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Hyperspectral Imaging Analysis of Cellular Heterogeneity Between and Across Populations

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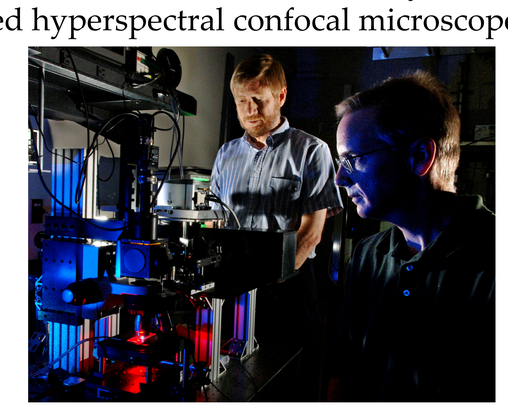
Objective

- To detect unknown viral pathogens without the use of specific affinity reagents

Methods

Hyperspectral Microscopy

Sandia National Laboratories currently has an in-house developed hyperspectral confocal microscope.[1]



We are in the process of developing a hyperspectral stimulated emission depletion (STED) microscope,[2] which will combine the advantages of super-resolution microscopy and hyperspectral imaging. Additionally, by employing a supercontinuum laser, the hyperspectral STED will support both a wider range of fluorophores as well as excitation-emission mapping.

Multivariate Curve Resolution

Multivariate curve resolution (MCR) extracts both the spectral components and their relative concentrations without requiring either to be known *a priori*. MCR can distinguish overlapping fluorescence signals, including separately identifying autofluorescence contributions. Sandia National Laboratories has developed highly efficient algorithms[3] suitable for large-scale MCR.

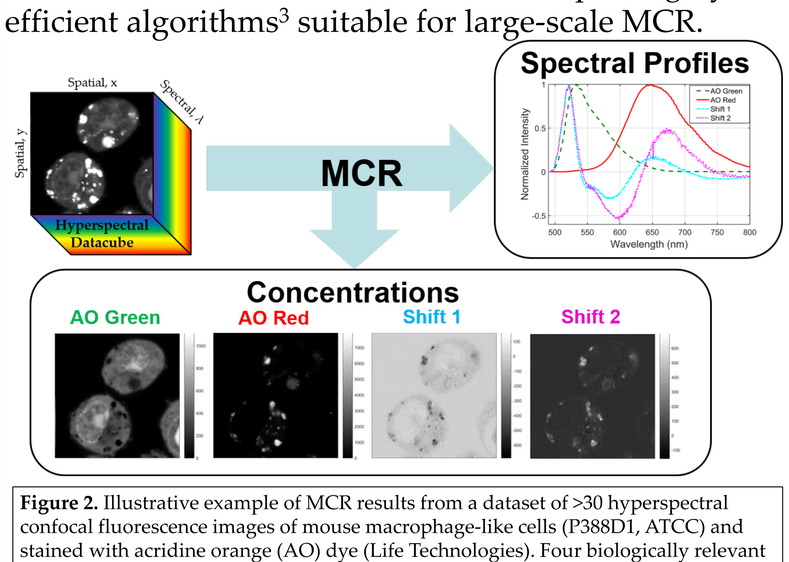


Figure 2. Illustrative example of MCR results from a dataset of >30 hyperspectral confocal fluorescence images of mouse macrophage-like cells (P388D1, ATCC) and stained with acridine orange (AO) dye (Life Technologies). Four biologically relevant spectral components were identified in the MCR model. An instrument offset was also used in the model but was omitted here for simplicity. Information from the supplier suggests that the AO green fluorescence spectrum corresponds to AO intercalating into dsDNA while the AO red fluorescence spectrum corresponds to AO binding to ssDNA or RNA. The shift spectral components are required to account for solvatochromatic effects, shifts and broadening of the AO spectra due to changes in the local environment.

Results

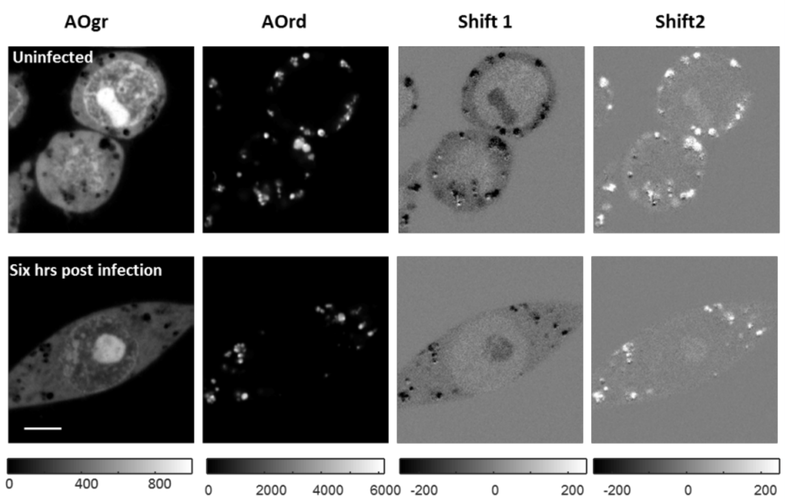


Figure 3. MCR identified concentration maps for each spectral component for a representative uninfected P388 cell and a P388 cell infected with human adenovirus 5. The intensities indicated by the grayscale correspond to the relative abundance of that component in each image voxel.

