SuperResolution-aided Recognition of Cytoskeletons in Scanning Probe Microscopy Images

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Abstract: In this paper, we discuss the possibility to adopt SuperResolution (SR) methods as an important preparatory step to Pattern Recognition, so as to improve the accuracy of image content recognition and identification. Actually, SR mainly deals with the task of deriving a high-resolution image from one or multiple low resolution images of the same scene. The high-resolved image corresponds to a *more precise* image whose content is enriched with information *hidden* among the pixels of the original low resolution image(s), and corresponds to a more faithfully representation of the imaged scene. Such enriched content obviously represents a better sample of the scene which can be profitably used by Pattern Recognition algorithms. A real application scenario is discussed dealing with the recognition of cell skeletons in Scanning Probe Microscopy (SPM) single image SR. Results show that the SR allows us to detect and recognize important information barely visible in the original low-resolution image.

1 INTRODUCTION

Recent advances in SuperResolution (SR) methods are fostering an increasing interest in the possibility to apply SR processing to improve the accuracy of image content recognition. The most frequent applications in this direction are oriented to video surveillance and intelligent traffic control (Shih-Ming et al., 2011; Suresh et al., 2007; Aliyan S., Broumandnia, 2012), though, obviously, any image based task can profitably benefit from such a technique.

Actually, SR mainly deals with the task of deriving a high-resolution image from one or multiple low resolution images of the same scene (the multiple images have usually very slight difference from one another since corresponding to following frames of a video). High resolution is meant both as an improvement of content precision, thanks to denoising and content enhancement, and as spatial enlargement.

The result in both cases is a *more precise* image whose content is enriched with information *hidden* among the pixels of the original low resolution image or multiple images, which correspond more faithfully to the imaged scene. Such enriched content obviously represents a better sample of the scene which can be profitably used by Pattern Recognition (PR) algorithms.

Starting from this statement, we argue that SR and PR can be valuably combined in a computational framework to recognize and understand image content.

In this paper, we briefly introduce this framework and then show an example of its application to the recognition of cytoskeleton in Scanning Probe Microscopy (SPM) images.

Indeed, in recent years, the study of Mesenchimal Stem Cells (MSCs) has attracted a lot of attention in tissue engineering and regenerative medicine thanks to MSCs ability to be committed, along several lineages, through chemical and physical stimuli. MSCs are usually analyzed via Atomic Force Microscopy (AFM), one of the often preferred SPM imaging techniques used to obtain mechanical information on cell surfaces and deposited extra-cellular matrix molecules (Danti et al., 2006).

The goal is to correlate morphological, functional, and mechanical aspects of human MSCs to obtain a deeper understanding of their effects on cells functions, metabolism and finally shape. These

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aspects can be revealed, from a microscopy point of view, by identifying the cytoskeletal components and organs (see Figure 1).

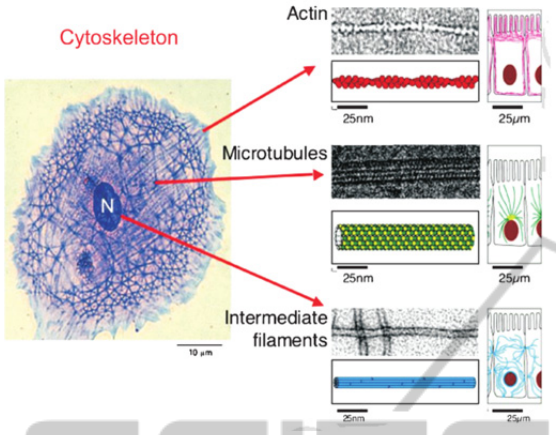

Figure 1: Cell cytoskeleton consists of microtubules (approximately, 25 nm in diameter), actin filaments (5–7 nm in diameter), intermediate filaments (8–12 nm in diameter), and other binding proteins.

With the support of biologists of the BioLab located at CNR in Pisa, the cytoskeleton was prepared according to a method (Hawkins *et al.*, 2013) which allowed us to work with images containing stabilized microtubule filaments.

However, the identification of such constituent microtubules is generally non trivial due to physiological variations in fiber surface properties and to AFM acquisition modality, which affect image visual appearance, such as tip-cell contact. In this frame, our solution, based on the use of SR to improve the image definition, can be a viable approach to semi-automatic identification and recognition of cytoskeleton components in AFM single image SR. The method improves spatial and photometric resolution, thus allowing the effective image recognition. In particular, the method highlights the hidden underlying biological structures.

The paper is organized as follows: Section 2 reports a brief overview of the computational framework for the combination of SR and PR techniques; hence, Section 3 focuses on the recognition of cytoskeleton in AFM images, and, finally, results and discussion are reported in Section 4.

