Designing a physiological research laboratory and developing its control program at the University of Manchester

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Summary – In this document, I will describe the design of the physiology laboratory I have planned at the University of Manchester. To this end, I will briefly review the physiological background related to the task, then present the implementation of the laboratory designed on this basis. During my presentation, I will focus on the MATLAB application I developed, which controls the measuring instruments and records the measured data.

1 Introduction

Some functional brain imaging techniques are based on the observation that there is a close relationship between neural activity in the brain and vascular responses, such as increased blood flow and oxygenation. The task of basic physiological research today is to bring researchers closer to understanding this process and thereby facilitate a more thorough investigation of the functioning of the human brain.

My research combines multi-wavelength optical imaging and electrophysiological data recording, which is expected to reveal the relationship between spectral components and the recorded electrode signal.

By illuminating the tissue with narrow-bandwidth light, even very small changes in the surface of the brain can be detected with a high-resolution monochrome camera, enabling the examination of brain activity. The sources of these optical

signals are blood volume, oxyhaemoglobin and deoxyhaemoglobin concentration, and tissue scattering.

The first step in my research was to design a laboratory suitable for studying the neural processes outlined above.

2 Physiological basics

A basic prerequisite of any engineering work is that the designer must be familiar, even if only partially, with the scientific knowledge without which the set goal, in this case the design of the laboratory, cannot be achieved. Thus, in the first chapter, I will briefly and simply summarise the relevant physiological knowledge.

2.1 Electrical activity of nerve cells, encoding of information

Living creatures are unique in that they use receptors to recognise their environment. These receptors produce chemical substances in response to mechanical stimuli. When these chemical products reach the nerve cells, they activate ion pumps located in the cell wall, which specifically pump potassium, sodium, calcium, and/or chlorine ions through the cell membrane [1]. The resulting potential difference was first measured by [2]. Figure 1 shows that the potential difference rises steeply from a resting potential of -40-50 mV to an action potential of +40 mV. This is followed by a rapid hyperpolarization phase, during which the potential drops to a value 20-30 mV below the resting potential. During this hyperpolarization phase, the nerve cell cannot be stimulated.

The potential change that occurs when a nerve cell is stimulated is referred to as an action potential in the literature. The amplitude of the action potential depends only on the type of nerve cell and is not influenced by the strength of the stimulus. This raises the question of how the degree of sensation is encoded in the nervous system. From an engineering perspective, the answer is simple. If we assign a binary value of 1 to the action potential and 0 to the resting potential, we already have a coding algorithm. Of course, it is not that simple, as the same stimulus will not produce the same code every time. The simplest way to describe neural coding is by the frequency of successive action potentials. A nerve cell responds to the same stimulus with a statistically well-described frequency of action potentials. [3]

Of course, it is never a single nerve cell is stimulated, but rather a group of nerve cells innervating the same area that collectively transmits the information. Accordingly, multiple nerve cells are stimulated in the brain. An electrode placed in the space between brain nerve cells can record the potential change created

jointly by several nerve cells, which appears in the form of spikes with different peak values. Figure 2 shows a detail extracted from an electrode signal I recorded, showing spike trains from different nerve cells (signals of nearly identical amplitude originate from the same cell).

