Multivariate Hyperspectral Raman Imaging Using Compressive Detection

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Supporting Information

ABSTRACT: A multivariate hyperspectral imaging (MHI) instrument has been designed and constructed to achieve greatly increased Raman imaging speeds by utilizing a compressive spectral detection strategy. The instrument may be viewed as a generalized spectrometer, which can function either as a conventional monochromator or in a wide variety of other hyperspectral modalities. The MHI utilizes a spatial light modulator (SLM) to produce programmable optical filters to rapidly detect and map particular sample components. A sequence of Hadamard-transform or random filter functions may be used to regenerate full Raman spectra. Compressive detection is achieved either using multivariate signal processing filter functions or the actual component spectra. Compressive detection is shown to be capable of achieving sampling speeds exceeding 1 ms per image pixel and the collection of chemical images in less than a minute.

Increasing the speed at which Raman chemical imaging is performed requires a shift in paradigm. The last major advances in Raman imaging speed occurred with the introduction of multiplexing1–2 and optical array detectors.5 The recent development of compressive sampling data collection strategies6–12 which have been used to create such devices as single pixel cameras,13 has set the stage for the creation of fast hyperspectral imaging instruments. Although previous studies have demonstrated the feasibility of such a hyperspectral detection paradigm,5,8,11,12,14 none of these have realized its potential speed advantages. Here we describe the design and performance of a new multivariate hyperspectral imaging (MHI) instrument which is optimized to facilitate the rapid collection of chemical images using a low-noise single-channel compressive detection strategy.

Despite ongoing improvements in optical array detector technologies (such as charge-coupled device, CCD, and electron-multiplying-CCD cameras, as well as time-delayed integration, TDI, strategies),5,17 the time required to collect hyperspectral images remains an obstacle to the wider application of Raman spectroscopy for chemical imaging. More specifically, point scanning with CCD-based spectral detection methods typically requires of the order of 1 s per spectrum and thus is impractical for the collection of large spectral images (as the collection of a 1 megapixel image would require 10^2 s or ~12 days). Global illumination strategies with a tunable detection band-pass filter and a two-dimensional (e.g., CCD camera) detector can be used to rapidly collect single wavelength images but do so at the expense of discarding the Raman scattered photons outside the wavelength detection band. Thus, tunable band-pass filter imaging is fundamentally less efficient (slower) than full-spectral detection. Line scanning5,18 can provide a superior alternative, by distributing the laser power and simultaneously collecting full spectra from each point along a line. Both point and line scanning methods simultaneously collect full spectral information from each image pixel with a signal-to-noise (S/N) that is limited only by the sample’s Raman scattering cross section, excitation laser power, and integration time.

A key advantage of the MHI detection strategy described in this work is provided by using a single channel detector to simultaneously detect all the photons transmitted by a multivariate optical filter of arbitrary programmable spectral shape. Thus, for example, a Raman spectrum with a total intensity of the order of 100 detected photons (counts) would have a S/N ~ 10 if all the photons were detected on an ideal single channel detector. However, if those same photons were spread over >100 wavelength channels (using a CCD detector), they would be practically undetectable, given that the signal (~1 count/channel) is less than the typical read-noise (of a few counts/channel). This is the fundamental reason why the MHI detection strategy can outperform conventional optical array detection methods. Moreover, the MHI detection strategy could potentially be implemented using low-cost SLM and single channel detector components12 (although the research grade components used in the present system are comparable in price to a CCD/spectrograph, as further described in the Experimental Section and the Supporting Information).

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It is important to stress that the full speed advantages of the MHI are only realized after pretraining to establish the optimal programmable filter functions for a given imaging application, with no pretraining the MHI can reproduce the functionality and speed of a conventional array detection scheme. Moreover, the MHI detection strategy is most advantageous (relative to full-spectral detection methods) under very high speed (or low signal) conditions, such that the total number of detected photons are comparable to (or smaller than) the number of full-spectral wavelength channels.

