Braz, A., López-López, M. & García-Ruiz, C., 2015. Raman imaging for determining the sequence of blue pen ink crossings. Forensic Science International, 249, pp.92-100.

Available at https://doi.org/10.1016/j.forsciint.2015.01.023

(Article begins on next page)

Accepted Manuscript

Title: Raman imaging for determining the sequence of blue pen ink crossings

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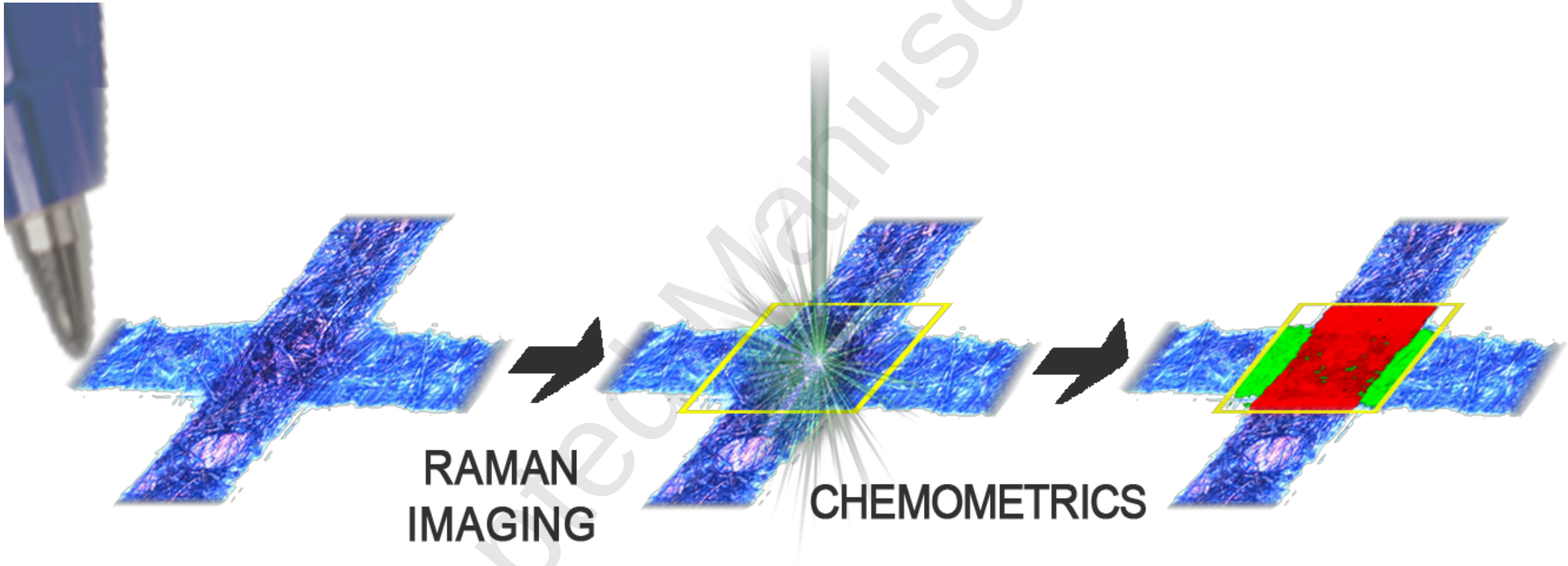
PII: S0379-0738(15)00037-7
DOI: http://dx.doi.org/doi:10.1016/j.forsciint.2015.01.023
Reference: FSI 7886

To appear in: *FSI*

Received date: 28-10-2014
Revised date: 14-1-2015
Accepted date: 23-1-2015

Please cite this article as: A. Braz, M. López-López, C. García-Ruiz, Raman imaging for determining the sequence of blue pen ink crossings, *Forensic Science International* (2015), http://dx.doi.org/10.1016/j.forsciint.2015.01.023

Graphical Abstract (for review)



Highlights

- Successful use of Raman imaging to determine the sequence of blue pen crossings
- Imaging of large crossing areas was rapid and non-destructive.
- Different times separating the crossing of lines produced similar results.
- A net-like pattern in the crossing area suggested partial skipping of last line.
- Different papers did not influence distribution or determination of crossings.

Raman imaging for determining the sequence of blue pen ink crossings

Raman imaging for determining the sequence of blue pen ink crossings

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Abstract

This manuscript presents a preliminary investigation on the applicability of Raman imaging for non-destructive and rapid analysis of blue crossing ink lines. The MCR method was used to facilitate visualization of the distribution of inks of the same colour and the most predominant Raman signature at the crossing was used to interpret the order of application of inks. Different pen ink types, different times separating the application of the two ink lines and different paper substrates were used. From the 90 Raman images examined, the correct order of application was determined in more than 60 % by direct observation. The remainder cases were not as clear due to the uneven distribution of inks and the empty spaces similar to a net-like pattern observed at the crossing. This pattern was possibly caused by physical impediments (the first ink applied acting as a physical barrier) or chemical impediments (the two inks did not stick). Such impediments were more strongly observed in the crossings involving the U. Eye pen ink, causing the complete skipping of this ink line. Moreover, most crossings showed some mixing between the two inks and it was more accentuated when the times separating the application of the inks were shorter, since the ink was fresher. The use of white or certificate papers did not seem to influence in the inks distribution nor determining the order of the inks. Although this study provided useful insights regarding crossing ink lines, future statistic studies may be helpful for more objective examinations.

KEYWORDS: Ink; questioned document; Raman spectroscopy; Raman imaging; intersecting lines; crossing

1. Introduction

Determining the order of crossing ink lines is a “hot” topic in Questioned Document Examinations that has been around for many years and it is often related to cases regarding the authenticity of a document or simply to confirm or disprove the credibility of a suspected claim related to another dispute. Examination of such cases must be non-destructive in order to undergo multiple analysis and to preserve the documents evidential value. Several methods have been proposed in the past, some being more invasive than others, such as lifting techniques, chemical reactions, Scanning Electron Microscopy (SEM), spectrophotometry as well as microscopic, photographic, indented impression and electrostatic techniques [1]. Nowadays, high-powered microscopic examinations with different magnifying lenses and illumination settings that rely on differences in gloss and specular reflection are the easiest and most rapid methods that can give solution to a great number of cases [2, 3]. However, these methods remain subjective to human interpretation which may lead to many inconclusive or incorrect results, especially with inks that have similar optical properties or crossings that are optically deceptive [4]. Some microscopy based techniques, such as Atomic Force Microscopy [5] and Laser Scanning Confocal Microscopy [6] have provided higher resolving powers and depth of focus when compared to optical microscopy, and can produce the same qualitative results as SEM but without the need for vacuum or conductive coating of specimens. However, both techniques are only successful with inks which are not absorbed into the paper fibers. The video spectral comparator combines video images with lights in the ultraviolet, visible and infrared as well as different excitation filters for examining various characteristics of inks, such as the absorption and reflectance properties. This equipment has been useful to determine differences in inks on documents and examine alterations and obliterations but has failed with most crossings with exception of those involving ballpoint ink and toner [7, 8]. One of the vibrational techniques, Attenuated Total Reflectance Fourier-Transform Infrared spectroscopy (ATR-FTIR) showed to be useful in some studies [9-11]. Some authors also reported improved results when additional spatial information was obtained using the imaging configuration, especially with crossings between ballpoint pens and toners [9]. Nevertheless, this technique failed with many crossings, like those involving ballpoint, gel, roller ball and felt-tip pen inks. Moreover, the authors reported that paper contributions greatly obstructed the measurements. Alternatively, Raman spectroscopy has shown to be a very promising technique for crossing ink lines [12] and more advantageous than FTIR because paper substrates have little or no interferences in the inks spectra. Similarly to FTIR, the Raman technique provides chemical and spatial information from the surface, but also in profundity, when equipped with the confocal mode that allows analyzing inner parts of the intersection. The literature reported some successful cases [13-15] but most studies only analyzed a few points in the crossing area, which is not representative of the crossing area and the authors failed to demonstrate the repeatability and reproducibility of the results to other intersections. In sum, all of the methods referred before have shown great analytical performance for solving many cases regarding crossing ink lines, some being more informative and objective than others and each having their advantages and limitations. However, they fail in a great number of cases, especially when two very similar ink lines are present.