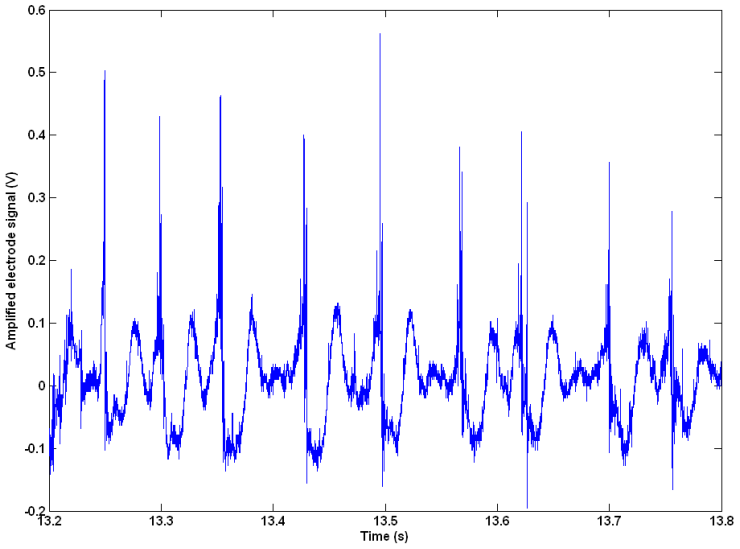


Figure 1. Electrode signal. A series of consecutive spikes. The signal, which shows the potential change generated jointly by several nerve cells (spikes with different peak values), was recorded with an electrode placed in the intercellular space.

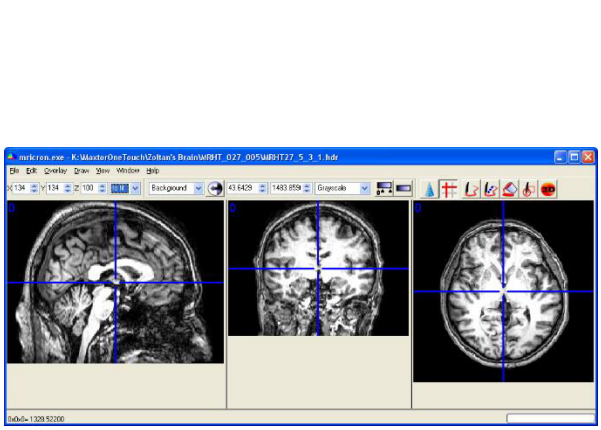
The signal transmitted by peripheral nerve cells reaches the cerebral cortex through the neural pathway. In the brain cortical nerve cells process the information and then send response signals to the periphery. This processing is a very energy- demanding process. The weight of a human brain operates in an 'oxygen cloud' and the incomplete combustion that occurs in muscles under high stress is not permitted here. This is also shown by the following data. The weight of a human brain is about 2% of ourthe body weight, but its oxygen consumption is 20% and its glucose (energy) consumption is 25%, which can increase several times over during intense mental activity, such as learning. This results that the blood and oxygen supply increases in the active areas. The question is, how can this higher signal level be detected? I will review this in the next section.

2.2 Brain imaging techniques

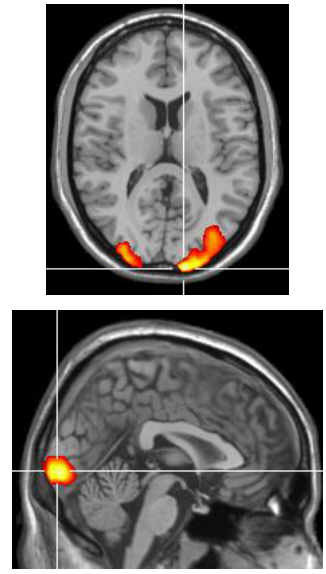
People have always been interested in how the living brain works. The problem is that our brain is inaccessible, as nature has understandably placed it in a well-protected hard shell (the skull). The electroencephalograph (EEG), invented in the

first quarter of the twentieth century, was the first device that could be used to examine the brain from the outside. The EEG has very good temporal resolution, but it cannot precisely determine which area of the brain the signal is being received from.

The next imaging tool was the positron emission tomography (PET) scanner, which was introduced in the 1970s. Finally, magnetic resonance imaging (MRI) appeared in the 1980s. Figure 2.a) shows an MR image taken by a 3T MR machine at the University of Manchester. Both methods (PET and MRI) measure haemodynamic changes in the brain rather than neural activity directly. The two techniques have a spatial resolution of around 1 mm, but their temporal resolution is only in the range of seconds. Another problem arises in relation to their application, as these two methods cannot be used to determine the nature of the neural function associated with the detected haemodynamic change, e.g. the activity of a given brain area may result in an inhibitory or excitatory neural response.



a.)



b.)

