Reducing Physiological Noise in Laser Scans of Neonatal Ferrets' Visual Cortex

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May 3, 2007

1 The Clients and Background

For this research project we are working in collaboration with Bill Eddy, Department of Statistics, Carnegie Mellon University, and Justin Crowley, Department of Biological Sciences, Carnegie Mellon University.

The overall goal for this project is to uncover the causes behind the physiology and anatomy of the visual system. Specifically, the optic nerve originates from the eyes, crosses in the lateral geniculate body, and then connects with the visual cortex in the brain. However, the reason for this peculiar structure is currently unknown. Further, the specific patterns of neuron growth in the visual cortex is unclear and also of interest.

The subjects for this research are neonatal ferrets. Neonatal ferrets were selected as they are born blind without fully developed optic nerves; thus the entire growth and connection of their optic nerves to the visual cortex can be observed. Observations regarding the growth are made by comparing images of the visual cortex at different timepoints of the ferrets' development. However, movement caused by the ferrets' physiology during the capturing of the images has made it difficult to compare these images. Our goal is to find and implement techniques that will reduce this physiological noise in the images.

2 Project Overview

Neuron development in a ferret's visual cortex is tracked by comparing specialized three-dimensional images taken at different stages of its life. In order to capture these images, a special protein - green fluorescent protein (GFP) - is injected into the ferret, which causes its neurons to fluoresce when subjected to extra energy provided by outside photons. A two-photon laser scanning confocal microscope is used to emit the photons as well as capture these images. As the laser scans the visual cortex, it fires one photon from each of two lasers, and then captures the image precisely at the point that the two photons meet in the visual cortex. If a neuron is present at that point, the microscope will capture it

by recording the luminosity of the green glow. By emitting two photons to different layers of the visual cortex and only capturing the spot where they meet, the microscope is able to record the image three-dimensionally. An example of a two-dimensional slice of an image in grayscale can be seen in Figure 1. The researchers intend to take images repeatedly over time, and subtract pairs of them to document the growth of the neurons in the visual cortex.

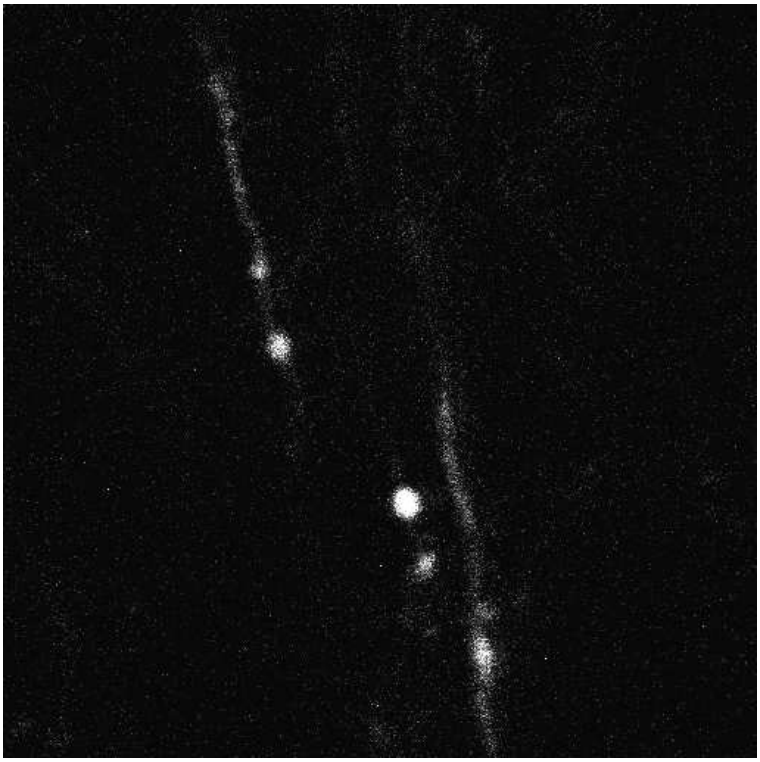

Figure 1: This is an example of a two-dimensional slice of an image recorded by the laser scanning microscope. The linear clustering of white pixels represent the presence of a neuron.

