BLIND DECONVOLUTION IN 3D BIOLOGICAL MICROSCOPY

1 Detailed description of the research topic involving the state of the art

The bibliography is given in section 7.

Confocal microscopy offers several advantages over conventional optical microscopy with its small depth-of-field, its reduction of out-of-focus blur, and its full three-dimensional (3D) image scanning ability. For biomedical applications, it can also acquire images of living cells, usually labeled with one or more fluorescent probes. The confocal laser scanning microscope (CLSM) is an optical fluorescence microscope associated to a laser that scans the specimen in 3D and uses a pinhole to reject most out-of-focus light. The ability of CLSM to image optical sections of thick specimens explains its rapidly increasing use in biological research [15].

Despite the advantages of the CLSM, the quality of confocal microscopy images suffers from two basic physical limitations. First, out-of-focus blur due to the diffraction-limited nature of optical microscopy remains substantial, even though it is reduced compared to widefield microscopy. Second, the confocal pinhole drastically reduces the amount of light detected by the photomultiplier, leading to Poisson noise [15]. The images produced by CLSM can therefore benefit from postprocessing by reconstruction methods designed to reduce blur and/or noise.

The aim of this proposal is two folds :
First, we want to propose methods for image deconvolution, assuming we know the point spread function (PSF), which models the degradation of the optical acquisition system. We also assume the noise is known (Poisson noise or approximated Gaussian noise with known standard deviation).
Second, we want to propose solutions for the real case, when the degradation is not exactly known. So we have to estimate both the degradation and the restored image from the observations only.

Many deconvolution methods have already been proposed for 3D microscopy, such as Agard and Sedat [1, 3], Tikhonov-Miller inverse filter [21], Carrington [19] and Richardson-Lucy (RL) algorithms [14, 17]. The latter has been used extensively in astrophysical or microscopic imaging [21], and is of particular interest for confocal microscopy because it is adapted to Poisson noise. An important drawback of RL deconvolution, however, is that it amplifies noise after a few iterations. This sensitivity to noise can be avoided with the help of regularization constraints, leading to much improved results. Conchello et al. [5] and van Kempen et al. [19, 20] have presented a RL algorithm using

energy-based regularization applied to biological images. Conchello's regularization term introduces oscillations enhanced with the number of RL iterations in homogeneous areas. Tikhonov-Miller based term, on the contrary, regularizes too much, resulting in smoothed edges. We have recently proposed a RL algorithm with TV (Total Variation) regularization which provides smoothing in homogeneous areas while preserving edges [7].

A second class of restoration methods contains multiresolution models. In particular, wavelet denoising offers an alternative method of regularization for deconvolution. Boutet de Monvel et al. [6] propose a denoising method for confocal image stacks, using Daubechies' wavelets for each direction, before applying a MAP or a non-regularized Richardson-Lucy method for deconvolution. In [22], Willett et al. use a multiscale approach based on platelets to denoise 2D images in the presence of a Poisson noise. Platelets are localized functions at various scales, locations, and orientations, which produce piecewise linear image approximations. This platelet-based method is very well suited to Poisson noise and preserves edges in the images. In [13], Kervann and Trubuil propose a method to denoise confocal images as a pre-processing before using a deconvolution algorithm based on Richardson-Lucy. The denoising method is based on a locally piecewise constant modelling of the image with an adaptive choice of window around each pixel [16]. They apply the Anscombe transform to transform the Poisson noise into a noise distribution with stabilized variance. We have recently developped a denoising method based on a 3D Complex Wavelet Transform (CWT) [4]. The CWT was proposed by N. Kingsbury in order to obtain a shift and rotation invariant transform with reduced redundancy (2^d if d is the dimension of the space, $d = 3$ in confocal microscopy application). The denoising results show that this transform is well adapted to biological 3D image restoration compared to real wavelet transforms.

