

## A New Method of Identifying Writing Sequence with the Laser Scanning Confocal Microscope\*

**REFERENCE:** Cheng KC, Chao CH, Jeng BS, Lee ST. A new method of identifying writing sequence with the laser scanning confocal microscope. *J Forensic Sci* 1998;43(2):348-352.

**ABSTRACT:** This paper describes and establishes a new method that examines the sequence of two crossing strokes in different colors in a questioned document. In order to identify the writing sequence, an empirical method is usually used to find out some physical evidence by human eyes with a microscope. However, owing to the phenomenon of mixed inks in the intersection, there is still some drawbacks with the conventional approaches. Hence, in addition to the empirical method, a new method using Laser Scanning Confocal Microscope (LSCM) is adopted. Unlike the conventional light source, the LSCM system uses a krypton argon laser source to emit a laser beam of three different wavelengths. The beam goes through a scanning control unit to act as the function of light scanning, and passes through the objective lens of microscope to the surface of crossing strokes. Reflected light goes the same pathway back to the scanning control unit and is collected by a photomultiplier where the image is reconstructed from a series of signals derived from the photomultiplier output, and saved onto a personal computer. It is necessary to scan the point probe over the field of view and capture sectional images in various depths. By overlapping every sectional image followed by computerized reconstruction, we can observe the evidence to help identify the writing sequence in the questioned document by means of a stereo spectacles. Experimental results show that with the help of the LSCM, together with the method we developed, more accurate judgments are achieved.

**KEYWORDS:** forensic science, questioned documents, writing sequence, laser scanning confocal microscope, krypton argon laser

Three methods are used in our laboratory to determine the writing sequences in a questioned document. First, an empirical method is usually used to find out some physical evidence by human eyes with a microscope. The physical evidence phenomenon is described as follows: It is very easy to find the evidence of the intersecting points where the last ink overlaps the first one for ball-pen drawing. Sometimes the second line gets narrower at the point crossing the first line (1). A simple method is used to determine the writing sequence of ball pen lines from the embossing phenomenon at the back of the paper (2). The continuity of the first line

at the point of intersection is always interrupted by the second line (the line on top) which helps to determine the writing sequence (3). Observation under ultra-violet light can only give decisive results when the two inks fluorescence are quite different. Unfortunately, this did not occur in any specimens we examined until we find LSCM method.

Second, "a typewriter's sticky lifter of the type" technique is used to lift the intersection of ball-pen strokes since the first line at the point of intersection is always interrupted by the second line. Normal writing paper proved to be acceptable if care was taken in applying and lifting the tape so that the paper fibers were not removed during the process. But when lines were drawn with various degrees of pressure, incorrect interpretations of line sequence results. For example, if the first line is drawn harder than the second intersecting line, the resulting lift gives the wrong sequence interpretation. That is, the first line seems to be drawn last. This illusion results when the second line get narrower and can not reach the bottom of the first line, causing a deeper furrow at their intersection. In effect, the lighter line skips over the deep furrow and therefore fails to lay down an ink layer (4).

The third method is determination of the writing sequence by measuring the hue error at the intersecting point and sample duplication (5). However, it is very difficult to collect the same writing instruments for sample duplication. Problems encounter in determining of the writing sequence include: when two ink lines intersect or are joined whilst the ink is still wet, the excess ink in both lines will merge at the intersection so that when dry, there will be no evidence from which the order of writing can be determined (6,7); regarding inks that dissolve into each other, the order in which two intersecting lines were drawn can never be established with certainty (8); and it is sometimes impossible to show which of the two was drawn first, especially if they are both lightly drawn and pale in color (8).

In order to tackle these problems, we have conducted experiments to establish a new method for examining the sequence of two different colored crossing strokes in a questioned document by using LSCM, which is detailed in the following sections.

### Materials and Methods

#### Materials

A Bio-Rad MRC 600 model & Zeiss Axioplan upright microscope and Comos MRC 680/11 version 6.05 software were used (see Fig. 1). The electronic parts of an LSCM are the light (krypton argon laser source) detector (PMT & discrete semiconductor sensor, integrated semiconductor sensor array), scan controller, analog signal processor, digital signal processor, image analyzer, host

<sup>1</sup>Scientific and Technical Research Center, MJIB; P.O. Box 3562, Taipei, Taiwan, R.O.C.

<sup>2</sup>Institute of Optical Sciences, National Central University; Chung-Li Taiwan, R.O.C.

<sup>3</sup>Institute of Graphic Arts, Chinese Culture University; Yang-ming-shan, Taipei, Taiwan, R.O.C.

