Digital Image Inpainting and Microscopy Imaging

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ABSTRACT A considerable amount of image processing techniques known as inpainting techniques have been recently developed aiming to provide solutions for filling in missing or damaged regions in a digital image. Typical such techniques reconstruct a defined area by using information from its neighborhood, for example, by completing inside the missing region the isophote lines arriving at its boundaries. In this article, we show that inpainting techniques have considerable potential usefulness in microscopy imaging, even though experimenting and using them in this domain has been almost entirely neglected up until now. In this purpose, we experiment the "curvature-preserving" partial differential equations as a solution to inpainting regions in images collected by several optical and scanning probe microscopy techniques. The results achieved are presented along with a discussion on typical problematic scenarios of microscopy imaging for which this type of techniques can provide a viable solution. *Microsc. Res. Tech. 74:1049–1057, 2011.* © 2011 Wiley Periodicals, Inc.

INTRODUCTION

In the last decade, much emphasis has been put on the development of automated digital inpainting techniques. Inpainting can be regarded as an interpolation problem with a wide range of applications in image processing and computer vision. This family of techniques has proven its usefulness in applications regarding the recovery of scene information obstructed by visible points, a situation known as disocclusion or error concealment. In fact, the digital inpainting techniques are inspired by the work of professional restoration artists who have been restoring paintings affected by defects, including missing regions, for centuries (Emile-Male, 1976; Walden, 1985). Digital algorithms developed in the last decade, such as (Bertalmio et al., 2000, 2001; Caselles et al., 2008; Telea, 2004) aim to develop and implement mathematical models derived from the works of such artists so that the recovery or modification of digital images could be performed automatically, providing good results at low computational costs. In medical imaging, inpainting has proven its usefulness in several experiments (Green et al., 2008; Manjon et al., 2009; Yuan and Shi, 2004), but there is however little evidence on the potential usefulness of inpainting techniques in microscopy imaging at this time. Applying inpainting to microscopy images can offer considerable advantages in several scenarios, which we will highlight in this article.

The fundamentals of inpainting can be defined as follows: Having a missing or deteriorated region in an image, to be known as Ω, inpainting algorithms try to reconstruct Ω by filling it using information from this region's neighborhood (Fig. 1). A typical reconstruction method consists in prolonging the isophote lines (lines of equal gray value) arriving at $\delta\Omega$ inward into Ω, while maintaining the angle of incidence and in the same time curving the prolongation lines progressively in order to prevent them from crossing each other (Bertalmio et al., 2000).

In this article, we show that inpainting can represent a very powerful and flexible tool for microscopy imaging. In this purpose, we experiment the "curvature-preserving" partial differential equations (PDE's) technique (Tschumperlé, 2006) as a solution for inpainting missing regions in images collected by several optical and scanning probe microscopy techniques: Confocal scanning laser microscopy (CSLM), differential interference contrast microscopy (DIC), fluorescence resonance energy transfer (FRET), atomic force microscopy (AFM), scanning tunneling microscopy (STM), and scattering-type scanning near-field optical microscopy (s-SNOM). Potential problematic scenarios of microscopy imaging in which inpainting can constitute a viable solid solution are presented as well. These scenarios are mostly related to the restoration of images affected by acquisition artifacts and to the approximation of interest areas for which acquisition is not possible. Another field in which inpainting can offer advantages is related to telemicroscopy. The recovery of telemicroscopy images in which certain blocks have been transmitted defectively or not at all due to channel fading can be performed by inpainting (Rane et al., 2003) as an alternative to the use of transmission protocols that may have a big contribution to the network congestion.

