

Raspberry Pi Platform for Multi-Modal Biosensing: Electrochemiluminescent and Colorimetric Analysis

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Summary:

A Raspberry Pi-based imaging platform is presented to characterise camera modules and imaging parameters for two contrasting optical detection modes: light-emitting (electrochemiluminescence, ECL) and light-absorbing (ELISA) biochemical assays. A monochrome and a color camera without IR filter are evaluated for ECL, while a color camera with IR filter is characterised for absorbance detection. Key imaging parameters (focus, resolution, gain, exposure), file format, and image analysis are systematically optimised for each mode, delivering a unified framework for versatile optical biosensing.

Keywords: Raspberry Pi, optical biosensing, electrochemiluminescence, ELISA, biosensor.

Background, Motivation and Objective

Optical readout is central to many biochemical assays, but not all assays generate signals in the same way. In sandwich immunoassays, the detection antibody label determines the readout modality: ECL [1] and chemiluminescence (CLIA) generate photons requiring low-light detection, while colorimetric assays such as ELISA rely on enzyme-mediated absorption or transmitted color changes [2]. These contrasting principles impose distinct requirements on imaging hardware, acquisition settings, and analysis strategy.

Recent advances in smartphone cameras, single-board computers, and compact optics have enabled low-cost, portable imaging systems for point-of-care applications [1,2,3]. However, most platforms allow a single detection mode, limiting flexibility when workflows require switching between emission and absorption readouts.

This work presents a unified Raspberry Pi-based imaging platform [1] capable of supporting both luminiscence and colorimetric assays within a single instrument, and establishes an experimentally validated framework to guide the selection of camera configuration, image format, and analysis strategy according to the optical nature of the assay signal.

Description of the New Method or System

A Raspberry Pi 4B controller integrated with a PalmSens EmStat4s potentiostat and interchangeable camera modules (monochrome OV9281 and color IMX477 with and without IR

filter) coupled to a 16 mm industrial lens with adjustable focus by tuning sensor-lens distance, was validated for both ECL [1] and colorimetric ELISA detection.

For ECL, all camera configurations were compared using a custom screen-printed electrode loaded with IL-8 IgG antibody labelled with Ru(bpy)₃-NHS ester (6 µg/mL, 2.5 µL), with emission triggered by chronoamperometry at 2 V for 5 s in 180 mM TPrA, 0.3 M phosphate buffer (pH 6.8) [1]. Background and signal frames were acquired inside a dark enclosure under fixed conditions (maximum analogue gain, 8 s exposure, auto white balance disabled, high resolution). Images were recorded in 16-bit TIFF, and 8-bit linear and gamma-encoded PNG formats. Quantification was performed using ROI-integrated background-corrected intensity, and signal-to-noise ratio (SNR) was calculated as the mean signal divided by the standard deviation of the background (n = 3).

For colorimetric ELISA, the IR-filtered color camera was used to record TMB substrate development in a TNF-α ELISA (DY210) before and after reaction stopping under fixed acquisition conditions (low resolution, 30 fps, auto white balance enabled). Four pixel aggregation strategies (mean, median, trimmed mean, and threshold mean) were compared across 16-bit TIFF, 8-bit linear and gamma PNG. Standard curves were fitted for blank (no analyte) corrected data using a four-parameter logistic model [2] ($4PL, Y = A + (D - A) / (1 + (C/x)^B)$), where A and D are the lower and upper asymptotes, B the Hill slope, and C the

concentration at inflexion point); limit of detection (LOD) was defined as a signal three standard deviations above the blank, SNR at the lowest standard relative to blank variability, and performance benchmarked against a plate reader absorbance (450–570 nm).

Results

For ECL detection of Ru-labelled antibodies emitting at ~620 nm (Figure 1), the signal was quantified as integrated intensity over the full electrode area to capture emission from spatially distributed labels. The monochrome camera achieved the highest signal per pixel and stable SNR across formats (47–57) by collecting all incident photons without spectral losses. For quantitative analysis, both 16-bit TIFF and 8-bit linear PNG are suitable, with higher bit depth providing improved dynamic range at low signal levels. When using non-linear gamma-encoded PNG, which enhances low-intensity signals, the tone response must be explicitly modelled [3].