Figure 2. MRI and fMRI

Figure a) shows an MR image. Figure b) clearly shows the areas activated by the fMRI technique.

In modern brain research to map brain functions, functional magnetic resonance imaging (fMRI) based on MR is most commonly used. The essence of this technique is to compare an MR image of the patient's brain at rest with an MR image recorded during a well-defined activity (e.g., tracking moving signals with the eyes). The difference signal is then projected onto the image of the brain, see figure 2. b.).

The methods described in the previous paragraphs have insufficient temporal or spatial resolution to answer the question of what relationship exists between neural activity in the brain and the haemodynamic response. One possible way to answer this question is to observe the brain directly using optical imaging while measuring neural activity with electrodes.

2.3. Partial direct optical examination of the brain surface

The skulls of some small rodents are thin and translucent enough to allow images of the brain surface to be recorded through them in living subjects. Four physiological characteristics influence the amount of reflected monochrome light that is recorded. [4] The four components shown in Figure 3 are:

1. Haemoglobin saturation. This factor comes from the colour difference between oxygen-saturated arterial blood (oxyhaemoglobin) and venous blood (deoxyhaemoglobin).
2. Blood Volume. The blood vessels in the activated areas of the brain dilate, causing a decrease in the amount of reflected light.
3. Tissue Scattering. The shape of excited nerve cells changes due to the inflow and outflow of ions, which modifies the amount of light absorbed.
4. The amount of a special chemical substance (cytochrome oxidase) present in nerve cells also influences the degree of light reflection. The role of cytochrome oxidase is currently unknown.
5. Water content, which is not shown in the figure, only causes changes in the infrared light range. [5]

The combined change in light absorption of the factors listed above results in a change in the amount of light reflected and thus recorded. Hillman presents the change in light absorption of these components as a function of the wavelength of the illuminating light in his summary article. [5] With this data, it is possible to select the required wavelength of the illuminating light.

Since four main components cause the change, in practice it is standard to use at least four different wavelengths of light at approximately the same time. Hillman outlines a measurement setup in which white light is passed through filters arranged on a wheel. By rotating the wheel, light sources of different wavelengths can be produced. The problem is that the filter wheel must be synchronized with the camera, i.e., the rotation of the wheel must be synchronized with the camera's exposure time. [5]

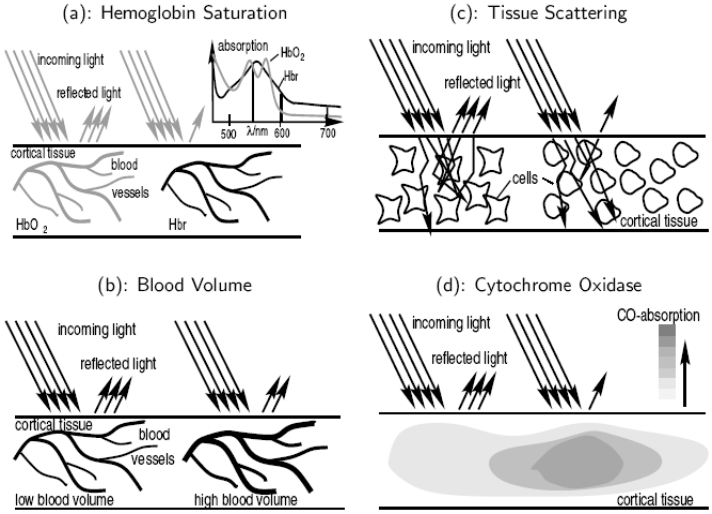
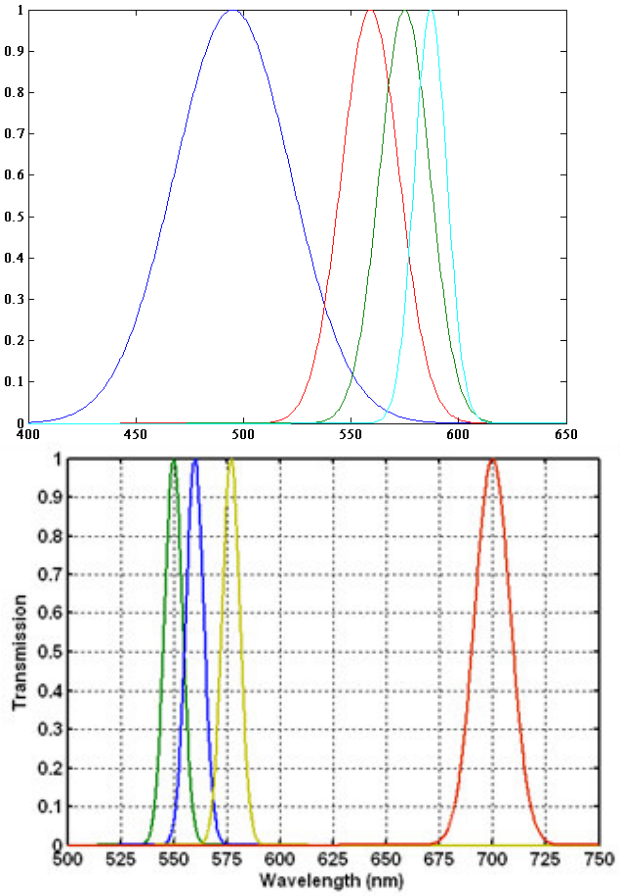


