Multiplex Detection with Multispectral Sensors and Machine Learning

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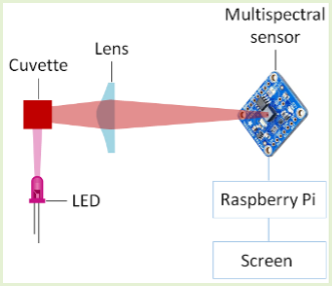
***Abstract*— Multiplex assays usually use probes with different spectroscopic signatures to detect two or more analytes simultaneously. By allowing researchers to test for multiple markers per sample, they give a more reliable picture of what is happening. Consider, for example, a patient coming to 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 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 appraoches, 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.**



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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. 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

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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 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 appraoches, 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

kkk In order to make rapid predictions with some acquired training data, we use some popular machine learning algorithms. Machine learning can improve sensor performance in different ways 4, 5. It makes a sensor system intelligent through the detection, quantification, and classification of past/current events or prediction of future ones. Statistical learning allows for the modelling of complicated relationships between the input and output of a sensor 6, (self-)calibration 7, 8, and improving the overall reliability and accuracy of a sensor system 9, 10. Machine learning algorithms can be staggered for improved performance. For instance, in 11 the performance of capacitive proximity sensors was enhanced through multiple signal processing levels. After detecting the presence of an object (model-based), the system determined its shape (categorization) and then utilized a library of measured values to accurately estimate the distance to the object (regression). This combination of deterministic and statistical models was shown to improve sensor accuracy significantly. In sensing applications, the ML algorithms are typically implemented digitally on general-purpose microprocessors. Recently, the concept of reservoir computing (RC) has emerged as an opportunity to recognize context based on the physical response of the systems rather than nonlinear transformation

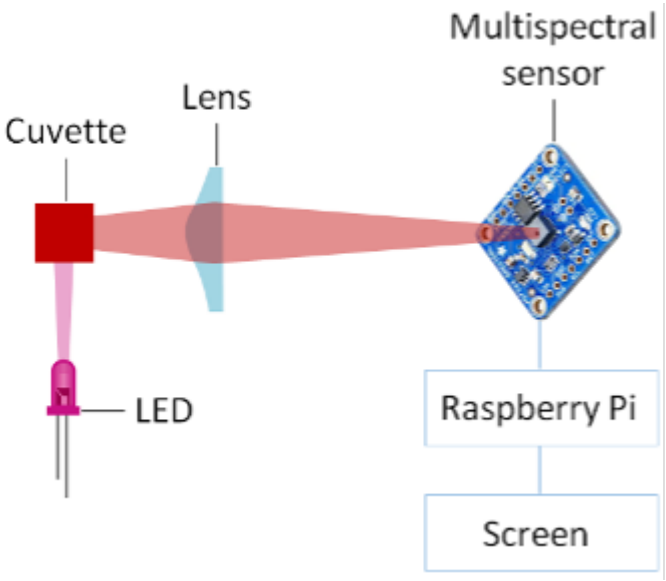

Fig. 1. Magnetization as a function of applied field. It is good practice to explain the significance of the figure in the caption.

of features in the digital domain 12–14. Careful system design

II. Methods

Use either SI (MKS) or CGS as primary units. (SI units are strongly encouraged.) English units may be used as secondary units (in parentheses). This applies to papers in data storage. For example, write "15 Gb/cm^2 (100 Gb/in^2)." An exception is when English units are used as identifiers in trade, such as "3½-in disk drive." Avoid combining SI and CGS units, such as current in amperes and magnetic field in oersteds. This often leads to confusion because equations do not balance dimensionally. If you must use mixed units, clearly state the units for each quantity in an equation.

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

C. Description of Machine learning algorithms

We have used supervised machine learning to predict the QD concentration from the eight wavelength intensities. For each observation x_i for $i = 1, 2, .., n$ 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 used 10fold-cross validation scheme.

We have tried 5 distinct types of 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) discuss multivariate and univariate here no need for distinct section

1) Model Definition: One possible approach to predict multiple QD types is to train a model for each emitter. During inference time we run both models in sequence.