OVERVIEW OF DEVELOPED INPAINTING ALGORITHMS

Most common inpainting techniques attempt to reconstruct an image region by continuing the iso-

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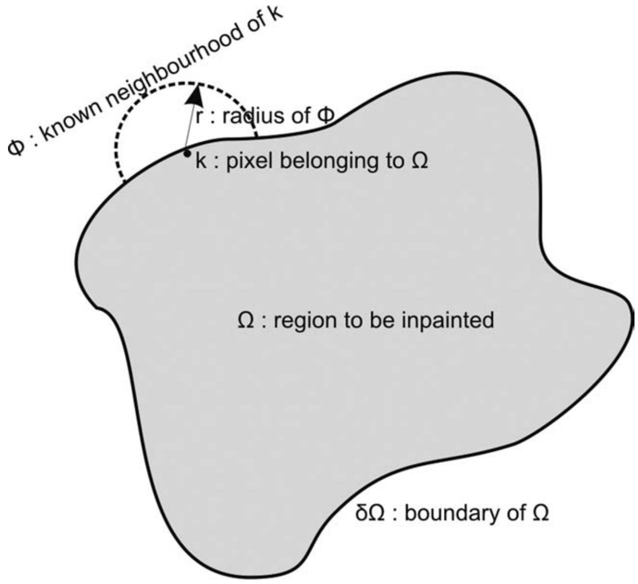


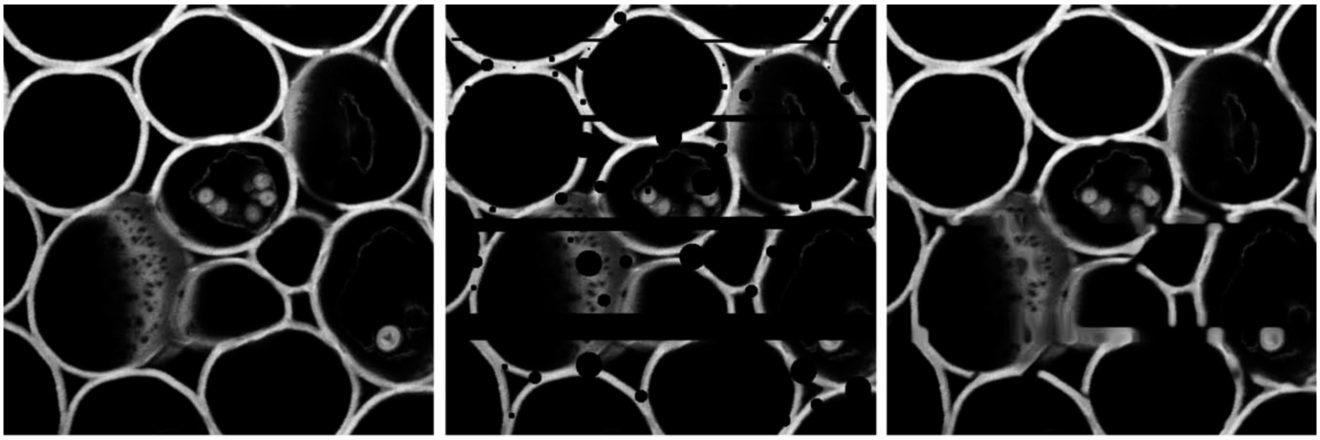
Fig. 1. The values of the pixels in the region to be inpainted (Ω) are calculated as a function of their neighborhood starting with the pixels adjacent to the boundary (δΩ). For example, k is calculated as a function of its neighborhood (Φ). Once a pixel in Ω has been inpainted it no longer belongs to this region, the area of Ω becoming smaller each time one of its pixels has been assigned a value.