\*This paper was presented at the 49th Annual Meeting of the American Academy of Forensic Sciences, New York, NY, Feb. 1997.

Received 24 March 1997; and in revised form 29 May and 1 July 1997; accepted 2 July 1997.

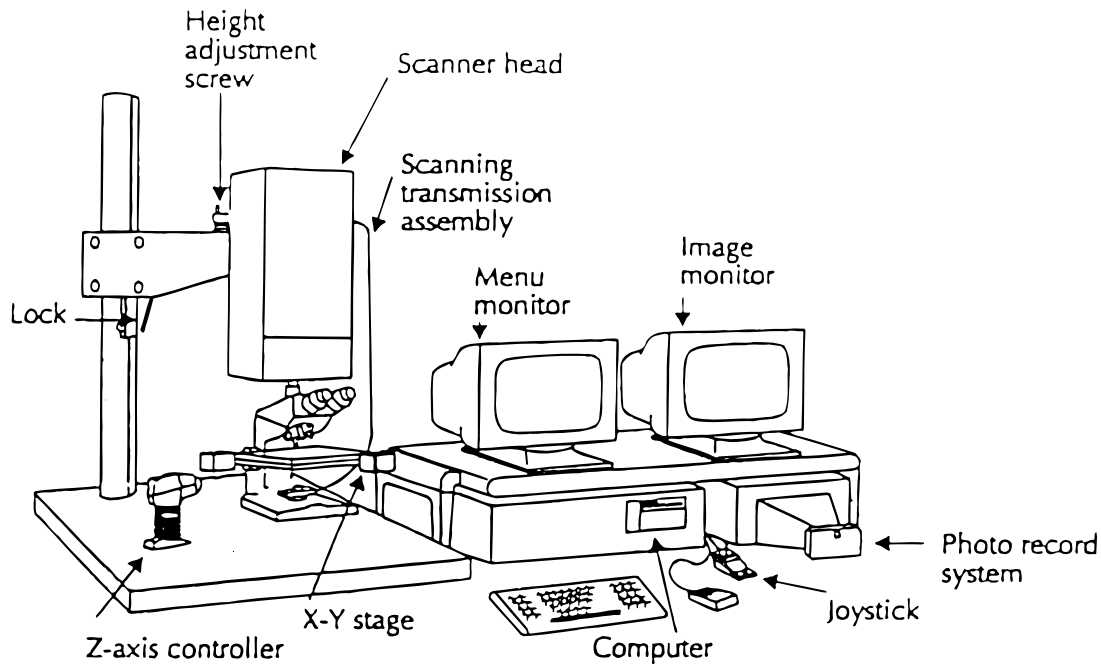


FIG. 1—BIO-RAD MRC 600 model and Zeiss Axioplan upright microscope.

computer and the peripherals. The scan controller, signals processors and image analyzer must communicate with each other under the control of the host computer, and finally we can examine the writing sequence by a pair of stereo spectacles.

In this experiment, 60 crossing line samples (30 homogeneous & 30 heterogeneous) were obtained from using 48 different color & 12 same color ball-pens and fiber-pens, all crossing were requested to make the line crossings using different writing pressures. We also used three colors of writing instruments, i.e., red, blue and black, and three kinds of filter block (BHS, YHS, RHS) together with each filter wavelength light (Filter No. 0–5, Neutral Density #0–3) for this experiments.

Note—homogeneous: The overlapping inks of two pens were the same based (glycol-based or water-based) ink.

Note—heterogeneous: The overlapping inks of two pens were the different based (one is glycol-based and the other is water-based) ink.

### Methods

The following describes the method for observing ball-pen ink crossings by LSCM. This technique does not damage or alter the document. It scans intersecting line areas probe over the field of view and capture sectional images in a series of varied depth (Z-

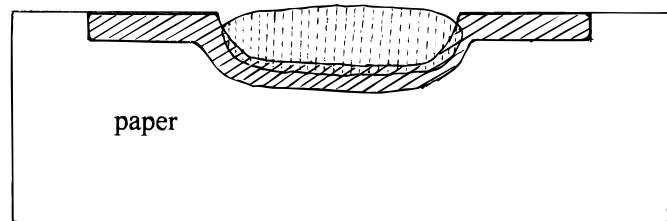


FIG. 2—The surface of paper is engraved in by a ball-pen tip and looks like a furrow. The ink of two colors is also in sequence absorbed by the fiber of paper.

