

Integrating Bioluminescence Imaging with OpenIVIS for Open-Source, Low-Cost, High-Level Biological Imaging

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1. Introduction

In vivo imaging systems (IVIS) allow researchers to non-invasively study living organisms in real time using optical methods, primarily bioluminescence and fluorescence imaging. These platforms are highly sensitive and widely applied in disease diagnosis, drug development, and treatment evaluation [1]. However, with costs often exceeding \$100,000, IVIS systems remain inaccessible to many small research groups [2].



Fig 1. Newton FT-900 CCD Imager [2]

To address this challenge, OpenIVIS, in collaboration with the Rathbun Lab at Claremont McKenna College (CMC), is developing a low-cost, open-source bioluminescence imaging platform. The Rathbun Lab focuses on engineering bioluminescent enzymes to study cellular interactions but lacks access to commercial IVIS technology due to its prohibitive cost.

Bioluminescence imaging (BLI), a core IVIS capability, captures light naturally emitted from living cells via luciferase enzymes rather than requiring external illumination. This reduces background noise but also produces very faint signals, necessitating the use of sensitive cameras to detect low-intensity bioluminescence [3].

2. System Setup

The OpenIVIS box is constructed from laser-cut black acrylic sheets, designed with interlocking jigsaw edges to form a light-tight sealed enclosure. The OpenIVIS system is powered by a Raspberry Pi 4. The camera used to take all images is the Raspberry Pi Camera Module 3 NoIR and was chosen for a number of reasons: affordability and accessibility, no IR filter improves low-light performance, and camera sensor Sony IMX708 has great sensitivity and signal-to-noise performance in dim conditions [4]. The total cost of the OpenIVIS system was under \$210, making it significantly more affordable than commercial imaging systems.

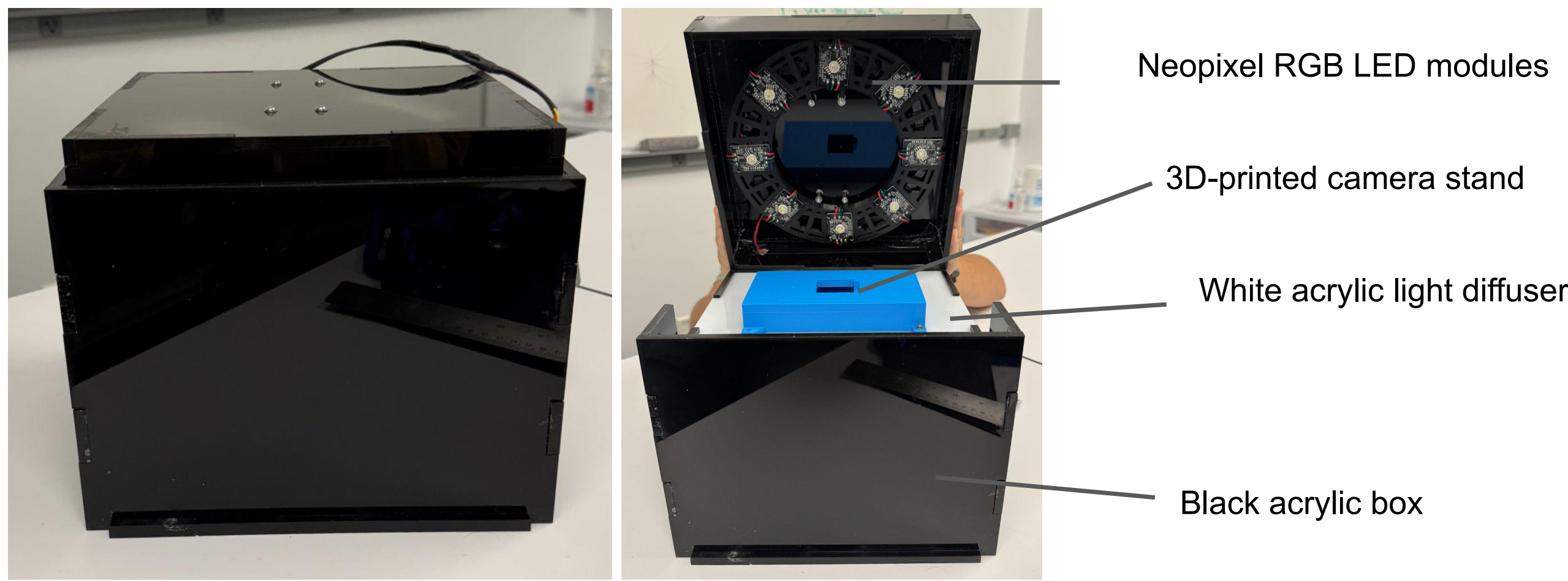


Fig 2. OpenIVIS Box with Bioluminescence Module

3. Experiments

iLux Agar Plate Imaging

The OpenIVIS bioluminescence module was tested with iLux, a genetically engineered *E. coli* system from the Rathbun Lab that emits light increasing with temperature. Bacteria were plated on agar plates, and time-lapse imaging was performed over 16 hours at room temperature, with brightness expected to rise with temperature [5].

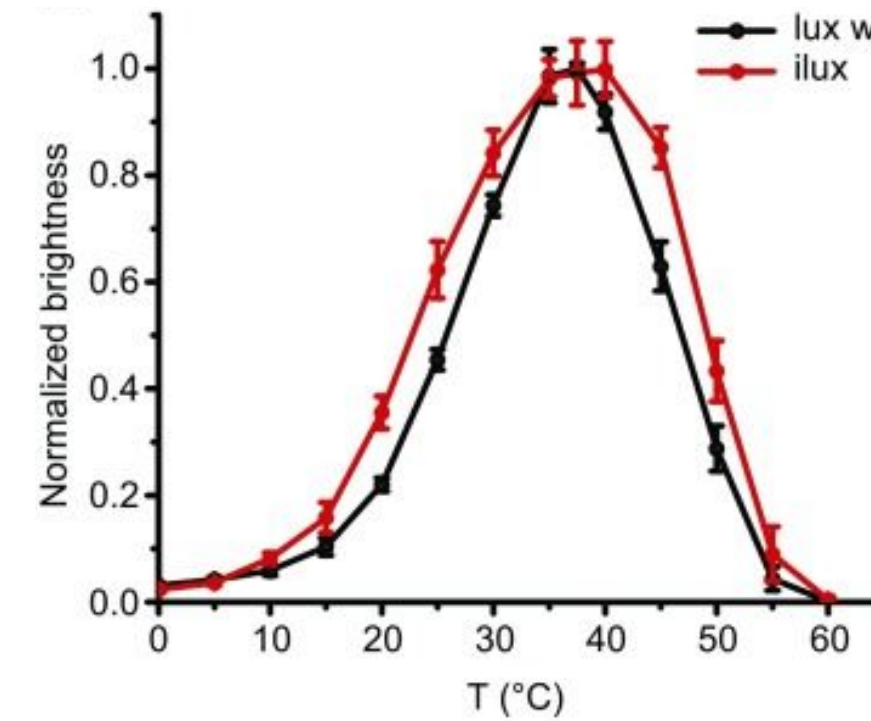


Fig 3. iLux Brightness vs. Temperature [5]

iLux 96-Well Plate Imaging

iLux bacteria were also plated in a 96-well plate at varying concentrations. Photon counts were measured as a baseline, then OpenIVIS captured images of the plate. The intensity values from OpenIVIS were compared to the photon counts to evaluate the system's sensitivity and signal detection performance.

4. Methods

System Calibration Protocol

To calibrate the system, the LED ring inside the box was used to illuminate the sample for focusing, not as a light source for excitation and a Python program focus.py returned the optimal lens position for manual focus.

Time Lapse Imaging Protocol

To analyze bioluminescent expression over time, a time-lapse imaging protocol was executed, taking an input of focused lens position, the time interval between image captures, the exposure times captured at each trial, and the total number of iterations.

Dark Frame Subtraction Protocol

A dark frame is a baseline image that captures the noise pattern of the camera sensor coming from fixed pattern noise or ambient light. For noise reduction and enhanced image visualization, the dark mean is subtracted pixel-by-pixel from the raw experimental image.

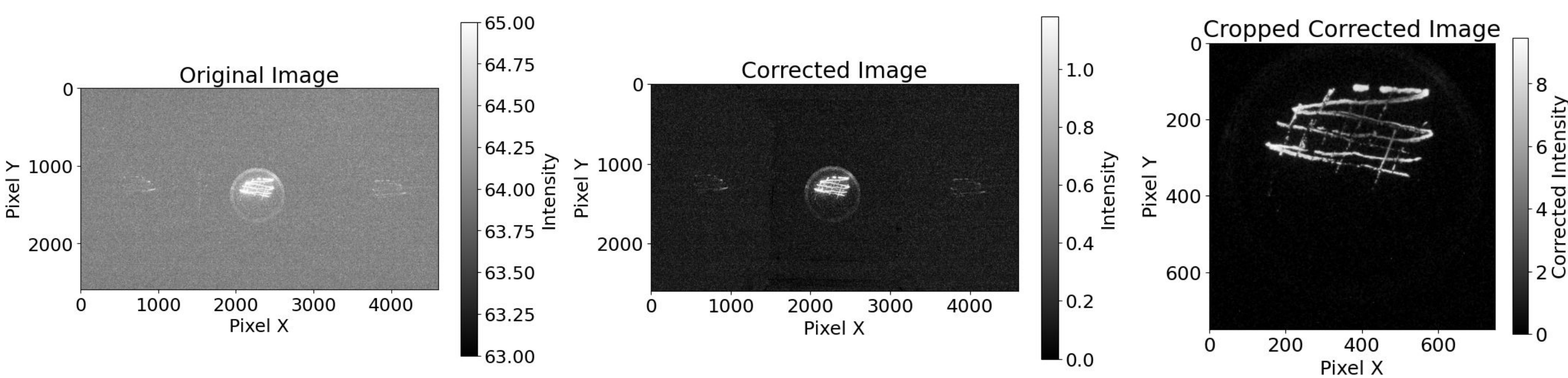


Fig 4. Dark Frame Subtraction Image Visualization Pipeline

Intensity vs. Time Protocol

A python script processes extracts the signal intensity for the brightest circular region of interest, then plots the total and mean intensity as a function of time.

Intensity Extraction Protocol (96-well Plate)

Regions of interest are manually defined for a 96-well plate uniform grid and a program computes and records both the total and mean intensities within each ROI.

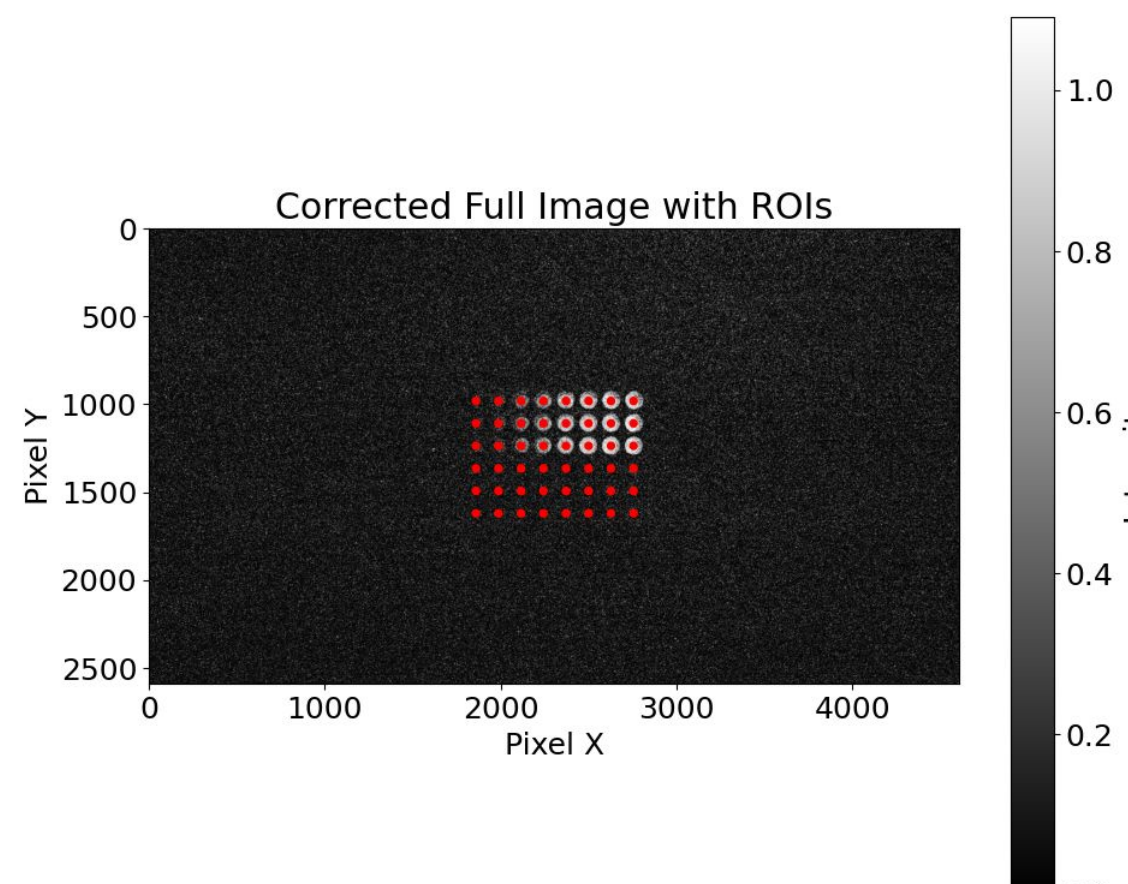


Fig 5. Intensity Extraction from 96-Well Plate

Intensity vs Photon Count and Intensity vs Concentration Protocol

Once intensity values are extracted, they are processed by a program that plots intensity vs. photon count, intensity vs. iLux concentration, and concentration vs. photon count. For nonlinear trends, the program applies a power law fit, outputting the fitted curve and R^2 value. When the data shows a nonlinear trend, the program applies appropriate curve-fitting techniques using power law, given by

$$y = kx^a$$

where x and y are the variables, k is the scalar constant, and a is the exponential factor [6]. Unlike a linear relationship which assumes a constant rate of change, a power law describes how the response increases at a nonlinear rate as the concentration increases.

