ABSTRACT

CODED SPECTROSCOPY FOR ETHANOL DETECTION IN DIFFUSE, FLUORESCENT MEDIA

by

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Rebecca Willett

An abstract of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Electrical and Computer Engineering in the Graduate School of Duke University

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Abstract

Optical sensing in the visible and near-infrared regions of the electromagnetic spectrum has many useful applications. One particularly interesting one is the non-invasive analysis of tissue since a high penetration depth is possible. With the use of Raman spectroscopy, a high degree of chemical specificity is available with laser powers that are harmless to living tissue. Such systems, however, are plagued by the low efficiency of the Raman scattering process by molecules and the intense background fluorescence from some biological materials. To address these drawbacks, we have investigated the use of coded spectroscopy to make Raman spectroscopy more feasible in routine use. By coding the input aperture of a dispersive spectrometer, throughput gains of 10-100 are possible over a traditional slit spectrometer. The theory, design, and performance characteristics of this static aperture coding will be discussed in this thesis. In addition, by coding the excitation light sources one can filter out the shifting Raman signals from the stationary fluorescent background. The theory and implementation of an expectation maximization algorithm for Raman signal reconstruction will be analyzed. In addition, the design of a multi-excitation, coded-aperture Raman spectrometer will be described, which uses both of the coding mechanisms described.
Acknowledgements

I have many people to thank for both the opportunity to study at Duke and for the great experiences I had there. My wife Robin has been a wonderful partner and extremely supportive during the end of my dissertation work. The rest of my family, especially my parents, have always been supportive of my various endeavors throughout the years. While at Duke it has been a pleasure to work with Dr. David J. Brady, whose guidance and brilliant ideas have been very critical in my development as an independent researcher. Some other colleagues at Duke who I have learned much from include Michael Gehm, Bob Guenther, and Michael Sullivan. Our many discussions, lunch meetings, and Coca-Cola sessions kept things from ever getting boring. The many other members of the DISP group have made it a great group to be a part of—Steve Feller, Evan Cull, Mohan Shankar, Andrew Portnoy, Cristina Fernandez, Yanqia Wang, Renu John, Prasant Potuluri, Nikos Pitsianis, and the rest.
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Chapter 1

Introduction

Current blood alcohol concentration (BAC) measurement techniques are insufficient for the needs of the alcohol abuse research, treatment, and enforcement communities. [1,2] Taking blood samples is the “gold standard”, however this method is very invasive by its nature, impractical for continuous monitoring, and troublesome from a biohazard and transportation standpoint for law enforcement. Breath alcohol concentration (BrAC) measurements are a common alternative, however they have their drawbacks as well. The blood-breath partition coefficient, which relates BrAC to BAC, varies among the population, and unless it is known a priori for a person, the accuracy of the technique suffers. [3] The subject must be conscious and recent alcohol consumption and regurgitation interfere with the accuracy of the measurements.

Optical spectroscopy offers a unique possible solution to this problem— non-invasive, continuous measurements with a high degree of accuracy. The optical field is routinely used in many biological diagnostics— from the microscopic study of biopsies to the visible examination of a patient by a doctor. There is a wealth of information that can be transmitted via the optical field. Optical instruments which measure these properties, however, are typically designed in a fashion that is not always compatible with biological sources. If in vivo samples are desired, no sample purification or isolation is possible, and signals typically lack sensitivity and/or specificity due to the complicated structure and composition of most biological sources.

Instead of taking optical sensor systems and applying them directly to such sources, we have applied a design methodology termed “computational sensing” in
order to better optimize the measurement process. With computational sensing, new mappings from the source space, in this case light scattered by the tissue, to the detector space, the actual intensity measurements of the system, can be investigated. The measurements are then digitally processed in order to recover the relevant information about the source. This integrated approach allows for new sensor implementations that can lead to higher performance, more capabilities, and/or lower cost.

One such optical sensor system that I will describe in this thesis is a coded-aperture, coded-excitation Raman spectrometer optimized for optical tissue diagnostics. The two novel aspects of the system are the use of a coded aperture instead of a conventional slit in order to perform high throughput spectral measurement, and coded-excitation to isolate the shift-variant Raman signal from background interferences. The ultimate goal of the design is to have a non-invasive blood alcohol concentration (BAC) sensor that has high sensitivity, low cost, and small total volume.

1.1 Raman Spectroscopy Background

One optical diagnostic technique that offers many possibilities for diagnostics is Raman spectroscopy, first discovered by C.V. Raman in 1928. [4] The “Raman effect” is an inelastic photon scattering mechanism, wherein a molecule is excited to a virtual energy state by a photon and then relaxes to a different molecular vibrational/rotational state than in which it started, and in doing so emits a photon of a shifted energy. The energy difference in the incoming and outgoing photon is termed the “Raman shift” and is equal to the difference in energy between two of the vibrational/rotational states of the molecule. A simplified energy diagram for relevant forms of light scattering is shown in Fig. 1.1.
Figure 1.1: Energy level diagram for light scattering. In Rayleigh scattering the energy of the incident photon, $E_{EX}$, is equal to that of scattered photon, $E_{RS}$. In Stokes Raman scattering, the incident photon transfers energy $E_{EX} - E_{SRS}$ to the molecule, and a photon of energy $E_{SRS}$ is emitted. In anti-Stokes Raman scattering, the excitation photon causes the molecule to lose energy $E_{ASRS} - E_{EX}$, and then a photon of energy $E_{ASRS}$ is emitted.
The vast majority of scattered light undergoes no change in energy, a process termed Rayleigh scattering, while a very small fraction exhibits a Raman shift. When the net change in energy of the molecule is higher, and thus the scattered photon has lower energy, the event is termed Stokes Raman scattering. [5] Anti-Stokes Raman scattering is the opposite—the molecule loses energy and the scattered photon has a higher energy. Due to the Boltzmann factor associated with the occupation of higher energy states, the Stokes Raman is more probable than the anti-Stokes Raman at room temperature. [6] The fundamental cause for a molecule to exhibit Raman scattering is the interaction of the oscillating electromagnetic field with a Raman-active transition, one with a polarizability that changes during a particular rotation/vibration. [7] The common energy units for Raman shifts are wavenumbers (cm$^{-1}$) equal to $1/\lambda$, where $\lambda$ is the wavelength of the photon in centimeters. The “fingerprint region” of Raman spectroscopy, where molecules have very unique spectra, lies between $\approx$400-1800 cm$^{-1}$. [8]

The motivation for using Raman spectroscopy for optical diagnostics is its high specificity. The vibrational/rotational energy spacing in molecules is highly dependent on the number, type, and orientation of chemical bonds in the molecule. [9] Additionally, since the amount of the Raman shift in energy is independent of the excitation wavelength, it is possible to operate in the ultraviolet (UV), visible (VIS), and near-infrared (NIR) regions of the electromagnetic spectrum. Thus the excitation light can be chosen to coincide with the “therapeutic window” of biological materials shown in Fig. 1.2. [10] In the NIR regime ($\approx$ 0.75-1.4 µm), photons can easily travel deep within tissue due to the low amount of absorption by water and other tissue components.

Raman spectroscopy has been shown to offer many possibilities for in vivo measurement—analyte detection and measurement, cancer diagnosis, and disease
diagnosis. [11] In this thesis I will focus on its ability to perform robust chemometrics, i.e. the quantitative analysis of chemicals in a sample. With the advent of high performance charge-coupled device (CCD) detectors, NIR diode lasers, and high-throughput spectrographs, systems have been demonstrated that detect various biological analytes at physiological levels in aqueous humor, urine, and filtered blood serum. [12–14] In highly-scattering media like blood and tissue, Raman-based diagnostics are more challenging, but in vivo applications are becoming more realizable with recent progress in instrumentation and data analysis. [15–17] There are certain aspects of tissue Raman spectroscopy that make the accurate detection of analytes in vivo very difficult.
1.2 Light Scattering by Tissue

In optical tissue diagnostics, the NIR regime is typically chosen due to the low absorption by tissue components. In Raman spectroscopy, it is also advantageous as a result of the reduced intensity of the background fluorescence. In this wavelength region in tissue such as skin, scattering rather than absorption dominates photon transport and thus the diffusion approximation can be made. The time-independent diffusion can be expressed as [8]

\[ \nabla^2 \Phi_d(r) - \kappa_d^2 \Phi_d(r) = 0, \tag{1.1} \]

where \( \Phi_d \) is the photon fluence, \( r \) is the radial distance from the source, and \( 1/\kappa_d \) is the diffusion length. The diffusion length can be expressed as

\[ \frac{1}{\kappa_d} = \left[ \frac{1}{3\mu_a[\mu_a + \mu_s(1-g)]} \right]^{1/2}. \tag{1.2} \]

The scattering and absorption constants, \( \mu_s \) and \( \mu_a \) respectively, as well as \( g \), the anisotropic scattering parameter are readily available for various biological components. [8] In order to roughly estimate the photon distribution in tissue, the solution for a point source is used:

\[ \frac{\Phi_d(r)}{h\nu c_m} = \frac{N\kappa_d^2}{4\pi} \frac{\exp(-\kappa_d r)}{r}. \tag{1.3} \]

Here \( N \) is the number of injected photons and \( c_m \) is the phase velocity in the medium. This equation is explicitly integrated to determine the approximate photon distribution of light as a function of the material parameters. The solution can be expressed as

\[ N(r) = N_{\text{tot}}[1 - (1 + \kappa_d r) \exp(-\kappa_d r)]. \tag{1.4} \]
Figure 1.3: The percentage of enclosed photons as a function of radial distance from a point source is shown above for the scattering-dominant limit in a uniform medium. The tissue phantom has $\mu_a = 0.23$ cm$^{-1}$, $\mu_s = 217.5$ cm$^{-1}$, and $g = 0.589$. [18] The blood model has $\mu_a = 12.5$ cm$^{-1}$, $\mu_s = 22.2$ cm$^{-1}$, and $g = 0.99$. [19] The skin model has $\mu_a = 0.63$ cm$^{-1}$, $\mu_s = 265$ cm$^{-1}$, and $g = 0.8$. [8]

To accurately calculate the distribution of the Raman photons requires a simulation that includes the Raman cross sections of tissue components and the boundary conditions of the medium. However, to a good approximation, the Raman photon density is proportional to the excitation photon density. In place of a simulation, an experiment was performed using a Ti:Sapphire laser and a photodiode array to measure the remitted photon distribution of a human forearm. A calibrated linear array of CCD detectors pushed up against the skin was used to measure the remitted light of the laser at 840 nm. The percentage of the total remitted light distribution is shown, along with calculated photon distributions in Fig. 1.3. Clearly, for skin, there is an excellent agreement between the model and experiment.

From the model, we find that in blood the photons stay relatively localized, with
90% of the photons staying within 1 millimeter of the excitation source. However in tissue the spot size of the radiation vastly increases—only 25% of the photons are distributed within 1 millimeter of the source.

1.3 Challenges of Raman Measurements

The first difficulty with Raman spectroscopy is the very low efficiency of the process, $1 \times 10^{-8} - 10^{-10}$, compared to absorption or fluorescence, $\approx 1 - 10\%$. [7] While techniques such as Coherent Anti-Stokes Raman spectroscopy (CARS) and surface-enhanced Raman spectroscopy (SERS) increase this efficiency orders of magnitude, these techniques are impractical for routine in vivo use. CARS requires costly tunable lasers, and its coherence requirement limits the penetration depth. SERS is unsuitable for in vivo use due to the requirement for the sample to exist on a tailored substrate. To deal with the low efficiency of the Raman process, high laser powers, efficient detectors, and high throughput optical systems are required.

Designs of these systems for tissue use are also hindered by the turbidity, or cloudiness, of the samples. With spontaneous Raman spectroscopy, photons are generated completely incoherently, and radiate in all possible directions. When a highly scattering sample is used, such as tissue in the NIR wavelength range, this creates sources which have an étendue greater than that can be measured by conventional instruments. This requires that only a portion of the generated photons can be measured, thus hindering the detection of the Raman signal.

Another obstacle in Raman spectroscopy is the fluorescent background that is excited along with the Raman signal in many samples. Even with NIR excitation, this fluorescence is orders of magnitude higher than the Raman spectra of many biological analytes at physiological levels. To further diminish the fluorescence, excitation wavelengths can be changed to $>1 \mu$m, however the efficiencies of CCDs are
prohibitively low at this wavelength, and Fourier Transform Infrared Spectroscopy (FTIR) must be used which requires much longer exposure times due to the reduced signal to noise ratio (SNR) of the technique. [11]

1.4 Computational Sensor Design

In order to design systems that are more robust against the issues described in the previous section, low signal levels and high background fluorescence, the strategy of computational sensing will be used. Two new techniques, static multimodal-multiplex spectroscopy (MMS) and coded-excitation Raman spectroscopy, have been developed in the course of the thesis research. Computational sensing realizes that the physical design process and digital post-processing of measurements are tightly interrelated. [20] The idea is to not completely rely either on an optical system or on the digital post-processing to optimize performance, but rather to balance processing on both parts of the system design. Some of the possibilities afforded by this concept include extended depth-of-field imaging using wavefront coding [21], multiplex imaging to perform efficient principal component identification [22], and optical multiplexing to perform efficient motion tracking [23]. Isomorphic mappings between the source and detector, such as an imaging system where a lens forms an image of a scene on a piece of film, are no longer necessary due to the ability to rapidly process the detector data. While such a design concept is by no means new, (e.g. Fourier transform spectrometers have been around for many years), the use of different mathematical transforms and their implementation in optical sensor systems is a very rich field for investigation.

The two new computational sensor design aspects of the thesis are— reconstructing a spectrum from the spatially multiplexed coded aperture spectrometer, and coding the excitation source of a Raman system to filter out the Raman signal. The
static aperture coding design of MMS allows for an efficient method of performing spectral measurements on sources of large étendue. By replacing the conventional slit of a dispersive spectrometer with an aperture code with independent columns, the allowable étendue is increased by $10^{−100\times}$, thus making Raman spectroscopic measurements of tissue more feasible. The multiplexed data on the detector is processed to recover a high fidelity estimate of the source spectrum. Previous approaches using aperture coded spectrometers were all either scanning devices or poorly conditioned inverse problems leading to greater noise sensitivity.

The second novel design concept is that of coded-excitation Raman spectroscopy. Since the Raman signal is shift-variant with respect to the excitation wavelength, it can be separated from the non-shift-variant fluorescent background by excitation at different wavelengths. By using either one tunable laser or an array of lasers all at slightly different excitation wavelengths, multiple spectra can be acquired which can be later digitally processed to isolate the Raman component of the signal.

1.5 Outline

Chapter 2 of this thesis details the motivation and theory of MMS systems. A review of multiplex spectroscopy will be discussed, as well as some of the specific MMS aperture codes and simulations to investigate the noise performance of such systems.

More of the engineering details of designing and constructing MMS spectrometers will be discussed in Chapter 3, and experimental validation of the throughput advantage will be analyzed.

The MMS Raman system, which was the centerpiece of the experimental work of the thesis, will be described in detail in Chapter 4. The results of applying the the Raman MMS system to multivariate chemometric analysis of ethanol in a tissue phantom will be shown.
In Chapter 5 the background, theory, and implementation of coded-excitation Raman spectroscopy will be shown. Experimental results of the approach using fluorescent dye, chemometric analysis, and tissue analysis will be analyzed.

While most of the work presented in this thesis is original work by the author, there are some portions where other people’s contributions are necessary to point out. In Chapter 2, much of the theory was formulated by Dr. M. E. Gehm. Much of the material there is reprinted with permission from a journal article co-authored by Dr. Gehm and the thesis author. [24] Much of the material in Chapter 4 comes with permission from a journal article authored by the thesis author. [25] The mathematical framework of Chapter 5 was developed by Dr. R. Willett.
Chapter 2

Coded-Aperture Spectroscopy of Diffuse Sources

As mentioned in Chapter 1, a critical component to any Raman spectroscopy-based tissue analysis is a high throughput, high fidelity spectrometer. Multiplexing allows for a system to have either an increased throughput and/or less sensitivity to certain types of noise, thus making it a valuable idea for such systems. We will first review the most successful applications of multiplexing in the design of spectrometers. The key differences between previous systems and our static MMS design will then be identified, followed by a detailed mathematical framework and analysis of our design.

