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Novel Fluorescence EEM Spectroscopy Method

Novel Fluorescence Excitation-Emission Matrix Spectroscopy method improves both data acquisition rate and spectral resolution

Reference: Fluorescence EEM

FreeProd, stock.adobe.com

IP Status

Patented, Patent application submitted

Seeking

Development partner, Commercial partner, Licensing, Seeking investment

About **Queen's University**

Queen's University in Kingston, Ontario is one of Canada's leading research-intensive institutions, combining quality and intensity in research with excellence in undergraduate and graduate education.

Background

Fluorescence spectroscopy is a highly sensitive, nearly background-free technique for chemical detection. This method is mainly limited by the fluorescence quantum yield of the analyte and the complexity of the matrix, which may produce interference, quenching, or autofluorescence.

Fluorescence detection has typically been achieved by either single-line UV excitation and dispersion of the entire fluorescence emission spectrum, or by tuning the excitation source over a wide wavelength range and detecting the entire spectrum of emitted light as a function of excitation wavelength with a broad band detector. In liquid samples, excitation and emission bands are broad, making it difficult to distinguish fluorophores in mixtures[1].

Excitation emission matrix (EEM) spectroscopy combines both these techniques. The excitation wavelength is scanned and an EEM is produced by recording a fluorescence emission spectrum at each of the many excitation wavelengths. This process generates a two-dimensional spectrum that allows analytes to be distinguished in a mixture of fluorophores. This technique is commonly used in the “fingerprint” analysis of foodstuff, environmental samples, industrial products and scientific samples[2].

Previously, most commercial fluorescence EEM spectrometers consisted of two scanning monochromators. The excitation and emission wavelengths were each selected using a scanning grating and a slit, and a broadband photodetector was used to measure light intensity. In this method, the data is obtained separately for each pair of excitation and emission wavelengths, which results in slower data acquisition rates which are not suitable for samples which are changing in real time. There is a need for commercial instruments which are capable of recording second-order fluorescence matrices with ever-increasing spectral resolution, sensitivity and speed.

1. Andrews, NLP et al. Hadamard-Transform Fluorescence Excitation-Emission-Matrix Spectroscopy. Anal. Chem. 2017. 89(16):8554–8564.
2. Bernicky, A et al. Simultaneous Double-Pass Absorption and Fluorescence Excitation–Emission Matrix Spectroscopy for Measurements of Reaction Kinetics. Anal. Chem. 92:12489-12497.

Tech Overview

Queen’s researchers have developed a novel technique to acquire fluorescence EEMs with a combination of spectral resolution and data acquisition rate which is unmatched by current methods. In this method, light from a bright white light source is spectrally dispersed onto a digital micromirror array (DMA), which allows the user to select any wavelength or combination of wavelengths for fluorescence excitation[1].

This method uses Hadamard-transform spectroscopy, a multiplexing technique which allows the user to modulate each wavelength with a unique pattern. This enables the use of the entire spectrum of the light source to excite the sample, generating higher-quality spectra while requiring less integration time (Figure 1).

Figure 1: Comparison of Hadamard Transformation and conventional sequential scanning.

The Hadamard Transform (HT) may be regarded as the binary counterpart of the Fourier Transform (FT). The HT uses a set of Walsh functions with binary values to form the Hadamard matrix, which is used to multiply signals. Practically speaking, the binary nature of HT-encoded signals allows them to be decoded much faster, often in real time[2].

The result of this method is a fully programmable light source which allows the user to direct large numbers of unique excitation wavelengths to the sample in a matter of microseconds to milliseconds. Each "barcode" of excitation light wavelength produces a unique emission spectrum, and this sequence of barcodes can be encoded through Walsh functions to produce an EEM spectrum.

This high-speed approach also allows for an improved excitation line width of 6.6 nm (Figure 2). When using this method with a DMA alongside a fiber-coupled LED as a light source, our researchers were able to record EEMs with 127 excitation wavelengths in as little as 1.6 s[1]. This increased data acquisition rate could be used to enable faster quality control or environmental analysis in many different industries. This high-speed approach can also allow for improved real-time analysis of chemical reactions, including providing additional mechanistic insight.

Figure 2: Excitation linewidth comparison after system improvements.

This method also works when combining absorption and fluorescence measurements, allowing for further insights into reaction mechanisms. Queen's researchers used absorption and EEM measurements to study the conversion of pheophytin-a, a fluorescent reactant, into copper-chlorophyll-a, a nonfluorescent but strongly absorbing product. This setup was able to resolve several intermediate steps in the reaction by correlating the fluorescence and absorption spectra. With the use of parallel factor analysis (PARAFAC), this setup even provided evidence for a new reaction intermediate which has the same fluorescence as $Pheo_a$ but with a larger absorption cross section[1].

