Enhanced Image Sensor Module for Head-Mounted Microscopes *

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***Abstract*— Several research groups have developed head-mounted fluorescence microscopes as a modality for recording neural activity in freely behaving mice. The current designs have shown exciting results from in vivo imaging of the bright dynamics of genetically encoded calcium indicators (GECI). However, despite their potential, head-mounted microscopes are not in use with genetically encoded voltage indicators (GEVI) or bioluminescence indicators. Due to its ability to match the temporal resolution of neuron spiking, GEVIs offer great benefits to experiments designed to provide feedback after real-time detection of specific neural activity such as the less than 250ms replay events that can occur in the hippocampus. Orthogonally, the emerging bioluminescence activity reporters have the potential to eliminate autofluorescence and photobleaching that can occur in fluorescence imaging. There are two important properties of the head-mounted microscope's image sensor effecting the ability to image GEVIs and bioluminescence indicators. First, the low signal to noise ratio (SNR) characteristics of GEVIs and bioluminescent indicators make signal detection difficult. Second, in order to take advantage of the GEVIs faster fluorescence kinetics, the image sensor must be capable of matching frame rates. Here, we present the design of a new imaging module for head-mounted microscopes incorporating the latest CMOS sensor technology aimed at increasing image sensor sensitivity and frame rates for use in real-time detection experiments. The design builds off an existing open-source project and can integrate into the existing data acquisition hardware and microscope housing.**

I. Introduction

It is well understood that most systems neuroscience experiments would be significantly improved by increasing the number of neurons which can be simultaneously recorded. Beyond this, there are many scenarios in which observing the activity of the same neurons across long periods of time would allow for critical questions involving learning, memory, and/or other circuit plasticity-related functions to be answered. Imaging neurons genetically modified to express optical activity reporting proteins promises to address both of these challenges, and several groups have reported ultra-lightweight (less than 3 grams) head-mounted fluorescence microscopes which enable the brains of mice to be imaged during free behavior. The most successful experiments to date (e.g., [Ziv]) have used genetically engineered calcium indicators (GECIs). While reliable expression and high brightness make recent GECIs very useful, they have slow kinetics — ~1-second-long transients in response to the 1-10ms spike times [2]. This limits their utility in closed loop experiments in which it is desired to perturb neural circuits with low latency in response to transient events such as the 250ms replay events that occur in the hippocampus.

Genetically encoded voltage indicators (GEVIs) offer fluorescence kinetics that can accurately track the temporal resolution of neuron spiking activity. Thus, GEVIs have the potential to work in tandem with head-mounted microscopes for real-time detection of replay events. Current GEVIs have insufficient SNR for these applications, but rapid development suggests improved reports may soon be available. Fluorescent activity indicators (e.g. voltage or calcium) suffer from problems with autofluorescence and photo-bleaching issues. Thus, the development of genetically encoded activity dependent bioluminescence has great potential to improve signal and increase experimentation time by eliminating the need for fluorescence excitation.

Currently, GEVIS and bioluminescent indicators suffer from lower signal to noise ratios (SNR) compared to GECIs [3], [4]. As research developments aim to improve the low SNR characteristics of the indicators, in parallel, it is critical to improve the microscope hardware for enhanced sensitivity by upgrading the image sensor to the latest CMOS image sensor technology. Furthermore, these sensors can offer faster imaging frame rates, which are necessary to take advantage of the GEVIs' temporal resolution. In addition, for imaging with GECIs, enhanced sensitivity enables reduction of excitation light, which in turn leads to longer experimentation time without photobleaching.

Along these lines, we introduce a new image sensor module that incorporates the latest CMOS image sensor technology while building upon an existing open-source head-mounted microscope design. The new image sensor was chosen with specifications aimed at addressing the application of imaging GEVIs and bioluminescence indicators. The design of our sensor module is compatible with the current Miniscope optics, housing and data acquisition unit. Finally, we validate performance by quantitively comparing samples of bead phantoms and tissue sections. The design for the new image sensor module is on GitHub available for open-source use.

