TLC Analyzer

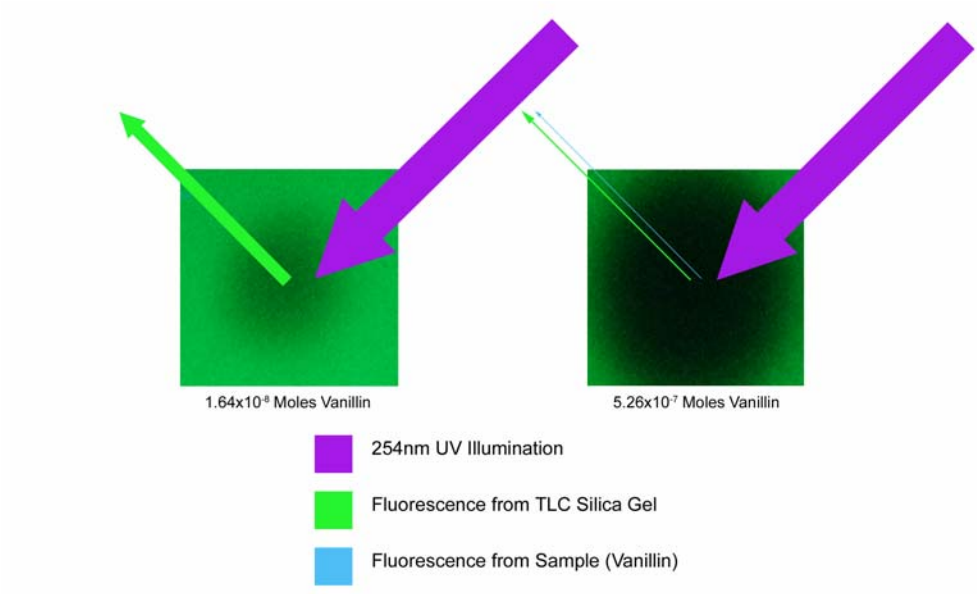
TLC Analyzer is a computer program that analyzes digital images of TLC plates to help you create multispectral scans, densitograms, and calibration curves. To learn more and/or to download a copy, visit this website:

http://www.sciencebuddies.org/science-research-papers/tlc_analyzer.shtml

Supplemental Information

The TLC Plate under UV Illumination

To understand the results of DE TLC, it is important to consider what happens to a fluorescent TLC plate subjected to UV light. The UV lamp emits 254 nm light that can either hit the silica gel on the plate or the sample on its surface. The silica gel will fluoresce green light, while the sample will generally absorb the light and block the light from reaching the silica gel. Of course, in order for the sample to absorb UV light and block it from the silica gel, the sample must have a high extinction coefficient (the amount of light absorbed and scattered) near 254 nm. Some chemical samples will themselves fluoresce like the silica gel on the plate, but at different wavelengths. The amount of the sample in the spot determines how much of the silica gel that the UV light is able to reach. If the concentration of a sample on the plate is relatively high, the chemical's spot will be dark under UV light. The UV light can also go between some of the molecules in the sample, hitting the silica gel. Thus, if the concentration of a sample is relatively low, the spot will appear greenish gray under UV light. In summary, the fluorescent green light that is seen within the sample's spot occurs when the UV light goes between spaces in the chemical and excites the silica gel on the plate.



The lower the concentration of the sample on a TLC plate, the more 254 nm UV light that can go between molecules of the sample to reach the green fluorescing compound in the silica gel on the plate. As the concentration of the sample increases, less and less UV light reaches the silica gel and the spot becomes darker (unless it is an extremely bright fluorescent compound in its own right).

