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**A NEW MATCHING IMAGE PREPROCESSING FOR IMAGE DATA FUSION**

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**ABSTRACT**

Hyperspectral images collected with different spectroscopic techniques can be combined to benefit from complementary information and to improve the general description of chemical systems. The simultaneous analysis of images collected by different spectroscopic platforms can only be carried out when images are spatially matched with each other (i.e., different pixel sizes should be balanced and translation/rotation/scaling transformations should be done if required).

The main goal of this work is the proposal of a general methodology to match image spatial properties that uses all pixels acquired in the images and, therefore, avoids the step of selecting analogous reference pixels to be compared. The effect of working with different kinds of image starting information on the robustness of the retrieved optimal translation and rotation parameters has also been assessed. The study has been tested in two different representative situations, namely: a) imaged sample with a clear contour b) imaged sample without defined contour. A final study on the effect of proper image matching is performed by applying Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) to the multiset formed by the appended images from different spectroscopic platforms before and after matching.

**Keywords**

Imaging matching, multitechnique image analysis, Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS).

1. **INTRODUCTION**

Hyperspectral imaging techniques are widely used in different areas of study such as polymer research, materials science, biomedical diagnostic, pharmaceutical industry, analytical chemistry, process control and environmental analysis[1–6]. The spatial and spectral information linked to these images provides chemical information and detailed knowledge of the distribution of the sample constituents in the surface (or volume) scanned [6].

Hyperspectral images can be acquired by different techniques, such as IR, Raman, fluorescence or mass spectrometry, among others. The combined information from different techniques is an excellent option to improve the capacity to differentiate among compounds because of the complementary information that they offer. Data fusion of different imaging techniques allows a comprehensive description of the sample, but joining images collected from different techniques is complex due to differences in spatial properties and signal patterns. A matching procedure is necessary to ensure coherence among the fused images and, as a consequence, a correct interpretation of the system studied. Chemometric tools are required for reliable image matching and subsequent multitechnique image analysis.

The preprocessing step required to build multiset structures with images collected by different spectroscopic platforms requires that different pixel sizes be balanced and translation/rotation/scaling transformations be performed when required. Spatial transformations among images are a problem easily solved when images have a clear contour or a clear structure with common landmarks, e.g., in remote sensing [7,8]. It is less straightforward when this is not the case, e.g., in chemical images, because the signal intensity pattern and the compounds detected may vary among techniques and equivalent morphological sample patterns are less clear. Few works about fusion of imaging techniques into a single multitechnique structure have been published and they are either based on a qualitative comparison of the information obtained separately with the different techniques or on studies where spatial matching is done from the selection of several equivalent points among images[9][10].

In this work, we propose a new methodology oriented to the search of optimized translation and rotation parameters for image matching. The novelty of the procedure is that it uses all pixels of the images for image matching. This strategy has two main advantages: a) avoiding mistakes associated with the wrong selection of analogous reference pixels among images to be aligned and b) a more robust image alignment, since much more information is actively used to search the optimized rotation/translation parameters needed to match the images. The procedure has been tested in two different kinds of scenarios: a) images of samples with a clearly defined contour and b) images of samples without contour.

Optimizing translation and rotation parameters for image matching often starts by identifying equivalent points among images to be matched. Since chemical images obtained with different techniques do not allow easily for this task, the procedure proposed uses all available pixels in the images to be matched and a SIMPLEX algorithm to find the optimal parameters for the matching problem. Special attention is needed to find comparable representative information among images from different techniques. In images with a clear contour, e.g. images of isolated objects on a support, this morphological information can be used for matching. When a contour is missing and there are no clear landmarks in the sample surface, the problem becomes more complex. In this scenario, several alternatives can be tested as initial image matching information, such as global intensity maps, singular value plots obtained from image local rank analysis and pure compound distribution maps resolved by Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) on each of the images to be matched. Information obtained from distribution maps obtained by MCR-ALS has been previously used for other data analysis purposes[3,11–13] and has been proven to be also the most useful for spatial matching compared with other image representations.

The approach proposed has been applied to images of a pharmaceutical mixture acquired with Raman and IR. Two pairs of Raman and MIR images have been matched: one formed by Raman and MIR images acquired on a whole sample with a clear contour shape and the other pair acquired on a sample surface without contour, i.e., without clear marker points linked to sample orientation and position. In this way, the methodology described covers the different situations found to match images with different spatial properties for multitechnique image analysis.

