtraction Imaging (KES) |
| **Equipment:** | Anesthesia equipment  
Microscope  
Vital Signs monitoring  
Warning Pads |
| **Energy Required:** | 30 keV |
| **Resolution Expected:** | 30 micron |
| **Labs Required:** | BMIT Sample Preparation Lab (Room 1113) |
| **Supplies, Consumables, and Tools Needed:** | Test tubes, scalpels, hollow point needles, syringes, porcupine quills, tissue containers, gloves, eye protection glasses, sodium iodide |

### Setup Requirements

<table>
<thead>
<tr>
<th>Sample Name</th>
<th># Samples</th>
<th>State</th>
<th>Media Surrounding Sample</th>
<th>Image Details</th>
<th>Sample Details</th>
<th>Container Details</th>
</tr>
</thead>
</table>
| Mouse       | 2         | Fresh | Air                      | Area: 5 x 20  
# / Sample: 5  
Single Exp. Time: 40` | Life Time: 3 hrs Prep Time: 30 min | Yes | Dimensions:  
Weight:  
Thickness:  
Density: |

### Priority Areas:

**Impact human health or the practice of medicine**

The priority area that best describes this research is that it will impact human health and the practice of medicine. The research we will be conducting in the area of pain reduction for vaccination in children has the ability to have a tremendous impact upon the way that pediatric medicine is practiced. If we are able to show a reduction in tissue and cell trauma based upon the natural shape of a porcupine quill this information could be used to revolutionize the needle industry. By concurrently showing that the BMIT beamline is able to track and visualize the path of a vaccine as it progresses through the body we will be able to show the VIDO-intervac team that a partnership with the CLS could be beneficial to both parties. This research can only be performed at the BMIT facility because of their capability to handle live animal imaging as well as high quality tissue sample imaging. The detail we are able to obtain about tissue deformation is much greater than what can be seen in clinical CT imaging as we have determined from our preliminary research. The access to tissue preparation facilities as well as the equipment to do K-edge subtraction and diffraction enhanced imaging is a feature that only the BMIT beamline has and is necessary to complete our proposed research.

### Scheduling Requirements

**Total # shifts for entire proposal:** 2
<table>
<thead>
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<th>Anticipated Timeline</th>
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<td>Preferred dates (current cycle)</td>
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<tr>
<td>Unacceptable dates (current cycle)</td>
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</tr>
<tr>
<td>None</td>
<td>2014-02-15 to 2014-02-23</td>
</tr>
</tbody>
</table>
SAFETY AND MATERIALS

Chemical Information
Are you bringing any hazardous chemical materials to the CLS? No

Biological Information
Are you bringing any biological materials to the CLS? Yes

Does this research require biological material to be imported into Canada? No

Does this research involve human tissue and/or biological fluids? No

Does this research involve the study of aboriginal people’s culture? No

Does this research involve: Live animals? Yes

All research performed at the Canadian Light Source facility involving animals (use of non-human vertebrates and cephalopods) requires review and protocol approval by the user’s home institution and/or appropriate animal care committee and the University of Saskatchewan’s Animal Research Ethics Board.

Who will be caring/handling animals while at CLS? Gurpreet Atulak - University of Saskatchewan, College of Medicine

Animal tissue and/or biological fluids from live animals? Yes

All research performed at the Canadian Light Source facility involving animal tissues or biological fluids from animals requires an approved University of Saskatchewan (UoS) Animal Use Protocol (AUP) Category A Short Form.

Biological Materials:

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Name</th>
<th>Units</th>
<th>Quantity</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>Live animals</td>
<td>Mouse</td>
<td>2</td>
<td>2</td>
<td>None</td>
<td>Charles River</td>
</tr>
</tbody>
</table>

Briefly describe method of anaesthesia:
The mice will be anaesthetized by oral anaesthesia using isoflurane administered by an over the face mask.

