Final Report
P2R Franco-Israeli Program 2007-2008

Blind deconvolution and 3D PSF modeling in biological microscopy

1. Participants:

French Side: ***Institut Pasteur*** (J.C. Olivo-Marin (PI)); ***ARIANA***, joint INRIA/CNRS/UNSA research group (L. Blanc-Féraud and J. Zerubia),

Israeli Side: ***Technion*** (A. Feuer (PI)) ***Weizmann Institute*** (Z. Kam),

2. Abstract:

The aims of this proposal are two-fold: first, derive analytical PSFs expressions for modelling the degradation of the images by an aberrated microscope and find accurate Gaussian approximations to be used for deconvolution purposes. Second, derive a blind deconvolution algorithm that makes use of the PSFs models above by estimating the parameters of the approximate PSFs thanks to an information-theoretic approach.

One of the most limiting factor of microscopy in biology is the fact that the full resolution of the microscope cannot be realized for three-dimensional thick samples. The reason is that imaging without aberrations (in practice, with aberrations smaller than the diffraction-limit resolution) can only be achieved under well defined conditions. For biological microscopy these are set for samples just under a cover-slide of well defined thickness. As soon as the focus of the objective is moved into the sample depth, the resolution of the optical system degrades.

It is the purpose of this project to develop computational methods that are compatible with modern three-dimensional microscope imaging procedures and make them usable in routine practice.

Three-dimensional image deconvolution is a post-acquisition method that uses the known properties of the microscope optics to reconstruct better images. However, in 3D microscopy, most of the deconvolution algorithms fail to function well when the optical characteristics in the working conditions deviate from the assumed model. These characteristics are usually represented by the Point Spread Function (PSF) which is the image of a sub-resolution point source.

For deconvolution methods in microscopic imagery, it is important to know precisely the degradation function (i.e. the PSF) which characterizes the microscope in several experimental contexts and which defines the degradations induced by all the elements of the set optical system/sample. For high accuracy, simple models of the non aberrated PSF are however not precise enough. Our goal is to devise accurate modelling of PSFs with aberrations and to find accurate approximations with analytical expressions depending only of a small number of parameters. This is sought for both confocal and wide field PSF models.

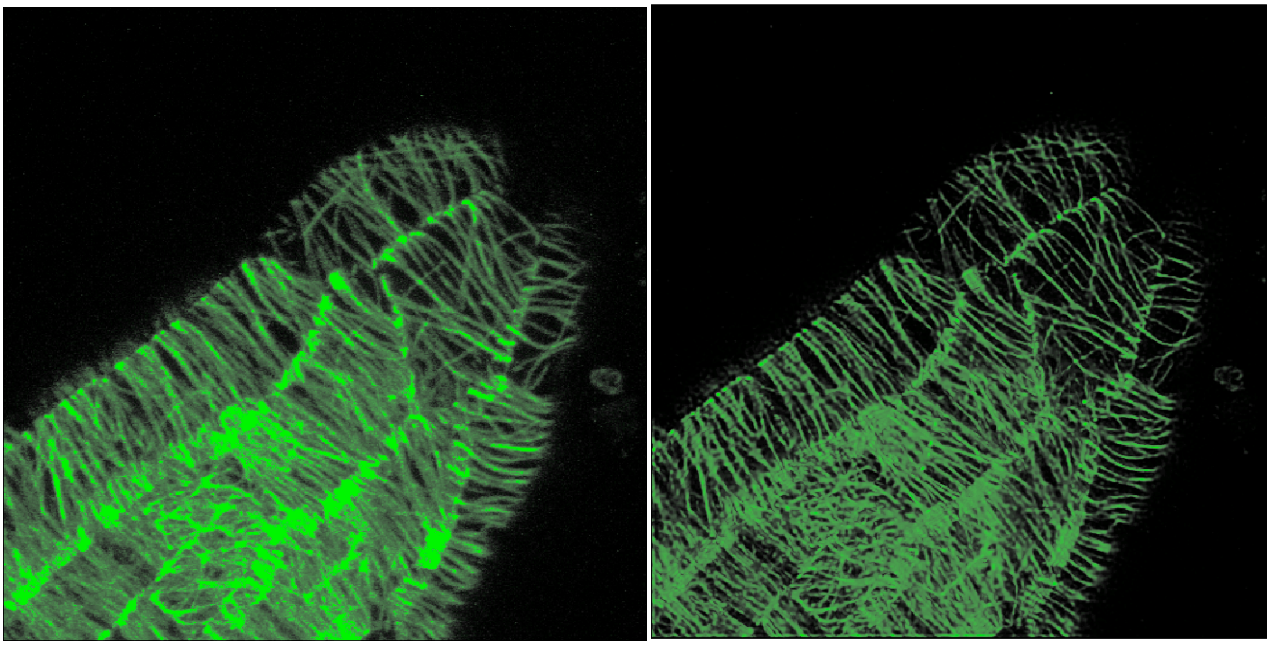
The proposed project develops deconvolution software that uses the modelling of aberrated PSFs to reconstruct the three-dimensional image and the PSF under aberrated conditions. To define the best model according to the degraded image, we use the approach of estimation theory.