The work includes a thorough study about the effect of the initial information used for matching on the robustness of the retrieved translation/rotation parameters. Besides, the effect of a proper image matching on subsequent multiset analysis is shown through the results of MCR-ALS applied to image multitechnique structures before and after matching.

1. **EXPERIMENTAL**

**Simulated data.** Simulated data have been generated to test the new image matchingprocedure. A binarized image, shown in figure 1a, has been used as a reference image. Two translated and rotated images have been generated by transforming appropriately the reference image (figure 1b). The translation and rotation parameters (dx,dy,Ө) used to generate each of the images in figure 1b have been (4, 3, -1) and (8, 3, 6), respectively. The first two figures are pixel shifts in the *x* and *y* direction and the third figure is the rotated angle in degrees. This set of images is only used to check the accuracy and robustness of the algorithm to recover the true translation and rotation parameters in an image matching problem.

**Sample preparation.** The analyzed sample has been a mixture of caffeine, acetylsalicylic acid (ASA) and starch. Caffeine and ASA simulate the APIs (active pharmaceutical ingredients) of a pharmaceutical formulation and starch acts as an excipient. The mixture of the pharmaceutical components was compacted and compressed by using a diamond cell in order to obtain a compact, flat and translucent sample, necessary for the correct acquisition of the transmission MIR and Raman images on the same sample area. All substances were from Sigma Aldrich (a.r) and were used without further purification.

**Infrared imaging.** The sample was imaged first by FT-IR using a Nicolet iN10 MX (Thermo). The image size is 38 × 32 × 1725, the first two figures being number of pixels in the *x* and *y* directions and the third number of spectral channels. The image was collected in point mapping mode where the pixel size is 15x15μm2. MIR spectra were the result of 64 accumulations, recorded in the spectral range 675-4000cm-1 with a 4cm-1 spectral resolution. The top left plot in figure 2 shows the global intensity map of the whole MIR pharmaceutical image. The zone marked in red represents the Region Of Interest (ROI) that will be used as example of image without contour. ROI image was 27×16 pixels.

**Raman imaging.** Raman spectra were acquired using a HR800 LabRAM **(**Horiba Jovin Yvon, Kyoto, Japan). A 532.058 nm laser was used as a light source and the Raman spectra were recorded with a 5s acquisition time in the spectral range going from 100 to 1800cm-1. Raman hyperspectral images have been acquired by point mapping with a pixel resolution of 15×15 μm2. The image size was 38 × 32 × 1745. The bottom left plot in figure 2 shows the global intensity map of the whole Raman pharmaceutical image with contour The zone marked in red represents the Region Of Interest (ROI) that will be used as example of image without contour, sized 27×16 pixels.

**Figure 1**

**3.DATA ANALYSIS**

* 1. **Data preprocessing**

Signal preprocessing removes spectral variance due to non-chemical information. Raman spectra showed a strong intense band at ~1332 cm-1 related to the diamond cell used as a support measurement, which was higher than the rest of the spectrum bands. The signal of the diamond Raman band was replaced by interpolating Raman intensities of neighboring spectral channels, since no other compounds gave signal in this region. Asymmetric Least-Squares (AsLS) was used to remove the irregular, intense and curved baselines present in Raman and IR spectra[12,14–16]. AsLS is based on a recursive local fitting of the whole spectrum with a baseline obtained by using a Whittaker smoother[15]. Two parameters are tuned according to the shape of the baseline, one linked to the smoothness (λ) of the fit and one to the penalty imposed to the points giving positive residuals in the fit (p)[14–16]. The use of AsLS requires a significant difference in frequencies among the spectral features and the baseline to be removed. This is the case among the narrow Raman features and the broader fluorescence baseline contribution. The same situation happens when comparing IR features and the broader Mie scattering contribution for this sample. Figure 2 shows the global intensity maps of Raman and MIR images with a clearly defined contour of a pharmaceutical mixture and the related AsLS-corrected Raman and MIR spectra. Raw Raman and MIR spectra are shown in supplementary figure S1.

