ICMOS: Intensified CMOS Camera for Biological Applications

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Abstract

Quality imaging is a critical component in many scientific experiments. However, the imaging devices currently in use consist mainly of either point-detectors, which have poor spatial information, or large area detectors, which have poor timing information. A device which combines fast non-imaging and slow imaging techniques is ideal for scientific research in which there are low light levels and a need for position and timing information. Such a device would be a powerful tool especially in the biological sciences where data obtained from microscopy is transferred in photons and where more powerful optoelectronic technology would be groundbreaking. We are in the initial stages of developing an ultra-fast and ultra-sensitive intensified CMOS camera which will hopefully exceed existing technology. Several tests, including incorporation with ongoing biology experiments at UCLA, were executed to increase understanding of the hardware and the improvements which will be necessary to achieve our specified imaging goals [1].

1. Introduction

Imaging biological processes has become increasingly appealing as methods involving photons (such as fluorescent dyes and quantum dots) have become more advanced. These techniques, which include FCS (Fluorescence Correlation Spectroscopy), FRET (Fluorescence Resonance Energy Transfer), TCSPC (Time-Correlated Single Photon Counting) and FLIM (Fluorescence Lifetime Imaging Microscopy) could proffer more insight into the structure and dynamics of biological molecules given an excellent photon detector or imaging device.

The types of photo detectors used in microscopy can be divided into two general categories: imaging devices such as the CCD or EMCCD cameras, and point detectors such as PMT (Photomultiplier Tube) or SPAD (Single Photon Avalanche Diode) among others. Current CCD based imaging devices are very sensitive, however, they are frame rate limited (~30Hz) by the readout speed and electronic noise. Conversely, point detectors can detect single photons at very high-rates (~MHz) but do not transmit position or image information efficiently.

Thus, a device which combines both high quality imaging capabilities and fast timing is ideal. We hope to create a device with can visualize live-cells with a high frame rate and single photon sensitivity; this has never before been achieved.

2. ICMOS Theory

The proposed ICMOS camera consists of two components: a CMOS camera and an image intensifier.

2.1 Image Intensifier

An image intensifier is a device which amplifies photons so that low light level signals can be detected. Image intensifiers were initially developed for military applications as night vision goggles. There are several variations on the standard image intensifier; however, the major components are a photocathode, phosphor screen and microchannel plate (MCP).

The image intensifier works by converting incident photons into electrons at the photocathode via the photoelectric effect. The coating on the photocathode allows for this conversion; good quantum efficiency of this component is therefore critical as photons not captured by the

photocathode are lost from the final signal. After the photocathode, the electrons are multiplied through the MCP by the application of very high voltages. Physically, the MCP is a slightly conductive glass substrate containing many parallel traversing channels which act analogously to a photomultiplier tube. The electron gain is dependent on the voltage supplied to the MCP and can be easily adjusted. The electrons exiting the MCP are accelerated by a constant voltage and hit the phosphor screen, where they are converted back into photons which are collected by the CMOS camera.

Phosphor is a chemical substance that fluoresces when excited. Several types of phosphor screens are commercially available and are characterized by their decay time, peak wavelength and conversion efficiency. Decay time is an important consideration in ultra fast imaging as a long decay length could translate as residual light on the ensuing frame. A basic image intensifier is pictured below.

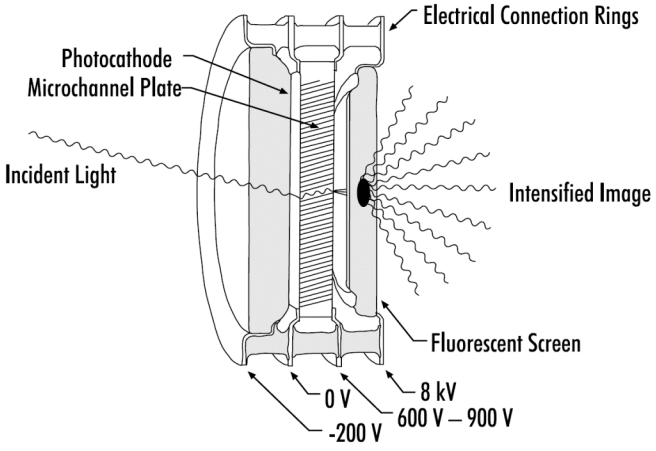


Figure 1: The layout of a basic image intensifier; a single incident photon is converted to many photons via the photocathode, microchannel plate and phosphor screen [2].