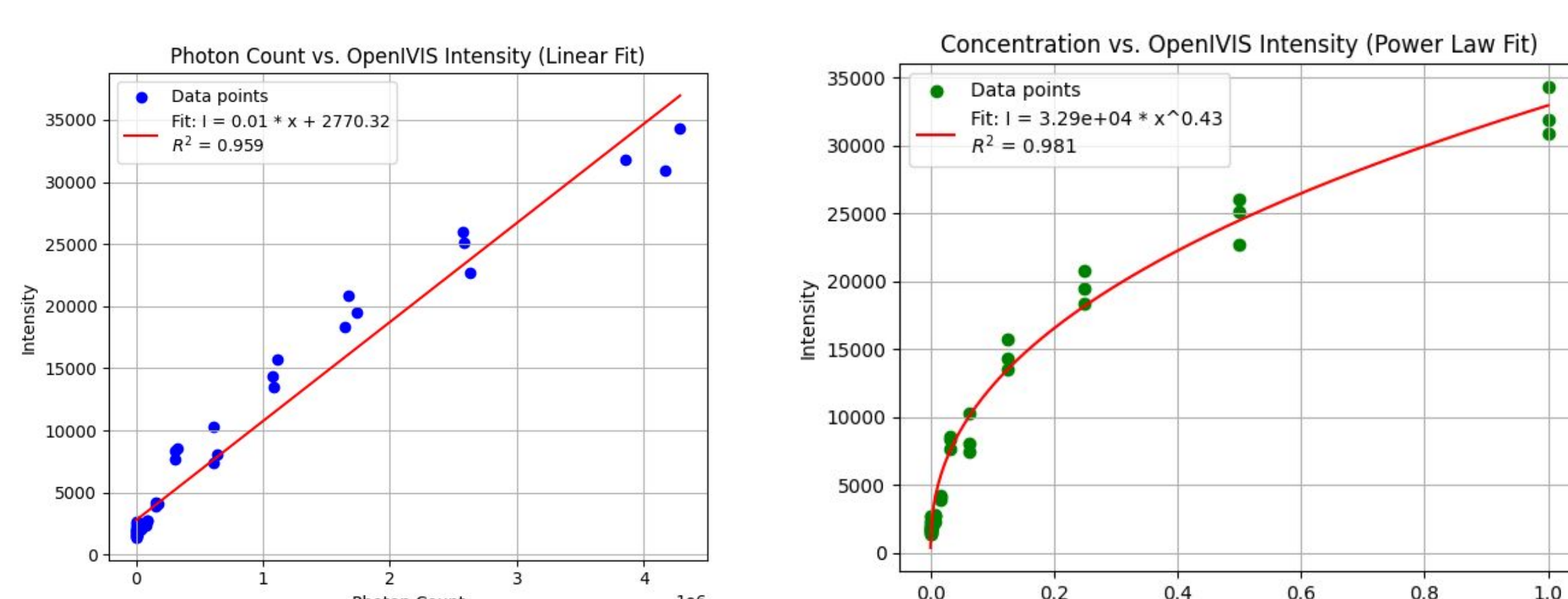


Fig 6. Intensity vs. Photon Count and Intensity vs. Concentration Graph

5. Results

Bioluminescence Intensity as a Function of Time

As expected, the iLux samples increased in brightness as the sample's temperature rose, confirming both the biological behavior of iLux and the sensitivity of our imaging system.

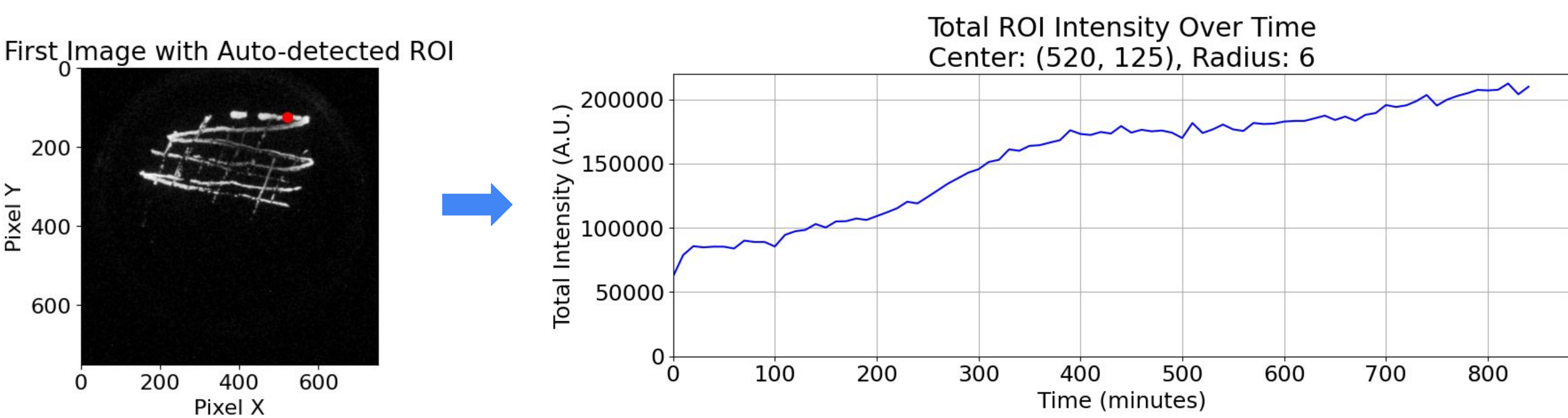


Fig 8. Bioluminescent Region of Interest (ROI)

Fig 9. iLux Intensity vs. Time for an Auto-Detected ROI

Concentration vs. Photon Count

To validate the relationship between iLux concentration and bioluminescent signal, photon counts from the commercial plate reader in the CMC Rathbun Lab were compared to concentrations prepared by researcher Evelyn Harrington using a 96-well plate of iLux dilutions. A correlation of 0.997 confirms a strong relationship between the two.

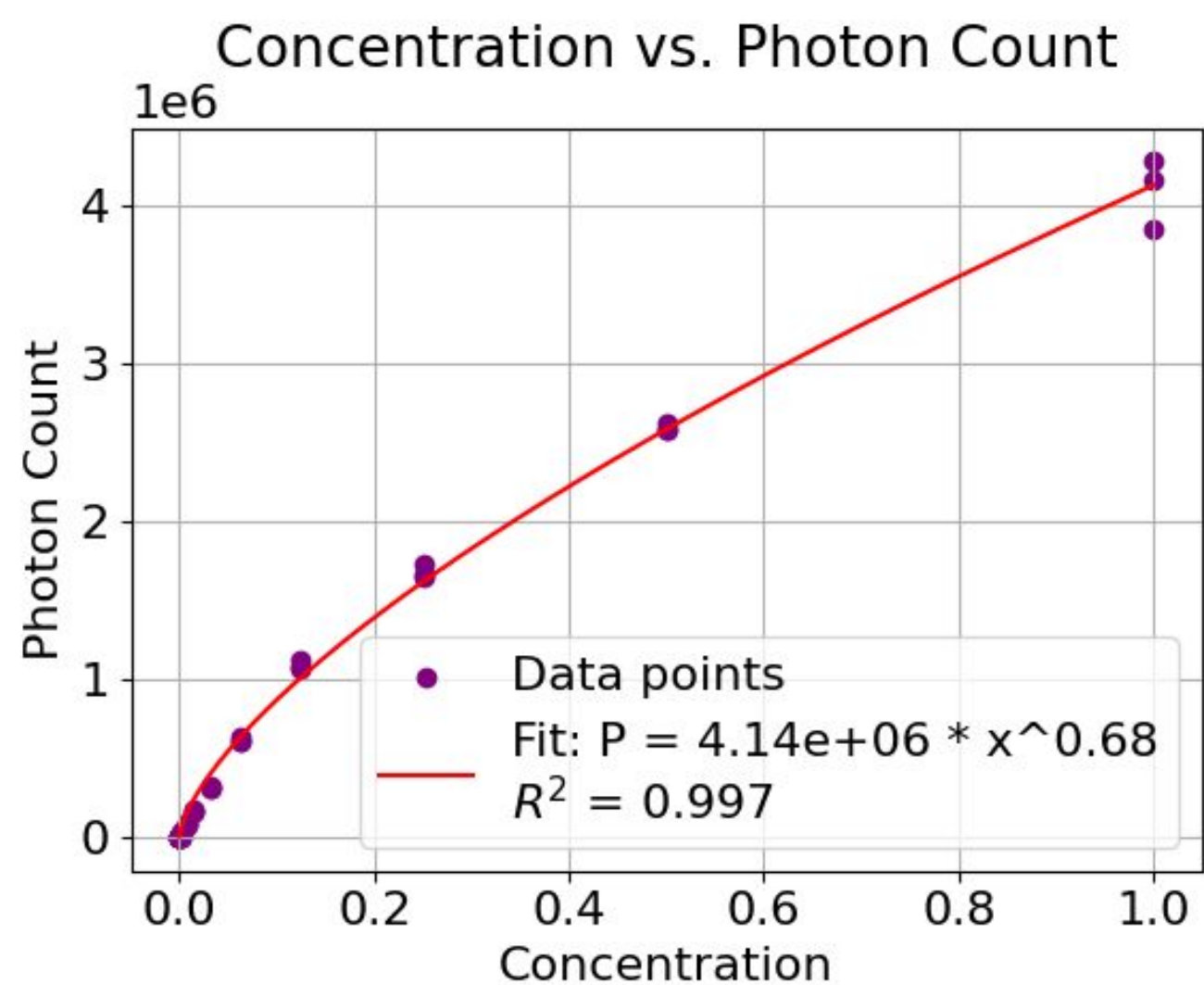


Fig 10. iLux Photon Count vs. Concentration

Photon Count vs. OpenIVIS Intensity

Photon counts from the commercial reader were compared to intensities extracted from OpenIVIS images of the 96-well plate. The two showed a strong correlation of 0.959, confirming that OpenIVIS reliably detected bioluminescent signals across a range of photon outputs.

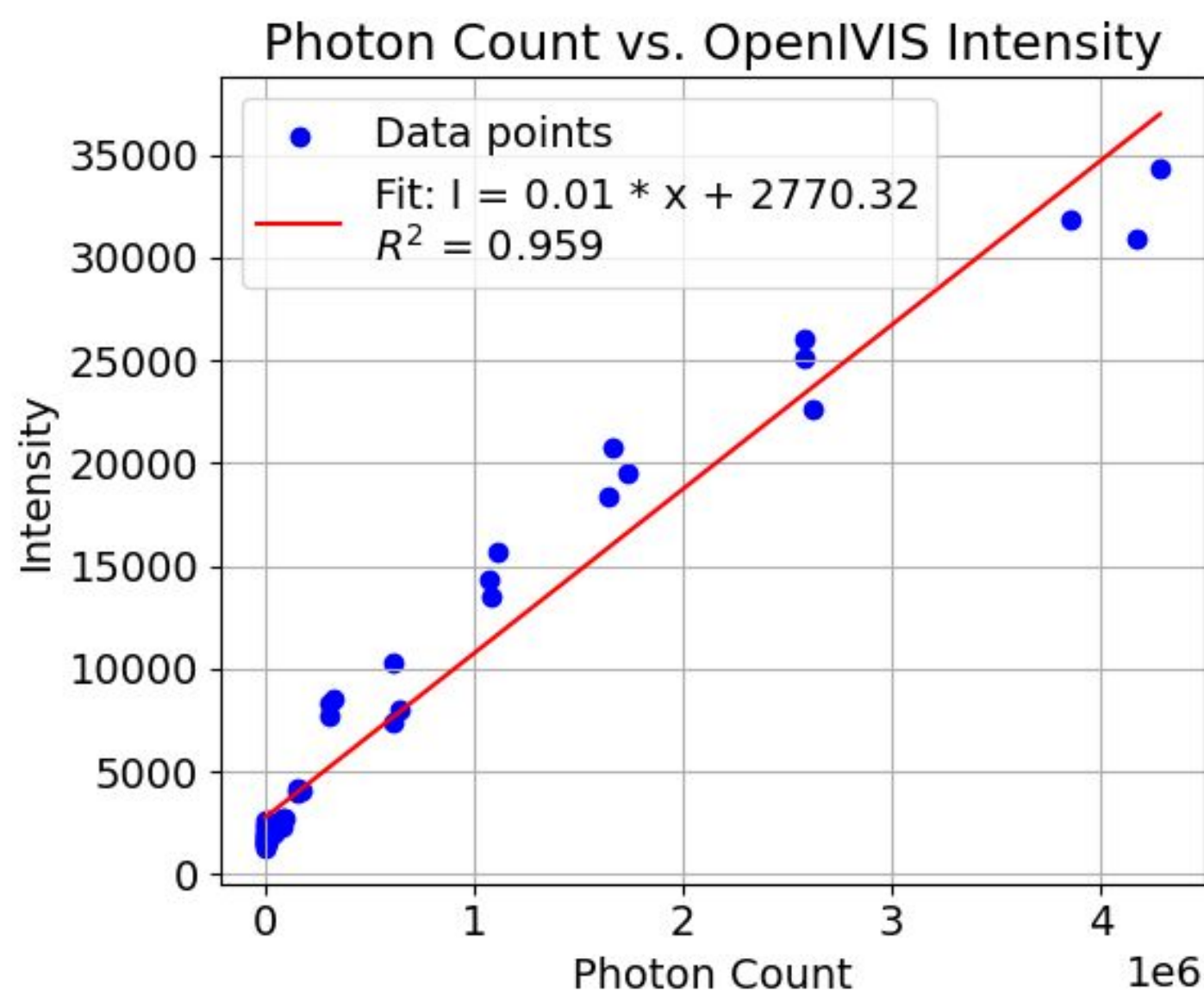


Fig 11. Open-IVIS Intensity vs. Photon Count

Concentration vs. OpenIVIS Intensity

Using concentration as the independent variable revealed a strong positive relationship with $R^2 = 0.981$. The exponential factor of 0.43 indicates sublinear growth, but the high correlation confirms that OpenIVIS effectively captures changes in bioluminescent intensity across concentrations.

