Image denoising based on Singular-Spectrum-Analysis (SSA) in femtosecond stimulated Raman scattering microscopy

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Abstract. Stimulated Raman scattering (SRS) microscopy is able to perform high sensitivity biological imaging with high spatial and spectral resolution and image acquisition time of a few seconds. Nevertheless, SRS images often suffer from low SNR, due to the weak Raman cross-section of biomolecules. Therefore, methods aiming to improve image quality are mandatory. In this paper, the performances of a 2D denoising algorithm based on SSA is analysed. SRS imaging of lipids droplets have been used in order to validate our algorithms.

1 Introduction

Image denoising plays a vital role in a wide range of applications such as image restoration, visual tracking, image segmentation and image classification. [1-3].
The most effective strategies for denoising rely on convolutional neural networks (CNNs). However, CNNs are characterized by an unknown input-output relationship, and their output is biased to the training set. This increases the uncertainty of a diagnostic result. A different class of algorithms, characterized by well-defined input-output relationships, are preferable for applications in medicine, such as Stimulated-Raman-Scattering (SRS) microscopy. However, only a few studies exist on this topic. The Singular-Spectrum-Analysis (SSA) is a powerful tool for denoising, with a well-known input output relationship [4][5]. This work delves into the application of SSA for denoising SRS images.
In SSA, the "trajectory matrix" of a (1D) signal is used to separate it into ita spectral components: when the noise is *independent-identically-distributed* (i.i.d.), it is then possible to perform denoising by discarding those components associated with the upper region of the spectrum. We adapted SSA for denoising 2D SRS images. Our strategy consists of filtering SRS images columnwise -where the noise was found i.i.d. [6] – and we use a correlation coefficient to recognize different spectral components in each column. This is a novel approach for denoising, which attempts at automating the procedure of visually inspecting the singular vectors [4].
The correlation coefficient was defined as:

$$Ci = \sum_j Yi(j)Yi(j+1)$$

Where Yi is the singular vector associated to the i-th singular value of the SVD of the trajectory matrix of the signal. All the components of the trajectory matrix resulting in $Ci \leq 0.8$ were associated to noise and suppressed (discarded).

Herewith, we report the results of applying our 2D denoising strategy to SRS images of biological samples. This paper is organized as follows. In the second section, we describe our home-built SRS microscope- i.e. the experimental setup. In the third section, we report the results of denoising we discuss them briefly. In the last section, we draw the conclusions.

2 IMAGE PROCESSING

In SRS, a slight variation in an intense laser beam (the transmitted probe or pump) on the scale of 10^{-5} to 10^{-4} has to be detected, which can be contaminated by laser intensity noise. Since, the SRS output signal and excitation pulses have the same wavelengths, their spectral separation is impossible, so most SRS studies take advantage of high-frequency optical modulation and phase-sensitive detection. The optical architecture of our implemented SRS microscope, covering the C H region (above 2500 cm^{-1}) of the Raman spectrum, was reported in our earlier articles. It was briefly obtained by integrating a femtosecond stimulated Raman spectroscopy with an inverted Nikon Ti-Eclipse microscope equipped with a scan head [7-13].

The image processing analysis was performed on several SRS images of lipid droplets (LDs) inside adipocyte cells. For investigation of the C H2 bond of lipids— having a Raman shift of 2845 cm^{-1} —our pump signal was set to

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810 nm and Stokes signal at 1053 nm. For all samples, the power of the pump and Stokes incident onto the sample was set to 11 mW. The LIA time constant was fixed to 100 μs with an 18 dB/oct slope and a sensitivity of 10 μV. Each SRS acquired image is a single collection of 512px x 512px, and the acquisition time was 16 s.

Figure 1 compares raw and filtered SRS images. We note the ability of SSA of suppressing noise in flat regions of the image, while accurately resolving the edges of lipid droplets.