2.2 CMOS Sensor

Digital cameras, including CCD and CMOS, consist of an array of photodiodes which are electrically biased so that the pixels generate and store electrons when exposed to light. The CCD is the more developed and sensitive sensor with higher quantum efficiency and resolution, however, CCD has much slower readout electronics than CMOS. Although there are many readout configurations, in general, a CCD must shift all data forward one line at a time to process the information to data storage. This method creates the limit of 33Hz for a standard CCD [3]. The CMOS sensor differs from the CCD sensors in several important ways. A weakness of CCD is that low noise is only achieved with slow readout speeds and is further limited by the readout protocol. CMOS cameras, however, can achieve high readout speeds because each column of photosensors has an amplifier associated with it and a row of pixels can be readout in parallel. By incorporating parallel readout, a CMOS camera obtains high readout speeds [4].

3. Design Specifications and Goals

The basic specifications that we hope to achieve with the ICMOS include a standard 1kHz frame rate, a 1,024 x 1,024 pixel image with a wide dynamic range, single photon sensitivity and excellent timing resolution. The proposed frame rate of 1kHz would exceed the maximum frame rate in the best available imaging technology, namely the EMCCD, by a factor of at least two. It is essential that the frame rate is at least 1000 Hz in order to observe fast phenomena such as calcium signals. Furthermore, a 1 Megapixel image with a wide dynamic range is essential as these features are already inherent to most CCD devices. Single photon sensitivity and excellent timing resolution would widely increase the appeal of this device as this information is currently obtainable only by a photodiode, SPAD or PMT.

The design of the intensified CMOS camera has evolved from its conception as our understanding has increased. In this manner, two entirely different prototypes have been assembled and tested.

The image intensifier utilized in the initial prototype, which we will refer to as the old image intensifier, consists of a GaAsP photocathode with

greater than 40% quantum efficiency at 400-600 nm attached to two micro-channel plates (gain 10^4) and a phosphor screen made of P43 with 1 msec decay time. This image intensifier is attached via a 1:1 lens coupling to the Southern Vision Systems SpectraView camera which contains a 1.3 Mega pixel CMOS Active-Pixel Digital Image Sensor made by Micron. The camera can record in 10-bit monochrome and possesses a pixel size of 12um.

The image intensifier used in the second prototype also contains a GaAsP Photocathode, however, the material P24 is utilized as a Phosphor screen (decay 20usec), 3 MCP are included and the limiting resistivity of the MCP is reduced to <3MΩ per layer. This image intensifier is attached to the Photron 512 PCI camera which uses 10-bit monochrome with 16um pixel size to achieve high light sensitivity. This information is summarized in the following tables.

Image Intensifier	**Old**	**New**
SERIAL No.	QK0185	QL0017
Photo Cathode	GaAsP	GaAsP
Sk	710 µA/lm	674 µA/lm
No. of MCP	2	3
Total Resistivity of MCP	646 MΩ	70 MΩ
Resistivity per MCP	323 MΩ	23 MΩ
MCP Gain	1.12E+4 (at 1800V)	1.42E+6 (at 2700V)
Luminous Gain	5.72E+5 (at 1800V)	2.57E+6 (at 2700V)
Phosphor Screen	P43	P24
Decay Time	1 msec	20 µsec
Optical Efficiency	22600 nit/Am/m2	7800 nit/A/m2
Image Resolution	36 Lp/mm	28.5 Lp/mm

Table 1: Comparison of the old and new image intensifiers. The major changes include difference in the number of MCP and reduction of MCP resistivity.