A few previous studies have demonstrated the implementation of hardware based spectral compression strategies. Such instruments all use a single channel detector to integrate the light transmitted through optical filters of various spectral shapes. For example, the filters may be designed to reproduce the eigenfunctions obtained from chemometric techniques such as principal component analysis (PCA) or partial least-squares (PLS). In other words, such hardware spectral compression methods utilize spectral eigenvectors (or loadings) for detection rather than for postprocessing of full-spectral data (or for compressed data storage). An early implementation of this strategy by Myrick and co-workers used static optical interference filters with customized transmission spectra for multivariate imaging and optical computing. However, since the required filters are sample specific, this approach requires manufacturing different filters for each chemical imaging application. Moreover, with dependence on the complexity of the filter function, many dielectric layers may be needed, thus increasing the cost and decreasing the maximum optical throughput of the required filters.

Spatial light modulators (SLM) offer an attractive alternative, as they provide a means of producing variable programmable filter functions. Recent implementations of this strategy have utilized various types of SLM devices, such as liquid crystal panels and digital micromirror device (DMD) arrays. LC-based SLMs use optical polarization to produce either phase or amplitude modulated variable spectral filter functions. DMD arrays provide binary filtering states, as each mirror is either “on” (reflecting toward the detector) or “off” (reflecting away). Liquid crystal (LC) based SLMs, on the other hand, provide a variable transmittance (or reflectance) gray scale and thus may readily be used to produce spectral filter functions of arbitrary shape (although a DMD can also reproduce this functionality by controlling the number and duration of mirrors that are in the “on” state during the data collection time). Previous applications using transmissive LC SLMs for spectral compression have suffered from low signal throughput (~20%). Recently developed reflectance based LC-SLMs provide much higher throughput (>80%) by increasing the reflectivity and fill factor of the LC array and thus have been selected for use in the present MHI instrument.

The unprecedented hyperspectral imaging speed of the MHI derives not only from the increased throughput and high contrast of the SLM but also from the use of a high-throughput volume holographic grating and a low noise avalanche photodiode detector. The MHI can be used either for full spectral detection, using band-pass or Hadamard-transform filters, or high speed compressive detection, using PLS or spectral angle mapping calibration and/or classification.

### Experimental Section

**Instrument Description.** The MHI is built around a Raman microscope with backscattering collection geometry. The schematic of the MHI detection optics is shown in Figure 1, and a detailed parts list is provided as Supporting Information. A 785 nm (single mode, 100 mW, Δλ = 0.026 nm) laser diode module (Innovative Photonic Solutions, Inc.) is used as the excitation source. The laser is directed through a 20 × (NA 0.40) NIR objective (Olympus, LMPL20XIR) using a 45° dichroic long pass filter (Semrock, LPD01-785RU-25 × 36 × 2.0) which passes the collected (Stokes-shifted) Raman scattered light from the sample. Additional Rayleigh scatter rejection is achieved using a 785 nm notch filter (Semrock, NF01-785U-25) placed outside of the microscope.

After scattering from the sample, the Raman signal passes into the MHI detection optics, as illustrated in Figure 1. The first component in the detection optical path is a half waveplate, which is used to rotate the polarization of the Raman light and thus determining whether the V or H Raman scattering component is detected. The second component is a Glan-Laser polarizing beam splitter which transmits the p-polarized light toward the SLM, and reflects s-polarized light coming back from the SLM (with polarization contrast of at least >1000:1). The second half-waveplate, between the lens and SLM is required in order to align the light’s polarization to match the liquid crystal axis of the SLM for optimal reflectance modulation. The MHI filter functions are produced by using the SLM phase modulation to control the degree to which the input p-polarized signal is rotated to s-polarization and thus reflected by the Glan-Laser polarizer into the detection optical path. The volume holographic grating (1200 L/mm, center wavelength 830 nm, Edmund Optics, 48-590) and an achromatic lens with a focal length of f = 100 mm (Edmund Optics, 47-317) are used to disperse light and focus different wavelengths onto different pixels of the SLM. Note that the distances between the holographic grating, lens, and SLM are matched to f (see Figure 1) to ensure that the light reflected by the SLM retraces the same optical path as the incoming light, although its polarization is modulated in accordance with the filter function applied to the SLM.