2.1 Multiplex Spectroscopy

2.1.1 Limitations of Slit-based Systems

Designing a spectrometer to efficiently collect light from diffuse sources such as scattered light by tissue is difficult. The conventional slit-based dispersive systems achieve a high spectral resolution through the use of a narrow slit, which can severely restrict the light available from such sources.

A simple schematic of a slit spectrometer is shown in Fig. 2.1. Relay optics and a dispersive element image the slit at wavelength-dependent locations on the detector plane. The spectral resolution will be determined by the minimum detectable separation of the slit images. As the slit width, $\delta x$, is widened, this will then increase the width of the spectral resolution element $\delta \lambda$.

The light throughput of an optical system is defined by its étendue, also known
Figure 2.1: Schematic of a traditional slit-based spectrometer. The relay optics image the slit at wavelength dependent-locations on the output detector. Spectral resolution depends on the ability to distinguish adjacent wavelength channels, which is reduced as the slit is widened.

as the optical invariant, which can be approximated by the product of the solid angle allowed by the optical system and the area of the input aperture,

$$G \simeq A \Omega. \quad (2.1)$$

The acceptance angle is fixed for a given instrument, and thus to increase the light gathering capacity the only option is to increase the input aperture area $A$, however this comes at a loss of spectral resolution as described earlier. For weak sources of large étendue this can be problematic, since only a small portion of the source will be available for a measurement thus decreasing the signal-to-noise ratio(SNR).

2.1.2 Previous Work on Multiplex Spectroscopy

The problem of the throughput-resolution trade-off of slit-based systems is by no means new, and has been solved by a variety of means. [26, 27] The one common thread to all of these methods is that of a “multiplex” sensor, one in which combina-
tions of desired quantities are measured instead of the actual quantities themselves. Multiplex sensing can allow for measurements to have higher signal levels without needing to increase the spectral brightness, thus allowing for measurements with a much higher SNR in the presence of signal-independent noise. [28]

In spectroscopy, the SNR improvements of multiplexing were first applied in Fourier Transform Infrared (FTIR) absorption spectroscopy. The standard technique of absorption spectroscopy was a scanning monochromator and a single detector in order to record the absorption of a certain sample at some range of wavelengths. [29] A monochromator is similar to the dispersive spectrometer described earlier, except an exit slit is placed in the detector plane in order to only allow a narrow wavelength band to reach a single detector. While the light source and detector were both large in area, the narrow entrance and exit slits of the monochromator meant most of the available light was lost at the jaws of the slits. Coupled with the high background noise levels of the detector elements, new approaches were sought to improve the measurements.

The FTIR systems involved coupling the light source into a Michelson interferometer with a scanning arm and a single detector. By acquiring the intensity pattern as a function of scan distance the autocorrelation is measured and by then performing a Fourier transform, the spectral density can be acquired. [30] The spectral resolution then comes from the fine scanning in the interferometer arm instead of from the narrow slits of the dispersive systems. The first advantage to such a technique was identified as the Fellgett (multiplex) advantage. [27] Since at each step of the interferometer arm all the different spectral channels are being measured at once, the average signal on the detector is much higher than for a single channel measurement. In early systems this was an especially great advantage since the majority of the detector noise was additive in the form of thermal fluctuations and readout noise.
The other advantage of the FTIR systems is the Jacquinot (throughput) advantage, originally discussed in the context of a Fabry-Perot spectrometer. [31] This is a result of the large étendue available to interferometric instruments for a given resolving power compared to a slit-based system.

In parallel to the interferometric method development, many researchers realized that by replacing the input and/or exit slits of a slit spectrometer with more complicated spatial filters, that both the multiplex and throughput advantages could be realized without a loss of resolution. Golay proposed modulating different “virtual slits” at the entrance and exit apertures with sinusoidal patterns of different frequencies on spinning disks. [26] This system realized a throughput gain since the entrance slit could be drastically increased, however it measured only one wavelength channel on the detector element so did not exhibit the multiplex advantage. Similar systems were explored by other groups. [32–34] Harwit realized by replacing both the entrance and exit slits with binary masks, usually a rectangular pattern with elements that are either transmissive or opaque, and scanning both the entrance and exit slits, both the throughput and multiplex advantages could be realized. [35] One of the important design decisions was the choice of codes to use, and this led to the use of the Hadamard matrices [36]. The majority of coded-aperture spectrometers became known as Hadamard Transform (HT) spectrometers. [37]

The motivation for using the Hadamard matrices becomes clear when the multiplex measurement is related to a previously studied problem of weighing groups of objects. [37, 38] The advantages of using the Hadamard matrices are the well-conditioned nature of the inversion problems that result from the HT spectrometers and the simplicity of binary mask manufacturing.

The initial HT spectrometers focused on single-channel detectors since these were the only detectors available at the time. With the advent of multi-channel detectors,
high throughput multiplexed imaging spectrometers were developed with the use of liquid crystal spatial light modulators [39, 40] and micro-electromechanical systems (MEMS) arrays. [41] In both systems, a three-dimensional data set (two spatial and one spectral) was formed by processing multiple images each taken with a different spatial multiplexing code.

When a source is diffuse, however, spatial multiplexing can allow for the static measurement of the spectrum with limited spatial information. Such an approach was shown to be effective at obtaining a spectrum of a diffuse source by replacing the slit of a spectrometer with a multiple-slit mask and then performing a 1D deconvolution of the detector data. [42] This approach suffers, however, from not being as well-conditioned computationally as the multiple measurement methods.

Since the multiple-slit mask and wide open slit approaches are similar to the 2D aperture coding approach we have developed, it should be informative to look into that approach with more detail. For these approaches, the spectrometer measurement, a vector $M$, can be treated as a 1D convolution of the input aperture function and $S$, the spectrum of the source. This can be expressed in matrix form by constructing the proper circulant matrix. For example, for a simple aperture code of [101] measured with 7 detector elements the relationship becomes

$$
\begin{bmatrix}
1 & 0 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 & 0 \\
1 & 0 & 1 & 0 & 0 \\
0 & 1 & 0 & 1 & 0 \\
0 & 0 & 1 & 0 & 1 \\
0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 1
\end{bmatrix}
\times S = M.
$$ (2.2)

The aperture code is placed in each column of the matrix, with a vertical shift equal to the column index. The spectral estimation then involves processing the
measured vector $M$ into a spectral estimate, $\hat{S}$, usually by least squares inversion. This type of inversion problem for this case, however, is somewhat ill-posed and its solution can be sensitive to noise in the measurements. [43] As an alternative, one could also remove the slit pattern entirely, corresponding to an aperture code of all 1’s. While this would add more throughput to the system, the inversion problem becomes even more ill-posed. The important aspect of the static MMS approach is that by using a 2D code much more control is gained in the form of the matrix problem to be inverted, and thus can be designed to be more well-conditioned.

### 2.1.3 Static MMS Design

The design that we have developed is static, well-conditioned, and allows for both the Jacquinot and Fellgett advantages for measuring the average spectrum of a diffuse source. This is accomplished by replacing the slit of a spectrometer with a 2D aperture pattern and measuring the output plane of the spectrometer with a 2D detector. We will refer to this design as static multimodal multiplex spectroscopy, static MMS. The mathematical background for choosing aperture patterns will be described along with a description of certain families of aperture patterns will be described.

### 2.2 Mathematical treatment

We follow the mathematical treatment in Gehm et al. [24] to analyze the static MMS implementation.

#### 2.2.1 System model

We begin by considering the following simplified model of a dispersive spectrometer:

$$I(x', y') = \iiint d\lambda dx dy H(x, x', y, y'; \lambda) T(x, y) S(x, y; \lambda).$$  \hspace{1cm} (2.3)
Here, $H(x, x', y, y'; \lambda)$ is the kernel describing propagation through the spectrometer, $T(x, y)$ is a transmission function describing the input aperture and $S(x, y; \lambda)$ is the input spectral density at position $(x, y)$. We use unprimed variables for quantities defined in the input plane while primed variables are used for the quantities in the detector plane. A simple schematic of these coordinates is shown in Fig. 2.2.

We take $H(x, y; \lambda) = \delta(y-y')\delta(x-(x'+\alpha(\lambda-\lambda_c)))$ as the propagation kernel. This kernel represents a basic dispersive spectrometer with unity-magnification optics, a linear dispersion $\alpha$ in the $x$-direction, and a center wavelength of $\lambda_c$ for an aperture at $x = 0$. Inserting this in Eq. 2.3 and performing the $\lambda$- and $y$-integrals yields

$$I(x', y') = \int dx \, T(x, y') \, S(x, y'; \frac{x-x'}{\alpha} + \lambda_c). \tag{2.4}$$
A traditional slit-spectrometer takes the input aperture as \( T(x, y) = \delta(x) \), so that

\[
I(x', y') = S \left( 0, y' ; \lambda_c - \frac{x'}{\alpha} \right).
\] (2.5)

Thus the intensity profile in the detector plane is a direct estimate of the spectral density at the slit location.

However, as discussed above, the drawback to such an approach is that the throughput of the system is severely curtailed. We wish to consider more complicated aperture patterns so that we can increase the photon collection efficiency of the system.

Our fundamental goal is to develop an aperture code which allows us to estimate the mean spectrum across an extended aperture, which we define as

\[
S_{\text{mean}}(\lambda) \propto \int \int dx \, dy \, S(x, y; \lambda).
\] (2.6)

### 2.2.2 The coding approach

In the more general case, to convert the intensity profile of Eq. 2.4 into an estimate of the mean spectrum, we multiply it by an analysis function \( \tilde{T}(x'', y') \) and integrate over the extent of the patterns in \( y' \):

\[
E(x', x'') = \int_{y''_{\text{min}}}^{y''_{\text{max}}} dy' \, \tilde{T}(x'', y') \, I(x', y')
\]

\[
= \int_{y''_{\text{min}}}^{y''_{\text{max}}} dy' \int dx \, \tilde{T}(x'', y') \, T(x, y') \, S \left( x, y' ; \frac{x - x'}{\alpha} + \lambda_c \right).
\] (2.7)

We then make the assumption that \( S(x, y'; \lambda) \) is constant, or slowly varying in \( y' \).

We can write this as

\[
S(x, y'; \lambda) \approx I(y') \, S(x; \lambda).
\] (2.8)
Inserting this in Eq. 2.7, we find

\[ E(x', x'') \approx \int_{y'_{\text{min}}}^{y'_{\text{max}}} dy' \int dx \, \tilde{T}(x'', y') T(x, y') I(y') S \left( x; \frac{x-x'}{\alpha} + \lambda_c \right). \quad (2.9) \]

If \( T(x, y') \) and \( \tilde{T}(x'', y') \) are constructed such that

\[ \int_{y'_{\text{min}}}^{y'_{\text{max}}} dy' \, \tilde{T}(x'', y') T(x, y') I(y') = \beta \delta(x - x''), \quad (2.10) \]

then our estimate becomes

\[ E(x', x'') \approx \beta \int dx \, \delta(x - x'') S \left( x; \frac{x-x'}{\alpha} + \lambda_c \right) \approx \beta S \left( x''; \frac{x''-x'}{\alpha} + \lambda_c \right). \quad (2.11) \]

This result can be interpreted as a two-dimensional function containing estimates of the input spectrum at different input locations. A slice through this function at a constant value of \( x'' \) corresponds to the input spectrum at a particular value of \( x \). In other words, if we halt our analysis at this point, we have created a 1D imaging spectrometer. We will discuss the implications of this imaging capability in the future.

In our analysis, we wish to proceed further and convert \( E(x', x'') \) into an estimate of \( S_{\text{mean}}(\lambda) \).

Since the spectral estimates of Eq. 2.11 are shifted with respect to each other, to calculate the mean spectrum we must integrate along the line \( x' = \lambda \alpha + x'' \):

\[ S_{\text{mean}}(\lambda_c - \lambda) \propto \int dx'' \, dx' \, \delta [x' - (\lambda \alpha + x'')] \, E(x'x'') \]

\[ \propto \int dx'' \, S(x''; \lambda_c - \lambda). \quad (2.12) \]

Thus with appropriately designed input apertures and analysis functions, we can
convert an intensity profile at the detector plane into an estimate of the input spectrum. But how does one perform this design subject to the constraint of Eq. 2.10?

2.2.3 Orthogonal/independent column codes

If we rewrite Eq. 2.10 in a form where \( x \) and \( x'' \) are not coordinates, but instead parameters

\[
\int_{y_{\text{min}}}^{y_{\text{max}}} dy' \, \tilde{T}_{x''}(y') \, T_x(y') \, I(y') = \beta \delta(x - x''),
\]

(2.13)

we arrive at an equation that is identical to the orthogonality constraint for eigenfunctions in Sturm-Liouville theory, [44] with \( I(y) \) playing the role of the weighting function and \( \beta \) as the norm. Therefore, we can meet the design requirement by basing the input aperture pattern on any family of orthogonal functions.

Using the language of Sturm-Liouville theory, if \( T \) and \( \tilde{T} \) are the same set of codes, we say that the system is \textit{self-adjoint}. In this case, the complete set of codes in \( T \) can be viewed as abstract vectors defining an orthogonal basis on a Hilbert space, and we refer to a family of this type as an \textit{orthogonal column code}.

If \( T \) and \( \tilde{T} \) are not the same set of codes, the system is said to be \textit{non-self-adjoint}. Here the complete set of codes in \( T \) can be viewed as abstract vectors defining a non-orthogonal basis on a Hilbert space. We refer to a family of this type as an \textit{independent column code}.

In Eq. 2.13, \( x \) and \( x'' \) can be either continuous or discrete parameters depending on the eigenvalue spectrum of the chosen family of functions. In the discrete case, the Dirac delta function \( \delta(x - x'') \) is properly replaced with the Kronecker delta \( \delta_{x,x''} \). Further, in this case the input mask and analysis pattern will be pixelated in the \( x \) and \( x'' \) directions, respectively.
2.2.4 **Heuristic treatment**

Considerable insight can be gained from a heuristic view of orthogonal and independent column coding. From Eq. 2.4, we see that for the case of uniform input intensity, the output intensity distribution is a convolution of the input aperture and the input spectrum:

\[
I(x', y') = \int dx \, T(x, y') \, S\left(\frac{x - x'}{\alpha}\right).
\]  \hspace{1cm} (2.14)

Thus, the light falling at a given value of \(x'\) in the detector plane arises from a combination of different wavelengths passing through different locations on the input aperture. A well-designed code allows us to break this ambiguity and determine the spectral content of the light. By choosing a family of functions as our transmission mask, we provide a unique code to each possible \(x\)-location in the input plane. We can view the transmission pattern at position \(x\) as an abstract vector \(|T_x\rangle\). The full family of transmission patterns then forms a basis \(\{ |T_x\rangle \}\). If we consider the light distribution falling at a given \(x'\)-location in the detector plane as the abstract vector \(|I_{x'}\rangle\), the contribution from position \(x\) on the input aperture is simply given by \(\langle T_x | I_{x'} \rangle\), the projection of \(|I_{x'}\rangle\) onto the adjoint of the corresponding vector \(|T_x\rangle\) \((\langle T_x | \equiv |T_x\rangle^\dagger)\). Because only light of wavelength \(\lambda_{x,x'} = (x - x')/\alpha + \lambda_c\) can propagate from \(x\) to \(x'\), this inner product also represents an estimate of \(S(x, \lambda_{x,x'})\). Forming the set of all inner products of the form \(\langle T_x | I_{x'} \rangle\), yields the 2D spectral estimate function \(E\).

2.3 **Specific mask families**

The section above demonstrates the appeal of using orthogonal or independent column codes as aperture mask patterns in dispersive spectroscopy. The number of possible families is, of course, infinite. The following sections describe certain specific
families of interest.