For deconvolution methods in microscopic imagery, it is important to know precisely the degradation function (ie. PSF) which caracterizes the microscope in several experimental contexts and which defines the degradations induced by all the elements of the set optical system/sample. Several approaches can be used to obtain this function. One is experimental. The PSF is given by the image acquisition of small (a hundred of nanometers) fluorescent beads. Each microsphere defines a point source and its image gives the PSF of the optical system. However the image of one bead is very noisy and it is recommended to average over several beads. Another approach consists in using a parametric physical model. The PSF we have used in previous work modelizes the diffraction of the light with the default of focus and the effect of the finite size of the pinhole [2, 8]. Some physical parameters of the model are known from the experiment, other are tuned to defined the "best" model according to the degraded image. This last step can be optimized and automatized by using estimation theory. Based on the experience acquired in such estimation methods for satellite images [11], we will propose a blind deconvolution algorithm for confocal microscopic images.

2 Scientific and technological background

The team comprises two groups that are world-wide recognized leaders in specific image processing topics (wavelet, variational and stochastic processes, denoising, deconvolution, super-resolution), and two groups that are working in image processing applications for cell biology of infectious diseases and drug screening in two world-wide recognized

biological institutes. The global structure of the project reflects the multi-disciplinarity of the team. This guaranties to foster collaborations and to make the best use of the complementarity of the research teams. Moreover, as some the project teams are either affiliated to or have active collaborations with major biological institutions, the evaluation of the methodology will be done on real-life applications.

Ariana team has a long experience in ill-posed problems, using variational or stochastic methods and also wavelet analysis. For satellite image deconvolution, Ariana team has developed in the past restoration methods by using non-linear regularization with maximum likelihood parameter estimation [9], or by using complex wavelet packet decomposition [10]. Very recent competitive methods, including ones developed in Ariana, combine wavelets and edge-preserving energy-based minimization. A collaboration between Ariana, Pasteur and Weizmann teams yields a 3D modified Richardson-Lucy deconvolution method which takes into account the specificity of the Poisson noise of the observed microscopic images and uses a non-linear regularization technique based on φ-functions [7]. Ariana team has used the Richardson-Lucy approach with various regularization constraints. This team also recently developed a denoising and deconvolution method using 3D complex wavelet decomposition for biological imagery [4]. Previous work on super-resolution for satellite and aerial images jointly developed by Ariana and a team of the Computer Science Department of the Hebrew University of Jerusalem was successfully tested by the French Space Agency and by the Remote Sensing Center of the West European Union Forces in Madrid [18].

The Ariana team has also proposed a blind image deconvolution method for satellite images [11]. This method has been successfully tested by the Space French Agency. A patent deposit was done in 2001 in France and in 2002 in several other countries.

The Quantitative Image Analysis Unit develops image processing methods and programs for the automatic analysis and quantitation of microscopic images. The main research topics include dynamic object segmentation, spot and particle tracking in dynamic microscopy, fluorescence quantification as well as detection methods for temporal sequences.

The Weizmann team have recently developed deconvolutions to create synthetic projections from "sweeping focus" acquisition mode, in order to obtain microscope imaging of thick samples in very fast high-throughput cell screening experiments. The method is useful for subcellular localization of fluorescently-labeled molecules in high-magnification microscopy applications for drug development.

The Technion team is specialized in image processing and restoration from uncomplete data, super-resolution and adaptive filtering of multidimensional data.

3 Conformity of the project to the priority research fields

The proposed project is submitted within area 3 of the CFP (ie. Novel Fields of Medical and Biological applications). It proposes to use the synergy of two leader groups in mathematical image processing (one located at INRIA and the other at Technion) and two outstanding research groups working in image processing applications for cell biology of infectious disease and drugs screening (one located at Institut Pasteur and the other at Weizmann Institute). The proposed research program will promote new fields of applica-

tion such as cell based assays for therapeutic research, and multidimensional microscopy assays in cell biology.