II. Design of New Sensor Module

A. Background

Currently, there are several published head-mounted fluorescent microscopes. The two most widely used are versions from the private company Inscopix and the Miniscope Team [5], [6]. The Inscopix design, based off the original Schnitzer's Lab microscope, is not publically

*This work was funded by an NSF CAREER award (CBET-1351692) and a HFSP Young Investigators award (RGY0088).

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available. Conversely, the Miniscope has become a benchmark in the open-source imaging community with extensive documentation, design and support available through the Miniscope website. Thus, the Miniscope image sensor module is the standard in this paper for comparison as well as the basis of the design for the new sensor module.

The Miniscope system is comprised of two main components, which are the data acquisition unit (DAQ) and the head-mounted microscope [6]. The DAQ connects to a PC through a USB 3.0 interface where it communicates with a GUI. The application GUI allows the user to interactively change the cameras settings, view/record the image data and change the excitation LED values. A feature of the system is that one single lightweight flexible coax cable provides the link between the microscope and the DAQ. This is possible from a serializer/deserializer system that encodes the image sensor data, power and a backchannel serial interface for transmission along the coax cable. The main design blocks of the image sensor module are the CMOS image sensor, power regulators, serializer and LED control.

B. Image Sensor Choice

Table 1 shows the datasheet specifications for the current Miniscope image sensor and the new sensor. The new image sensor was chosen with several factors in consideration. First, for ease of integration into the current Miniscope system, the new image sensor maintains compatibility with the serializer/deserializer system implementing the parallel port for the image data. However, this has the downside of limiting the max frame rate to 60 frames per second (fps) at HD720p format. A strategy to improve the frame rate is to use window cropping which can significantly increase the frame rate of a desired region of interest (ROI). This can offer an advantage during real-time detection experiments where the small field of view (FOV) can provide a quicker processing time for each frame. A future upgrade is to redesign the DAQ unit and the image sensor module to use the LVDS interface to achieve the maximum data rates. Second, the new sensor offers dramatic improvements in sensitivity and dynamic range. Furthermore, the analog gain capability the new sensor is of great advantage during low light settings. Third, the larger active image size allows for the use of larger optic lenses. Due to a switch from global shutter to rolling shutter, one possible limitation of the new sensor is potential for motion artifacts when imaging *in vivo*. On the other hand, this keeps the image sensor price very affordable.

C. Image Sensor Printed Circuit Board Design

The new printed circuit board (PCB) design integrates the new image sensor while still utilizing the existing circuits for the serializer and LED control. Implementing the new sensor into the same PCB footprint created a challenge due to the larger sensor footprint and increase in number of capacitors and power sources required. Thus, the new PCB still maintains the 13mm width, but the length is 22.1mm making it only 5.6mm longer. In addition, it can still fit the current Miniscope optic's housing. Another improvement is the voltage level that sets the analog value for the excitation LED has a wider range offering larger LED excitation levels. The low range is not a problem when imaging GCaMP because the LED values are typically low. However, large excitation values are likely to be required for imaging GEVIs to achieve a higher signal value. To enable future testing of stimulation circuits, connection points are available for the backchannel serial interface, a general-purpose input/output and power sources. The design of the board is located at https://github.com/kemerelab/EMBC2018.

TABLE I.

CMOS Image Sensor Specification Comparison		
Description	***On Semi MT9V032 (Miniscope)***	***Sony IMX290LLR (New Sensor)***
Sensor Type	Mono	Mono
Optical Format	1/3" (5.35mm)	1/2.8" (6.45mm)
Pixel Size	6um x 6um	2.9um x 2.9um
Shutter Type	Global	Rolling
Max Full Resolution	752 x 480	1920 x 1080
Max Frame Rate (fps)	60	Parallel CMOS – 60 (720p) LVDS – 120 (1080p)
Max Analog Gain (db)	12	30
Dynamic Range (db)	52	66 [7]
SNR (db)	33	40.5 [7]
Temporal Dark Noise (e-)	Not Available	5 [7]
Absolute Sensitivity (p)	Not Available	8.5 [7]