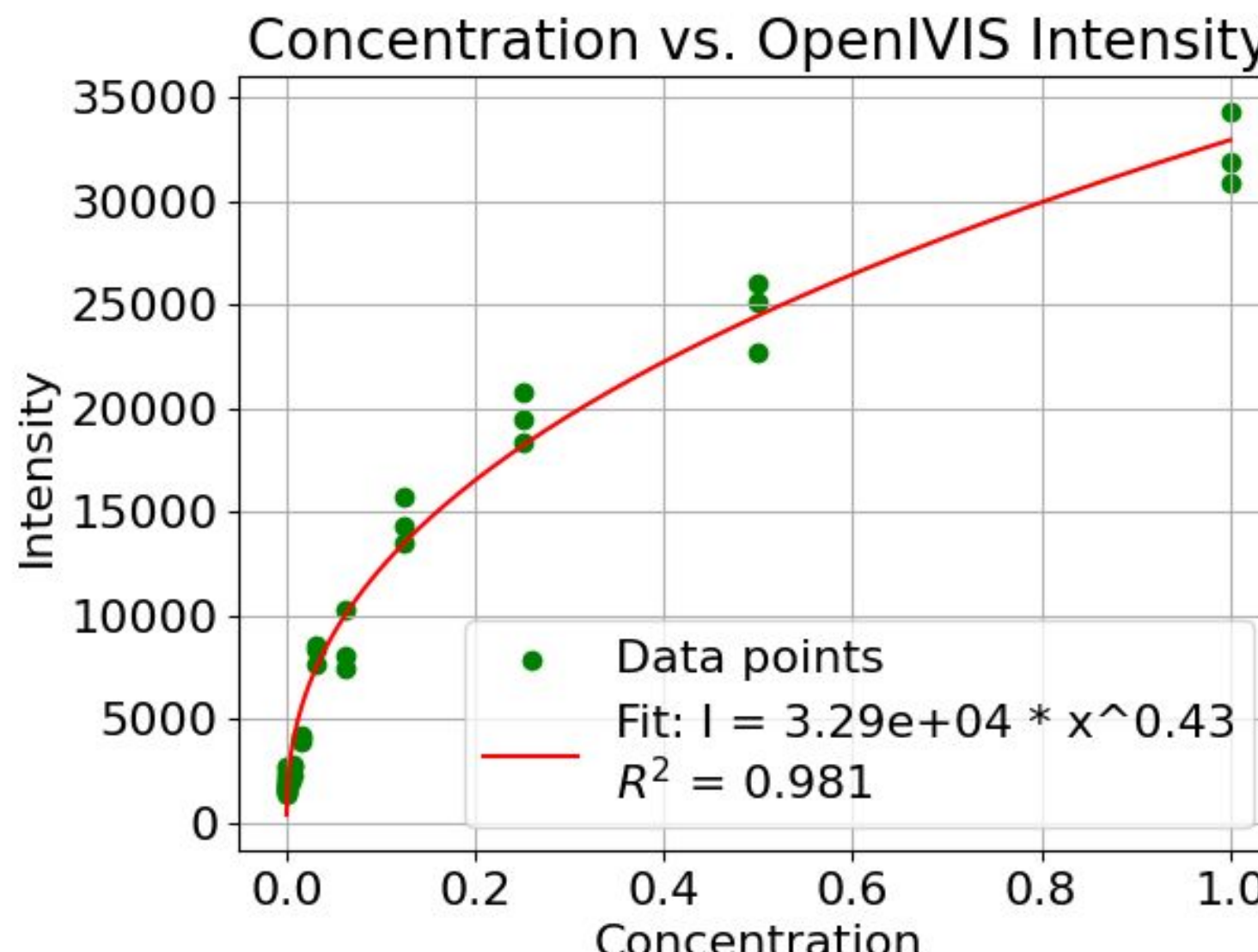


Fig 12. iLux Concentration vs. OpenIVIS Intensity

Intensity vs. Pixel Count

To track spatial changes in bioluminescent signal over time, intensity histograms were generated for the first and last images. The initial histogram shows few high-intensity pixels, indicating low expression, while the final histogram has a longer tail, reflecting more pixels at higher intensities.

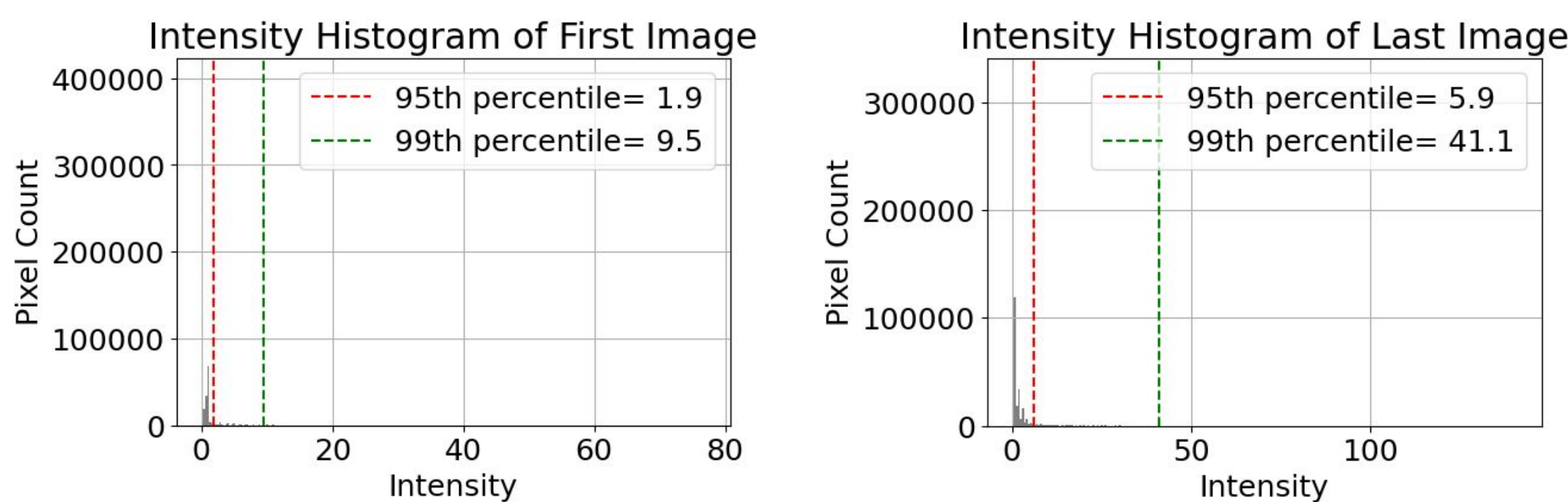


Fig 13. iLux Intensity vs. Pixel Count for an Auto-Detected ROI

6. Analysis

While OpenIVIS effectively captured bioluminescent intensity, its performance was limited by glare from ambient light. Even with the box closed, small seam leaks introduced noise into the signal. This issue is being mitigated with blackout material and improved sealing.

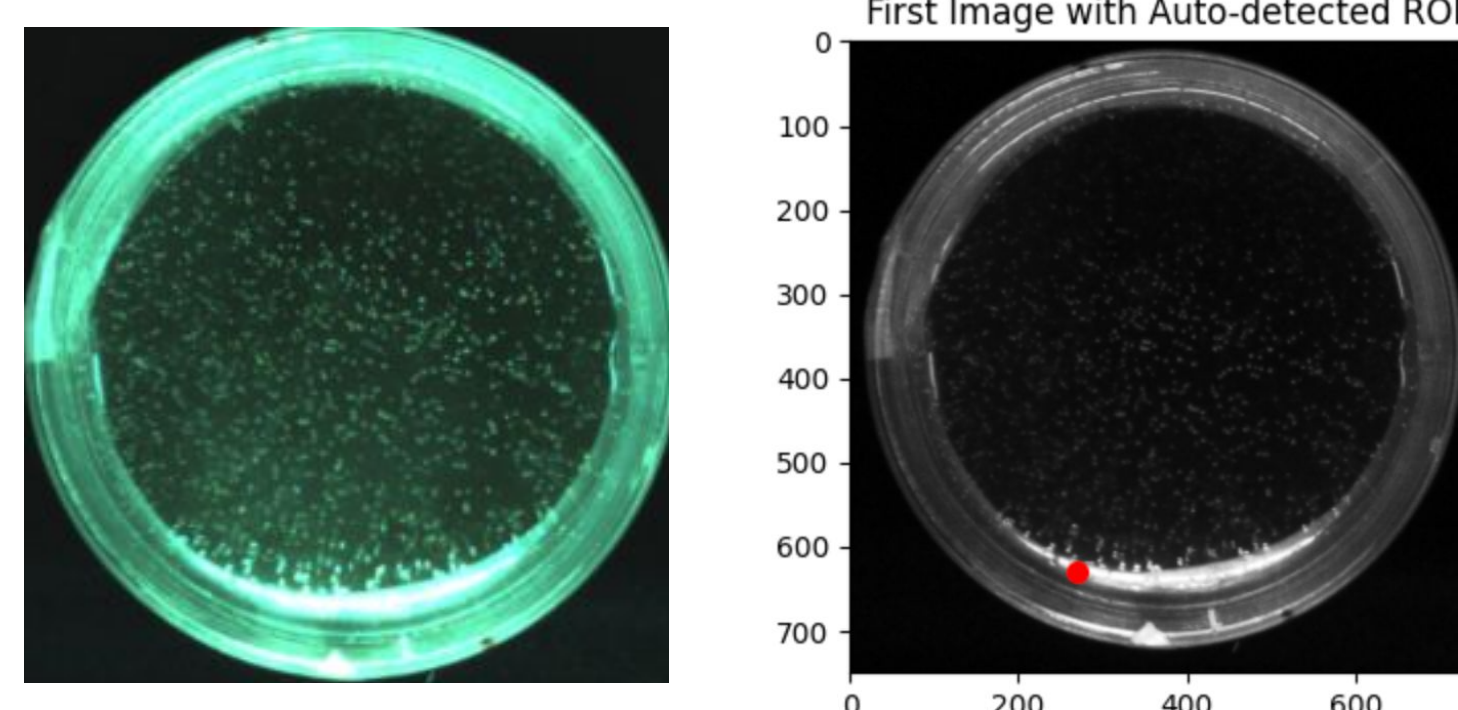


Fig 14. Glare-affected Agar Plate

Low signal intensity required longer exposures, which increased sensor noise. Dark frame subtraction reduced but did not eliminate this background. Despite these limitations, OpenIVIS intensities correlated strongly with photon counts and iLux concentrations, demonstrating its ability to provide quantitative imaging with accessible hardware.

7. Conclusion

Building on the existing OpenIVIS design, this work introduces a low-light bioluminescence imaging module capable of capturing signals across varying concentrations. OpenIVIS provides a cost-effective alternative to commercial in vivo systems, expanding access to advanced imaging for institutions with limited resources, such as the Rathbun Lab at CMC. Its modular design also supports customization for diverse imaging applications.

Future steps include developing a Python pipeline to characterize images by signal-to-noise ratio, spatial resolution, and minimum detectable signal; integrating a thermistor for real-time temperature monitoring to better correlate iLux brightness; and releasing all scripts and documentation through the HMC Biophotonics GitHub to enable broader adoption of OpenIVIS.

8. Acknowledgements

This work was supported by the Silicon Valley Community Foundation under Grant No. 2022-251322. The authors would like to thank Harvey Mudd College, Jacob Stampiel, and Sydney Torrey. We also thank the Rathbun Lab at Claremont McKenna College for generously providing iLux bacterial strains and experimental data for system validation.

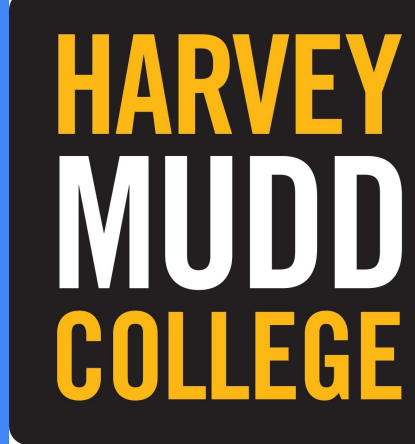
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9. References

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1. Introduction

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Fig 1. Newton FT-900 CCD Imager [2]

To perform bioluminescence imaging, OpenIVIS is in collaboration with the Rathbun Lab at Claremont McKenna College (CMC). Their work is focused on engineering bioluminescent enzymes to study cellular interactions. The challenge is that CMC lacks access to commercial IVIS systems, which are prohibitively expensive. OpenIVIS's motivation is to develop a low-cost, open-source bioluminescent imaging platform to support biology research at CMC and similar institutions.

Bioluminescence imaging (BLI) is a key functionality of an IVIS and is a noninvasive optical imaging technique that allows researchers to study biological processes in living organisms. Instead of illuminating a sample and detecting the emitted light, bioluminescence captures light that is naturally produced within living cells through luciferase enzymes. Since no external light source is required, background noise is significantly reduced. However, this also means the emitted light is extremely faint, so BLI systems typically use sensitive cameras to detect the low-intensity signals [3].

2. System Setup

The OpenIVIS box is constructed from laser-cut black acrylic sheets, designed with interlocking jigsaw edges to form a light-tight sealed enclosure. The front panel functions as a sliding door that lifts upward and locks into place. The lid for bioluminescence imaging consists of a 3D printed ring to hold an array of Neopixel LEDs and beneath that lays a white acrylic sheet is used as a light diffuser to provide even illumination when necessary. A 3D-printed camera stand is mounted on the diffusion plate to securely hold the camera in a fixed position above the sample.

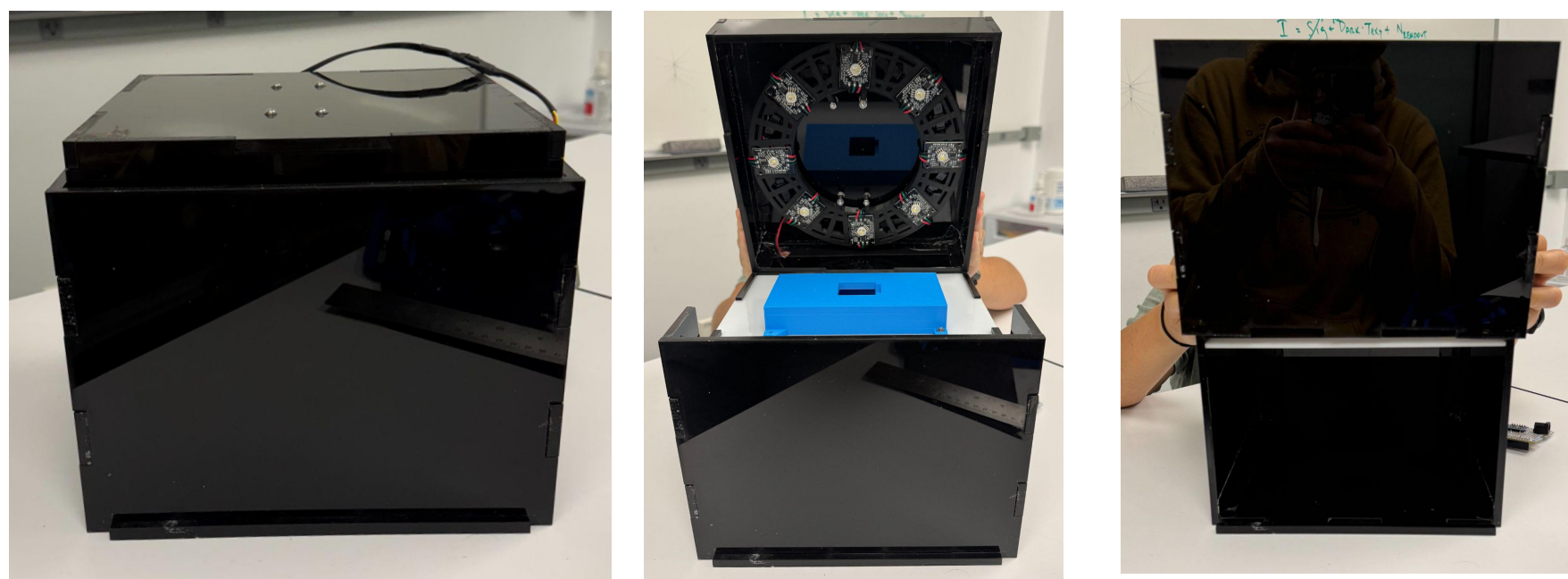


Fig 2. OpenIVIS Box with Bioluminescence Module

The OpenIVIS system is powered by a Raspberry Pi 4. The camera used to take all images is the Raspberry Pi Camera Module 3 NoIR and was chosen for a number of reasons: affordability and accessibility, no IR filter improves low-light performance, and camera sensor Sony IMX708 has great sensitivity and signal-to-noise performance in dim conditions [4]. Lastly, the camera connects to the Raspberry Pi 4, allowing for seamless real-time Python imaging.