The interaction between an ink and a paper substrate, especially printing inks, has been covered in the literature from different standpoints and applications [16-20]. It is a complex process, where physical and chemical reactions take place, such as evaporation of volatiles, polymerization, oxidation, cross-linking and even paper corrosion [21]. Concerning writing pens, when an ink line is applied, it disperses throughout the paper and is absorbed into it. Oil-based inks have the tendency for being only partially absorbed and most of the ink stays adherent to the surface of the paper, due to its high viscosity. Water-based inks on the other-hand, have the tendency for soaking into the paper fibers, like water into a sponge [6]. In contrast, the interaction between two crossing ink lines in a paper substrate has been mostly understudied. In principle, when a second ink is applied over another, it can disperse across the void spaces as well as the other ink. Nevertheless, the distribution resulting from the crossing between two ink lines has never been visualized *per se*. A former study reported by our research group [22] has shown cross-sections of painted overlapping layers of inks for the purpose of visualizing and understanding a process that is very similar to real crossing ink lines. Three different configurations were observed: the two ink layers formed a double layer distinctly on top of each other and the ink layer at the top was dispersed in the ink layer at the bottom, forming either a partial or a complete mixture. Several factors could determine the formation of either of these configurations: i) the ink's chemical composition, which is patented and usually unknown to the forensic examiner; ii) the amount of ink applied, which mostly depends on writing pressures as well as the type of point and pen used; iii) the time that separates the application of both inks, which is directly related to the inks drying processes; iv) the structure and surface of the paper substrate, that determines the hydraulic conductivity and absorption of the ink; and v) climatic factors such as heat, humidity and light, which may also play a role. In that study [22], red and blue overlapping layers of painted ink of 1 cm^2 were used to facilitate microscopic visualization. Although this might not entirely reflect the size of real ink crossings, nor should be reproducible to crossings involving other ink colours, the authors concluded that the paper and the writing pressure showed no significant influence on the inks distribution and that the type of ink as well as the drying times were very important factors that should be studied further with real crossing ink lines. Surprisingly, little is known about the influence of each of these factors in the distribution of crossing ink lines. This manuscript discusses a preliminary investigation on the applicability of Raman imaging for non-destructive and rapid examination of real crossing lines made with blue pen inks when different pen ink types, different times separating the application of the two ink lines and different paper substrates are used. These parameters have been studied to understand the distribution of the two inks at the crossing as well as their influence in determining the correct sequence of crossings.

2. Experimental

2.1 Sampling

Six blue pens were purchased at local markets in Madrid (Spain) (Table 1). Crossings were prepared by the same person in the following manner: the first ink was always a horizontal line applied from left to right and the second ink line was crossed vertically to the first from top to bottom. The writing pressure was not controlled. The second ink line was then applied at different times: immediately (zero minutes), thirty minutes, five hours, two days and one week. The same UPM office copy/print white paper (80 $g.m^{-2}$) was always used, except in the study of the effect of different papers, where all samples were prepared in a Natur recycled paper (80 $g.m^{-2}$) and a Conqueror laid brilliant white paper (100 $g.m^{-2}$). In this part of the study, two days was the time between applications of inks. To test the effect of the amount of ink in oil-oil crossings, three superimposed lines were applied for extra amounts of ink. All crossings were allowed to dry at least for a few hours under the same laboratory climatic conditions before analyses.

2.2 Instrumentation

Samples were examined with a Thermo Scientific DXR™xi Raman Imaging Microscope (Waltham, United States) with an Electron Multiplying Charged-Coupled Device (EMCCD) detector and controlled by the Thermo Scientific OMNICxi Raman Imaging 1.0.0.2427 software. The green laser emitting at 532 nm was used for all measurements with a power set at 1.0 mW on the sample. A grating of 1200 lines per mm and a confocal pinhole of 25 µm were used. The microscope was set to 10× magnification under bright field illumination. Laser exposure times were 14.29 milliseconds (i.e. 70 spectra acquired per second) in a total of 30 scans. The mapping areas were defined in a "mosaic" image tile of approx. 600 µm × 600 µm including the intersection area and part of each individual ink line, depending on each crossing dimensions. With a step size between two successive measurements of 8 µm, approximately 5,000 – 7,000 spectra were registered for each sample, which resulted in total measurement times of less than 50 minutes. The wavenumber range measured was from 85 to 3500 cm^{-1}. The Multivariate Curve Resolution (MCR) method with two components and a 4th order polynomial baseline correction was automatically applied to the spectral data using the equipment's software.

2.3 Blind testing

For blind testing, the 90 Raman images obtained from MCR analysis of crossings with different times separating the application of inks were examined by two persons non-involved in this study. They were asked to report on their visual detection of inks distribution at the crossing.

3. Results and discussion

The Raman imaging technique can analyse the entire crossing area under short measurement times due to fast-readout detectors and fast automatic stages, in addition to identifying some of the inks chemical components like in conventional Raman spectroscopy. The imaging configuration delivers a chemical image from thousands of spectral locations (pixels) of the crossing which shows the spatial distributions of the inks chemical components at the crossing [23]. Regarding the instrumental conditions, the speed of analyses was considered an important experimental parameter if this technique is to be used routinely in forensic casework. In turn, this required the optimization of some experimental parameters in order to provide the most representative and highest quality chemical images in shorter times as possible. The laser excitation wavelength selected was 532 nm because it provides good quality spectra, as most blue inks absorb in the visible region. The laser power, exposure times and number of scans were optimized to provide the best S/N ratio for all samples and without causing sample burning. The area selected for imaging was about 600 µm × 600 µm, and included the entire crossing as well as part of the ink lines separately. Measuring the ink lines separately was important in order to examine the continuity in the lines, which could give information of the inks distribution at the crossing. The step size selected was 8 µm in order to cover the maximum area while maintaining the time of analyses as short as possible. Consequently, the 10× magnification was preferred because the resulting spot size would cover a more representative amount of ink rather than higher magnifications that would focus very precise points. Additionally, this magnification may also minimize spectral variances due to the paper's irregular topography.

The criterion for establishing the proof of concept in this study consisted in selecting samples that have a different spectrum from each other and from the paper substrate in at least one band. However, it is important to remark that this might not always occur in real case scenarios and other methodological approaches should be developed in this case. Figure 1 shows the spectra of the blue pen inks selected. In Raman spectroscopy, the ink`s colorants (dyes and pigments) are more active than most of the other components, thus they are the components typically detected. According to the literature [24], the main colorants identified for each pen ink sample analysed were: Cristal Violet dye (B. Cristal), Victoria Pure Blue BO dye (P. SGrip), Copper Phthalocyanine pigment (B. Atlantis and U. Eye) and Victoria Blue B dye (P. G2 and P. V5). The B. Atlantis and the U. Eye showed similar spectra and no differences were identified. For this reason, crossing combinations involving these two pens were not performed. That being said, several combinations of crossings were prepared with these pens and each crossing was also prepared in the opposite permutation, originating a total of eighteen combinations. To facilitate the interpretation, the first line applied was always horizontal and the last line was always vertical. The MCR method was used to identify and highlight the spectral signatures corresponding to each ink at the crossing and differentiate between them. It is one of the most popular resolution methods for image analyses where the data set is decomposed into components, usually by rank analysis methods that repeatedly perform Principal Component Analysis (PCA) [25, 26]. The resulting image shows the spectral contributions of the component contained in each pixel of sample, providing this way a reliable distribution map and simultaneously the chemical characterization of the surface of the crossing. The great advantage of this method is that it does not require priori information about the system nor uses any behaviour/calibration model. Thus, the data visualized will follow physically or chemically meaningful constraints, rather than mathematical or statistical constraints [27]. The spectral contributions of the ink lines separately helped obtaining a better

MCR model of the crossing area. Additionally, reducing the spectral range to the most informative region where only bands were observed (from 400-1650 cm^{-1}) also contributed to better results. The two components in the images were given a colour. The green colour was given to the spectral signature of the horizontal ink line and red was given to spectral signature of the vertical ink line. The paper substrate, which did not show any bands, was seen in black, by default. The methodology used for determining the sequence of the crossing consisted in visually identifying the most predominant Raman signature in the crossing area, which may indicate the ink that was applied last; at the same time, examination of the continuity or discontinuity of that signature in the ink lines separately and through the crossing area could further reveal information on the physical and chemical distribution of the inks at the crossing.