"**Gaussian process regression** is nonparametric (i.e. not limited by a functional form), so rather than calculating the probability distribution of parameters of a specific function, GPR calculates the probability distribution over all admissible functions that fit the data. However, similar to the above, we specify a prior (on the function space), calculate the posterior using the training data, and compute the predictive posterior distribution on our points of interest."

"**Regression NN** predict an output variable as a function of the inputs. The input features (independent variables) can be categorical or numeric types, however, for regression NN, we require a numeric dependent variable. If the output variable is a categorical variable (or binary) the ANN will function as a classifier"

" **random forest** is a meta estimator that 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. The sub-sample size is controlled with the max samples parameter if bootstrap=True (default), otherwise the whole dataset is used to build each tree"

" **Support Vector Regression** is a supervised learning algorithm that is used to predict discrete values. Support Vector Regression uses the same principle as the SVMs. The basic idea behind SVR is to find the best fit line. In SVR, the best fit line is the hyperplane that has the maximum number of points."

"**Linear regression** is 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 (x), the method is referred to as simple linear regression. When there are multiple input variables, literature from statistics often refers to the method as multiple linear regression."

2) 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

TABLE I
MACHINE LEARNING MODELS

Model	Type	Accuracy*	Trainable Parameters
Gaussian Process Reggression	Univariate	4.5	N/A**
Linear Regression	Univariate	4.5	1
Bayesian Regression	Univariate	4.5	1
Random Forest Regession	Univariate	4.5	1
Neural Network	Univariate	4.5	1
Neural Network	Multiivariate	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 univaraite models accuracy is the average between the two models
**Bayesian methods are non-parametric and therefore have no trainable parameters

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

B. Model Selection

1) Metrics: kk 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.

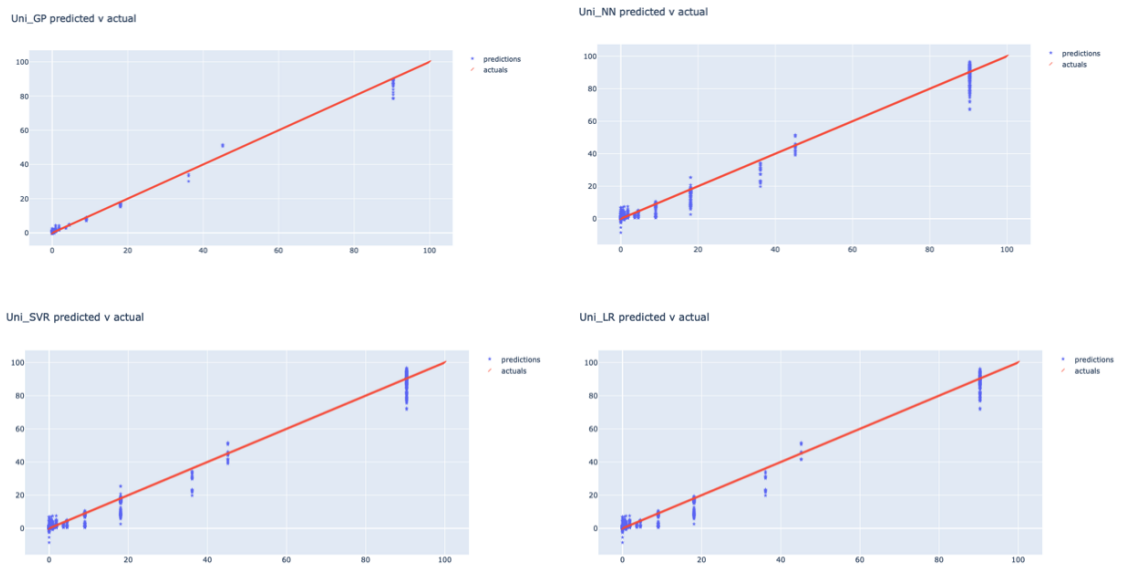

Fig. 2. Magnetization as a function of applied field. It is good practice to explain the significance of the figure in the caption.

IV. Conclusion

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Appendixes, if needed, appear before the acknowledgment.

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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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- *Example when using et al.:*

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