The SLM (Boulder Nonlinear Systems Inc.) is composed of a linear array of 12,288 pixels. Each pixel is a separately addressable optical phase modulator which is used to rotate the polarization of the detected light between 0° (p-polarization) and 90° (s-polarization), only light that is rotated away from 0° will be reflected into the detection optical path by the Glan-Laser polarizing cube. The degree of polarization rotation of each SLM pixel is set with an eight-bit control voltage. The eight-bit

![Figure 1. Schematic diagram of the MHI detection optics (see text and Supporting Information for details).](image-url)
Figure 2. SLM reflectance calibration. (A) The measured collected light intensity at 850 nm (normalized to unit full scale) is plotted as a function of the voltage applied to the SLM. The thick dashed segment highlights the voltage range used for setting the % T of the corresponding SLM pixel. (B) Calibrated 100% T (dotted) and 0% T (dashed) SLM voltage settings are plotted as a function of both SLM pixel number (bottom axis) and wavelength (top axis).

The reflectance scale of the SLM pixels allows the MHI to produce programmable spectral filter functions of variable shape, as illustrated in Figure 1. The birefringence of each SLM pixel is temperature and wavelength dependent. The relation between the SLM voltage setting and percent transmission as a function of wavelength is shown in Figure 2 (and further details concerning the SLM transmission vs wavelength calibration are provided as Supporting Information).

The s-polarized signal which is reflected into the detection path is collected with an 18 mm focal length aspheric lens (Edmund Optics) and focused into a 200 μm core optical fiber (NA 0.22, with SMA terminals, Thor Laboratories Inc.) that is directly coupled to the APD detector (Hamamatsu, C4777-01). The APD module has a built-in temperature control and amplifier and was chosen for its low noise and high photosensitivity between 800 and 950 nm. The APD has an active sensor diameter of 3 mm and a measured photosensitivity of ~1.2 e9 V/W. This current-integrating detector was selected for the MHI (rather than a photon-counting APD) as MHI Raman signals may exceed 10 MHz photon count rates. However, a photon-counting APD can provide lower noise for MHI signals corresponding to detection rates of the order 1 MHz or less (as further described in the Supporting Information).

The voltage output of the APD is connected to a USB data acquisition device (National Instruments USB-DAQ 6211) for digitization and computer readout. The DAQ unit has a maximum read rate of 250 kHz and can store 250 000 samples before analog-to-digital conversion. The integration time of the measurements using the APD-DAQ is set by controlling the number of samples that are collected and signal averaged. The spatial, spectral, and time resolution performance of the MHI is further described in the Results and Discussion section (as well as in the Supporting Information).

**MHI Detection Strategies.** The MHI may be viewed as a generalized spectrometer which is capable of functioning either as a conventional scanning spectrometer or using other more efficient spectral detection strategies. Different detection modalities utilize different spectral filter functions. For example, one may reproduce the functionality of a simple scanning spectrometer by using the SLM to produce band-pass filters with variable center wavelengths. The efficiency of the latter strategy is lower than that of a CCD-based full spectral instrument, since most of the Raman scattered light (away from the band-pass) is rejected by each filter. Alternatively, one may more efficiently collect full spectra using either Hadamard or random (compressive sampling) filter functions. The efficiency (speed) of the Hadamard transform detection method can approach that of a CCD-based point scanning, or line scanning, instrument (as half of the Raman scattered light is detected by each filter). By using random (compressive sampling) filters, one can in principle achieve higher speeds than when using Hadamard filters, since fewer filters are required to regenerate a complete spectrum. Far more efficient (and faster) MHI spectral imaging data collection may be achieved using trained filter functions which are tuned to optimally detect particular components of interest. For example, this can be achieved using filter functions whose shape is the same as that of the component spectra, or using filter functions whose shape reproduces loadings (eigenfunctions) optimized for a particular imaging application. In other words, high speed chemical imaging requires pretraining to construct MHI filter functions. Two such methods are demonstrated in this paper. One is spectral angle mapping using component spectral filter functions, and the other utilizes PLS loading vectors as filter functions. In both cases, the full Raman spectra used for training were obtained using the MHI with a Hadamard transform detection scheme.

For most applications, the measured SLM signals (i.e., APD voltages obtained when using a particular SLM filter function) are normalized by the integrated “all-on” signal, obtained by setting all of the SLM pixels to 100% T (in order to maximize the reflectivity of the SLM). In other words, the all-on measurement is used to normalize each SLM signal so that it represents the fraction of the total number of Raman scattered photons which are detected when using the corresponding SLM filter function.