The total cost of the OpenIVIS system was under \$210, making it significantly more affordable.

- Black and white acrylic and the acrylic cement: \$97
- Raspberry Pi 4 Model B and Camera Module 3 NoIR: \$60
- 8 Ultra-Bright NeoPixel RGBW LEDs: \$44
- Adafruit Perma-Proto HAT: \$5

3. Experiments

iLux Agar Plate Imaging

The OpenIVIS bioluminescence module was tested using iLux, a genetically engineered *E. coli* system developed in collaboration with the Rathbun Lab. iLux emits a bioluminescent signal that increases with temperature. The bacteria were plated in both agar plates, and time-lapse imaging was conducted over 16 hours at room temperature. The expectation was that brightness would increase with temperature [5].

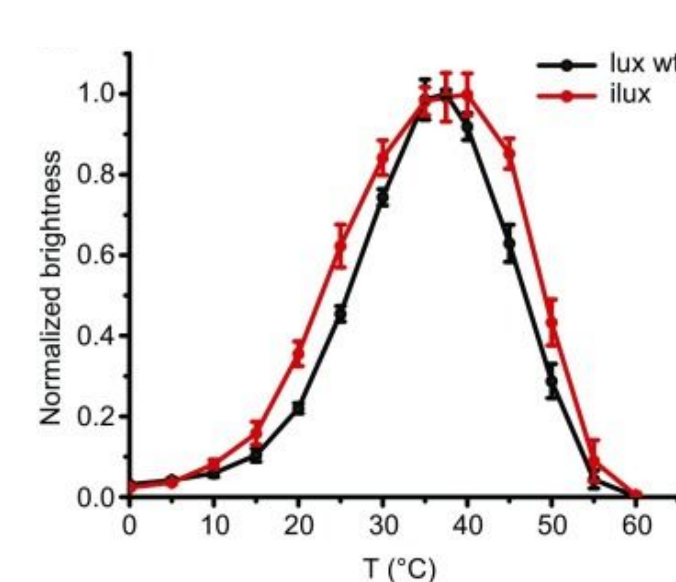


Fig 3. iLux Brightness vs. Temperature [5]

iLux 96-Well Plate Imaging

Additionally, the iLux bacteria was plated in a 96-well plate with varying concentrations and their photon counts were measured before imaging with OpenIVIS. These photon counts served as a baseline for evaluating the sensitivity of the OpenIVIS system. Then, the OpenIVIS system was then used to capture bioluminescent images of the well plate and the intensity values of each well were compared to the measured photon counts to assess signal detection performance.

4. Methods

System Calibration Protocol

To calibrate the system, the LED ring inside the box was used to illuminate the sample for focusing, not as a light source for excitation using script LEDOn.py. Python program focus.py was used to find the optimal lens position for manual focus and the same value was used during image capture with timeLapseBio.py.

Time Lapse Imaging Protocol

To analyze bioluminescent expression over time, a time-lapse imaging protocol was executed using the timeLapseBio.py python program. This function takes an input of lens position (determined by focus.py), the time interval between image captures, the exposure times captured at each trial, and the total number of iterations.

Dark Frame Subtraction Protocol

A dark frame is a baseline image captured under identical imaging conditions (exposure time and camera settings) but without any imaging subject present. This frame captures the noise pattern of the camera sensor coming from fixed pattern noise or ambient light. For reliable dark frame subtraction, ten dark frames were captured and the mean of the dark frames was output. For noise reduction and enhanced image visualization, the dark mean is subtracted pixel-by-pixel from the raw experimental image.

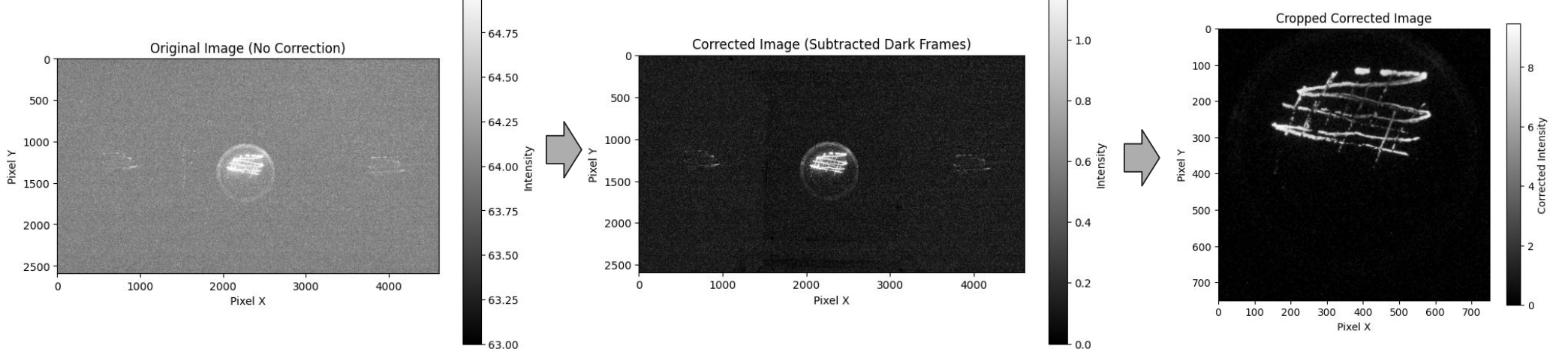


Fig 4. Dark Frame Subtraction Image Visualization Pipeline

Intensity vs. Time Protocol

A python script processes a folder of .npy images taken at successive time points and extracts the signal intensity for a circular region of interest, then plots the total and mean intensity as a function of time. To find the circular region of interest, the helper functions were used to locate the brightest region of interest, which is emphasized because it most likely reflects the area of peak bioluminescent activity, providing the clearest signal for quantifying changes over time.

Intensity Extraction Protocol (for 96-well Plate)

The program first performs dark frame subtraction to minimize noise, then the regions of interest are manually defined based on the well center coordinates of the top right well defined as (x,y) then creates a grid of center coordinates, since a 96-well plate is a uniform grid. Then, the program computes and records both the total and mean intensities within each ROI. These values are used for quantitative analysis, including the Intensity vs. Photon Count and Intensity vs. Concentration regressions.

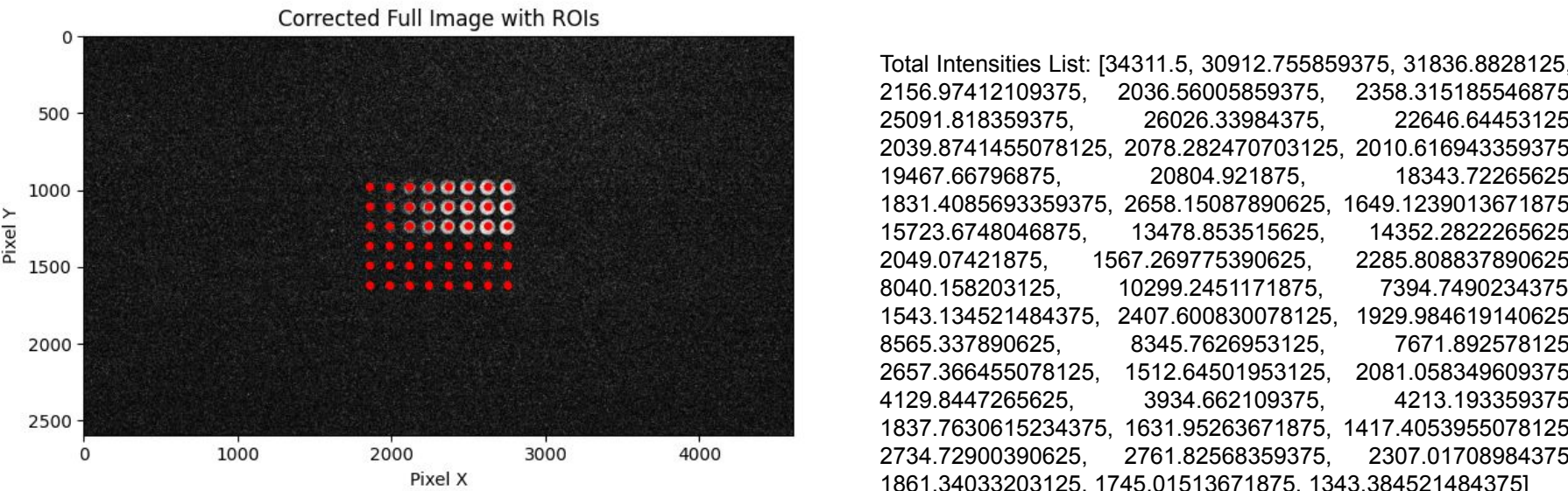


Fig 5. Intensity Extraction from 96-Well Plate

Intensity vs. Photon Count and Intensity vs. Concentration Protocol

Once intensity values are extracted, they are inputted into a program that plots intensity versus photon count, comparing OpenIVIS-derived intensity measurements to photon counts recorded by the commercial plate reader. The resulting linear regression line and R² value are outputted to assess the strength of the relationship.

Next, the program plots intensity versus iLux concentration and concentration versus photon counts following a similar process. When the data shows a nonlinear trend, the program applies appropriate curve-fitting techniques using power law. The fitted curve and corresponding R² value are outputted. The power law fit used in this graph models the relationship between bioluminescence intensity and concentration using the equation

$$y = k \cdot x^a$$

where x and y are the variables, k is the scalar constant, and a is the exponential factor [6]. Unlike a linear relationship which assumes a constant rate of change, a power law describes how the response increases at a nonlinear rate as the concentration increases.

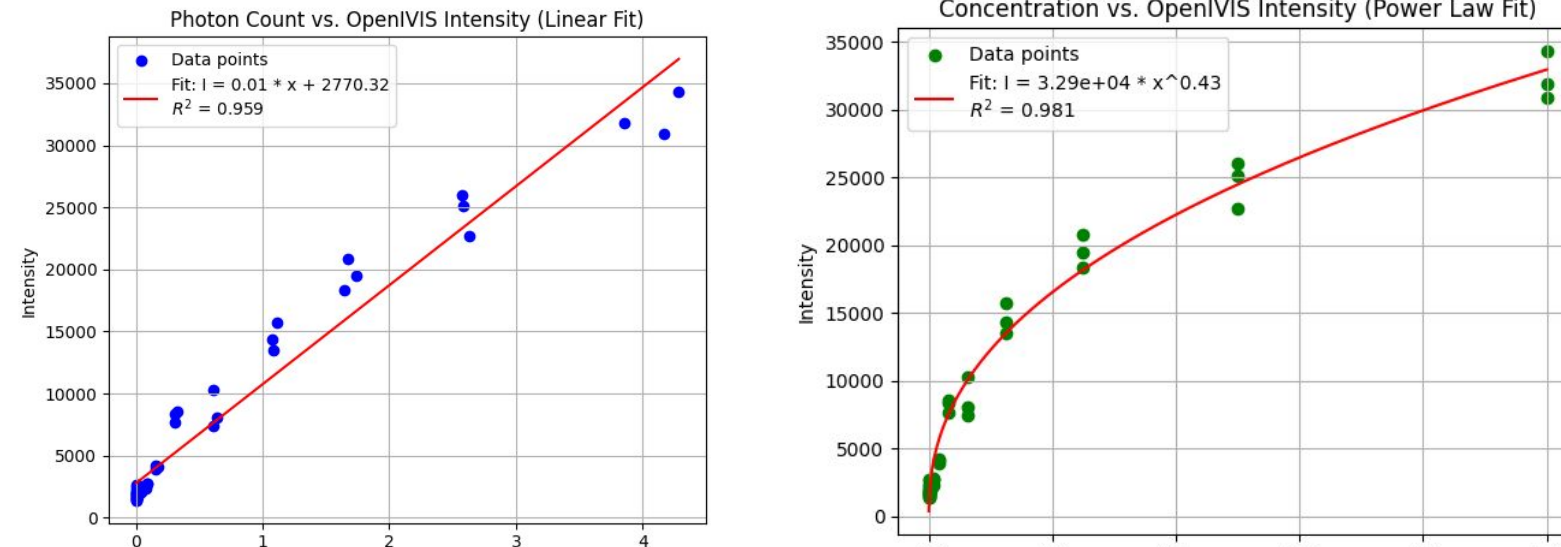


Fig 6. Intensity vs. Photon Count and Intensity vs. Concentration Graph

5. Results

Bioluminescence Intensity as a Function of Time

As expected, the iLux samples increased in brightness as room temperature rose. Intensity plots generated with intensity vs. time function confirmed both the biological behavior of iLux and the sensitivity of our imaging system. As time increases, the total and mean intensity increase, indicating that bioluminescent output increases over time. This increase in intensity makes sense based on our predictions of temperature starting around 5 degrees Celsius to 22 degrees Celsius.