photes arriving its boundary as smoothly as possible. This approach delivers a perceptually plausible reconstruction of the missing area, which in many cases is very close to the original information that is missing or is damaged. In (Bertalmio et al., 2000), the image smoothness information, estimated by the image Laplacian, is propagated along the isophotes directions, which are established by a time-varying estimation that is coarse at the beginning but which progressively achieves the desired continuity. In the approach presented in (Bertalmio et al., 2001), ideas from classical fluid dynamics are implemented in order to propagate isophote lines continuously from the exterior into the region to be inpainted. In this method, the Laplacian of the image intensity plays the role of the vorticity of the fluid, Navier–Stokes equations, which govern the dynamics of the Newtonian incompressible fluids, being used for achieving the desired results. Euler–Lagrange equation coupled with anisotropic diffusion is used in the total variational model (Chan and Shen, 2000a) in order to maintain the isophotes' directions and the same authors propose later a secondary algorithm based on the curvature-driven diffusion model (Chan and Shen, 2000b), which enhances their previous method by providing the possibility to inpaint larger regions. A method based on simple 3×3 filters, which repeatedly convolved over the missing regions diffuse known image information to the missing pixels, is introduced in (Oliveira et al., 2001). Another noticeable method is the algorithm presented in (Telea, 2004), in which the author estimates the image smoothness as a weighted average over a known image neighborhood of the pixel to inpaint. In this approach, the missing regions are regarded as level sets and the author uses the fast marching method described in (Sethian, 1996) to propagate the image information. In (Chen et al., 2005), inpainting is performed by progressive method of processing the image based on multiresolution analysis. In (Celia et al., 2007), a model for completing missing parts using the geodesic path continuation to perform the filling-in of the inpainting domain is presented. Later on, in (Barcelos et al., 2007) a "geometrical variational model" approach is presented that can fill-in surface holes by choosing the completing surface as one that minimizes a power of the mean curvature, while in (Chui, 2009) the author applies a method that is based on combining the directional derivatives of the heat kernels with respect to the inner normal vectors as integral kernels of the "propagation" operators, with the line of the notion of diffusion maps introduced in (Coifman and Lafon, 2006).

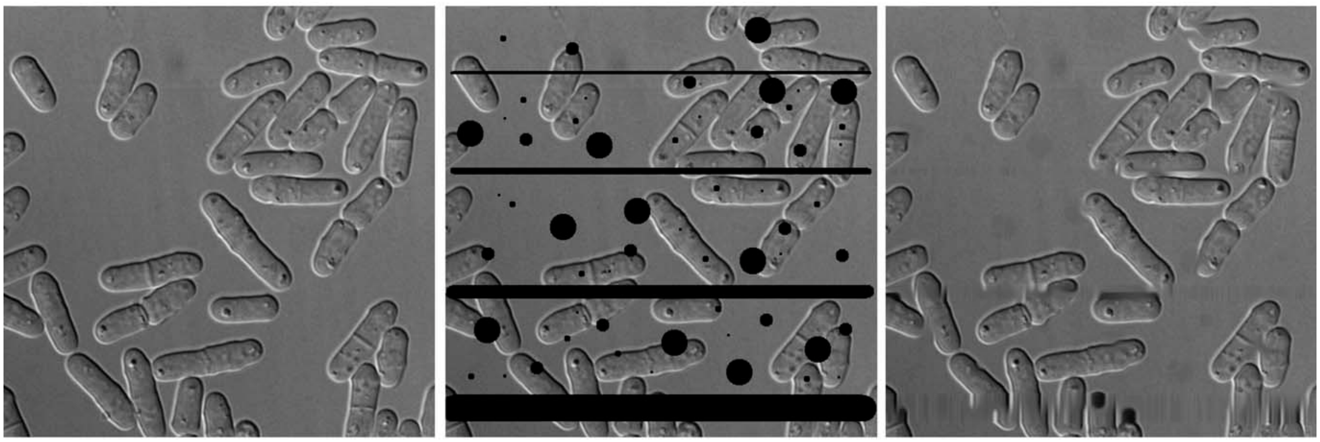
One of the problems that most inpainting algorithms encounter and have difficulties overcoming is inpainting textured areas, or combined structured and textured areas. In the case of textured areas, the classic idea of inpainting algorithms consisting in the smooth continuation of the isophotes arriving at the boundary of the region to be inpainted will not work. In (Efros and Leung, 1999), a texture synthesis algorithm is proposed as a solution to the problem of filling in textured regions. The idea of this algorithm is however in its turn not suitable for filling in structured regions. In regard to this problem, we notice two proposed possible solutions. In (Rane et al., 2003), the authors propose to classify a missing or defective area as being textured or structured. The blocks classified as structured are processed by texture synthesis, while the blocks classified as structured are filled in by inpainting. This algorithm faces however limitations when dealing with regions that contain both textured and structured information. The method presented in (Bertalmio et al., 2003) proposes as a solution to the same problem the decomposition of the original image into the sum of two functions: a first function used in the decomposition is of bounded variation, representing the underlying image structure, while the second function captures the texture and possible noise (Vese and Osher, 2002). The two functions are reconstructed afterward separately with structure and texture filling-in algorithms (by inpainting, respectively texture synthesis algorithms). After the two functions are reconstructed, these are combined back together and thus the restoration of an image containing both structured and textured missing areas is achieved.