Briefly describe method of monitoring anaesthetized animal (pulse, blood oxygen level, etc.):
Mice would be continuously monitored during imaging. Once anaesthesia is administered, confirmation through pedal reflexes would be done and the mouse is in the imaging hutch, there would be real time visual camera aided monitoring from the control room.

Briefly describe method of restraint in hutch:
The mouse will be restrained and anaesthetized in a test tube using a protocol that has been developed by Dean Chapman, Ken Gagnon and Gurpreet Atulakh.

Briefly describe each sample and state (fixed, in suspension, frozen, etc.) and its off site preparation procedure:
The mouse will be individually restrained and anaesthetized in a test tube at the CLS. This protocol has been commonly used on the BM1T beamline.

Describe how the samples will be transported to the CLS:
The mice will be delivered by the animal care staff of the University of Saskatchewan vivarium.
Will any sample preparation be done at the CLS? Yes
Include details on the procedure, the use of any safety controls, personal protection equipment, mode of disinfection, etc.:
The mice will be placed into a test tube at the BMIT beamline at the CLS facility and will then be anaesthetized by oral inhalation. Gurpreeet will perform this procedure and will be constantly monitoring the mouse using a humane intervention point checklist. She will be wearing proper safety equipment, such as golves.
Will any waste (biological and/or sharps) be generated Yes while at the CLS?
a) Solids containing or contaminated with biological materials (i.e., petri dishes, disposable pipette tips, agar etc.):
The tissue sections obtained from the mice will be kept for study, and then disposed of using the proper protocol.
b) Liquids containing or contaminated with biological materials (i.e., cultures, buffers, media etc.):
N/A
c) Sharps contaminated with biological materials (i.e., syringes, scalpel blades, glass pipettes etc.):
The needles which are used to inject the mice will be disposed of in a sharps container.
d) Sharps not contaminated with biological materials:
e) Pathological materials (i.e., animal carcasses, tissues, etc.):
The animal carcasses will be disposed of using standard operating protocols.
Identify any other additional risks, concerns or safety controls related to the biological materials and/or tasks to be performed that have not already been adequately identified or explained. These may include recombinant risks, environmental risks, regulatory requirements, methods for preventing beamline work area contamination etc.:
No, there are no additional risks so long as the vaccines are not recombinant. This will be ensured with the VIDO science team prior to experimentation.
Does your work involve Genetically Modified Organisms, Genetically Modified Microorganisms or Transgenic Organisms? No

Radioactive Material Information
Are you bringing anything radioactive? No

Nano Material Information
Are you bringing any nanomaterials to the CLS? No

Pesticide Information
Are you bringing pesticides to the CLS? No

Will any sample preparation be done while at the CLS regardless of simplicity (i.e. transferring a few mg of material into a sample holder must be disclosed)? Yes
Briefly describe the sample preparation procedure (regardless of simplicity):
The mouse will be anaesthetized at the CLS. This will follow the procedure stated in the standard operating procedure which is attached to this proposal. This SOP was followed during our initial clinical CT imaging and was proven to work well.
<table>
<thead>
<tr>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you bringing any equipment to the CLS to assist you with this experiment?</td>
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</tbody>
</table>
University Committee on Animal Care and Supply  
(UCACS)

UNIVERSITY OF SASKATCHEWAN

<table>
<thead>
<tr>
<th>SOP #</th>
<th>Title</th>
</tr>
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<tbody>
<tr>
<td>SOP_20130041</td>
<td>K-edge Subtraction Imaging of Intramuscular and Subcutaneous Injection Dynamics in Mice</td>
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</tbody>
</table>

To describe intramuscular and subcutaneous injection procedures in mice and monitoring during imaging.

This policy is in place to ensure the safe and ethical treatment of the animals being studied as well as the safety of the researchers.

