Temporal Hyperspectral Imaging of Multiple Fluorophores Expressed by Yeast Colonies

Howland D. T. Jones[1], Mark H. Wall[1], Megan Halfmann[2]

[1]Middleton Spectral Vision, Middleton, WI, [2]Stowers Institute, Kansas City, MO

Introduction/Background

Biological colony growth can be influenced by the growth conditions which they are subjected to. Temperature, growth media, the environment and contamination all can have an effect on the growth and viability of colonies under study. It has also been shown that protein aggregation within a cell can either be beneficial to the viability of a cell or cause detrimental effects leading to cell death[1]. To understand the effects that these conditions play on the colony growth and viability, techniques are needed that allow for fast and effective characterization of the composition of the colonies present after incubation. Fluorescence hyperspectral imaging provides an effective pathway to identify biological diversity in the growth colonies[2]. As a prelude to such studies we show here the feasibility of imaging colony growth that exhibits multiple distinct fluorescence emission species.

A number of colonies transfected with the ability to express fluorescent proteins were investigated with the Middleton Spectral Vision MacroPhor™ Fluorescence Imaging System. The MacroPhor with its hyperspectral detection is well suited for this application, providing easy and fast full spectral image acquisition. The MacroPhor has powerful analysis tools that are used to extract valuable information about colony growth based upon the spectral diversity present in the collected fluorescent image. This study interrogated the growth of six yeast colonies that differed in transfection with different fluorescent proteins (mKate2, LSS mOrange, mCitrine, and mOrange). The fluorescent images collected over the course of the study were analyzed using Multivariate Curve Resolution (MCR) to reveal the unique fluorescence signatures, their distribution, and to quantify the relative intensities of those signatures. The analysis illustrates the type of information that can be extracted and applied to study of multi-fluorophore samples.

Methods

MacroPhor™ Fluorescence Imaging System

Fluorescence imaging is a valuable technique for studying colony growth on agar media in petri dishes. Middleton Spectral Vision has developed MacroPhor™ Fluorescence Imaging System for macro-scale scanning of biological systems. Image collection begins by illuminating the specimen under investigation with line illumination from a laser. The sample under investigation responds to this illumination by emitting fluorescence light.

The fluorescence emission from this line illumination is collected in a 180-degree geometry and is directed to an imaging

Figure 1. Middleton Spectral Vision MacroPhor™ Fluorescence Imaging system

spectrograph equipped with a sensitive CMOS camera. The spectrograph disperses the fluorescence along the Y axis of the C detector/ camera. Hence, a single collected fluorescence line sent to the imaging spectrograph gives information about the spectral diversity at discreet spatial points on the collected x axis emission line. Successive image lines are collected and sent to the imaging spectrograph and detector / camera to build up a full image of the specimen by, in this case, translating the specimen in the y direction via an automated stage in a so-called "push broom" manner. This push broom line imaging system excites the sample with either of the available laser wavelengths, 405, 488, 540 or 640 nm, and collects the emission in the 400 – 800 nm region. For this study the 488nm laser light was used. The end result of the image collection is the generation of a hypercube of data that contains information related to spatial axis x, y, and the spectral axis (wavelength).

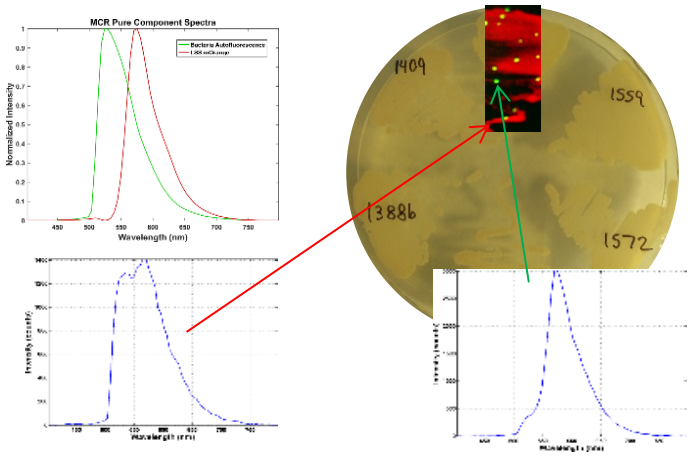


Figure 2: This figure shows the results of the MCR applied to the yeast colony at the 12 o' clock position in the petri dish. The MCR image shows two distinct populations. The spectra shown are the MCR determined pure components and the observed spectra in the map correlated to the MCR results. The two distinct population distributions in the fluorescence image have been artificially colored red and green.