2.3.1 Harmonic masks

Above, we allude to the fact that the intensity profile $I(y)$ takes the role of the weighting function in Sturm-Liouville theory and, in conjunction with the integration limits, controls the nature of the orthogonal functions. If we consider a uniform input intensity, symmetric integration limits ($y_{\text{min}} = -Y$, $y_{\text{max}} = Y$), and a discrete eigenvalue spectrum, we get the constraint (for the remainder of this section we use $y$ in place of $y'$ as there is no chance of confusion)

$$
\int_{-Y}^{Y} dy \tilde{T}_{x'}(y) T_x(y) = \beta \delta_{x,x'}
$$

(2.15)

which is satisfied by the well-known harmonic functions. For example (using $\mathbb{Z}^*$ to represent the nonnegative integers),

$$
T_x, \tilde{T}_{x'} \in \left\{ \cos \left( \frac{m y \pi}{Y} \right) \right\}, \quad m \in \mathbb{Z}^*,
$$

(2.16)

is an obviously self-adjoint solution to Eq. 2.15.

However, there is a problem with this set of functions. Because we are working with incoherent illumination, $T_x$ can only modulate the intensity of the light, not the field. As a result, we are forced to consider only functions with values in the interval $[0, 1]$.

This has a significant impact on the nature of the solutions that we may find. It is not possible to find a self-adjoint set of continuous functions that meets this requirement. Since negative values are not allowed, the inner product between any two such functions is positive definite. Hence the functions in $T_x$ cannot also be the functions in $\tilde{T}_{x'}$. We are forced to consider an independent column code.
Figure 2.3: (a) Aperture pattern for an independent column code based on harmonic functions. Note that the pattern is continuous vertically, but discrete horizontally, (b) Aperture pattern for an independent column code based on Legendre polynomials. The codes have been chosen such that the transmission has physically-realizable values in the interval $[0, 1]$. Note that the patterns are continuous vertically, but discrete horizontally.

One possible independent column code based on harmonic functions is:

$$T(x) \in \left\{ \frac{1}{2} \left( 1 + \cos \left( m \frac{\pi}{Y} \right) \right) \right\}, \quad m \in \mathbb{Z}^*. \quad (2.17)$$

The corresponding analysis codes are then:

$$\hat{T}(x') \in \left\{ 2 \cos \left( m \frac{\pi}{Y} \right) \right\}, \quad m \in \mathbb{Z}^*. \quad (2.18)$$

An aperture mask based on this independent column code with $m = 1 \text{--} 64$ is shown in Fig. 2.3 a.
2.3.2 Legendre masks

There is another set of famous orthogonal functions that satisfies the constraint of Eq. 2.15—the Legendre polynomials

\[ P_n(y) = \frac{1}{2^n} \sum_{m=0}^{\lfloor n/2 \rfloor} (-1)^m \binom{n}{m} \binom{2n-2m}{n} y^{n-2m}, \quad (2.19) \]

where

\[ \binom{a}{b} = \frac{a!}{(a - b)! b!}. \quad (2.20) \]

As was the case with the harmonic masks, the functions form a self-adjoint set of codes:

\[ T_x, \tilde{T}_x'' \in \left\{ P_m \left( \frac{y}{Y} \right) \right\}, \quad m \in \mathbb{Z}*. \quad (2.21) \]

However, as above, these codes involve modulation values which are not physically possible in an incoherent system. Scaling to produce physically-realizable values results in an independent column code. One possible version is

\[ T_x \in \left\{ \frac{1}{2} \left( 1 + P_m \left( \frac{y}{Y} \right) \right) \right\}, \quad m \in \mathbb{Z}*. \quad (2.22) \]

The corresponding analysis codes are then:

\[ \tilde{T}_x'' \in \left\{ 2P_m \left( \frac{y}{Y} \right) \right\}, \quad m \in \mathbb{Z}*. \quad (2.23) \]

An aperture mask based on this independent column code with \( m = 1-64 \) is shown in Fig. 2.3 b.
2.3.3 Hadamard masks

In the previous sections we consider only continuous functions of \( y \) as possible code families. Based on the heuristic insights of Sec. 2.2.4, it seems reasonable to also consider discrete functions of \( y \). A particularly good choice are pixelated functions based on Hadamard matrices. [36] We define \( H_n \) as an order-\( n \) Hadamard matrix, and use the symbols \( H_n(:,m) \) and \( H_n(m,:) \) to refer to the \( m \)th column and row of \( H_n \), respectively. Then

\[
T_x, \tilde{T}_{x'} \in \{H_n(:,m)\}, \quad m \in \mathbb{Z}^*; m \leq n
\]  

(2.24)

is a self-adjoint set of codes. Given that the elements of a Hadamard matrix are either 1 or \(-1\), this is again not realizable with incoherent illumination. Shifting and scaling the code values results in a non-self-adjoint independent column code

\[
T_x \in \left\{ \frac{1}{2} (1 - H_n(:,m)) \right\}, \quad m \in \mathbb{Z}^*; m \leq n.
\]  

(2.25)

With the corresponding analysis code

\[
\tilde{T}_{x'} \in \{2H_n(:,m)\}, \quad m \in \mathbb{Z}^*; m \leq n.
\]  

(2.26)

This particular choice is known as an S-matrix in the traditional Hadamard literature. An aperture based on an S-matrix code is shown in Fig. 2.4 a.

In all of the aperture masks so far, we shift and scale the code values to achieve a physically-realizable modulation. In every case, the application of a shift turns an orthogonal column code into an independent column code. However, if we had a method for identifying the sign of a code value, then we could apply the sign in software (by multiplying the measured value by \(-1\) where appropriate). By adding this extra computational step, we could achieve a physically-realizable aperture while
Figures 2.4: (a) Aperture pattern for an independent column code based on a Hadamard S-matrix, (b) Aperture pattern for an orthogonal column code (in conjunction with processing of the measured intensity) based on a row-doubled Hadamard matrix. The codes have been chosen such that the transmission has physically-realizable values in the interval $[0, 1]$. Note that the pattern is discrete both horizontally and vertically.

avoiding the need for a shift and have a self-adjoint set of codes.

Unfortunately, any row of the code contains both positive and negative values. The multiplex nature of the system then ensures that light from these different regions are combined on the detector plane, making it impossible to apply the appropriate weighting in software. However, if we could segregate positive and negative regions of the code onto separate rows, then we could apply a weighting to entire rows in the detector plane and achieve our goal. We refer to codes that have been modified in this manner as row-doubled.

To row-double a Hadamard matrix, we replace each original row $H_n(m, :)$ with two rows:

$$H_n(m, :) \rightarrow \begin{bmatrix} \frac{1}{2} (1 + H_n(m, :)) \\ \frac{1}{2} (1 - H_n(m, :)) \end{bmatrix}.$$  \hspace{1cm} \text{(2.27)}
If we denote a row-doubled version of $H_n$ as $\hat{H}_n$, then

$$T_x, \hat{T}_x^{\prime\prime} \in \left\{ \hat{H}_n(:, m) \right\}, \ m \in \mathbb{Z}^*; m \leq n$$

(2.28)

is a physically-realizable orthogonal column code when combined with the now-possible computational step of weighting the appropriate rows in the measurement by $-1$. An aperture based on a row-doubled Hadamard matrix is shown in Fig. 2.4 b.

### 2.3.4 Continuous vs. discrete codes

There is an important difference between the continuous mask codes (harmonic and Legendre) and the discrete codes (S-matrix and row-doubled Hadamard). In the case of the continuous code families, there are an infinite number of possible codes ($m \in \mathbb{Z}^*$). This means the underlying Hilbert space is infinite-dimensional. Any physical aperture based on these codes must choose only a subset of the possible code patterns. As a result, the implemented basis is not complete, and Parseval’s relation will not hold. In short, in the presence of noise, the total power associated with the different apertures after processing will not necessarily equal the total power measured on the detector plane.

For the discrete codes, however, there is only a finite number of code patterns in any given family ($m \leq n$). The underlying Hilbert space is then $n$-dimensional, and an aperture can be designed that contains all of the codes. In this case, Parseval’s relation will hold and power is necessarily conserved during the processing.

### 2.4 Simulations of MMS system

To investigate the signal-to-noise properties of the MMS masks, a simulation with realistic parameters was done. The dominant noise sources in a spectrometer can be grouped into two categories— signal-independent noise and signal-dependent noise.
The primary signal-independent noise sources in CCDs comes from the readout noise in the electronics that is always present, and the “dark” noise caused by thermally generated electrons that accumulate in the detector pixels. [45] Thermal noise can be reduced to a very negligible amount by extreme cooling of the detector, and readout noise can be reduced but never eliminated by using slow readout electronics. The signal-dependent noise comes from the Poisson statistics of the photon arrivals at the detector.

A frequent concern in multiplexing instruments is the influence of the different types of noise on the performance of the instrument. Early single-channel HT designs described by Harwit [37] claimed only to have an advantage over slit-based instruments with signal-independent noise. With Mende’s work [42], he realized that by using a multi-channel detector an SNR gain could be gained even in the presence of signal-dependent noise with a sparse spectrum. This gain, however, was at the peaks of the spectra, whereas in the rest of the spectrum the noise of the measurements increased. To investigate these issues with the MMS design, a simulation using a \( N = 32 \) S-matrix code was used. A simplistic model of the spectrometer was used—simply a discrete x-convolution of a test spectrum containing 5 spikes with the aperture pattern. To simulate the slit response, a slit of equivalent width and height to one column of the S-matrix code was also convolved with the test spectrum. Poisson noise of the proper amount was then added to each image, readout noise then added, and then the data was processed. A least-squares solution to the S-matrix data was used to reconstruct the spectrum, and a summation of the slit data along the non-dispersion direction was used to process the slit data.

In Fig. 2.5 simulated reconstructions for a S-matrix coded aperture, of order 31, and for a slit of equivalent resolution are shown. The test signal is 5 spikes with a smooth background, similar to a Raman spectrum that might be acquired. 100
Figure 2.5: (a-d) Simulated spectral reconstructions of 5 spike spectrum with smooth background with 2D MMS code. Exposure time increases from a to d. (e-f) Simulated spectra of same 5 spike spectrum from slit of equivalent resolution as mask in (a-d). Exposure time for a the same as e, b the same as f etc.
iterations were used, with 10 root-mean-squared counts of readout noise, and added Poisson noise. At a short exposure time, \( \approx 50 \) counts/pixel, Fig. 2.5a/e, shows that the 2D coded aperture system reveals the peaks with much less uncertainty. As the exposure time is increased, in both the slit and coded aperture systems the 5 peaks are clearly visible.

To estimate the ability of the coded aperture and the slit to accurately determine the height of a given peak, necessary for quantitative analysis, the ratio of the signal at a given wavelength channel to its variation was calculated. Over 100 simulations at different noise situations, the average value at each spectral channel was divided by the standard deviation of the measured value. In Fig. 2.6, the different situations are shown. The coded aperture clearly performs better at estimating peak heights in the absence of a background signal and in the presence of detector noise. For Poisson-noise limited situations with intense backgrounds, however, the coded aperture system performs similar to the slit system at the peaks in the spectrum and slightly worse at the background regions. This agrees well with Mende’s observations, and can be explained by the increase in signal being offset by the Poisson noise in the signal.

A further simulation was done to compare the 2D MMS approach to the multiple-slit circulant S-matrix approach described in Sec. 2.1.2. [42] In the multiple-slit approach, a 1D pattern of slits replaces the single slit of a spectrometer, and the result is deconvolved. A test spectrum used for simulations, and the detector images for both approaches is shown in Fig. 2.7.

The spectral reconstructions for 10 simulations with different exposure times are shown in Fig. 2.8. Only 10 simulations were done due to the increased computation time needed to process the 1D coded data. As is clear from the figure, at low exposure times the 2D MMS system is able to reconstruct the spectrum with a lower sensitivity
Figure 2.6: Plots showing ratio of signal level to standard deviation of signal level over 100 simulations of slit and 2D coded mask system. (a) Signals are 5 spikes with no background, very weak signals so read noise is the dominant source. (b) Signals are 5 spikes with smooth background, very weak signals so read noise is the dominant noise source. (c) Signals are 5 spikes with no background, strong signals so Poisson noise dominates. (d) Signals are 5 spikes with smooth background, strong signals so Poisson noise dominates.
Figure 2.7: (a) Test spectrum for 2D/1D coding comparison. (b) Detector image of spectrum in (a) using a $N=31$ 1D S matrix circulant code. (c) Detector image of spectrum in (a) using a $N=31$ S-matrix 2D MMS code to noise. This seems to fit with the idea that the 1D approach suffers from being more ill-conditioned than the 2D approach.
Figure 2.8: (a-c) Simulated spectral reconstructions of 6 spike spectrum with smooth background with 2D MMS code. Exposure time increases from a to c. (d-f) Simulated spectra of same 6 spike spectrum from 1D S-matrix code of equivalent resolution as mask in (a-d). Exposure time for a the same as d, b the same as e etc.
Chapter 3

MMS System design and performance

The goal of this chapter is to describe the design process for MMS systems and report results on constructed systems. The optical design process involves a complicated interplay of many parameters—desired resolution, aperture size, aberrations, acceptance angle, field size, system weight etc. The most important goals for the MMS designs are a high-throughput system, since that has been the whole premise of their use, and a high enough quality imaging system in order to implement the aperture codes described earlier. We will first identify the design equations for a slit spectrometer, since they share many similarities with the MMS spectrometers. Important differences exist, however, and these will be pointed out and shown how they affect the designs. We will then analyze experimental data taken with an MMS system to show the signal-to-noise and throughput advantages.

3.1 Spectrometer Design

3.1.1 Dispersive Spectrometer Design

The goal of any spectrometer is to perform a measurement of some light field’s power spectral density, referred to more commonly as the spectrum. Currently, Raman spectroscopy is most prevalent at wavelengths in the UV-VIS-NIR region of the spectrum (200-1300 nm) due to the availability of high intensity, high spectral purity light sources from gas, diode, and solid-state lasers. Detectors with high dynamic ranges and sensitivities are also available, with the most popular being single-channel photodiodes and multi-channel CCD detectors. Most Raman spectrometers in this wavelength range fall into two categories—dispersive-based instruments, and scan-
ning interferometric instruments. Scanning interferometric instruments are typically used for their higher resolution capability albeit at the cost of lengthy scans. Dispersive designs are commonly used in wavelength ranges where silicon CCDs are sensitive (200-1000 nm) due to the fast acquisition times of a multi-channel detector. [11]

For the wavelength-separating element prisms or gratings are typically used, with gratings being the preferred choice for high dispersion applications. A grating is an optical element with a periodically varying index of refraction at periods near the wavelength of the light used. The frequency of the index modulation, known as groove density since original gratings were scribed into metal, \( d \), will be defined as the number of periods per unit distance (typically mm). A grating then diffracts light corresponding to the well-known grating equation,

\[
nd\lambda = \sin\alpha + \sin\beta, \quad (3.1)
\]

where \( \alpha \) is the angle of an incident plane wave measured relative to the grating normal, \( \beta \) is the angle of diffraction measured relative to the grating normal, and \( n \) is the order of diffraction, typically +1 or -1 to reduce the overlap of unwanted orders. By differentiating Eqn. 3.1 and keeping the incident angle \( \alpha \) constant, an expression for the angular dispersion is found,

\[
\frac{d\beta}{d\lambda} = \frac{nd}{\cos \beta}. \quad (3.2)
\]

Lenses or curved mirrors of focal length \( f \) are placed one focal length away from a narrow slit in order to form a plane wave. A schematic of a dispersive spectrometer with lenses and a transmission grating geometry is shown in Fig. 3.1. The analysis is valid for reflective gratings and mirrors as well. An additional optic is placed after
Figure 3.1: Simplified diagram of a dispersive spectrometer with a multi-channel detector at the output plane. Light at input angle $\alpha$ to a grating with period $1/d$ is diffracted at an angle $\beta$ according to the grating equation. Lenses of focal length $f$ at distances $f$ from the input and output planes form an approximately 1:1 imaging system. An input slit of width $w$ assures light from only a narrow range of angles is incident on the grating. Light at wavelengths from $\lambda_{\text{min}}$ to $\lambda_{\text{max}}$ are measured by a multi-channel intensity detector.
the grating in order to image the diffracted plane waves. For our analysis we will assume identical optics in the front and back of the optical system. An ideal plane wave entering a lens at angle $\theta$ will form a diffraction limited spot at a distance $x \simeq \theta f$ from the optical axis. [46] Thus, we can relate the angular dispersion to a linear dispersion at the output plane, usually expressed in wavelength units per unit distance,

$$\frac{d\lambda}{dx} = \frac{\cos \beta}{ndf}.$$ (3.3)

For an ideal system, the closest detectable separation of two wavelengths will be limited by the size of the Airy disc. Adopting the Rayleigh criterion, the minimum separation distance is $1.22\lambda f/D$, where $f$ is again the focal length of lens and $D$ is the diameter of the illuminated area of the lens, which combined with Eqn. 3.3 determines the minimum wavelength separation detectable,

$$\Delta \lambda_{\text{min}} = \frac{1.22\lambda \cos \beta}{ndD}.$$ (3.4)

The analysis so far has been for an infinitely small input point, whereas a slit or pinhole of finite width $W$ is always placed in the input aperture of the spectrometer. This leads to an instrumental “bandpass” $B$ defined by multiplying the width of the image of the slit by the linear dispersion of Eqn. 3.3,

$$B = \frac{W \cos \alpha}{ndf}.$$ (3.5)

This bandpass roughly corresponds to the measured full-width-half-maximum
(FWHM) of a monochromatic light source, assuming the slit width is larger than
the diffraction limit of the optical system, and thus will be used interchangeably with
the the term spectral resolution. There would now seem to be a large design space
in order to achieve a desired spectral resolution \( B \) over a certain spectral bandwidth
\( \Lambda = \lambda_{\text{min}} \ldots \lambda_{\text{max}} \).