In addition to the image data, there is a separate set of data that includes the physiological characteristics of the neonatal ferret. As images are being taken, the ferret is anesthetized; its heart is beating at a rate of 5 beats per second and it is breathing at a rate of 3 breaths per second. The microscope takes approximately two seconds to scan each slice of an image. The combination of the time that it takes to scan an image along with the physiology of the ferret causes brain movement, and in turn creates a large effect on the appearance of the image. These physiological effects manifest themselves in pixel movement rather than pixel intensity. Each individual pixel can move up to 10 pixels in any combination of the three image dimensions. The movement of each pixel

caused by the physiological effects, keeps the researchers from successfully lining up the images. For example, if the brain is shifted slightly upward because of increased blood flow caused by a heartbeat, a pixel that is recorded in one slice may actually be two slices below.

Our goal is to create an image that is free of movement caused by the heartrate and respiration of the ferret. Initially, we were going to create a statistical model that moved each individual pixel to its correct location in the image. However, all models were shown to be inadequate in solving this particular problem. Thus we took a new approach, which is to use replications of images to create one correct image by combining the parts of the replicates that were least affected by the ferrets' physiology. By creating correct images, the researchers will be able to line up images taken at different times and carry out their ultimate goal of learning about the growth of neurons in the visual cortex.

3 The Data

3.1 Image Data

The image data consist of the pixels of the image and their associated three-dimensional coordinates as recorded by the laser scanning microscope. Specifically, each pixel is given:

- an integer from 1 to 4095 corresponding to a grayscale, where the darker the pixel the smaller the number
- an integer from 1 to 512 corresponding to the pixel location in the x plane or "row"
- an integer from 1 to 512 corresponding to the pixel location in the y plane or "column", and
- an integer from 1 to roughly 32 corresponding to the location or "slice" of visual cortex in the z plane.

Additionally, each plane is scanned multiple times before moving to the next z plane, thus adding an additional variable of time.

3.2 Physiological Data

Before, during, and after the visual cortex is scanned, physiological data and experimental data is recorded. The physiological data includes: EKG, a recording of the electrical signal from the heart, which is a proxy measure of blood flow; pulse oximeter, a measure of the oxygen level in the blood - also an approximate measure of the blood flow; and respiration, measured as the expansion of the chest. Experimental data includes various pulses from the laser scanning microscope signaling when scanning is taking place. These are referred to as synchronizations, or line synch pulses.

The physiological data is collected at a rate of 5000 samples per second, whereas the microscope scans intermittently due to position changes. Since the physiological, experimental, and image data are recorded concurrently, we are able to use the experiment data, or the synch pulses, to match the physiological observations with the position, or pixels of the image.

4 Working with the Data

4.1 FIASCO

Based on the size and type of the data, all of our work is done with the computer software FIASCO, which stands for Functional Image Analysis Software - Computational Olio (Lazar, 2001). FIASCO can compute many types of mathematical operations and statistical methods on images and larger data sets such as our physiology data set. Consequently, FIASCO is indispensable to our work.

4.2 Matching Dimensions

To work with the image and physiological data sets together, first we needed to merge them, more specifically match each pixel with a physiological observation. As stated earlier, the image data is measured in three dimensions plus time. Often, the researchers will scan each xy plane three times before moving on to the next z slice. Since we want to find the pixels that are associated with the least movement, we want to have a physiological value to correspond with each pixel in an image. To obtain these values, we worked closely with the synch pulses. After some initial exploratory analysis, there appeared to be roughly three physiological samples recorded for every line scanned in an image. This can be seen in Figure 2, where the x-axis measures the physiological sample and the y-axis measures the synch value (from 0 to 2047). [1] When the synch value is at 2047, this signifies that the sample was taken while the microscope was scanning. There are generally three consecutive samples with this synch value followed by six or seven that are recorded while the microscope was repositioning. This six-three pattern was shown to be rather consistent throughout the data; that is within each slice.

It takes approximately 500 microseconds to scan a single y line (1 microsecond per pixel), and since during this time there is only .24% of a single heartbeat and .15% of a single breath, we can think of physiology of the ferret as nearly constant. For this reason, it makes sense to average the three physiological sample values for each line. From there, we can assign that single physiological value to each pixel in the corresponding y line, and thus successfully match up the dimensions of the two data sets.