CMOS Camera	**Old**	**New**
Company	Southern Vision System	Photron
Type	Spectraview	FASTCAM 512 P
No. of Pixels	1280 x 1024 pixel	512 x 512 pixel
Pixel Size	12 µm	16 µm
Effective Area	15.3 x 12.2 µm	8.2 x 8.2 µm
Readout Speed	2 µsec per line	1 µsec per line
Frame Rate (full frame)	500 fps	2000 fps
Memory	4MB	1.3 GB
Lens Coupling	1:1	2:1
Price	~$10k	~$25k

Table 2: Comparison of Photron FastCam and Southern Vision Systems SpectraView .

4. Old vs. New Experimental Comparison

PMT tubes already available in the lab (Hamamatsu CA5952/CA5953) were employed to test the gain of each image intensifier with the aim to understand the intrinsic capabilities of this important piece of hardware. The PMT was calibrated for various operating voltages (ranging from 650V-1750V) given a constant light intensity of an LED. The output current increases non-linearly as the operating voltage is increased in a well known manner.

Because the range of the PMT tube is limited, neutral density filters are implemented to achieve the output range necessary as NDF decrease the amount of photons into the PMT by a known factor. The light intensity (increased approximately by an order of magnitude each data point) and PMT voltage (chosen by finding a voltage that gives an output current to dark current ratio of approximately 10%) were then tuned so that the number of photons into the PMT could be ascertained. The image intensifier is placed directly in front of the PMT (to decrease any geometrical factors) with a 10^-6 neutral density filter to eventually determine the number of photons out. The output current is calculated from neutral density filter value and implementation of the PMT relative calibration curve. Many conclusions were made from the systematic gain analysis. The theoretical output limit of 8.5 x 10^14 photons/sec was experimentally confirmed for the old image intensifier. These results are shown in Figure 2. The vertical black lines forming the box represent the range of photons necessary for good image quality and single photon sensitivity from 1 PE/msec/pixel to 1000 PE/msec/pixel. The uppermost horizontal line represents the theoretical MCP limit of output photon intensity. However, it is noted that saturation begins not at this limit but at an order of magnitude lower. Finally, the lower horizontal line corresponds to the lowest ADC sensitivity. The CMOS camera operates in 10 bits and therefore has a range of 1 to 1024. The total square region is thus what needs to be covered by

the image intensifier; ideally the image intensifier should transverse this in a straight line without saturation.

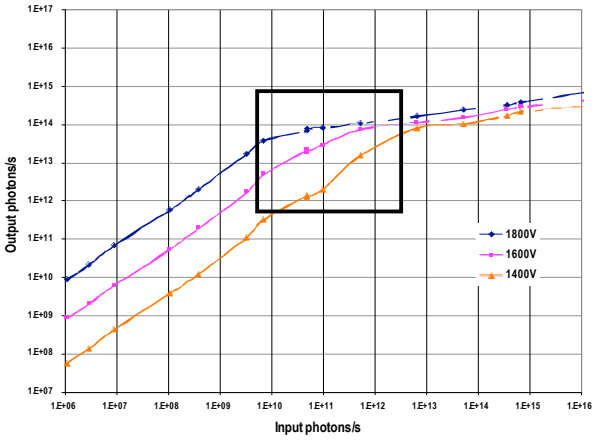


Figure 2: Output versus Input photons/per second for the Old Image Intensifier.

The new image intensifier was tested in the same manner; the results are shown in Figure 3. The 2100V setting is close to the ideal case but is still an order of magnitude too low. This test demonstrates that one MCP with a gain of ~100 is enough for single photon sensitivity for our camera because the CMOS has ~<50 electron noise floor and an ADC count of 60 electrons. Also, it was determined that the amount of DC current on the MCP or the resistivity is a limiting factor to the amount of electrons which can be produced. Because standard image intensifiers have been optimized for CCD cameras in the past, the resistivity of the MCP must be reduced by a factor of 30 to obtain the faster frame rates that the CMOS is capable of achieving. In other words, once the frame rate is sped up to 1 kHz, the image intensifiers can not maintain the same image quality. Therefore, the amount of photons per unit time must be increased which in turn saturates the MCP.