Figure 3. Four factors influencing the amount of light reflected from the surface of the brain. [6]

The filters used must be sufficiently narrowband so that the spectral signals received do not overlap. In practice, the centre frequency of the filters is usually selected according to the following criteria:

1. Isobetic points, where the graphs of the oxy- and deoxyhaemoglobin components intersect. When using illumination light with a wavelength corresponding to the isobetic point, the recorded signal essentially depends only on changes in blood volume. Examples of such light are light with wavelengths of 520, 530 and 570 nm.
2. The points where the ratio of oxyhaemoglobin and deoxyhaemoglobin components are highest. For example, at 560 nm, the deoxyhaemoglobin component is dominant, while at 577 nm, the oxyhaemoglobin component is dominant.
3. At near-infrared frequencies, tissue scattering becomes dominant (700-750 nm).

Figure 4a) illustrates the problem of overlap mentioned above. The distance (FWHM) between the -6 dB points of the three filters (between 550 and 600 nm) used by Berwick et al. [7] is ±16 nm, ±14 nm and ±9 nm, so even though the centre frequencies were well chosen, the resulting signals overlap significantly. The ±5 nm FWHM of the filters I used allows for a significant reduction in this overlap (Figure 4 b).



a.) b.)

Figure 4 Characteristics of filters used.
a.) Characteristics of filters used by Berwick et al. [7]. b.) Characteristics of filters used by me. The horizontal axis shows the wavelength of transmitted light, while the vertical axis shows the normalised intensity of filtered light.

2.4 Choosing an animal model

Laboratory rats are commonly used as subjects in similar experiments, as the whisker system of these animals is a very good model. The whisker system is matrix-shaped, with rows (A-E) and columns (1-7). [1] This whisker matrix corresponds to a distinct region of the brain (barrel cortex), the structure of which is shown in Figure 5.