In this experiment, EEM and absorption spectra were collected every 30 seconds, using 67-channel modulation with an integration time of 450 ms per mask. This improved data acquisition rate allows for greater real-time analysis of reactions (Figure 3). The improved data acquisition rate makes it possible to take "snapshots" of active chemical reactions, as the entire spectrum can be captured much faster than in other methods. This also reduces the need to quench such reactions[3], as far more data can be collected in real-time.

Figure 3: Selected absorption spectra

As mentioned above, this method could be used for much faster quality control and quality assurance. It could also be used in monitoring chemical reactions in real-time which could not be accurately monitored by commercially available alternatives. This combination of speed and resolution creates many new possibilities for the use of fluorescence EEM spectroscopy.

1. Bernicky, A et al. Simultaneous Double-Pass Absorption and Fluorescence Excitation–Emission Matrix Spectroscopy for Measurements of Reaction Kinetics. Anal. Chem. 92:12489-12497.
2. Andrews, NLP et al. Hadamard-Transform Fluorescence Excitation-Emission-Matrix Spectroscopy. Anal. Chem. 2017. 89(16):8554–8564.
3. Andrews, NLP et al. Determination of the thermal, oxidative and photochemical degradation rates of scintillator liquid by fluorescence EEM spectroscopy. Phys. Chem. Chem. Phys. 2017. 19:73–81.

Benefits

- Multiplexing technique allows for much faster throughput of high resolution spectra
- Able to process EEM data much faster than existing methods, often in real time
- Higher resolution combined with PARAFAC analysis can help filter out unwanted noise or reactions

Applications

- “Fingerprint” analysis of food, wine, environmental samples, industrial products, scientific samples, etc.
- Can provide mechanistic insights into chemical reactions
- Separation science

Opportunity

Seeking exclusive licensee, research collaborator or venture investment.

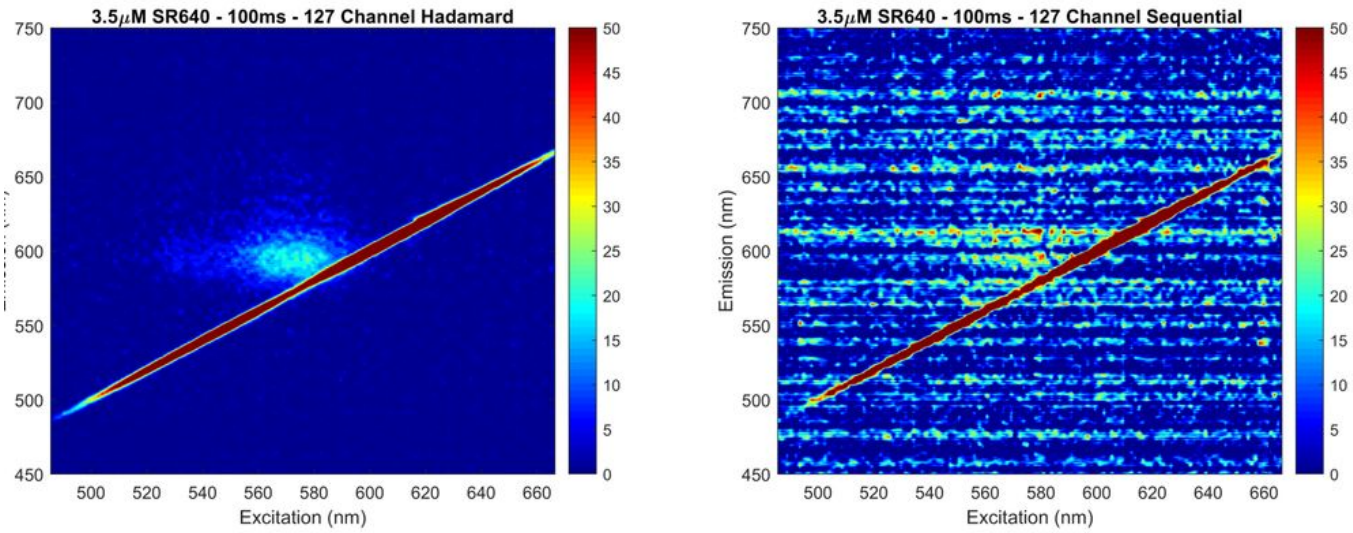
Patents

- Granted US Patent US10,481,092 “Multiplexed excitation emission matrix spectroscopy”
- Canadian patent application has been filed

Appendix 1

Figure 1: Comparison of Hadamard Transformation and conventional sequential scanning.

Comparison of EEM spectra generated using the Hadamard Transform method (left) and sequential scanning (right). Both EEM spectra are of fluorescent dye sulforhodamine 640 and use a very short integration time (100 ms).