Inpainting methods do not resume to the above ones; however, the aim of this article is not to compare existing inpainting methods but to introduce inpainting as a tool with considerable potential usefulness for microscopy imaging.

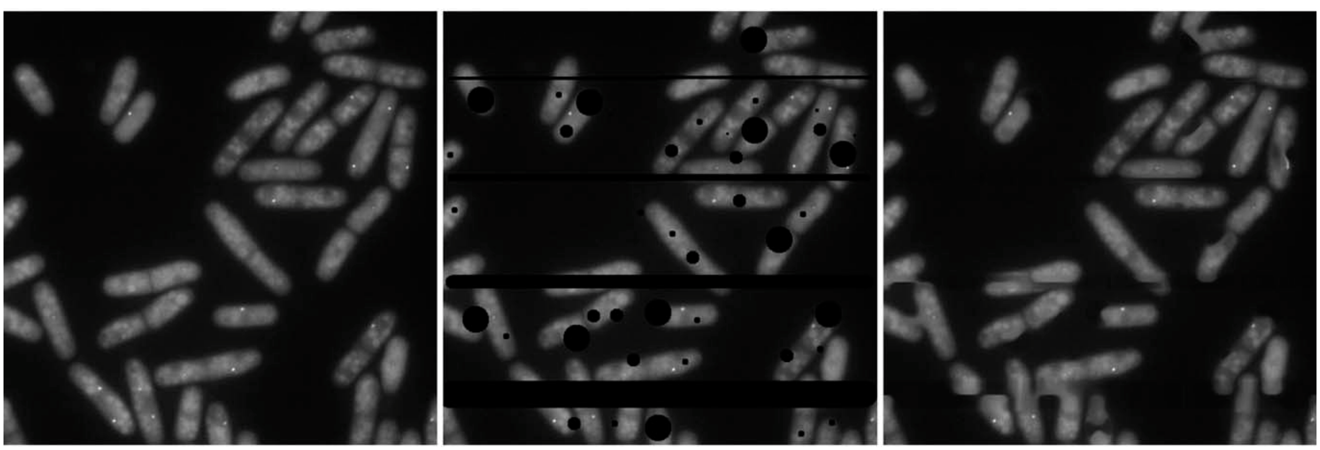
In (Tschumperlé, 2006), the author proposes a tensor-driven diffusion PDE that regularizes multivalued images while respecting specific curvature constraints. A theoretical interpretation of the curvature-constrained formalism in terms of line integral convolutions (Cabral and Leedom, 1993) is introduced along with a numerical scheme that implements the proposed PDE by successive integrations of pixel values along integral lines. The advantages of this iterative scheme compared to classical PDE's implementations refer to the preservation of the orientations in the case



(a)



(b)



(c)

Fig. 2. Original, masked, and restored images of (**a**) *Convalaria majalis* collected by CSLM (512 × 512 pixels); *Schizosaccharomyces pombe* collected by (**b**) DIC and (**c**) FRET (512 × 512 pixels); (**d**) plastic replica calibration grating collected by AFM (600 × 600 pixels); gold layer collected by (**e**) STM (256 × 256 pixels) and (**f**), s-SNOM (300 × 300 pixels). (left—original images; middle—masked images; right—images restored by curvature driven PDE's inpainting).