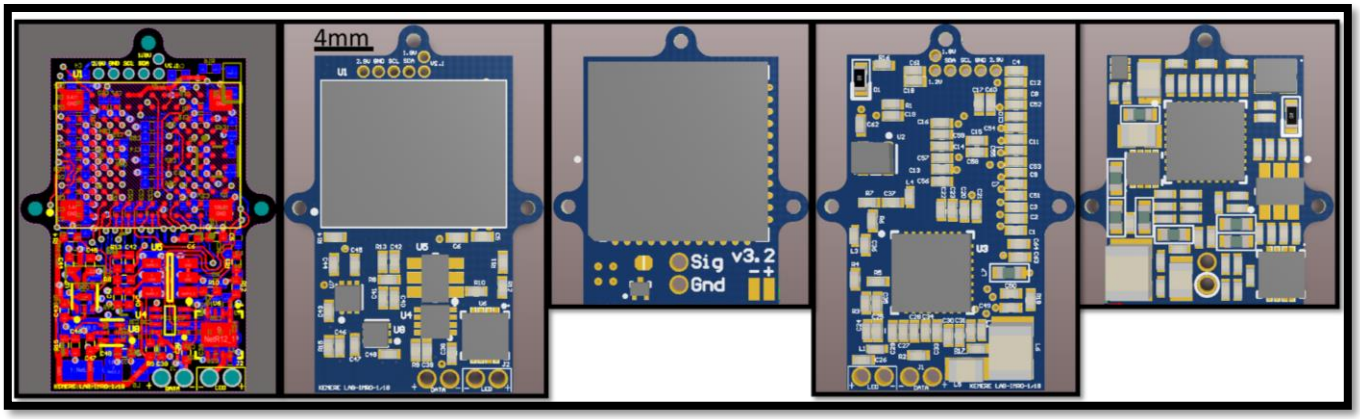


Figure 1. From Left to Right: New Image Sensor PCB Layout, New Image PCB Top View, Current Miniscope Image PCB Top View, New Image PCB Bottom View, Current Miniscope Image Sensor PCB Bottom View

III. Performance Evaluation

Due to continued fabrication and testing on the new sensor module, a performance evaluation on the new sensor was conducted with a development board from the company IDS. The board, model number UI-3862LE-M, combines the new image sensor with a USB 3.1 interface along with computer software that provides access to all features of the image sensor. In order to use the sensor with the Miniscope optics, we secured the Miniscope optic housing to the sensor. Shown here are results of comparisons of SNR calculations and in vitro GCaMP6f slides. In addition, further demonstration of the new sensor performance is shown through imaging coverslips of the GEVI Arc Light expressed in human neural progenitor cells.

A. SNR Comparison

With the aim of comparing the new sensor with the current Miniscope sensor, we imaged Invitrogen Fluorescent 6um Microsphere Slides and calculated SNR values. The SNR value is the average true signal intensity divided by the standard deviation of the peak signal intensity over 20 images. For both sensors we set the gain settings to the max Miniscope gain of 12db and used the same Miniscope optics and LED. In addition, we tested two different frame rates and two different low settings of the LED Value. The LED Value is the digital value (0-100) set by the user on the Miniscope software and it corresponds to the intensity of the LED excitation light. See Experimental Methods section for more information. Table II shows the results. As can be seen, the sensors perform similarly at the 12db gain setting. Significantly, the new sensor has a much higher analog gain capability at 30db.

B. GCaMP Slide Comparison

We prepared 50um histological sections of the brains of mice in which were expressing GCaMP6f in the area of the CA1 region of the hippocampus. For each sensor, we imaged matching field of views (FOV) on the slide with the same Miniscope optics and LED. The window area for each sensor FOV was approximately the same and shown in Figure 2. Next, for each sensor, we plotted histograms of images from the same FOV taken at 60fps with different LED values. Figure 3 shows the results. Overall, the new sensor imaging at higher gain values is able to achieve overall higher intensity value. This is important for in vivo imaging with GCaMP in that it could lead to lower excitation LED values, thus allowing for longer experimentation time without photobleaching.

