

Quantitative Hyperspectral Imaging Technique for Measuring Material Degradation Effects and Analyzing TLC Plate Traces

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In forensic document analysis, multi-spectral reflectance and luminescence imaging techniques are routinely used for distinguishing inks and for enhancing the legibility of faint or invisible writing. The transition from conventional, qualitative spectral imaging to quantitative hyperspectral imaging (QHSI) made possible by the SENTINEL instrument facilitates and enhances the applicability of the technique to less common tasks. Several simple demonstration experiments were carried out to illustrate how the QHSI technique can be used in 2 application areas, the study of degradation effects in materials and the analysis of thin layer chromatography (TLC) plates. As examples for the 1st application area, the changes in the reflectance and luminescence characteristics of paper and writing induced by exposure to sunlight and strong UV light were measured with the QHSI instrument. As an example for the 2nd application area, the SENTINEL instrument was used to measure a TLC plate with ink samples. Based on the large number of calibrated reflectance and luminescence images, one can generate false-color images that facilitate the visual comparison of the positions and intensity of bands. A more detailed analysis is possible by extracting numeric cross-section data along the different sample traces.

Introduction

Spectral imaging devices are routinely used for the nondestructive analysis of the reflectance and luminescence characteristics of questioned documents. The new generation of quantitative hyperspectral imagers differs from conventional spectral imaging devices in 2 important ways. First, the new instruments feature a considerable increase in the number of independent spectral bands which can amount up to several hundreds. This larger number of independent bands increases the chances of finding differences between the compared features. The use of multi-channel processing on hyperspectral reflectance images for differentiating inks and deciphering obliterated entries is demonstrated in Attas (2004) and Ostrum (2006).

The 2nd, more important improvement as compared to conventional devices is that a quantitative hyperspectral imager provides a calibrated spectral curve for every pixel of the recorded scene. This has several important

advantages for the way in which the data can be analyzed.

The 1st advantage is that spectral curves can be easily extracted from all desired locations in the image, plotted together in diagrams, and processed numerically so that any differences between the curves can be established in a quantitative way. This means that a more objective comparison can be made as compared to the somewhat subjective visual comparison based on the conventional spectral images. In addition, a proper calibration of the hyperspectral data makes it possible to compare features in samples that are not available for simultaneous measurement. Because the calibration links the measurement results to general physical quantities such as the spectral reflectance, even the results from different instruments can be compared.

The 2nd advantage of quantitative hyperspectral imaging is the possibility to optimize the workflow of a case investigation. The tasks of measuring a document sample and of analyzing the results with respect to particular forensic

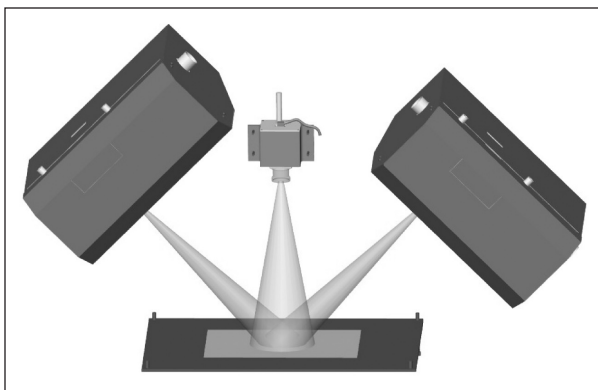


Figure 1 Schematic setup of the SENTINEL quantitative hyperspectral imager

questions have become quite independent of each other. After having measured all available spectral bands of a document in a single, relatively short session, the forensic analysis on the collected data can be performed off-line. This means that the document itself is no longer required and can be forwarded for further processing with other, possibly destructive analysis techniques. The measurement data can

be analyzed simultaneously by several experts in order to reduce the total processing time or to address different forensic questions. It is even possible to revisit the measurement data at a much later stage in the case to follow hints that may come from other analysis techniques or new questions that may arise from other circumstances of the case.

Methods and Materials

For the work described in this article, an experimental prototype instrument of the SENTINEL Quantitative Hyperspectral Imager was used. The instrument is developed and commercialized by the company DEMCON (DEMCON Advanced Mechatronics b.v., Zupthenstraat 25, 7575 EJ Oldenzaal, The Netherlands, www.demcon.nl; www.hsi-sentinel.nl). The construction of a similar prototype instrument for recording hyperspectral reflectance images of historical documents and techniques applied for calibrating and analyzing the hyperspectral data are described in detail in Klein, et al. (2008).

The imaging configuration of the SENTINEL instrument is shown schematically in Figure 1. The examined document is illuminated by 2