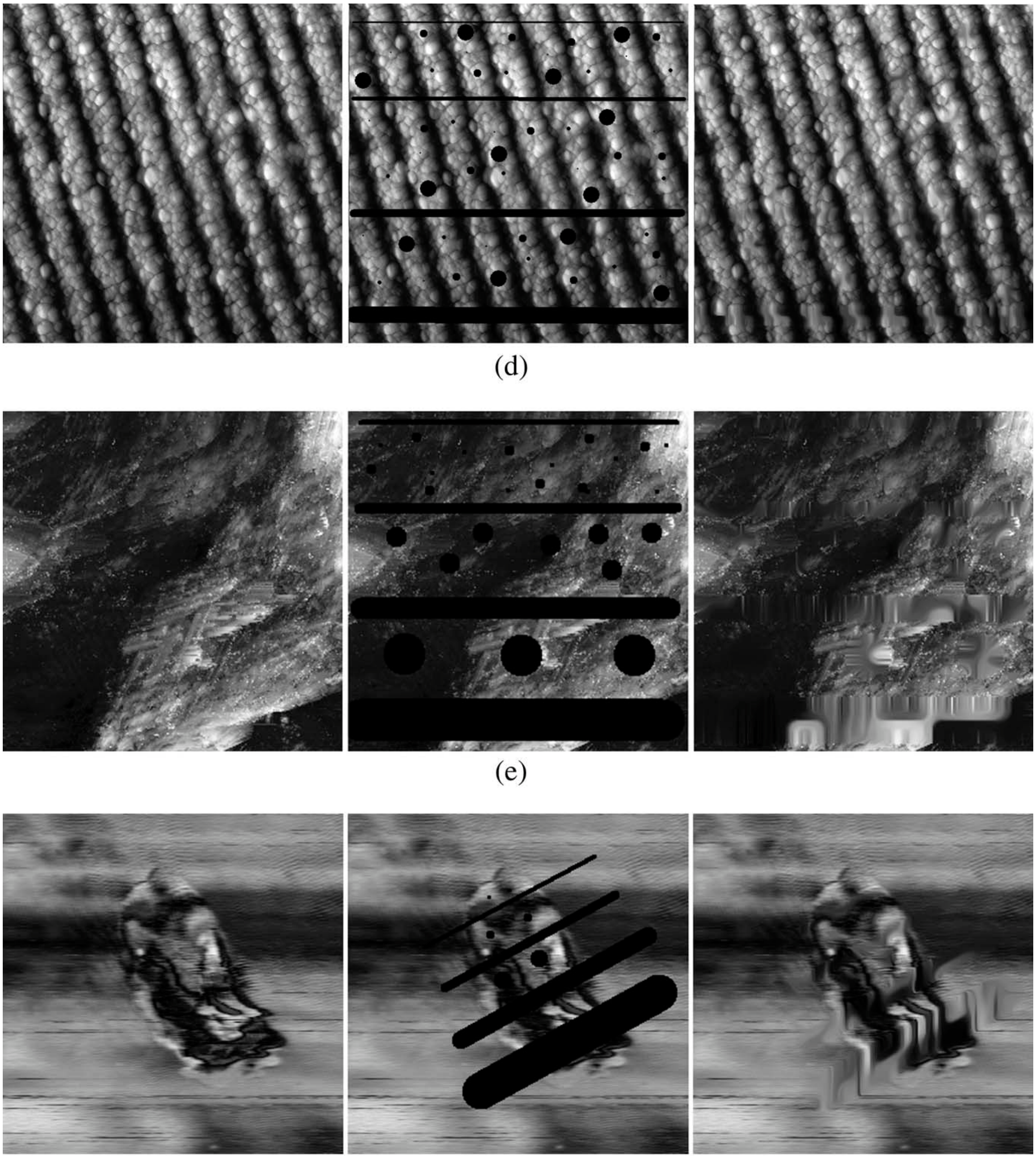


Fig. 2. (continued)

of thin image structures, and low computation times. The author presents the inpainting process as a direct application of his proposed curvature-preserving PDE. In this case, inpainting is performed by applying the diffusion equation only on the regions to inpaint, which allows the neighbor pixels to diffuse inside these regions. In this way, the missing parts of an image can be reconstructed through the nonlinear completion of the image data along isophotes directions. We have chosen to use this method in order to reconstruct the microscopy images on which we experiment because the quality of the provided results in terms of reconstructed image consistency, reconstruction accuracy, and runtime was superior to other techniques that we had tested.