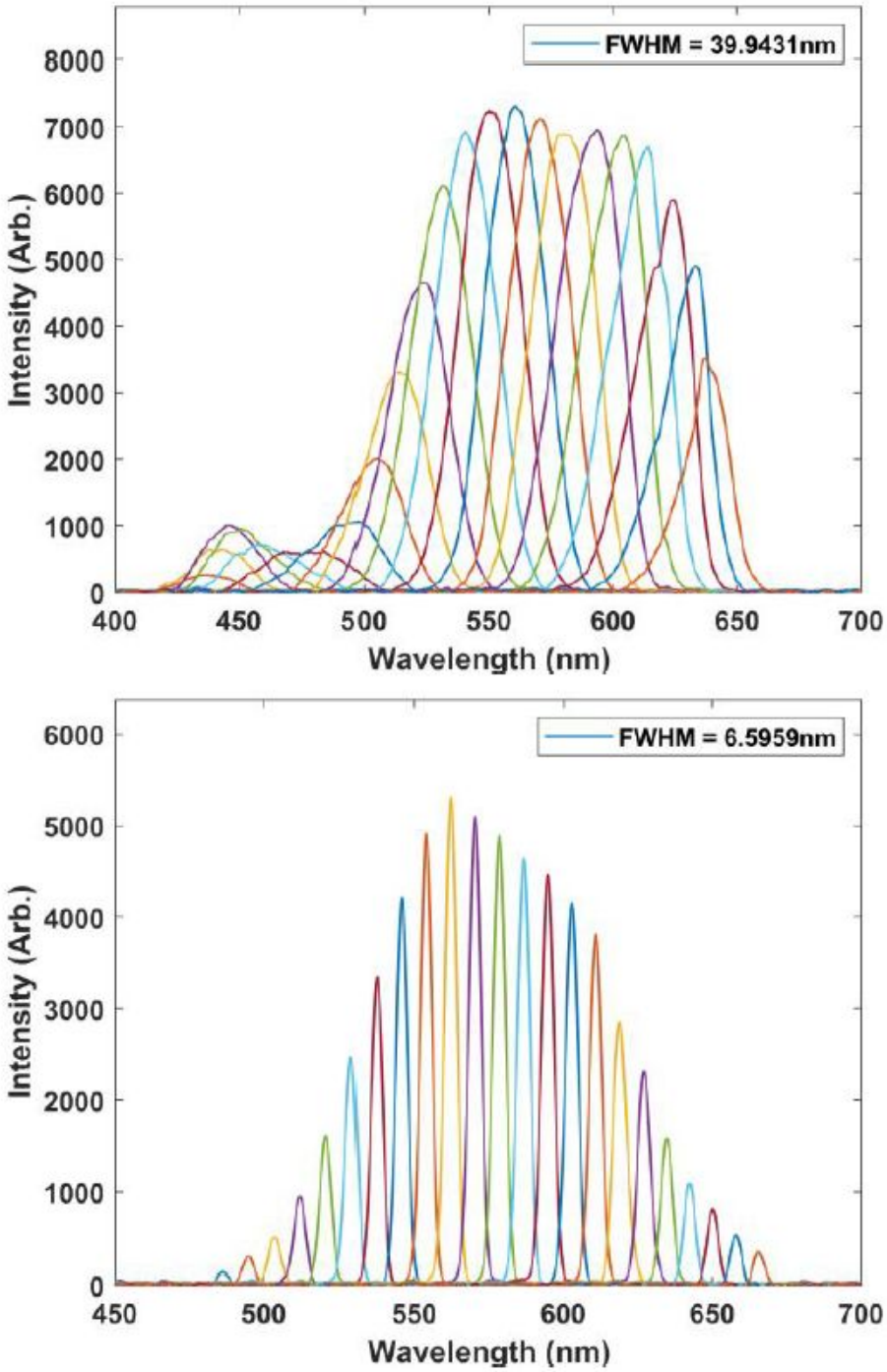


Appendix 2

Figure 2: Excitation linewidth comparison after system improvements.

Excitation linewidth comparison. (Top) A 67 channel calibration series displaying every third mirror column prior to instrument modifications (Bottom) A 67 channel calibration series displaying every third mirror column after modifications.

Note that the peak width of the excitation channels was reduced from 40 nm to 6.6 nm after the recent improvements of the technique.



Appendix 3

Figure 3: Selected absorption spectra

Selected absorption spectra associated with the conversion of $Pheo_a$ to Cu-Chl_a. The displayed spectra were collected every 5 min. This increased data acquisition rate provides superior insights into the mechanism of this reaction, including the discovery of a previously-unknown reaction intermediate.