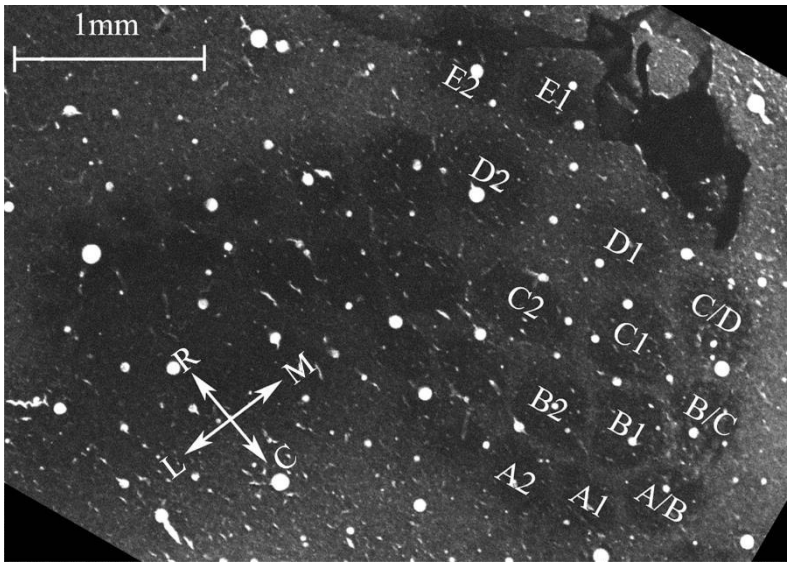


Figure 5. The barrel cortex, stained section.

An additional advantage of using this model is that its operation has already been well characterized in electrophysiological experiments. In their summary paper, Kleinfeld et al. found that there is a logarithmic relationship between the frequency of mechanical stimulation of the moustache (fing) and the spike frequency (fspike) in the corresponding barrel cortex area, which can be described by the following simple equation: fspike=2 ln(fing)+c, where c is a constant. [8]

This connection allows us to look for correlations between the haemodynamic response and the spike frequency.

After reviewing the physiological background and some of the technical issues involved, I will discuss the design and construction of the laboratory.

3 The structure of the laboratory

During the design process, I had to take the following aspects into consideration:

- Detection of three or four different components
- Resolution of approximately 10 μm
- Computer-controlled stimulation
- Computerized data recording
- Stable power supply for life support devices
- High-precision power supply for illumination
- High-speed filter changer
- High-speed computer
- High-speed, high-capacity storage
- Operation under MATLAB
- Filtering of electrical interference signals
- Elimination of mechanical vibrations

The logical diagram shown in Figure 6 was developed taking the above points into consideration. The computer used in the design runs on the fastest processor available at the time of the design (2 Pentium®4 3.20GHz CPUs) and has 2 GB of memory. The write speed achieved by connecting the two 500 GB hard drives in RAID 0 allows image recording throughout the entire duration of the experiment series. This is important because, due to slow background storage and MATLAB's 1 GB memory limit, previous series of experiments were unable to receive video signals during the time interval marked in pink in Figure 9.b. [6] The problem was caused by the fact that the speed of saving the digitized image information to the background storage was slower than the speed of receiving it, so the MATLAB program finished saving the data stored in memory during this time interval.

The camera used operates with a 12-bit A/D converter, has a maximum resolution of 1024x1024 pixels, and can take up to 30 images per second. The filter changer can handle four filters, and tests have shown that it can switch from one filter to another at a frame rate of 28 frames per second (4x7) while the camera optics are closed.

The stimulating signal and the camera synchronization signal are provided by two D/A converters (A0 and A1 in the figure). The amplified signals from the electrodes can be received by a 16-channel A/D converter. I used the AI0 A/D converter to record the stimulating signal. This signal will play an important role in data processing, as it can be used to calculate the exact timing.