PPE: Lab coat or gown, Gloves, Close toed shoes  
Isoflurane anesthesia system (with medical grade oxygen)  
Anesthetic work station (with induction chamber and nose pieces for mice)  
Heat lamp  
Eye ointment – lacrelibe  
1 ml syringes  
27 and 25 gauge needles  
0.15 g NaI/ml PBS [1 mM]  
Bleach/Disinfectant in spray bottle

All injections must be performed using sterile needles and syringes. A new needle and syringe should be used for each cage of mice. Draw up into the syringe and needle, the amount of solution to be administered. Line the bevel of the needle with the numbers on the syringe.

<table>
<thead>
<tr>
<th>INJECTION SITES AND VOLUMES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ROUTE</strong></td>
</tr>
<tr>
<td>SQ</td>
</tr>
<tr>
<td>IM</td>
</tr>
</tbody>
</table>

**INTRAMUSCULAR (IM) INJECTION**

Restrain the mouse by the scruff method with your non-dominant hand. Secure the rear foot nearest to you beneath your little finger and lower thumb. Swab the area to be injected with 70% ethanol. Insert the needle, bevel up, into the caudal thigh at a 45° angle. Aspirate to ensure that you have not entered a blood vessel. If no blood is seen, slowly inject the material. A tail first restrainer can also be used for IM injections. Gently pull the foot of the leg to be injected through the restrainer and locate the caudal thigh muscle.

**SUBCUTANEOUS (SQ) INJECTION**

Restrain the mouse by the scruff method. Use your thumb and forefinger to make a tent of skin over the scruff. Prep the area with 70% ethanol. Insert the needle, bevel up, at the base of the tent. The needle should be inserted parallel to the skin and should be directed toward the posterior of the animal. Aspirate to ensure proper placement and inject the material.
Preparation and Handling of Animals during Synchrotron Imaging

- Mouse would be transferred to the imaging hutch (BMIT POE-2) in transfer cage.
- Mouse would then be anesthetized using 5% isoflurane induction by inhalation in the acrylic induction chamber. Mouse would be checked for loss of pedal and blinking reflexes.
- Sodium iodide will be administered to anesthetized mouse by an IM/SQ injection (27-25 gauge needle) 0.15 mg NaI/ml isotonic phosphate buffered saline [1 mM, pH 7.4].
- After NaI administration, the mouse will be restrained for safety in a specially designed holder with free flowing air and maintained under anaesthesia with 1.5 - 2% isoflurane. Mice will remain immobilized for the 1-30 minutes required for K-edge subtraction imaging. Two closed circuit cameras trained on the mouse, a digital thermometer and pulse-oximeter will be used to monitor body temperature as well as mouse breathing, signs of animal distress, and ambient room temperature. The eyes would be lubricated with lacticube and isotonic sterile saline would be injected subcutaneously at 10 mL/kg B.W./hour to avoid dehydration during anaesthesia.
- If deemed appropriate, the mouse will also be imaged by x-ray CT while maintained under continuous flow of isoflurane/oxygen.
- At the end of imaging period, the mouse will be returned to the acrylic induction chamber and given a lethal dose of isoflurane for 5 min, followed by cervical dislocation.
- Animals will be returned from the BMIT POE-2 hutch to the animal preparation area via the acrylic induction chamber.
- The tissue of interest would be excised and fixed in 4% formaldehyde in the bio-safety hood.
- Induction chamber, mouse holders, and animal preparation area will be disinfected.

Regardless of the method used for intramuscular injections, it must be noted that the sciatic nerve runs along the length of the femur. It is very important to avoid injuring this nerve. This is best accomplished by pointing the needle, caudally rather than cranially, into the caudal thigh muscles.

Dr. Melanie Van der Loop would be present at the time of imaging. Any adverse situation would be accordingly handled.

Bleeding at injection site – apply pressure until the bleeding stops and clean with gauze and sterile water

U.S. National Institute of Health
SOP#2 - K-edge subtraction and computed tomography imaging of live mice
Intraperitoneal (IP) Injection in Rats and Mice SOP