**Figure 2**

**3.2. Image matching procedure**

Images registered on the same sample by different spectroscopic techniques need spatial transformations, i.e., pixel translations in *x* and *y* directions and rotations, to be matched. This can be achieved by different procedures, many of them based on the previous selection of equivalent pixels among images [9,17]. As mentioned before, this selection task is not trivial for chemical hyperspectral images obtained in different platforms, in which the spatial pattern is not well defined and differences in signal intensity behavior among techniques can hinder this correspondence. To avoid this problem, the procedure proposed to match images collected from different spectroscopic platforms uses information from all available pixels and works as follows.

In order to align two (or more) images the following steps are applied (a supporting graphical scheme is provided in Figure 3). First, one image is chosen as a reference, *Ar*, while the other, secondary image, *A****s***, is moved with respect to the first. In essence this is a symmetric process, it does not matter much which one is chosen as the reference. There are three parameters that define the displacement of the secondary image: the angle Θ of rotation about the center of the image and two shift parameters dx and dy defining the translation along the x- and y-axes. In the algorithm the rotation is performed before the translational shift. After this transformation the pixels do no longer align, therefore the signal amplitudes are interpolated linearly onto the pixel positions of the reference image (we use the matlab function interp2.m for the 2D interpolation). The difference between the two choices of reference and secondary images lies in small differences of these interpolations. No significant differences were detected in several example computations.

(i,j where there is overlap)

The objective function, defined in equation (1), is the sum over all squares of the differences between the signals of the reference image and the interpolated signals of the secondary image. The summation only covers the overlapping region of the two images after the rotation/translation transformations. In this context, *A(i,j)* represents any single data measurement (intensity, singular value, concentration, see later for explanation) associated with a particular pixel with Cartesian coordinates *i,j*. To minimize the objective function in equation (1) and find the optimal translation and rotation parameters (dx, dy and Θ), a SIMPLEX-based optimization algorithm is used.

Two different situations are studied. In images with a clear contour, information based on the contour shape is used to match images. In images without contour, we have compared different kinds of starting information among images, namely, global intensity maps, singular value maps obtained from local rank analysis[18] or information based on the distribution maps obtained by MCR-ALS analysis on each individual image (see sections 3.2 and 4 for more detail on how these kinds of information are obtained). Although equation 1 is defined for comparison of a single 2D frame in each image (reference and image to be aligned), whenever the information is formed by a set of analogous frames among the reference and the image to be aligned, they can be pairwisely compared and equation 1 will be the pooled sum of squares obtained from all performed comparisons. In the latter case, since frames come from the same pair of images, a single set of dx, dy and Θ will be optimized.

**Figure 3**

**3.3 Hyperspectral image resolution (MCR-ALS)**

Although image resolution is not the main goal of this work, the algorithm used will be explained since it is a methodology to provide initial information for image matching and it has also been used as a final step to assess the effects of proper image matching in multitechnique image analysis.

The information in an image is structured as a data cube where two dimensions design the pixel coordinates (*x* and *y*) and the third the spectral channels. In order to understand the underlying bilinear model related to this measurement, the hyperspectral image cube must be unfolded into a data matrix where the rows contain all the pixel spectra one under the other and the columns design the spectral channels. Now the **D** matrix follows the bilinear model shown in eq 2:

**D=CST+E Eq.2**

Where **D** contains the raw spectra of the image, **C** is the matrix of concentration profiles and **ST** contains the pure spectra of image constituents. **E** is the matrix of experimental error or unexplained variance by the MCR model. The distribution maps of each particular image constituent can be obtained folding back each column of the **C** matrix so that the original two-dimensional (2-D) configuration of the sample surface is recovered [3,6,11,19]. This bilinear model is the expression of the Beer-Lambert law in matrix form.

Hyperspectral image resolution methods provide the distribution maps and pure spectra related to image constituents from the sole information contained in the raw image measurement[6,11,18,20–23]. Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) is an iterative method oriented to recover the bilinear model described in equation 2 through the following basic steps:

1. Determination of the number of components present in the raw image **D** (e.g. by Singular Value Decomposition, SVD)
2. Generation of initial estimates based on the selection of the purest pixel spectra, e.g. by the use of a SIMPLISMA-based method[24].
3. Alternating optimization of **C** and **ST** matrices by least squares under constraints until convergence is achieved.