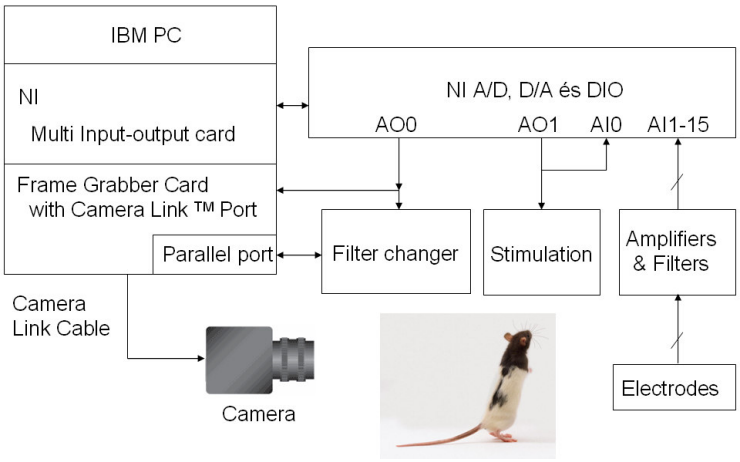
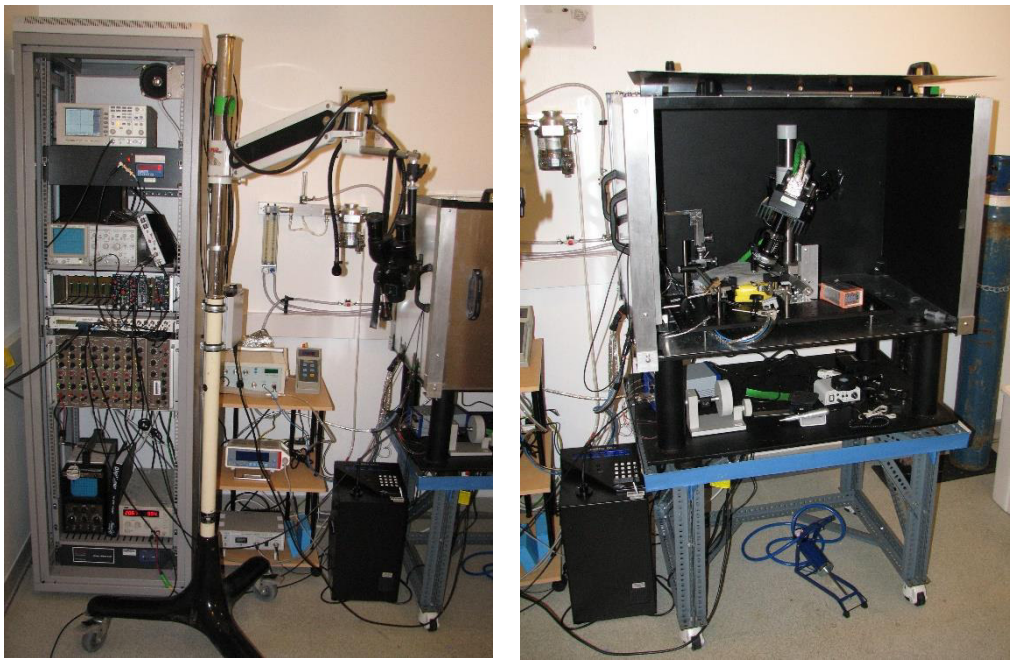


Figure 6. Schematic diagram of the laboratory setup



a.) b.)

Figure 7. Implementation of the laboratory in practice. The tower on the left side of Figure 7.a houses the UPS, the high-stability DC power supply, the 16-channel high-sensitivity amplifier, the National Instruments A/D, D/A rack, a Neurolog amplifier and filter unit, three oscilloscopes (1 digital storage

and 2 analog), the Humbag (50 Hz adaptive filter), and the counter to measure the heart rate. A mobile microscope can be seen in the foreground of the image, with the life support instruments in the background. The filter changer, which contains the high-intensity xenon lamp and four filters, is located at the bottom right. Figure 7.b shows the lockable box resting on the stand, three sides of which (left, top and front) can be removed. The box is made of aluminum, so it not only blocks out external interfering light, but also acts as a Faraday cage. Four inflatable rubber mounts between the dexion stand and the cast iron support element ensure the filtering of mechanical vibrations (the necessary pump is visible under the stand). The gray/blue box located on the back left of the support element generates the variable DC voltage required for the ceramic vibrating element connected to the whisker. The gray rotating wheel in front of it allows the vertical position of the brain electrode to be controlled with micron precision without having to open the aluminum box. The high-resolution monochrome camera is located inside the aluminum box. An extra heat sink is located at the front of the camera, which serves to reduce thermal shot noise. The camera itself is located on a heavy, stable stand. The weight of the stand provides additional protection against mechanical vibrations. The support rail visible under the camera is used to place the electrode holder and positioning head, which is located in the background of the image. In addition to the preamplifier box located on the right edge, additional fastening elements are also visible inside the box. Some of these hold the animal stable, while others hold the whisker stimulation device.