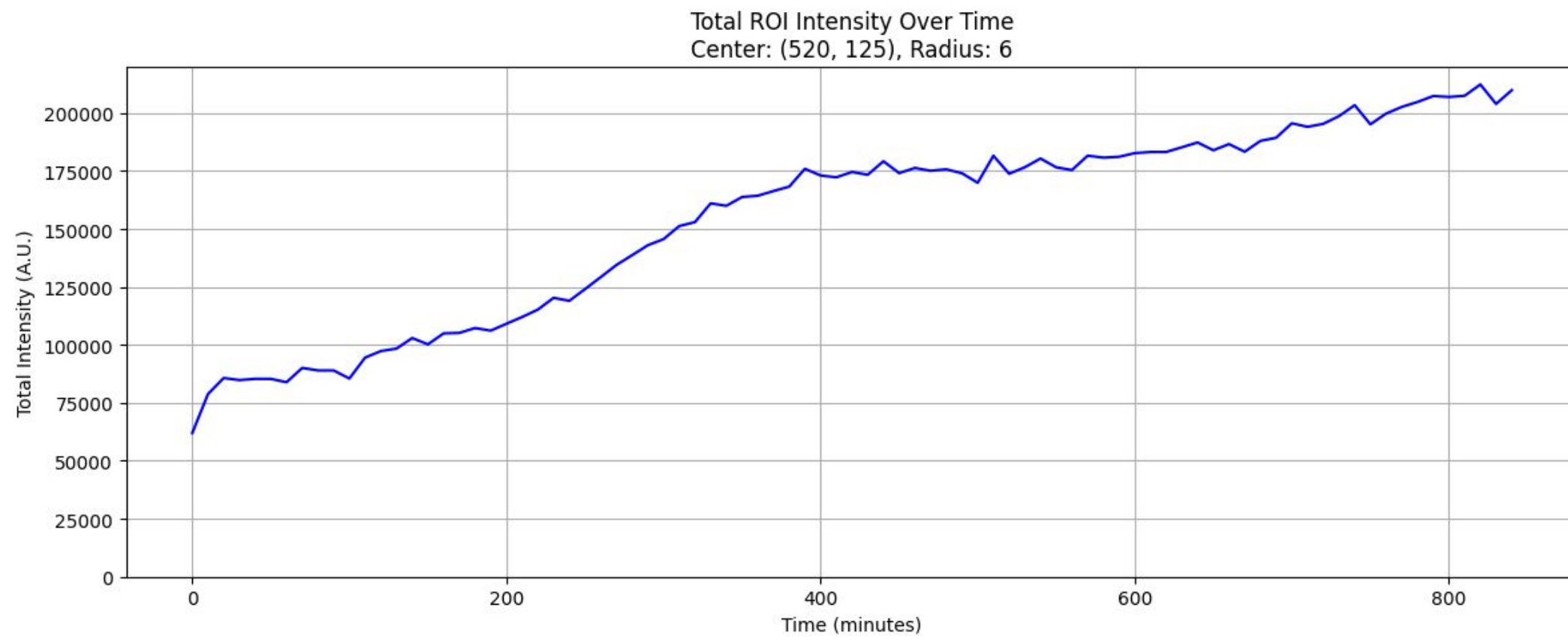


Fig 7. iLux Intensity vs. Time for an Auto-Detected ROI

To visualize how bioluminescent signal intensity changed spatially over time, intensity histograms (intensity vs. pixel count) were generated. These histograms visualize pixel intensity distributions in the first and last images of the time series. The histogram of the first image suggests either a low initial expression of bioluminescence because there are less pixels at higher intensities. The histogram of the last image seems to have a longer tail, suggesting that more pixels contain higher intensities.

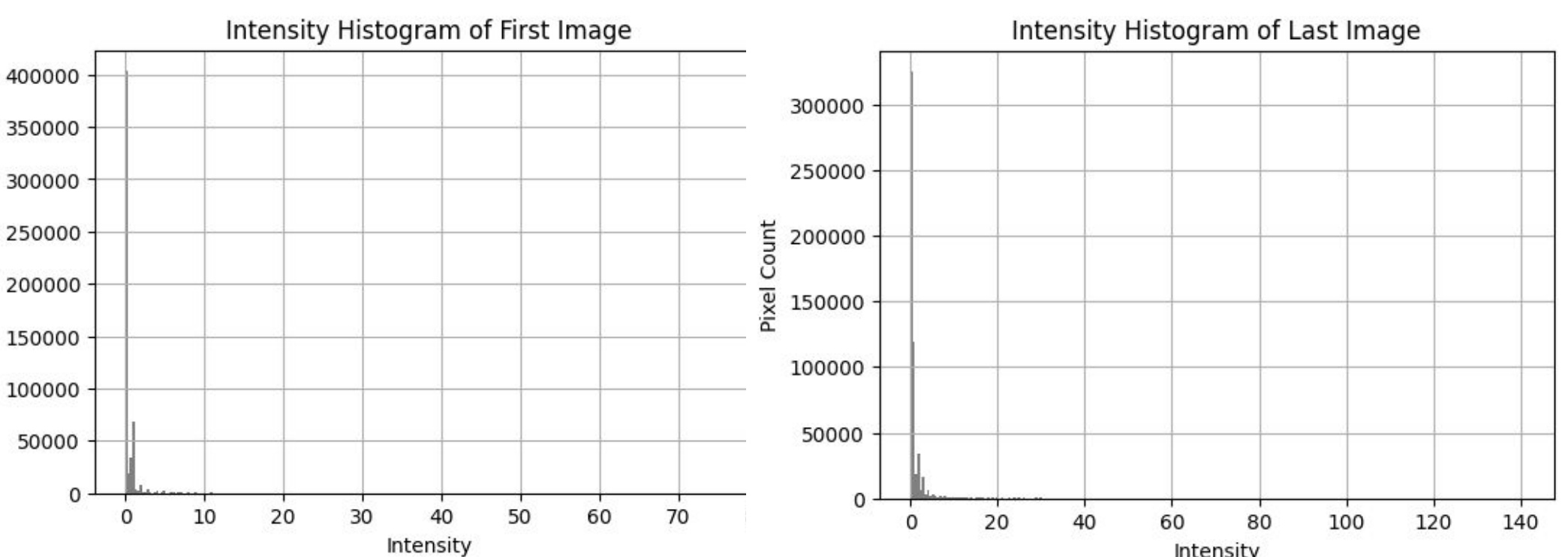


Fig 8. iLux Intensity vs. Pixel Count for an Auto-Detected ROI

Concentration vs. Photon Count

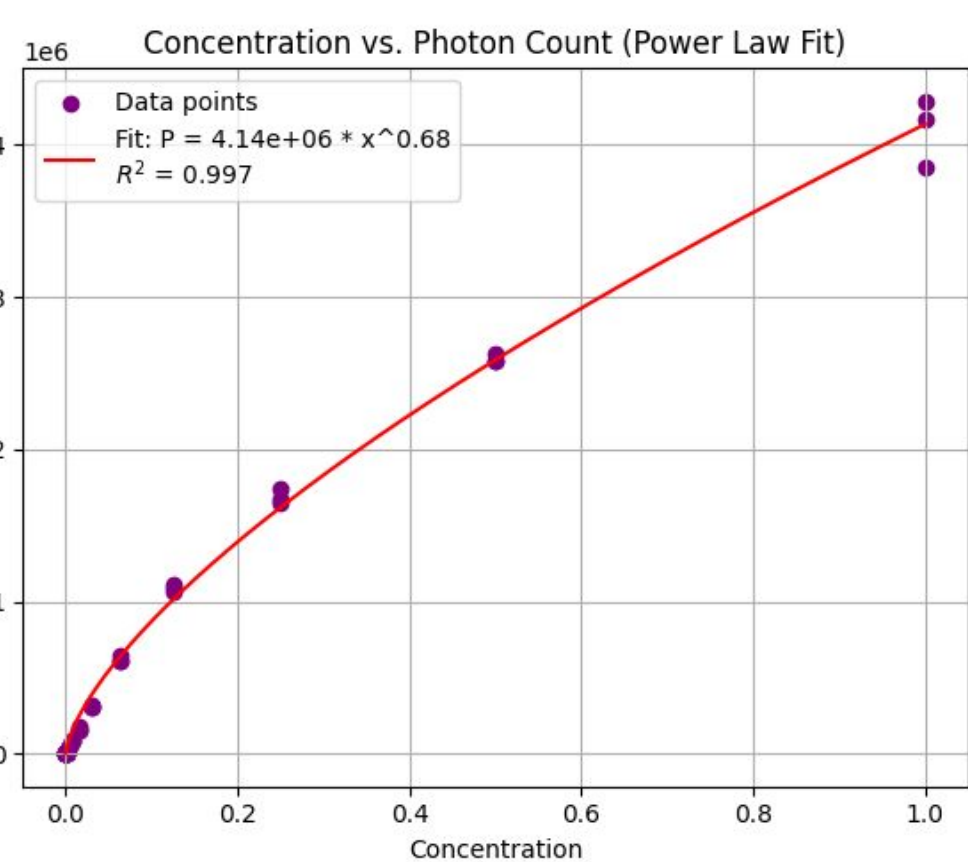


Fig 9. iLux Photon Count vs. Concentration

With a correlation value of 0.997, this confirms the strong, positive trend that with increasing concentration results in an increased bioluminescent signal in photon counts, even nonlinearly. In this case, an exponential factor of 0.68 indicates that the relationship between concentration and photon count is sublinear growth, which means that as concentration increases, photon count also increases, but at a decreasing rate.

Photon Count vs. OpenIVIS Intensity

To measure the sensitivity of the OpenIVIS system, the photon counts can be compared to intensity values extracted from OpenIVIS images of the 96-well plate using the Intensity Extraction Protocol. When comparing the OpenIVIS-derived intensities to the photon counts from the commercial reader, the system showed a strong correlation.

Graphing the two variables revealed a strong positive correlation with an R² value of 0.959. This means that the system reliably detected bioluminescent signals across a range of photon outputs. Next, a relationship between concentration and intensity can be made by graphing the two variables, with concentration as the independent variable.

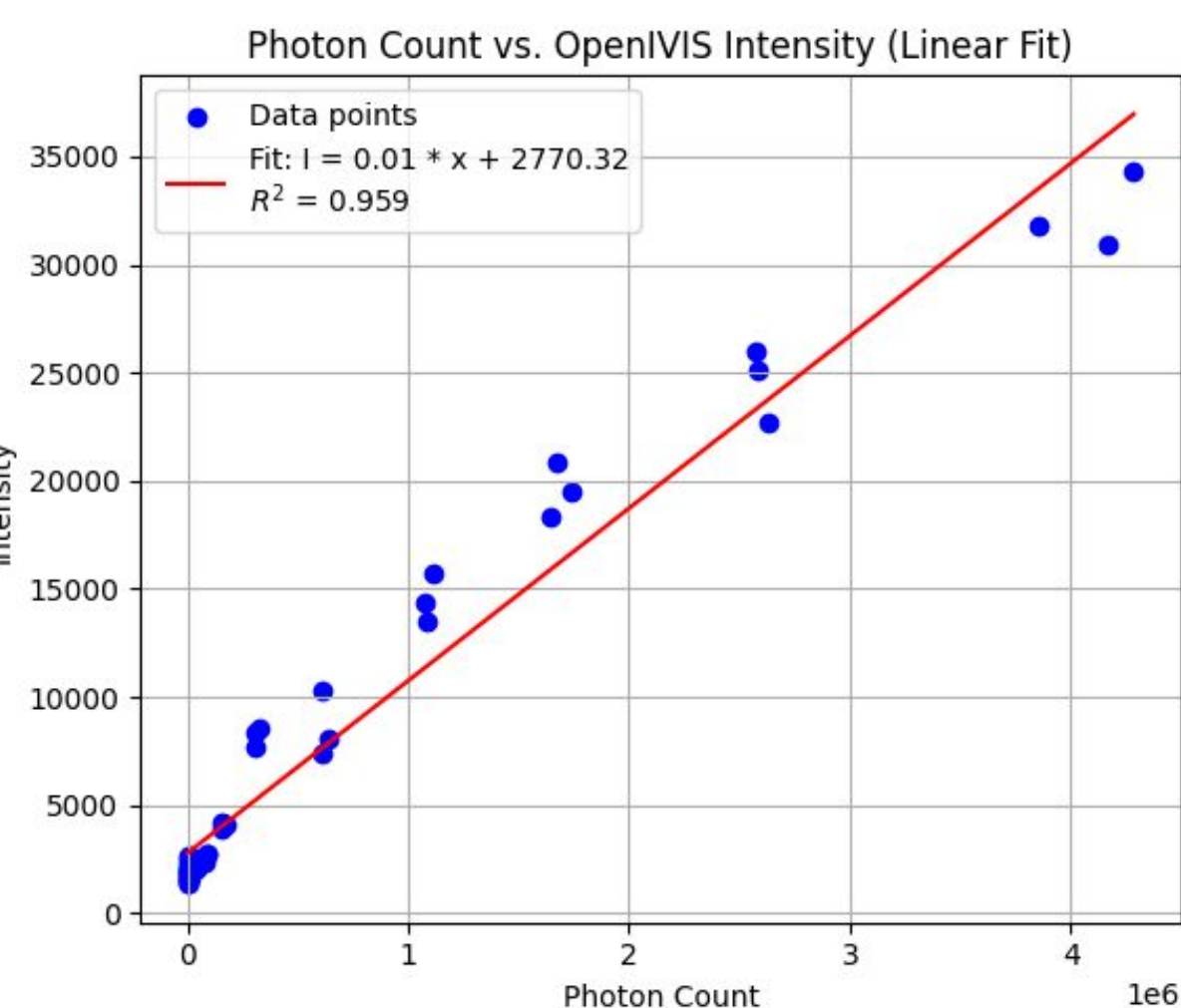


Fig 10. Open-IVIS Intensity vs. Photon Count

Concentration vs. OpenIVIS Intensity

Finally, to establish the relationship between iLux concentration and intensity, an analysis was performed using concentration as the independent variable. This resulted in a strong, positive relationship with an R² value of 0.981. In this case, an exponential factor of 0.43 indicates that the relationship between concentration and photon count is sublinear growth. Although the power law relationship differs from a linear fit, the high correlation shows that the OpenIVIS system effectively captures changes in bioluminescent intensity with varying concentrations.