Other factors now need to be considered to in order to optimize a design. For
Raman applications, light throughput is an important criteria since the efficiency of
the process is very low. Étendue, as described earlier, is an important concept that
describes how spread out in space and angle a light source is. For example even a
large diameter laser beam would have a small étendue due to its very narrow angular
distribution, whereas a fluorescent lamp would have a much higher étendue since the
light would be emanating from a large area into many angles. We can define the
étendue, \( G \), of a source as

\[
G = \int_A \int_\Omega dA d\Omega, \quad (3.6)
\]

where \( A \) is the emitting area of the source, and \( \Omega \) is the solid angle distribution
of the emission.

The étendue of an optical system can be similarly defined as the product of the
area of its input aperture and the solid angle allowed by the optics. The étendue of
the slit-based spectrometer, \( G_s \), then becomes the product of the area of the slit and
the solid angle allowed by the optics,

\[
G_s = hw \sin^2 \Omega_a. \quad (3.7)
\]

Here \( h \) and \( w \) are the height and width of the entrance slit and the \( \sin^2 \Omega_a \) comes
Figure 3.2: Étendue for a light source is calculated by integrating the differential solid angle, $d\Omega$, and differential area, $dA$, over the entire source.

From the solid angle of a cone with half-angle $\Omega_a$. We can relate this to $f/\#$, the term for the light gathering power of a lens, since $f/\# = 1/(2\sin \Omega_a)$,

$$G_s = \frac{hw}{(f/\#)^2}. \quad (3.8)$$

The input slit also defines the instrumental bandpass, as shown earlier in Eq. 3.5, so we can also write the spectrometer étendue as,

$$G_s = \frac{hnA fB}{(f/\#)^2 \cos \alpha}. \quad (3.9)$$

In order to maximize the light throughput of a spectrometer, its étendue should be matched or greater than that of the light source. Thus for a given bandpass, the slit height, grating frequency, focal length, and $f/\#$ are the main design parameters. For a static instrument, however, with a multi-channel detector, the linear dispersion is chosen to fill entire the length of the CCD with the desired spectral range. Decreasing the linear dispersion would reduce the throughput due to the reduction in slit width
needed to maintain the bandpass and thus is not really an option. Thus, to increase the throughput without a loss of bandpass the only option is to increase the slit height, increase the size of the detector, and reduce $f/#$ of the system, all of which can not be continued without bound due to physical limitations in the optics and detectors, leading to the situation of the next section of designing spectrometers for high étendue sources.

### 3.1.2 Dispersive Spectroscopy of Diffuse Sources

Dispersive spectroscopy of diffuse sources poses problems due to the relationship between throughput and resolution as seen in the Eqn. 3.9. Thus, the experimenter is faced with three possibilities:

1. Allow the input slit of the spectrometer to act as a spatial filter and thus reject the majority of the available photons.

2. Use a structured fiber bundle or other non-imaging technique to remap the diffuse source into a slit shape.

3. Remove the slit entirely and deconvolve the spectra using a known instrument response function.

4. Design a coded aperture spectrometer with an input aperture matched to the size of the source.

For weak, diffuse sources, such as those common in biological systems, the first option will result in a photon starved measurement and extremely poor SNR. In the second option, to avoid rejecting photons, the resulting slit must have the same area as the original source. In order to completely couple large sources, this can result in slit lengths which are unreasonable. Further, the method introduces fiber coupling and
transmission losses. However, despite these problems, it is a straightforward approach that is favored by many leading research groups. [15, 47, 48]

Another option is to remove the slit entirely and then digitally post-process the 2D detector data in order to remove the blurring due to the wide input aperture. This approach has been shown to be only moderately effective for either spectrally sparse or spatially uniform sources, since artifacts are present due to the ill-conditioned nature of the problem. [49]

We choose the last option, and will use the mathematical framework of Chapter 3 in order to design high throughput spectrometers for analysis of diffuse sources.

3.1.3 Design Implications of MMS Spectroscopy

It is now enlightening to revisit the design equations of Section 3.1.1 with the MMS methodology. We will define the width of each column of an independent column code mask as $w'$, leading to a mask of total width of $Nw'$ where $N$ is the number of independent columns in the code. The bandpass of the instrument will be defined by $w'$, while the throughput of the system will increase by $N/2$ compared to a slit of equal height as the mask. In situations where an increase in throughput is not necessary, the MMS can have other advantages since the slit width and throughput are decoupled. In looking at the parameters determining the bandpass in Eqn.3.5, it becomes clear that the focal length can be decreased and/or the groove frequency decreased without a loss of resolution. In cases where the bandpass is limited by diffraction these gains would not be possible, however in many cases the input slits limit the spectral resolution. By reducing the focal length more compact systems can be designed with equivalent resolution of bulkier systems. The decrease in groove frequency allows for smaller detectors to be used by the increase in linear dispersion, leading to a further decrease in total sensor volume.
3.2 Implementation issues of MMS systems

There are a variety of MMS implementation issues where the performance of the real-world system deviate from the idealizations we considered in Ch. 2. The following sections address the most important of these issues.

3.2.1 Pixelization of the detector plane

In Sec. 2.2 we assume that we have access to the detector plane intensity distribution $I(x', y')$. In reality, we do not. Our measurement of the intensity profile is downsampled by the pixel size on the detector array. This has several important implications for the system. First, for the continuous codes, Eq. 2.15 is no longer strictly true. It remains approximately true, however, as long as we include only codes that contain spatial frequencies below the Nyquist limit defined by the pixel size.

Second, for the discrete codes, the aperture must be designed so that when imaged onto the detector, the features involve integral numbers of pixels in the $y'$ direction. This places performance requirements on the manufacturing accuracy of the aperture and the magnification of the imaging optics in the spectrometer. Additionally, an aperture involving a discrete code must be aligned with respect to the detector plane such that the vertical divisions between features align with divisions between pixels. This requires sub-pixel positioning ability on the input aperture during construction and alignment. This requirement can be relaxed, however, by increasing the number of pixels used per mask element. The detector data is then downsampled appropriately to form a data matrix with as many rows as the original aperture code.

3.2.2 Manufacturing grayscale patterns

Physical realities in the previous section limit us to coding patterns with values in the interval $[0, 1]$. However, the fact that a given modulation pattern can be physically
imprinted on the input intensity has no bearing on the manufacturability of the required input aperture.

Arbitrarily-patterned, continuous-tone masks with transmissions ranging from 0-100% are indeed possible. However, given the complexity of most orthogonal column code patterns, the cost to manufacture transmission masks to the required precision is prohibitive. One alternative is to convert the designed continuous-tone mask into a half-toned version. A small region of the continuous-tone pattern is subdivided into an array of even smaller subregions. Each of these subregions is assigned a transmission of either 0 or 100%, such that the net transmission in the region matches the grayscale value of the continuous-tone pattern. Provided that the conversion happens on a spatial scale that is smaller than the pixelization of the detector plane, no significant difference should be detectable.

There are a variety of halftoning algorithms available for optimizing the conversion. We have recently acquired a halftoned version of our harmonic mask pattern described above, and have begun testing.

3.2.3 Optical distortions and corrections

The internal optics of the spectrometer can have a significant effect on the performance of the system. The optical properties of a static MMS deviate from a traditional instrument in a critical manner. Because the MMS encodes spectral information across the detector plane in a highly non-local way, optical errors anywhere have a non-local effect on the reconstruction, introducing noise and errors at regions throughout the spectral range.

What are the primary optical errors we must worry about? We assume above that the incoherent imaging kernel is given by $H(x, x', y, y'; \lambda) = \delta(y - y')\delta(x - (x' + \alpha(\lambda - \lambda_c)))$. Significant deviation from this assumption leads to degraded (or erroneous)
spectral reconstructions. Thus we have three primary optical requirements

1. The spectral resolution of the instrument should be limited by the width of a feature on the input mask $\Delta x$. This requires that the size of the incoherent impulse response be small compared to $\Delta x$. Further, the size of the impulse response should not vary significantly across the input and output fields.

2. The impulse responses in the $x$ and $y$ directions should be uncorrelated. This requires that the optical system have low distortion across the input and output fields.

3. The input intensity profile should be unaffected by propagation through the system (aside from a wavelength-dependent shift in $x$ direction). This requires that there be no field-dependent intensity modulations (vignetting) in the system.

There is an additional way that our ideal imaging kernel can break down. Unfortunately, this issue exists even for an ideal optical system and must either be dealt with through special modifications to the hardware or through software corrections of the detector image prior to spectral reconstruction.

It is well known that imaging an aperture through a diffraction grating results in an image that is curved in the direction of the dispersion. [50] In terms of our imaging kernel, this manifests as a $\lambda_c$ that is $y$-dependent. This curvature, which is sometimes referred to as smile distortion, is the result of the particular geometry of the wave-normal sphere. For high-$f/#$ systems, the curvature is minimal and can be ignored. However, since we are concerned with maximizing étendue, a static MMS is almost always constructed at very low-$f/#$. As a result, the curvature is significant, as can be seen in Fig. 3.3 a. This curvature can be corrected in two possible ways: either the input aperture can be pre-distorted to compensate for the applied curvature, or
Figure 3.3: (a) Raw intensity image captured at the focal plane. The smile distortion is clearly visible. The spectral source has only sharp spectral lines, so the image contains only a few, crisp images of the mask pattern. The corrected intensity image after smile distortion has been removed via software processing. The leftmost edge of the sharp mask image in a was fit to a parabola to determine the amount of shift to be applied to each row of the image. (b) The corrected image after shifting image in a to account for smile distortion.

de the collected image can be computationally processed to straighten the patterns prior to reconstruction. We choose the later approach. The smile distortion is a simple quadratic. During the calibration phase, we fit the leading edge of an aperture image to a parabola and determine the required circular shift to apply to each row of the image to straighten the patterns. The result of the procedure is shown in Fig. 3.3 b.

3.3 MMS Experimental Results

This section presents a series of experimental results collected on one of the static MMS systems. The primary goal is to clearly demonstrate the existence of the Jacquinot and Fellgett advantages and show that the performance scales as expected.
3.3.1 Optical System

As described in Sec. 3.2.3, the optical design in an MMS system needs to have a field independent point spread function (PSF), and a small enough PSF to adequately resolve the mask features on the detector plane. Such designs are almost always done using a ray tracing software package, such as ZEMAX. In this way, the parameters of the CCD, linear dispersion, and field size can be entered into the program and the lens system can be optimized to minimize aberrations. For the very low $f/\#$ systems that are desirable for their high throughput, the minimization of these aberrations becomes very difficult. Multi-element lenses allow for the balancing of all the different types of aberrations—spherical, coma, astigmatism, and field curvature. [51] For near diffraction-limited performance, a custom lens design is frequently required, and this was the path taken for the first MMS systems.

The optical system, shown in Fig. 3.4 a, consists of two identical pairs of lenses to form a 1:1 imaging system from the aperture mask to the detector plane with a transmissive grating between the lens pairs. Each lens pair consists of two positive meniscus lenses and two positive doublets. By the design only having two unique lenses, the manufacturing complexity and costs were reduced. The lens diameters are 22 mm and the limiting aperture is a 13 mm aperture stop on the grating. The working $f/\#$ is $\simeq f/2$. The diffraction grating is a volume-phase holographic grating composed of a thin layer of dichromated gelatin sandwiched between two pieces of glass. The thin grating, $\simeq 8 \mu m$, allows for a high diffraction efficiency, $> 80\%$ over a wide range of angles and wavelengths. The 1000 lines/mm of the grating provides a linear dispersion of 13 nm/mm at the detector plane.

The detector is composed of a 765×510 pixel Kodak KAF-0402ME CCD, with 9 × 9 $\mu m$ pixels, housed in a Santa Barbara Instruments Group (SBIG) ST-7XME camera. This was chosen due to high quantum efficiency, $\simeq 35\%$ at 850 nm, compact
form factor, and low cost. The optical design’s “spot diagram”, essentially the PSF of the system at a given wavelength and field position is shown in Fig. 3.5 a. The reference black boxes in the figure are the dimensions of $2 \times 2$ pixels, and the reference black circle is the diffraction limit for the given $f/\#$. The four different spots are for four different field positions to verify the quality of the imaging system over the entire field. While the optical system is not quite at its diffraction limit, the complexity of the system would have had to be increased to better the performance by increasing the number of lens elements or using larger optics, both of which were not seen as being worth the minor performance gain. To compensate for the spread of the spot diagrams, the mask features are chosen to be $4 \times 4$ pixels so they can be resolved. The modulation transfer function (MTF) plot for the system is shown in Fig. 3.5 b. The MTF is a measure of contrast at different spatial frequencies in the object, and for the 4 pixel mask features, corresponding to spatial frequency of 13.8 lines/mm, the average MTF is over 0.9. Since the mask features have sharp edges, this will definitely degrade the contrast, however the optical system has adequate contrast at the primary spatial frequency of the aperture mask.

The optical train is housed in tubes and mounts on a frame which bolts onto the top of the camera as shown in Fig. 3.4 b. The entire structure is fabricated using a stereolithography machine, also known as a “3D Printer”, which builds the entire structure layer by layer. This system is fabricated in a nickel-coated ceramic in order to provide mechanical stability. All of the structures are designed in a 3D CAD software package, such as Solidworks, and then the stereolithography system translates the design files into the appropriate slices to construct the desired parts.
Figure 3.4: (a) Optical ray-trace of first generation system. The multi-element lens design images the mask plane onto the detector with a wavelength-dependent shift due to the diffraction grating. Lens shape and glass type are optimized in a software design program to achieve desired dispersion and minimize aberrations. (b) Mechanical housing of optical system. Structure is rapid prototyped on a stereo-lithography system with a nickel-coated ceramic material to assure mechanical stability. The entire optical train bolts onto front face of camera enclosure.
Figure 3.5: a-Spot diagram for first generation system showing ray-trace positions for different field points. The boxes are 18 mm on a side, corresponding to a $2 \times 2$ pixel area. b-Modulation transfer function plot at various field positions and wavelengths for first generation system.