3. Scientific achievements:

This project has resulted in the publication of one journal paper [4] and five international conference papers [2-3, 5-7]. One additional paper has been submitted recently on the latest results achieved by the team [8].

3.1 Blind deconvolution by an Alternate Minimization algorithm (P. Pankajakshan et al.)

In continuing with the first phase of ARIANA's work, we developed and validated an Alternate Minimization (AM) algorithm for jointly estimating the parameters of the diffraction-limited Point Spread Function (PSF) of a Confocal Laser Scanning Microscope (CLSM), and the specimen fluorescence distribution function. The optical section of a 3D specimen under focus suffers from two primary physical limitations. The diffraction-limited nature of the optical system, and the reduced amount of light detected by the photomultiplier cause blur and photon counting noise respectively. Thus each optical section has the in-focus plane and also out-of-focus contributions from other regions of the object. These images can hence benefit from post-processing restoration methods based on deconvolution. Estimating the object and the PSF simultaneously using blind deconvolution is a very ill-posed problem as one can always find many possible solution combinations of the PSF and the object that can satisfy equally the given observation. A model on the image acquisition physical process is necessary for band-limiting the PSF estimation results and in restricting the degrees of freedom to the number of free parameters of the model. A 3D separable Gaussian model is suitable for thin-layered specimens with media of matched refractive indices. However, the parameters of such a model may vary during the course of experimentation, and so they have to be estimated directly from the observational data. Similarly, a priori knowledge on the specimen introduced through a Total Variation (TV) constraint permits stabilization of the deconvolution algorithm and favors its convergence. The novelty of the approach is its application to restoring 3D biological image data by assuming that the statistical variation of the photon counting process follows a Poissonian statistics. The results obtained by the proposed algorithm on the PSF and the specimen from the simulated data show that the PSF could be estimated to a high degree of accuracy, and those on real data show better deconvolution as compared to a theoretically modelled PSF.



Maximum Intensity Projection (MIP) of (left) the root apex of an Arabidopsis Thaliana with a volume 56.32 µm x 56.448 µm x 13.64 µm (©INRA) and (right) of the restored image slices (©Ariana-INRIA/I3S).

3.2 Gaussian approximations of microscope PSFs (B. Zhang et al.)

We have comprehensively studied the least squares Gaussian approximations of the diffraction-limited 2D/3D paraxial/non-paraxial point spread functions (PSFs) of wide-field

fluorescence microscope (WFFM), laser-scanning confocal microscope (LSCM) and disk-scanning confocal microscope (DSCM) described using the Debye diffraction integrals. Optimal Gaussian parameters were derived for the 2D paraxial WFFM PSF, under both the L^∞ and L^1 normalizations. For the other PSFs, with the L^∞ normalization, near-optimal parameters in explicit forms are derived using Maclaurin series matching. Numerical results show that the 2D approximations are very accurate; the 3D approximations are average for WFFM, accurate for DSCM and LSCM with small and reasonably large pinholes respectively, and are nearly perfect for LSCM with small pinholes. These Gaussian approximate PSF models allow fast computation and greatly simplify the modeling of biological objects under these microscopes.

3.3 Improving particle localization with empirical PSFs (M. Marim et al.)

Building on approximations of PSFs with Gaussian kernels, we developed a method to improve the localization accuracy of single particles in fluorescence microscopy. Accurate computational localization of single fluorescent particles is of interest to many biophysical studies and underlies recent approaches to high resolution microscopy using photo-switchable fluorophores. The position of individual particles is typically computed by least-squares fitting of a gaussian intensity profile to the image, whose band-width is either derived from an idealized theoretical model of the point spread function (PSF), or itself fitted to the image. we measure the real PSF bandwidth using fluorescent beads as calibration probes, and use this new bandwidth in a Gaussian model fitting algorithm. We have shown that by measuring the real PSF bandwidth using fluorescent beads as calibration probes, and use this new bandwidth in a Gaussian model fitting algorithm, we could improve significantly the 3D localization accuracy in the nanometer range.