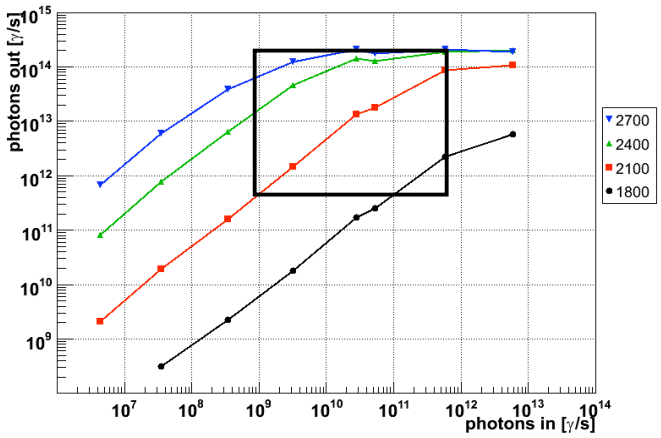


Figure 3: Output photons vs. Input photons measured by New ICMOS. Data points are shown at four high voltages where saturation is seen at the higher voltages.

There are several parameters that are important when assessing imaging devices including quantum efficiency, sensitivity, noise, dynamic range, spatial resolution and frame rate. Quantum efficiency is a measure of the ability of a device to produce electric charge as a percentage of the total number of incident photons. The sensitivity of a camera, or the minimum detectable light signal, is limited by the noise of the camera; a camera can not be more sensitive than the noise level. Noise occurs from several intrinsic limitations including thermal (dark noise), light (shot noise), and electronic (read noise) factors. Dynamic range refers to the maximum and minimum intensities (often expressed as ADC Value) which can be detected in the same image. Spatial resolution refers to the inherent finite limit of spatial information a camera can capture; this is often expressed as the amount of pixels available. Finally, frame rate is a defined as the fastest recording rate and is an important and one of the most appealing parameters in the development an ICMOS camera [4]. These factors were assessed through various imaging tests.
Further, the relative sensitivities of the camera were tested by doing a side by side comparison. Given the same conditions of frame rate and frame size, the Photron was found to be more sensitive according to the Figure 4. The increased

sensitivity is likely due to different CMOS technology and the larger pixel size in the Photron camera. (Larger pixels have lower spatial resolution but usually offer higher dynamic range due to an increased full well capacity.) The noise level and dynamic range in each camera was comparable in 8-bit mode. Please refer to Table 2 for a complete comparison.

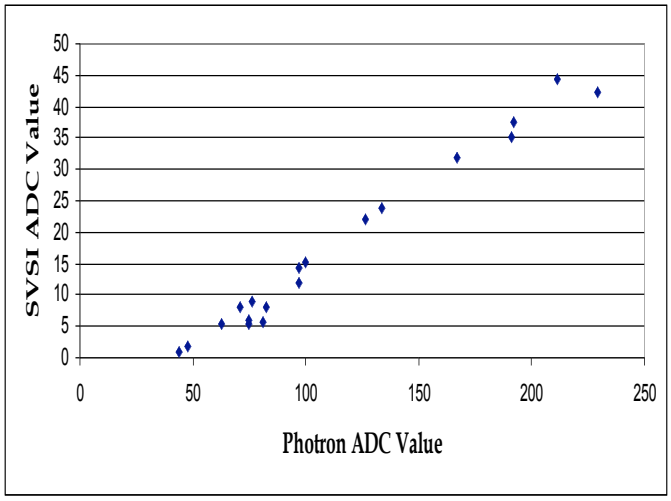


Figure 4: The raw data of average SpectraView ADC Value versus average FastCam 512 PCI ADC Value. The points were collected over a range of frame rates with varying geometrical setups using a blue LED of 430nm light source.