There are several factors that can affect the inks' distribution at the crossing; the writing pressure was not controlled in this preliminary study, and all crossings were performed by the same person. The amount of ink applied in a single line was also not controlled, although the line widths applied by each pen were in a similar range (from 0.3 to 0.4 mm). Additionally, the laboratory climatic conditions did not vary significantly during experiments. On the contrary, the time separating the crossing of the two ink lines was investigated comprehensively. It was hypothesized that the fresher the first ink line at the moment of crossing of the second ink line, the more likely that the two inks mix. By contrast, the longer the time separating the crossing of the two ink lines, the dryer the first one, thus the more likely that the two inks do not mix. In order to investigate this, all crossings were prepared with different times separating the crossing of the two ink lines. The times studied were chosen after consultation with the local police forces and the type of cases they frequently encounter. According to them, the common casuistry involves crossings that were performed with short times apart rather than at later stages. Thus, the times selected were immediately (zero minutes), thirty minutes, five hours, two days and one week, resulting in a total of 90 crossing samples. It is important to note that cases of crossings performed with longer times apart (2-3 years later) may also be encountered and therefore should be investigated in future studies. The results with the crossings using pens with the same ink type are described and discussed next.

Gel-gel crossings

Regarding the gel-gel crossing P. G2 over B. Atlantis (Figure 2a), results showed a predominance of the Raman signature of the P. G2 ink at the surface of the crossing (in red) for the several times measured. In these crossings, some occasional gaps similar to a net-like pattern were visible in the crossing area, in which the spectral contributions of the B. Atlantic ink underneath (in green) were also visible. This pattern was not observed in the ink lines separately. Additionally, some darker shades in the crossing areas of all images can be seen, and their spectral examination showed that they contained contributions from both inks, as exemplified in Figure 2b. The dashed lines indicates the spectrum of the mixture (in grey) and it can be depicted band contributions from both inks, suggesting the mixing of both inks occurred in those areas. Comparing the images obtained at different times, the net-like pattern and the dark shades became less notice as the times separating the application of inks were larger. In fact, the crossing image of 1 week showed a dense predominance of the P. G2 ink (in red) and very little net-like pattern and dark shades were observed. In the opposite crossing (B. Atlantis over P. G2), it was observed a predominance of the Raman signature of the P. G2 ink (in green) in the central part of the crossing, while the lateral parts were dominated by contributions of the B. Atlantis ink (in red). The continuity of the B. Atlantis ink line separately and throughout the crossing area suggests that this ink line was distributed over the P. G2 ink, in spite of the predominance of the P. G2 ink in the crossing area. This is in agreement with the correct sequence of application of inks. Additionally, the few gaps that were observed in the B. Atlantis ink line at the crossing area as well as separately may suggest a possible failure of that pen's mechanism. Therefore, a failure of a pen's mechanism such as the gaps observed or even leakage of the ink can cause uneven ink-flow which will affect interpretation of the results and should be taken into consideration during examinations.

Oil-oil crossings

Regarding the oil-oil crossings involving the B. Cristal and the P. SGrip (Figure 3a), results showed a net-like pattern in the crossing area that influenced a clear visualization and determination of the predominance of either inks. The net-like pattern, which could also be found in both pen ink lines separately, is typical of oil-based ballpoint pens, where the ink is controlled and applied by frictional forces of a ball as it rolls on the paper surface; given the fact these inks are very viscous, only a controlled amount gets out, resulting in occasional empty spaces in the lines, like the net-like pattern observed. Although rollerball pens use a similar mechanism, the type of ink used in these pens is more fluid and releases a greater amount of ink. Thus, the net-like pattern is not observed with ink lines made by liquid and gel pen inks. In order to understand whether the amount of ink applied at the crossing could influence the physical distribution of the oil-oil inks in that area, an additional experiment was carried out in which the same crossings were prepared again, in both permutations, but instead of a single line, three superimposed lines representing more amount of ink. It is important to remark that this configuration does not reflect real case scenarios and was only considered in order to better understand the phenomenon occurring with these pen inks. The time separating ink applications for this additional experiment was 2 days, for the first ink to dry. Different combinations were prepared: three superimposed lines of both inks, three superimposed lines only of the ink applied first and three superimposed lines only of the ink applied last, resulting in a total of six crossing samples. These crossings were then compared with the crossings made with only a single line of both inks. In Figure 3b, the Raman images obtained showed that when there was more ink in both lines, the net-like pattern was no longer visible in the ink lines separately but it was still present in the crossing area, in both crossings. In that area, the predominance of the Raman signature of the ink applied last (in red) at the surface was more evident. Similar results were observed when there was more ink only in the line applied last (in red). When there was more ink only in the line applied first (in green), it was still possible to visualize the predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing in the crossing B. Cristal over P. SGrip in spite of the reduced amount of this ink at the crossing. However, in the opposite crossing, the predominance of the Raman signature of the ink applied first (in green) could induce in error by mere visual examination. Therefore, these results indicate that the amount of ink in the oil-based ink samples studied can determine the degree of net-like pattern that can be observed in those ink lines separately and also at the crossing area, which influences visualization of the most predominant Raman signature at the crossing area.

Liquid-liquid crossings

Regarding the liquid-liquid crossings involving the P. V5 and the U. Eye pens (Figure 4), results showed a predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing, for the several times of crossing but only in the crossing P. V5 over U. Eye. In this case, the correct sequence of application of inks was obtained for the several different times separating the application of the ink lines. Additionally, many dark shades and a net-like pattern similar to what was described previously were depicted in every image in both crossings, indicating the tendency for these inks to mix. This pattern became less noticeable as the times separating the application of the inks were longer. Interestingly, in the opposite crossing (U. Eye over P. V5), the Raman signature of the U. Eye pen ink (green) was seen over the other ink, suggesting the inverse order of application. In order to assure that this was not an isolated occurrence, these crossings were repeated and analysed once again. The same results were consistently obtained with each time separating the application of the ink lines, indicating that the distribution of these two inks is seen in the inverse order and that the time separating the application of these two ink lines had no significant influence in their distribution at the crossing (data not shown). These results suggest the complete skipping of the U. Eye pen ink line at the crossing.

The results obtained with the crossings using pens with different ink types are described and discussed next.

Gel-liquid crossings

In Figure 5, the Raman images of the gel-liquid crossings involving the P. G2 and the U. Eye pens showed the predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing for the several times of crossing but only in the crossing P. G2 over U. Eye. This is in agreement with the correct sequence of application of inks and the times separating the application of the ink lines showed no significant influence in the inks distribution at the crossing. However, in the opposite crossing (U. Eye over P. G2), the sequence of the inks was the inverse, similarly to the crossings described before with the U. Eye pen. No significant differences were observed in the crossings with different times, apart from some net-like patterns observed in the crossing area. The Raman images of the gel-liquid crossings involving the P. G2 and the P. V5 pens showed the predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing, for the several times of crossing and in both crossings. Many dark shades corresponding to the mixing of the two inks can be seen, as described previously. Additionally, the net-like pattern was observed in every crossing area but not in the ink lines separately. Nonetheless, the continuity in the ink line applied last (in red) separately and throughout the crossing areas evidences the correct sequence of application of inks, contrary to the crossings involving the other liquid-based, the U. Eye pen.

Gel-oil crossings

Regarding the gel-oil crossings involving the P. G2 and both oil-based pens, B. Cristal and P. SGrip (Figure 6), results showed the predominance of the Raman signature of the ink applied last (in red) at the surface of the crossing for the several times and in both crossings. Although the net-like pattern observed could greatly influence the visualization of the predominance of the inks at the crossing, examination of the continuity in the ink lines separately and throughout the crossing area indicated more clearly the ink that was applied last. The only exception was observed in the crossing P. G2 over B. Cristal with 5 hours separating the application of inks where the amount of P. G2 ink appreciated in the crossing area was reduced and could induce visualization of the inverse sequence of application of inks. Furthermore, some mixing of the two inks also occurred in both permutations as the dark shades indicate in all of the crossing images.

