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

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March 5, 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 Biology, Carnegie Mellon University. The motivation for this project comes from the fact that scientists know that in the visual system, the optic nerve comes from the eyes, crosses in the lateral geniculate body and then connects with the visual cortex. However, they do not know why, or what causes this growth. Neonatal ferrets were selected for this particular study because they are born without fully developed optic nerves; thus the entire growth and connection of the optic nerve to the visual cortex can be observed.

The project described in this paper, as with much of Professor Eddy's research, presents a general problem requiring a novel statistical solution rather than standard statistical analysis and/or classification. Specifically, we are trying to use statistical techniques to reduce the noise in the images of the visual cortex brought about by physiological aspects of the neonatal ferrets.

2 The Data

2.1 Image Data

Neuron development in a ferret's visual cortex is tracked by comparing specialized three-dimensional images taken at different stages of its life, usually at two-week intervals. 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.

Subsequently, our data consist of the pixels of the image and their associated three-dimensional coordinates. 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 32 corresponding to the location or "slice" of visual cortex in the Z plane. Additionally, each plane is scanned three times before moving to the next Z plane; so there is an additional time variable, taking on the values 1, 2, and 3.

2.2 Physiological Data

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 only anesthetized so its heart is beating at a rate of 5 beats per second and it is breathing at a rate of 3 breaths per second. Each slice takes two seconds for the microscope to scan. Combined, the physiological characteristics have a large effect on the appearance of the images since they make the ferrets' brain move. 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 x, y and z directions.

We are fortunate enough that the lab researchers are able to record the physiological data in a manner that it can be lined up with the images. We are mainly interested in three of the channels recorded, one for the heart rate (ekg), one for the respiration (rsp) and one that is called syn which gives us line synchronization. The physiological data is collected continuously at a rate of 5000 samples per second, whereas the scanner is running intermittently due to position changes. This specific channel syn, indicates which of the samples of the physiological data are taken when the microscope is scanning. A sample plot seen in Figure 1 is an example of a subset of the ekg channel plotted continuously with vertical lines at the time points where the samples line up with microscope scanning.

3 Project Overview

What the researchers intend to do is take these images repeatedly over time and then line up two images, subtract them and document the growth of the neurons in the visual cortex. The problem with doing this is that the movement of each pixel caused by the physiological data, keeps the researchers from successfully lining up the images. For example, if the brain is shifted slightly upward at the peak of a heartbeat, a pixel that is recorded in one slice, say $Z = 3$, may actually be two slices below, in this case $Z = 5$. Again, this movement can be up to 10 pixels not only in the Z plane, but in the X and Y planes as well. Looking

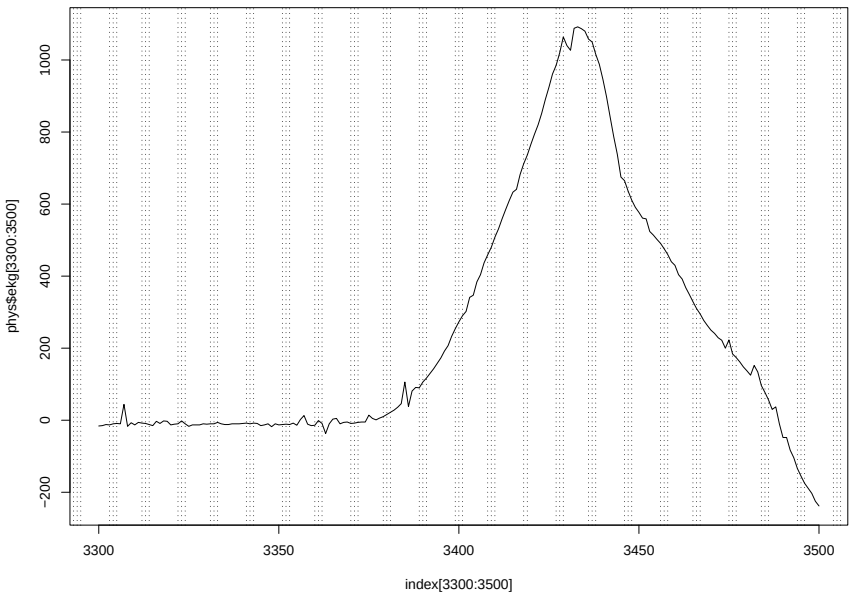

Figure 1: This is an image of the ekg channel plotted continuously over approximately four hundredths of a second with vertical dotted lines at the times where the ekg was sampled and the microscope was scanning.

closely at the images, the difference in pixel location over the three times taken just seconds apart is apparent. The small movements can be seen in Figure 2, by looking closely at the positions of the features in the images. To remedy this movement, we are going to create a statistical model that moves the pixels to their correct location in the image. By doing this, the researchers will be able to carry out their ultimate goal of learning about the growth of neurons in the visual cortex.