3.3.2 Spectral Reconstruction Process

The 2D CCD intensity data from the CCD is digitally post-processed to recover a 1D average spectrum of the source. In order to be able to calibrate various parameters of the reconstruction, a source with sharp, narrow features is used such as a rare gas pen lamp. This calibration data is then saved, and used for reconstructions of unknown sources.

The first step in the data processing is to correct the smile distortion discussed in Sec. 3.2.3. The leading edge of the mask pattern for a sharp peak is fit to a 2nd order polynomial to determine the pixel shifts required to eliminate the curvature. The polynomial coefficients from a calibration sample are saved and thus the fitting is not done with every spectrum.

The corrected data is then selectively binned vertically to form a $2N$-row data matrix as shown in Fig. 3.6. This is necessary for two reasons— the mask features are usually many pixels high and the magnification is typically not exactly 1:1. The
Figure 3.6: Raw data from the detector is binned in order to downsample the data to the proper number of mask rows. For the row-doubled Hadamard cases, the complementary rows are subtracted in order to implement the negative weighting. A non-negative least squares inversion then recovers the spectrum at each of the input aperture columns. These spectral estimates are then shifted and averaged to obtain a SNR gain.
rows used for this downsampling are the CCD rows that correspond to the mask rows. The complementary rows (rows added for the row doubling process) are then subtracted from their complement to form a data matrix that has \( N \) rows, shown in Fig. 3.6. This matrix is now the discrete \( x \)-convolution of the Hadamard code with the input spectrum.

While the analysis functions discussed in Ch. 2 could now be applied, since noise is always present a least squares solution is rather pursued. The relationship between the detector data and the unknown spectrum can be mathematically expressed as

\[
\mathbf{H}_{N \times N} \mathbf{S}_{N \times M} = \mathbf{R}_{N \times M}.
\]

(3.10)

Here \( \mathbf{H} \) is the Hadamard matrix of order \( N \), \( \mathbf{S} \) is a matrix whose rows represent the input spectra at the columns of the input aperture, and \( \mathbf{R} \) is a matrix whose rows are the row-subtracted CCD data. Inversion for \( \mathbf{S} \) is then \( M \) matrix problems of size \( N \times N \). We use non-negative least-squares algorithms, since photon counts are always positive. [52] While iterative algorithms could also be applied, the least-squares methods were used due to their ease of implementation and fast results. Exposure times used for this system led to detector measurements with moderate SNR; in noisier situations these iterative techniques could be especially useful. The matrix from the least-squares inversion is shown in Fig. 3.6. The rows of \( \mathbf{S} \) have shifted spectral origins, reflecting the shifted input positions. Ideally, each adjacent estimate would be shifted exactly one horizontal mask element width, however non-unity magnification, and various other imperfections cause the shift to vary slightly. We perform a simple correlation to determine the relative shift between the rows. Using these shifts, we align the rows as shown in Fig. 3.6. The \( N \) estimates are then summed to provide a further SNR advantage. The variation in dispersion with input angle to the diffraction grating, clear from Eq. 3.2, leads to there not being
a constant pixel shift of the spectral estimates for every wavelength. This can be compensated for by calibrating each spectral estimate to absolute wavelength units, and then resampling the estimates appropriately. This process prevents a slight loss of resolution at the edges of the spectrum, and was not a significant issue for our narrow spectral range. For more broadband instruments, however, this should be always taken into account.

An important observation is that the inversion process is a series of least-squares inversions using in this case a Hadamard matrix of size $N \times N$. The number of inversions needed is the number of columns on the detector plane. For the described system, $N$ is 32, leading to very fast inversions. On a 1 GHz PC with 512 MB of memory a complete spectral inversion from 2D detector data to a 1D spectrum takes less than a second.

### 3.3.3 Throughput Experiments

In all the experiments below, the spectral source for the experiments was a xenon discharge lamp operated in conjunction with a diffuser. The light from the diffuser was allowed to fall directly on the mask aperture—no relay optics of any kind were used. Unless otherwise noted, the CCD integration time was 160 ms. The particular spectrometer has a spectral range of $\Delta \Lambda \approx 775 - 900$ nm. The spectral resolution depends on the mask, and for the majority of the masks used, was $\delta \lambda \approx 0.65$ nm. The smallest mask feature was 36 $\mu$m, corresponding to 4 pixels on the CCD. The reconstruction parameters changed with each mask, since the removal and addition of the aperture mask caused the center of the mask aperture to move slightly. Thus, the calibration parameters were determined for one of the images for each mask.

Fig. 3.7a compares the spectrum reconstructed using the mask generated from $\hat{H}_{40}$ and from a slit with a width (36 $\mu$m) equal to the feature size of the mask.
Figure 3.7: (a) Comparison between the reconstructed spectrum from a mask based on $\hat{H}_{40}$ and a slit aperture. The mask aperture clearly captures significantly more light while maintaining equivalent spectral resolution. (b) Comparison of reconstructed spectra from row-doubled Hadamard masks of various orders ($n = 40, 32, 24, 16, 12$). The system throughput increases as the mask order increases, as expected. The codes increase system throughput without affecting spectral resolution.

Figure 3.8: (a) Throughput gain achieved by order-$N$ masks compared to slits of equivalent height. Theoretically, the gain should scale as $N/2$. We observe approximately $N/4$. For reasons discussed in the text, we attribute this discrepancy to the MTF of the optical system. Note that even with this discrepancy, a mask of moderate complexity is able to increase throughput by an order of magnitude without sacrificing spectral resolution. (b) Comparison of a small spectral peak as reconstructed by a mask based on $\hat{H}_{40}$ and a slit. The SNR gain with the mask is consistent with the measured throughput gain.
Clearly, the coded aperture collects significantly more light, without sacrificing spectral resolution.

We tested row-doubled Hadamard masks, $\hat{H}_n$, of a variety of orders ($n=40, 32, 24, 16, 12$). In Fig. 3.7 b, we plot the results from the different masks. The signal strength increases as the mask order increases as we would expect. However, determining the throughput advantage is complicated by the fact that as the mask order increases, there is an increase in not only the number of openings on a given row of the mask, but also in the number of mask rows as well. To check the throughput scaling, we normalize the total counts collected for a given mask by dividing by the total counts collected with a slit that occupies an equal number of rows on the CCD. In a row-doubled Hadamard mask, there are $N/2$ openings on any row. As such, we would expect the normalized counts to also scale by this amount. The results are plotted in Fig. 3.8 a.

We see that the observed scaling is approximately $N/4$, rather than the expected $N/2$. We believe the discrepancy can be attributed to the optical system in the spectrometer. Because the reduction in light collection is a constant factor of $\approx 2$, regardless of mask size, we can rule out vignetting as the cause. Rather, we believe the effect arises from the modulation transfer function (MTF) of the optics. In the horizontal ($x$) direction, the Hadamard masks and the slit have the same range of spatial frequencies. In the vertical ($y$) direction, however, the slit contains only a DC component, while the masks contain high spatial-frequencies from the row-doubling. Experimentally, when we compare the counts on a single row of the CCD between the mask and the slit, we observe a ratio of approximately $N/4$ as measured for the entire pattern. If we instead compare the counts on a row between the mask and a square pinhole, we observe a ratio of approximately $N/2$ as theory would predict. Thus we conclude that the discrepancy is related to the MTF of the optical system.
Finally, we attempt to quantify the improvement in signal-to-noise-ratio SNR that accompanies the increase in throughput. Fig. 3.8 b shows a region of the xenon spectrum containing a very small peak (so weak that it is not visible at the scales of the previous figures). The top graph of Fig. 3.8 b shows the peak as reconstructed by the row-doubled, order-40 Hadamard mask. The bottom plot is the peak as measured by the slit aperture. If we define the SNR of the peak to be its height divided by the RMS value of the region near the peak, we find that the SNR for the mask aperture is \( \approx 23.7 \) while the SNR for the slit is \( \approx 7.0 \). This is an SNR gain of \( 23.7/7.0 \approx 3.4 \).

From Fig. 3.8 b, we see that the mask provided a throughput advantage of \( \approx 10.3 \). For a shot-noise process we would expect this throughput gain to result in an SNR gain of \( \sqrt{10.3} \approx 3.2 \), which is indeed close to the observed value.

The ability to dramatically increase the throughput of the system clearly demonstrates the Jacquinot advantage of the system. The presence of the Fellgett advantage is also easily deduced. By having multiple openings on a row of the input mask, any detector pixel sees a combination of spectral channels. As such, the signal level on the pixel is increased over what it would be in the absence of multiplexing. Since the additive detector noise remains constant, the SNR on the pixel must increase. Thus our system also exhibits the Fellgett advantage.

It must be noted, however, that for sparse signals, such as the pen lamp peaks used, the increased throughput leads to an SNR increase as also shown by Mende in their implementation of a static coded-aperture spectrograph. [42] For continuum sources, however, the approach should lead to no SNR advantage for quantum noise (shot noise) limited systems due to the increased noise resulting from the increased throughput. With more sparse signals, the increased shot noise is distributed throughout the spectrum, while the signal is increased on the peaks, leading to an overall SNR gain. For Raman spectroscopy applications the spectra are not contin-
uum sources, the signals are a series of spikes and thus MMS systems are a good choice. With signal-independent noise, however, the MMS approach should lead to an additional SNR gain due to the increased signal on the pixels. For our CCD system, this was difficult to ascertain due to the low noise levels in the camera system. In other wavelength regions where detector noise is more of an issue, such as the mid-wave infrared or long-wave infrared, the multiplex advantage might be more apparent.
Chapter 4

Raman MMS Design and Performance

In this chapter we describe an MMS Raman system for chemometrics of diffuse samples. While the spectrograph portion of the system is similar to the one in Ch. 3, some improvements to the coding mask implementation and the mechanical structure will be discussed. One important design criteria for the system is the ability to use multiple excitation lasers, however, this will be discussed more in detail in a following chapter. All of the system results discussed in this chapter will be for only using one excitation laser. Chemometric performance for ethanol measurement in a tissue phantom will be analyzed.

4.1 Optical Design

For the described system, excitation at 808 nm provides low background autofluorescence and puts the chemical fingerprint region of Raman features (500-2000 cm$^{-1}$) at $\simeq$ 840-960 nm, within the high quantum efficiency range of our CCD. The illumination portion delivers light to the sample cuvettes, and relays the Raman scattered light to the spectrometer. The spectrometer portion consists of an MMS spectrograph using a row-doubled Hadamard code and a cooled CCD.

4.1.1 Excitation and collection system

The optical layout of the system is shown in Fig. 4.1. The excitation source is a butterfly packaged, fiber-coupled, single longitudinal- and transverse-mode semiconductor diode laser (QPhotonics QFLD-808-100S) operating at 808 nm with 60 mW incident on the sample. The butterfly package is current- and temperature-regulated,
Figure 4.1: The schematic of the Raman MMS system. The excitation laser light passes through the band-pass filter (BPF), is then collimated by a lens (L1), transmitted through the dichroic filter (DF), then focused on to the sample by a lens (L2). The diffuse source (DS) is then imaged onto the aperture mask (AM) by 2 lenses (L2,L3) and the reflection by the dichroic filter (DF). The cylindrical lens (CL) Fourier transforms the source vertically to achieve uniform illumination across the mask columns. The aperture mask then serves as the input to the spectrograph, consisting of two lenses (L4,L5) which image the aperture mask onto the focal plane array (FPA) after passing through a long-pass filter (LPF) to reduce the Rayleigh scattered excitation light and the volume phase holographic grating (VPH) to disperse the source along the rows of the focal plane array.

but there is no external stabilization of the laser wavelength, resulting in a linewidth of ≈0.3 nm.

The output of the laser is collimated with a 10 mm focal length, aspheric, plastic lens and passes through a thin-film bandpass filter (Omega Optical custom: transmits 800-810 nm otherwise optical density of 5) to reject out-of-band spontaneous emission. The relay section of the system provides two functions—delivering the excitation laser light to the sample, and relaying the scattered light from the sample to
the aperture mask. The excitation light passes through a rectangular dichroic filter (Omega Optical custom: transmits 800-810 nm, reflects 850-950 nm in a 45° geometry), and is focused onto the sample by an \( f/0.8 \) equivalent lens group to a spot of \( \approx 200 \mu m \). This is shown in Fig. 4.3 ba. In Fig. 4.3 b the scattered light from the source is collimated by the \( f/0.8 \) lens group, reflected off the dichroic filter and imaged onto the aperture mask by an \( f/2 \) lens group. The lenses provide a 60° collection full-angle at the sample, and then magnify the source \( \approx 2\times \) onto the mask. This matches the angular extent of the source to the spectrograph (\( f/2 \) or 30° collection full-angle). The lenses are all 50 mm diameter and anti-reflection (AR) coated at 800-950 nm to increase the throughput. The \( f/0.8 \) collection lens group are all made of UV grade fused silica glass to reduce the autofluorescence that is prevalent in most glasses.
Figure 4.3: (a) Ray-trace of excitation laser path. The laser wavelength, 808 nm, passes through the dichroic filter in the 45 degree geometry. A short focal length lens collimates the laser beam, and the collection lenses focus and center the beam onto the sample. (b) Multi-element lenses image the scattered light from the sample onto the input aperture of the spectrometer. The dichroic filter reflects light at wavelengths >820 nm. The source is magnified $\approx 2.4$ by the lens system.

A 100 mm focal length cylindrical lens is placed between the final relay lens and the aperture mask in order to Fourier transform the field in the $y$-direction to improve spatial uniformity. Since the aperture code assumes uniform spectra along the columns, a much better reconstruction fidelity is seen with such an anamorphic setup for weakly scattering samples. For highly scattering samples there is a negligible effect on the fidelity, and the cylindrical lens is removed for highly scattering samples.

4.1.2 MMS spectrograph design

The patterned mask, described later, then forms the input aperture of an axial transmissive Raman spectrograph with a center wavelength of 900 nm. The design goal of the spectrograph was to have high throughput, moderate spectral resolution, and sufficient image quality to easily resolve the mask features. A transmissive volume
phase holographic grating (Wasatch Photonics) with 1000 line pairs per millimeter provides the dispersion. Combining the grating with two 75 mm focal length \(f/1.4\) lenses (Pentax B7514C), a 36 mm diameter aperture stop at the grating, and the 54 \(\mu m\) minimum mask feature size, results in an overall \(f/2.1\) system with \(\approx 0.6\) nm over the 4.30 mm \(\times\) 1.73 mm input aperture. The spectral range of interest, 850-950 nm, under-fills the detector plane in the dispersion direction in order that every spectral estimate is fully sampled by the detector plane.

In the process of assembling MMS systems it became clear that the alignment of the mask, grating, and CCD detector is very critical. In order for the mask registration on the detector to be within a pixel for all wavelengths, the CCD rows, the grating dispersion, and the mask rows need to be all within a small tolerance. The impact of the different misalignments are shown in Fig. 4.4. Since the different wavelength channels are mixed on the detector plane, a software correction would be very difficult to implement. This led to a careful design of the mask and grating mounts, shown in Fig. 4.5 to allow for fine adjustability of the mask tilt, grating tilt, and mask position.

In the spectrograph a long-pass-filter (Omega Optical), 850-1000 nm pass-band with optical density of 6 at the laser wavelength, provides rejection of the Rayleigh scattered laser light. The focal plane detector is a deep-depletion back-illuminated CCD array (Andor Technologies DU440-BR-DD), with a 2048 \(\times\) 512 array of 13.5 \(\mu m\) square pixels. The high-resolution CCD allows for higher-order masks to be used in the system. The camera is operated at \(-60^\circ C\) to decrease the high dark current that is common with deep depletion CCDs. No water cooling or liquid nitrogen is required to keep the CCD stabilized at this temperature. The dark current at this temperature is \(\approx 1\) e\(^{-}\)/pix/s, however the signal counts per pixel from sample autofluorescence are at least two orders of magnitude higher.
Figure 4.4: (a) Image of MMS detector when mask is tilted with respect to the CCD detector and the direction of dispersion. (b) Image of MMS detector when grating dispersion is tilted with respect to the CCD detector and the mask.