4 The operating software

The measurement control software was developed entirely in MATLAB. Before starting a measurement series, two graphical user interfaces (GUI) can be used to set the parameters of each hardware element.

The first GUI (Figure 8) is used to set the camera parameters. Three drop-down menus allow you to set the camera's frame rate, gain, and binning, which converts multiple pixels into a single pixel. The use of the latter may be necessary in low light conditions, the disadvantage is that it reduces the resolution (with 2x2 binning, the resolution of 1024x1024 pixels deteriorates to 512x512 pixels). Since it is not necessary to save the entire region during the recording of the image (space saving), the program takes advantage of the fact that the camera's control card provides the option to set the Region of Interest (ROI). The ROI can either be selected with the mouse on the image (red rubber box), or it can be set in the left-hand menu with the coordinates, width and height of the upper left point. Since four different filters are used during the measurement, it is advisable to check what images are obtained with the different filters. The currently used filter can be changed using the Filter button. As an additional help, the program displays the maximum brightness pixels with a value of 4096 in blue. The other buttons and the Binning menu can only be accessed using the Stop button, as this requires stopping the camera operation. The Image button saves the current image to a file. The Stable button displays the difference between consecutive images, allowing

you to check the noise level of the image. Finally, the Histogram button draws a histogram of the currently displayed image.

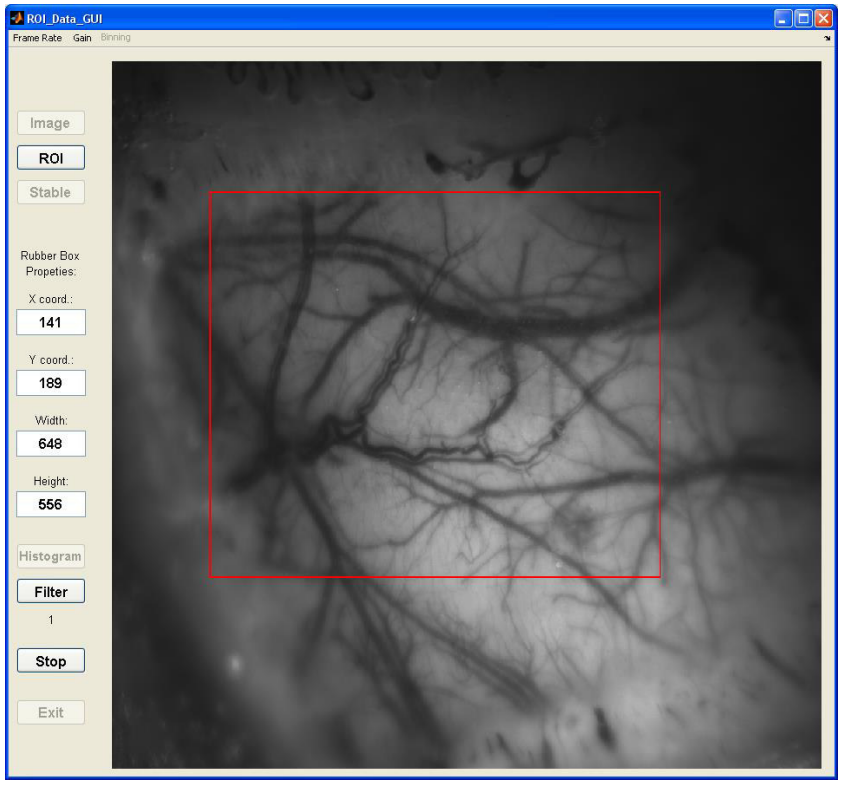
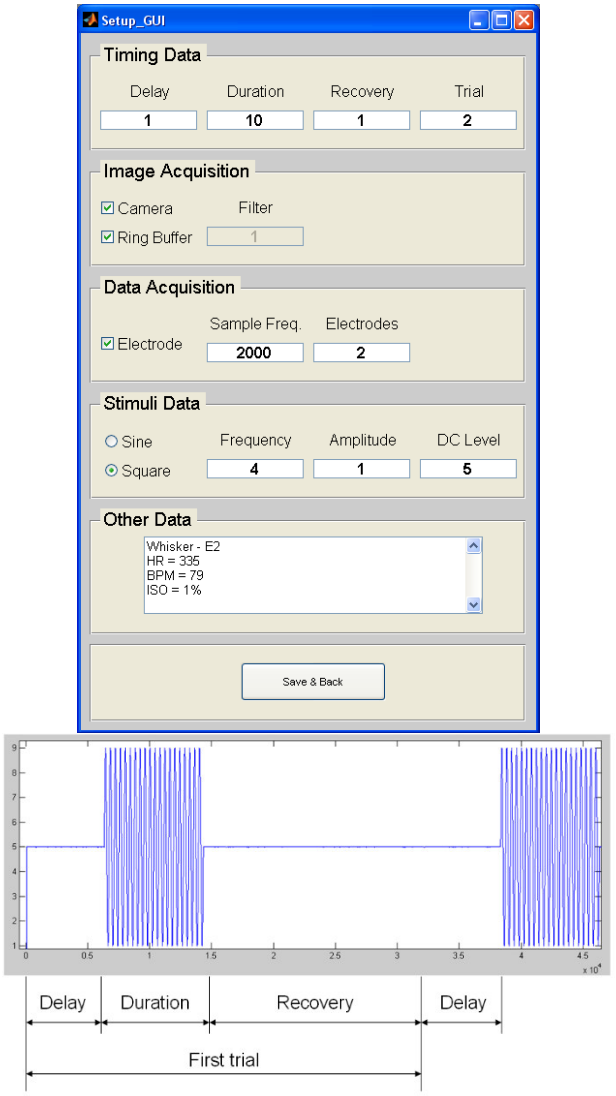