Liquid-oil crossings

In Figure 7, the Raman images of the liquid-oil crossings involving the U. Eye and both oil-based pens, B. Cristal and P. SGrip, showed the predominance of the Raman signatures of the oil-based inks at the surface of the crossing, for the several times of crossing and in both crossings, even when they were applied first. This result is similar to the ones previously observed with the U. Eye pen ink. However, in this case, the spectral contributions of the U. Eye were still observed since the oil-based pens left empty spaces in the paper to which the U. Eye ink absorbed to the paper fibres. Overall, no significant differences were observed in the crossings with the different times separating the application of the ink lines. These results were comparable to the ones obtained with the gel-oil crossings.

Influence of paper

To study the influence that the use of different papers can have in the dynamics of crossing ink lines and in the determination of the correct sequence of application of the inks, all crossing samples were prepared in two other different papers because the type of paper can influence both the inks' penetration and drying process. A recycled office paper and a white paper mostly used to print certificates and official documents were used and so, the three papers differed in terms of colour, thickness and roughness. The inks absorption to the paper is regulated by capillary effects and also depends on the papers porosity [28] but since only a qualitative assessment was intended in this study, additional information regarding the papers porosity was disregarded. The time separating the application of the ink lines was two days, for the first ink to dry, and these results were compared with the same crossings described before in regular white office paper. Regarding the crossings in recycled paper, the Raman signal of the inks separately was very low and at the crossings, all samples either gave signals of intense fluorescence, possibly from the paper (data not shown). The laser power and exposure times were adjusted in order to avoid the fluorescence problems but this lead to sample burning. Since no quality spectra were obtained, these samples were discarded from the study. Regarding the crossings in certificate paper, results obtained were identical to the ones obtained with white office paper, in terms of predominance of the Raman signature, the net-like pattern in some crossings and the inverse sequence observed with the U. Eye pen ink.

Blind testing

For the double blind testing, all images for each crossing prepared with different times separating the application of inks were accounted as individual samples. Each operator had to report the colour (either red or green) that was the most predominant at the crossing, which would indicate the distribution and sequence of inks. However, none of the operators knew beforehand that the correct sequence of inks was when the red colour over the green one. They were also instructed to report inconclusive when they were not confident with expressing either result. Consequently, the two operators were able to determine the correct sequence of inks at the crossing (red over green) in 69 % and 61 % of the cases, respectively. However, 31 % and 32 % of incorrect results were reported, respectively. Additionally, one operator reported inconclusive in about 7 % of the crossings. Worthy mentioning, the crossings where the U. Eye pen ink was applied over but it was observed under the other ink, which corresponded to 22 %, were also included in the incorrect results reported. Thus, the U. Eye result is intrinsic to the crossings involving that particular pen ink and is independent of the analyses and the operator's interpretation. The remainder 9 % and 10 % of incorrect results corresponded to some crossings where the net-like pattern optically deceived the operators, particularly those where the oil-based ballpoint pen inks were involved.

4. Conclusions

This manuscript has presented the potential of Raman imaging in combination with MCR chemometrics to analyse the surface of a crossing and determine the correct sequence of application of blue ink lines. The advantage of Raman imaging is that it provides discriminating chemical information of two crossing ink lines of the same colour in a non-destructively and rapidly way. The MCR approach was found a good method for the identification and localisation of each ink contribution at the crossing area. The two-colour scale allowed the direct visualization of the distribution of inks which facilitated the interpretation of the sequence of crossing ink lines of the same colour. It was also found that its ability to resolve the mixture depends on the quality of the spectra, the noise level and the chemical differences between inks. The visual interpretation of the most predominant Raman signature at the crossing area was the main limitation of the methodology used in this study because determination of the sequence of application of inks was unclear in many cases and was subjected to the examiner´s interpretation. To overcome this, the development of objective methodologies that are able to quantify the Raman signatures in the images by probabilistic/statistics approaches is necessary in future research.

The combination of Raman imaging with other analytical and chemometric techniques is a future trend that could give solution to a greater number of cases regarding crossing ink lines. Notwithstanding, this manuscript discussed two very interesting phenomena occurring at the crossing, such as the net-like pattern observed in many samples and the complete skipping of the Uniball Eye pen ink line. These were evidence that the nature of the interaction between two crossing ink lines is an understudied subject which determines obtaining more correct results. Thus, further research is necessary in order to better understand the physical and chemical processes taking place at the crossing. Additionally, the influence of other factors affecting the distribution of inks at the crossing, such as the writing pressure, the amount of ink, other writing configurations (that simulate left-handed writing for example), different crossing angles and papers and uneven distribution of inks should be investigated further in order to apply the methodology proposed in real forensic cases.

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Table 1. Detailed information regarding the pen samples used.

Pen ID	Brand and model	Ink-based type	Pen mechanism	Line width (mm)
B. Cristal	Bic Cristal Medium	Oil	ballpoint	0.4
B. Atlantis	Bic Atlantis Gel	Gel	rollerball	0.4
P. SGrip	Pilot Super Grip M	Oil	ballpoint	0.31
P. G2	Pilot G2 gel	Gel	rollerball	0.39
P. V5	Pilot V5 Hi-Tech Needlepoint	Liquid	rollerball	0.3
U. Eye	Uniball Eye Micro	Liquid	rollerball	0.3

Figure Captions

Fig. 1. Raman spectra of the blue pen ink samples measured in points of the lines separately.

Fig. 2. a) Raman images of the crossings using gel-gel blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green). **b)** Raman spectra of single points in the P. G2 (red) and the B. Atlantis (green) ink line separately and of a dark shade point at the crossing area (grey), of the crossing sample P. G2 over B. Atlantis.

Fig. 3. a) Raman images of the crossings using oil-oil blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green). **b.** Raman images of the crossings using different amounts of oil-oil pen inks and using two days separating the application of each ink line, where the horizontal line was applied first (green).

Fig. 4. Raman images of the crossings using liquid-liquid blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

Fig. 5. Raman images of the crossings using gel-liquid blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

Fig. 6. Raman images of the crossings using gel-oil blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

Fig. 7. Raman images of the crossings using liquid-oil blue pen inks at different times separating the application of each ink line, where the horizontal line was applied first (green).