5. Application

5.1 Muscle

Because the ICMOS is intended for biological applications, it is necessary to implement the camera into a microscopy based experiment to ascertain the true capabilities of the instrument. Thus, an ongoing inquiry into the spatiotemporal characteristics of the calcium release process in mouse skeletal muscle, as conducted by Mario DiFranco and Julio Vergara, is selected for its expected compatibility with this imaging system. (For further detail see Ref. 5 and 6.)
The ICMOS camera was moved to the Vergara lab and attached to the current setup as shown in the following picture.

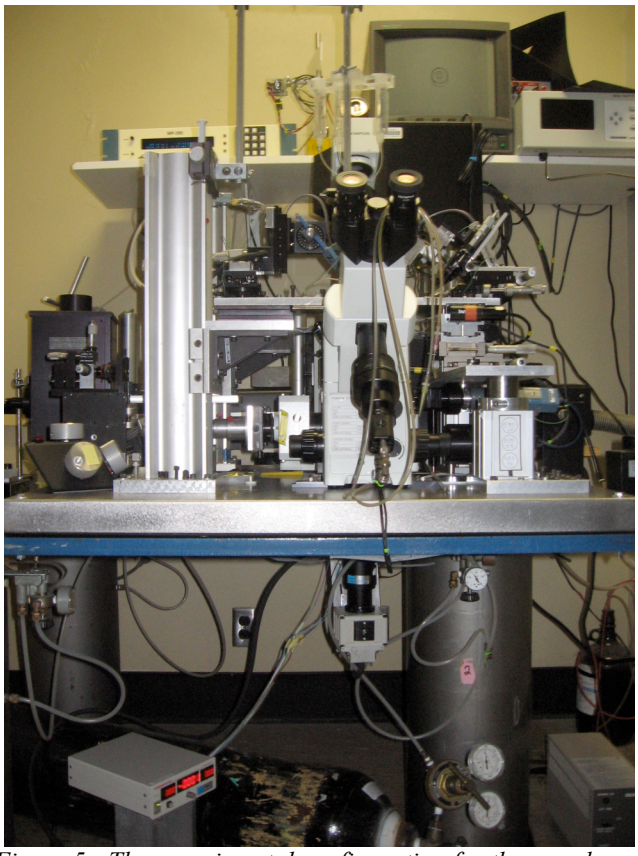
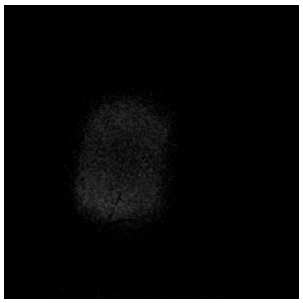
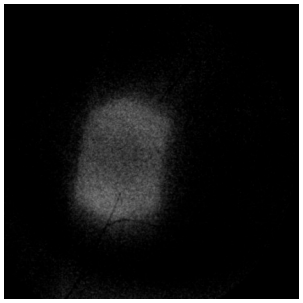


Figure 5: The experimental configuration for the muscle experiment. The ICMOS camera is attached to the microscope below the table. The high voltage supply rests on the floor to the left.

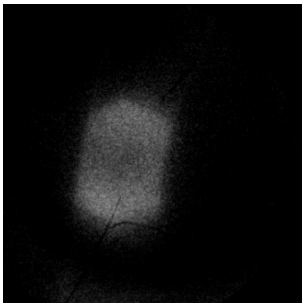
This experiment is modified to produce a consistent photon intense signal; the calcium signal is optimized to produce a very high signal to noise ratio (~4 times the noise floor). The fluorescent dyes, fluo-3 and di-8-ANEPPS, are used to indicate the calcium. Fluo-3 possesses a lower affinity and is therefore the more reliable indicator (less signal distortion), however, it is substantially dimmer than di-8-ANEPPS. Data collected di-8-ANEPPS as an indicator confirms that a signal can be easily captured using the brightest dye. The capabilities of the ICMOS new prototype exceed expectations by also capturing fluo-3 signals as shown in the following frames.