3.4 Fluorescence image denoising (B. Zhang et al.)

Following on the work at Institut Pasteur of Bo Zhang (PhD student funded by BDI CNRS) in collaboration with scientists at CEA-Saclay and CNRS-Caen, we validated a denoising algorithm for fluorescence images. Images produced by LSCM and DSCM have either a Poisson or a mixed-Poisson-Gaussian (MPG) statistical nature according to different function modes of the microscope. We have proposed two approaches for Poisson noise removal. One method is based on biorthogonal Haar-domain hypothesis tests, which is particularly suitable for fast estimating smooth intensities from large datasets. Our second method makes use of a well designed variance stabilizing transform (VST) allowing to Gaussianize and stabilize a filtered Poisson process. This VST can be combined with most multi-scale transforms yielding multi-scale VSTs (MS-VST). We have shown that this MS-VST approach provides a very effective denoiser capable of recovering important structures of various (isotropic, line-like and curvilinear) shapes in (very) low-count images.

Although we wanted to test the efficiency of this algorithm as a preliminary denoising step before performing the deconvolution in collaboration with the groups at Technion and ARIANA, this part of the project could not be performed as B. Zhang left the team after graduating.

3.5 Combined denoising and deconvolution (C. Chaux et al.)

We developed a method on the use of an iterative algorithm for 3D confocal microcopy image restoration based on 3D wavelets [5], that has shown very promising results that would need now be tested more extensively on confocal images coming from Institut Pasteur and Weizmann Institute. This will be done in 2009 as an extension of the project.

4. Budget for the French side:

The French side initially asked for a yearly budget of 24k euros per year, and finally got 14,7k euros per year. To facilitate the management of this relatively small amount, it was agreed among the two French partners and approved by the French Ministry, that the teams would receive

the full budget (14,7k euros) for a full year at once. Therefore, in 2007 the Institut Pasteur team received 14,7k euros and the ARIANA team will receive 14,7k euros by the end of 2008 that will reimburse the costs incurred by the team. By Sept. 2008, 75 % of the global French budget had been spent or engaged for trips to visit the different members of the team in France and in Israel and to attend conferences. The remainning money will be used during the year 2009 to cover pending costs of the Ariana team (in particular the invitation of some of the PhD committee members for P. Pankashakshan in Sept 09).

A scientific meeting was held at INRIA Sophia Antipolis on Oct 15-16 with the participation of all the teams (http://www-sop.inria.fr/ariana/Projets/P2R/brainstorming2007.html). The goal was to have a brainstorming session on the next steps and also to put in place strategies to coordinate the actions for the coming months. This was a very successful and constructive meeting. The cost was 1300 euros and it was completely covered by a separate budget from INRIA, while the travel expenses for the Israeli and Pasteur teams were covered from their budget.

Ariana hired a PhD student (M. Praveen Pankajackshan) funded by INRIA, who started in December 2006, to work full time on this project. His future work will be developed and tested during the third year of his thesis in collaboration with Technion, Pasteur and Weizmann, and with Dr Caroline Chaux, a former postdoc at ARIANA funded by INRIA from November 2006 to September 2007, and now at CNRS-Marne la Vallée.

5. Publications

1. P. Pankajakshan, B. Zhang, L. Blanc-Féraud, Z. Kam, J.C. Olivo-Marin and J. Zerubia, "Parametric Blind Deconvolution for Confocal Laser Scanning Microscopy (CLSM)," Research Report, No. 6493, INRIA, France, April 2008.
2. P. Pankajakshan, B. Zhang, L. Blanc-Féraud, Z. Kam, J.C. Olivo-Marin and J. Zerubia "Blind deconvolution for diffraction-limited fluorescence microscopy," In Proc. IEEE Intern. Symp. on Biomedical Imaging, ISBI 2008, Paris, pp. 740-743, May 2008.
3. Marim, M., Zhang, B., Olivo-Marin, J.-C. and Zimmer, C. (2008) Improving single particle localization with an empirically calibrated gaussian kernel, In Proc. IEEE Intern. Symp. on Biomedical Imaging, ISBI 2008, Paris, May 2008, pp. 1003–1006
4. Zhang, B., Zerubia, J., and Olivo-Marin, J.-C. (2007) Gaussian approximations of fluorescence microscope PSF models, *Applied Optics*, 46, 10, pp.1819-1829
5. Pankajakshan, P., Zhang, B., Blanc-Féraud, L., Kam, Z., Olivo-Marin, J.-C., and Zerubia, J. (2007) Parametric Blind Deconvolution for Confocal Laser Scanning Microscopy, *IEEE Intern. Conf. on Engineering in Biology and Medicine 2007, Lyon, August. 2007*
6. Chaux, P., Blanc-Féraud, L., and Zerubia, J. (2007) Wavelet-based restoration methods: application to 3D confocal microscopy images, *SPIE, International Conference on Wavelets XII 2007, San Diego, August 2007* (invited paper)
7. Zhang, B., Fahdili, J., Starck, J.-L. and Olivo-Marin, J.-C. (2007) Multiscale variance-stabilizing transform for mixed-Poisson-Gaussian processes and its applications in bioimaging*, IEEE Intern. Conf. on Image Processing 2007, San Antonio, Sept. 2007*
8. P. Pankajakshan, B. Zhang, L. Blanc-Féraud, Z. Kam, J.C. Olivo-Marin and J. Zerubia, "Parametric Blind Deconvolution for Confocal Laser Scanning Microscopy (CLSM)," Journal of Optical Society of America (JOSA) (submitted).