The operations **C=DS(STS)-1**and **ST=(CTC)-1CTD** are involved in the model optimization by alternating least squares (step 3). The parameter used to evaluate the fit quality of the final MCR model is the lack of fit defined as follows:

Lof (%)= **Eq. 3**

where *dij* is the element of the original data matrix in row *i* and column *j* and *eij* is the residual obtained from the difference between the element dij of the original data set and the analogous element obtained from the MCR-ALS model.

MCR-ALS allows introducing constraints during the optimization step. The use of constraints during the process provides chemical meaning to the concentration and pure spectra profiles and decreases the ambiguity effects in the final results obtained. For image analysis, non-negativity constraint has been applied since the concentration profiles as well as the spectroscopic readings of IR and Raman spectra are, by nature, positive[20,25,26] Normalization of pure spectra in **ST** has been used to avoid scale fluctuations in the profiles during optimization.

Often, the simultaneous analysis of several images can be of interest. In this case multiset structures or augmented data matrices **D** are built that contain different submatrices **Di**, linked to different individual images. In this work, multiset structures are formed by images obtained with different spectroscopic platforms on the same area surface. For this situation, a row-wise augmented data matrix is built putting the spectra of each image next to each other. The construction of multitechnique multiset structures is complex because the pixel mode must be common among images. This means that images (**Di**) must have the same pixel size and the same spatial orientation for the correct interpretation of the multiset structure. Multitechnique multisets also obey the bilinear model based on the Lambert-Beer Law (see eq2). The decomposition **D=CST** provides a single **C** matrix, valid for the fused images describing the same sample, and an augmented matrix **ST**, formed by as many submatrices as techniques used in the images forming the data set. The profiles of the **C** matrix can be refolded conveniently to recover the single set of distribution maps, each related to a particular image constituent (see figure 4).

**Figure 4**

**3.4. Software**

Image matching and MCR-ALS routines are in-house written MATLAB routines. MCR-ALS for single image analysis and complete multisets has been performed with the GUI freely downloadable at: [www.mcrals.info](http://www.mcrals.info).

**4.RESULTS AND DISCUSSION**

**4.1. Simulated data**

First, the matching image procedure proposed has been tested on the simulated data. The binarized image, shown in figure 1a, has been used as reference image and simulated images, shown in figure 1b, have been used as images to be aligned. Different sets of initial guesses for translation and rotation parameters have been used to check the accuracy and robustness of the algorithm to find the optimal values for these parameters. Optimized parameters obtained are very similar to the translated and rotated parameters used to simulated images to be aligned, despite the variation in the initial estimates used. Differences between optimized translation and rotation parameters and the reference values applied to obtain the simulated images are less than one unit (see table 1 for results). The negative signs indicate that the second image has to be moved in this way to undo the spatial transformations performed and match spatially the reference image. Inview of these results, we consider that the new image matching procedure is properly validated and can be used to perform this task on real data.

**4.2. Image matching**

The original Raman and MIR images of pharmaceutical mixtures described in section 2 have the same pixel size; therefore, balancing translation and rotation among images is the only matching step required to have coherent images that allow performing data fusion. A more complex approach devoted to fuse and unmix images with different spatial resolution will be presented in a future work.

The image matching algorithm proposed in section 3.2 has been tested on different types of starting image information, namely global intensity maps (raw and binarized), singular value plots obtained from local rank analysis and distribution maps of individual image MCR-ALS analysis (raw and binarized), to see which option provides the most robust results in terms of retrieval of rotation and translation parameters. The study was performed on the two pairs of images previously described, with and without contour.

Below, the starting information used and the way it has been obtained is briefly described.

**Global intensity maps**

The global intensity maps are 2D representations displaying the sum of all Raman and MIR intensities of each pixel spectrum in the image. Figure 5a and 6a show the raw global intensity maps of Raman (image to be aligned) and MIR (reference image) images with and without contour, respectively. Global intensity maps can be binarized when images present a clearly defined contour, since it is easy to visually set a threshold between significant and negligible sample signal. Figure 5b shows the binarized version of global intensity maps of Raman and MIR images with a clear contour. In this case, the binarized global intensity maps have been codified regarding the presence (1, white color) and absence (0, black color) of sample signal.