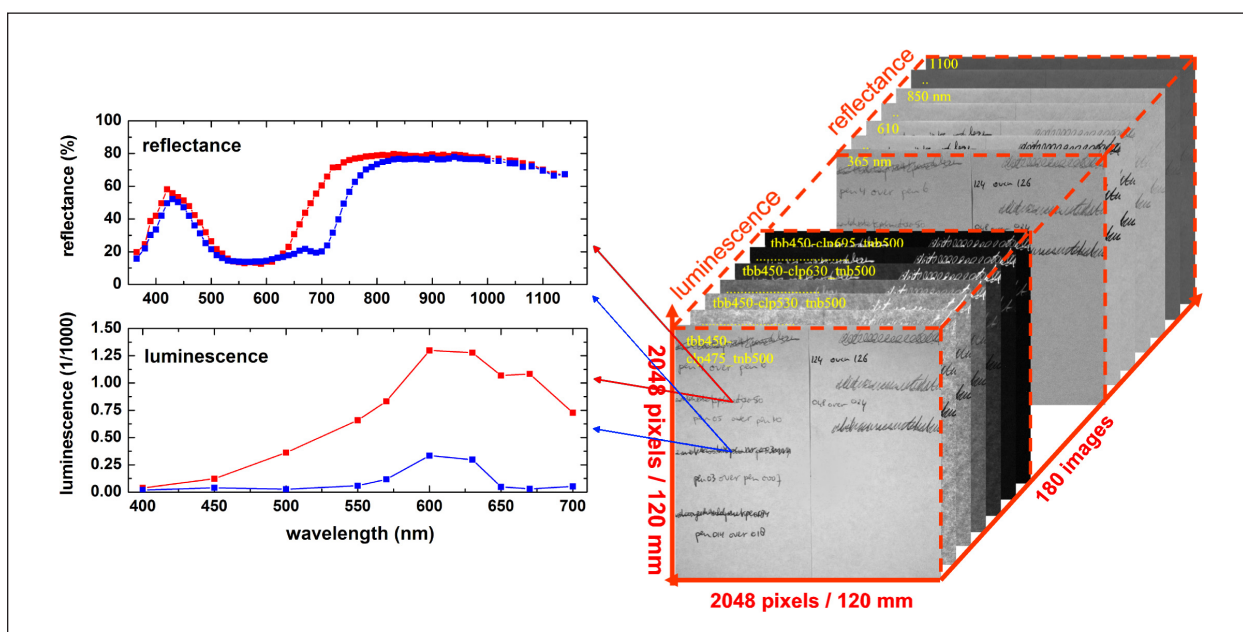


Figure 2 The hyperspectral data cube consists of the calibrated spectral reflectance and luminescence images of the recorded document area. For each point on the document, the entire reflectance and luminescence curves can be extracted. The illustration indicates the spectral reflectance and luminescence curves for 2 ink areas as blue and red curves. The data cube of a standard measurement with the SENTINEL instrument contains 180 images, each with 4 megapixel resolution.

wavelength tunable light sources and imaged by a 4-megapixel CCD camera equipped with a filter wheel. In the light sources, as well as in the camera, spectral discrimination is achieved by using discrete optical filters. As compared to electronically tunable components such as liquid-crystal or acousto-optic tunable filters, discrete optical filters have considerably higher light transmission at a given spectral bandwidth, which translates to a lower noise and corresponding higher sensitivity of the measurements.

By combining the many excitation wavelength bands provided by the light sources with the various spectral filters of the camera, 73 reflectance and 107 independent fluorescence/luminescence spectral bands are realized. The high-resolution images are recorded in the wavelength range from 365 to 1160 nm, thus covering the near-ultraviolet, visible, and near-infrared parts of the light spectrum.

During a typical measurement, the device software automatically steps through an entire sequence of spectral bands preselected by the user out of the list of available bands in the graphical user interface of the instrument control software. For each band the camera focus, exposure time, and other acquisition parameters are adjusted before recording and storing the image. These raw images can be used for the conventional qualitative analysis of the document.

In order to accurately calibrate these images for further quantitative analysis, spectral images of a homogeneous white reference surface with well known spectral characteristics are recorded with the same settings as were used for the document recording. Based on the raw images, the reference images, and further instrument calibration data such as the sensor dark levels, the software then calculates the calibrated spectral images.

Due to the calibration, the pixel values in the resulting images—unlike those in the raw spectral images used for conventional analysis—become independent of the specific light distribution and sensitivity of the CCD camera in the different bands. A pixel value in, for example, a calibrated reflectance image gives the exact spectral reflectance value of the corresponding point on the document at a specific wavelength.

The complete set of calibrated spectral images resulting from a measurement is called the *hyperspectral data cube* (Figure 2). The standard hyperspectral data cube from the SENTINEL instrument contains for each pixel coordinate 180 reflectance and luminescence spectral values

of the corresponding location on the document, which amounts to 1.5 gigabytes of data. The analysis of the data was carried out on conventional PCs (dualcore or quadcore processors, 2-3 GHz) using the prototype instrument software and prototype analysis functions (MathWorks Matlab®) for the calibration calculations and further processing.

Quantitative Analysis

The numerical data contained in the hyperspectral data cube forms the basis for the quantitative spectral analysis of the questioned document. For example, in order to compare different ink areas, one can define regions of interest (ROIs) of arbitrary shapes in these areas. With the processing software, the mean spectral curves of the pixels in each ROI can then be calculated. It is also possible to have the software automatically search the entire data cube for those images that show the maximum contrast between the marked ink areas. A statistical analysis of the pixel spectral values in the different areas can provide information about how significant any observed difference of the mean values is.

All spectral images contained in the hyperspectral data cube are registered; i.e., a certain pixel coordinate corresponds to exactly the same location on the document in all images. Using software such as Adobe® Photoshop® one can, therefore, easily combine any of the grayscale reflectance or luminescence images to artificial-color images in which varying spectral characteristics are visualized as different color shades.

A very important advantage of using the calibrated spectral data for the analysis of a questioned document is the possibility to apply powerful image processing algorithms (Berger and others 2006, 2009; Weaver 2008) that detect statistical correlations of the data in multiple bands, which is not possible by simple visual comparison of the images. In satellite imagery, such algorithms are routinely used to detect objects having anomalous reflectance spectra and map the distribution of different surface types in the recorded scene. In the field of questioned document analysis, such multichannel processing can be used to improve the capability of distinguishing inks and to enhance the visibility of faint features significantly as compared to the conventional single-channel analysis.