axis) of the surface (XY-axis) for crossing strokes (see Fig. 2). The laser output power required for LSCM is usually in the 1 ~ 10 mW range, which is adequate for imaging in reflection mode and is acceptable for most specimens. For fluorescence applications, frequently the laser emission line doesn't coincide with the absorption maxima of many popular fluorochromes. These fluorochromes have been routinely used with the conventional fluorescence microscopes equipped with mercury burners. In order to overcome these problems, multi-line laser (ex., KrAr & HeNe lasers) or multi-lasers can be used for these fluorescence applications. Fluorescence or light reflection goes the same pathway back to the scanning control unit and was collected by a photomultiplier, then image is generated electronically from series signal derived from the photomultiplier output, finally saved onto a personal computer (9). By overlapping every sectional image (merging first image Fig. 3 & second image Fig. 4) followed by computerized reconstruction, we can see the evidence of the writing sequence in the questioned document by means of a stereo spectacles (10).

### Results

The results of the experiments are given in Table 1. It is evident from Table 1 that the writing sequence of the colored crossing can be clearly and correctly determined by the LSCM method and observed under the stereo spectacles in about 75% (45 samples) of the case. In the remaining 25% (15 samples), 3 samples could not be determined correctly because one or both inks of the strokes cannot be illuminated further. The other 12 samples are of the same color. Therefore, in the latter case the ink is dissolved into each other. Hence it is not clear enough for us to save the illuminated images in varied depths.

Three sets of two intersecting lines of writing used 3 kinds of filter block (BHS, YHS, RHS) together with each light source (Filter No. 0–5 & Neutral Density #0–3) for test to find most suitable experimental condition. The result is listed below in Tables 2–4 (Objective lens 20 × Eyes lens 10).

Enhance → Project Z series (OK)  
 From 1 to 22 → Save 8 bits(256 colors) < File name >

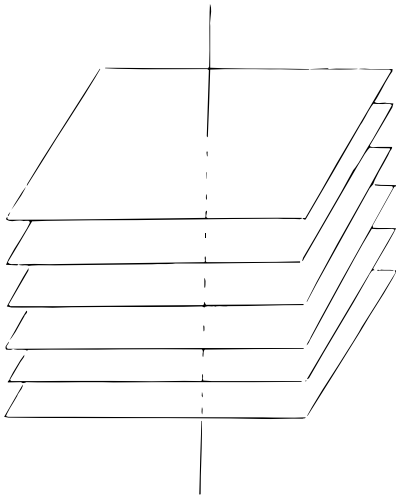
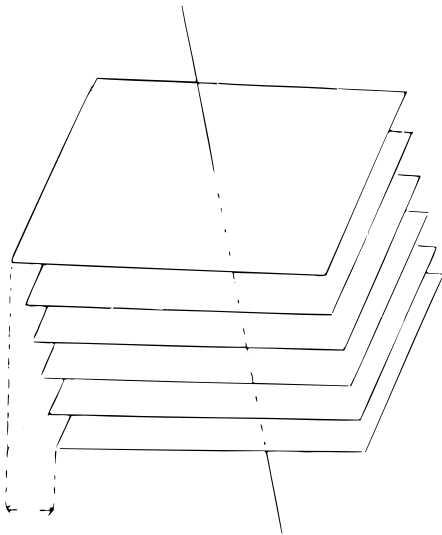


FIG. 3—First image was reconstructed from overlapping 22 images of varied depths of paper and ink by computer.

Enhance → Project Z series  
 From 1 to 22 change pixel shift → Save 8 bits(256 colors)



Shift difference = 20 pixels  $20/22 \approx 1.00$  pixel

FIG. 4—Second image was reconstructed from overlapping 22 images of varied depths and change about one pixel shift of paper and ink by computer.

TABLE 1—Results of 60 samples are examined by LSCM method.

Type of Crossing	Correct	Incorrect	Undetermined	Correct Rates
Homogeneous 30	23	2	5(same color)	76.6%
Heterogeneous 30	22	1	7(same color)	73.4%
Total 60	45	3	12(same color)	75%

TABLE 2—YHS filter block together with light source (Filter No. 3 Neutral Density #2) is best for the intersection of blue and red ink examined by LSCM method. BHS filter block (Filter No. 1 & N.D. #2) is best for red ink but worst for blue ink. RHS filter block (Filter No. 4 & N.D. #1) is best for blue ink but worst for red ink.