2 SUPERRESOLUTION-AIDED PATTERN RECOGNITION – AN OVERVIEW OF THE METHODOLOGY

Pattern Recognition (PR) applied to image content can be roughly defined as the "art" of detecting and identifying relevant structures and/or their relationships present in an image, usually with the final aim to (semi-)automatically perform an image-based task.

PR techniques heavily rely on the quality of the visual appearance of the image, i.e. on the definition and precision of the structures imaged in it. In this frame, PR can dramatically benefit from SR processing aimed at enhancing the visual quality of images as well as magnifying their spatial resolution so as to enlarge and highlight relevant structures barely visible and recognizable in the original low-resolution images.

Indeed, a pre-processing step, usually intended to image enhancement and restoration, is normally included in PR processing chain. In this frame, systematic SR is a viable solution, focused on image content enrichment based on the recovery of missing high-resolution details that are not explicitly found in low-resolution images. This is what we are going to illustrate in this paper.

In particular, we here concentrate on single images; this means that both PR and SR techniques are applied to a still image (in literature, this case is also referred to as single-frame SR). Further work will deal with PR in images from video or multiple imagery data (i.e., multiple-frame SR). In this case, the SR processing can benefit from the presence of multiple images of the same scene and then exploit the information hidden in such a pack of data.

In the following, we report an overview of the framework already introduced in (D'Acunto *et al.*, 2013).

Formally, we assume the following image acquisition model (Liu et al, 2008).

$$L(x,y) = S(x',y') * H(x'-x, y'-y) + N(x,y) \quad (1)$$

where $L(x,y)$ is the acquired image, $S(x',y')$ is the *Point Spread Function* (PSF), $H(x'\text{-}x, y'\text{-}y)$ is the ideal image and $N(x,y)$ is the noise.

The PSF is strictly correlated to the image acquisition instrument and the degree of *spreading* (i.e., blurring) of a point object actually measures the quality of the imaging system. In many cases, PSF is a complex function depending on instruments characteristics and limits as well as possible artefacts

introduced during the acquisition.

For instance, in SPM imaging, PSF results from all the artefacts introduced by the AFM tip-sample contact, the tip-ample convolution or finite tip radius, and sample changing stiffness under tip pressure. Another source of artefact during the scan of biological sample is the temperature change, which could introduce drifts due to piezo-tube with subsequent sample structure deformation (D'Acunto and Salvetti, 2011).

In the general framework we propose, the main idea is to reconstruct the ideal image $H(\mathrm{x}' - \mathrm{x}, \mathrm{y}' - \mathrm{y})$ by firstly de-noising the acquired image L, so as to eliminate the noise component $N(\mathrm{x}, \mathrm{y})$; and then by reducing the SPF in two ways: by (*i*) eliminating the acquisition artefacts and (*ii*) super-resolving the artefact- and noise-free image.

The latter step allows us to recover an image as close as possible to the ideal image, including scarcely visible details that are not explicitly found in the original acquired low-resolution image L.

Once recovered such an image, PR techniques can be applied to understand image content, and hence solve the specific image-based task at hand.

2.1 SuperResolution Method

A SR method gets the original low-resolution still image as input and creates the high-resolution image by filling the new image grid with all the available low-resolution image pixels. During this filling process, the SR algorithm leaves some empty pixels, whose values are then estimated by a filling function.

According to the approach followed to define this function, existing methods can be categorized in (*a*) interpolation-based, (*b*) reconstruction-based, and (*c*) example-based.

Interpolation-based SR methods assume that images are spatially smooth and can be adequately approximated by polynomials such as bilinear, bicubic or level-set functions (Park *et al*., 2003; Morse and Schwartzwald, 2001; Fattal, 2007). This assumption is usually inaccurate for natural images and thus over-smoothed edges as well as visual artifacts often exist in the reconstructed high-resolution images.

The reconstruction-based approach faces SR as an inverse problem consisting in recovering the original high-resolution image by fusing multiple low-resolution images, based on certain assumed prior knowledge of an observation model that maps the high-resolution image to the low resolution images (Irani and Peleg, 1991; Lin and Shum, 2004). Each low-resolution image imposes a set of linear constraints on the unknown high-resolution pixel values. When a sufficient number of low-resolution images are available, the inverse problem becomes over-determined and can be solved to recover the high-resolution image. However, it has been shown that the reconstruction-based approaches are numerically limited to a scaling factor of two (Lin and Shum, 2004).

