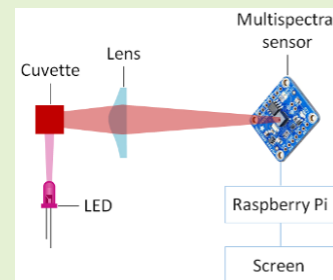


Multiplex Detection with Multispectral Sensors and Machine Learning

First A. Author, *Fellow, IEEE*, Second B. Author, and Third C. Author, Jr., *Member, IEEE*

Abstract—Multiplex assays usually use probes with different spectroscopic signatures to detect two or more analytes simultaneously. By allowing researchers to test for multiple markers per sample, they give a more reliable picture of what is happening. Being available in multiple colors and able to be conjugated to biomolecules, quantum dots are ideal as labels in multiplex assays. As a proof-of-concept, we demonstrate the capability of these multispectral sensors, using various machine learning approaches, for multiplex detection by using mixtures of quantum dots of two different colors. We show that the sensors can detect quantum dot concentrations relevant to biological applications by combining our sensor readings with ML algorithm running a Raspberry Pi Zero. We implement a 5 distinct types of ML algorithms to predict the QD concentration, along with various tools for training, evaluation, and utilizing a model.

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I. INTRODUCTION

COLORIMETRIC and luminescent assays are tests that generate colored reactants or fluorescence when an analyte of interest is present in a sample. They are widely used in biosensing, such as in disease diagnosis, food safety testing, environmental monitoring, and quality control. Since their signals are obtained through the spectral analysis of light, the detectors needed for these assays can be complex, bulky, and expensive. They often require trained personnel to maintain and operate them. These factors can slow, or even prevent, diagnosis in resource-limited regions. Imaging-based technologies such as smartphone spectrometers have been touted as solutions to make biosensing accessible worldwide [1]. However, they need complicated procedures for calibrating the wavelength scale and converting digital images into spectra. (details about what is complicated about the procedures) Moreover, users have limited control over camera parameters. It restricts their

application to areas where people have the technical know-how to set them up properly in order to get reproducible image quality. Inexpensive multispectral sensors have recently been introduced commercially, and they have none of these constraints of imaging-based technologies. They require no calibration by the user as they come fitted with Gaussian interference filters and can monitor the following wavelengths simultaneously, with a full-width at half-maximum of 40 nm: 450 nm, 500 nm, 550 nm, 570 nm, 600 nm, and 650 nm. This set of wavelengths is adequate for many colorimetric, fluorescent, and luminescent assays. Being easy to assemble, troubleshoot and operate, these multispectral sensors are appealing to resource-limited laboratories. They have, for example, been interfaced with a Raspberry Pi Zero to produce a simple colorimetric reader for bio-assays. They are also versatile and can be found in applications ranging from wireless capsule endoscopy prototypes to systems characterizing microfluidic chips. In this work, we demonstrate their potential for multiplex detection. kkk

A. Multiplex Applications of QDs

Multiplex assays usually use probes with different spectroscopic signatures to detect two or more analytes simultaneously. By allowing researchers to test for multiple markers per sample, they give a more reliable picture of what is happening. Consider, for example, a patient coming to a clinic with respiratory symptoms. The physician may take a sample from the patient, and if they have access to single-plex tests only, they will test for the pathogen they suspect is most likely responsible for the respiratory problem. If the test comes out

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negative, they will need another sample and will have to test for another pathogen. In essence, the physician is using an elimination process for diagnosis. A multiplex test that screens the patient for multiple pathogens simultaneously will expedite the diagnosis process. Being available in multiple colors and able to be conjugated to biomolecules, quantum dots are ideal as labels in multiplex assays. They have, for example, been used to quantify the amount of cholera toxin, ricin, shiga-like toxin 1, and staphylococcal enterotoxin B in a sample. Multiplex analysis based on quantum dots have also been developed to identify illicit substances such as dexamethasone, gentamicin, clonazepam, medroxyprogesterone acetate, and ceftiofur. More recently, they have been applied to detect multiple tumor markers, or to monitor the activity of protease enzymes such as trypsin, chymotrypsin and enterokinase. As a proof-of-concept, we demonstrate the capability of these multispectral sensors, using various machine learning approaches, for multiplex detection by using mixtures of quantum dots of two different colors. We show that the sensors can detect quantum dot concentrations relevant to biological applications.

