EXCITATION-EMISSION CHARACTERIZATION OF ICG IN BIOLOGICALLY RELEVANT SOLUTIONS

Sophie Lyon, QUEL Imaging
Sophie@QUELimaging.com
Ethan P. M. LaRochelle PhD, QUEL Imaging
Kimberley S. Samkoe PhD, Dartmouth College
Alberto J. Ruiz PhD, QUEL Imaging

Key Words: Fluorescence guided surgery, excitation emission matrix, red-edge shift, indocyanine green

As fluorescence guided surgery (FGS) gains popularity, it is important to understand not only the spectral characteristics of the fluorophores being used but the effect of the chemical environment on the spectra. Indocyanine green (ICG) has become a popular fluorophore for FGS, due to its near-infrared (NIR) excitation and emission, low toxicity, and established use in clinical applications for over 50 years.

The typical characterization available for fluorophores such as ICG is lacking in a number of ways. Oftentimes, reference spectra are collected at concentrations orders of magnitude higher than is clinically relevant. Many of these spectra are taken in solvents optimized for low scattering, which bear little resemblance to the *in vivo* chemical environment. Additionally, fluorescence emission spectra are generally reported from only one excitation wavelength. While this basic information has utility, more thorough characterization data provides the opportunity to advance the clinical translation of new imaging technologies and improve the standard of care.

Excitation emission matrices (EEM) are three-dimensional data sets of fluorescence intensity over a range of excitation and emission values. To demonstrate the utility of the EEM with a clinically relevant fluorophore, an ICG-equivalent laser dye (IR-125) was characterized. IR-125 is a stereoisomer of ICG, making it an appropriate analog for the collection of spectral data *in vitro*. A UV-Vis-NIR spectrofluorometer (Horiba, Duetta) enables the rapid collection of EEM data for each sample with minimal photobleaching. EEMs were acquired with an excitation range of 650 to 900 nm in 1 nm step increments while collecting emission data from 700-1000 nm. A biologically-relevant fluorophore concentration of 1 μM was analyzed. Solutions were made in dimethyl sulfoxide (DMSO), bovine plasma in Na-EDTA, or 1 – 50% bovine serum albumin (BSA).

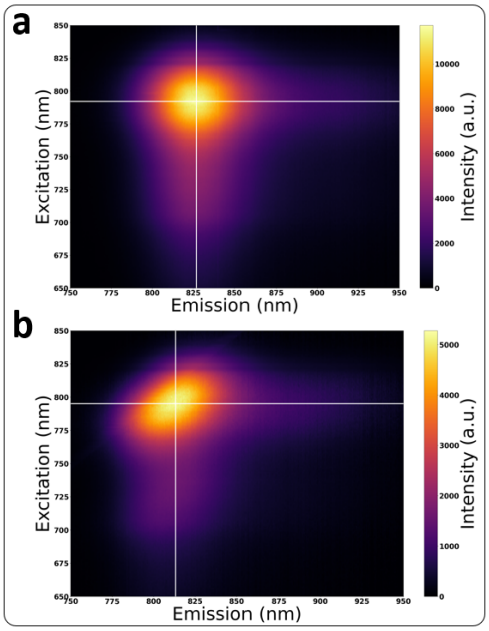

Figure 1 – EEM spectra of IR-125: a) in DMSO; b) in BSA with red-edge shift

The resulting EEM data was analyzed using a Python script to identify the peak excitation and emission wavelengths for each solution as well as characterize the observed rotational shift in longer wavelength excitation. The peaks were first approximated using the maximum intensity values and then a Gaussian fit over a 20 nm window was applied to reduce uncertainty. The clockwise rotation of an EEM trace (Figure 1) observed in the presence of plasma and BSA, was characterized by calculating the slope from the peak emission and excitation shifts. The peak excitation emission of 1 μM IR-125 in DMSO, 5% BSA, or plasma, is (792, 827 nm), (795, 813 nm), or (798, 810 nm), respectively. The rotational shift for the same solutions is 0.0, 0.4, and 0.7 nm/nm.

It is important to note, this non-intuitive spectrum would go unnoticed when only a single excitation wavelength is used. The increased fluorescence emission wavelength maxima at higher excitation wavelengths, known as red-edge shift (RES), is recognized as a solvent effect, yet is regularly disregarded as it is caused by the microenvironment of the fluorophore. Biologically relevant scattering agents, such as albumin, often lead to RES, necessitating a further understanding of this spectral phenomenon to optimize FGS.

With the expansive growth of the field over the past decade, new fluorophores such as OTL38 (Cytalux), have recently been approved. While this work focuses primarily on ICG, the analysis framework can be extended to other fluorophores, providing a foundation to optimize the performance of new applications in fluorescence guided surgery.