[1] The maximum value of the synch channel comes from the total number of bits used for recording the physiological data. Here, $2047 = 2^{11} - 1$, indicating that there was a 12 signed bit channel used in recording. The number of bits used may change during different sessions of recording.

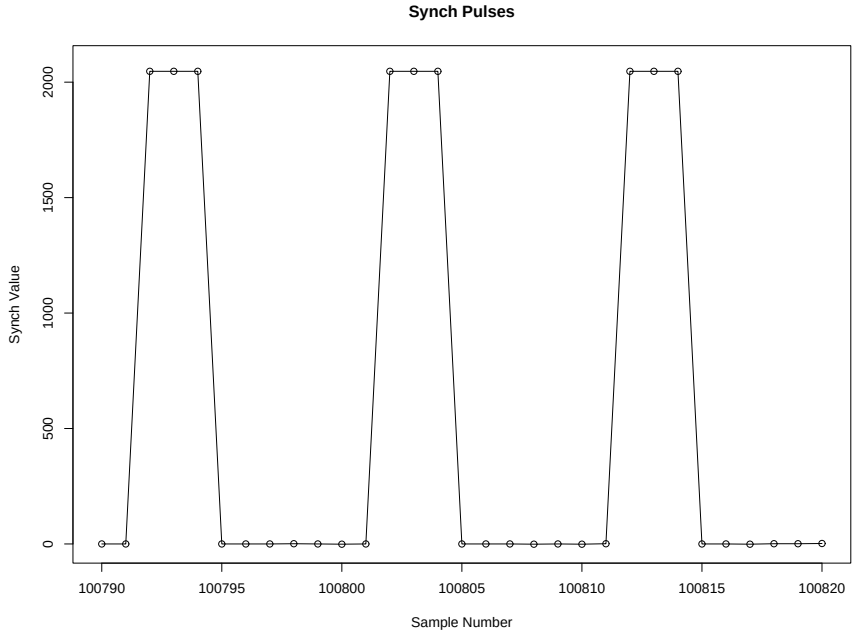

Figure 2: This is an image of the synch pulses plotted for a small amount of physiological samples. The x-axis is the physiological sample number and the y-axis gives the synch value. The values at the top signify the times when a sample was recorded and the microscope was scanning. Notice the six-three pattern with six or seven observations at 0 and three up at 2047.

4.3 Missing Physiological Data

Upon further investigation of the physiology data, we discovered a systematic error in the recording of the physiological and experimental data. Approximately .02% of the physiological and experiment data were not recorded, hindering our ability to match each image y line with a physiological observation. In the line synch pulses, the missing observations manifested itself both in a deviation from the six-three pattern as discussed, as well as a count of less than 512 synch pulse triplicates in a z slice. Figure 3 shows one of these dropped synch pulses.

To correct this, physiological data had to be imputed with observed data. Data that is missing in the middle of a slice is apparent and easily corrected. The first three known data points after the missing data are used to replace the missing values. That is, the physiological data corresponding to the subsequent y image line is used for the previous image line.

Physiological data that is missing at the beginning of the slice or at the end of a slice poses a larger problem. As it is not clear whether the missing data is at the beginning or at the end of the slice from the line synch pulses, an additional *slice* synch pulse must be examined. The slice synch pulse signals the start of