The advantages of using a calibrated *hyperspectral data cube* will be shown with 2 different applications.

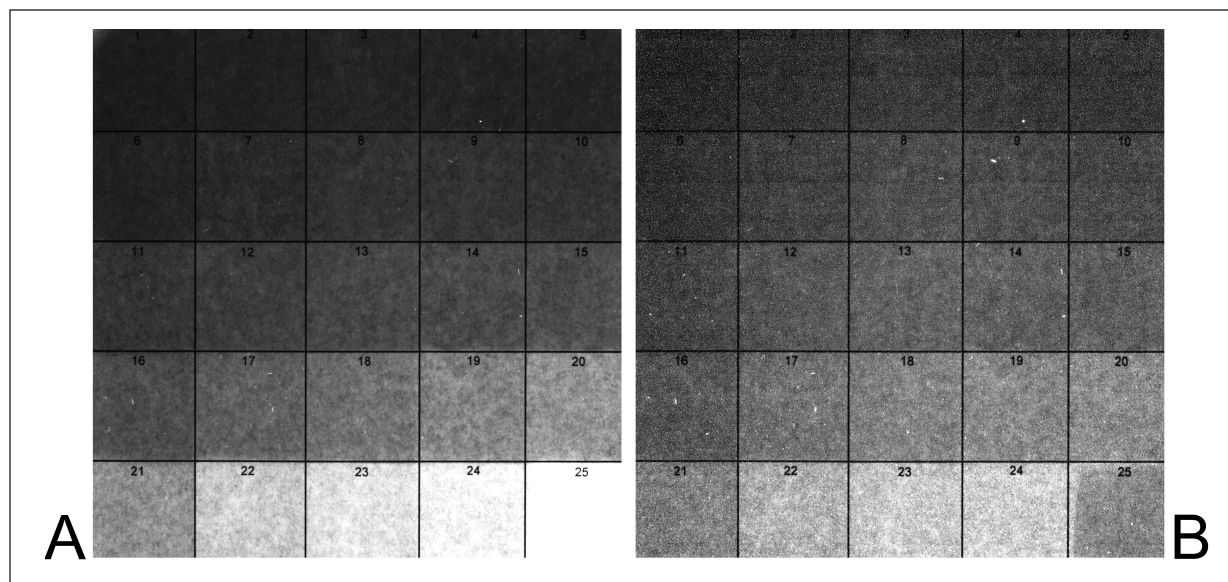


Figure 3 Calibrated luminescence images of a white laser printer paper sample aged by exposure to daylight for the duration of 0 days (square #25; bottom right) to 24 days (square #1; top left). A: Excitation wavelength 570 nm / imaging long-pass filter 630 nm. B: Excitation wavelength 650 nm / imaging long-pass filter 715 nm. Within the 1st day of exposure (square #25 to #24), the luminescence changes in opposite directions for both channels.

Application 1:

Degradation Effects of Paper and Ink

It is, of course, very well known that when paper or ink is exposed to light for extended periods of time its spectral characteristics can change. In fact, using the hyperspectral imager, one can measure that considerable changes can occur, especially in the fluorescence/luminescence spectra within only a few hours of exposing a document; e.g., on an office desk well illuminated by daylight.

In most cases, the luminescence signal decreases with increasing light exposure as the induced photochemical processes decompose the fluorescing agents. However, depending on the material, some luminescence bands may increase due to the formation of degradation materials. To demonstrate these effects, we carried out a few simple light-exposure experiments.

A sample was made from white laser printer paper which was divided into 25 squares. The sample was put on an office desk and exposed (through the window) to daylight for a total of 24 days. On the 1st day, all squares except square #1 were covered by light-proof cardboard; and on every following day, an additional square was exposed. Square #25 was not exposed at all.

In a 2nd experiment, 9 different inks were exposed to daylight (transmitted through a window) for time spans ranging from 0 to 15 days.

The 3rd experiment consisted of exposing different ink writing samples to a high-intensity UV source (365 nm) for a total period of 173 hours, performing a quantitative hyperspectral reflectance measurement every 10 to 20 hours.

Application 2:

Analysis of TLC Plates

When using thin-layer chromatography (TLC), e.g., for analyzing ink samples, it is common practice to begin with a straightforward visual comparison of the position, relative intensity, and color of the TLC bands and to use a conventional digital camera or flatbed scanner for documentation. As a 2nd step, sometimes spectral reflectance and fluorescence images of the TLC plate are taken with available multi-spectral instruments. Especially in the fluorescence mode, additional bands may become visible and help to distinguish different inks. Where available, a special TLC-scanning device (e.g., Camag TLC scanner) is used that provides spectral intensity cross-sections along the sample columns (tracks) on the TLC

plates and also reflectance curves at desired locations in the bands. This quantitative data helps to detect small differences in the samples more reliably and objectively. As an alternative to such a special TLC-scanning device, we used the calibrated reflectance and luminescence data provided by the hyperspectral imager to analyze a TLC plate. The spectral curves contained in the hyperspectral data cube for all locations along the columns allow a quantitative comparison that can reveal very subtle differences between the different columns that are not obvious at 1st glance.