Image Data Analysis

Collected hyperspectral image cubes contain a vast amount of spectral information. Data mining this vast amount of data is required to extract the information of interest. In the present case the relevant information desired is the unique fluorescence spectra and their distribution. One technique that is very useful for identifying/ classifying spectral information in large data sets is Multivariate Curve Resolution. The Middleton Spectral Vision KemoQuant™ is a powerful analysis package, which allows MCR to be rapidly applied to the very large hyperspectral image files. MCR is a multivariate spectral analysis technique that is capable of determining all independently varying spectral signatures present in the hyperspectral image data and their corresponding intensities. To ensure that MCR identified the most representative emission signatures present in the yeast colonies, all images collected from the entire study were combined and analyzed together.

Results

The MCR analysis results for the hyperspectral image obtained for yeast colony 1710 positioned at the 12 o'clock in a petri dish is shown in Figure 2. The MCR results clearly give two distinct (i.e. pure) components that correlate to two distinct fluorescing populations. The MCR-based spectra of the pure components and the observed experimental spectra of these distinct populations are shown in Figure 2. In the resultant MCR image (shown in Figure 2) the pure components have been colored green and red to give added contrast between the two unique populations. The red component correlates highly with the spectra of LSS mOrange and can be confidently identified as the LLS mOrange labeled yeast colony, whereas the minor second component can be identified as a possible bacterial contamination.

The MCR pure components determined by combining the images together and performing the MCR calculation on the entire set of spectra yielded six principle or pure components which are shown in Figure 3.

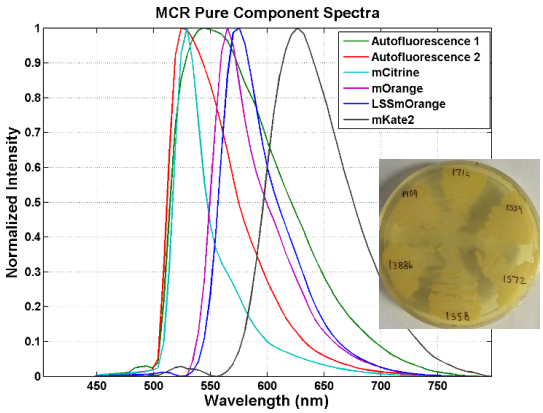


Fig 3. The MCR-derived pure spectra for the entire set of images.

The MCR results on the colony adjacent to the colony in figure 2 were performed with results showing three distinct fluorescent signals. The spectra and distribution maps associated with these MCR components are shown in Figure 4. In this colony there is a clear indication that this colony shows the presence of yeast labeled mKate2 as the major component. Assignment of this component to mKate 2 was determined by the close match of this component to the emission spectrum of mKate 2. However, there are two other unique components which can be ascribed to two related autofluorescences from the native yeast. The distribution map for the autofluorescence 1 exhibits a similar distribution as the mKate2 and could be assigned to the natural autofluorescence from the native yeast.