Spectral angle mapping uses the analyte spectrum itself as the filter function. This method is quite simple to implement as it requires only scaling the spectrum to a full intensity range of 0 to 1 for use as an SLM transmittance filter function. Note that any such SLM filter function can be represented by an n-dimensional vector, and the same is true for the Raman scattered light intensity at different wavelengths. Spectral angle mapping effectively measures the correlation coefficient (dot-product) of the latter two vectors by measuring the amount of the Raman scattered light which is reflected toward the detector by the SLM. The Raman scattered light (spectral vector) emerging from a given point in a sample is thus classified based on its correlation coefficient with each SLM filter function (detection vector), with appropriate classification cutoff values.

Alternatively, pretraining using PLS regression may be used to determine optimal MHI filter functions. This method requires more computation effort prior to data acquisition than spectral angle mapping but can be advantageous as PLS regression maximizes the covariance between the spectra of each species and their concentrations, thus establishing a quantitative concentration metric built into the scaling of the loadings (filter spectral vectors). PLS also has increased selectivity since the PLS loadings are constructed such that they maximize the variance between the components of interest.
Materials and Samples. The following liquid samples were used in order to validate the chemical classification and quantitation performance of the MHI: n-hexane (Mallinkrodt, 5189, 99.4%), n-hexanol (Sigma Aldrich, reagent grade, 98%, H13303), and cyclohexane (Sigma Aldrich, 99.9+% HPLC, 27 062-8). The high-speed chemical imaging performance of the MHI is demonstrated using the following pharmaceutical composite and a powder containing two types of sugar microcrystals. The pharmaceutical composite sample was produced using an aspirin tablet (Equate, lot no. 3CE0649) in which three small craters were created and packed with theophylline anhydrate (AMEND Drug and Chemical Co., lot no. Z52258K16). The theophylline powder was heated at 80 °C for 24 h to ensure that it was in the anhydrate form. The surface of the aspirin-theophylline tablet was shaved with a razor blade to create a flat imaging surface. The sugar imaging sample was created by distributing powders of D(-) fructose (Sigma-Aldrich, F-0127) and sucrose (Mallinkrodt Chemicals, 8360-04) granules over a glass microscope slide. The spatial resolution of the MHI was determined using a standard 1951 USAF test target (Edmund Optics Inc.), consisting of a 2 in. square clear (soda-lime glass) substrate with a chrome USAF test target pattern.

RESULTS AND DISCUSSION

Validation of MHI Imaging Performance. Signal-to-Noise and Resolution. The total signal throughput of the MHI detection optics is greater than 50%, as determined by comparing the intensity of incoming horizontally polarized incoming light before the MHI Glan-Laser polarizer, with that emerging from the detection optical fiber (which is coupled to the APD detector), as shown in Figure 1. These throughput measurements were performed at 785, 850, and 915 nm using diode-laser light sources. The resulting MHI throughput of >50% is significantly higher than that previously reported when using an transmission LC-SLM (with maximum transmittance of ~20%), before including the additional losses associated with other components in the detection path.11,28

The following measurements were performed in order to quantify the sensitivity and noise trade-offs associated with the MHI detection system, under typical experimental Raman detection conditions. The USB-DAQ device has a noise of ±12 μV per read, as measured using a constant input voltage (obtained from a battery with resistive voltage-divider). The dark voltage and noise (standard deviation) of the APD is ±0.21 mV, as measured with the APD completely shielded from light. The latter dark noise is equivalent to an optical power of approximately ±182 μW (at ~850 nm). The response time of the APD was determined to be approximately consistent with the manufacturer specifications of 57 μs. More specifically, the measured APD response time is 55 ± 16 μs (determined as described in the Supporting Information). The manufacturer specified APD photosensitivity of 2.7 × 10^-10 V s/photon (at 850 nm) was also found to be consistent with our independent experimental measurements.

The actual S/N performance of the APD was determined to be approximately consistent with the manufacturer specifications of 67 μs. More specifically, the measured APD response time is 55 ± 16 μs (determined as described in the Supporting Information). The manufacturer specified APD photosensitivity of 2.7 × 10^-10 V s/photon (at 850 nm) was also found to be consistent with our independent experimental measurements.