B. Machine learning background

In order to make rapid predictions with some acquired training data, we use some popular machine learning algorithms. Machine learning has been used for detection, quantification, and classification of past/current events, prediction of future ones or even self-calibration (cite 1,2,3,4). For instance, *give a few examples*. In sensing applications, the ML algorithms are typically implemented digitally on general-purpose microprocessors (cite ML for sensor data), or (smart phone application?). In this paper we implement combine our sensor readings with ML algorithm using a cheap and widely accessible SBC: Raspberry Pi Zero. We implement a 5 distinct types of ML algorithms to predict the QD concentration, along with various tools for training, evaluation, and utilizing a model.

II. METHODS

A. Sensor System

Our system uses a commercially available multispectral sensor (Adafruit AS7341). Its data are read and processed with a standard I2C bus on an appropriate microcontroller, which was a Raspberry Pi Zero in our case. The system can be tailored to different applications since the software to run it is written in the Python language and can easily be modified. The microcontroller gives users the ability to attach a screen to view the data, or to transfer the results to another location via WiFi.

B. Set up for testing our sensing system

The SI unit for magnetic field strength

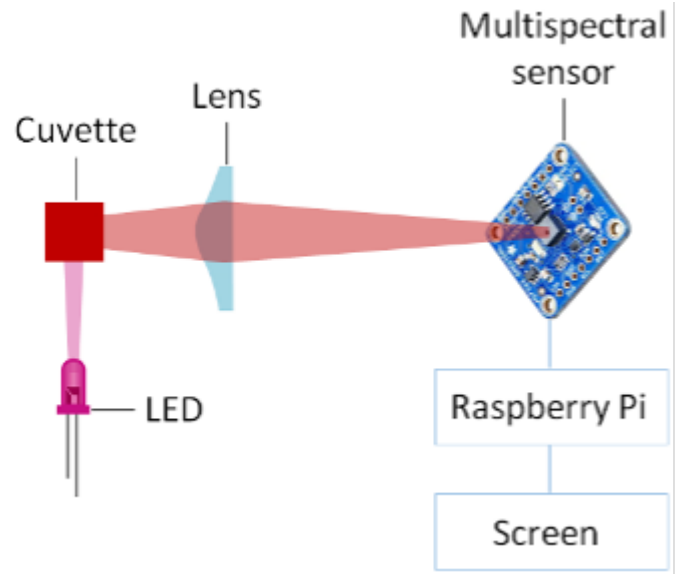


Fig. 1. Magnetization as a function of applied field. It is good practice to explain the significance of the figure in the caption.

C. Description of Machine learning algorithms

We use supervised machine learning to predict the QD concentration from the eight wavelength intensities. For each observation x_i for $i = 1, 2, \dots, n$ there is an associated response measurement y_i . The goal during training, is to approximate a mapping function from the input x to the output y variable, so that when given a new set of unknown input data, the algorithm can correctly predict the correct output variable. To avoid potential overfitting of the data, we have use a 10 fold-cross validation scheme. (some info about regression vs classification?) We have tried 5 distinct types of common ML algorithms to predict the QD concentration. In this section we provide a shot description and key properties of each ML algorithm. (following the style of Subhendu Pandit paper)

One possible approach to predict multiple QD types is to train a model for each emitter of interest, this approach is flexible but more computationally and memory expensive. If using this scheme, during inference time we run all models in sequence. Alternatively, we could train our models to predict in a multivariate fashion, we discuss this possibility according to each algorithm.

Gaussian process regression: is nonparametric, meaning the data is not assumed to belong to any particular parametric family of probability distributions (cite x), so rather than calculating the probability distribution of parameters of a specific function, gaussian process regression calculates the probability distribution over all admissible functions that fit the data. However, we specify a function space prior, calculate the posterior using the training data, and compute the predictive posterior distribution on our points of interest. GP has been proven to be an effective method for nonlinear problems due to many desirable properties, such as a clear structure with Bayesian interpretation, a simple integrated approach of obtaining and expressing uncertainty in predictions and the capability of capturing a wide variety of data feature by hyper-

parameters [13, 2]. The classical GP model can be only used to deal with a single output or single response problem however there are various ways to apply gaussian process regression with multiple response variables (cite)

Regression MLP: Multi-level Perceptron (MLP) is a neural network connecting several layers of input nodes as a directed graph, tasks with predicting an output variable as a function of the inputs. The input features can be categorical or numeric types, however, for regression NN, we require a numeric dependent variable. The multiple layers and non-linear activation allow the MLP to learn non linear features. To make multivariate predictions we simply increase number of neurons in our output layer.

Random Forest: A random forest fits a number of classifying decision trees on various sub-samples of the dataset and uses averaging to improve the predictive accuracy and control over-fitting. Random forests also require less hyper-parameter tuning than other methods discussed here, with the exception of Linear regression. To make multivariate predictions we simply increase the output layer

SVM Regression: Support Vector Machine is a supervised learning algorithm that is used to predict discrete values. Support Vector Regression uses the same principle as the SVMs. A support-vector machine constructs a hyperplane or set of hyperplanes in a high- or infinite-dimensional space, which can be used for classification, regression, or other tasks like outliers detection (cite). Im actually not sure how to do multivariate for this example yet.