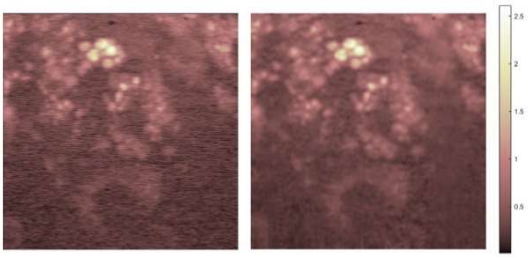

Figure 1. on the left an example of raw SRS image, on the right the image obtained denoising the raw image by SSA

Figure 2 contains a more detailed comparison in a column of the image. The result confirms previous observations. We note that peaks and valleys in the signal were accurately reproduced, while noise was heavily repressed. This mainly indicates that the choice of the threshold $Ci \leq 0.8$ is acceptable, and it is likely that setting a lower threshold would yield to the conservation of spurious components of noise, while setting an higher threshold would lead to inability to correctly resolve the edges.

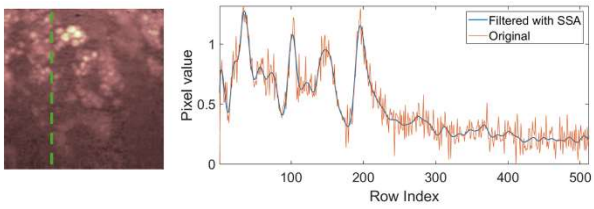

Figure 2. Qualitative evaluation of the SSA filter in a column of the image (column 142). On the left, the filtered image. On the right, a comparison of raw and filtered signal.

3 CONCLUSIONS

In this paper, the performance of a 2D image processing algorithm based on SSA in denoising SRS images have been investigated. The algorithm was found suitable for denoising SRS images, being able to heavily suppress noise, while preserving edges and details in the image. We believe that our findings could be generalizable to the variety of nonlinear microscopes readers may use, and they could provide a widely usable and cost free methodology to improve biological imaging quality.

References

1. M. A. Flower, Webbs Physics of Medical Imaging, Taylor & Francis, 2012
2. R. C. Gonzalez and R. E. Woods, Digital Image Processing, 2nd edn. (Prentice-Hall, Inc., 2002).
3. M.Sonka, V. Hlavac, R. Boyle, Image Processing, Analysis and Machine Vision, 4th edition, Cengage Learning, 2015
4. Golyandina et al. ,Analysis of Time Series Structure: SSA and related techniques, Champman-Hall,2001
5. A.M. Tomé, D. Malafaia, A.R. Teixeira, E.W. Lang, On the use of Singular Spectrum Analysis, in arXiv:1807.10679,2018,pp.1-23
6. Ranjan, R., Costa, G., Ferrara, M. A., Sansone, M., Sirleto, L. Noises investigations and image denoising in femtosecond stimulated Raman scattering microscopy. J. Biophotonics 2022, 15(6),e202100379https://doi.org/10.1002/jbio.2021 00379.
7. B. Manifold, E. Thomas, A. T. Francis, A. H. Hill, F. Dan, Biomed. Opt. Express 2019, 10(8), 3860.
8. P. Abdolghader, A. Ridsdale, T. Grammatikopoulos, G. Resch, F. Légaré, A. Stolow, A. F. Pegoraro, I. Tamblyn, Opt. Express 2021, 29(21), 34205.
9. H. Lin, H. J. Lee, N. Tague, J. B. Lugagne, C. Zong, F. Deng, J. Shin, L. Tian, W. Wong, M. J. Dunlop, J. X. Cheng, Nat. Commun. 2021, 12, 3052.
10. A. Zumbusch, W. Langbein, and P. Borri: Nonlinear vibrational microscopy applied to lipid biology, Prog. Lipid Res., vol. 52, pp. 615-632, 2013.
11. A. D'Arco, N. Brancati, M. A. Ferrara, M. Indolfi, M. Frucci, and L. Sirleto: Subcellular chemical and morphological analysis by stimulated Raman scattering microscopy and image analysis techniques, Biomedical Optics Express, vol. 7, pp. 1853-1864, 2016.
12. R. Ranjan, A. D'arco, M. A. Ferrara, M. Indolfi, M. Larobina, and L. Sirleto: Integration of stimulated Raman gain and stimulated Raman losses detection modes in a single nonlinear microscope, Optics Express, vol. 26, no. 20, pp. 26317-26326, 2018.
13. Ranjan, R.; Costa, G.; Ferrara, M.A.; Sansone, M.; Sirleto, L. Noise Measurements and Noise Statistical Properties Investigations in a Stimulated Raman Scattering Microscope Based on Three Femtoseconds Laser Sources. Photonics, 9, 910, 2022.