Figure 1

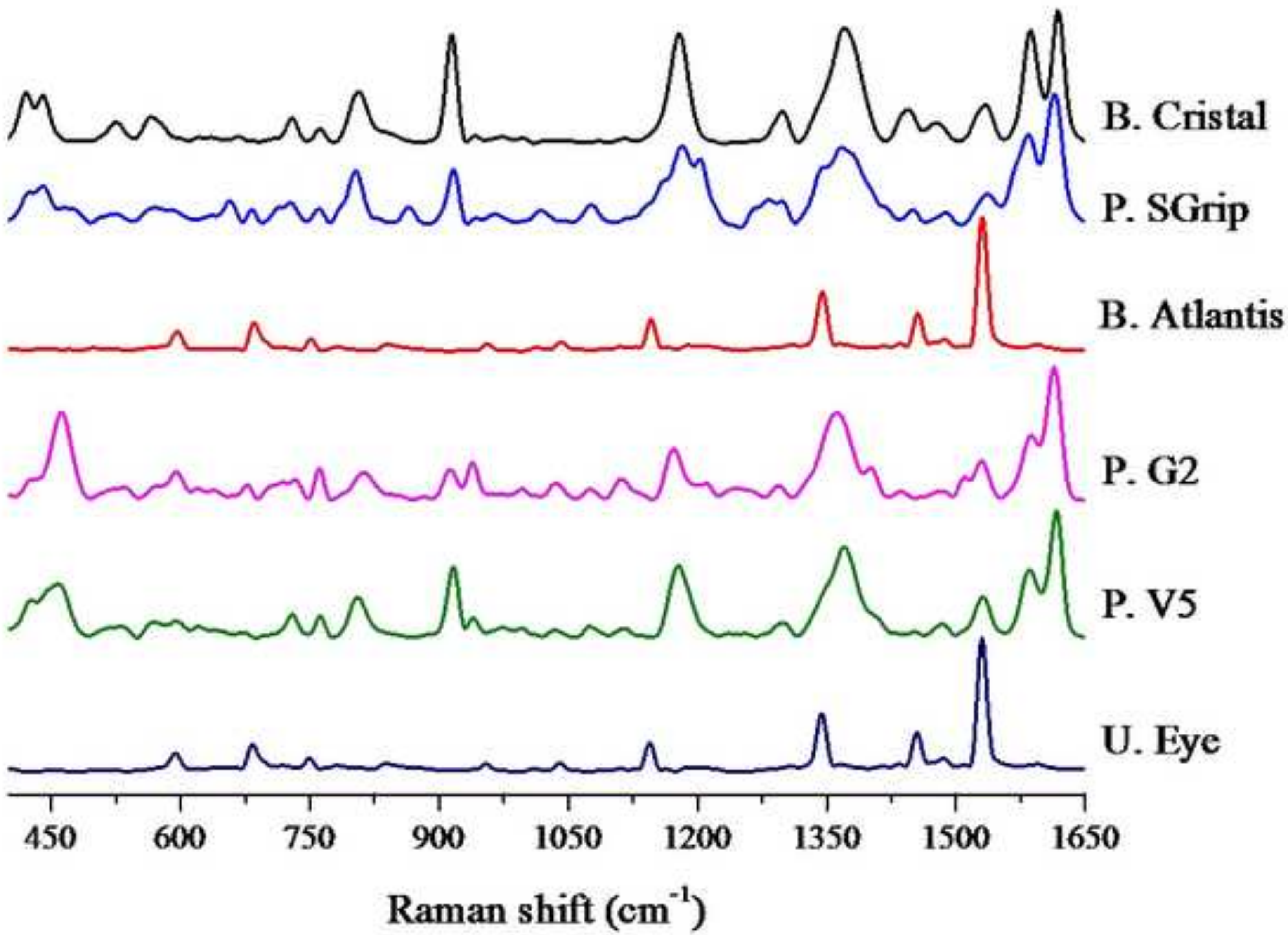
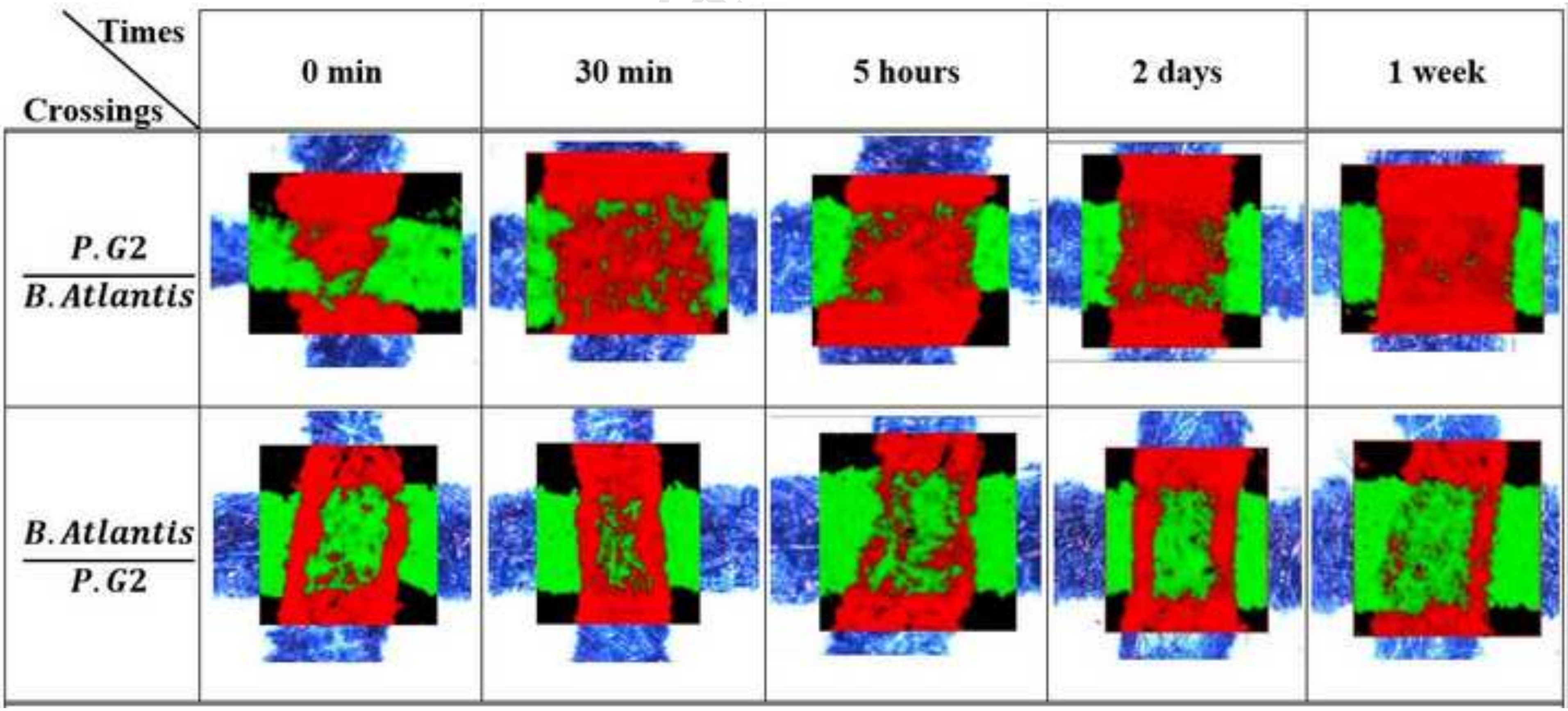
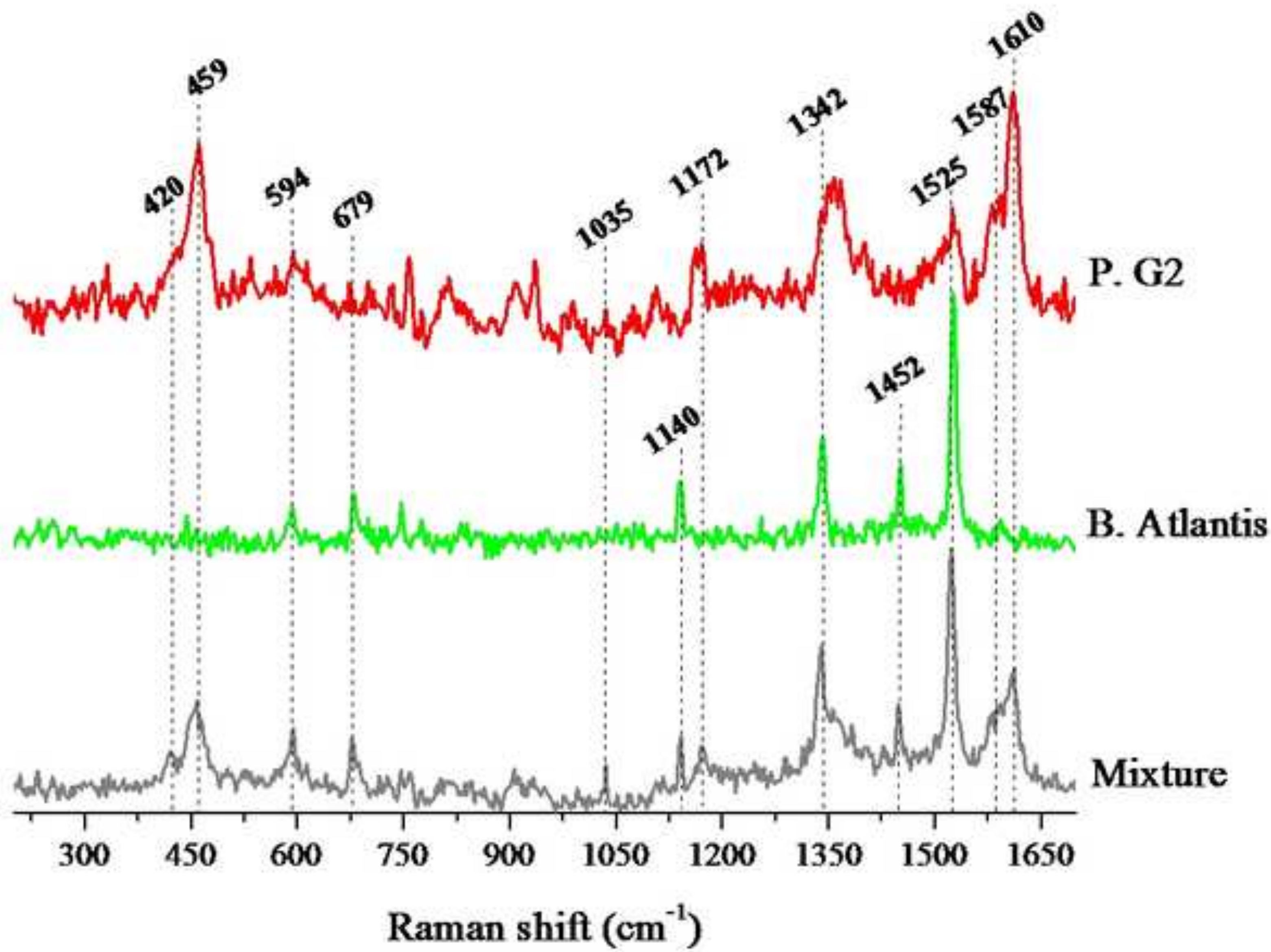