6. Meetings:

- September 24, 2007 - Visit of Bo Zhang at INRIA Sophia Antipolis - Méditérranée.
- October 15-16, 2007 - P2R Brainstorming, INRIA, Sophia-Antipolis - Méditérranée, participants: J.-C. Olivo Marin, Z. Kam, A. Feuer, J. Zerubia, L. Blanc-Féraud, C. Chaux (CNRS-Université Paris-est) and P. Pankajakshan.
- Dec. 18-19, 2007- P2R seminar on medical and biological imaging, Novotel, Jerusalem, Israel, participants: J.-C. Olivo-Marin, Z. Kam and A. Feuer.
- Jan. 17, 2008 - Meeting at Marne La Vallée, participants: L. Blanc-Féraud and C. Chaux.

- Feb. 26, 2008 - Meeting at INRIA, Sophia-Antipolis – Méditérranée, participants: J. Zerubia, L. Blanc-Féraud, C. Chaux and P. Pankajakshan.
- Mar. 18, 2008 - Meeting at Marne La Vallée, participants: L. Blanc-Féraud and C. Chaux.
- May 6, 2008 - Meeting at Marne La Vallée, participants: L. Blanc-Féraud and C. Chaux.
- June 19, 2008 - P2R prospective meeting at INRIA, Sophia-Antipolis - Méditérranée, participants: J.-C. Olivo Marin, A. Dieterlen (UHA), G. Engler (INRA), J. Zerubia, L. Blanc-Féraud, C. Chaux and P. Pankajakshan.
- July 16, 2008 - Meeting at Institute Pasteur, Paris, participants: J.-C. Olivo Marin, J. Zerubia.
- July 16, 2008 - Meeting at Marne La Vallée, participants: L. Blanc-Féraud and C. Chaux.
- August 1, 2008 - Meeting at INRIA, Sophia-Antipolis – Méditérranée, Participants: A. Feuer, L. Blanc-Féraud and J. Zerubia.
- August 28, 2008 - Meeting at INRIA, Sophia-Antipolis – Méditérranée, participants: L. Blanc-Féraud, J. Zerubia and P. Pankajakshan.
- Sept. 16, 2008 – Leica seminar at Institute Pasteur, particpant: J. Zerubia.
- Sept. 23, 2008- Meeting at Marne La Vallée, participants: L. Blanc-Féraud and C. Chaux.
- Oct. 1, 2008 - P2R prospective meeting at Institut Pasteur, participants: J.-C. Olivo Marin, A. Dieterlen (UHA), G. Engler (INRA), J. Zerubia (INRIA), L. Blanc-Féraud (INRIA), C. Chaux (CNRS-Université Paris-est) and P. Pankajakshan (INRIA).
- Nov. 17, 2008 - P2R general meeting in Institute Pasteur, Paris, participants: L. Blanc-Féraud, P. Pankajakshan, A. Feuer, Z. Kam and J.-C. Olivo-Marin.

7. Talks:

- B. Zhang, “Multiscale Variance Stabilizing Transform for Noise Removal and Spot Detection in Fluorescence Confocal Microscopy,” Quantitative Analysis Unit, Institute Pasteur, Paris, at INRIA Sophia Antipolis-Méditérranée, Sept. 24, 2007.
- P. Pankajakshan, “Blind deconvolution for Confocal Laser Scanning Microscopy (CLSM),” INRIA/CNRS Ph.D. seminar, Sophia-Antipolis, France, Nov. 15, 2007.
- P. Pankajakshan, “Inverse Problems in Image Processing,” Indian Institute of Science Math Initiative (IMI), Bangalore, India, Aug. 6, 2008.
- A. Dieterlen, “Quantification in 3-D fluorescence microscopy,” MIPS Laboratory, Université de Haute-Alsace, Mulhouse, at INRIA Sophia Antipolis-Méditérranée, Feb. 25, 2008.