Figure 4.5: (a) CAD rendering of rotatable mount for transmission grating allowing for the dispersion axis to be aligned with the detector plane and aperture mask. Part was fabricated on a stereolithography system. (b) CAD rendering of adjustable mask mount for Raman MMS system. Part was custom fabricated in aluminum for stability. Mask is rotatable and height adjustable by two screws to allow the mask image to be centered on the detector plane with no tilt.
4.1.3 MMS mask

For this instrument we used the row-doubled Hadamard codes described in Chapter 2. The aperture mask is lithographically patterned chrome on quartz (Applied Image Group), allowing for > 90% transmission and a blocking optical density of ≈ 4. An \( N = 32 \) row-doubled Hadamard code with 54 \( \mu \)m square features allows for a system resolution of \( \simeq 0.6 \) nm. A scale replica of the code is shown in Fig. 4.6. The rows and columns of the row-doubled Hadamard code are randomly permuted to reduce spurious correlations observed in earlier experiments. There is a 4:1 ratio between the mask feature size to CCD pixel size. This avoids the need for sub-pixel positioning accuracy of the mask. Completely opaque rows of 1 CCD pixel height are placed between each row in the code. These *dead rows* reduce crosstalk between adjacent codes of the mask, resulting from the the PSF of the optical system. The height of the mask is thus \((2N \times 4 + 2N - 1) \times 13.5 \mu \)m = 4.30 mm. The width of the mask is simply \( N \times 4 \times 13.5 \mu \)m = 1.73 mm. Since the row-doubled Hadamard codes average half open, and the dead rows reduce the average vertical throughput by 20%, the total open area is 2.98 mm\(^2\). To achieve a similar collection area in a slit-based spectrometer that uses a fiber to remap the source would require a slit height of 55 mm, if the resolution of the two systems were equal. Clearly this is significantly larger than typical detector arrays, and as such, only a coded-aperture system could achieve a collection area of this size.

### 4.2 Performance characteristics

The processing of the detector images into spectra is similar to that in Sec. 3.3.2. A xenon pen lamp is used to calibrate the parameters of the reconstruction, and then these parameters are saved to reconstruct unknown samples.
4.2.1 Resolution of system

The resolution of the instrument was analyzed to first determine that the mask provided the expected resolution and second that the overall Raman MMS system’s resolution was not broader than the linewidth of the chemical used later for quantitative analysis, ethanol. The instrument response of a Raman system is a combination of the resolution of the bare spectrometer combined with the linewidth of the excitation laser. To determine the resolution of the bare spectrometer, we measure the spectrum of an Argon discharge lamp. The raw CCD image and processed spectrum for the lamp are shown in Figure 4.7. Fitting a Lorentzian to the strongest peak of the spectrum allows us to determine a FWHM of 4.00 pixels, which corresponds to $\simeq 0.58$ nm. Since the width of the mask features is 4 CCD pixels, we see that we are indeed resolution limited by the mask feature size. Thus, we have increased our throughput without sacrificing resolution.
Figure 4.7: (A) CCD image of an Argon emission spectrum (1 s exposure). (B) Reconstructed emission spectrum of Argon.
To determine the full instrument response, we then use the instrument to measure the Raman spectrum of cyclohexane. Both the CCD image and reconstructed Raman spectrum are shown in Fig. 4.8. Cyclohexane has an extremely narrow Raman line—so narrow that it surely can not be resolved by our instrument. The measured linewidth, then, is approximately the full instrument response. Fitting a Lorentzian to the narrowest peak of the cyclohexane spectrum allows us to determine a FWHM of 5.26 pixels, which corresponds to $\approx 0.89$ nm. This results in a wave-number resolution that varies from $12.8 \text{ cm}^{-1}$ at $500 \text{ cm}^{-1}$ to $9.8 \text{ cm}^{-1}$ at $2000 \text{ cm}^{-1}$. Therefore we see the that the full instrument response is broadened as a result of the linewidth of the excitation laser.

The final question is how the full instrument response compares to the linewidth of ethanol. Measuring the Raman spectrum of ethanol, we find a FWHM of 9.03 pixels. As this is less than double the Raman linewidth of the instrument, we conclude that the linewidth of ethanol is comparable to the instrument response of the MMS Raman spectrometer.

### 4.2.2 Concentration Estimation Experiment

In order to explore the use of MMS Raman spectroscopy as a non-invasive molecular estimation tool, ethanol concentration estimation in a diffuse, fluorescent medium was performed. A phantom of Intralipid was designed to have scattering properties similar to that of tissue. [18] In addition to its scattering properties, it has a significant amount of autofluorescence as well as Raman features that overlap with ethanol. Consequently, it is an excellent model for some of the challenges faced in real tissue.

Our goal is to use the system to estimate the ethanol concentration in the Intralipid phantom. Cuvettes containing varying concentrations of ethanol by weight ($0.008\% = 1.7 \text{ mmol/L}$, 0.016%, 0.04%, 0.08%, 0.16%, 0.4%, 0.8%) are prepared, and
Figure 4.8: (A) CCD image of Raman spectrum of cyclohexane (10 s exposure at 60 mW excitation power). (B) Reconstructed Raman spectrum of cyclohexane.
then Raman spectra of are measured with the instrument. Exposure times of four minutes provided a high SNR, and 6 trials are performed with each sample. Data collection is automated with a Labview software program, and then the CCD images are processed into spectra by our custom codes written in Matlab. This procedure is repeated again with new samples three days later.

4.2.3 Regression Model and Cross-Validation

Analysis of the spectra begins with simple pre-processing. The spectra are normalized to a unit sum, since the primary cause of fluctuation in the signal is autofluorescence, which should be proportional to the laser power. The spectrum are then zero-meaned to improve the modeling performance. The spectra from day 1 (before and after preprocessing) are shown in Fig. 4.9.

Analysis of the data proceeds by constructing a PLS model of a training data set and then using that model to predict the concentrations in a test data set. The goal of the model construction is to find a regression vector \( \mathbf{w} \) such that \( \mathbf{S} \cdot \mathbf{w} = c \), where \( c \) is the concentration of the sample with spectrum \( \mathbf{S} \). [53] A sample regression vector as well as the Raman spectrum of pure ethanol is shown in Fig. 4.10. The method is clearly selecting wavelengths corresponding to the Raman peaks of ethanol as the primary contributors to the regression vector. The performance of the model is initially tested via leave-one-out cross-validation. In this method, a data point is selected from the data set, and a model is built from all of the remaining data points. This model is then used to estimate the concentration of the left-out data point. This process is repeated for all points in the data set.

More rigorous performance testing uses blind cross-validation. In this method a set of data points is used to form a model, and this model is then used to estimate the concentrations of a completely separate data set. In all cases, excellent performance is
Figure 4.9: (A) Raw, reconstructed Raman spectra of varying ethanol concentrations in Intralipid phantom. (B) Spectra after normalization and zero-mean scaling.
achieved with regression vectors formed from between 6 and 8 principal components, indicating that the algorithm is not over-fitting to noise. Examples of results from leave-one-out and blind cross-validation are shown in Fig. 4.11. Perfect performance of the model would have the data points falling exactly on the diagonal line. We see that leave-one-out cross-validation leads to a broader spread of the data points, while blind cross-validation leads to an offset of the data points from the target line (which could potentially be corrected with a more complicated model).

For each analysis method, we compute the traditional metrics of $r^2$ and RMSE, as well as several relative error statistics which we feel are more accurate predictors of detection limits. The RMSE is defined as

$$\text{RMSE} = \left[ \frac{1}{k} \sum (c_{\text{est}} - c_{\text{act}})^2 \right]^{1/2},$$

(4.1)

where $c_{\text{est}}$ is the estimated concentration, $c_{\text{act}}$ is the actual concentration, and $k$ is
Figure 4.11: (A) Leave-one-out cross-validation data for day 1. (B) Blind cross-validation for day 2, using regression vector from day 1. Dashed lines indicate one common legal intoxication limit (17.4 mmol/L=0.08% wt.).
the number of samples. The relative error (RE) for a given data point is defined as

\[
RE = \frac{|c_{est} - c_{act}|}{c_{act}}. 
\]  

(4.2)

We then compute the mean and standard deviation of the RE at each concentration, as well as the mean RE (MRE) for concentrations between 8 and 173.9 mmol/L. A summary of all the results for various combinations training and test data sets are shown in Table I. A plot of the mean RE with error bars at ±1 standard deviation for leave-one-out and blind cross-validation are shown in Fig. 4.12. Data points for concentrations below 8.7 mmol/L were suppressed on the plots due to their large relative errors. Depending on the amount of relative error one is willing to accept, we can determine a detection threshold from these plots. Making the arbitrary choice of 20% error as our threshold, we see that the system has a detection limit of between 8.7 and 17.4 mmol/L, corresponding to a concentration of between 0.04 and 0.08 % by weight. For the blind cross-validation, the error is primarily from the offset observed in Fig. 4.11. Although the error is extremely large at the lowest concentration, the data points are still clustered in such a way to make them distinguishable from the higher concentrations. This is reflected in the high \( r^2 \) value.

To further check the consistency of the models, we performed two more analyses. First, we combined the data from both days into a single data set and performed leave-one-out cross-validation. The results were comparable to leave-one-out cross-validation from either of the single days, and required a comparable number of principal components in model formation. Second, we randomly segmented the data from both days into a training data set and test data set and used blind cross-validation. This showed improvement over blind cross-validation from either single day, and demonstrates the further improvement possible when the algorithm is exposed to all forms of system variation.
Figure 4.12: (A) Leave-one-out cross-validation relative errors. (B) Blind cross-validation relative errors.
Table I: Performance statistics for data models formed with PLS regression. The first column identifies the data sets used for model generation and testing. When the two are identical, evaluation is by leave-one-out cross-validation, otherwise blind cross-validation was used. In the last row, a random set comprising half of the data from both days was used for model generation, and the remaining data was used for evaluation. The second column indicates the number of principal components used in constructing the model. The third column indicates the $r^2$ correlation of the predicted values. The remaining columns show the RMSE and MRE for the full concentration range as well as the RE at two particular concentrations.

<table>
<thead>
<tr>
<th>Train/Test</th>
<th>N</th>
<th>$r^2$</th>
<th>RMSE</th>
<th>MRE (%)</th>
<th>RE (%)</th>
<th>RE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dataset mmol/L 8-174 mmol/L @8.7 mmol/L @17.4 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/1</td>
<td>8</td>
<td>.99</td>
<td>2.87</td>
<td>11.1 ± 22.0</td>
<td>31.8 ± 43.7</td>
<td>14.8 ± 9.6</td>
</tr>
<tr>
<td>1/2</td>
<td>8</td>
<td>.99</td>
<td>6.96</td>
<td>15.3 ± 15.2</td>
<td>33.9 ± 26.5</td>
<td>11.1 ± 4.6</td>
</tr>
<tr>
<td>2/2</td>
<td>6</td>
<td>.99</td>
<td>2.64</td>
<td>9.4 ± 10.1</td>
<td>22.5 ± 9.86</td>
<td>12.6 ± 8.9</td>
</tr>
<tr>
<td>2/1</td>
<td>6</td>
<td>.98</td>
<td>15.20</td>
<td>63.4 ± 58.3</td>
<td>114.1 ± 41.0</td>
<td>139.8 ± 6.2</td>
</tr>
<tr>
<td>1+2/1+2</td>
<td>8</td>
<td>.99</td>
<td>3.62</td>
<td>12.3 ± 31.3</td>
<td>40.8 ± 63.3</td>
<td>10.4 ± 6.7</td>
</tr>
<tr>
<td>Rand./Comp.</td>
<td>8</td>
<td>.99</td>
<td>3.55</td>
<td>9.2 ± 9.8</td>
<td>21.0 ± 12.2</td>
<td>12.4 ± 9.6</td>
</tr>
</tbody>
</table>
Chapter 5

Coded-excitation Raman spectroscopy

The MMS systems described earlier provide for a high fidelity measurement of the Raman spectrum of a given sample. One issue encountered, however, is the background fluorescence of most samples. Frequently, large exposure times are required, in order to decrease the effects of the shot noise of this background on the weak Raman peaks that are at same wavelength channels. While NIR excitation reduces this fluorescence significantly from UV or visible excitation, many biological substances such as collagen exhibit a strong fluorescent background compared to the Raman signal of most molecules.

In this chapter, we will describe a method filtering out the Raman signal from the fluorescent background through the use of multiple excitation lasers and an iterative algorithm. While the idea of using more than one excitation wavelength has been done previously, our approach is different due to the use of more than two excitations and the use of a statistical-based reconstruction algorithm.

5.1 Previous work on Raman signal filtering

Various techniques have been developed to isolate the Raman features that involve both system design and data post-processing. [54–60] Time-gating takes advantage of the near-instantaneous response of Raman scattering and the longer lifetimes of fluorescence by employing pulse-gating [54] and optical Kerr-gating. [57] While effective, time-gating at fast enough intervals to avoid fluorescence from molecules with shorter lifetimes creates a difficult and costly experimental setup.

The narrow features of Raman lines compared to the broad fluorescent background
can also be used to digitally process spectra after being collected. Through FFT-filtering and edge detection [56] spectra can be high-pass filtered to isolate the Raman features, however this can lead to artifacts and distortions of the spectrum, especially in noisy situations. A polynomial fit of the background is a standard technique which allows for minimal distortion of the Raman spectrum [59] and is straight-forward to implement, but typically adds some smoothing operations to reduce high frequency noise in low signal conditions.

The shift-nature of the Raman effect allows another mechanism for removing the fluorescent background. By Kasha’s rule [61], small changes in excitation wavelength will have almost no effect on the fluorescence emission. For Raman spectra, however, the entire spectrum will shift in energy by the amount of excitation shift. This property has been used by many groups under the terms of “Shifted Excitation Raman Difference Spectroscopy” (SERDS). [55,58,60] In these implementations, two excitations of a sample at slightly different wavelengths are acquired, and then these are subtracted and then processed to estimate the Raman spectrum of the sample. Curve-fitting [55] and linear deconvolution [58,60] are the main approaches in the signal processing of the difference spectra.

5.2 Multi-wavelength excitation for Raman signal filtering

We are investigating an extension of SERDS with two new approaches— the use of more than two excitation frequencies and Raman signal estimation using an expectation-maximization (EM) algorithm. The use of more excitation frequencies allows for more complex excitation patterns which in our simulations has shown to increase the performance of the signal estimation. The multiple frequencies could come from either a single tunable laser, or a bank of lasers at slightly spaced wavelengths. Our current sys-
tem uses eight separate lasers in order that excitations with multiple laser frequencies could be investigated as well. In order to incorporate data from multiple excitations, a more general solution technique has been developed. Expectation-maximization algorithms, originally used to super-resolve and denoise images in astronomy [62,63] and medical imaging [64], have been successful in tackling other inverse problems in spectroscopy [65,66]. Coupled with efficient regularization techniques [67], the EM algorithm provides a powerful framework for solving Poisson inverse problems in the presence of noise. We show that for low signal conditions, the EM algorithm is more effective than background subtraction techniques at extracting the Raman spectrum from a sample with a highly fluorescent dye. The efficacy of the algorithm increases with the number of excitation frequencies used, even when holding the total exposure energy deposited the same showing the advantage of more than two lasers.