**Figure 5**

**Singular value plots from local rank analysis**

Information related to compound overlap may be potentially more similar than signal pattern among images collected with different techniques. To explore this possibility, local rank information was obtained by applying the algorithm Fixed Size Image Window-Evolving Factor Analysis[18]. FSIW-EFA works performing local PCA analyses on 2D pixel windows of the same size moved across the full image surface[18]. Once this is done, all first singular values, second singular values and so on are displayed in related 2D plots reflecting the structure of the sample surface.

Figures 5c and 6b show the second and the third singular value plots of Raman and MIR images obtained from FSIW-EFA performed on the whole Raman and MIR images with a clear contour and on the ROI images with no contour. Second and third singular value plots of Raman and MIR of both couples of images were the only singular values used in the matching procedure because they were showing the most similar pattern among the images compared. The first singular value plot showed essentially changes in signal intensity (different among spectroscopic techniques), whereas second and third singular value plots were more oriented to enhance image zones with compound overlap.

**Figure 6**

**Distribution maps of MCR-ALS on single Raman and MIR images**

Distribution maps are excellent representations of the pixel space of the pure compounds of the image. The spatial distribution of each pure compound in a sample is consistent information, which can be similarly detected among images registered with different techniques. To obtain this information, MCR-ALS was applied to analyze separately each of the Raman and MIR images used in this study. MCR-ALS analysis of the MIR image with and without contour and the Raman image without contour resolved three components (caffeine, ASA and starch), whereas four components were resolved for the Raman image with contour (the three chemical compounds and an additional contribution linked to the very low residual signal from the diamond support remaining after correction). In all images, resolution was applied under the constraints of non-negativity in concentration profiles and spectra and with spectra normalization of matrix **ST**. Table 2 shows the model fit parameters for all images. Resolution of all compounds in these images was unique due to the conditions of local rank/selectivity related to the favourable compound overlap pattern [6]. This fact has been confirmed by assessing ambiguity of the final results with the approach proposed by Tauler [27]. At this point, it is worth commenting that resolution results of hyperspectral images are often unique due to the large amount of diverse information in terms of composition and compound overlap provided by the thousands of pixels analyzed.

**Table 2**

Figures 7a and 7b show the final results of distribution maps and pure spectra for the Raman and MIR images of the whole sample area scanned, respectively. Pure spectra of caffeine, ASA and starch (right side of figures 7a and 7b) were assigned to the suitable compounds by comparison of Raman and MIR spectral features in pure spectra with literature reference spectra. Distribution maps of Raman and MIR analysis are similar morphologically, although they show some differences in the concentration pattern.

**Figure 7**

Figures 5d and 6c display the raw distribution maps of the three pure sample constituents (caffeine, ASA and starch), which are the sole information used for image matching, since these are the compounds detected by the two techniques. The use of distribution maps allows selecting only the compounds common to the different techniques to be compared. This is an advantage over other kinds of starting information, which do not offer this possibility and may suffer from differences in the compounds detected and, as a consequence, in the related global signal acquired. This would also allow discarding the use of maps of components resolved with a high ambiguity, if that was the case, to avoid problems in the image matching procedure.

Raw distribution maps can also be binarized, according to the presence (1, white color) or absence (0, black color) of the pure compound. Figure 5e and 6d show the binarized distribution maps of Raman and MIR images with and without contour, respectively. In this case, only the morphological information linked to presence/absence of a particular compound is kept.

Once the initial information for image matching was obtained, different initial estimates of translation and rotation parameters (dx, dy and Θ) were used to test the robustness of the optimized parameters for the different kinds of starting information used.

Tables 2 and 3 summarize the results of the image matching procedure in all the scenarios tested. Initial and optimized values for the translation and rotation parameters are provided. Different trends can be seen in the optimized parameters when images with and without contour are compared.

**Table 3**

For Raman and MIR images with a clear contour, the use of this morphological information is clearly helpful for image matching purposes. Such an information is best represented in the binarized global intensity maps, which only rely on enhancing the sample contour information vs. the support. The use of binarized intensity global maps for image matching presents robustness in the translation and rotation parameters found (dx, dy, Θ) because the pattern in presence/absence of chemical sample contribution is very consistent among images (see figure 5b). It is interesting to note that optimized parameters of table 3 lack robustness when raw global intensity maps are used as starting image information instead of their binarized version. The reason is that MIR and Raman images show different patterns of signal intensity and this has a negative effect when comparing information among images (figure 5a).