TABLE II.

SNR Calculation Comparison		
Setup Description	***On Semi MT9V032 (Miniscope) SNR***	***Sony IMX290LLR (New Sensor) SNR***
Frame Rate = 30fps, LED Value = 1	17.2	23.7
Frame Rate = 30fps, LED Value = 2	33.5	35.0
Frame Rate = 60fps, LED Value = 1	6.0	23.3
Frame Rate = 60fps, LED Value = 2	17.4	22.9

Figure 2. (Left) Miniscope Senor, 60fps, Gain 12db, LED Value = 10 (Right) New Sensor, 60fps, Gain 26db, LED Value = 5

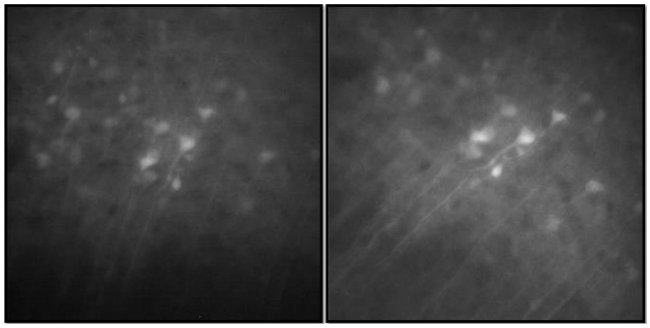


Figure 3. Histograms for GCaMP images taken with Miniscope sensor at 60fps, a constant gain and different excitation LED values (Note that some pixel intensities are not experienced, likely due to sensor rounding)

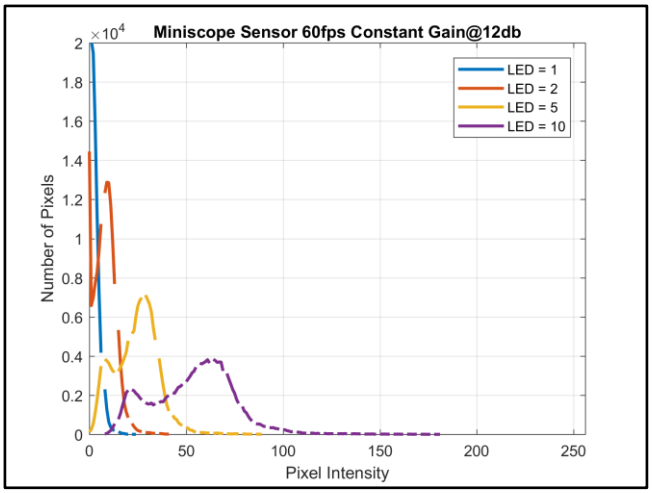
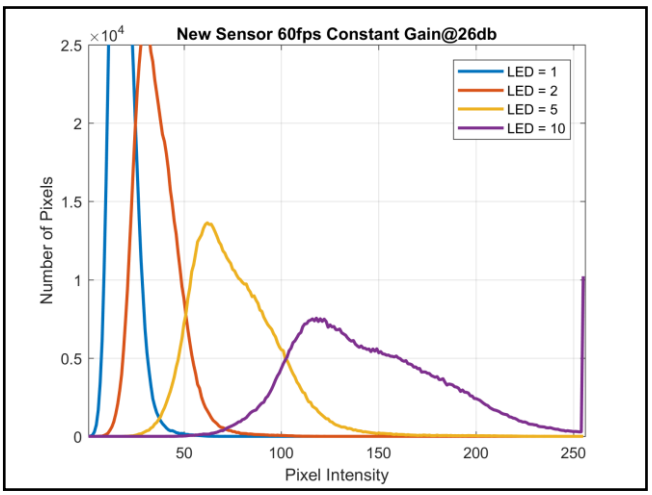


Figure 4. Histograms for GCaMP images taken with new sensor at 60fps, a constant gain and different excitation LED values



C. Neuron ArcLight Slide Test

Next, we prepared human neural progenitor cells transfected with pLenti-CMV-ArcLight vector. The new image sensor secured to the Miniscope optics and LED was

used to image the coverslips containing the cells. Results are shown in Figures 5 and 6.