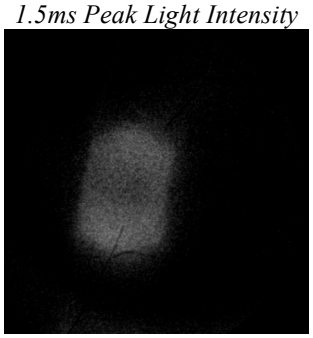
.5ms



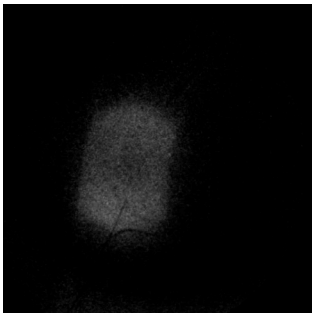
1ms



1.5ms Peak Light Intensity



2.0ms



2.5ms

Figure 6: Unprocessed frame sequence of calcium signal as captured at 2000 fps over 5 frames.

The intensity versus time information (also captured simultaneously on an analog device) is obtained by post processing images in ImageJ, a Java-based image processing program developed at National Institutes of Health, using the ADC value data. The following graphs show the intensity vs. time analysis for a 1 x 1 signal area and 10 x 10 area of the signal (chosen arbitrarily from the bright center of the observable signal). Because the pixels are averaged in the second graph, the noise decreases accordingly.

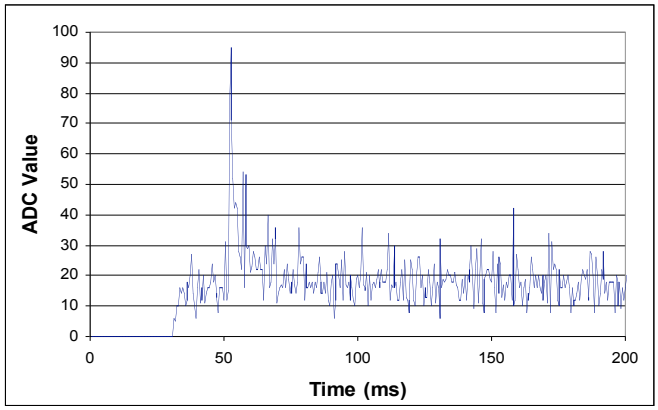


Figure 7: An intensity versus time analysis of 1 pixel of the imaged signal.

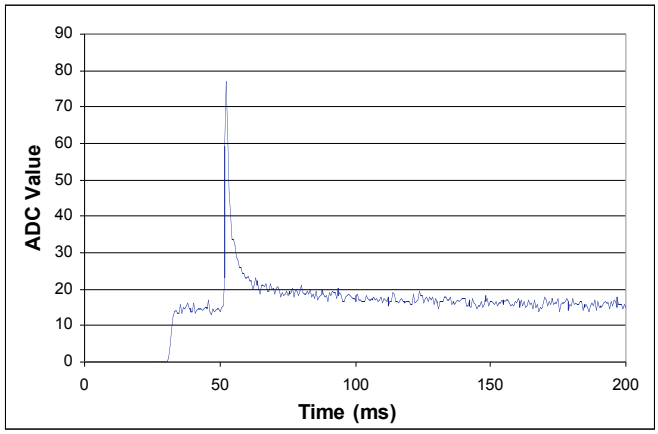


Figure 8: A 10 x 10 pixel area of the same signal as Figure 1. The noise decreases as more pixels are evaluated due to the effects of averaging.

Despite these promising findings, our detector still lacks quality spatial resolution. The T-tubules, which slice the muscle fiber are easily visible to CCD, however, the CMOS can only capture these

structural details in a bright field image (where shutter is open for the whole field or entire fiber thus letting in more light).