5.2.1 Signal Model

Raman scattering is an inelastic photon scattering event which transfers some of the energy from an arriving photon to a rotational/vibrational mode of a molecule. The scattered photon thus experiences an energy shift, the Raman shift, making the process shift-variant with respect to the excitation frequency. Frequency, $\nu$, will be used to describe the photon energy since it is directly proportional to energy. The total Raman signal measured, $S_R$, is

$$S_R = \int h_R(\nu - \nu')S_e(\nu')d\nu'. \tag{5.1}$$

where $\nu$ is the measured optical frequency, $\nu'$ is the excitation frequency, $S_e$ is the spectrum of the excitation laser in optical frequency, and $h_R$ is the Raman spectral impulse response. In fluorescent media, however, there also exists a non-Raman
impulse response which for small shifts in excitation frequency can be approximated by an impulse response $h_{NR}$ which has no dependence on excitation frequency $\nu'$. The measured non-Raman signal can be approximated as

$$S_{NR} \approx \int h_{NR}(\nu)S_e(\nu')d\nu'.$$

(5.2)

With only one measured spectrum the shift nature of the Raman signal can not be exploited; however, by making multiple measurements with different excitation signals the problem of Raman signal estimation can be solved. In SERDS systems, two measurements at slightly spaced wavelengths are taken, thus forming a pseudo-derivative of the Raman signal. This pseudo-derivative is then used to estimate Raman signal by using curve-fitting, Fourier deconvolution, or discrete integration. These techniques are limited, however, since the curve-fitting is limited to simple spectra, the deconvolution is poorly conditioned and can amplify noise, and discrete integration leads to a broadening of the Raman features and a sloping baseline. The previously stated techniques are also limited to only two excitation frequencies. Also, more robust techniques are required for noisy situations, since reduced exposure time and laser power could be useful in many applications. To estimate the Raman spectrum given multiple excitation frequencies, the problem will first be formulated in matrix form. The measurements $y$ are the recorded spectra, each of length $N$, for each of the $K$ excitations,

$$y \equiv [y_1^T, y_2^T \ldots y_K^T]^T.$$  \hspace{1cm} (5.3)

The Raman and non-Raman signal are defined as $S \equiv [S_{NR}^T S_R^T]$, with length $2N$, leading to a complete description of the problem as an inverse problem in the form
of

\[ y \sim \text{Poisson}(HS), \quad (5.4) \]

where \( H \) is a matrix of size \( KN \times 2N \). \( H \) can be formed with knowledge of the excitation spectrum of each laser used and the form of the impulse responses \( h_R \) and \( h_{NR} \) described earlier. In discrete form, the impulse responses become an identity matrix for \( H_N \) and an off-diagonal identity matrix \( H_{Rk} \) with the amount of off-diagonal corresponding to the relative shift of laser \( k \). The total operator \( H \) then becomes

\[
H = \begin{bmatrix}
H_{NR} & H_{R1} \\
\vdots & \vdots \\
H_{NR} & H_{Rk}
\end{bmatrix} \quad (5.5)
\]

Since the uncertainty in the measurements are typically dominated by photon noise due to the strong nature of the fluorescence, the task of estimating the Raman signal \( S_R \) is a Poisson inverse problem. A modified expectation-maximization (EM) algorithm with maximum penalized likelihood estimation is used for signal estimation. [68] The estimate for \( S \), defined as \( \hat{S} \), is iterated upon by a Lucy-Richardson formula [62,63],

\[
\hat{S}(t + 1) = \hat{S}(t) \times (H^T(y \cdot / (H\hat{S}(t)))) \quad (5.6)
\]

where \( t \) is the iteration number and the dots indicate element-wise multiplication and division. A multiscale Poisson denoising shown to be effective in noisy Poisson estimation problems can be applied separately to the Raman and non-Raman com-
ponents of $S$ at each iteration. The described technique is completely general in regards to the excitation spectra of the lasers used. As a starting point for analysis, the scheme chosen is to collect spectra at slightly shifted excitation frequencies with one laser per excitation.

5.3 Experimental setup for multi-wavelength Raman experiments

In the course of the thesis research, two multi-wavelength experimental setups were constructed. Both used 8 fiber-coupled lasers as their light sources, however the excitation and collection geometries and optics were different. The first setup worked well, however improvements that became apparent during its use predicated the design and use of the second system. Experimental results from both setups will be discussed later.

5.3.1 Dichroic filter-based multi-wavelength system

The optical setup described in Sec. 4.1.1 was the first approach implemented. Eight excitation lasers (manufactured by QPhotonics), at wavelengths from $\approx 800\text{-}808\text{ nm}$ are tunable by temperature and current to provide a uniform wavelength spacing. The lasers are semiconductor diode lasers mounted in 14-pin industry-standard butterfly packages, and are housed in a Newport Model 708 8-Channel Laser Diode Telcom Mount as shown in Fig. 5.1. The fibers from each laser enter into a fiber-coupled mechanical shutter which allows each laser to be modulated on and off while still keeping the diode active. The shutters are computer controlled via a digital input/output board in a computer allowing for synchronization between the laser switching and the camera exposures. A laser diode controller (Newport Model 8008) provides the thermo-electric cooler (TEC) control and drive current control. The
Lasers are temperature and current controlled to stabilize their output wavelength. Temperatures are tuned to 20-35°C to space the laser wavelengths ≃1 nm apart.

A fiber then connects the output of the shutters to a ring of fiber connectors shown in Fig. 5.1 b. The output of each laser passes through a bandpass filter and collimating lens as described in Sec. 4.1.1. The eight beams are all then focused to the center of the optical axis of the lens system by the group of lenses near the sample. The shutters ensure that only one of the excitation beams is present on the sample.

5.3.2 Fiber-bundle based multi-wavelength system

While the dichroic-filter based system was able to acquire Raman spectra at multiple wavelengths, certain shortcomings of the system were identified and then a new design was implemented accordingly.

One issue was the insertion of loss of the fiber-optic shutter and the dichroic filter combined with the maximum 150 mW of output power from each excitation laser,
which resulted in only 30 mW of power incident on the sample. To remedy this, the laser system was replaced with 8 wavelength stabilized diode lasers with center wavelengths of 782.6, 784.1, 784.4, 786.8, 788.6, 790.7, 793.6 and 794.3 nm (Innovative Photonic Solutions). This choice of wavelengths required the custom manufacturing of only 3 lasers. The stabilization of each laser’s wavelength is provided by an integrated volume-phase holographic grating which results in a narrow linewidth, $< 0.2$ nm, and high power operation, $> 300$ mW per laser. [69] The external stabilization allows for the elimination of any warm-up time that was necessary previously. Since the lasers could be activated in times less than one second, the shutters are able to be eliminated. Combined with the higher output of the lasers, an excitation power of 150 mw is possible with each excitation laser, a $5 \times$ improvement.

The alignment of the ring of lasers was another shortcoming of the initial design. Each laser needed to have its excitation spot centered to micron-level resolution, since different excitation profiles would create a distortion of the spectra. The thin-film laser rejection filters are very angularly sensitive in terms of their wavelength transmission profiles, and this seemed to be one cause of the spectral distortions. In addition, the dichroic filter emitted an autofluorescence which was at the level of the Raman signals emitted from some of the samples analyzed. Since this autofluorescence came from different portions of the dichroic filter, it created another varying background between the different excitation lasers.

To simplify the excitation system, a custom fiber bundle (RoMack Fiber Optics) composed of eight 100 µm core fibers was used. On one end of the bundle the fibers are closely packed into one SMA connector, and on the other end the fibers fan out into eight FC connectors for the laser sources. The closely packed end then attaches via a SMA connector to a 400 µm core fiber, sized appropriately to allow virtually all the light from the eight fibers in the bundle. This larger diameter fiber is $\simeq 30$ m
Figure 5.2: (a) Excitation and collection design for fiber-bundle based multi-wavelength Raman system. Output fibers from the 8 excitation lasers are tightly packed in a bundle, then coupled into a large core fiber. The mode-mixer (MM) randomizes the light distribution to provide the same excitation profile for each laser. Beam is collimated by lens L1, short-pass filtered by filter F1, then reflects off mirror M1 which is epoxied on a glass window to steer the beam towards the sample. Lens group L2 focuses the excitation beam onto the sample and along with lens group L3 images the source onto the input aperture of the spectrometer. Filter F2 rejects the Rayleigh scattered light from the laser. (b) CAD rendering of system in a. Thorlabs cage and tube mounts made of anodized aluminum form the basis of the structure. Adjustments on the fiber mounting plate and the window-mounted prism allow for precise adjustments. The exploded view is for illustrative purposes only.
long, and wound into a tight loop in order to randomize the distribution of light from all the different excitation lasers. This “mode mixer” has an output SMA connector to couple the uniform excitation light onto a 400 μm core fiber to the sample. A diagram of the setup is shown in Fig. 5.2. Alignment now becomes a much simpler task since all of the excitation lasers can be aimed with the use of a single adjustment. The dichroic filter can be replaced with a small mirror to steer the excitation light towards the sample and provide a minimal obscuration to the collected light of the spectrometer. This has the added advantage of diverting the Fresnel reflections from the laser beam away from the collection optics of the spectrometer.

While the lens groups for relaying the scattered light from the sample to the spectrometer are the same as described in Sec. 4.1.1, the collimating lens for the laser needed to be changed due to the larger core area of the excitation fiber. Since the reflecting prism needs to be as small as possible to avoid obscuring the spectrometer’s collection area, a short focal length lens would seem to be the best choice. The large area of the fiber, however, will make the off-axis rays enlarge the extent of the beam with a very short focal length. As a compromise, with a 16 mm focal length, 8 mm diameter achromatic doublet, the extent of the beam was able to be reflected by a \( [10 \times 10 \text{mm}] \) prism reflector, as shown in Fig. 5.3. Since the collection optics are 50 mm in diameter, only 5% of the collected is obscured. A short-pass filter is inserted after the collimating lens to reduce any overlapping emission generated by the optical fiber. In order to compensate for the reduction in laser filtering by removing the dichroic filter, an addition thin-film filter is used before the back half of the relay optics.

### 5.4 Data Analysis

The data processing of both experimental setups is identical. To collect data for a given sample, each excitation is turned on in sequence and CCD images are captured
Figure 5.3: Ray-trace of prism-mirror excitation design. The hypotenuse of a right angle prism reflects the excitation beam towards the sample. The prism’s cross section is $10\times10\text{mm}^2$ in reference to the collected light, creating a 5% obscuration of the 50 mm diameter collection optics.

accordingly. These images are turned into spectra by the processing methods described in Sec. 3.3.2. From a multi-wavelength processing perspective, the method of generating the spectra is not important. Thus, the analysis shown is applicable to slit-based spectrometers, or any other device that can capture the spectrum of a sample at multiple excitation wavelengths.

5.4.1 System Forward Model Formation

In order to implement the EM algorithm, the $H$ and $H^T$ operators of Sec. 5.2.1 need to be determined from a calibration of the spectrometer and excitation lasers. An important detail of the spectrometer is that the channel spacing is non-uniform in energy units, so the matrix definitions used earlier need to be modified. In order to simplify the process, functions using interpolations are chosen instead of matrices. The other driving factor for developing the functions is to have a forward model that allows for direct comparison of the calculated estimate and the measured data. While a pre-processing step of interpolating the measured data on a spacing which is uniform in wave-number would allow for simple shifts to implement the operators,
this would alter the statistics of the observations.

Each measured spectrum $y_k$ contains $N$ channels, each corresponding to a wave-number value which are non-uniformly spaced in $N$. This relationship can be determined by recording the spectrum of a known source. The peaks in the measured spectrum of an argon discharge lamp are fit to the wave-number values of the emission lines with a second-order polynomial. This polynomial is evaluated at each channel providing a vector containing the wave-number value of each channel, $w(n)$. In order to determine the wave-number value of each laser, a sample of naphthalene is measured with each excitation laser, and its strong peak at 1382.2 cm$^{-1}$ can be used to determine the shift of each laser, as shown in Fig. 5.4. The peak channel in each spectrum, $p_k$ for $k = 1..K$ number of lasers, can then be used to determine each laser frequency in wave-number, $l_k = w(p_k) - 1382.2$.

The Raman signal estimate, $S_R$, will then be referenced to the spectrum from the first excitation laser. The relative shift of each laser compared to the first is then, $\Delta(k) = l_k - l_1$, allowing for the Raman signal for the rest of the lasers to be generated by shifting them $\Delta(k)$. A spline interpolation is used to perform the shift. With this

**Figure 5.4**: Naphthalene spectra collected with each excitation laser
calibration information, the forward function $H$ can be written as,

$$H(S_{NR}, S_R) = [S_{NR} + S_{NR}^w, S_{NR} + S_{NR}^w(w(n) + \Delta_2), \cdots, S_{NR} + S_{NR}^w(w(n) + \Delta_K)]$$ (5.7)

where $S_{NR}^w$ is the $S_R$ signal in wave-number coordinates,

$$S_{NR}^w(w(n)) = S_R(n).$$ (5.8)

Thus, the $S_R$ and $S_{NR}$ signals have the same channel spacing and number of channels as each of the original measurements. By writing out the idealized $H^T$ matrix, it is clear that similar functions can be used to implement an $H^T$ matrix. The “unshifted” spectra become

$$z_k = y_k^w(w(n) - \Delta_k),$$ (5.9)

with an example of the values of $z$ for the naphthalene spectra shown in Fig. 5.5. The “Raman Shift” axis is simply the difference between the wave-number axis $w(n)$ and the first laser’s frequency in wave-number, $l_1$. This shifting in wave-number is again carried out by a spline interpolation. The transpose function can then be written as

$$H^T(y_1, \cdots, y_K) = \left[ \frac{1}{K} \sum_{k=1}^K y_{kn}, \frac{1}{K} \sum_{k=1}^K z_{kn} \right],$$ (5.10)

which performs an averaging of $y_K$ for the $S_{NR}$ correction term and an averaging of the “unshifted” $y_K$ for the $S_R$ correction term. By saving the parameters for the
Figure 5.5: Unshifted naphthalene spectra showing the effectiveness of the calibration to align the Raman signals from all the excitation laser.

interpolations in the $H$ and $H^T$ operators, an unknown sample can be processed without any need of further calibration.

5.5 Experimental results

To test out the ability of the multi-wavelength Raman system to extract Raman features from unknown samples, a variety of experiments have been undertaken. Both experimental setups— the dichroic-filter based one, and the fiber-bundle based one, were used. Thus, these results will report on data from both. To compare the EM algorithm to the conventional linear processing techniques, a fluorescent dye dissolved in ethanol was used since the Raman spectrum of ethanol is well known. Multi-wavelength chemometric analysis was also attempted, similar to that of Sec. 4.2.3 except using the output of the EM algorithm instead of the spectra acquired from one laser. Finally, to see if the multi-wavelength system could recover Raman features of ethanol in real tissue, a simple animal experiment with a rat was undertaken.
5.5.1 Multi-wavelength Raman analysis of fluorescent dye

To investigate the performance of the EM algorithm, a trace amount of the laser dye HITC perchlorate was dissolved in pure ethanol at a concentration of \( \simeq 1 \times 10^{-8}M \) to form a fluorescent sample with a known Raman spectrum. Since the concentration of the ethanol is many orders of magnitude larger than the dye, it is assumed that the dye’s contribution to the Raman spectrum is negligible. A cuvette filled with the dye mixture was then placed into the sample holder of the spectrometer and measured at various exposure times and laser excitations. At 50 mW of excitation power, the peak counts on the CCD are \( \simeq 25,000 \) for a 1 second exposure. This was chosen as the exposure time to use since the maximum counts on the detector were 65,536. Exposures at each of the 8 lasers were taken 16 times to allow for different signal-to-noise ratios by summing different numbers of spectra taken with the same laser. The 2D CCD data is formed into an 800 channel spectrum by custom software written in MATLAB, as described earlier. One of the measured spectra for each laser are shown in Fig. 5.6 a. While the incident power on the sample is approximately equal for each sample, the dye’s absorption changes over the excitation wavelength range leading to the difference in measured intensities. The spectra are normalized to a unit sum before processing to estimate the Raman features. Raman features of ethanol are clearly visible at this concentration and exposure time. In addition, a sample with a higher concentration of dye, \( 1 \times 10^{-6} \) M, was measured to provide a lower SNR situation. At this higher dye concentration the CCD saturated at the 50 mW power level. A 5 mW power level was used to achieve the same count level as with the lower dye concentration. Spectra were acquired again at 1 second exposures 16 times with each laser, and 1 spectrum from each laser are shown in Fig. 5.6 b. In these spectra the higher dye concentration and lower laser power make the Raman features of ethanol no longer visible. For the higher concentration dye data, the data’s
background was corrected due to the inability of a unit sum normalization to remedy the absorption changes by the sample. [70] In the future, we would like to implement this correction by modeling the change in fluorescence with excitation wavelength in the forward model of the estimation algorithm.

