Confocal fluorescence microscopy and force-volume imaging in atomic force microscopy: A signal processing perspective

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Abstract—This lecture deals with signal processing methods dedicated to hyperspectral data recorded in confocal fluorescence microscopy and atomic force microscopy (AFM), with applications to biological imaging. We first address two typical inverse problems arising in confocal microscopy, namely hyperspectral image restoration (or deconvolution) and hyperspectral unmixing. The second part of the lecture is dedicated to the analysis of force curves and force-volume images recorded in AFM. Their interpretation is done using physico-chemical models, e.g., electrostatic and mechanical models. The quantitative estimation of parameters from experimental force curves is based on fully automated tools. This leads us to reconstruct a set of images at the nanoscale, each corresponding to a specific physico-chemical parameter.

I. HYPERSONTAL IMAGE PROCESSING IN CONFOCAL FLUORESCENCE MICROSCOPY

Confocal fluorescence microscopy is well-adapted to biological imaging, since it is now possible to visualize biological specimens in vivo at the subcellular scale. Moreover, these observations can be made at different wavelengths [1] leading to three-dimensional data, in which the first two dimensions \((x, y)\) are spatial and the third \((\lambda)\) denotes the wavelength. Depending on the number of wavelengths, the datacube is referred to as multispectral or hyperspectral.

Images recorded by a microscope are usually both noisy and blurred due to diffraction and photon-to-electron conversion, respectively [2]. We propose a method for hyperspectral image restoration relying on a regularized least-squares formulation. Two kinds of prior information are taken into account: spatial and spectral smoothness of the hyperspectral image, and positivity of the voxel values. The algorithms proposed in [3], [4] are based on a fast numerical optimization procedure that is well-suited to large dimensional datacubes.

Hyperspectral unmixing is another challenging problem encountered in many applications ranging from biology, geoscience, remote sensing to planetology [5]. Here, the datacube is seen as a 2D collection of 1D spectra, each spectrum \(\lambda \mapsto f(x, y, \lambda)\) reading as a mixture of a few pure materials, called endmembers. Hyperspectral unmixing amounts to recovering these endmembers together with their fractional weights (called abundances) at each pixel of the datacube. We present an application in geoscience where the recorded images represent genetically engineered bacterial cells [6]. The bacterial cells, acting as whole-cell biosensors, are increasingly being employed for in situ studies in microbial ecology [7].

II. QUANTITATIVE ANALYSIS OF FORCE CURVES IN AFM

AFM has now become a powerful technique for imaging samples at the nanoscale, in particular living samples. This technology allows one to scan cell surfaces directly on their natural support and to get topographic information, but also to measure the force of adhesion of cells [8]. During the past decade, AFM has been widely used in biology, in particular in the cancer research area [9]–[11] where one can reconstruct highly resolved images of a single cell.

In AFM, the analysis of complex biological systems can be performed through force-volume imaging (FVI) in which a set of force curves \(z \mapsto f(z)\) are being recorded over a spatial grid \((x, y)\) defined on a given sample surface. Approach and retraction force curves are being recorded while approaching the tip towards the sample (until contact) and then retracting the tip. The resulting force-volume images are thus 3D datacubes \(f(x, y, z)\), where the third dimension \(z\) represents the tip-to-sample distance.

The analysis of such FVI aims at providing a mapping, i.e., a spatial distribution of the relevant physical parameters pertaining to the biological sample. The main signal processing bottleneck is to segment force curves in order to determine the regions of interest on which the physical (mechanical) models are valid. In [12], we proposed an advanced automated method for force curve segmentation. This method is applied to the analysis of bacteria and biological cells.

REFERENCES