When images to be aligned present a clear contour, the use of binarized global intensity maps is the best and simplest option for image matching. However, it is interesting to observe how other kinds of information can work in this situation.

The use of singular value plots coming from local PCA analysis do not show very consistent results because, although in a more indirect way than raw global intensity maps, differences in signal intensity among compounds in the different techniques may affect the capacity of detection of regions of compound overlap.

The results linked to using distribution maps provide similar conclusions as when raw and binarized global intensity maps were compared. Although raw distribution maps from common compounds provide robust results in the translation parameters found (dx, dy), some problems remain in finding Θ due to the slightly different concentration patterns in chemical pixels among maps of different images (see figure 5d). However, the optimal parameters of the binarized version of distribution maps are robust due to the fact that the pattern in presence/absence of compounds, which is the sole information contained in binarized distribution maps and that merely preserves the map morphology, is very consistent among images (see figure 5e). The optimal parameters obtained from the use of binarized distribution maps are very similar to the optimized parameters got from the binarized version of global intensity maps. This fact shows consistency of the matching procedure.

A more challenging scenario is found when images do not have a sample contour, i.e., the sample covers all the surface analyzed (see table 4). In this case, the variation of optimized parameters when changing the initial estimates becomes clearly higher when raw global intensity maps, singular value plots or even raw distribution maps are used as starting information for the image matching procedure. This phenomenon becomes more evident because, even if these differences existed in the images with sample contour, the area linked to the absence of sample was positively helping in all image matching procedures, independently of the rest of information used. When images lack contour, optimized parameters are only consistent when binarized distribution maps are used. In comparison with the results shown in table 3, optimized parameters values are not exactly the same because the ROI Raman and MIR pharmaceutical mixture images are not mismatched in the same way as images with contour.

**Table 4**

After the study of the effect of starting image information on the image matching procedure in both couples of images, binarized global intensity maps have proven to be the best option to obtain a robust matching among images with a clear contour, while the use of binarized distribution maps is the choice for the matching procedure among images without contour. The use of binarized distribution maps as starting information for matching images presents several advantages, namely:

* Consistent compound presence/absence and morphology patterns, independent of the spectroscopy used.
* The information of compounds is separated in the resolved maps. This allows selecting only the maps that are comparable among images for proper matching. Note that this advantage is absent in the rest of starting information tested, i.e. global intensity maps or singular value plots, where all compounds (common or specifically detected by a particular technique) contribute to the information compared.

Regarding the effect of morphological structure of the sample imaged, the matching procedure proposed has worked in all the scenarios tested, even if there were no clear landmarks for comparison among the images matched.

**4.3 Multitechnique MCR-ALS image analysis**

Once optimal translation and rotation parameters are found (using binarized distribution maps for Raman and MIR images without contour and binarized global intensity maps for images with contour), suitable spatial transformations are applied to match the images that should be analyzed together. Spatial transformations can generate regions with empty pixels in both images. These regions have been removed from both images before data fusion.

Matched MIR and Raman images are joined in a multitechnique multiset structure setting one image next to each other building a row-wise augmented data matrix. MCR-ALS method was carried out in both aligned multitechnique multiset structure using three constituents under the same constraints used in the single MCR-ALS analysis. In this case, support background pixels, i.e., pixels related to the residual diamond cell signal, were removed before the MCR-ALS analysis in the case of images with contour. For comparison and assessment of the effect of image matching on the final MCR model, multiset analysis was performed on the multitset structure formed by Raman and MIR images before and after image matching. For the multiset of Raman and MIR images with contour, the lack of fit was 13.7% for the matched multiset structure, while lack of fit was 24.4% for the mismatched multiset. In the case of the multiset formed by Raman and MIR images without contour, the lack of fit was 7.54% and 15.01% for the matched and mismatched multisets structures, respectively. The fit quality of the MCR-ALS results obtained improves significantly after performing the image matching procedure in both cases.

Figure 8 shows MCR-ALS results for the matched multitechnique multiset of Raman and MIR images with contour.