Example-based methods learn the mapping between low-resolution and high-resolution image patches from a representative set of image pairs, and then the learned mapping is applied to super resolve the image at hand. The underlying assumption is that the missing high-resolution details can be learned and inferred from the low-resolution image and a representative training set. Numerous methods have been proposed for learning the mapping between low-resolution and high-resolution image pairs with promising results (Freeman et al., 2002; Sun et al., 2003; Chang et al., 2004; Sun et al., 2008; Yang et al., 2008; Xiong et al., 2009).

With the initial intent to verify that our idea has real potentialities, we have selected the most promising SR method among a set of single-frame state-of-the-art techniques. In particular, the SR method proposed in (Kim and Kwon, 2010) is an application-agnostic example-based SR method. It works in the spatial domain and consists in a multi-step procedure that merges interpolation and learning. More precisely, after a first step of cubic spline interpolation to obtain the image at the desired scale, the method estimates the missing values by generating a set of *candidate* high-resolution images according to a local patch-based regressive approach. This candidate images are then combined to form a final high-resolution image. More precisely, for each image location (x, y), the pixel value is obtained as the convex combination of the *N* candidates according to the following *softmax* scheme:

$$H(x,y) = \sum_{i=1,\ldots,N} \omega_i(x,y) L(x,y,i) \tag{2}$$

where

$$\omega_j(\mathrm{x},\mathrm{y}) = \frac{e^{-\frac{|d_i(x,y)|}{\sigma_C}}}{\sum_{j=1,\ldots,N} e^{-\frac{|d_j(x,y)|}{\sigma_C}}} \tag{3}$$

and $\{d_i(\mathrm{x},\mathrm{y})\}_i$=1..N is the estimation of distances between the unknown considered pixel and each candidate. This estimate is calculated using a set of

linear regressors:

$$d_i(x,y) = |PL(x,y)^T W_i| \quad i = 1, \dots, N \qquad (4)$$

where $PL(x,y)$ is a vector constructed using the concatenation of all columns of a spatial patch of L centred at (x,y) and the parameters $\{W_i\}$ are optimized based on the patch-based regression results L for a subset of training images.

A final post-processing step is included so as to improve edge appearance.

3 CYTOSKELETON RECOGNITION IN SPM IMAGES

The study of Mesenchimal Stem Cells (MSCs) relies on the identification of their skeletal components and organs. These have usually a microtubule shape with a particular distribution pattern, as shown in Figure 1. Indeed, it is well-known that living cells are in general very soft and mechanically inhomogeneous; hence the corresponding cytoskeleton forms a rigid network that controls and supports both the cell shape and the cell movement.

AFM is usually the most used SPM technique to investigate cell skeletons. The AFM works using a probe to image the cell sample. Such tiny probe can be considered as a paraboloid with a final sphere (normally the radius of the sphere is 10-20nm) in permanent or intermittent contact with the sample generally considered flat (this corresponds to two different modes of acquisition).

Based on the contact force between the probe and the cell sample, the image recorded with an AFM present a shot of the cell cytoskeleton. Being the cytoskeleton composed by a complex network of different cell components, such as actin filaments, microtubules, proteins etc, it can be a rather complex challenge to identify the different cyto-components (see Figure 2).

Nevertheless, SR processing can significantly improve the identification of such components (as recently shown also by Chacko et al. 2013).

In this sense, we applied our framework to semi-automatically identify the microtubule structures of cytoskeletons depicted in AFM images.

As evident in Figure 2, besides clearly visible filaments, many other structures are barely visible and distinguishable in the microscopy image. SR processing is a viable solution to face this issue.

According to the general framework introduced above, a multi-step procedure is applied to identify the different microtubules and filaments:

- image correction and denoising;
- image contrast improvement;
- image resolution improvement;
- microtubule recognition.

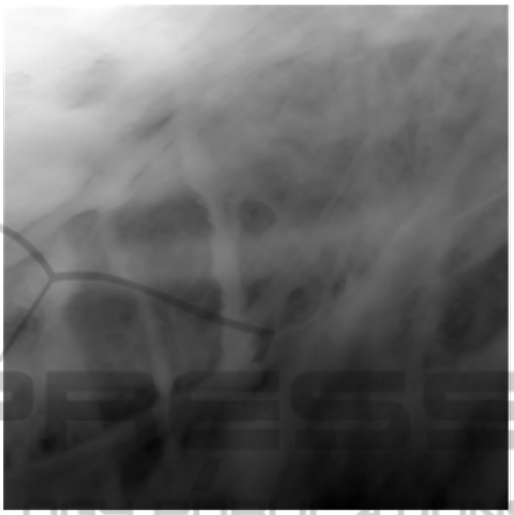

Figure 2: An AFM image depicting the microtubule structures of an MSC skeleton.