IMAGED SAMPLES AND MICROSCOPY TECHNIQUES USED

In this article, we observe the results of digital image inpainting on images collected by several microscopy techniques: CSLM (Sheppard et al., 1997), DIC (Murphy, 2001), FRET (Clegg, 1996), AFM (Binnig et al., 1986), STM (Binnig et al., 1982), and s-SNOM (Hillenbrand et al., 2001). Inpainting is performed on images representing the following: a typical reference sample for CSLM, *Convalaria majalis*, visualized in fluorescence mode by using a Leica TCS SP system; subcellular structures of *Schizosaccharomyces pombe* visualized by DIC and FRET [images available in the (Riffle and Davis, 2010) public database], a plastic replica calibration grating visualized in contact mode AFM by using a Quesant 350 SPM system; a gold layer investigated by using a Nanosurf Easyscan STM and by using a homemade s-SNOM system developed at University Politehnica of Bucharest (Stoichita et al., 2010). The images contain structured and textured content specific to microscopy imaging, and random image regions have been excluded from the original images in order to observe the nature of the results that can be obtained by digital image inpainting. Original, altered, and inpainted version of the images are presented in the next section along with discussion of the results and application notes.

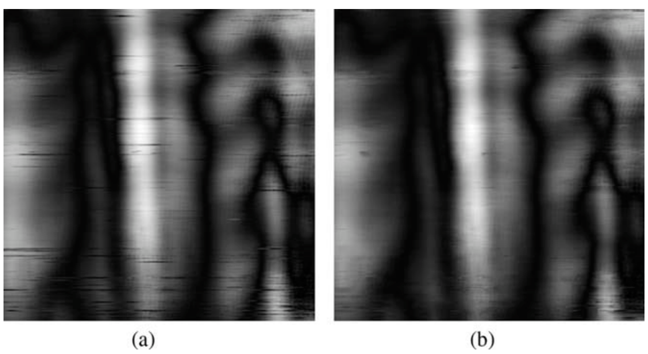


Fig. 3. s-SNOM image of brass sample (**a**) affected by acquisition artifacts due to exposure and focus inconsistencies to which the scanning tip is subjected (**b**) reconstructed by inpainting.

RESULTS, DISCUSSION, AND APPLICATION NOTES

In this section, we present results of digital image inpainting when applied to images acquired by several microscopy techniques CSLM, DIC, FRET, AFM, STM, and s-SNOM. In the original images, we introduce a mask that obstructs certain regions and further on we observe the effects of inpainting these defined regions by the curvature-driven PDE's method presented in (Tschumperlé, 2006), Figure 2. The mask that marks the regions to be inpainted consists of four horizontal lines, and several circular regions, having the following thickness/diameter: 3, 7, 15, and 30 pixels.

We can observe in Figure 2 that the results achieved by inpainting the masked regions depend on the dimension of the area to be inpainted. If the mask covers only partially an object, inpainting provides good results in the sense that the reconstructed morphology of the object is very similar to what can be observed in the original image. In the case when the region to be inpainted completely covers a distinct object surrounded by background, the information corresponding to the background content will be extended to the region to be inpainted resulting in a reconstructed region with an aspect similar to the background, which does not correspond to the original. Concealing image