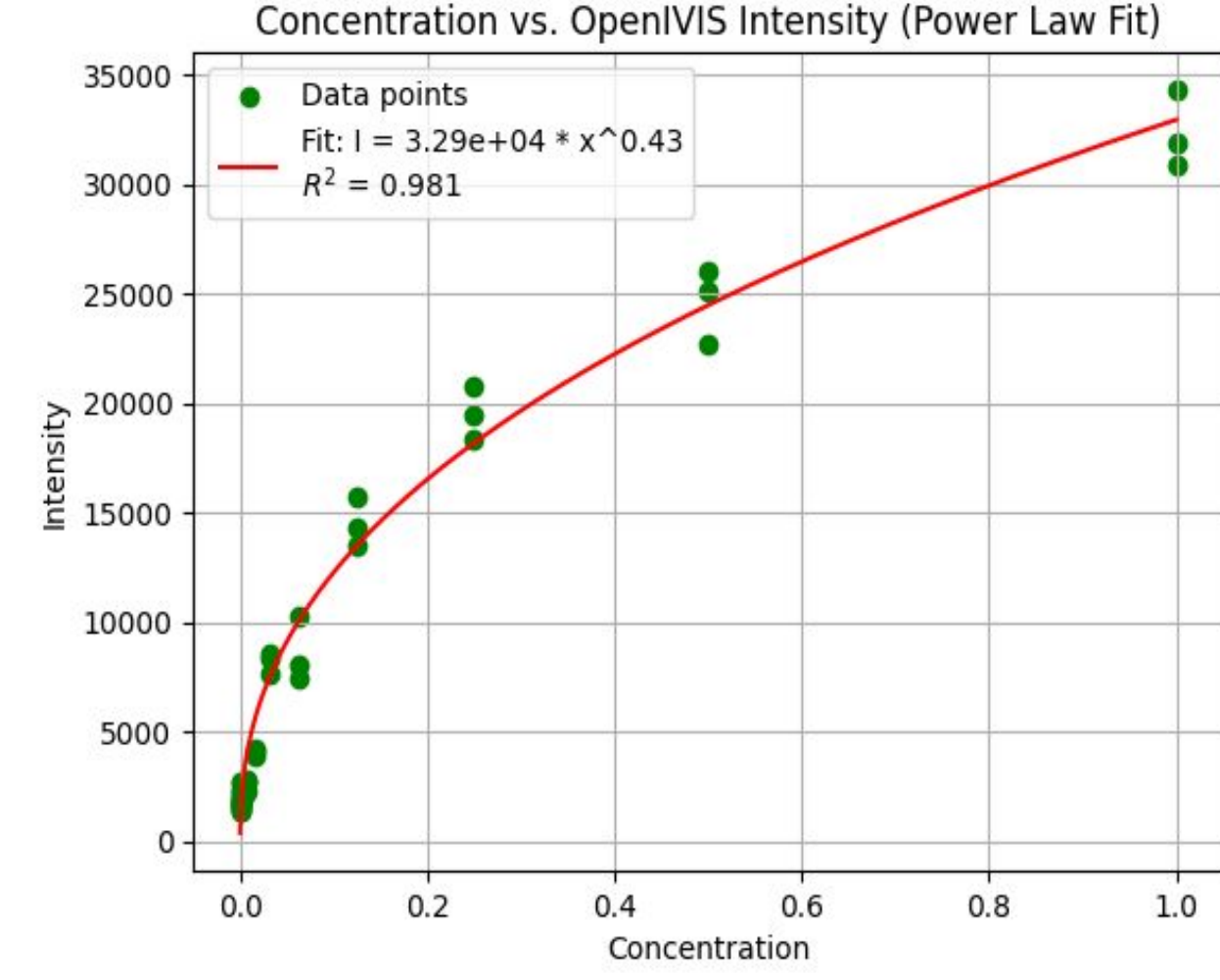


Fig 11. iLux Concentration vs. OpenIVIS Intensity

6. Analysis

While the OpenIVIS system successfully captured changes in bioluminescent intensity, several challenges limited its performance. The most prominent issue was glare from external light sources, especially in rooms ambient lighting. Even with the box closed, small leaks in the seams can introduce enough light to affect the signal. Currently, this problem is being addressed by using a blackout material over the box and improving the box seal.

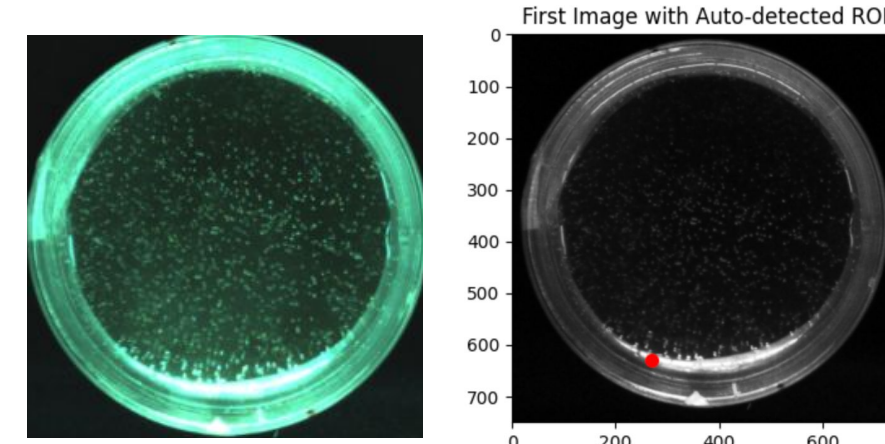


Fig 12. Glare-affected Agar Plate

The low intensity of bioluminescent signals meant longer exposure times were often necessary to capture the signal, increasing sensor readout noise. Although dark frame subtraction reduced background noise, residual noise remained.

Despite these challenges, the OpenIVIS system showed a strong correlation between OpenIVIS-derived intensities, photon counts from a commercial reader, and known iLux concentrations. This demonstrates that the OpenIVIS system can achieve high-fidelity, quantitative biological imaging using accessible hardware.

7. Conclusion

Building on the existing OpenIVIS design, this work presents a low-light detection bioluminescence imaging module. The system successfully captures low-intensity bioluminescent signals with varying concentrations.

OpenIVIS aims to provide a cost-effective alternative to commercial in vivo imaging systems, opening access to advanced biological imaging techniques for educational institutions, community colleges, and small research groups with limited funding like the Rathbun Lab at CMC. Its modular components allow for customization of diverse imaging modalities.

Moving forward, OpenIVIS plans to write a Python program pipeline to characterize images with signal to noise ratio, spatial resolution, and minimum detectable signal to provide biologists with image characteristics. In addition, integration of a thermistor to measure the temperature of the agar plates in real time and correlate it precisely with iLux brightness will provide more accurate and precise results. Lastly, OpenIVIS plans to release all Python scripts on the HMC Biophotonics public GitHub repository and documentation website to help others build and use OpenIVIS themselves.

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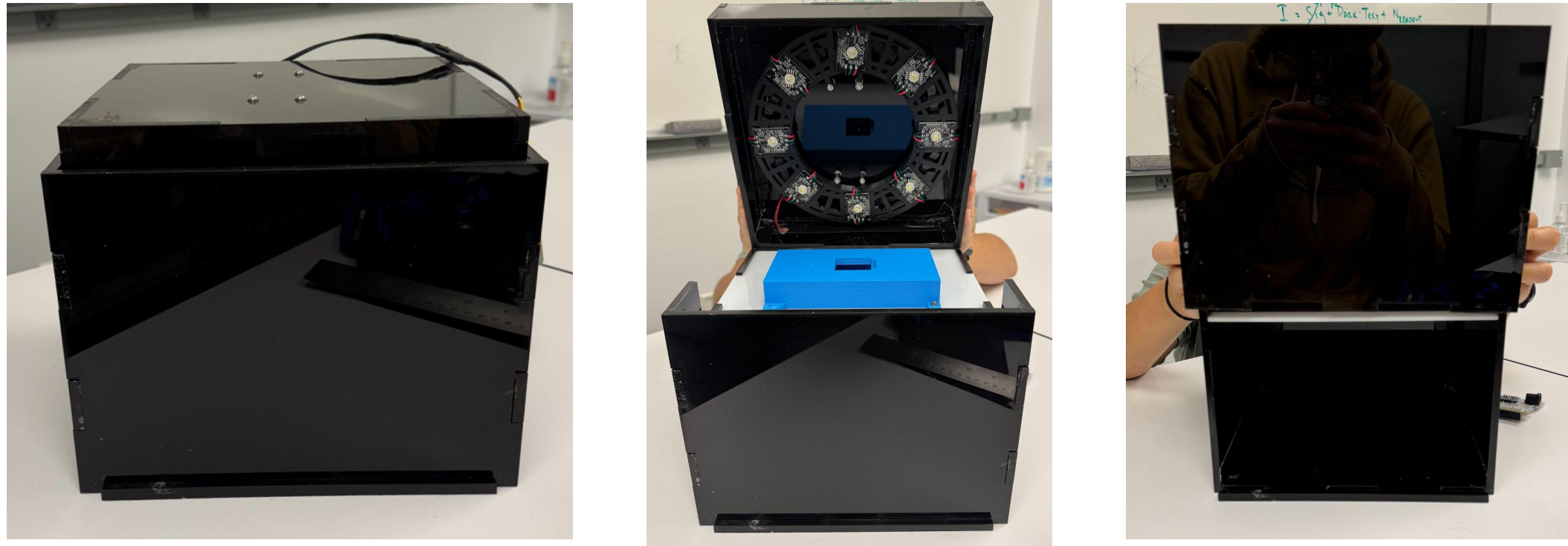


Fig 2. OpenIVIS Box with Bioluminescence Module

The OpenIVIS system is powered by a Raspberry Pi 4. The camera used to take all images is the Raspberry Pi Camera Module 3 NoIR and was chosen for a number of reasons. Affordability and accessibility is crucial for OpenIVIS, so the camera being priced around \$30 is ideal. Having no IR filter improves its low-light performance which is ideal for bioluminescence imaging and the camera sensor Sony IMX708 has great sensitivity and signal-to-noise performance in dim conditions [4]. Lastly, the camera connects to the Raspberry Pi 4, allowing for seamless real-time Python imaging scripts directly on the Pi.

Cost Breakdown
The total cost of the OpenIVIS system was under \$210, making it significantly more affordable than commercially available IVIS systems.

- Black and white acrylic and the acrylic cement: \$97
- Raspberry Pi 4 Model B: \$35
- Raspberry Pi Camera Module 3 NoIR: \$25
- 8 Ultra-Bright NeoPixel RGBW LEDs: \$44.
- Adafruit Perma-Proto HAT: \$5

3. Experiments

iLux Agar Plate Imaging

The OpenIVIS bioluminescence module was tested using iLux, a genetically engineered *E. coli* system developed in collaboration with the Rathbun Lab. iLux emits a bioluminescent signal that increases with temperature. The bacteria were plated in both agar plates, and time-lapse imaging was conducted over 16 hours at room temperature. The expectation was that brightness would increase with temperature [5].

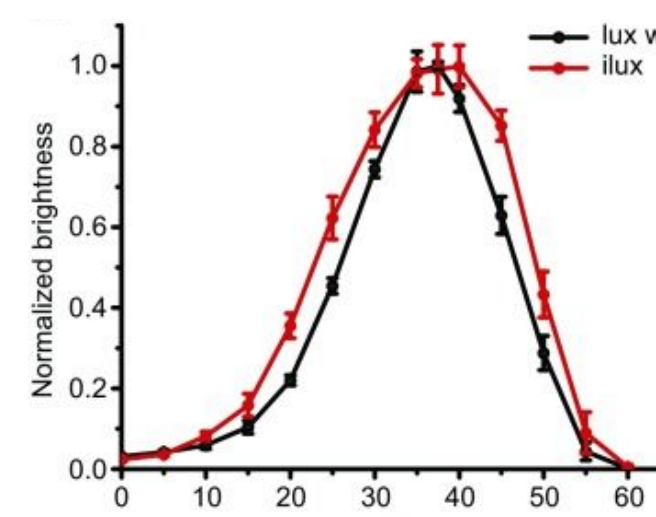


Fig 6. iLux Brightness vs. Temperature [7]

iLux 96-Well Plate Imaging

Additionally, the iLux bacteria was plated in a 96-well plate with varying concentrations and their photon counts were measured before imaging with OpenIVIS. These photon counts served as a baseline for evaluating the sensitivity of the OpenIVIS system. Then, the OpenIVIS system was then used to capture bioluminescent images of the well plate and the intensity values of each well were compared to the measured photon counts to assess signal detection performance.

4. Methods

System Calibration Protocol

To calibrate the system, the LED ring inside the box was used to illuminate the sample for focusing, not as a light source for excitation using script LEDOn.py. Python program focus.py was used to find the optimal lens position for manual focus and the same value was used during image capture with timeLapseBio.py.

Time Lapse Imaging Protocol

To analyze bioluminescent expression over time, a time-lapse imaging protocol was executed using the timeLapseBio.py python program. This function takes an input of lens position (determined by focus.py), the time interval between image captures, the exposure times captured at each trial, and the total number of iterations.

Dark Frame Subtraction Protocol

A dark frame is a baseline image captured under identical imaging conditions (exposure time and camera settings) but without any imaging subject present. This frame captures the noise pattern of the camera sensor coming from fixed pattern noise or ambient light. For reliable dark frame subtraction, ten dark frames were captured and the mean of the dark frames was output. For noise reduction and enhanced image visualization, the dark mean is subtracted pixel-by-pixel from the raw experimental image.

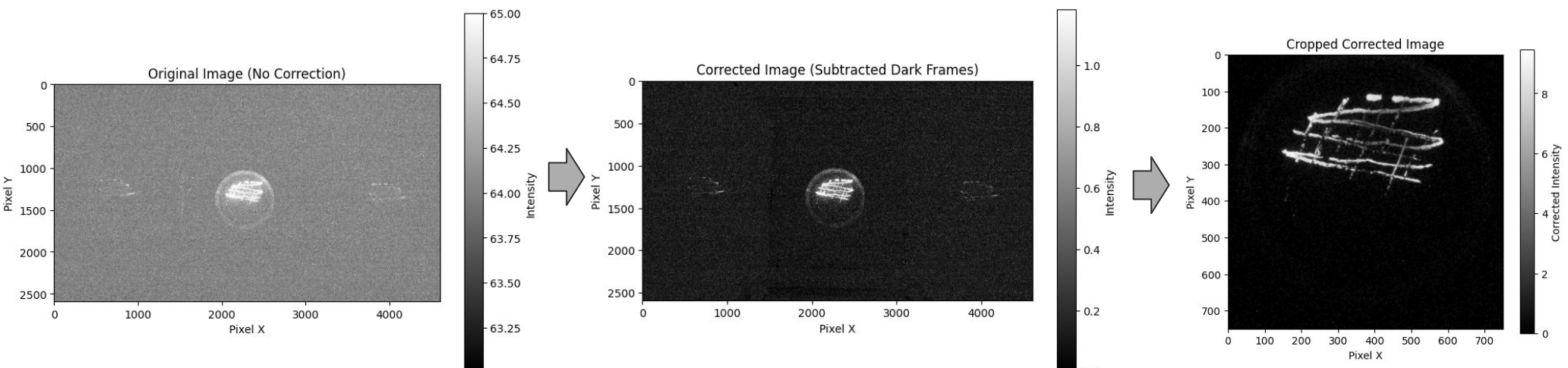


Fig 3. Dark Frame Subtraction Image Visualization Pipeline

Intensity vs. Time Protocol

A python script processes a folder of .npy images taken at successive time points and extracts the signal intensity for a circular region of interest, then plots the total and mean intensity as a function of time. To find the circular region of interest, the helper functions were used to locate the brightest region of interest, which is emphasized because it most likely reflects the area of peak bioluminescent activity, providing the clearest signal for quantifying changes over time.