Results

Application 1:

Degradation Effects of Paper and Ink

Figure 3 shows 2 calibrated luminescence images of a sample made from white laser printer paper, which was divided into 25 squares which were sequentially exposed to daylight over a period of 24 days. The luminescence image in Figure 3A, which was recorded at a wavelength combination of 570 / 630 nm (excitation / long-pass imaging), shows that within a single day (square #25 to square #24) the luminescence signal was reduced drastically. However, the 650 / 715 nm image in Figure 3B demonstrates that at a different luminescence band, the signal actually *increased* significantly in the same period. For longer exposures, the signal in both channels decreased gradually.

Figure 4 shows for both bands the average luminescence signal strength of a square as a function of the exposure time. The curves confirm that the 650 / 715 nm signal increased by about 40% after the 1st day of exposure, while the 570 / 630 nm signal decreased monotonously from the beginning of the exposure or the paper to daylight.

A possible explanation is that chemical reactions induced by the light have as an intermediate product an agent that shows luminescence at the 650 / 715 nm band. This agent itself then further degrades, which results in a reduction of the luminescence in the course of the following days of exposure.

To investigate how the spectral characteristics of inks can change by exposure to light, an experiment was performed in which inks were exposed for various time spans. Nine different ink samples were arranged in 16 identical 3x3 arrays on the paper (Mondi BioTop 3, 80g/m²). Each of the 16 square arrays was exposed to sunlight for

a different number of days. As can be seen in the real-color image in Figure 5A, some of the inks exhibit considerable bleaching and discoloration in the visible wavelength range. Similar to the light-aging of paper, the luminescence signal may either decrease, increase, or remain constant with increasing light dosage, depending on the spectral band and on the ink. The artificial-color image in Figure 5B, which is composed from 3 calibrated luminescence images, shows a luminescence reduction for the ink that strongly luminesces in the 550 / 750 nm band (excitation / luminescence, artificial green), an increase for the ink luminescing at the 450 / 600 nm band (artificial red), and remains constant for the ink luminescing at the 400 / 550 nm band (artificial blue) with increasing exposure to the daylight.

In Figure 6, the signal at 3 luminescence channels for 1 particular ink (left diagram) and for 4 blue inks at the 400 / 550 nm luminescence channel (right diagram) are plotted as a function of the number of days the corresponding samples were exposed. It should be noted that the light dosage per day was not controlled in this experiment, so that the absolute rates with which the luminescence signals changed as a function of the absorbed light energy cannot be derived from these graphs. However, for any given day, all 9 ink samples received the same dosage, so that the development of their luminescence signals over the exposure days can be compared to each other. Whether the luminescence remains constant, increases, or decreases, and with which rate it changes depends on both the type of ink, on the measured luminescence channel, and on the number of exposure days. It can be concluded from these results that there is no general rule how exposure to light may have altered the luminescence signal of an ink in a given situation.

The strong sensitivity of the luminescence characteristics of paper and ink to light exposure poses, therefore, a considerable risk of misinterpreting the measurement results when luminescence signals are used to compare and identify samples of unknown light-exposure history. In such cases, light-aging studies of reference samples may be required to establish correlated changes of the spectral bands that can help to find evidence for existing relations between the samples. The quantitative hyperspectral imaging technique can simplify and improve the reliability of such studies considerably because the spectral values are comparable even if the samples cannot be measured simultaneously.

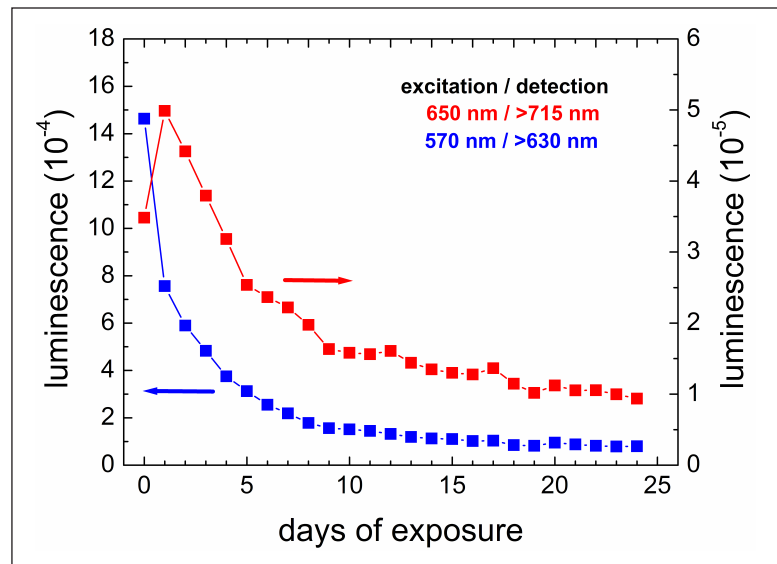


Figure 4 Average luminescence signal of the white paper as a function of the exposure time. Red (values on right y-axis): The 650 nm / 715 nm signal increases for the 1st day, then decreases. Blue (values on left y-axis): The 570 nm / 630 nm signal decreases monotonously.