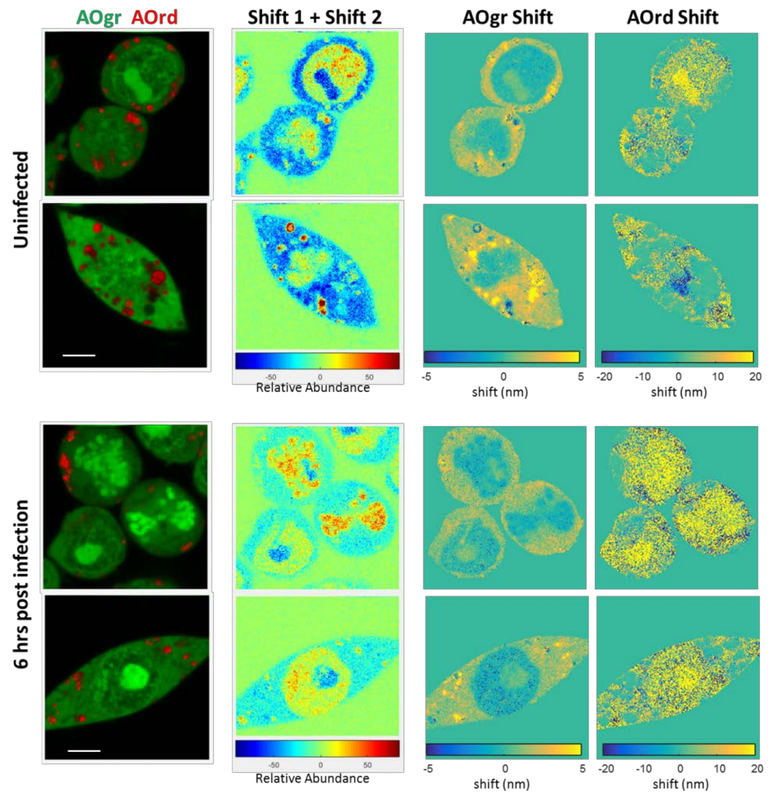


Figure 4. Effect of subcellular environment on AO spectral properties for two representative images of infected and uninfected cells. Left panel: Composite image of AOrd and AOgr concentrations. Red and green color scales have been independently optimized for viewing. Second Panel: Images of the combined shift components (Shift1 + Shift2). Right most two panels: Images showing the concentration and location of shift components represented as degree of shift from the emission maximum of 534 nm (AOgr shift) and 649 nm (AOrd shift). Scale bar = 5 um.

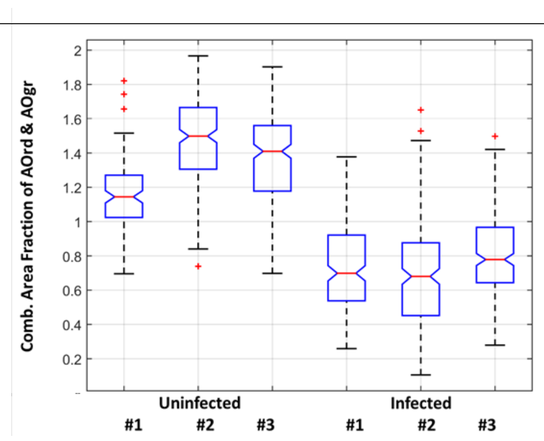


Figure 5. Illustration of one of the classification variables employed, the combined fraction of the cell where the red and green spectral components exceed a threshold value. Each category contains an average of 190 cells.

Figure 6. (Top) Scatter plot of the two cellular descriptors used to classify the cells as infected or uninfected. The scatter plot reveals regions that are resolved from each other as well as regions where infected and uninfected cells are not clearly distinguished. Five uninfected outliers extend beyond the axes of the scatter plot. **(Bottom)** Assigning probabilities that a given cell is infected using multivariate logistic regression. If logistic regression was performed using only the x-axis variable, the probabilities would lie perfectly on a logistic curve. Instead, multivariate logistic regression was used where inclusion of the additional variable tends to improve prediction, slightly enhancing the separation between infected and uninfected cells. The horizontal lines divide the cells into three bins: cells classified as infected, uninfected, and unclassified.

Conclusions

- Viral pathogens can be detected solely based upon cellular response without using specific affinity reagents.
- Hyperspectral imaging and MCR allow the evaluation of morphological and spectroscopic signatures of viral infection.
- Due to heterogeneity within the uninfected and infected populations, some cells cannot be unambiguously classified as either infected or uninfected.
- If ambiguous cells can be discarded, populations consisting entirely of just infected and uninfected cells can be extracted with arbitrarily high sensitivity and specificity (0.96 and 0.97 shown).

Acknowledgements

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References

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