The actual S/N performance of the APD was compared to that expected using the following expression.

\[ S/N_{APD} = \sqrt{n} \frac{S_L}{\sqrt{\sigma_D^2 + \sigma_L^2}} \]  

\[ S_L = V_{APD} \tau_{APD} / C_{APD} \] represents the light induced APD signal, expressed as a number of photons detected by the APD during its response time of \( \tau_{APD} \sim 55 \) μs, where \( V_{APD} \) is the APD output voltage and \( C_{APD} \sim 2.7 \times 10^{-10} \) V s/photon is the APD photosensitivity. The total detection noise includes both the APD dark noise \( \sigma_D \sim 43 \) (expressed as an equivalent number of photons) and photoelectron–hole (Poisson) noise \( \sigma_L = (2S_{APD})^{1/2} \), where the factor of 2 is included because each detected photon produces an electron–hole pair.5 In order to obtain the optimal signal-to-noise, the APD must be sampled at a rate that is greater than \( 1/\tau_{APD} \sim 20 \) kHz (and we typically sampled the APD at 250 kHz). Under these conditions \( n = \tau/\tau_{APD} \) represents the number statistically independent APD output voltages which are measured in a total read time.

The points in Figure 3 were each obtained from 30 replicate APD signal measurements of Raman scattered light from a liquid n-hexanol sample using different excitation laser intensities (ranging from 1 to 100 mW) and integration times (from bottom to top) of 100 μs, 1 ms, 10 ms, and 100 ms. The experimental S/N of these measurements was then compared to predictions obtained using eq 1, which was used to generate the solid curves in Figure 3. The dashed lines in Figure 3 represent predictions for an ideal electron–hole detector (with no dark noise \( \sigma_D = 0 \)). These results indicate that for APD voltages of 1 mV < \( V_{APD} < 10 \) mV, which are typical of the MHI Raman signal levels, the MHI signal-to-noise ratio is no more than about a factor of 2 less than that of an ideal electron–hole Poisson detector. A photon-counting APD may be used to further improve the MHI signal-to-noise performance, particularly under low signal conditions (as further discussed in the Supporting Information).

The spatial resolution of the MHI instrument is ~4 μm using the 20× objective, as illustrated in Figure 4 (see Supporting Information for further details).
dictated by the sample excitation spot size imaged on the SLM. The resolution was determined to be \( \sim 18 \, \text{cm}^{-1} \) by measuring the fwhm of the 991 cm\(^{-1} \) benzene peak and the 811.7 nm line from an argon lamp. The latter spectra were measured with the MHI using the Hadamard transform spectral collection procedure. This resolution corresponds to a spot size on the SLM of \( \sim 190 \, \mu\text{m} \), which is about a factor of 4 larger that expected under ideal imaging conditions (given the 4 \( \mu \text{m} \) spatial resolution at the sample and the 100/9\( \times \)11\( \times \) magnification of the collection optics at the SLM surface). The spectral range of the MHI is \( \sim 240 \, \text{cm}^{-1} \) to 2100 cm\(^{-1} \), which is dictated by the 19.6 mm width of the SLM (as well as the 1200 g/mm groove density of the holographic volume grating and the f = 100 mm focal length of the lens between the grating and the SLM, see Figure 1). Thus, the MHI effectively has \( \sim 103 \) independently addressable wavelength channels (as dictated by its full spectral range of 1860 cm\(^{-1} \) and resolution of \( \sim 18 \, \text{cm}^{-1} \)). Further improvement in spectral resolution may be obtained by introducing a vertical slit in the detection path (as further described in the Supporting Information).

**Liquid Classification and Concentration Measurements.** Figure 5 shows the MHI classification results obtained using liquid cyclohexane and n-hexane samples and either PLS or spectral equivalent (spectral angle mapping) MHI filters, with an APD signal averaging time of either 1 or 0.1 ms per filter. The MHI signals from each pure liquid have all been scaled to an average value of \( \sim 1 \). The line in Figure 5 was obtained using linear discriminant analysis (LDA), which provides a classifying metric for distinguishing these two compounds based on their MHI signals. Although similar performance was in this case obtained using both the PLS and spectral angle mapping detection strategies, the same is not the case for samples whose Raman spectra are much more similar to each other, such as n-hexane and n-hexanol. Similar tests performed using the latter two liquids demonstrated that accurate classification (with standard deviation error bars smaller than the distance between the n-hexane and n-hexanol points) is possible using PLS filters with a 10 ms APD signal averaging time but not when using spectral angle mapping with spectral equivalent filters (as further described below).