4 Statistical Methods

A model for putting the pixels into their correct location in an image can be done by regressing the image data on the physiological data. The statistical tools that may offer assistance in achieving this goal put forth by Professor Eddy are linear regression and a Taylor series approximation. Specifically, if the structure of the visual cortex is some unknown function, p, then each pixel color can be given by its location. That is, $g = p(\mathbf{X})$, where g is the color of the pixel in grayscale, p is the function given by the visual cortex structure, and $\mathbf{X}$, is a vector of the x, y, z coordinates within the visual cortex. However, since each pixel is recorded in a finite amount of time, $\mathbf{X}$ can also be thought of as

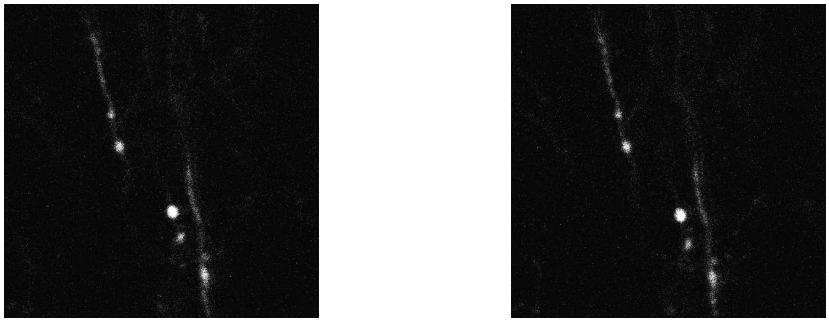

Figure 2: These are two images of a single x, y slice shown at two consecutive times. The small movement of the features shows the effects of the physiology of the ferret.

time. Because of this, the physiological characteristics may also be considered as a function, $\mathbf{C} = bh(\mathbf{X})$, where $\mathbf{C}$ is a vector consisting of the heart rate and respiration component, bh is a function of the heart rate and respiration given when the scanner is at the location $\mathbf{X}$. Using this functional notation, our image can be seen as:

$$g = p_1(\mathbf{X} + bh(\mathbf{X})) \tag{1}$$

that is, the color pixel we actually see, g is a function of where the pixel should be, plus the movement caused by the heart rate and breathing. Since a series of three images are taken, we have three functions, p_1, p_2, and p_3. Consequently, the analysis will be a simple linear regression, where we wish to minimize:

$$\left[p_1(\mathbf{X} + bh_1(\mathbf{X})) - p_2(\mathbf{X} + bh_2(\mathbf{X}))\right]^2 \tag{2}$$

with bh_1 and bh_2 being the standard β coefficients. However, since the functions are not linear, we will use a Taylor approximation to linearize them. Equation 1 becomes,

$$p_1(\mathbf{X} + bh_1(\mathbf{X})) = p_1(\mathbf{X}) + bh_1(\mathbf{X})p_1'(\mathbf{X}) \tag{3}$$

where $p_i'(\mathbf{X})$ is the gradient of the image, $p_i(\mathbf{X})$. Then from Equations 1 and 3, Equation 2 becomes:

$$\left[p_1(\mathbf{X}) + bh_1(\mathbf{X})p_1'(\mathbf{X}) - (p_2(\mathbf{X}) + bh_2(\mathbf{X})p_2'(\mathbf{X}))\right]^2 \tag{4}$$

By solving this regression problem, we should be able to obtain coefficients for bh_i, which will demonstrate how much movement the heart and breathing are causing. The pixels can then be moved back accordingly to get a truer and clearer image of the visual cortex.

5 Difficulties

5.1 Proposed Method

Since there is little time between the consecutive slice images, we can assume that $p_1(\mathbf{X}) = p_2(\mathbf{X})$. And therefore, $p_1'(\mathbf{X}) = p_2'(\mathbf{X})$. Consequently, the solution that minimizes Equation 4 is the trivial solution, that is $bh_1(\mathbf{X}) = bh_2(\mathbf{X}) = 0$. Because of this, an additional constraint must be incorporated into the model. However, at this time the correct constraint is unknown.

5.2 Instantaneous Scanning of the y Lines

Because of the speed at which the microscope scans a single line, we are treating each y line as recorded instantaneously. For each of the y lines, we have found from the syn channel that the physiological data is sampled on average three times. We have been successful at averaging these three consecutive samples taken during scanning and aligning them with their corresponding y line. Since each y line is instantaneous, there is not time for any substantial change in the physiological measurements, which is why it is logical to average them. By doing this we are assigning each of the 512 pixels of a y line, a single physiological value. This becomes a problem when we are looking at the gradient of each image, needed for Equation 4. In taking the gradient, we are looking at the change of pixel intensities in neighborhoods around each pixel in all three dimensions. However, by assigning each pixel within a y line a single value, we cannot observe any movement due to physiological effects from pixel to pixel, but rather movement of the entire line with respect to the single physiological value. This hinders us from finding unique coefficients of the physiological data to each pixel.

5.3 Data Size

One difficulty that we face is that the data that we have is extremely large. For instance, the number of pixels in one image that is $x \times y \times z$, has $512 \times 512 \times 32$ pixels, and then there are three of each x, y slice taken. Because of the sheer size of just the image data, we have to use a different computer program called FIASCO, which stands for Functional Image Analysis Software-Computational Olio. FIASCO is able to compute all types of operations and statistical methods on images, which is why it is so beneficial to our work on this project. Learning this software however, may create some complications in working with the data.

6 Developments

At this point we have aligned the two data sets and assigned each pixel of the image data, a physiological value. Further, we have successfully estimated the gradient of an image by taking the difference between the means of neighboring pixels. We are in the process of correcting our model by adding the necessary constraint to find the nontrivial solution that allows us to realign the pixels to their correct location. The details of this constraint however, may necessitate the modification of our goals and/or change the focus of our research. We understand that although our project is small, any work accomplished may be a critical part of very important research regarding neural structure and growth.