4 Expected scientific, social and economic contribution

Over the past ten years, a considerable part of research efforts in biology have been directed towards integrating large molecular data sets into in vivo systems and relating exhaustive genetic information to functional analysis. The recent development of new advanced in vivo microscopy techniques together with the possibility of manipulating and tagging proteins with, e.g., GFP-derived probes, have been instrumental in allowing for a shift in basic and applied biological research. As an example, large scale studies allow nowadays for a global and exhaustive documentation of biological processes within the context of living cellular or tissue systems, even though cell functions involve complex networks of many interacting molecules, many not yet identified. The use of the programs developed in the context of this project will enable to dig further into these directions and will be used in collaborating biological institutes to develop and strengthen the following topics :

- The data produced by cell-based assays will be of great interest for basic understanding of complex cell behavior, is expected to have direct implications in diagnostics and treatment of diseases highly relevant for pharmaceutical companies. Not only may the traditional drug discovery process adopt cell-based screening if it is shown fast and effective for sorting potential drug leads before going into slow and expensive animal studies, but avenues for new methods not possible before will also be opened. For example, synergistic drug cocktails are very seldomly designed systematically, although the multiple malfunctions associated with many diseases are well recognized. Sensitive and quantitative cell-based imaging assays bear the potential of becoming a paradigmatic platform for such drug cocktails development with lower concentrations of each compound therefore lower toxicity and side effects, yet with better specificity due to affects of multiple pathways that mediate the targeted malfunction.

- Multidimensional microscopy assays in cell biology. The most general source of visual information decoded in biological system concerns the dynamic topology of a target. Targets can be on the levels of single proteins, protein complexes, organelles or entire integrated systems, starting from viruses, prokaryotic cells, eukaryotic cells, parasites, etc. 3-D image analysis provides the required spatial organization that is complementary to the functional relations established by array technologies. Acquiring 3-D data of cells in vivo over time yields a 4D image sequence, which allows dynamic studies of biological processes.The novel tools developed to analyze 3-D multi-probe signals acquired by high-resolution multidimensional microscopy will permit us to quantitatively describe the organization of labelled probes and proteins in the cell during the cell cycle.

5 Detailed research program

In Ariana, we will develop new deconvolution techniques in 3D based on complex wavelet packet transform and test them on confocal microscopy images provided by

Pasteur as well as on widefield microscopy data provided by Weizmann. Another part of Ariana's research work will be done in collaboration with Technion for blind deconvolution. The idea will be to use MLE (Maximum Likelihood Estimation) and EM (Expectation Maximisation) methods to be able to recover the PSF of the microscope from observed images. Tests will be done on synthetic and real data provided by both Pasteur and Weizmann.

At Pasteur, we will develop a combined method of deconvolution and detection of biological objects based on the 3D complex wavelet packet framework developed by the Ariana team. We will pay particular attention to incorporating the physics-related parameters of acquisition into the algorithm in order to achieve a deconvolution scheme which is able to adapt to the acquisition set-up and to the class of objects being imaged. We will apply these algorithms to temporal image sequences coming from both widefield or confocal microscopy done at the Institut Pasteur and the Weizmann Institute in the context of cell biology or host-pathogen interaction studies.

At Weizmann deconvolutions are routinely applied to widefield microscopy. Within this research program, the Ariana algorithms will be used to deblur cytoskeleton images of both fixed and immunostained data and to live cell expressing fluorescent protein labeled components of the cytoskeleton and other cell organelles. Quantitative analyses of such images based on segmentation of labeled structures are expected to yield cleaner and more precise data needed for studying cell mechanisms and pathways. Deconvolution algorithms for the reconstruction of true 3D projections of thick samples using high-magnification microscopy will be developed and applied for high-throughput applications based on imaging of cells in multiwell microplates. Continuing previous work [12] the Weizmann team will also develop various models for theoretical description of the microscope point-spread function in ideal and in aberrated conditions, and will test these models using fluorescent microspheres and by incorporating them in the Ariana deconvolution algorithms, and comparing blind deconvolution results for the PSF with expected aberration in the real acquisition process (e.g. depth-dependent spherical aberration).

At Technion, the team will work on blind deconvolution using EM (Expectation-Maximisation) and MLE (Maximum Likelihood Expectation) methods in collaboration with Ariana. We will also use our knowledge on super-resolution and sampling to propose new algorithms and test them on synthetic and real data provided by Pasteur and Weizmann teams.