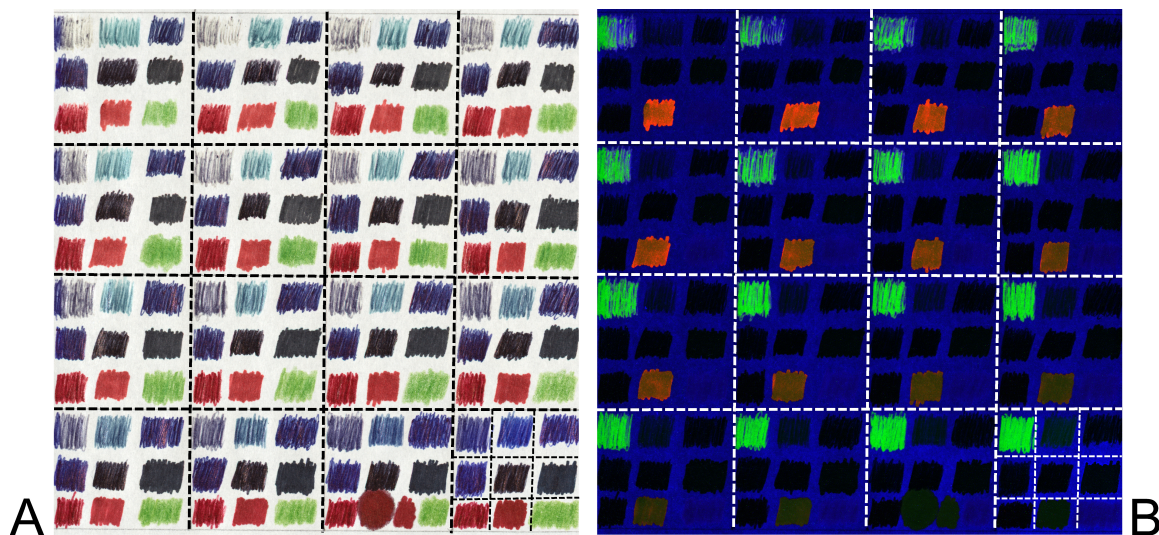


Figure 5 Paper with 16 identical 3x3 arrays of 9 ink samples aged by exposure to daylight for up to 15 days. The square sample array at the bottom-right was never exposed, the square sample array at the top-left for 15 days. Within each row the exposure increases from left to right. A: Real-color image showing bleaching and discoloration especially of the dark blue inks. B: False-color image from 3 calibrated luminescence channels (R: 450 / 600 nm; G: 550 / 750 nm; B: 400 / 550 nm) showing examples for inks with increasing (red), decreasing (green), and constant (blue) luminescence signal for increasing light dose.

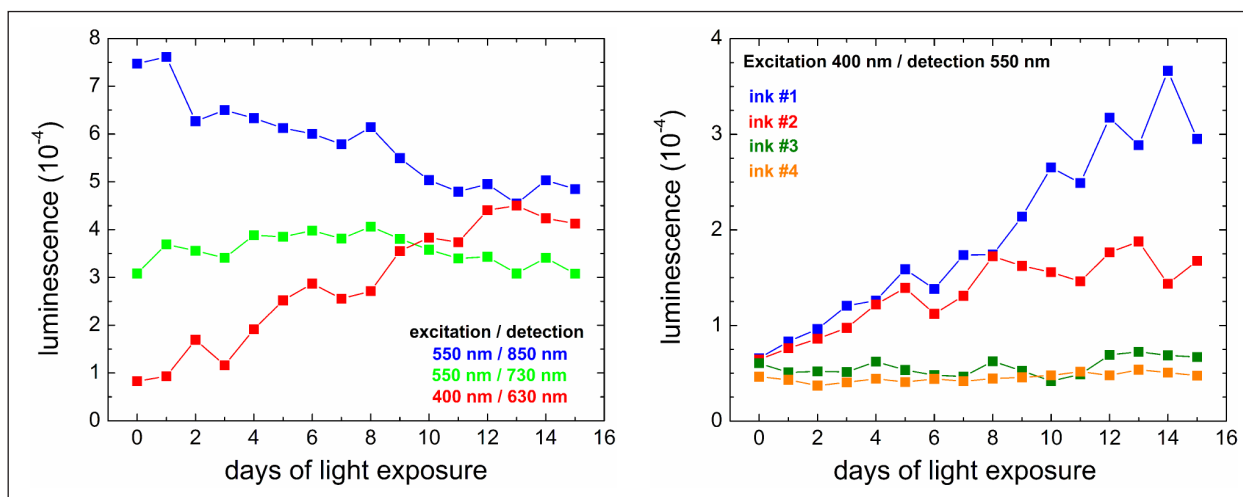


Figure 6 Left: Mean values of 3 different luminescence channels of areas of the same ink as a function of the number of days of exposure to daylight. Right: Mean values of 4 blue inks at 1 particular luminescence channel as a function of the number of exposure days. Whether the luminescence signal increases, decreases, or remains constant with the exposure depends on the type of ink, the luminescence channel, and the number of exposure days.