**Figure 8**

Pure spectra matrix represents Raman and MIR spectral signatures of each image constituent. Spectral signatures are very similar to reference spectra for these compounds and to those obtained from individual MCR-ALS image analysis of each technique. Recovering correct spectral signatures is a sign of a correct matching preprocessing among Raman and MIR images, since the only set of related distribution maps refers really to the same sample. Misaligned multisets provide less well defined maps and spectral signatures with mixed spectral features of compounds.

A last confirmation of the benefits connected with a correctly matched multiset can be observed in more detail when the residual maps of image multisets with contour before and after matching are compared (see figure 9). Residual maps are represented using the lack of fit scale, i.e., for every pixel *i* , the lack of fit value is obtained as:

**Figure 9**

where subindex *j* refers to the spectral channel. In this case, the residual map of the multiset before image matching shows higher residuals than the correctly matched image multiset structure, particularly in the zones of boundaries among different components, where consequences of the image mismatch become more evident.

**5.CONCLUSIONS**

Data fusion of chemical images acquired with different spectroscopic platforms is particularly complex because of the lack of clear analogous information among images. This is due to the often unclear morphological structure of chemical samples and to the variation in signal patterns provided by different spectroscopic techniques.

Coherence among images is of utmost importance when image fusion has to be carried out. The difficult choice of equivalent pixels in images from different techniques is avoided with a new procedure based on a SIMPLEX optimization that makes use of all pixels in the images to find the optimal translation and rotation parameters to match images. Morphological information, either linked to the full sample contour, displayed in the binarized global intensity map, or to compound-specific information, represented by the binarized distribution maps obtained after MCR image analysis, is a good choice to perform image matching among techniques. The reason behind is that patterns of presence/absence of sample (in the case of images with a clear contour) or pure compounds ( in the case of images with unclear contour) in the images are very consistent and are not affected by the technique used in the image acquisition.

An additional advantage of binarized distribution maps over other kinds of image information is that they are compound-specific and, as such, this allows selecting only the maps related to common compounds detected by the different techniques to perform the matching procedure. This is not doable when information coming from global signal or mixed principal component contributions is used.

The positive effect of accurate matching procedure is clearly seen when multitecnique image analysis is performed by MCR-ALS in the matched and mismatched multisets structures, respectively. The model fit parameters, the spatial structure of the residuals and the resolved spectra and distribution maps recovered confirm that multitechnique image analysis improves significantly after adequate image matching.

The methodology proposed might be considered as a general protocol to match images acquired from different spectroscopic imaging techniques with or without the presence of contours or clear landmarks. This preprocessing is essential to ensure the necessary quality in data analysis results associated with image data fusion.

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**ºTABLE CAPTIONS**

Table 1. Optimized translation and rotation parameters for simulated data image matching.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | |
| **Simulated data** | **Initial parameters**  Θ **dx dy** | | | **Optimized parameters**  Θ **dx dy** | | |
| (dx, dy, Θ)  4 3 -1 | 4 | 3 | -1 | -4.06 | -2.91 | 1.18 |
| 10 | 5 | -6 | -4.06 | -2.91 | 1.18 |
| 8 | 3 | 6 | 4.06 | -2.91 | 1.18 |
| 8 3 6 | 4 | 3 | -1 | -7.85 | -3.82 | -5.54 |
| 10 | 5 | -6 | -7.85 | -3.82 | -5.54 |
| 8 | 3 | 6 | -7.85 | -3.82 | -5.54 |

Table 2. Lack of fit (%) of MCR-ALS individual analysis of each Raman and MIR image with and without contour.

|  |  |  |
| --- | --- | --- |
|  | **Image with contour** | **Image without contour** |
| Raman image | 3.35% | 2.40% |
| MIR image | 5.81% | 3.41% |