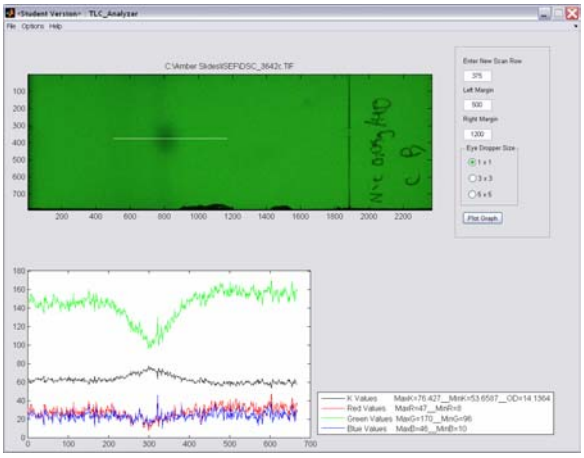
Extended Error Analysis

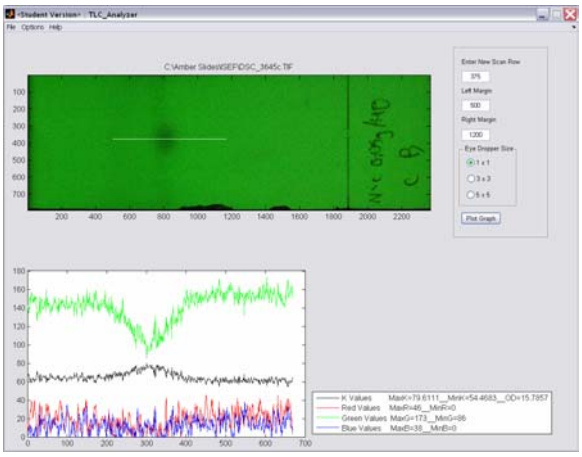
Table 3: Error Analysis Summary

Potential Source of Error	Potential Magnitude of Error	Magnitude of Error after Control	Comments
Errors Due to Improper TLC Technique			
Incorrect sample measurement	Can be large	+/- 1%	By using microcaps and using them correctly, this source of error is easily controlled.
Irregular shape of spot	Varies	Small	Should throw out bad spots and practice spotting.
Outside limits of detection	Can be large	Small	Make sure that the concentration is within the detection limits for DE TLC (this depends upon the compound).
Errors Due to Noise			In general, noise can be controlled by averaging pixel brightness values using the eyedropper in TLC Analyzer.
File compression	K= +/- 0-1% G= +/- 1-2% B= +/- 1-6%	Insignificant	JPEG compression is lossy and it creates noise in the image, so do not save JPEG images multiple times. The same image saved as a TIFF and a JPEG will have different brightness values. This research had good results using high-quality JPEGs (which actually have more noise than TIFFs).
Image detector	Noise can be extreme	Insignificant	The higher the ISO speed setting of the camera, the more noise. Note that the appropriate ISO speed varies for different cameras, but lower is always less noisy.
Irregularities in a plate	K= +/- 3% G= +/- 4% B= +/- 25%	Insignificant	TLC plates are not completely uniform, so the brightness values will vary across the plate.

Potential Source of Error	Potential Magnitude of Error	Magnitude of Error after Control	Comments
Irregularities between plates	?	?	This is hard to measure because all of the other variables are masking the error. However, it is a fact that each plate is different from another.
Error due to solvent ratio changes	?	?	Different solvents make the background of the TLC plates lighter or darker. This needs further research.
Errors Due to Photographic Technique			
Inconsistent exposure	Varies	Insignificant	The exposure must remain constant when taking pictures. This means that the lens aperture, focal length, exposure time, ISO speed, and focus should remain constant. This is why a camera with manual exposure must be used.
UV lamp not warmed up	+/- 10%	Small	Lamps should be warmed up for at least five minutes before taking pictures.
Non-uniform illumination	+/- 10%-20%	Small	The lamp, camera, and TLC plate must be kept the same distance from each other for each picture.
Lens light falloff	Varies	Insignificant	Light falloff can be controlled if the image is centered in the frame.
Errors Due to Analytical Technique			
Scan row misses the actual min/max	+/- 1%-3%	Insignificant	It can be difficult to locate the part of the spot with the absolute minimums and maximums for brightness values. To control for this error, TLC Analyzer optionally finds the minimums and maximums for RGBK values that are a certain number of rows above and below the specified scan row.