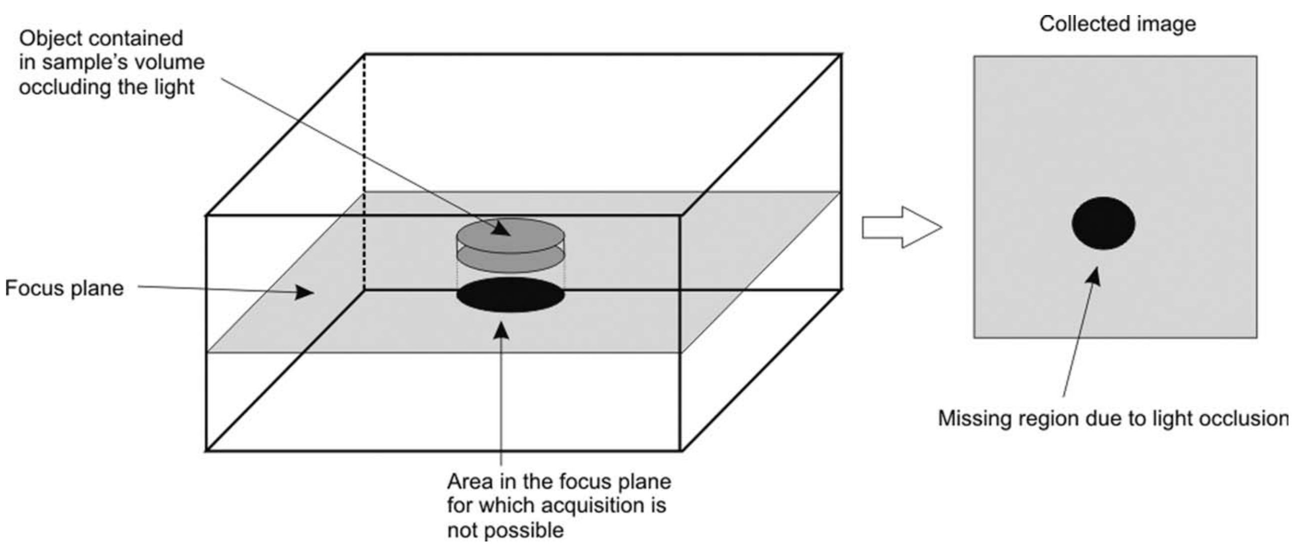


Fig. 4. Scenario in CSLM imaging in which acquisition is not possible for the entire field of view contained in the focus plane due to sample configuration.