The color camera with Bayer RGB and IR filters showed the poorest performance (SNR < 9), as the IR filter attenuates emission in the red/near-IR region where $\text{Ru}(\text{bpy})_3^{2+}$ emits, while the Bayer pattern further reduces effective per-pixel signal by splitting photons across RGB color channels. Removing the IR filter substantially improved sensitivity; in this configuration, only the red channel provides a meaningful signal (SNR 83 amplified by gamma-encoding), as green and blue channels contain negligible emission and noise predominates [3].

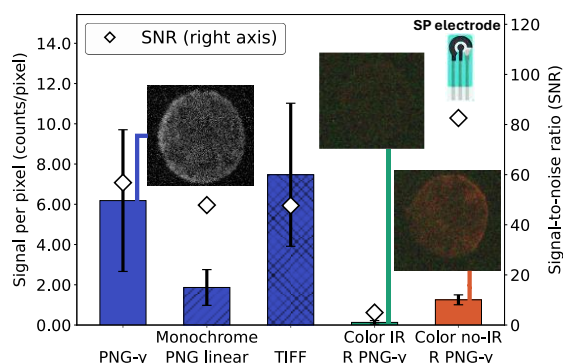


Fig. 1. Signal per pixel (bars) and SNR (diamond marker) for Ru-labelled IL-8 antibody detection across camera configurations and image formats. Error bars represent standard deviation ($n=3$). Representative ECL images are displayed with automatic brightness and contrast adjustment for visualization.

For colorimetric ELISA (Figure 2), the IR-filtered color camera was used, with pixel averaging over the colored well area as the core metric. Median aggregation outperformed all other strategies by suppressing outlier pixels from illumination inhomogeneity. Bit depth had no meaningful impact; 16- vs 8-bit linear formats were analytically indistinguishable for the

averaged signal. Channel selection followed complementary color logic: before stopping, the blue TMB substrate made the R channel optimal (LOD 9.3 pg/mL, $R^2 = 0.999997$, Hill slope 1.294 vs plate reader reference 1.303); after stopping, the color shift to yellow made the B channel optimal (LOD 7.8 pg/mL, $R^2 = 0.99991$). Under gamma encoding, saturation of the red channel, consistent with gamma amplifying low-intensity signals at the cost of saturating high-intensity ones, shifted the best performer to the G channel before stopping (LOD 10.3 pg/mL, $R^2 = 0.99996$). Despite comparable calibration metrics to the plate reader, image-based methods showed lower SNR (~4 vs 62.6), reflecting the higher precision of monochromatic photometric detection.

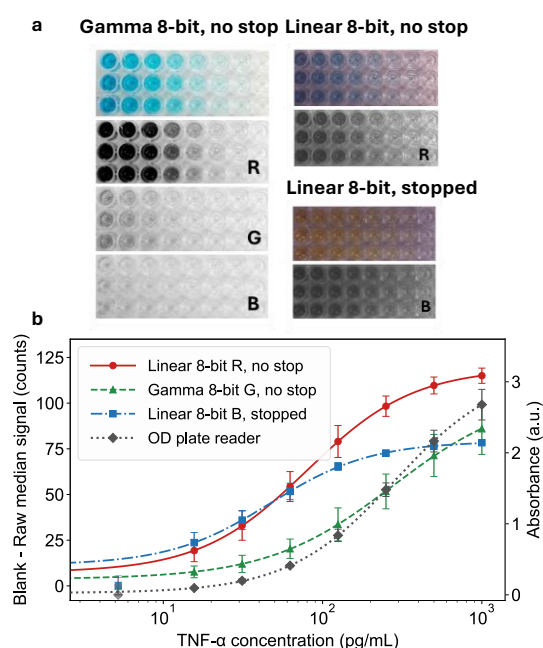


Fig. 2. Colorimetric TNF-α ELISA. (a) Representative 8-bit gamma and linear PNG image crops of the ELISA plate before and after reaction stopping, with R, G, and B channel splits. (b) 4PL standard curves for the best-performing image channels and plate reader absorbance.

References

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