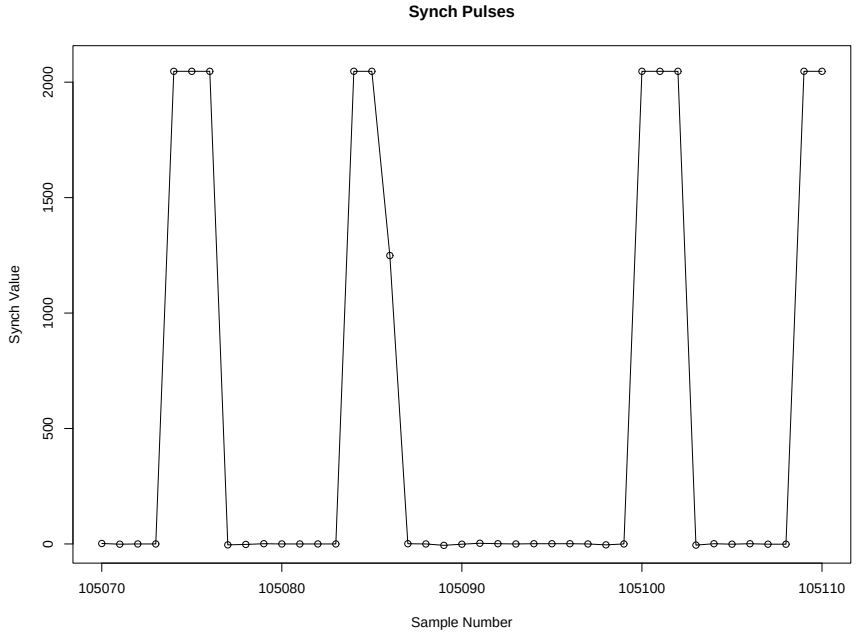

Figure 3: This is an image of the synch pulses plotted for a small amount of physiological samples. The x-axis is the physiological sample number and the y-axis gives the synch value. The values at the top signify the times when a sample was recorded and the microscope was scanning. Notice the large gap from approximately 105085 - 105098, indicating that some synch pulses have been dropped when the microscope was scanning.

the microscope scanning a new z slice. Therefore, it can be inferred that a slice with 511 line synch triplicates that is missing a slice synch pulse is missing physiological data at the beginning of the slice. This case is treated similarly to the case where the data is missing in the middle of the slice; that is, the first three known data points after the missing data are used. If the slice synch is present, the missing data is assumed to be at the end of the slice. Therefore, the last three known data points before the missing data are used to replace the missing. Because of the relatively small change in physiology between lines, this imputation should not affect the results.

5 Creating the True Image

5.1 General Idea

In order to create a true image it makes sense to look at the replication of an image that has the least movement. Even further, we can look at the phys-

iology effect between each line replication and use the one that has the least movement. In other words, we use the line with the minimum physiological value and presumably the least amount of brain movement associated with it to create an image.

5.2 Change of Data Acquisition

The researchers for this experiment have a process for beginning the recording of each slice of image data (Smith, 2006). This process involves triggering the start of a laser scan for a slice when the ferret is at the peak of its QRS cycle (the top of a heartbeat). The reason for triggering scanning is to minimize the variation between images caused by physiology. However, triggering each slice at the same point in the ferrets' physiology will not provide a replication that has the minimum physiological value. Thus, we have suggested that the researchers used a lagged triggering process that starts imaging for each replication of a slice at different points in the QRS cycle. Figure 4 shows EKG data plotted over three replications of a particular z slice, with a vertical line at the timepoint where the microscope was triggered to begin scanning. By using lagged triggering, we are guaranteed to have at least one y line replicate showing minimum movement due to physiology.