Figure 8. GUI for setting the parameters of the camera.

A series of experiments consists of several consecutive, repeated experiments with the same parameters, called trials (pronounced trajel). Figure 9.b shows the timing of a trial. The Delay is usually one or two seconds, and the signal recorded during this time serves as a baseline during data processing. The usual length of the Duration (stimulation) is 2-5 seconds. The Recovery time is usually given so that, on the one hand, the hemodynamic response can return to the initial value during this time, and on the other hand, the total duration of the trial exceeds 10 seconds. This is necessary because a low-frequency (~0.1 Hz) oscillation called the V signal occurs in the cerebral blood flow. [9] In the case of trials longer than 10 seconds, the initial phase of this oscillation is different, so that when they are summed, they cancel each other out.

The parameters of the experimental series can be set using the GUI shown in Figure 9.a. These parameters include the trial data described above, as well as the

values of the digitizing devices and the filter changer control. Any data characterizing the experimental series can be entered in the Other Data text box.



a.) b.)

Figure 9. The GUI shown in Figure a. allows to set the parameters of the stimulation, filter changer and D/A, A/D converters. Figure b. shows the parameters that can be set for each trial (an experimental series is made up of several consecutive trials). The pink rectangle shows the time range that could not be recorded during previous measurements.

Summary

Overall, I can say that during my nearly one-year work, I managed to build a laboratory that represented the cutting-edge technology of the time. This is also shown by the fact that the laboratory I designed was also built at the Shanghai University. With the help of the completed and already operational laboratory, I sought answers to the following questions:

- How do the physical parameters of the stimulating signal influence the brain response?
- Are there clearly separable spectral components?
- What is the relationship between the different spectral components of the optical signals and nerve impulses?

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