Figure 6 shows Raman spectra and MHI filter functions for liquid n-hexane and n-hexanol, whose Raman spectra look quite similar (in the fingerprint region from 400 to 2000 cm\(^{-1} \)). These results were obtained using PLS filter functions derived from the Hadamard transform training spectra of n-hexane and n-hexanol shown in Figure 6A. The latter training spectra for the PLS algorithm are normalized to unit area (with a concentration vector that codes n-hexane as 0 and n-hexanol as 1). The SIMPLS\(^{36} \) algorithm was used to perform the PLS analysis of the input spectra (using MATLAB along with the PLSToolbox, eigenvector Research, Inc.). The output from the SIMPLS algorithm for a two component system is a set of two regression vectors, as shown in Figure 6B; the solid curve in Figure 6B resembles the sum of the spectra of the two liquids, and the dashed curve in Figure 6B resembles the difference between the two spectra. Figure 6C illustrates the way that the latter PLS regression vector is split into two parts, each of which are non-negative functions.\(^{10} \) The latter two regression vectors are scaled to a maximum value of 1 (which corresponds to a maximum SLM transmittance) and the corresponding scaling constants, \( c^\dagger \) and \( c^- \), are subsequently used to regenerate the full PLS response. Further details concerning the generation of MHI filter functions are provided as Supporting Information (which also includes comparisons of MHI and CCD spectra of various liquid and solid samples in Figure S-1 in the Supporting Information).

Figure 7 shows MHI concentration quantification results obtained for various n-hexane/n-hexanol liquid mixtures, using PLS filter functions defined as described above (and shown in Figure 6), with APD signal averaging times ranging from 1 to 100 ms. These MHI based concentration measurement results are remarkably good considering the similarity of the fingerprint spectra of these two liquids as well as the fact that only the pure liquid spectra were used to train the PLS filter functions. The correlation coefficient of the linear fit to the data points shown in Figure 7 increased from 0.959 to 0.997, as the integration time (per SLM filter measurement) increased from 1 to 100 ms, respectively.

**Imaging of Solid Composites and Powders.** The chemical image shown in Figure 8 was obtained from the aspirin/theophylline composite sample. Initial (training) measurements were performed by collecting five Hadamard spectra of each of the two pure components with a high S/N (\( \sim 50:1 \)) using the

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**Figure 5.** PLS (square points) and spectral equivalent (circle points) filter classification of n-hexane (upper-left points) and cyclohexane (lower-right points) samples at integration times 1 ms (solid points) and 100 \( \mu \text{s} \) (open points). Spectral equivalent filter measurements are plotted as the response to the cyclohexane filter (left axis) versus the response to the n-hexane filter (bottom axis). PLS filters are plotted as the predicted \% n-hexane (right axis) versus the actual \% n-hexane (top axis).

**Figure 6.** PLS-derived SLM analyte filter algorithm. (A) Pure component Hadamard spectra of n-hexanol (solid) and n-hexane (dashed). (B) PLS output regression vectors using spectra from part A. (C) Splitting of the second PLS regression vector (solid curve in part B) into a positive part (solid) and the absolute value of the negative portion (dashed).
Figure 7. MHI concentration measurements are compared with the actual mixture concentrations results for n-hexane/n-hexanol liquid mixtures, with filter functions obtained using PLS and three different MHI integration times (per filter signal). The error bars (standard deviations of 10 repeated measurements) of the 100 ms integration results are similar to the size of the corresponding data points.

Figure 8. MHI chemical image (100 × 100 pixels) of an aspirin (blue) tablet with theophylline (red) packed craters at 1 ms per pixel per filter with a total integration time of 30 s. The image color ramp is continuous from blue (0) to green (0.5) to red (1).