To better evaluate the multi-wavelength EM algorithm, the data was processed three ways— by a polynomial fit background subtraction, SERDS using Fourier deconvolution, and the previously described EM algorithm approach. The polynomial fit background subtraction is implemented by fitting a 5th order polynomial to the regions of the spectrum from one of the lasers that have no Raman features. This smooth background is then subtracted from the original spectrum to recover a Raman spectrum free of the fluorescent background, shown in the top of Fig. 5.7a and b for the low and high fluorescent dye concentrations, respectively. For the low dye concentration this works well, resulting in a Raman spectrum similar to the reference spectrum of ethanol taken with one excitation laser on the same instrument at the bottom of Fig. 5.7a.
Figure 5.7: (a) Low concentration dye results. Top- Polynomial fit background subtraction of spectra from laser 1. Middle- Subtraction of spectra from lasers 1 and 2, and estimated Raman spectrum from Fourier deconvolution of the subtraction. Bottom- Pure ethanol measured with one of the excitation lasers (b) High concentration dye results. Top- Polynomial fit background subtraction of spectra from laser 1. Middle- Subtraction of spectra from lasers 1 and 2, and estimated Raman spectrum from Fourier deconvolution of the subtraction. Bottom- Pure ethanol measured with one of the excitation lasers

At the higher dye concentration, however, the polynomial background fit is not as successful, as shown in the top of Fig. 5.7b. The low frequency ripple is probably due to a combination of the filter responses of the laser blocking filters and the non-uniformity of the CCD detector response. Even with the smoothing that could done to minimize the effect of these features in the spectrum, not even the largest Raman feature of ethanol is prominent. The previously mentioned SERDS technique was also used to process spectra from 2 of the excitation lasers. By subtracting spectra taken with two different excitation wavelengths, a derivative-like signal is obtained as shown in the middle Fig. 5.7 a. The derivative signal can be estimated by solving the equation

\[ y_d = H_d \ast S_R, \]  

(5.11)
where $y_d$ is the difference spectrum, $H_d$ is a positive and negative impulse separated by the frequency difference of the two lasers, and the $*$ indicates a convolution. [58] Before being subtracted, the spectra are linearized in wave-number and intensity corrected in order to make the $H_d$ function be a constant function of frequency. The Raman signal $S_R$ can then be found by solving Eqn. 5.11 with Fourier analysis,

$$S_R = F^{-1}(F(y_D)/F(H_d)),$$

(5.12)

with $F$ and $F^{-1}$ defining the forward and inverse discrete Fourier transform, respectively. A further cosine apodization step helps reduce the Gibbs ringing in the reconstructed spectrum, and results in a low-pass filtering of the Raman estimate as well. [71] As shown in Fig. 5.7a, the derivative estimate has a high SNR, and the deconvolution estimate is very similar to the measured pure ethanol. There is a broadening of the features due to the wide spacing of the laser frequencies used. For the high concentration dye in Fig. 5.7 b, the SERDS processing is more effective at eliminating the low frequency ripples in the spectrum that was evident in the polynomial subtraction. The shot noise of the fluorescence is much more apparent in the derivative estimate in Fig. 5.7b. The primary peak of ethanol is easily identified as shown in the Figure, however the other ethanol features are not as apparent as in the low dye concentration case.

For the EM estimation with the fluorescent dye data, two analyses were pursued—a comparison of the EM approach with SERDS and polynomial background subtraction, and to see how the number of excitation lasers affected the estimation performance. In order to keep the total excitation energy constant, as the number of excitation frequencies doubled the number of 1 second exposure to use would be re-
Figure 5.8: (a) Low concentration dye EM reconstructions for 2, 4, and 8 excitation lasers, (b) High concentration dye EM reconstructions for 2, 4 and 8 excitation lasers produced by a factor of 2. Thus, the 3 cases used were using 2 lasers with a 4 second exposure time each, 4 lasers with a 2 second exposure time each, and 8 lasers with 1 second exposures. The EM algorithm was applied to each set of spectra using lasers 1 and 2 for the 2 laser case, lasers 1-4 for the 4 laser case, and all 8 lasers for the 8 laser case. There weren’t large differences in the reconstructions as long as the spacing of the lasers was not very large, such as using lasers 1 and 8 for the two laser case. This is probably due to the breaking down of the assumption of the measured fluorescence being completely insensitive to excitation wavelength for larger wavelength ranges. This is an issue with using all 8 excitation lasers since the lasers used spanned a large wavelength range, 12 nm. This led to some regions of the reconstructed spectrum not being completely stable as the number of iterations increased. Thus, a stopping criteria of a maximum difference between two iterations, usually $1 \times 10^{-8}$, had to be used for reconstructions from all 8 lasers for the low concentration dye spectra.

For the high concentration dye, however, the more lasers used, the better the reconstruction was, as shown in Fig. 5.8. The five ethanol peaks are clearly visible in the 8 laser reconstruction, whereas in the 2 and 4 laser reconstructions small artifacts in the reconstruction are of the same height as some of the ethanol peaks.
The stopping criteria was not as much an issue for this dataset, as can be seen in the error plot in Fig. 5.9. The error is calculated by comparing the reconstructed Raman spectrum to the reference spectrum of ethanol, acquired with one laser on the same system. Since ethanol by itself emits virtually no fluorescence, this measurement can be assumed to be the actual Raman spectrum of ethanol. By looking at the error plot, it also becomes clear that only the 8 laser excitation data shows increased fidelity with more iterations. This seems like it could be related to the situation in previous simulations—where more excitation wavelengths performed better reconstructions. It is suggested here that the conditioning of the inverse problem becomes better with a more complicated impulse response, by having more excitation lasers, thus the algorithm can refine the Raman estimate better.

In comparing the multi-wavelength reconstructions to the linear processing techniques, at the high concentration of dye the EM algorithm processing seems to do a much better job. The mean-squared-error of the linear techniques for the high concentration dye data was > 0.5, much higher than even the 2 wavelength EM error, 0.12.
5.5.2 Multi-wavelength chemometrics

As another application of the multi-wavelength excitation technique, chemometric analysis of ethanol in a tissue phantom was done similar to that of Sec.4.2.3. While the partial least squares regression (PLSR) does in some ways isolate the Raman signal, by determining what portions of the spectra are correlated with the concentration of a certain chemical, the idea was to see if using multiple lasers and the EM algorithm could help the PLSR achieve a lower prediction error.

Tissue phantom samples with ethanol concentrations ranging from 0 to 0.5 % by volume were prepared. Each sample was measured by all 8 excitation lasers 16 times with 5 second exposure times in order for different numbers of exposures to be summed together. After the images were processed into spectra, the summation process was determined by how many lasers would be used in the multi-wavelength reconstruction process. The multi-wavelength processing was compared with single excitation data as well by performing PLSR on the data from only one excitation laser. The grouping of the spectra was similar to that in Sec. 5.5.1 where the single-wavelength data used 8 spectra summed together, the multi-wavelength reconstructions used 4 spectra summed for the 2 laser case, 2 spectra summed for the 4 laser case, and no summing for the 8 laser case. In this way, the total exposure energy for all the concentration estimation data points is the same. Only 2 samples were used for each concentration for each exposure type in order to keep the analysis more consistent (this was the maximum number available for the single wavelength case).

After the multi-wavelength processing, the extracted Raman component was used to do the PLSR. In the absence of multi-day datasets, a more strict single day cross-validation for the PLSR was implemented by holding out the 2 data points at each concentration, and testing the model generated by the other data points on the left-out data. In this way, the testing and model-forming data sets had samples
Figure 5.10: (a) Actual and predicted concentration values for the single wavelength and multiple wavelength sample sets. (b) Root mean squared error of cross-validation as a function of number of lasers used. Error bars are used since multiple combinations of the 8 lasers are possible. The 8 laser case had only one combination available, thus there is no errorbar.

with independent concentration values. The concentration predictions for the multi-wavelength EM processed data and single wavelength data are shown in Fig. 5.10 a for one set of data from the 1, 2, 4, and 8 laser cases. There were multiple possibilities available for the 1, 2, and 4 laser cases in terms of which excitation lasers to use. The root-mean-squared-error for cross-validation (RMSECV) is shown in Fig. 5.10 b. Multiple combinations were used to calculate the RMSECV, and thus error bars were used to show the spread of the results. While there seems to be a clear trend in the error in reference to the multi-wavelength excitation processing, even the 8 laser case shows a minimum improvement compared to the average error for the single-wavelength PLSR. Since the spectra taken at multiple concentrations are available to PLSR, it seems the multi-wavelength processing’s extraction of the Raman portions of the signal is not necessary.
5.5.3 Multi-wavelength detection of ethanol in an animal

To have a more difficult ethanol detection task than a tissue phantom, some preliminary animal experiments were done to get a rough idea of the detectability of ethanol using a multi-wavelength excitation Raman system. The dichroic filter setup was used since that was the current setup in operation at the time of the animal tests. Thus, only 30 mW were available on the sample. The cuvette holder that frequently was used as the sample holder was removed, and a large plate fabricated on the stereolithography machine acted as a platform for the animal. The platform had one raised wall with a circular opening that the animal could be lightly pressed against to keep the excitation spot on the animal centered on the collection area of the spectrometer, as shown in Fig. 5.11 a.

In order to perform PLSR, the animal would have had to have its ethanol concentration changed and blood alcohol measured at various times. This was not practical at the time, and instead it was decided to inject the animal with ethanol at increasing concentrations until the ethanol peaks were visible in the spectrum. A intravenous line was set up in the femoral artery of the animal, and after being anesthetized saline was sent through the blood stream to clear out the circulation system. A spectrum of the paw of the animal was then acquired by using all 8 excitation lasers for 30 seconds each. The body of the animal exhibited an extremely strong autofluorescence, and thus the paw was used since its background was much weaker. The EM algorithm described previously was then used to estimate the Raman spectrum, shown in Fig. 5.11 b. These background features are due to the animal’s skin and connective tissue, etc. Injections of 10:90 and 20:80 mixtures of ethanol:water caused unnoticeable changes in the Raman spectrum. With the 50:50 ethanol water mixture, however, the ethanol features became clearly visible as shown in Fig. 5.11 c. When the spectra is compared with Fig. 5.11 d, many features that were not in the initial
spectrum of the rat paw that overlap with the ethanol features are present in the spectrum of the paw. Thus, it seems with reasonable confidence that the ethanol is being detected in the tissue by the multi-wavelength Raman system.
Figure 5.11: (a) Anesthetized rat in sample holder of multi-wavelength Raman system. (b) Multi-wavelength reconstruction of rat paw before injection of ethanol. (c) Multi-wavelength reconstruction of rat paw after injection of 50:50 ethanol/water mix (d) Reference spectrum of pure ethanol
Chapter 6

Conclusions

Raman spectroscopy has lots of potential for non-invasive diagnostics, however its poor efficiency places many stringent demands on an optical sensor system. With coded spectroscopy these demands can be relaxed by relying more on digital processing of the measured signals. Aperture coding allows for higher throughput dispersive spectrometers which can result in a system that can be more sensitive, compact, inexpensive, or a combination of all of them. Since the MMS method we developed is a static implementation, the added cost and complexity is minimal in terms of hardware. By coding the excitation source in a Raman system, the shifting Raman signal can be filtered out from a fluorescent background which is emitted by many types of samples. Once the multi-wavelength system is calibrated, no user intervention is needed to isolate the Raman signal unlike the conventional polynomial background subtraction techniques.

6.1 Summary of results

In Chapter 2 the motivation for coded aperture spectroscopy was identified and our static MMS implementation was given a mathematical foundation. The design implications of the approach was discussed in 3 along with the first custom MMS design used to validate the technique. Various implementation issues were identified, and the throughput gain was experimentally verified. In the signal-to-noise analysis for the static MMS approach, it became clear that one of two conditions were necessary to realize a SNR gain. In the presence of the detector noise and other signal independent noise, the multiplexing that occurs is advantageous since the average signal
on each pixel will increase for most sources. For shot-noise limited systems, however, the increased signal on the pixels is offset by the increased shot-noise. If the signal is sufficiently sparse, however, the increased throughput leads to an increased SNR at spectral peaks. This is a result of the signal increasing at the peaks, but its shot noise being distributed throughout the spectrum.

The application of the MMS design to a Raman system was discussed in Chapter 4. Some improvements to the implementation were identified, as well as the excitation and collection optics for the Raman system. Chemometric experiments of ethanol in a tissue phantom showed the ability of the MMS system to perform quantitative measurements at low chemical concentrations. The multi-wavelength excitation technique was then treated in Chapter 5 along with a description of the two experimental setups used. The performance of the EM algorithm developed to estimate the Raman signal was tested on various samples. For Raman signal estimation, the technique seemed to work well in fluorescent dye and tissue, however it was seen to have a marginal effect on chemometric analysis using the concentration estimation techniques of Chapter 4.

6.2 Future of Coded Spectroscopy

In looking at the knowledge gained in the process of the thesis, it seems appropriate to look at how the techniques developed could be applied to tissue spectroscopy and other areas. The higher sensitivity gained by the MMS technique along with improvements in components could lead to low-cost systems for routine diagnosis. The slow scan speed needed with conventional systems to scan large areas with a high spectral accuracy could make things like Raman skin analysis more practical. The exceptional performance of MMS at detecting weak peaks in the presence of little background could be helpful in Raman systems further out in the near-infrared,
> 1\(\mu m\). The lack of low noise detectors at this wavelength range would make the MMS systems even more attractive than slit-based systems. In addition, spontaneous anti-Stokes Raman applications might be a good fit, due to the huge reduction of fluorescence by observing energies higher than that of the excitation laser.

With coded-excitation Raman spectroscopy, the use of the technique with a tunable laser should make the technique more practical than the multiple-laser approach used in the thesis. As diode laser technology becomes more advanced, such tunable sources could become more widely available. In addition, since the approach is general in terms of the wavelength spectrum of excitation, a system that could use a light source broader in wavelength than a laser might be possible.
Bibliography


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Biography

Scott Thomas McCain was born in St. Louis, Missouri on January 19, 1978. He attended Parkway North High School in St. Louis, Missouri, and graduated in 1996.

Scott attended the University of Illinois at Urbana/Champaign, majoring in electrical engineering and was a James Scholar for all 8 semesters he was in attendance. At Illinois he was a member of the Eta Kappa Nu electrical engineering honor society and of Phi Eta Sigma, another national honor society. He graduated with honors in 2001 with a bachelors degree in electrical engineering. He remained at the University of Illinois at Urbana/Champaign and received a masters degree in electrical engineering in 2002. He was a research assistant in the Laboratory for Optical Physics Engineering under the direction of Dr. J. Gary Eden. His masters thesis title was “Sealed microdischarges fabricated in metal and glass”.

In 2003 Scott enrolled in the PhD program in electrical engineering at Duke University. Upon admission, he was offered a graduate school fellowship. There he performed research in optical sensing under the guidance of Dr. David J. Brady at Duke University’s Fitzpatrick Institute for Photonics. He was very active in the Optical Society of America (OSA) student chapter, serving as Treasurer, Vice-President, and President in his time there. He was successful at acquiring joint affiliation with SPIE, the International Society for Optical Engineering. In 2004 he was the Optics in the Southeast Annual Poster Competition Winner in Charlotte, NC. Then in 2005 he received the Optical Society of America’s Outstanding Researcher Award at the Optics in the Southeast Conference in Atlanta, GA. In 2006 he served in the delegation of the SPIE/OSA Congressional Visits Day Task Force. Scott will receive his PhD degree in May 2007.
Publications


Conference Talks


Patent Activity


