Spectral Unmixing of Indocyanine Green In the Vascular Network of a Sycamore Leaf

Bryan A. Smith[1], and W. Matthew Leevy[1]

[1]Department of Chemistry and Biochemistry, 236 Nieuwland Science Hall, University of Notre Dame, Notre Dame, IN 46556 USA

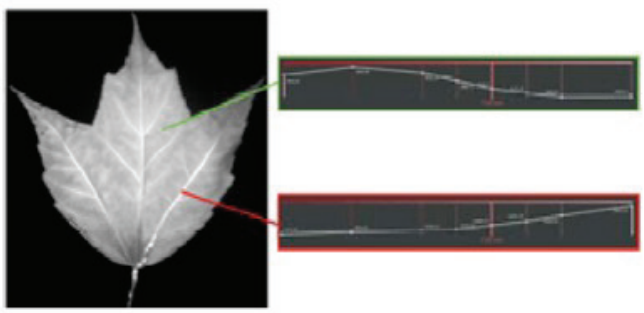
The vascular network of a leaf is essential for providing transport and mechanical support. Leaf venation patterns progress throughout growth, during which a hierarchy of discrete vein-size classes being to develop.[1] Vein classes are designated by the width of the veins at their point of branching, with higher-order veins exhibiting narrower widths than lower-order veins. Leaf vasculature varies between species; thus, understanding how these venation patterns develop could have important implications for understanding both plant physiology and evolution.[2] Traditional strategies for investigating leaf vein patterning include genetic modification,[3] pharmacological methods,[4] and theoretical modeling.[5] In this study, we tested the ability of a near-infrared dye, indocyanine green, to label the vasculature network of a sycamore leaf to allow for facile identification of the leaf architecture.

Imaging Probe and Biological Model

Indocyanine green (ICG) is a near-infrared cyanine dye that is FDA approved for determining cardiac output, hepatic function, and liver blood flow, as well as for ophthalmic angiography.[6] ICG exhibits a λ_{max} at 775-803 nm and emits between 820-830 nm, depending on its association with plasma proteins. The near infra-red (NIR) region of light (650-900 nm) offers an "optical window" for fluorescence imaging in complex biological systems, which is due to minimal light absorption by endogenous molecules and increased tissue penetration. In plants, however, there are a significant number of fluorophores that contribute to the autofluorescence even in the NIR; thus, making spectral unmixing critical in plant tissues even into the NIR. To investigate leaf venation patterns, researchers often utilize Arabidopsis leaves. Arabidopsis plants are the preferred model for studying plant vasculature given that the entire genome of some strains has been sequenced.

This allows researchers to genetically modify proteins with fluorescent reporters to examine spatial and temporal protein expression during vasculature development. These fluorescent-engineered plant proteins are also used as leaf vein biomarkers to identify venation patterns.[7] Fluorescence imaging is performed using either histochemistry or confocal laser-scanning microscopy, which involves processing or fixing of plant tissue. Here, we used a much larger sycamore leaf and showed that the vasculature can easily be identified through macroscopic, epi-fluorescence imaging without the need for genetic or physical manipulation.

Figure 1



Spectral unmixing of indocyanine green and autofluorescence emission signals from a sycamore leaf. The spectra in the green box represent the emission spectra of the leaf autofluorescence, while the spectra in the red box is the fluorescence emission spectra from indocyanine green in the leaf vasculature.

Imaging Protocol

Fluorescence Imaging

A sycamore leaf (Platanus occidentalis) was cut at the base of the stem. The stem was then placed into a vial containing 800 µL of ICG (1 mM in water). Twenty-four hours later, the leaf was imaged using an In-Vivo MS FX PRO Imaging System (Bruker BioSpin, Woodbridge, CT USA).

Spectral Unmixing

Using the MS FX PRO, the sycamore leaf was illuminated with filtered light at 650, 670, 690, 700, 710, 720, 730, and 750 ± 10 nm. Fluorescence emission was captured using a CCD camera equipped with an 830 ± 20 nm emission filter (f-stop = 2.51, FOV = 190 mm, binning = 2, 30 second image acquisition). Acquired images were opened in Multispectral software. Emission-based spectra were modeled and assigned for both the leaf autofluorescence and ICG. The assigned spectra were unmixed and the fluorescence signals representing autofluorescence, and ICG were pseudocolored as "Green" and "Magenta," respectively.

Discussion of Imaging Results and Associated Image Analysis

Macroscopic, epi-fluorescence images showed fluorescence emanating from both the leaf tissue and vasculature. To delineate the leaf autofluorescence from ICG signal, the different emission-based spectra were unmixed (Figure 1). The fluorescence images representing both the leaf autofluorescence and ICG were pseudocolored. Figure 2 shows that ICG effectively labeled the leaf vasculature, allowing for identification of venation patterns within the leaf. The highest fluorescence intensities, as depicted by the white color, could be seen in the primary veins with higher order veins branching off (Figure 2, middle panel). The ICG fluorescence could ultimately be used to quantify venation patterns such as vein density, free vein endings, vein loops, and the number of vein branch points.[2] This information would be vital for linking experimental and theoretical models of vascular patterning in leaves. In addition, the ability to separate different fluorescence spectra from an image could be useful for simultaneously monitoring expression of multiple-vasculature proteins or detecting the spatial location of a protein in relation to the entire vasculature network.

Figure 2

autofluorescence from the leaf was pseudocolored as green, while the indocyanine green fluorescence image was pseudocolored as magenta. The autofluorescence and indocyanine green images were overlaid (bottom panel) to provide a map of the leaf's vasculature network.

References

1. Kang, J.; Dengler, N. Int. J. Plant Sci. 2004, 165, 231-242
2. Rolland-Lagan, A.; Amin, M.; Pakulska, M. Plant J. 2009, 57, 195-205
3. Cnops, G.; Neyt, P.; Raes, J. et al. Plant Cell 2006, 18, 852-866
4. Wenzel, C. L.; Schuetz, M.; Yu, Q.; Mattsson, J. Plant J. 2007, 49, 387-398
5. Feugier, F. G.; Iwasa, Y. J. Theor. Biol. 2006, 243, 235-244
6. "Indocyanine green" National Cancer Institute. http://www.cancer.gov/Templates/drugdictionary.aspx?CdrID=540122
7. Scarpella, E.; Marcos, D.; Friml, J.; Berleth, T. Gene Dev. 2006, 20, 1015-1027

www.bruker.com **Bruker BioSpin**
info@bruker.com