6 Description of available research sources

6.1 FR1 : Ariana team

6.1.1 Research team members

- **Josiane Zerubia, Director of research, INRIA**

  Biography
  Josiane Zerubia is a permanent research scientist at INRIA since 1989. She has been director of research since July 1995. She was head of a remote sensing laboratory (PASTIS, INRIA Sophia-Antipolis) from mid-1995 to1997. Since January 1998, she

has been in charge of a new research group working on remote sensing (ARIANA, INRIA-CNRS-University of Nice). She has been adjunct professor at Sup'Aero (ENSAE) in Toulouse since 1999.
Before, she was with the Signal and Image Processing Institute of theUniversity of Southern California (USC) in Los-Angeles as a post-doc. She also worked as a researcher for the LASSY (University of Nice and CNRS) from 84 to 88 and in the Research Lab. of Hewlett Packard in France and in Palo-Alto (CA) from 82 to 84.
She got the MSc degree from the Department of Electrical Engineering at ENSIEG, Grenoble, France in 81, and a Doctor Engineer degree in 86, a Ph D in 88 and an "Habilitation à Diriger des Recherches" in 94, all from the University of Nice Sophia-Antipolis, France.
She is a Fellow of the IEEE. She was part of the IEEE IMDSP Technical Committee (SP Society) from 1997 to 2003, associate editor of IEEE Trans. on IP from 1998 to 2002. She has been member-at-large of the Board of Governors of IEEE SP Society since 2002, area editor of IEEE Trans. on IP since 2003 and guest co-editor of a special issue of IEEE Trans. on PAMI in 2003. She has also been a member of the editorial board of the French Society for Photogrammetry and Remote Sensing (SFPT) since 1998. She has been co-chair of two workshops on Energy Minimization Methods in Computer Vision and Pattern Recognition (EMMCVPR'01, Sophia Antipolis, France, and EMMCVPR'03, Lisbon, Portugal), co-chair of a workshop on Image Processing and Related Mathematical Fields (IPRM'02, Moscow, Russia), chair of a workshop on Photogrammetry and Remote Sensing for Urban Areas, Marne La Vallee, France, 2003.
Her current research interest is image processing using probabilistic models or variational methods. She also works on parameter estimation and optimization techniques.

Publications

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A. Jalobeanu, L. Blanc-Féraud, J. Zerubia. "Hyperparameter estimation for satellite image restoration using MCMC Maximum Likelihood method", Pattern Recognition, 2, 35, 2002.

- **Laure Blanc-Féraud, Director of research, CNRS**

Biography

Laure Blanc-Féraud received the phD degree in image restoration in 1989 and the "Habilitation à Diriger des Recherches" on inverse problems in image processing in

2000, both from the University of Nice-Sophia Antipolis, France. She is currently director of research at CNRS in Sophia Antipolis. Her research interests are inverse problems in image processing by deterministic approach using calculus of variation and PDEs. She is also interested in stochastic models for parameter estimation and their relationship with the deterministic approach. She is currently working in the Ariana research group (I3S/INRIA) which is focussed on Earth observation. She is vice-director of the I3S laboratory (CNRS and University of Nice-Sophia Antipolis) since 2003.

Publications
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G. Aubert, L. Blanc-Féraud, R. March "Γ-convergence of discrete functionals with nonconvex perturbation for image classification" SIAM Journal on Numerical Analysis, Volume 12(3), pp.1128-1145, 2004.

- **master student to be hired.**

6.1.2 Inventory of relevant equipment

15 PCs and 7 SUN stations are available as well as 8 laptops.
ENVI software is also available for image processing and IMARIS Software for vizualization of 3D biological images will be available at the end of 2004.

6.2 FR2 : Pasteur research group

6.2.1 Research team members

- **Jean-Christophe Olivo-Marin, Director of research, Institut PASTEUR**

Biography
Jean-Christophe Olivo-Marin received his Ph.D. in 1989 and the "Habilitation à Diriger des Recherches" in 1998 both from the Institut d'Optique Théorique et Appliquée, University of Paris-Orsay, France. He is the head of the Quantitative

Image Analysis Group at the Institut Pasteur, Paris. Since 2004, he is also Technology Director at the Institut Pasteur Korea, Seoul. Previous to that, he was a staff scientist at the European Molecular Biology Laboratory, Heidelberg, from 1990 to 1998. His research interests are in image processing and computer vision applied to biological image analysis, with special emphasis in multiresolution processing, image segmentation and video microscopy sequence analysis. He is a member of IEEE, SPIE and the Pattern Recognition Society.