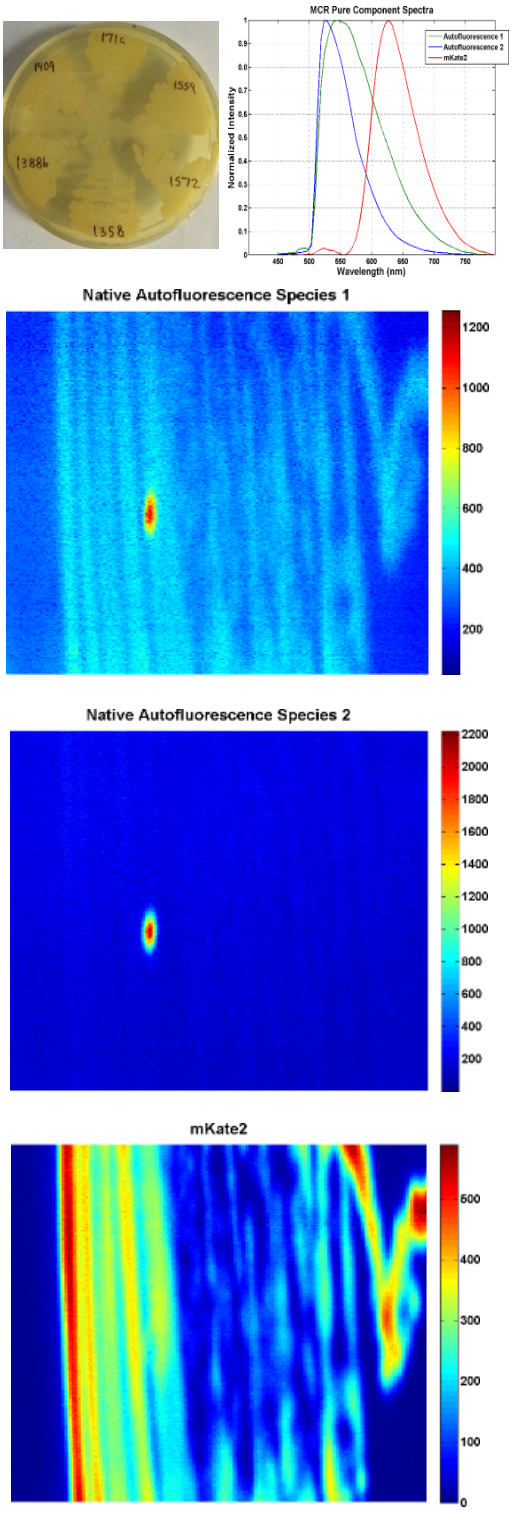


Figure 4: Upper image: video image showing the colony associated with the MCR image results; a) MCR derived Pure component emission spectrum b) Distribution of Autofluorescence 1, c) Distribution of Autofluorescence 2, D) Distribution of mKate2.

The individual fluorophore emission distribution images can be assigned to display colors such as assigning red to mKate2, green to auto fluorescence 1 and blue to autofluorescence 2. A composite image can then be produced showing the relative spatial distribution of each fluorescence emission. This is presented in Figure 5. This composite view allows the relationship between the three distinct fluorescence emissions to be easily seen.

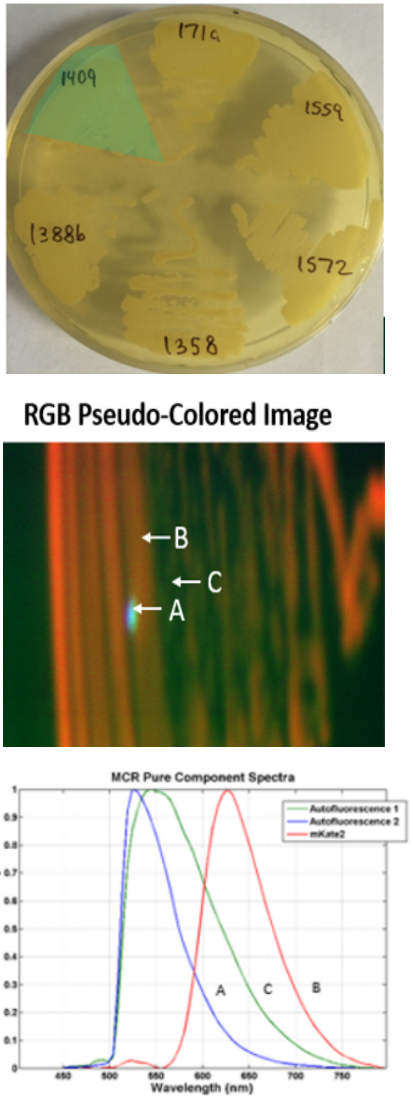
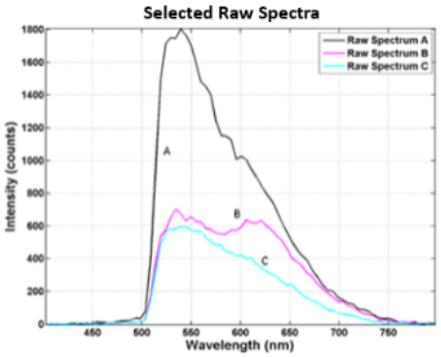


Figure 5. Photo of sample with colony analyzed shaded (top), Pseudo-colored fluorescent composite image of the yeast colony presented the MCR pure component spectra and the Raw image spectra at points A, B, C (bottom). This image provides a view of the relative spatial distribution of the three determined emissions species.



Conclusions

The use of hyperspectral imaging can aid the study of biological colony growth and behavior for colonies containing several distinct fluorescent labels. The Middleton Spectral MacroPhor Fluorescence Imaging System is a flexible imaging platform that streamlines the acquisition of hyperspectral fluorescence information. Through the KemoQuant™ Analysis Software, the collected hyperspectral data can be recast to independently varying components to give important insight into complex samples. It was shown here that yeast colony growth can reveal multiple species in a single colony.

References:

1) Holmes DL, Lancaster AK, Lindquist S, Halfmann R. *"Heritable remodeling of yeast multicellularity by an environmentally responsive prion"* Cell. 2013 Mar 28;153(1):153-65
2) Martin Hof, Rudolf Hutterer, V. Fidle Fluorescence Spectroscopy in Biology: Advanced Methods and their Applications to Membranes, Proteins, DNA, and Cells Springer 2004 pp. 304