Intensity Extraction Protocol (for 96-well Plate)

The program first performs dark frame subtraction to minimize noise, then the regions of interest are manually defined based on the well center coordinates of the top right well defined as (x,y) then creates a grid of center coordinates, since a 96-well plate is a uniform grid. Then, the program computes and records both the total and mean intensities within each ROI. These values are used for quantitative analysis, including the Intensity vs. Photon Count and Intensity vs. Concentration regressions.

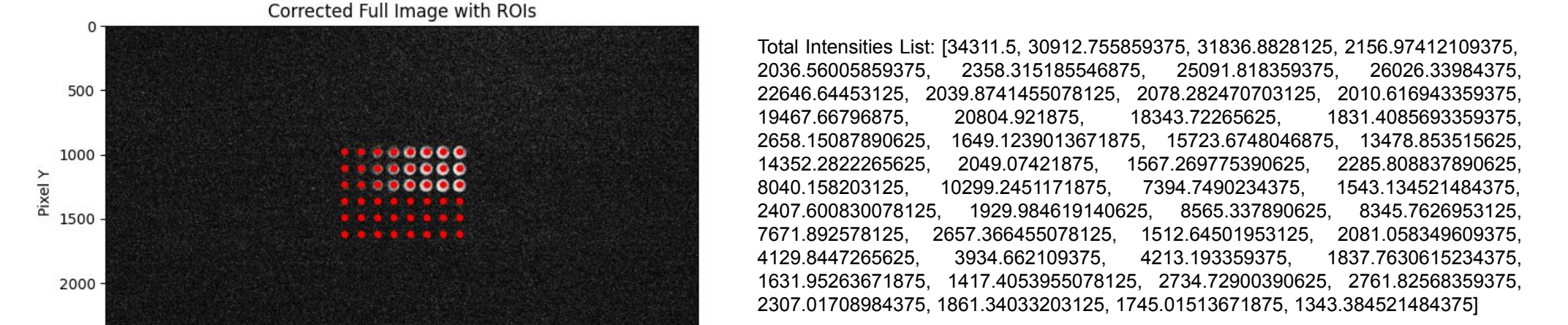


Fig 4. Intensity Extraction from 96-Well Plate

Intensity vs Photon Count and Intensity vs Concentration Protocol

Once intensity values are extracted, they are inputted into a program that plots intensity versus photon count, comparing OpenIVIS-derived intensity measurements to photon counts recorded by the commercial plate reader. The resulting linear regression line and R² value are outputted to assess the strength of the relationship.

Next, the program plots intensity versus iLux concentration and concentration versus photon counts following a similar process. When the data shows a nonlinear trend, the program applies appropriate curve-fitting techniques using power law. The fitted curve and corresponding R² value are outputted. The power law fit used in this graph models the relationship between bioluminescence intensity and concentration using the equation

$$y = k \cdot x^a$$

where x and y are the variables, k is the scalar constant, and a is the exponential factor [6]. Unlike a linear relationship which assumes a constant rate of change, a power law describes how the response increases at a nonlinear rate as the concentration increases.

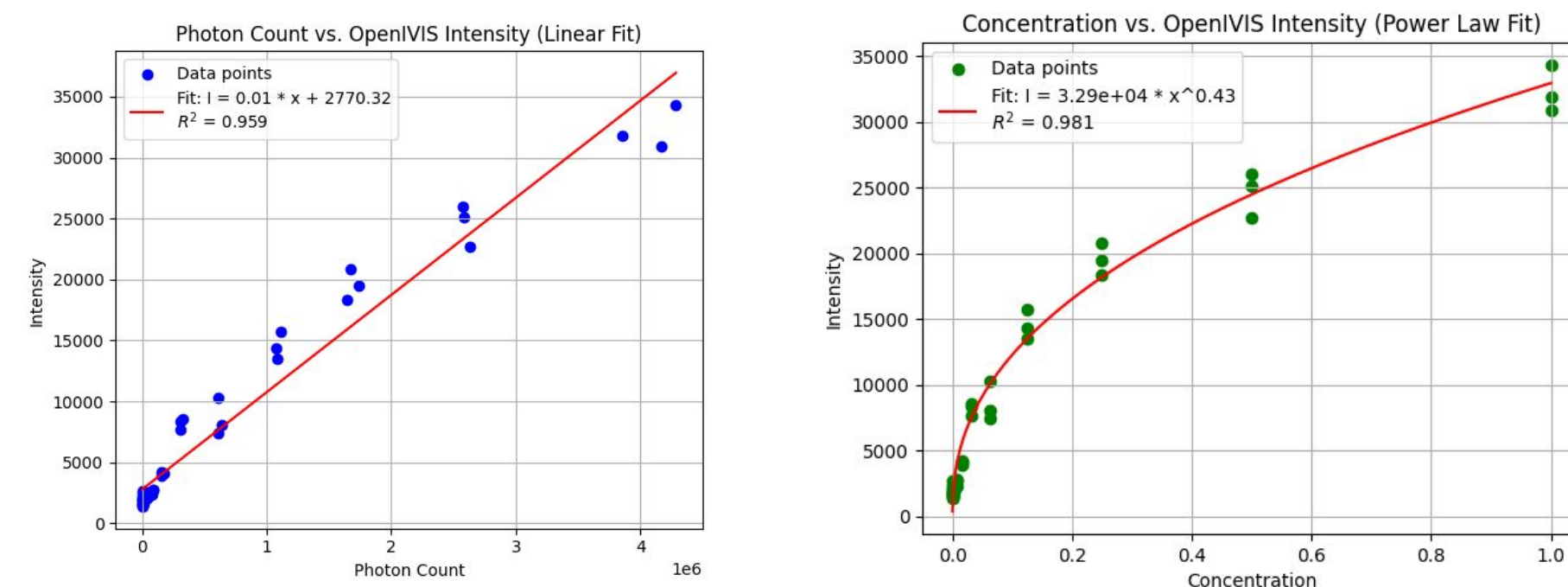


Fig 5. Intensity vs. Photon Count and Intensity vs. Concentration Graph

5. Results

Bioluminescence Intensity as a Function of Time

As expected, the iLux samples increased in brightness as room temperature rose. Intensity plots generated with intensity vs. time function confirmed both the biological behavior of iLux and the sensitivity of our imaging system. As time increases, the total and mean intensity increase, indicating that bioluminescent output increases over time. This increase in intensity makes sense based on our predictions of temperature starting around 5 degrees Celsius to 22 degrees Celsius.

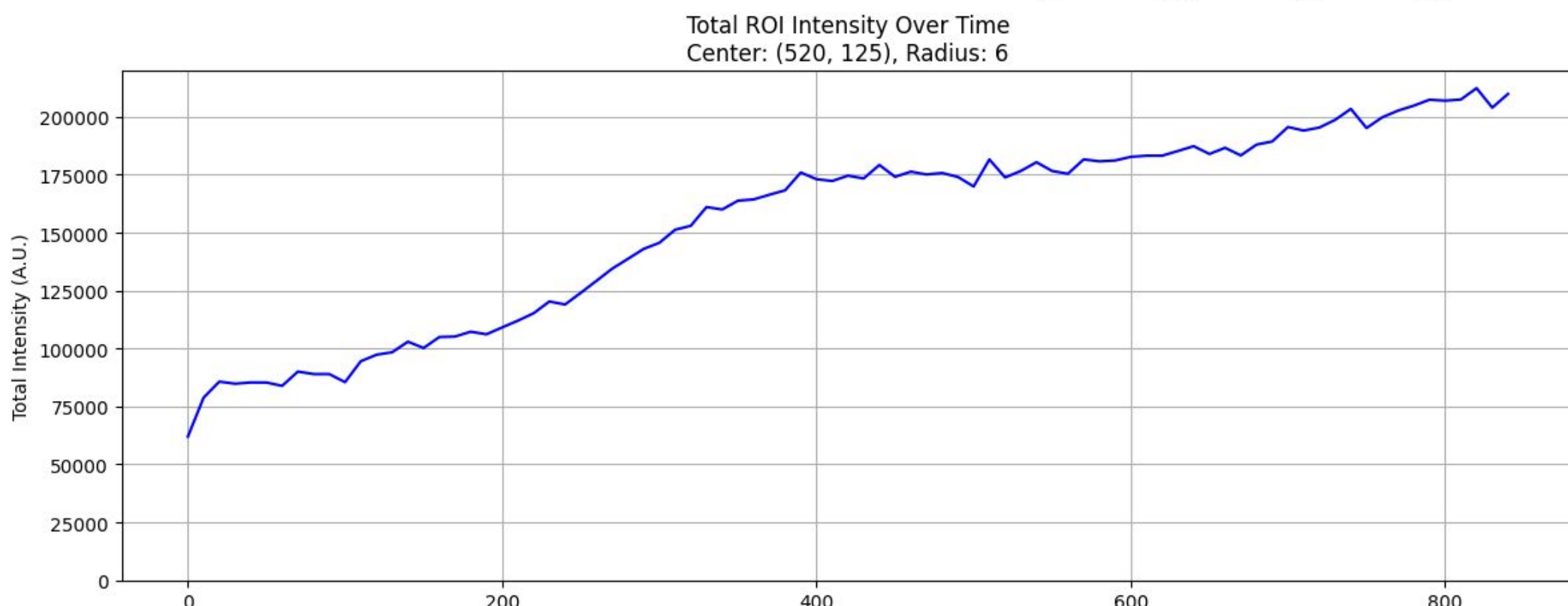
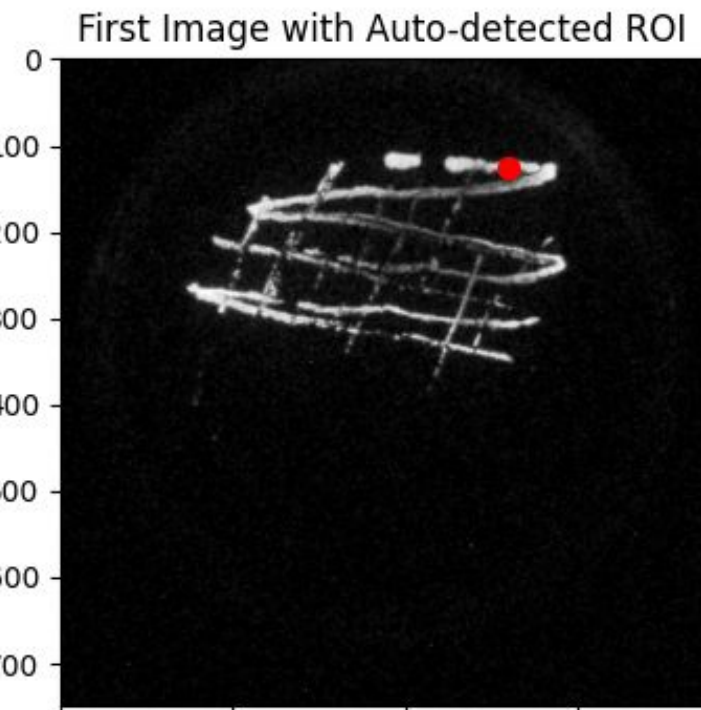


Fig 7. iLux Intensity vs. Time for an Auto-Detected ROI

To visualize how bioluminescent signal intensity changed spatially over time, intensity histograms (intensity vs. pixel count) were generated. These histograms visualize pixel intensity distributions in the first and last images of the time series. The histogram of the first image suggests either a low initial expression of bioluminescence because there are less pixels at higher intensities. The histogram of the last image seems to have a longer tail, suggesting that more pixels contain higher intensities.

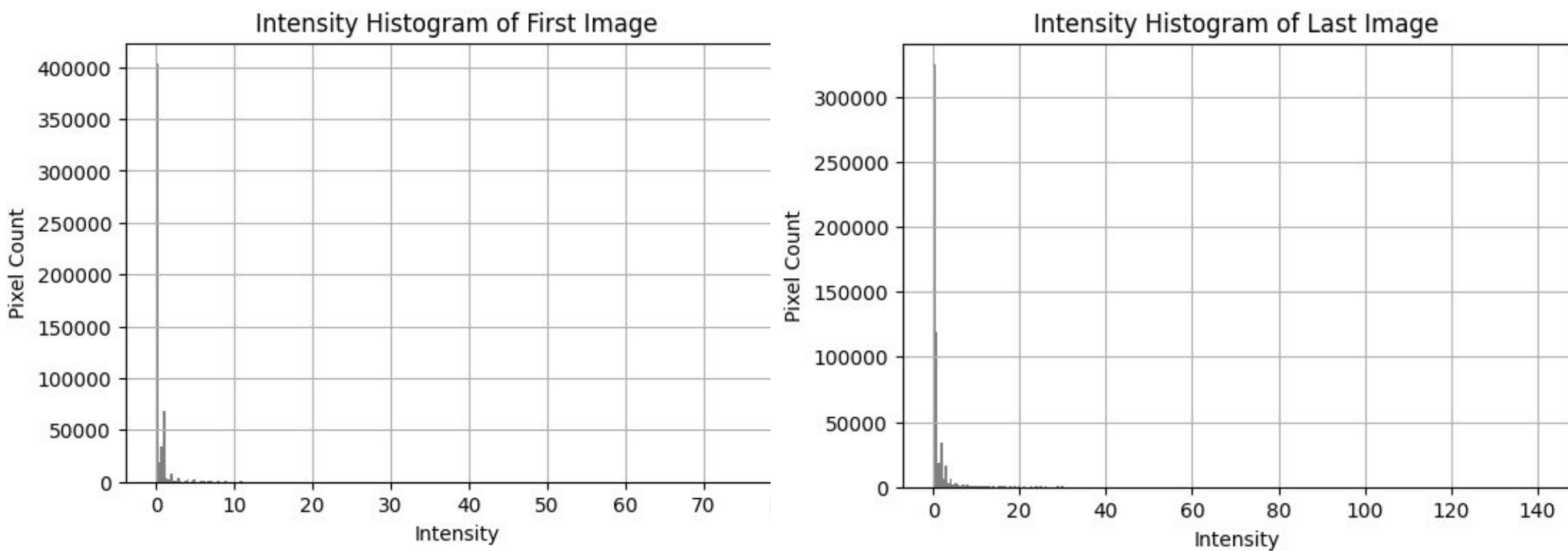


Fig 8. iLux Intensity vs. Pixel Count for an Auto-Detected ROI

Concentration vs. Photon Count

To validate the relationship between iLux concentration and the bioluminescent signal, the photon count measured by the commercial plate reader in the CMC Rathbun Lab were compared to the concentrations prepared by researcher Evelyn Harrington. The measurements were taken from a 96-well plate of rows A-H and columns 1-6. The rows and columns contained dilutions of the iLux reporter and the Rathbun Lab identified each well's concentration and corresponding photon count.