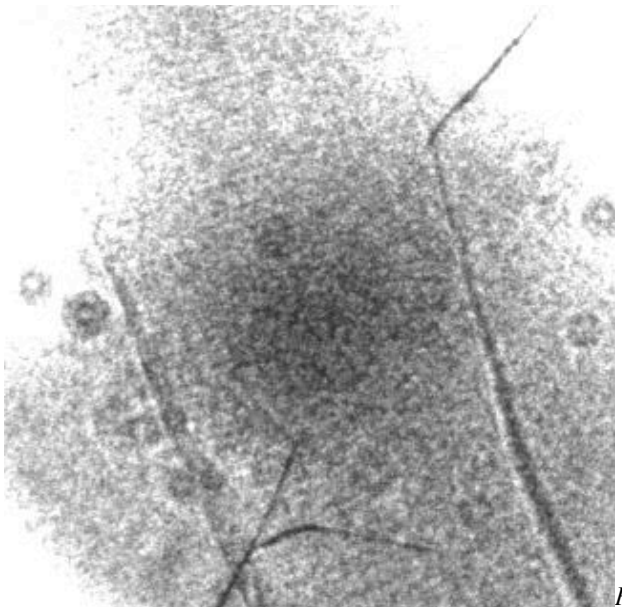


Figure 9: Bright Field View of Muscle Cell taken by new ICMOS camera in which the structures perpendicular to the muscle strand, T-tubules, can be resolved. Calcium transfer occurs between these discs.

Further, it was noted in this experiment that the image data possessed an intrinsic distortion. In the flat field image, which is obtained by capturing a homogenous sample, it was observed that amount of ADC decreases by about 150 ADC in the center of the image. The corruption of the image can be seen in Figure 8. The image is saturated around the edges and becomes increasingly dark toward the center. (The dark diagonal lines are not part of the image, but rather particles on the camera.) The quantum efficiency (QE) is also affected by this burn; the center yields a substantially reduced QE whereas the outside yields higher QE as evidenced by the increase in white saturated regions on the periphery of the image. The origins of the burn are likely from operator error where too much light was exposed on the photocathode. The image intensifier has a finite lifetime and aging process wherein the device will degrade over usage time; this may also have factored into its destruction. Fully understanding the operating limitations of the image intensifier is important due to the frailty and value of the equipment. A cut off switch could possibly be incorporated to ensure that the photocathode is not exposed to damaging light levels.

5.2 Neuron

A second experiment was also undertaken to further the proof of principle investigation. The ICMOS camera was introduced into an ongoing experiment, as conducted by Consuelo Morgado, with the aim to again gather position data of calcium transfer in the neuron taken from a rat brain. Previous data was taken with a photodiode and the bright field image information was obtained with a CCD. An external trigger links the software to the ICMOS camera which is placed on a mount on top of the microscope. The old and new prototypes yielded few results in this experiment. The signal strength and object size relative to the muscle experiment are much smaller. Refocusing the camera specifically for the conditions is negligible. The burn on the photocathode of the new camera further impedes reliable operation. The only semblance of a signal was obtained at lower frame rates. The following graphs shows a signal averaged over a 20 x 20 pixel spot taken on the bright signal of the image as shown in Figure 12. The overall signal was calculated from analog data to begin at 135ms; the frame rate is 60fps and the MCP Voltage is 2175V.

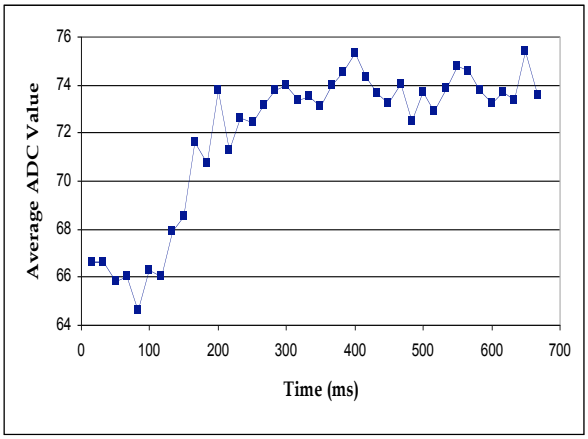


Figure 10: Average Analog to Digital Conversion Value versus Time for a supplied action potential to a neuron.

