DIY spectrophotometry: Rapid, low-cost RGB approach for vis-spectroscopy, OD600, and fluorescence quantification in profiling nosocomial antibiotic resistance (MRSA)

Wong, Darren Yong Sehng

2016

Wong, D. Y. S. (2016, March). DIY spectrophotometry: Rapid, low-cost RGB approach for vis-spectroscopy, OD600, and fluorescence quantification in profiling nosocomial antibiotic resistance (MRSA). Presented at Discover URECA @ NTU poster exhibition and competition, Nanyang Technological University, Singapore.

https://hdl.handle.net/10356/107548

NANYANG TECHNOLOGICAL UNIVERSITY

URECA
Undergraduate Research Experience on CAmpus

Category: Biomedical and Medicine
Lee Kong Chian School of Medicine
Student: Darren Wong Yong Sheng
Project ID: LKCM15012

DIY spectrophotometry: Rapid, Low-cost RGB approach for Vis-spectroscopy, OD_{600}, and Fluorescence Quantification in profiling nosocomial antibiotic resistance (MRSA)

INTRODUCTION

PROBLEM: Existing spectrophotometric assays are prohibitively expensive and incompatible with resource-limited settings (i.e. developing countries, citizen science and field testing), thereby necessitating a viable, economical alternative capable of yielding accurate and reproducible results.

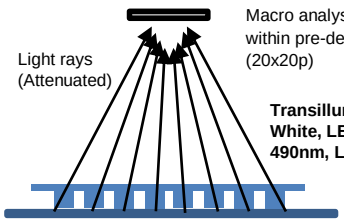
The increasing prevalence of multi-drug resistance has made the rapid & accurate determination of antibiotic susceptibility an imperative. In particular, Methicillin-resistant S. aureus (MRSA), a nosocomial pathogen, is responsible for significant inpatient morbidity and mortality [3.2 cases/1000 inpatient days] [1].

AIM: Herein, an affordable, high-throughout, semi-automated method of determining S. aureus antibiotic susceptibility via RGB analysis is described.

OD_{600} (MIC) values from broth microdilution, and end-point fluorescence values from strain-typing (PCR) were quantified, yielding absorbance readings comparable to commercial spectrophotometers (R2 correlation coefficient = 0.9931, 0.9539 respectively)

METHOD

SETUP: Image capture



Camera → **Image Processing:** Macro analyses pixel intensities within pre-defined area (20x20p)

Transillumination light source: **White, LED**: vis-spectro, OD_{600} **490nm, LED**: fluorescence

RGB Weighting:

- Existing RGB protocols approximate MPIs to a single channel → variable chromophore sensitivity, limited accuracy and reproducibility.
- A novel solution of is proposed herein: Each RGB channel is weighed (%) based on min/max channel-specific MPI values to yield weighted MPIs → greater R^2 correlation coefficients by effectively 'diluting' anomalous readings in any given channel & improves sensitivity to chromophores not overtly R/G/B.

$$Weightage\ Factor_{\ c} = \frac{|Max_{MPI} - Min_{MPI}|_c}{\sum_{i=c\,(R,G,B)}^{all\ c} |Max_{MPI} - Min_{MPI}|_c}$$

$$Weighted\ MPI = \sum_{i=c\,(R,G,B)}^{all\ c} MPI_c \times Weightage\ factor_c$$

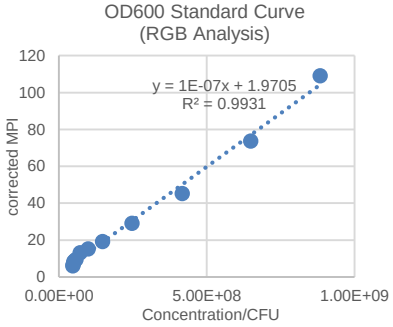
Blank Compensation for Vignetting, Variable Trans-illumination pathways: Correction factors facilitate the empirical compensation of visual aberrations.

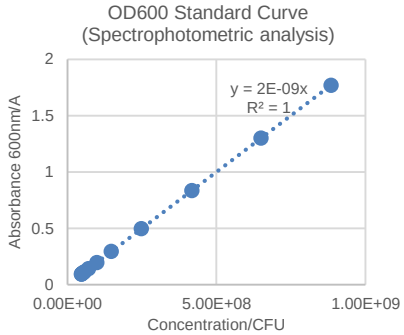
$$Correction\ Factor\ (well_n) = \frac{Max\ MPI_{(across\ all\ wells)}}{MPI_n}$$

$$Corrected\ MPI = Weighted\ MPI \times Correction\ Factor$$

RESULTS

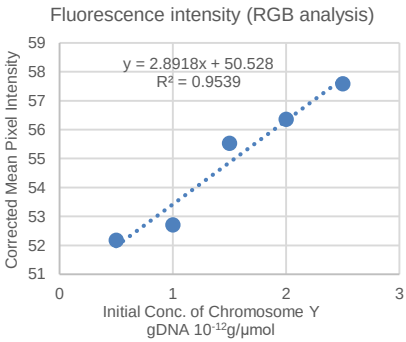
PHENOTYPIC AST: OD_{600} (Min. Inhibitory Concentration) – Comparison of **OD_{600}** S. aureus broth microdilutions processed using RGB analysis vs commercial spectrophotometry.

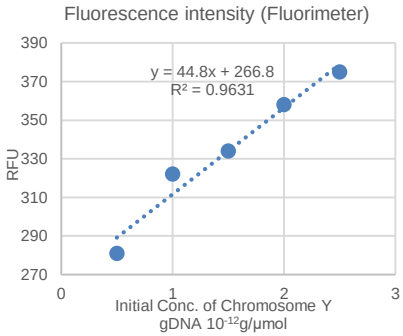




Conversion factor between Absorbance/A and MPI (mean pixel intensity): **1 OD_{600nm} = 57.9 MPI**

GENOTYPIC AST: End-point fluorescence Intensity – Comparison of end-point fluorescence of y-chromosome gDNA PCR amplicons (43 cycles). 485nm excitation and 525nm emission wavelengths used for commercial fluorimeter analysis.





DISCUSSION

The accuracy and reproducibility of RGB analysis is comparable to commercial spectrophotometry [R^2 of 0.9931 vs 1 for OD_{600}, R^2 of 0.9539 vs 0.9631 for fluorescence intensity]. Discrepancies observed at weaker (attenuated) light intensities is attributable to the superimposition of gamma-correction [$I_{out} = I_{in}^{1/2.2}$] causing non-linear amplification of RGB values. This disproportionately affects extremely low and high intensity MPI values, amplifying and attenuating them respectively.

The quantifiable range of OD_{600} values corresponds the detection range of **0.0975A – 1.2995A**; for fluorescence, this is **281-375 RFU**; this substantive, reproducible intra-polated range of values would enable the low-cost, rapid and reliable determination of S. aureus resistance profiles through both phenotype and genotype:

- High-throughput antibiotic (Methicillin) susceptibility tests in broth microdilutions
- End-point fluorescence of MRSA qPCR (mecA/ PBP2 gene expression)

FUTURE WORK: Integrated Attachment + App on platform (iOS)

> Portable, lightweight, passive attachment (incorporating diffraction grating/wavelength selection) + iSpectro app

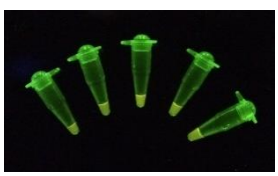


Figure 1: Fluorescent samples of human gDNA under 485nm trans-illumination