Linear regression: In a linear model, e.g. a model that assumes a linear relationship between the input variables (x) and the single output variable (y). More specifically, that y can be calculated from a linear combination of the input variables (x). When there is a single input variable, the method is referred to as simple linear regression otherwise we have multivariate example. Furthermore, to make multiple output predictions we extend linear regression however this can be shown to be same as independently solving a separate regression problem for each response variable.

1) *Frequentist vs Bayesian Models*: here we discuss the key differences in assumptions and applications between bayesian methods and frequentist methods. Also discuss confidence intervals, and some considerations when comparing models.

D. Training Details

Here we can discuss general training details. Epochs, libraries we used. and other compute details

1) *Hyper-parameter Optimization*: Every algorithm attempted here has various hyper-parameters that affect the corresponding optimization process. These can have a significant difference in learning, so its important we employ a search to find optimal hyper-parameters. I think we can make a table in appendix showing optimal hyperparameters

III. RESULTS

A. Characterizing the limit of detection

A conclusion section is not required. Although a conclusion may review the main points of the paper, do not replicate the

TABLE I
MACHINE LEARNING MODELS

Model	Type	Accuracy*	Trainable Parameters
Gaussian Process Regression	Univariate	4.5	N/A**
Linear Regression	Univariate	4.5	1
Bayesian Regression	Univariate	4.5	1
Random Forest Regression	Univariate	4.5	1
Neural Network	Univariate	4.5	1
Neural Network	Multivariate	4.5	1

*Accuracy is measure of MSE for 10-fold Cross val. All models were tested using the same cross validation dataset breakdown. For univariate models accuracy is the average between the two models

**Bayesian methods are non-parametric and therefore have no trainable parameters

abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

B. Model Selection

1) *Metrics*: A Cost Function is used to evaluate the performance of a Model on data. Mean squared error (MSE) is a common measure for evaluating the performance of a model which sums (or averages) the square of the difference between estimated and true values over the population. The main motivation for using MSE is its differentiability which simplifies the Cost Function minimization process. An alternative metric is the Mean absolute error (MAE) (i.e., the sum/average of absolute values of the differences between estimations and observed outputs) which is sometimes employed for sequential/temporal data processing. As mentioned before, training is achieved through by adjusting the weights (i.e., elements of vector ?? in Eq. 1) kk.

Here we discuss cross validation accuracy and BIC, AIC criterial?

2) *Confidence interval for Bayesian Methods*: Figures that are composed of only black lines and shapes. These figures should have no shades or half-tones of gray, only black and white.

C. Making Predictions

1) *Raspberry Pi Details*: Figures that are composed of only black lines and shapes. These figures should have no shades or half-tones of gray, only black and white.

2) *Confidence Intervals for retraining*: Figures that are composed of only black lines and shapes. These figures should have no shades or half-tones of gray, only black and white.

IV. CONCLUSION

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Appendixes, if needed, appear before the acknowledgment.