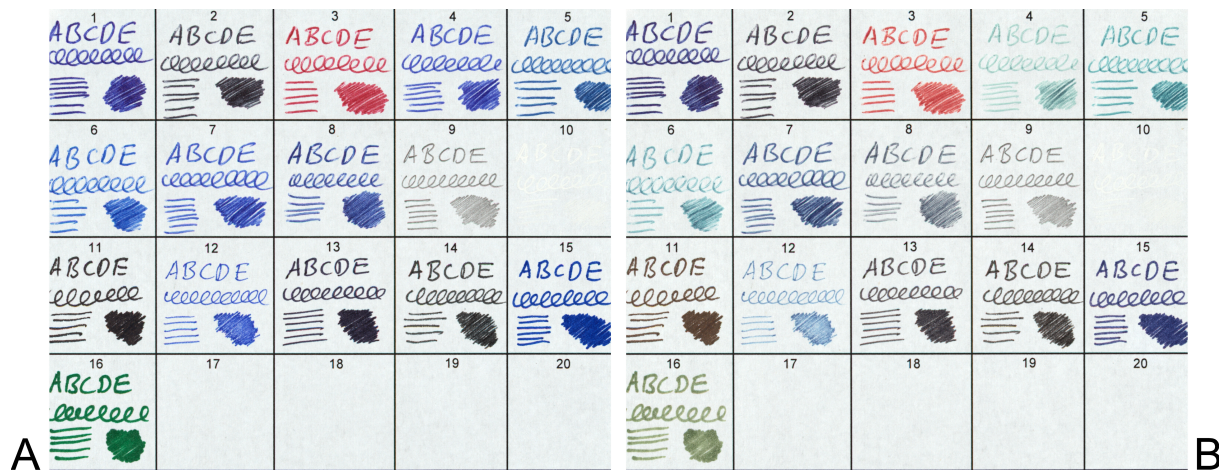


Figure 7 Real-color images of writing samples exposed to high-intensity 365 nm UV light source. A: Before exposure to UV light. B: After 173 hours of exposure. All writings have become fainter, and many have changed in color such as samples 4 and 5.

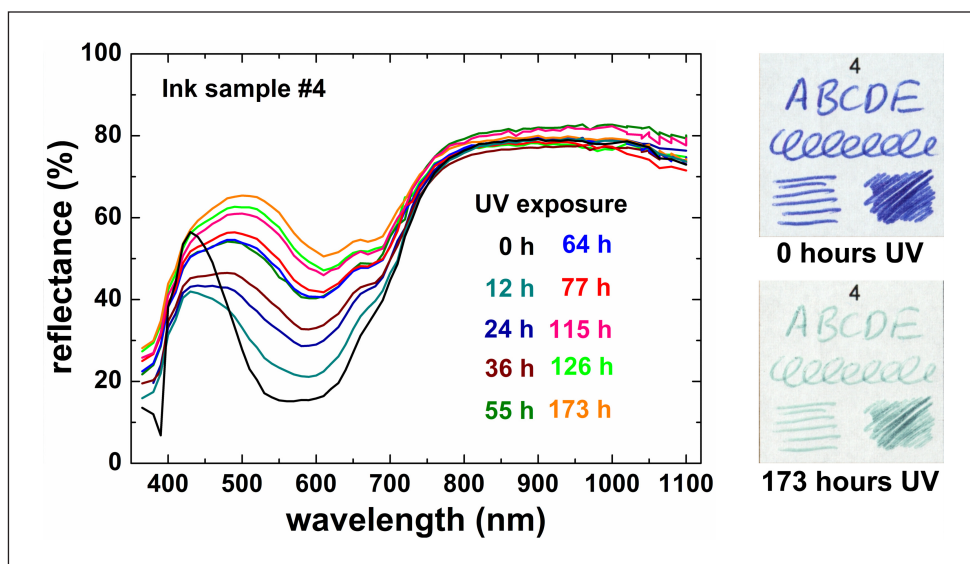


Figure 8 Calibrated reflectance curves of ink sample #4 measured after different exposures to a high-intensity UV-source. With increasing UV exposure, the curves describe higher reflectance values; i.e., the writing becomes fainter, and the curves change in shape, which means that the color of the ink changes. Both effects can be seen clearly in the real-color images taken before and after 173 hours of UV-exposure.

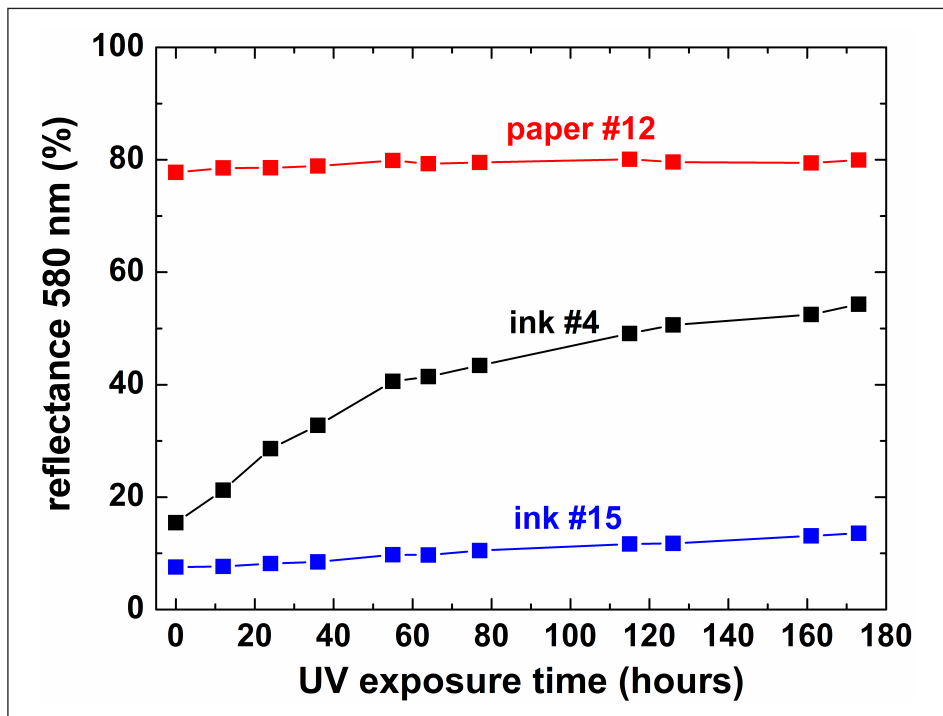


Figure 9 Reflectance values of 2 blue-ink samples and the paper substrate at 580 nm as a function of the UV exposure. At this particular wavelength, ink #4 bleaches much faster than ink #15.