	Blue Ink	Red Ink
BHS(blue) 488 nm Filter No. 1 N.D. #2	—	+++
YHS(yellow)* 568 nm Filter No. 3 N.D. #2	+ Fluor. Emission Normal	+ Fluor. Emission Normal
RHS(red) 647 nm Filter No. 4 N.D. #1	+++	—

\*Best.

TABLE 3—BHS filter block with filter No. 5 & N.D. #1 (all lines (B + Y + R)) is best for the intersection of red and black ink examined by LSCM method. YHS filter block (Filter No. 3 & N.D. #2) is good for red ink but worst for black ink. RHS filter block (Filter No. 4 & N.D. #1) is all worst for red & black ink.

	Red Ink	Black Ink
BHS(blue)* 488 nm Filter No. 5 N.D. #1	++ Fluor. All lines (B + Y + R)	++ Fluor. Emission Normal
YHS(yellow) 568 nm Filter No. 3 N.D. #2	+	---
RHS(red) 647 nm Filter No. 4 N.D. #1	—	---

\*Best.

TABLE 4—BHS filter block with filter No. 5 & N.D. #1 (all lines (B + Y + R)) is best for the intersection of blue and black ink examined by LSCM method. YHS filter block (Filter No. 3 & N.D. #2) is good for blue ink but worst for black ink. RHS filter block (Filter No. 4 & N.D. #1) is best for blue ink but worst for black ink.

	Blue Ink	Black Ink
BHS(blue)* 488 nm Filter No. 5 N.D. #1	+ Fluor. All lines (B + Y + R)	++ Fluor. Emission Normal
YHS(yellow) 568 nm Filter No. 3 N.D. #2	+	--
RHS(red) 647 nm Filter No. 4 N.D. #1	+++	--

\*Best.

**Discussion**

When the two inks of ball-pen line intersection are of the same color, they will dissolve with each other. Therefore, writing sequence can hardly be determined by LSCM. But, sometimes it can usually (not always) be determined by the continuity phenomenon of the last lines (physical evidence), and in all other cases we can easily find the evidence of the intersecting points of the last ink overlapping the first one by using Zeiss Axioplan microscope. When examine the writing sequence by LSCM method, we must test many times by changing filter block and light source and setting up buttons until the image is clear enough for the experiment. The most important thing is to adjust the two lines of intersecting strokes until the illuminated image is at its clearest. In other words, the two inks of the intersecting lines must be illuminated to induced



FIG. 5—BHS filter block together with all lines (B + Y + R) wavelength light can illuminate fluorescence for any color ink, whether water-based or glycol-based ink.

fluorescence by laser light. If one of them cannot be illuminated to induced fluorescence, we cannot examine the writing sequence by using this method. Generally, red ink of ball-pen is best for illuminated induced fluorescence but black ink of ball-pen is worst for illuminated induced fluorescence. The water-based fiber-pen is not easy to give illuminated induced clear fluorescence by using YHS & RHS filter block but the glycol-based ball-pen blue color ink is very easy to be illuminated induced strong fluorescence. That is, the more transparent the ink is, the clearer the fluorescence image is.

Using the YHS filter block shows that red & blue ink produced the best result and black ink produced the worst result. BHS filter block together with all lines (B + Y + R) wavelength light can induce fluorescence for all colors of writing ink (include black ink). Hence, written in any kind ink, whether water-based or glycol-based ink, can induce clear fluorescence under this condition (see Fig. 5). If the two intersecting lines of writing can induce fluorescence uniformly, then we can easily examine the sequence of writing by using 3D spectacles.

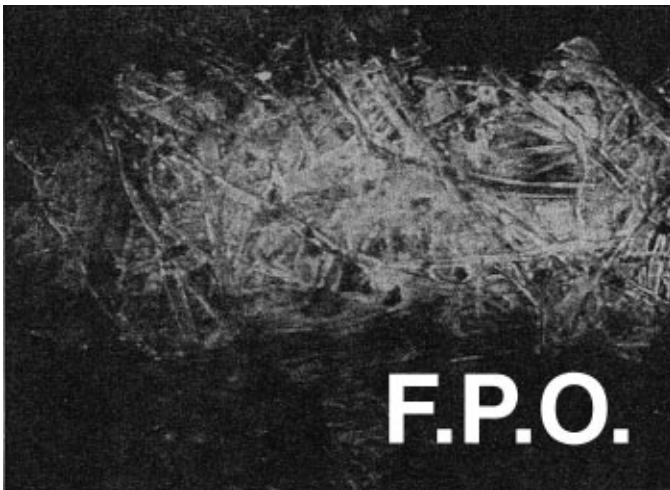


FIG. 6—Red and blue ink of intersecting lines suits YHS (508 nm) filter block together with filter No. 3 and neutral density #2.