Table 3. Optimized translation and rotation parameters for MIR and Raman image matching using different kinds of starting information. Raman and MIR images have a clear sample contour.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | |
| **Starting**  **information** | **Initial parameters**  Θ **dx dy** | | | **Optimized parameters**  Θ **dx dy** | | |
| Global  intensity maps | 4 | 3 | -1 | 4.08 | 2.67 | -1.04 |
| 10 | 5 | -6 | -0.07 | 3.25 | -0.44 |
| 8 | 3 | 6 | 4.41 | 2.58 | -0.74 |
| S.v. Plot | 4 | 3 | -1 | 5.86 | 3.20 | -1.56 |
| 10 | 5 | -6 | 18.33 | 4.92 | -5.68 |
| 8 | 3 | 6 | 5.71 | 4.46 | 5.97 |
| Distribution maps | 4 | 3 | -1 | 6.72 | 2.41 | -1.35 |
| 10 | 5 | -6 | 5.96 | 2.28 | -1.40 |
| 8 | 3 | 6 | 13.5 | 3.05 | -1.43 |
| Binarized distribution maps | 4 | 3 | -1 | 7.21 | 2.51 | -1.48 |
| 10 | 5 | -6 | 7.1 | 2.51 | -1.51 |
| 8 | 3 | 6 | 7.1 | 2.51 | -1.51 |
| Binarized global intensity maps | 4 | 3 | -1 | 7.07 | 2.47 | -1.66 |
| 10 | 5 | -6 | 7.07 | 2.47 | -1.66 |
| 8 | 3 | 6 | 7.07 | 2.47 | -1.66 |

Table 4. Optimized translation and rotation parameters for MIR and Raman image matching using different kinds of starting information. Raman and MIR images lack sample contour.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | |
| **Starting**  **information** | **Initial parameters**  Θ **dx dy** | | | **Optimized parameters**  Θ **dx dy** | | |
| Global  intensity maps | 4 | 3 | -1 | -6.40 | 17.75 | -2.93 |
| 10 | 5 | -6 | -24.0 | 15.70 | -21.01 |
| 8 | 3 | 6 | -1.11 | 4.76 | 25.12 |
| S.v. Plot | 4 | 3 | -1 | -0.69 | 3.55 | 9.40 |
| 10 | 5 | -6 | 9.43 | 5.54 | -5.99 |
| 8 | 3 | 6 | 5.78 | 1.13 | -0.86 |
| Distribution maps | 4 | 3 | -1 | 6.89 | 0.62 | -0.39 |
| 10 | 5 | -6 | 18.68 | 2.32 | -0.06 |
| 8 | 3 | 6 | 15.04 | 1.64 | -0.21 |
| Binarized distribution maps | 4 | 3 | -1 | 8.20 | 0.51 | -0.35 |
| 10 | 5 | -6 | 8.20 | 0.51 | -0.35 |
| 8 | 3 | 6 | 8.20 | 0.51 | -0.35 |

**FIGURE CAPTIONS**

Figure 1 Simulated data of binarized image. a) Binarized reference image b) Simulated translated and rotated binarized images to be aligned.

Figure 2. *Left plots:* global intensity maps of MIR and Raman image of pharmaceutical mixture. Red framed areas are ROI images. *Right plots*: MIR and Raman spectra of pharmaceutical images after baseline correction by Asymmetric Least Squares.

Figure 3. Ghrapical scheme of the new image matching procedure proposed.

Figure 4. a) Bilinear model coming from the resolution of a complete multitechnique image multiset structure.

Figure 5. Image starting information for matching procedure of Raman and MIR images with a clear structure. a) Global intensity plots. b) Second and third singular value plots from PCA local rank analysis. c) Distribution maps of ASA, Caffeine and Starch obtained from individual MCR-ALS image analysis. d) Binarized distribution maps of ASA, caffeine and starch e) Binarized global intensity maps

Figure 6 . Image starting information for matching procedure of Raman and MIR images without defined shape. a) Global intensity plots. b) Second and third singular value plots from PCA local rank analysis. c) Distribution maps of ASA, Caffeine and Starch obtained from individual MCR-ALS image analysis. d) Binarized distribution maps of ASA, caffeine and starch.

Figure 7. MCR-ALS results obtained from the single Raman and MIR image analysis of pharmaceutical mixture with a clear structure. a) Distribution maps and pure spectra of Raman image constituents (ASA, caffeine, starch and support).b) Distribution maps and pure spectra of MIR image constituents (ASA, caffeine and starch).

Figure 8. MCR-ALS results of multitechnique image analysis. Resolved distribution maps and pure spectra from MCR-ALS simultaneous analysis of Raman and MIR image on the same area of pharmaceutical mixture.

Figure 9 *Left plot*: Residual maps obtained from the multitechnique MCR-ALS analysis of misaligned multiset structure. *Right plot:* Residual map obtained from multitechnique MCR-ALS analysis of aligned multiset structure.

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