Figure 5. Human Neural Progenitor Cells expressing GEVI Arclight imaged with the new sensor at 30fps, Gain set to 30db, Excitation LED Value of 10

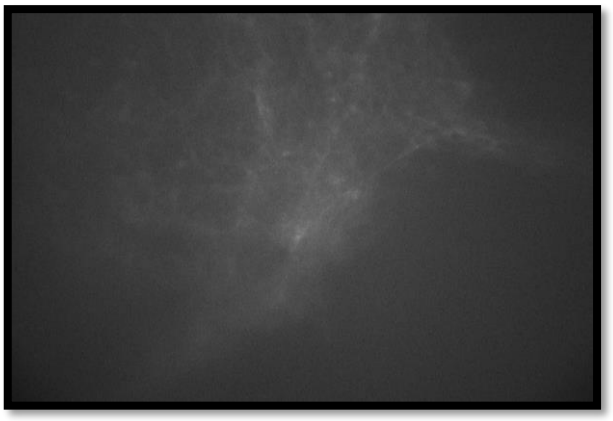
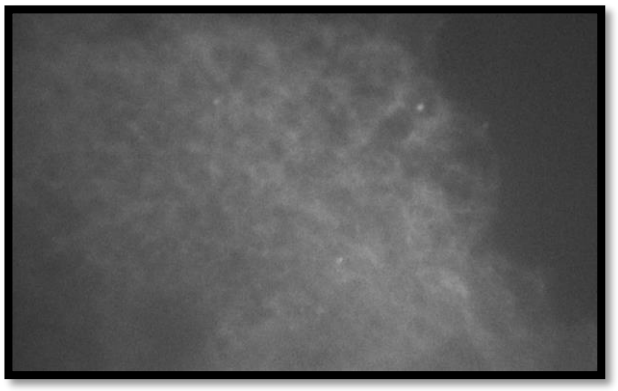


Figure 6. Human Neural Progenitor Cells expressing GEVI Arclight imaged with the new sensor at 30fps, Gain set to 30db, Excitation LED Value of 10



IV. Experimental methods

A. SNR Calculations

For the SNR Calculations, we implemented the following method. We imaged Invitrogen FocalCheck Fluorescent 6um Microsphere's Slide B and used the same FOV and bead phantom for all calculations. For each sensor and LED Excitation value, 20 images were taken. For each image, the center of the phantom bead was found. The average of a window around the center of the bead was calculated. A second average from a background window was calculated. The true signal was calculated as the average of the background subtracted from the average signal. Each true signal was then averaged over the 20 images. Next, for each image, the peak intensity of the phantom bead was found. The standard deviation of noise was calculated as the standard deviation of all of the peak intensities. Finally, the SNR was calculated as the average true signal divided by the standard deviation of noise.

B. GCaMP Slide Preparation

All experiments were approved by the Rice University IACUC. Mice were sacrificed 14 days post injection of AAV9-CamKII-GCaMP6f in the CA1 region of the hippocampus. The fixed brain tissue was sectioned in 50um increments, mounted on slides in Vectashield with DAPI and then sealed with coverslips.

C. Neuron Arclight Slide Test

Human Neural Progenitor Cells (ReNcells from EMD Millipore) were transduced by lentiviruses containing the pLenti-CMV-Arclight vector (obtained from the Milan Lab at Massachusetts General Hospital). Three days after transduction the cells expressing ArcLight were selected with Puromycin, added at a concentration of 1 ug/mL to the growth media. The ReNcells were maintained and passaged following published protocols [8]. The lentivirus was packaged in HEK293T cells transfected by psPAX2, pMD2.G and pLenti-CMV-ArcLight, and harvested from the cell culture media over the course of 72 hours post-transfection. The lentiviruses were concentrated by ultracentrifugation (120,000xg for 4 hours), resuspended in DPBS and titrated prior usage on ReN cells.

Acknowledgment

Alex Rodriguez thanks for the instruction and help with the GCaMP slide preparations.

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