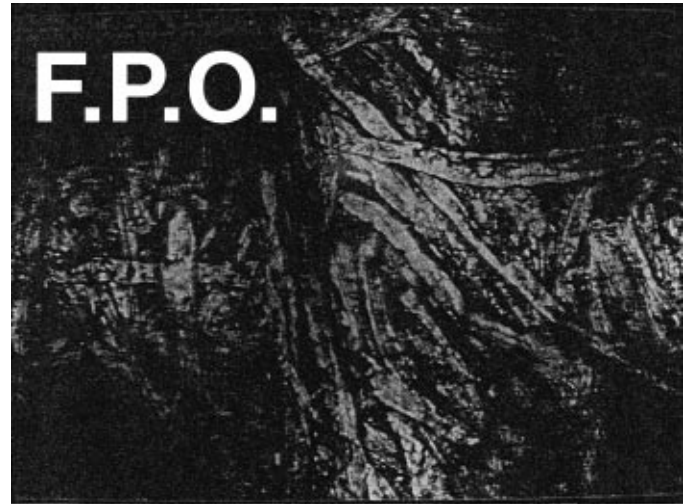


FIG. 7—Red & black ink of intersecting lines suits BHS (488 nm) filter block and all lines (Blue + Yellow + Red) wavelength together with filter No. 5 and N.D. #1.

### Conclusion

What method we use to examine the two inks of the intersecting lines that can be illuminated to produce fluorescence clearly depends on what color the intersecting strokes are. Table 2 shows that red & blue ink of intersecting lines suits YHS (508 nm) filter block together with filter No. 3 and neutral density #2 light source (see Fig. 6). Table 3 shows that red & black ink of intersecting lines suits BHS (488 nm) filter block together with filter No. 5 and neutral density #1 light source (all lines (Blue + Yellow + Red) wavelength) (see Fig. 7). Table 4 shows that blue & black ink of intersecting lines suits BHS (488 nm) filter block together with filter No. 5 and neutral density #1 light source (all lines wavelength) (see Fig. 8).

From the above, we conclude that by using all lines wavelength light and BHS filter block to induce fluorescence on the ink of the intersecting lines, we can also determine the writing sequence from the fiber direction without using 3D spectacles (see Fig. 9).

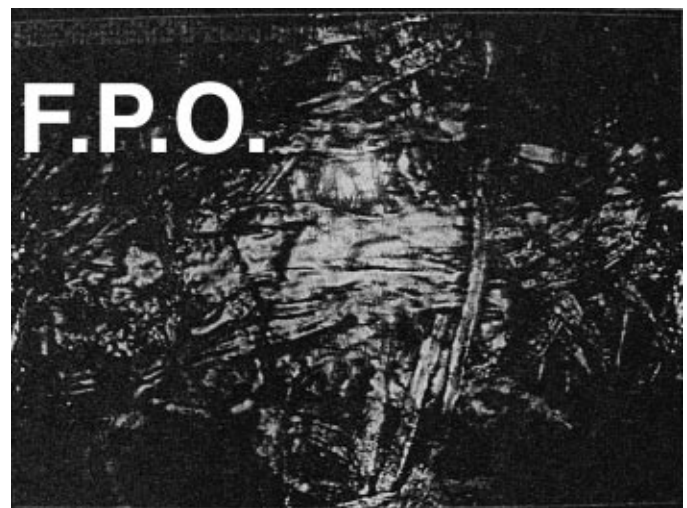


FIG. 8—Blue & black ink of intersecting lines suits BHS (488 nm) filter block and all lines (Blue + Yellow + Red) wavelength together with filter No. 5 and N.D. #1.



FIG. 9—By using all lines wavelength light (Blue + Yellow + Red) and BHS filters block to illuminate fluorescence on the ink of the intersecting lines. We can also determine the writing sequence from the fiber direction without using 3D spectacles.

This experiment was conducted by using 3 kinds of filter block (BHS, YHS, RHS). Out of 60 samples, we could determine the writing sequences for 75% of the samples. The result justifies the use of the LSCM system and proposed methods.

#### *Acknowledgment*

We would like to thank Jui-Ching Shyur of MOTC for providing English editorial expertise with this paper.

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Additional information and reprint requests to:

Kun-Chi Cheng, M.Sc.

Questioned documents examiner

Investigation Bureau, Ministry of Justice

P.O. Box 3562 Taipei County

Taiwan, R.O.C.