More precisely, due to the characteristics of the microscope imaging device, a tilt correction is initially required. Then, contrast enhancement is carried out according to Zuiderveld's method (Zuiderveld, K., 1994). As introduced above, SR processing relies on the application of the Kim-Kwon method.

Finally, the super-resolved image is processed using a patch-wise semi-automatic pattern recognition algorithm.

The aim is to identify a specific area $H_0(\mathrm{x}', \mathrm{y}') \subseteq H(\mathrm{x}, \mathrm{y})$ of the super-resolved image H corresponding to a microtubule.

Starting from a selected area of the image, the PR algorithm selects a central pixel p_0 and applies a kind of region growing method based on the gradient value of pixel neighbourhood. More precisely, the algorithm constructs a connected region corresponding to a microtubule by adding, neighbour by neighbour, a pixel connected with the previous if the derivate between these two pixels is lower a certain value (relative derivate Δ_R) and if the distance between the analysed pixel and the start pixel is lower than a certain value (absolute delta Δ_A).

Formally, starting from the selected pixel $\mathrm{p}_0 \in H_0$, a new pixel is inserted in H_0 if and only if:

$$\mathrm{p}_{i+1} \in \mathrm{I}_0 \Leftrightarrow \mathrm{p}_i \in \mathrm{I}_0, |\mathrm{p}_i - \mathrm{p}_{i+1}| < \Delta_R, \; |\mathrm{p}_o - \mathrm{p}_{i+1}| < \Delta_A \qquad (5)$$

4 RESULTS

The proposed multi-step procedure has been implemented in Matlab and applied to AFM images of MSC cytoskeletons, as the one shown in Figure 2.

Results show that, thanks to the SR methods, also filaments barely visible in the original low-resolution image have been identified.

Figure 3 shows an example of such result. A patch of the original low-resolution image has been selected, as shown in Figure 3.A and Figure 3.B shows its rough enlargement. The SR method allowed a 4X super-resolved image to be obtained, i.e., the one reported in Figure 3.C. This way, a "hidden" filament could be discovered and characterized. Indeed, the PR method was able to identify and delineate it as shown by the result in Figure 3.D.

Figure 4 shows another example on a different sample.

Measuring the dimension of the recognized patterns provided a quantitative confirmation of the results by consulting biologists of BioLab in Pisa. A pixel in super resolved images corresponded to about five nanometers. In the example of Figure 3, we recognized fourteen microtubule structures,

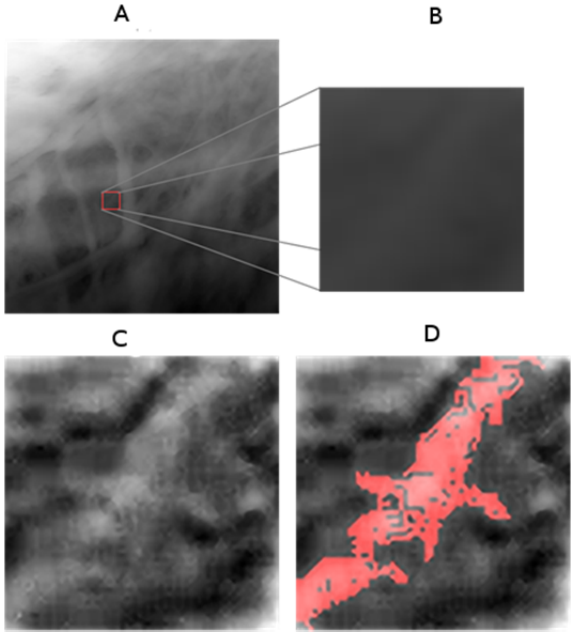

Figure 3: Results of the SR-aided pattern recognition method for the detection of microtubule cell structures. A: The original image and the selected patch. B: roughly enlargement of the original content of the selected patch. C: the 4x super-resolved image of the selected patch. D: the results of the pattern recognition method applied to the super-resolved image.