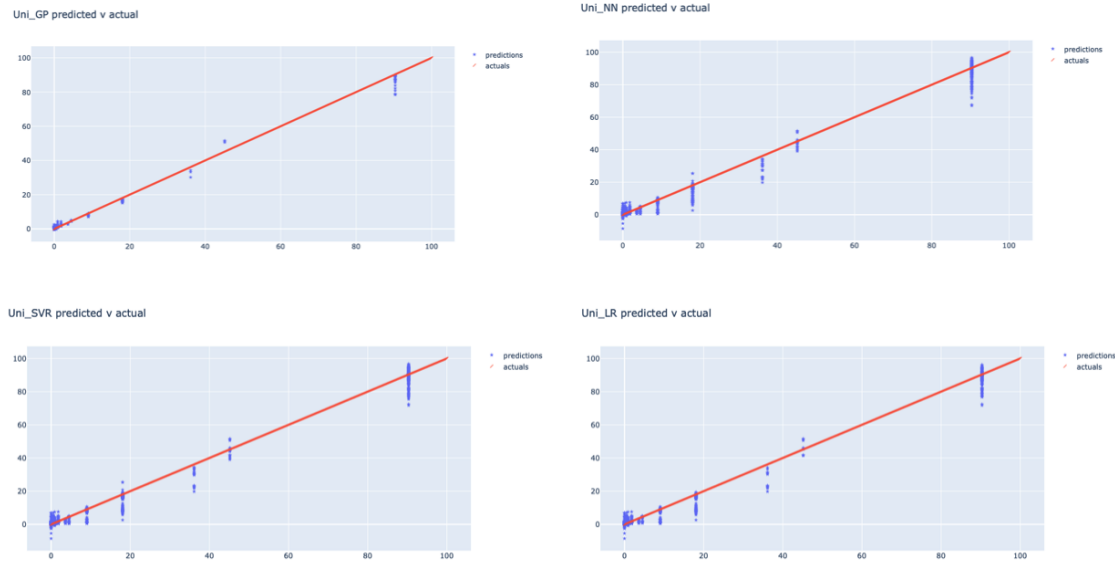


Fig. 2. Magnetization as a function of applied field. It is good practice to explain the significance of the figure in the caption.

ACKNOWLEDGMENT

The preferred spelling of the word “acknowledgment” in American English is without an “e” after the “g.” Use the singular heading even if you have many acknowledgments. Avoid expressions such as “One of us (S.B.A.) would like to thank” Instead, write “F. A. Author thanks” In most cases, sponsor and financial support acknowledgments are placed in the unnumbered footnote on the first page, not here.

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See [?], [?].
- *Basic format for periodicals:*
J. K. Author, "Name of paper," *Abbrev. Title of Periodical*, vol. x, no. x, pp. xxx-xxx, Abbrev. Month, year, DOI: 10.1109.XXX.123456.
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- *Basic format for handbooks:*
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See [?], [?].
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 - 2) J. K. Author, "Title of dissertation," Ph.D. dissertation, Abbrev. Dept., Abbrev. Univ., City of Univ., Abbrev. State, year.
- See [?], [?].
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 - 1) J. K. Author, private communication, Abbrev. Month, year.
 - 2) J. K. Author, "Title of paper," unpublished.
 - 3) J. K. Author, "Title of paper," to be published.
- See [?]-[?].
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 - 1) *Title of Standard*, Standard number, date.
 - 2) *Title of Standard*, Standard number, Corporate author, location, date.
- See [?], [?].
- *Article number in reference examples:*
See [?], [?].
- *Example when using et al.:*
See [?].

REFERENCES

- [1] L. Vonrueden, S. Mayer, K. Beckh, B. Georgiev, S. Giesselbach, R. Heese, B. Kirsch, M. Walczak, J. Pfrommer, A. Pick, R. Ramamurthy, J. Garcke, C. Bauckhage, and J. Schuecker, "Informed Machine Learning - A Taxonomy and Survey of Integrating Prior Knowledge into Learning Systems," *IEEE Transactions on Knowledge and Data Engineering*, pp. 1-20, 2021.



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Second B. Author was born in Greenwich Village, New York, NY, USA in 1977. He received the B.S. and M.S. degrees in aerospace engineering from the University of Virginia, Charlottesville, in 2001 and the Ph.D. degree in mechanical engineering from Drexel University, Philadelphia, PA, in 2008.

From 2001 to 2004, he was a Research Assistant with the Princeton Plasma Physics Laboratory. Since 2009, he has been an Assistant Professor with the Mechanical Engineering Department, Texas A&M University, College Station. He is the author of three books, more than 150 articles, and more than 70 inventions. His research interests include high-pressure and high-density nonthermal plasma discharge processes and applications, microscale plasma discharges, discharges in liquids, spectroscopic diagnostics, plasma propulsion, and innovation plasma applications. He is an Associate Editor of the journal *Earth, Moon, Planets*, and holds two patents.

Dr. Author was a recipient of the International Association of Geomagnetism and Aeronomy Young Scientist Award for Excellence in 2008, and the IEEE Electromagnetic Compatibility Society Best Symposium Paper Award in 2011.



Third C. Author, Jr. (M'87) received the B.S. degree in mechanical engineering from National Chung Cheng University, Chiayi, Taiwan, in 2004 and the M.S. degree in mechanical engineering from National Tsing Hua University, Hsinchu, Taiwan, in 2006. He is currently pursuing the Ph.D. degree in mechanical engineering at Texas A&M University, College Station, TX, USA.

From 2008 to 2009, he was a Research Assistant with the Institute of Physics, Academia Sinica, Taipei, Taiwan. His research interest includes the development of surface processing and biological/medical treatment techniques using nonthermal atmospheric pressure plasmas, fundamental study of plasma sources, and fabrication of micro- or nanostructured surfaces.

Mr. Author's awards and honors include the Frew Fellowship (Australian Academy of Science), the I. I. Rabi Prize (APS), the European Frequency and Time Forum Award, the Carl Zeiss Research Award, the William F. Meggers Award and the Adolph Lomb Medal (OSA).