B. Cristal
P. SGrip
B. Atlantis
P. G2
P. V5
U. Eye
450
600
750
900
1050
1200
1350
1500
1650
Raman shift (cm-1)

Figure 2 a)



Times
Crossings
0 min
30 min
5 hours
2 days
1 week
P.G2 / B.Atlantis
B.Atlantis / P.G2

Figure 2 b)



459
420
594
679
1035
1172
1342
1525
1587
1610
P. G2
1140
1452
B. Atlantis
Mixture
300
450
600
750
900
1050
1200
1350
1500
1650
Raman shift (cm-1)

Figure 3 a)

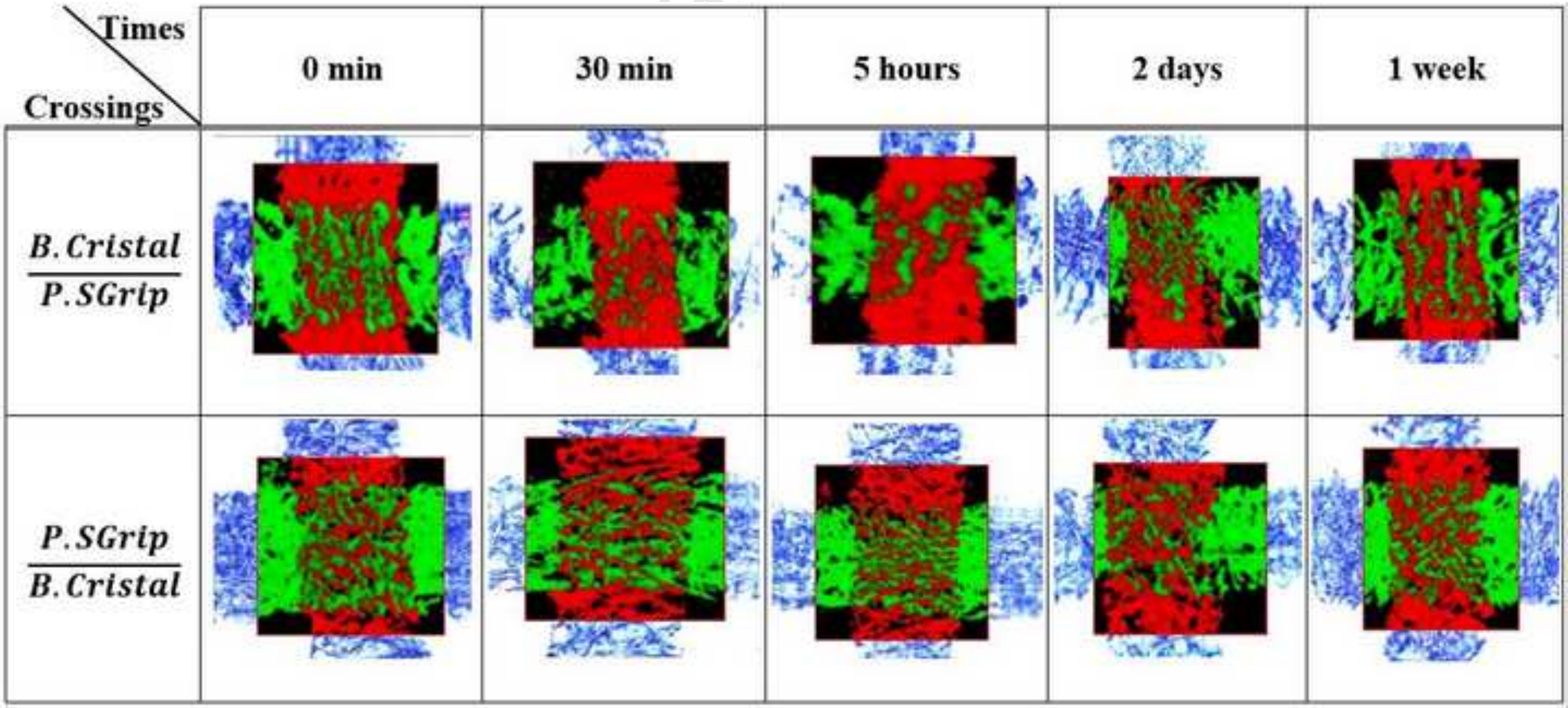

Times
Crossings
0 min
30 min
5 hours
2 days
1 week
B.Cristal / P.SGrip
P.SGrip / B.Cristal

Figure 3 b)

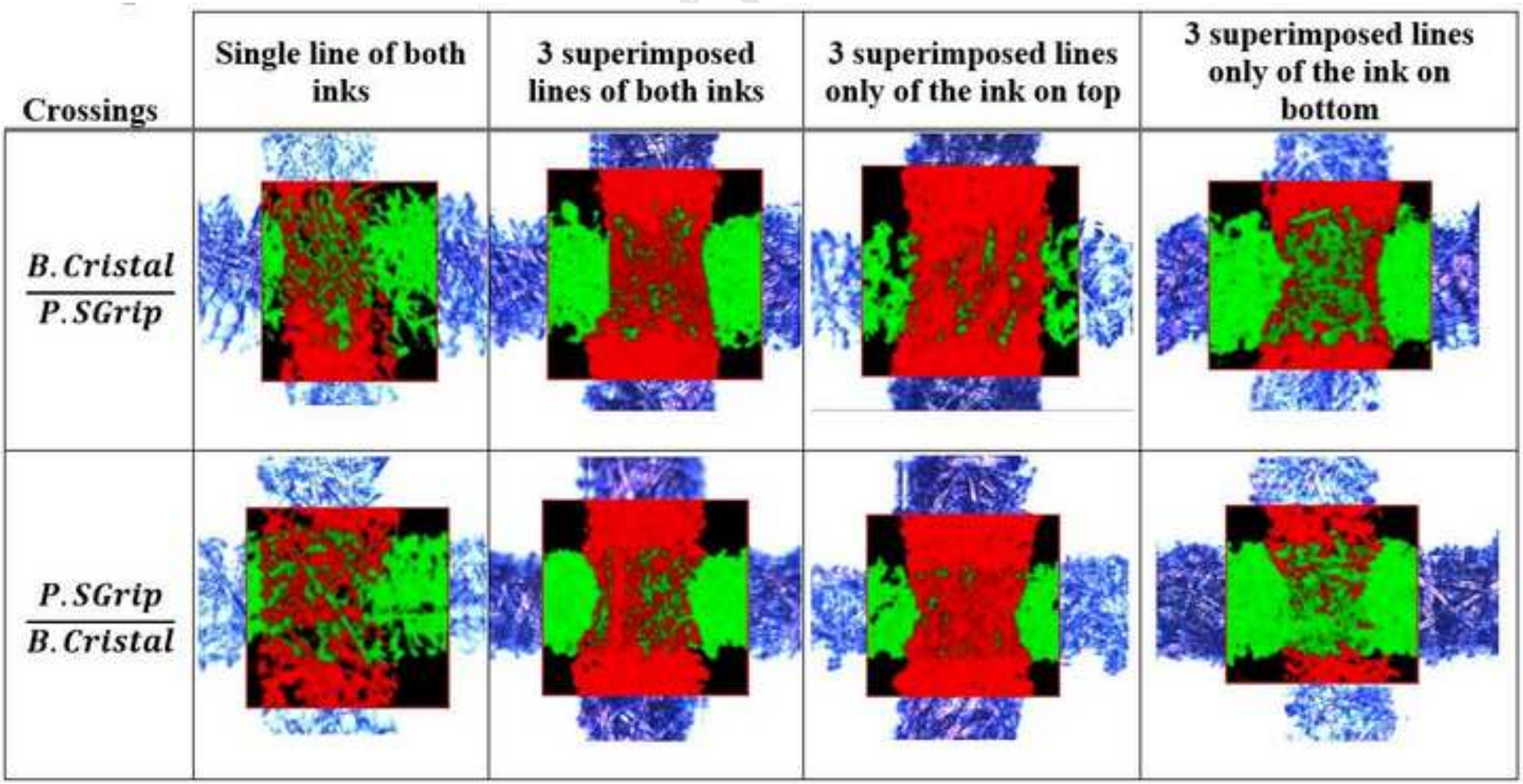

Crossings
Single line of both inks
3 superimposed lines of both inks
3 superimposed lines only of the ink on top
3 superimposed lines only of the ink on bottom
B.Cristal / P.SGrip
P.SGrip / B.Cristal