Publications

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Olivo-Marin, J.-C. (2002) Extraction of spots in biological images using multiscale products, Pattern Recognition, 35, 9, pp. 1989-1996.

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Deubler, J. and Olivo, J.-C. (1997) A wavelet-based multiresolution method to automatically register images. Journal of Mathematical Imaging and Vision , 7, 3, pp. 199-209.

- **Christophe Zimmer, Senior Research scientist, Pasteur**

Biography

Christophe Zimmer obtained a doctorate in astrophysics and space techniques from Université Paris 7 in 1997. From 1998 to 2000, he worked as Assistant Research Geophysicist under a NASA contract at University of California Los Angeles, on the analysis and modeling of Jupiter's magnetic field data. In 2000, he joined the Quantitative Image Analysis group of Institut Pasteur, where he develops automated methods for extracting biological information from dynamic microscopy data. He is a permanent researcher of Institut Pasteur since 2003.

Publications

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- **Bo Zhang, PhD student**

Biography
Bo Zhang received his engineer degree from Ecole Nationale Supérieure des Télécommunications (ENST) in 2003 and his Bsc degree from Nanjing University, China, in 2001. After completing a DEA "Mathématiques, Vision, Apprentissage" at the ENS Cachan, he is currently pursuing his PhD in the Quantitative Image Analysis Group at the Institut Pasteur, Paris.

Publications
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6.2.2 Inventory of relevant equipment

- 10 PC workstations
- 1 SGI server
- 1 automated Nikon microscope for transmission imaging
- several software packages for image analysis

6.3 IL1 : Weizman research group

6.3.1 Research team members

- **Zvi Kam, Professor**

  Biography

  Zvi Kam got his D.Sc. in Physics at the Technion, Haifa, and did his postdoctoral research in biophysics at the University of California, San Diego. He is a Professor at the Weizmann Institute of Science since 1981. He is developing methodologies for high-resolution microscopy, and applying light microscopy to cell biological research. His present research involve development and application of cell-based methods for drug development and genomic library screens. He has built probably the only high-magnification automated screening microscope operating today, which acquires 40-80Gbytes of image data each day, and is working on computerized analysis methods to score such experiments according to designed essays.

  Publications

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- **student to be named in future.**

6.3.2 Inventory of relevant equipment

The Weizmann laboratory is fully equipped for cell biological work, has 6 computerized microscope system for fixed samples, live samples and automated screens of cells in multiwell microplates. 20 various workstations (Silicon Graphics, Apple and PC) are available for image visualization, processing and large volume computation. Computer farms and Terabyte RAID disk storage serve these workstations. Multiple software packages are installed, and the major image visualization and interpretation platform used is Priism (www.msg.ucsf.edu/ive), within which a large volume of image processing and quantita-

tive analysis software has been written.

6.4 IL2 : Technion research group

6.4.1 Research team members

- **Arie Feuer, Professor**

  Biography
  Professor Feuer received his B.Sc. and M.Sc. in Mechanical engineering at the Technion, Haifa, Israel ('67 and '73 resp.) and his Ph.D. from Yale University, CT, in 1978. From 1967 to 1970 he was with Technomatics Inc. working on the design of automatic machines. From 1978 through 1983 he worked for Bell Labs in network performance evaluation. In 1983 he joined the faculty of Electrical Engineering at the Technion where he is currently a professor and head of the Control and Robotics lab. His research interests included adaptive systems (in control and in signal processing) and sampled data systems. Since 1992 he has been intensively involved in research in digital image enhancement, multidimensional sampling and signal representations. Specifically, some of the projects he worked on had to do with improving digital resolution capabilities of various image acquisition devices. These included scanners, digital cameras - still and video, and orthoscopic devices.
  Professor Feuer is a Fellow of the IEEE and a council member of IFAC.

  Publications
  M. Elad and A. Feuer, " Super - Resolution Restoration of Image Sequence : Adaptive Filtering Approach" *IEEE Trans. on Image Processing*, Vol.8, No. 3 (1999), pp 387-395
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6.4.2 Inventory of relevant equipment

Fully equipped for computerized image analysis work.

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