Figure 7 shows color images of different ink writing samples that were aged by exposing them to a high-intensity UV source (high pressure Hg-lamp Black-Ray Model B100AP, wavelength circa 365 nm). Figure 7A shows the samples before the UV exposure and Figure 7B after the maximum exposure of 173 hours. After every 10 to 20 hours of exposure, a quantitative hyperspectral reflectance measurement of the sample sheet was carried out. From the resulting hyperspectral data cubes, the spectral curves of all the ink samples and the substrate can be extracted and compared.

Figure 8 shows the spectral curves of the blue-ink sample #4 for the different UV exposure times. In the infrared beyond 800 nm, the ink is fairly transparent, both before and after the UV exposure, which can be seen by the high reflectance value caused by the paper substrate. However, in the visible range, with increasing UV exposure the curves describe higher spectral values and also change their shapes. In the real-color images of the sample, it can be seen that the writing becomes more transparent and also its color changes considerably.

Figure 9 shows the 580 nm spectral reflectance values as a function of the UV exposure for 2 blue-ink samples and the paper substrate. While the paper reflectance hardly changes, both blue inks show a bleaching effect at this wavelength. However, ink #4 changed much faster than the darker ink #15.

Application 2:

Analysis of TLC plates

By measuring a TLC plate with the quantitative hyperspectral imager, both qualitative (visual) and quantitative analyses of the samples are possible on all spectral bands. Figure 10A shows the real-color reflectance image of a TLC plate with analysis tracks from 5 blue and 5 black ballpoint ink samples taken from the same piece of paper. By visual inspection, the 1st and 3rd samples from the left cannot be distinguished. However, in the luminescence regime, chromatograms can look quite different from what can be seen in the visible, or more generally in the reflectance regime. A convenient way to visualize the information from several luminescence bands simultaneously is to combine them to an artificial-color image as shown in Figure 10B. In this image, the chromatograms of samples 1 and 3 look completely different, so that one can conclude that these are different inks or the same ink with a

different history. McKasson (1980) explains that non-luminescent inks may contain dyes which luminesce after separation due to dequenching. As a dye mixture, the luminescence may be quenched due the fact that dyes may absorb each other's luminescence. This is often more apparent in the denser writing areas.

In addition to a visual comparison of the spectral images and (artificial-)color images derived from them, the calibrated spectral data provided by the SENTINEL instrument makes it possible to extract the cross-sections of the chromatograms in any reflectance or luminescence image. Figures 11A and B show a comparison of the cross-sections of the samples 1 and 3 at the 570 nm reflectance band and the 500 / 600 nm luminescence band, respectively. As compared to the grayscale or color images of the TLC plate, the cross-sections make it easier to determine the exact positions, shapes, and intensity ratios of the peaks. The cross-sections of the reflectance image show peaks at exactly the same positions and with comparable peak heights for samples 1 and 3, so that they cannot be distinguished. In the 500 / 600 nm luminescence cross-section (green channel in the artificial-color image of Figure 10), sample 1 shows an enhanced peak at the 18 mm position and an additional large peak at the 24 mm position which is not present in the cross-sections of sample 3.

Conclusion

In this article, we demonstrate some of the possibilities of the quantitative hyperspectral imaging technique for applications in the field of questioned document analysis. As opposed to conventional spectral imaging, quantitative hyperspectral imaging provides a hyperspectral data cube which contains the calibrated spectral reflectance and luminescence curves for each pixel. The calibrated data can be used to study treatment and degradation effects on writing materials and to visualize the measurement results.

Commonly occurring degradation effects such as caused by exposure of documents to daylight over time spans of a few days can be very relevant for the interpretation of the results of forensic document analysis based on spectral imaging. In particular, the luminescence signal of inks and paper can change considerably due to exposure to daylight. Depending on the material and the specific luminescence band, the signal may increase, decrease, or remain constant with increasing light exposure. If the luminescence