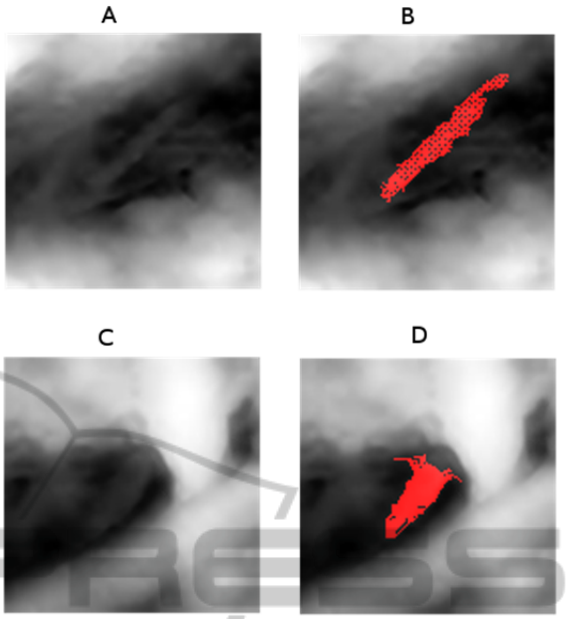

Figure 4: Another example of application of the SR-aided pattern recognition method. A and C are SR areas and B and D are the respective recognized patterns.

considering different square sub-images. In all these cases, both length and width of the recognized pattern were in agreement with typical values (Schaap et al., 2006) of microtubules.

These instances show how effectively SR processing can improve the original image and then facilitate the recognition of specific patterns.

We also tested our method by applying it to synthetic images containing a set of cylindrical shapes. We found that these shapes could be recognized after both reduction of resolution and addition of noise. We found that the percentage error (number of pixels either wrongly assigned or non-assigned to the pattern to identify) was 0.8% when the signal-to-noise ratio was 11.6 dB and was 7.4 % when the signal-to-noise ratio was 8.7 dB.

Figure 5.A gives the 3D representation of the high-resolution area shown in Figure 4.C, while Figure 5.B gives the 3D representation of the original area corresponding to Figure 4.C.

5 CONCLUSIONS

The method proposed consisted in the application of PR methods to single images enhanced by SR algorithms. The application we carried out to the recognition of cytoskeleton microtubules led to biologically significant results as confirmed by a

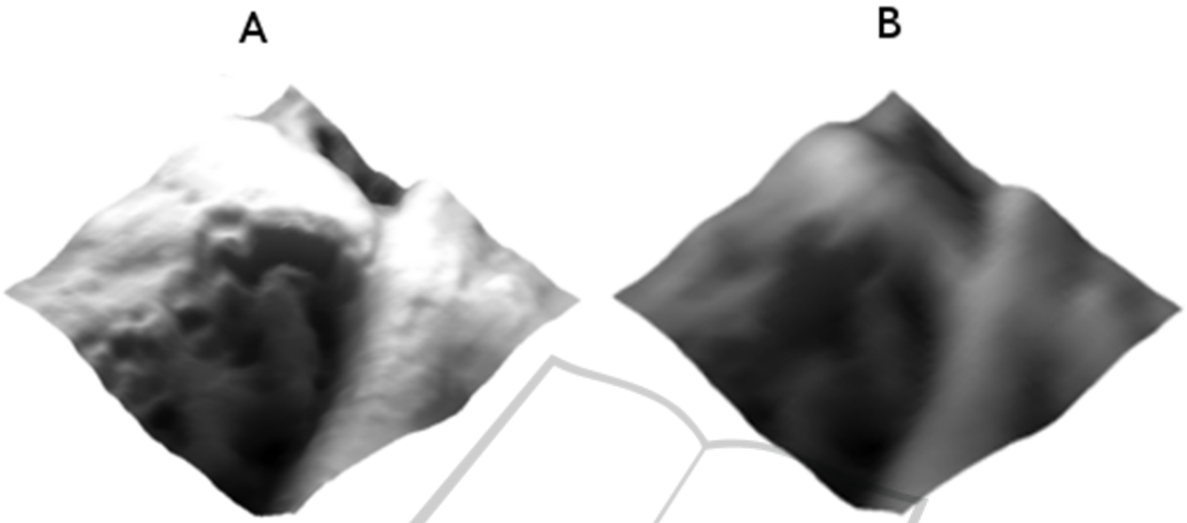

Figure 5: On the left a 3D perspective of the SR image, on the right a 3D perspective of the original image.

group of biologists. This confirmed the vast range of effectiveness of SR and allowed introducing a useful specific tool in the field of the recognition of biological structures.

Futures research will concern the following points. Firstly, the method will be applied to a greater number of experimental images. This will allow improving it according to the properties of new data and to better assess its validity.

Secondly, the stage of proper PR, following the stage of image enhancement, will be further tested and possibly improved.

Thirdly, more precise criteria will be given for the selection of appropriate sub-images, with the aim of possibly making this stage automatic.

Finally, other methods of image processing will be taken into account with the purpose of introducing modification and/or additions to our method. For instance, methods of filaments estimation should be considered (see, e.g., Genovese et al., 2012).

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