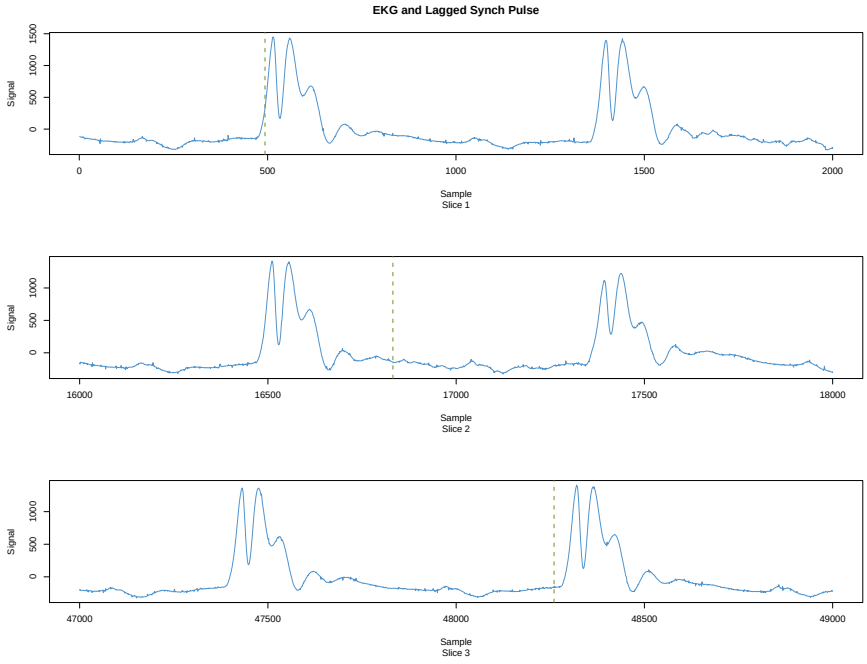

Figure 4: This is an example of lagged triggering. Each replication of the z slice is triggered to begin scanning at a different equally spaced timepoint in the QRS cycle. The vertical lines represent these timepoints for each replication.

5.3 Pulse Oximeter

We have two sources of measurement of blood flow, EKG and the pulse oximeter. The EKG is measured as an electric signal and is very accurate in the measuring of the heart rate and blood pressure. The pulse oximeter measures the color of the blood, with reder blood associated with a higher pressure of blood. Due to movement of the ferret as well as differences in the skin color of the ferret, the pulse oximeter is known to be less precise.

However, since the goal is to find the lines of an image that were captured during a period with minimal brain movement, the pulse oximeter may be the best available measure of the movement caused by the heart rate and subsequently blood pressure. The further the blood travels away from the heart, the more the blood pressure is slowed by viscous friction from the arteries. Therefore, a measurement of blood pressure that is taken equidistant from the heart as the brain to the heart would be more representative of the movement in the brain. Of the two measurements of the heart rate, the EKG is measured at the source, while the pulse oximeter is measured at the ferrets tongue or claw. Therefore, although the EKG may be a more accurate measure, the pulse oximeter will be used as the physiological point of interest to minimize movement. Figure 5 shows the lag in blood pressure measured by the EKG and the pulse oximeter.

6 Future Plans

Further coding in FIASCO must be done in order to replace all missing synch values in the physiology data. Once completed, we will have successfully matched the dimensions of the image and physiology data sets. From there, we must create a script to find each line with the corresponding minimum physiology value. Then we can amalgamate all of the lines for all of the slices to create one true image. Once we have a complete method for creating a true image, the researchers will be able to implement this method and to line up the corrected images taken at different timepoints in the ferrets' life to document neuron growth in the visual cortex.

7 References

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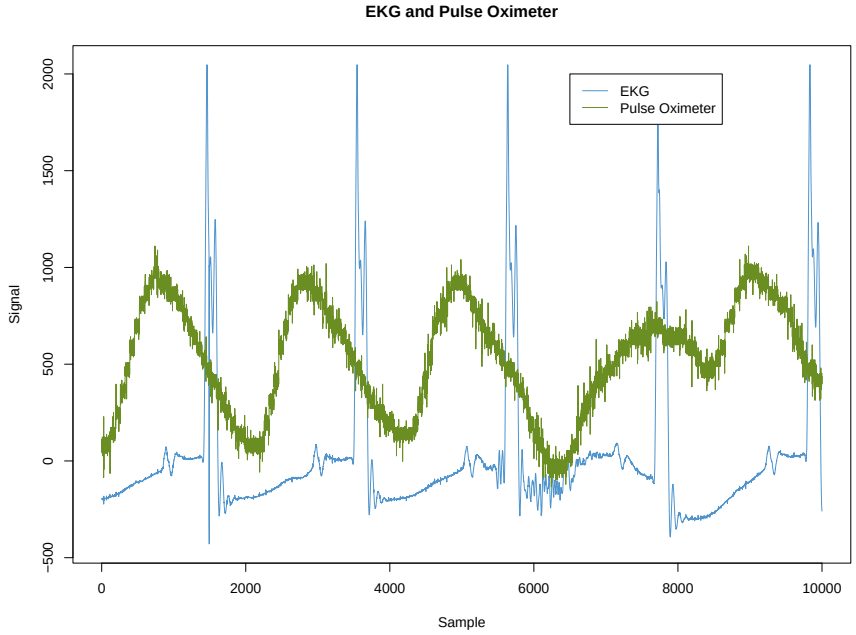

Figure 5: This is a plot of both the EKG and Pulse Oximeter signals for a ferret. The shape of the pulse oximter is more representative of the movement in the brain caused by heartrate.

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