Hadamard spectral collection strategy. The pure component spectra were then processed using PLS and classified with theophylline coded as 1 and aspirin coded as 0.

The chemical map shown in Figure 8 was obtained using a 1 ms APD signal averaging time with two PLS filters (for the positive and negative components of the second PLS loading vector) and one all-on filter. The total signal collection time was 30 s for the tablet chemical map in Figure 8. The average voltage response to the two PLS filters was typically 2–3 mV (with S/N ratios between 20:1 and 30:1, see Figure 4). When the integration time per filter was decreased to 100 μs, the total signal collection time decreased to 3 s, with a S/N ratio of ~10. The actual data measurement time may be longer than the signal collection time, depending on the dead-time associated with moving the sample stage and reading the signal. When reading the MHI signal continuously while linearly raster-scanning the stage we have collected MHI images in a total time that is less than 1.5 times longer than the associated total signal collection time (and further improvement is undoubtedly possible).

Sugar Crystal Mixture Imaging. Sugars have relatively weak Raman scattering intensities. The average APD voltage reading obtained from powders of sucrose and fructose (with the PLS filters) was ~300 μV, which is about 10 times lower than that obtained for aspirin and theophylline (as well as various liquid samples). A powder composed of mixture of sucrose and fructose microcrystals was dusted onto a glass microscope slide. The region that was imaged contained two large sucrose crystals separated by smaller crystals composed primarily of fructose, surrounded by smaller crystals composed primarily of fructose, with a total integration time of 30 s. The image color ramp is continuous from blue (0) to green (0.5) to red (1).

Figure 9. MHI chemical image (100 × 88 pixels) of sucrose (red) and fructose (violet) crystals spread on a glass microscope slide (green) at different integration times.

The fundamental advantages of the MHI detection strategy, relative to optical array (CCD) based micro-Raman detection schemes, derive from the lower noise and higher speed of the MHI signal continuously while linearly raster-scanning the stage we have collected MHI images in a total time that is less than 1.5 times longer than the associated total signal collection time (and further improvement is undoubtedly possible).

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CONCLUSIONS

The MHI instrument design described in this work utilizes a programmable optical filter to produce a high-throughput near-infrared micro-Raman spectrometer and hyperspectral imaging system. The results demonstrate that this instrument can either reproduce the functionality of optical array based spectrometers (using Hadamard transform filter functions) or far more rapidly collect hyperspectral images using either PLS or spectral angle mapping filter functions derived from the sample components of interest. The latter speed advantage can be of the order of 100 or more, as it is approximately equal to the ratio of the number of full-spectral wavelength channels and the number of MHI filters that are required for a particular imaging application.

Although we have focused on hyperspectral Raman imaging applications, the MHI detection strategy can readily be adapted to a wide variety of other high speed spectral detection applications. For example, the MHI may be used to increase the multiplexing capability of fluorescence-based bioarray sensing and high-speed sorting applications. More specifically, programmable fluorescence detection filters trained using multivariate signal processing algorithms may be used to distinguish chromophores with highly overlapping emission spectra and thus increase the multiplexing capability relative to that obtained using conventional fluorescence band-pass detection strategies. Moreover, the MHI compressive detection strategy may be used for chemical kinetics measurements with millisecond (or faster) time resolution. More specifically, programmable filters trained to project reactant, product, and/or intermediate species may be used to track time dependent concentration changes in liquids, solids, polymers, glasses, or biological samples.

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MHI. More specifically, the detection limit of a CCD-based point- or line-scanning micro-Raman system is limited by the CCD read-out speed and noise. The MHI detection scheme is expected to outperform a conventional micro-Raman detection strategy for signals which approach or are below the latter CCD detection limits. For example, if a Raman signal consisting of a total of ~3000 counts measured in an integration time of 1 ms were distributed over ~1000 pixels of a CCD with a read noise of ~3 counts/pixel, the resulting spectrum would have an average S/N of ~1. Equations 1 (and Figure 3) indicates that the same signal would produce an MHI APD voltage of ~0.8 mV and a S/N of ~15. Moreover, a photon counting APD detector may be used to further improve the MHI signal-to-noise and detection limit (as described in the Supporting Information).

■ ASSOCIATED CONTENT

**Supporting Information.** Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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