With a correlation value of 0.997, this confirms the strong, positive trend that with increasing concentration results in an increased bioluminescent signal in photon counts, even nonlinearly. In this case, an exponential factor of 0.68 indicates that the relationship between concentration and photon count is sublinear growth, which means that as concentration increases, photon count also increases, but at a decreasing rate.

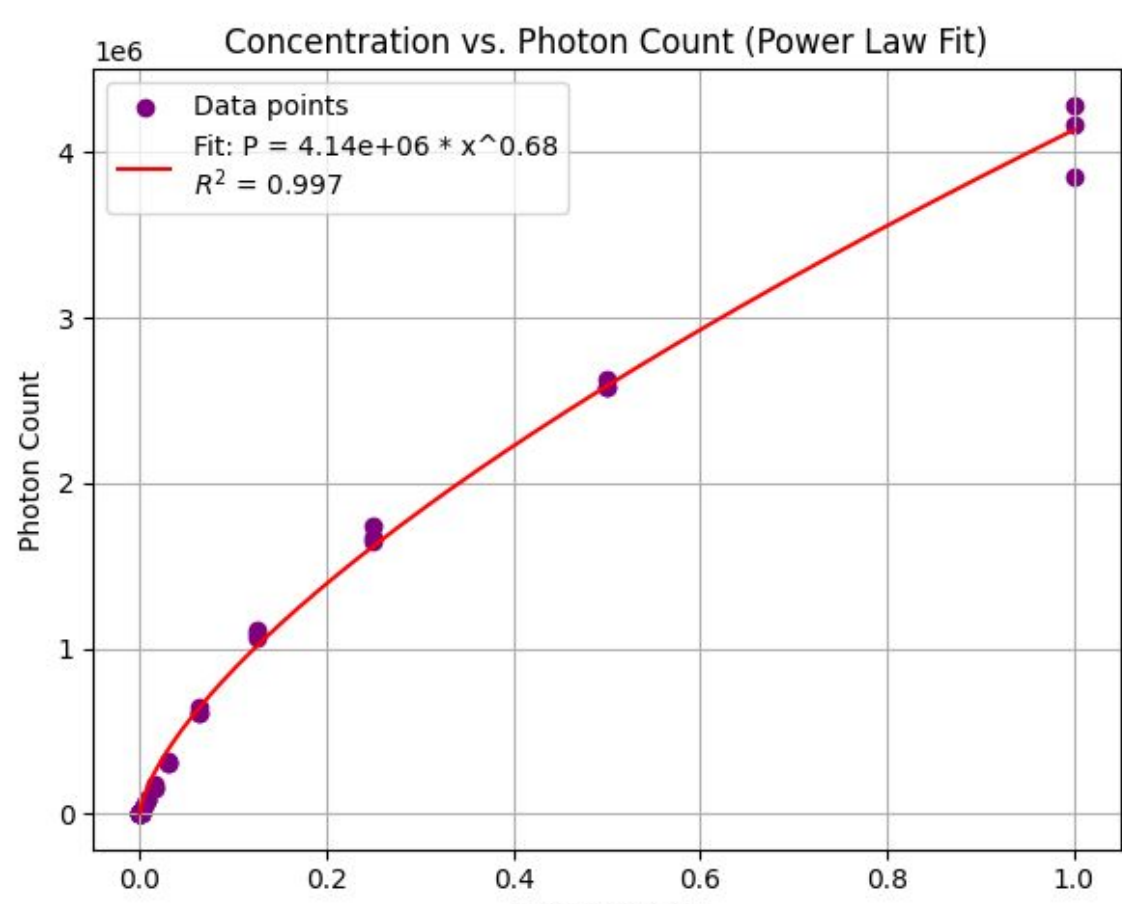


Fig 9. iLux Photon Count vs. Concentration

Photon Count vs. OpenIVIS Intensity

To measure the sensitivity of the OpenIVIS system, the photon counts can be compared to intensity values extracted from OpenIVIS images of the 96-well plate using the Intensity Extraction Protocol. When comparing the OpenIVIS-derived intensities to the photon counts from the commercial reader, the system showed a strong correlation.

Graphing the two variables revealed a strong positive correlation with an R³ value of 0.959. This means that the system reliably detected bioluminescent signals across a range of photon outputs. Next, a relationship between concentration and intensity can be made by graphing the two variables, with concentration as the independent variable.

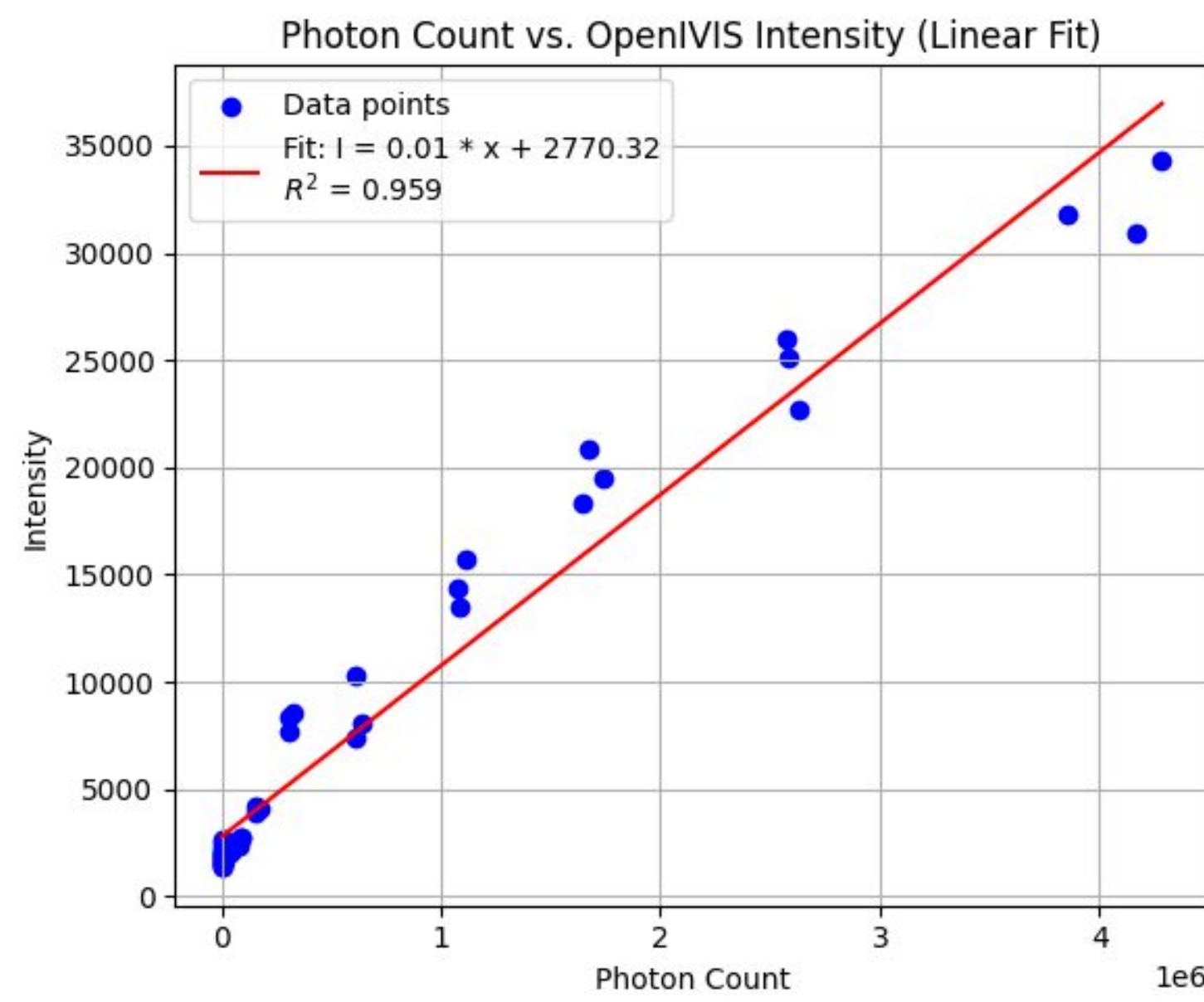


Fig 10. Open-IVIS Intensity vs. Photon Count

Concentration vs. OpenIVIS Intensity

Finally, to establish the relationship between iLux concentration and intensity, an analysis was performed using concentration as the independent variable.

This resulted in a strong, positive relationship with an R² value of 0.981. In this case, an exponential factor of 0.43 indicates that the relationship between concentration and photon count is sublinear growth. Although the power law relationship differs from a linear fit, the high correlation shows that the OpenIVIS system effectively captures changes in bioluminescent intensity with varying concentrations.

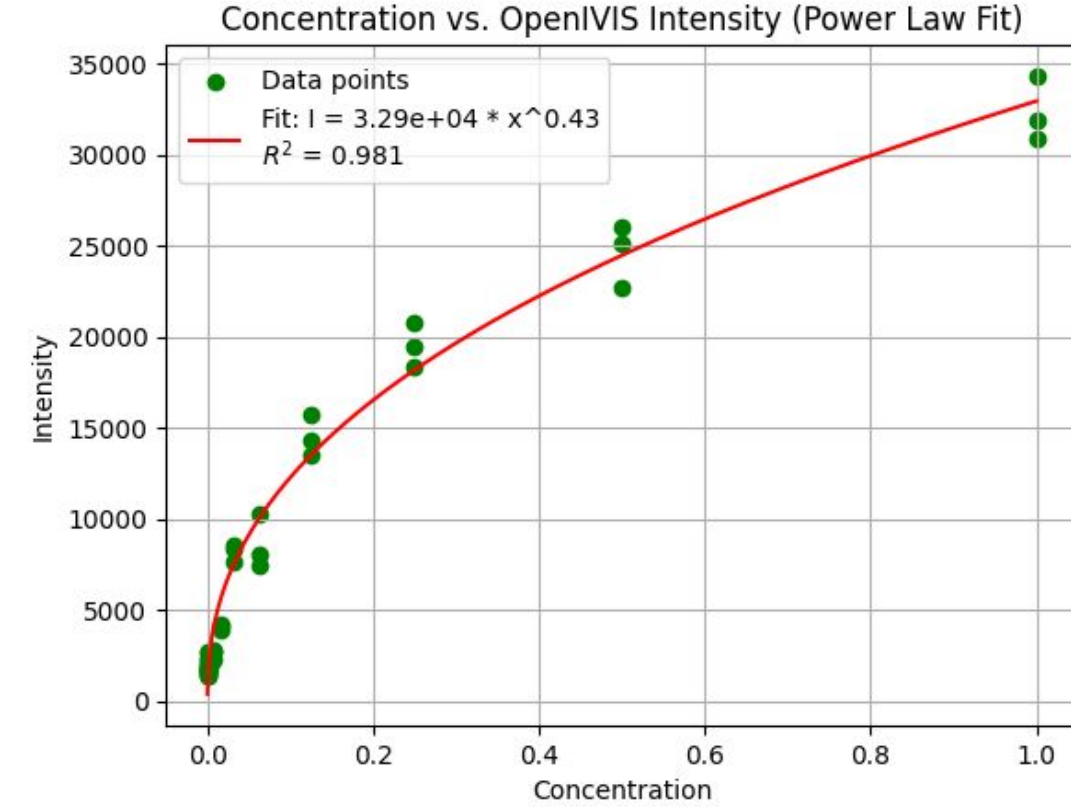


Fig 11. iLux Concentration vs. OpenIVIS Intensity

6. Analysis

While the OpenIVIS system successfully captured changes in bioluminescent intensity, several challenges limited its performance. The most prominent issue was glare from external light sources, especially in rooms ambient lighting. Even with the box closed, small leaks in the seams can introduce enough light to affect the signal. Currently, this problem is being addressed by using a blackout material over the box and improving the box seal.

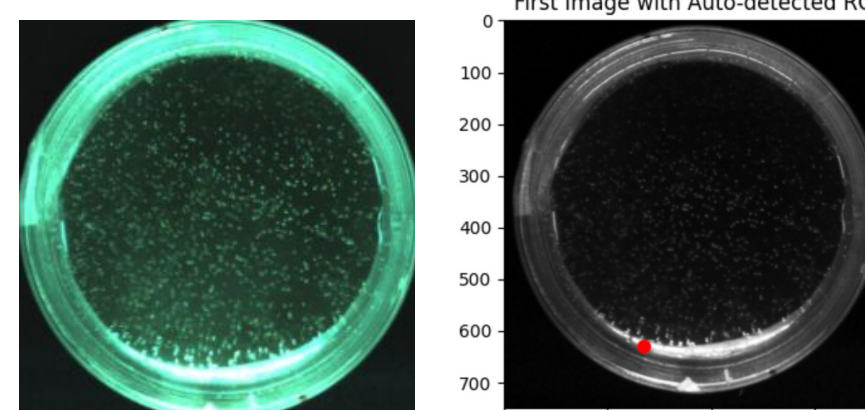


Fig 12. Glare-affected Agar Plate

The low intensity of bioluminescent signals meant longer exposure times were often necessary to capture the signal, increasing sensor readout noise. Although dark frame subtraction reduced background noise, residual noise remained.

Despite these challenges, the OpenIVIS system showed a strong correlation between OpenIVIS-derived intensities, photon counts from a commercial reader, and known iLux concentrations. This demonstrates that the OpenIVIS system can achieve high-fidelity, quantitative biological imaging using accessible hardware.

7. Conclusion

Building on the existing OpenIVIS design, this work presents a low-light detection bioluminescence imaging module. The system successfully captures low-intensity bioluminescent signals with varying concentrations.

OpenIVIS aims to provide a cost-effective alternative to commercial in vivo imaging systems, opening access to advanced biological imaging techniques for educational institutions, community colleges, and small research groups with limited funding like the Rathbun Lab at CMC. Its modular components allow for customization of diverse imaging modalities.

Moving forward, OpenIVIS plans to write a Python program pipeline to characterize images with signal to noise ratio, spatial resolution, and minimum detectable signal to provide biologists with image characteristics. In addition, integration of a thermistor to measure the temperature of the agar plates in real time and correlate it precisely with iLux brightness will provide more accurate and precise results. Lastly, OpenIVIS plans to release all Python scripts on the HMC Biophotonics public GitHub repository and documentation website to help others build and use OpenIVIS themselves.

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