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Blind Deconvolution for Confocal Laser Scanning Microscopy

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In the first phase of our work, we have developed a method for the joint estimation of the parameters of the diffraction-limited Point Spread Function (PSF) of a Confocal Laser Scanning Microscope (CLSM) and the specimen function under observation. The CLSM is an optical fluorescence microscope that scans a specimen in 3D and uses a pinhole to reject most of the out-of-focus light [1]. However, the quality of the images suffers from two basic 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 parts of the object. Although the out-of focus contribution in confocal images is greatly reduced, it is not totally eliminated, and in practice is dependent on the pinhole diameter. This reduces the contrast and complicates the direct quantitative analysis of the specimen. These images can hence benefit from post-processing restoration methods based on deconvolution. However, the main difficulty in restoring the 3D image is that the exact PSF is not precisely known, denoising the image can induce artifacts and restoration by deconvolution is an ill-posed problem.

An efficient method for parametric blind image deconvolution involves the simultaneous estimation of the specimen 3D distribution of fluorescent sources and the microscope PSF. Since the exact PSF is unknown and might vary during the course of the experimentation, it has to be estimated directly from the observation data. Since the diffraction-limited CLSM PSF has a circular symmetry about the lateral plane and mirror symmetry about the axial plane, such a PSF could be best approximated in the LSS by a 3D separable Gaussian model [2]. By assuming that the physical imaging acquisition process of the microscope could be thus modeled, we reduce the number of free parameters describing the PSF and simplify the estimation process. The novelty of the approach we had proposed is its application to restore 3D biological image data by assuming that the statistical variation of the photon counting process follows a Poissonian statistics.

An a priori model of the specimen is necessary to stabilize the alternate minimization algorithm and to converge to the desired solutions. The Gibbsian distribution captures the prior knowledge in this case, and is the regularization model. By using the Bayesian method for the joint restoration, the posterior distribution can be generated from the likelihood and the prior probabilities.

* Joint work with Laure Blanc-Féraud and Josiane Zerubia.

The specimen point source distribution and the PSF could then be restored by maximization the a posteriori (MAP) distribution. Direct maximization can yield many possible solutions. Since the cost function in such a case is not convex to both the specimen function and the parameters of the PSF, we minimize it first with respect to object and then use the estimate to determine the parameters.

We choose the Richardson-Lucy (RL) algorithm [3] [4] for the deconvolution process as it is best suited for the Poisson data, and Total Variation (TV) [5] as the regularization model. This estimate is then used by the Conjugate-Gradient algorithm for estimating the parameters. We have presented some results on simulated data, and the method gives good results both qualitatively and quantitatively. However, it should be noted that, all of the out-of-focus light cannot be rejected and some noticeable haze and axial smearing remains in the images.

In the next phase of this project, we aim to restore images that have been affected by the Spherical aberrations [6]. Spherical aberrations are often caused by the mismatch in the refractive index between a microscope objective lens immersion medium and the specimen embedded medium. Restoration of the images in such a case is often a difficult problem because the PSF is spatially variant in the axial direction. Future work is also aimed at improving the prior representation of the object to emulate biological specimens.

References

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Contours Actifs explicites polygonaux : Application à la segmentation d'images vidéo

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Abstract. Les contours actifs (snakes) sont utilisés depuis de nombreuses années pour la segmentation d'images vidéo. Dans cette présentation, on commencera par un historique de ces méthodes et on verra leur mise en oeuvre sur des exemples d'images ou de séquences. On décrira plus en détail les contours actifs explicites polygonaux(à l'opposé des implicites : level set) et on verra les problèmes rencontrés ainsi que les différentes solutions apportées. Finalement on présentera une approche originale de *'corner tracker snake'* qui à l'avantage de segmenter des régions de formes irrégulieres avec une représentation minimale.

Active contours (snakes) have been used in video image segmentation for several years. In this presentation, we will start by presenting their evolution. We will especially focus on explicit polygon snakes (as opposed to implicit snakes: level set) and we will show the problems encountered and the solutions proposed with this kind of snakes. Finally, we will present an original snake approach called *'corner tracker snake'*, which is well adapted to the segmentation of irregular region shape. This approach also employs information theory in order to find the minimal description of the contour.

Notes

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Les séminaires des doctorants STIC permettent aux futurs docteurs d'échanger leurs expériences dans leur travail de thèse, tant sur le plan scientifique que sur le plan professionnel et éducatif. Ces rencontres ont lieu mensuellement dans l'un des laboratoires STIC de Sophia Antipolis.

Un séminaire est l'occasion de deux à quatre interventions. Chaque intervention comporte un exposé technique d'une vingtaine de minutes suivi d'une période d'échanges et de retours d'expérience d'une dizaine de minutes.

Ces actes compilent les résumés en anglais des exposés techniques du séminaire doctorant du 15 November 2007.

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