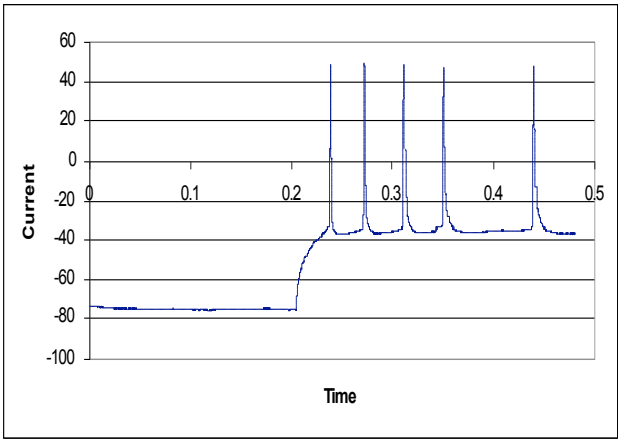


Figure 11: Current (Amps) versus Time (Seconds) of the same signal as shown in Figure 10 as captured by the photodiode.

The superiority of the photodiode for displaying this information is clearly illustrated in these graphs.

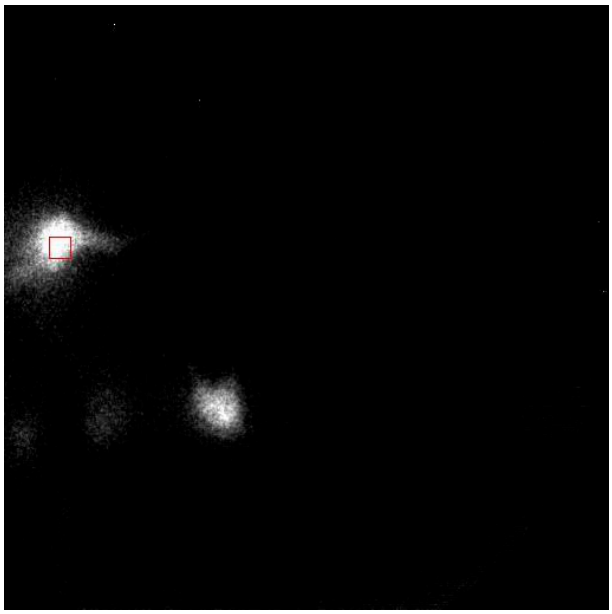


Figure 12: Brightness and Contrast Auto Enhanced Image of a neuron. The red 20 x 20 pixel box represents the area over which the ADC Value versus Time data was analyzed.

6. Future

From the data collected in the hardware and application tests, it is apparent that the old and new cameras will need to be improved further to obtain meaningful data from current biological applications. From the data obtained from this study, the next image intensifier will be designed with one MCP due to the inherent limitations of this component. Further, another design for the intensifier is being considered. This design includes a GaAsP photocathode followed by a P47 phosphor screen, two fiber optic plates, a bi-alkali photocathode (acceptable for second stage), a second P47 phosphor screen and finally a third fiber optic plate. This model will have 10^4 gain, a large dynamic range and EMCCD comparable energy resolution which is essential in collecting wide field of view image data.

The final specifications for the ICMOS camera will still be a 1000Hz frame rate at 1,024 x 1,024 pixel resolution (using Photron FastCam 1024PCI).

Once high-speed and sensitive imaging is realized, we can further optimize the system to biological applications by gating the signals to the image intensifier at sub-nano second speeds. In FLIM, for example, a decay time of fluorescence dyes on the order of 1-10 nsec can be resolved by pulsing the voltage between the photocathode and MCP with an ultra fast gating module synchronized with a pulsed laser. This has been done in the past with the ICCD [7].

Additional features will include a setup with the Yokogawa Confocal Microscope and an Optical Insights Quad-View. Finally, the Quad-View will produce four color images simultaneously.

7. Conclusions

The most critical issue in ICMOS development, thus far, has been the unexpectedly low dynamic range. The properties of the MCP are now well understood from the various hardware and application investigations as discussed above. The ICMOS camera is still a very much realizable concept and the exhibited capability of the camera is such that only slight enhancement should lead to a successful biological insight. The future is very promising for the ICMOS and subsequent models will no doubt produce a many scientific conclusions.

Acknowledgments

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