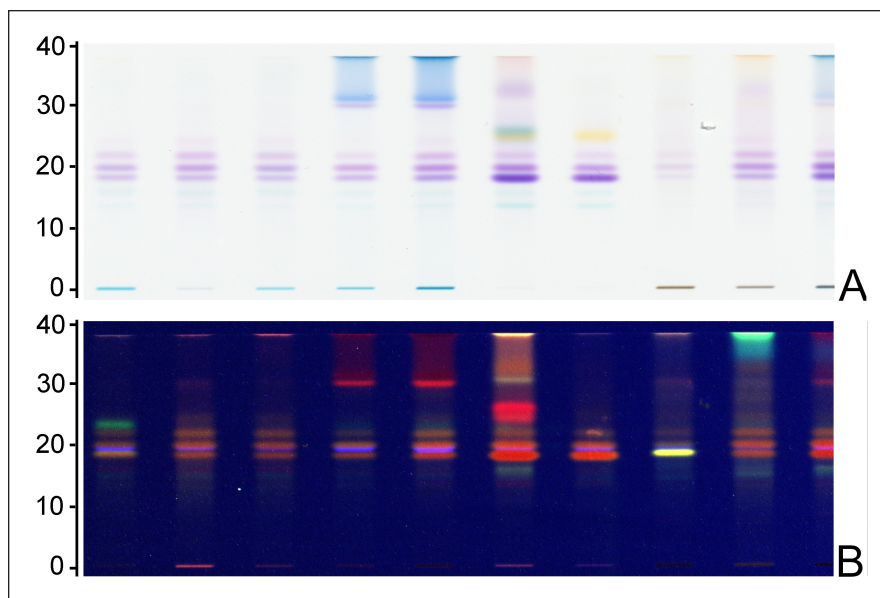


Figure 10 TLC separation of 5 blue and 5 black ballpoint pen inks on a LiChrospher Si 60 F254 TLC plate, extraction with pyridine, sample application using CAMAG ATS-4, solvent system: n-butanol/2-propanol/water/acetic acid – 20/10/10/1 (15 min.) followed by n-butanol/ethanol/water/acetic acid – 15/3/3.9/0.45 (30 min). The scales on the left indicate the position on the plate in millimetres. A: Real-color image in which the 1st and 3rd left-most columns look very similar. B: Artificial-color image composed from the 3 luminescence channels R: 550 / 650 nm; G: 500 / 600 nm; B: 400 / 500 nm. The luminescence of the ink on the far left is clearly different from that of the 3rd ink.

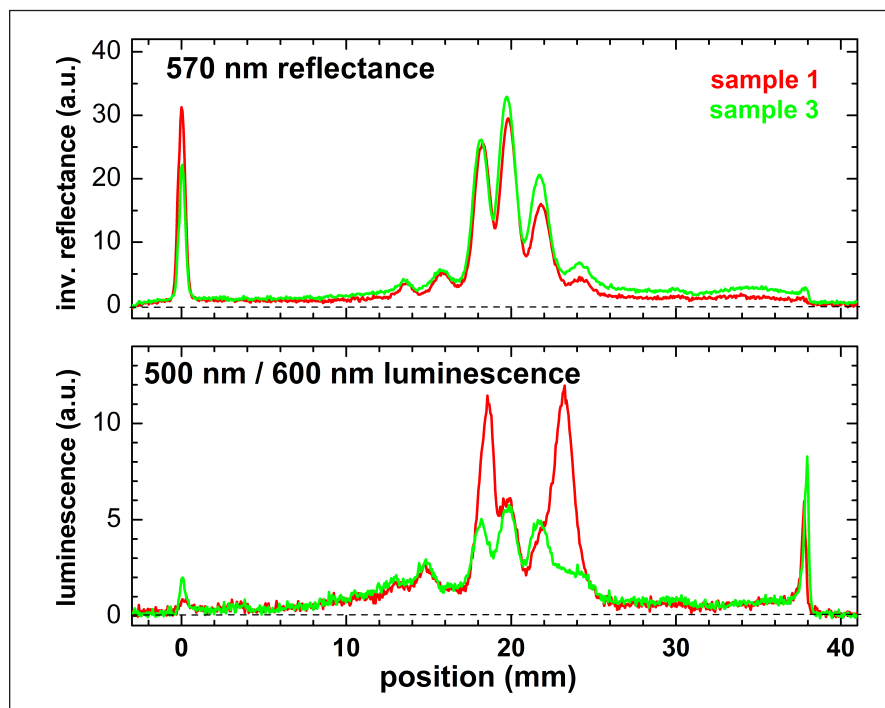


Figure 11 Cross-section of the (calibrated) spectral images of the TLC plate for the 1st and 3rd ink track from the left. A: Cross-sections in 570 nm reflectance image. The peaks of inks 1 and 3 appear at the same position and have comparable peak heights. B: Cross-sections in 500 / 600 nm luminescence image. Ink sample #1 has clearly different peak positions and relative peak heights as compared to #3.

of a sample is produced by unstable reaction products, the luminescence may even change in a non-monotonous way.

As a consequence, one has to be very careful with what can be concluded from the spectral analysis of a document if the light-exposure history is not known. For example, if a questioned document had been partly covered and partly exposed to light, the same ink can easily produce quite different luminescence spectra in the exposed and in the covered sections of the document, thus potentially suggesting the wrong conclusions.

In a case where a pen or paper is available that is suspected to match a questioned document, it may be required to verify a hypothesis about degradation or treatment-induced changes of the spectral characteristics by making a corresponding treatment study. The quantitative hyperspectral imaging technique is ideally suited to carry out such a study and to compare (in a nondestructive way) the resulting measurement data with the data from the original questioned document. This is because the recorded spectral images are all accurately calibrated to a general standard; and by this calibration the data become transferable, so that samples measured at different times or even at different places with different instruments can be compared.

Due to the numerical nature of the measurement data, the quantitative hyperspectral imaging technique can also support the analysis and the documentation of TLC plates which are commonly used to analyze inks. From the hyperspectral data cube, one can generate artificial-color images of the TLC plate which visualize, for example, the chromatograms recorded in different luminescence bands. It is also possible to extract the calibrated spectral values along cross-sections of the samples so that the positions, shapes, and relative heights of the peaks in the chromatograms can be compared. An additional advantage of the hyperspectral data cube is that it preserves the entire spectral information recorded from the TLC plate which itself may degrade within a short time.

In conclusion, our experiments with the SENTINEL quantitative hyperspectral imager demonstrated the possibilities to make the well established spectral imaging technique suitable for new applications in the field of forensic document analysis.

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