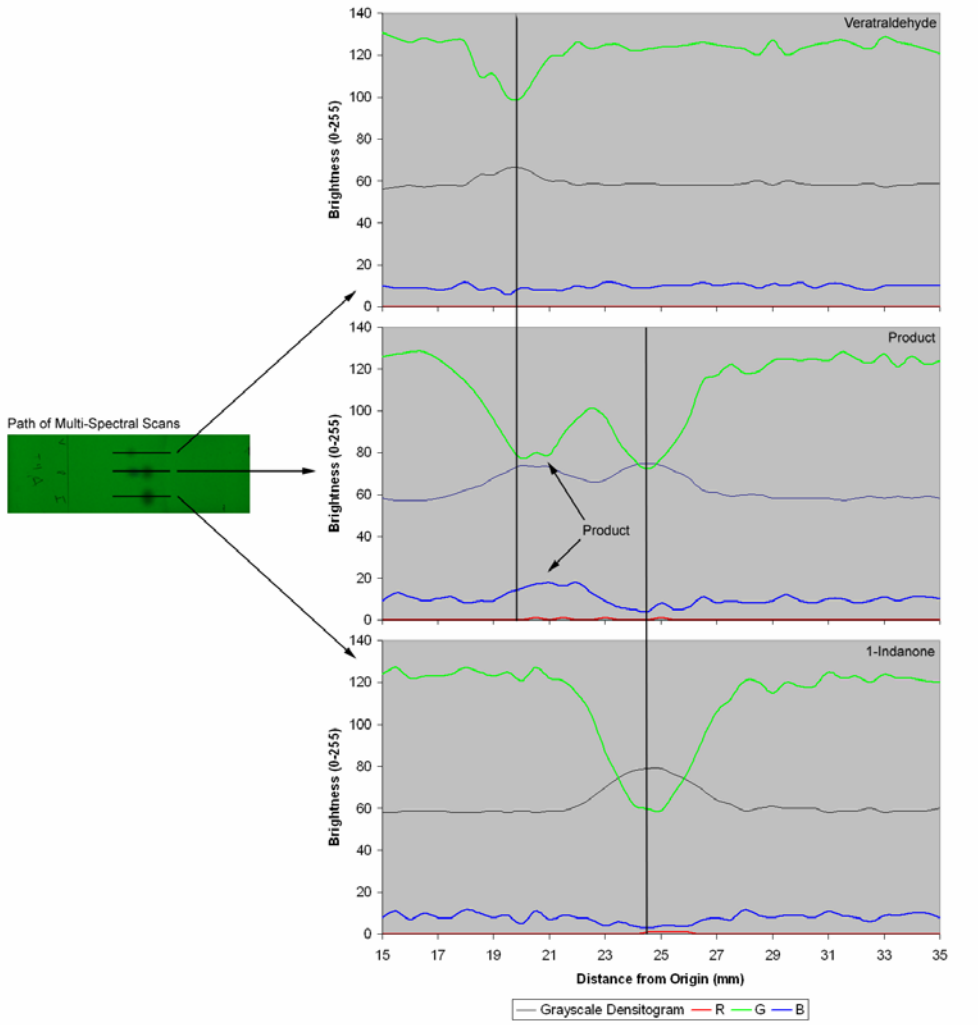
Potential Source of Error	Potential Magnitude of Error	Magnitude of Error after Control	Comments
Black and white densitogram hides certain phenomena	Varies	Small	Black and white calibration curves are comprised of an "average" of the red, green, and blue components. Sometimes one monochromatic curve is better than another monochromatic curve, or even the composite (black and white) curve. For example, for nicotinamide the green monochromatic calibration curve has a higher R^2 than the blue curve's. Since nicotinamide fluoresces blue light, I hypothesize that as the concentration increases, both the green and blue light from the plate decreases in intensity linearly. But the fluorescent blue light from the nicotinamide sample increases with concentration, so the blue brightness values do not decrease as linearly as the green values, giving the blue monochromatic curve a low R^2 value. To control this error, use the best curve, whether it be monochromatic or composite.





An example of noise: The ISO speed of the camera denotes how sensitive the image detector is to light. The higher the ISO speed setting of the camera, the more noise. Shown above is a multi-spectral scan from TLC Analyzer at ISO 400 (left) and ISO 3200 (right) for the same TLC plate.

Additional Example of a Multi-spectral Scan



Multi-spectral scan of a TLC plate created by plotting brightness values from Adobe Photoshop. Three different samples were put on the plate: the two on either side of the plate are the starting materials in a synthesis, while the spots in the middle are from a sample of the reactants after a few minutes of reaction time. The product is hidden by one of the starting materials (Veratraldehyde) because they have very similar R_f values. The product is fluorescent because it is a relatively rigid molecule, and by looking at a multi-spectral scan of the plate, the two spots can be differentiated.