Figure 4

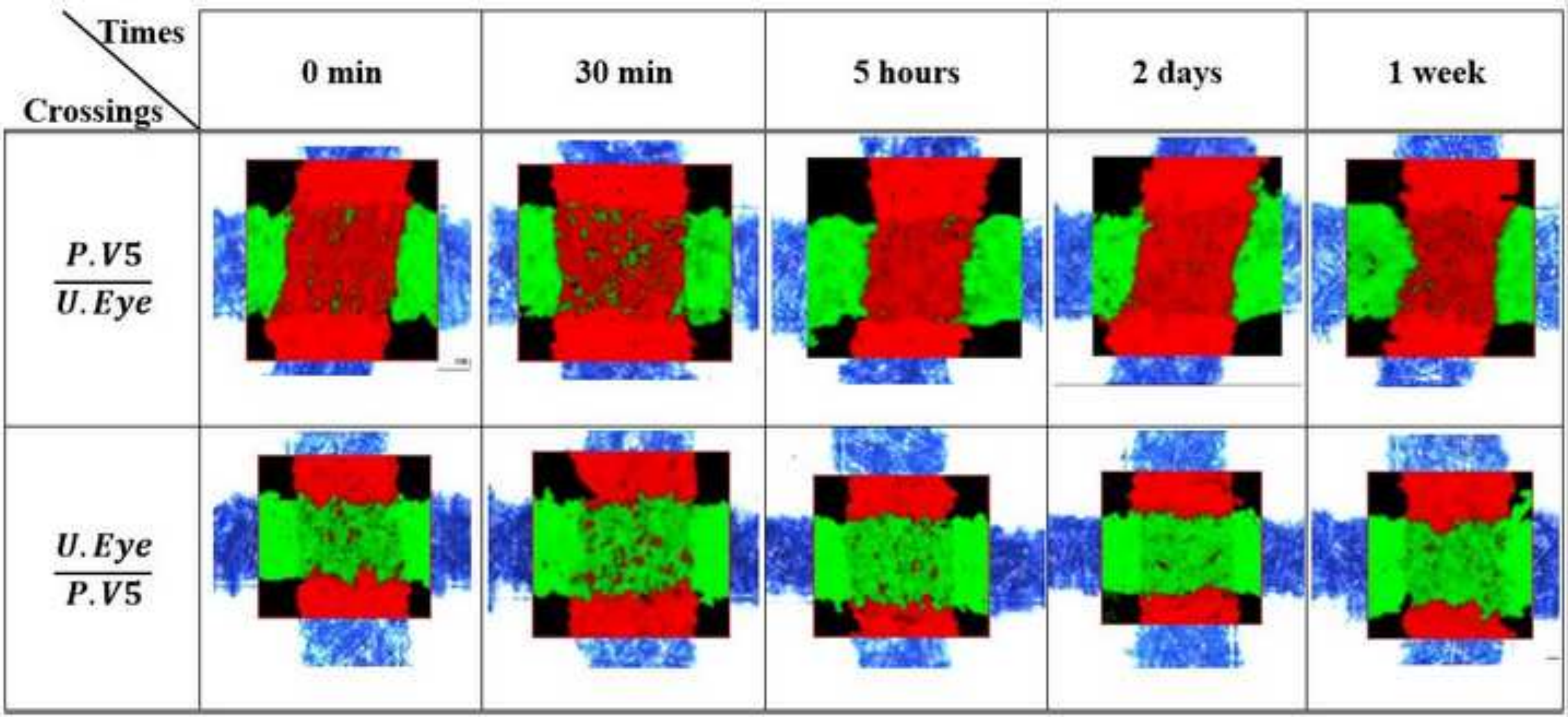

Times
Crossings
0 min
30 min
5 hours
2 days
1 week
P.V5 / U.Eye
U.Eye / P.V5

Figure 5

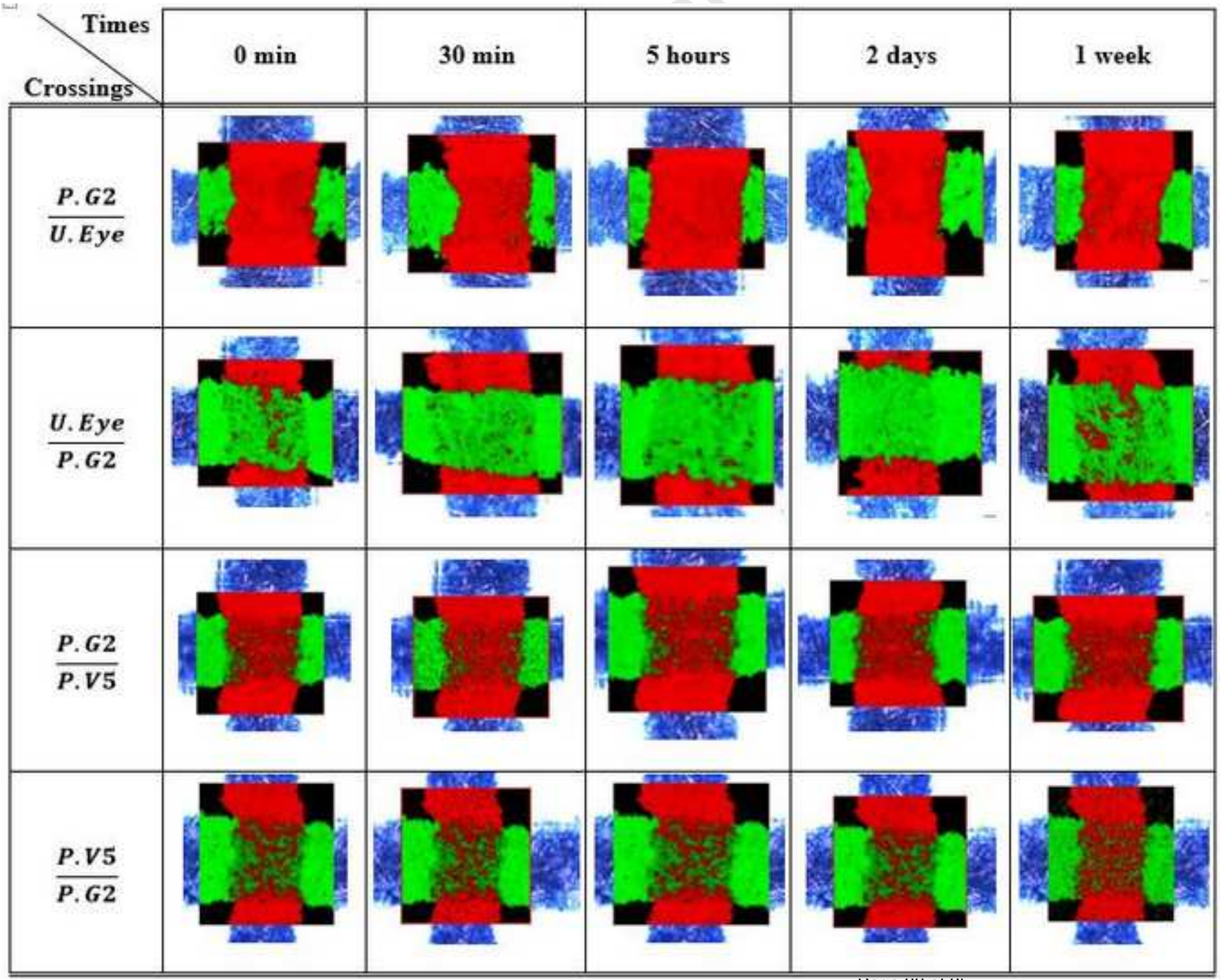
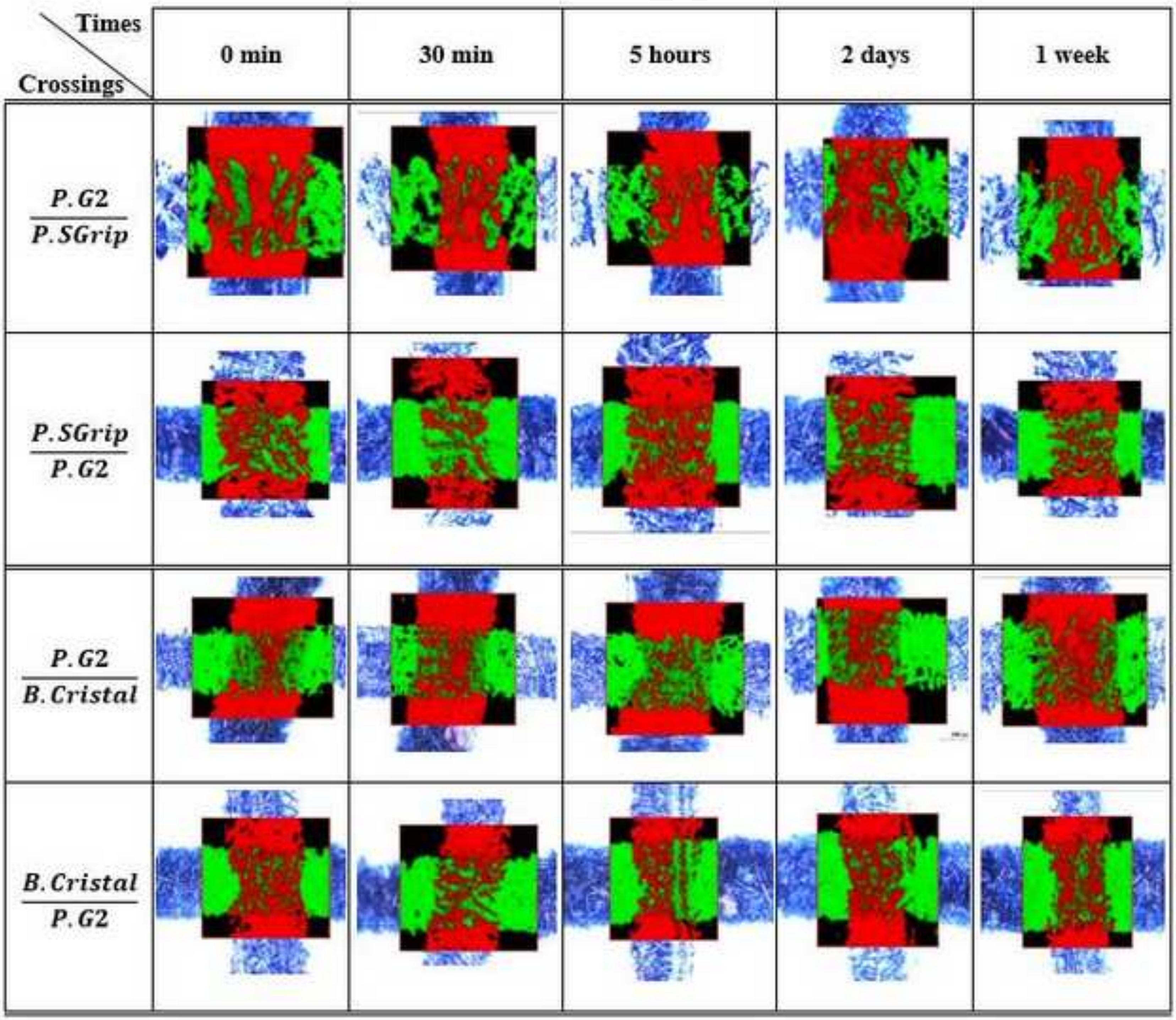
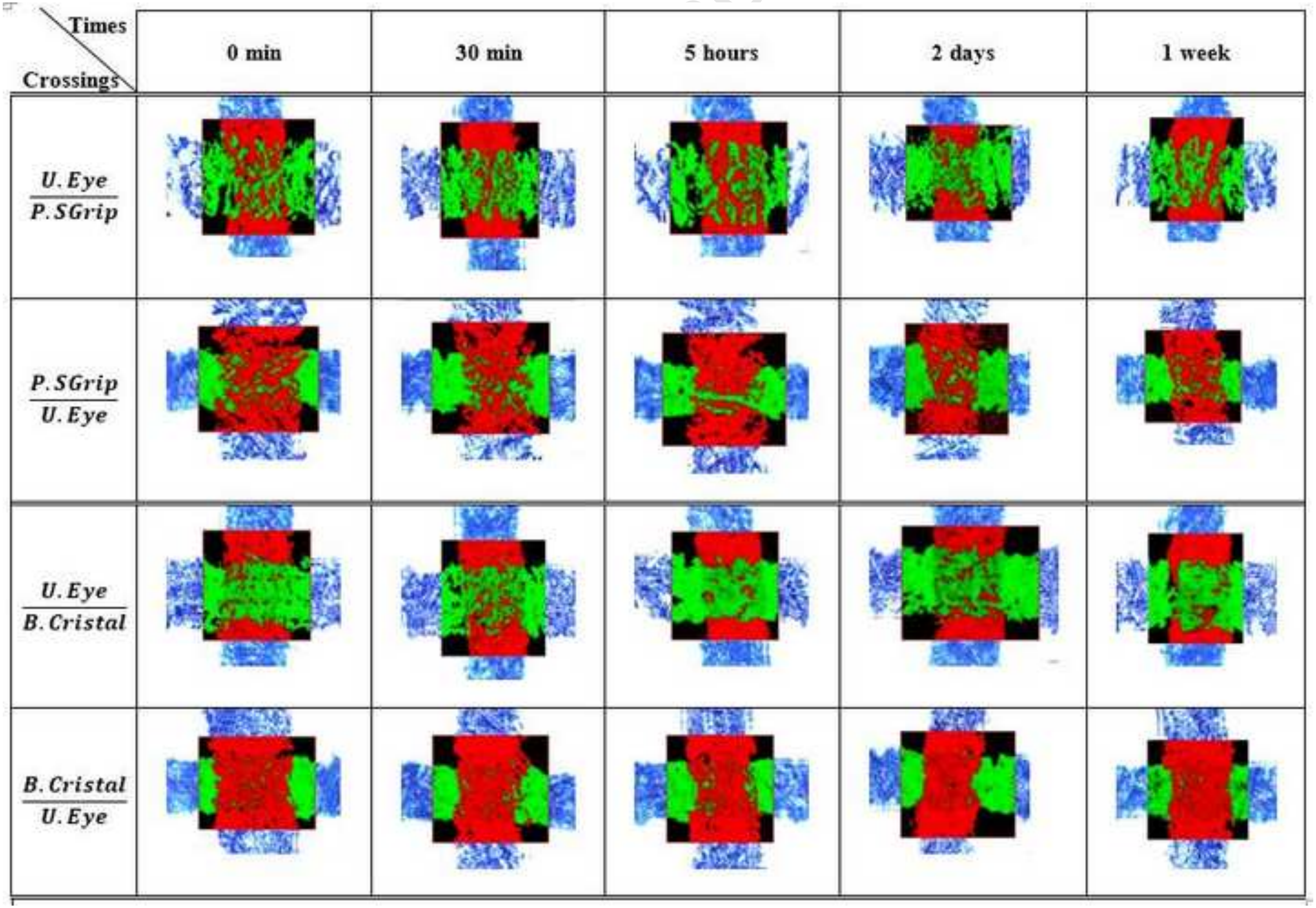

Times
Crossings
0 min
30 min
5 hours
2 days
1 week
P.G2 / U.Eye
U.Eye / P.G2
P.G2 / P.V5
P.V5 / P.G2

Figure 6



Times
Crossings
0 min
30 min
5 hours
2 days
1 week
P.G2 / P.SGrip
P.SGrip / P.G2
P.G2 / B.Cristal
B.Cristal / P.G2

Figure 7



Times
Crossings
0 min
30 min
5 hours
2 days
1 week
U.Eye / P.SGrip
P.SGrip / U.Eye
U.Eye / B.Cristal
B.Cristal / U.Eye