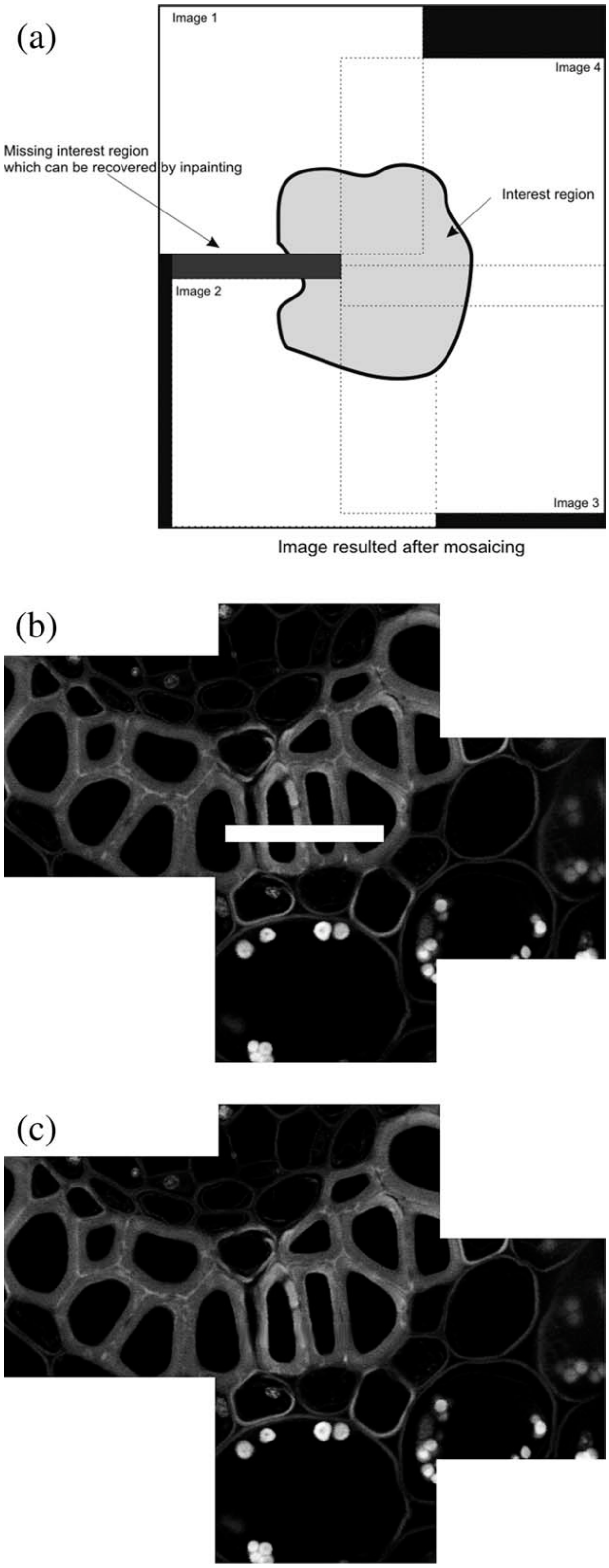


Fig. 5. Scenarios for compensating missing regions in image mosaics by inpainting. (**a**) Schematics of missing region in image resulted after stitching due to errors in the automated sample stage movement (**b**) image mosaic of images collected by CSLM on *Convalaria majalis* with missing part in its central region, (**c**) image mosaic with missing region approximated by inpainting.

objects by inpainting them with surrounding background, can however be used in conjunction with background estimation algorithms that play a key role in object tracking, and which may define a better background estimate based on a training set consisting of background-only images. Very good results are observed in the case when the inpainted region is neighbored in more than one cardinal direction by content belonging to the image object to be reconstructed (Figs. 2a–2c). In this case, the structure of the object is inpainted toward the inner part of the missing regions from different directions, which is similar to inpainting several regions of lower dimension. We can also observe that the results are better in the case of inpainting regions surrounded by regions with a strong gradient (Figs. 2a and 2b), than in the case of regions surrounded by content that would have a soft response to a gradient operator (Fig. 2c), as the blur introduced by the diffusion process is accentuated in this secondary case. In the case of the tested AFM image (Fig. 2d), even though we are dealing with image content that resembles to a pattern, inpainting provides a very accurate reconstruction for the masked regions that cover an area lower than the size of the texture elements. In the other case, when the masked region is higher than the texture elements the results consist in blurry patch that cannot be perceived as an accurate or plausible reconstruction. Unsatisfactory results are observed also in the case of reconstructing an image area of unstructured content, which does not present as well a pattern aspect (Fig. 2e, STM), here the algorithm results in patches similar in mean intensity to their surroundings but having a blurry aspect that does not approximate the original content. It can be observed as well a limitation of PDE-based digital image inpainting when experimented on images affected by mechanical noise, as in the case of the presented images collected by s-SNOM (Fig. 2f). In the case of s-SNOM as in the case of most SPM techniques, mechanical vibrations of high amplitude translate to a dithered aspect of the image. This means that when imaging an object with a geometry defined also by straight lines, these lines will be visualized as curved lines. In our case, this means that the isophotes belonging to the features affected by this situation will be continued toward the inner part of the reconstructed region at an angle that does not correspond to the real case. Further on we will discuss several problematic scenarios of microscopy imaging in which inpainting can be regarded as a solution.

Artifact Removal

In the case of any microscopy technique, unwanted artifacts introduced by various factors can take part in an image (Hecht et al., 1997; Jenkins et al., 1992; Kuhle et al., 1998; Markham and Conchello, 2001) leading to a defective aspect and to misinterpretations. Some of the artifacts that are specific to various microscopy techniques translate to missing or defective regions in the collected image. In Figure 3a, we present an image collected by s-SNOM on a brass sample. We can observe in the collected image acquisition artifacts in the form of horizontal lines, which occur due to the fact that the tip of the cantliver is subjected to focus or laser beam exposure inconsistencies. In this case, the

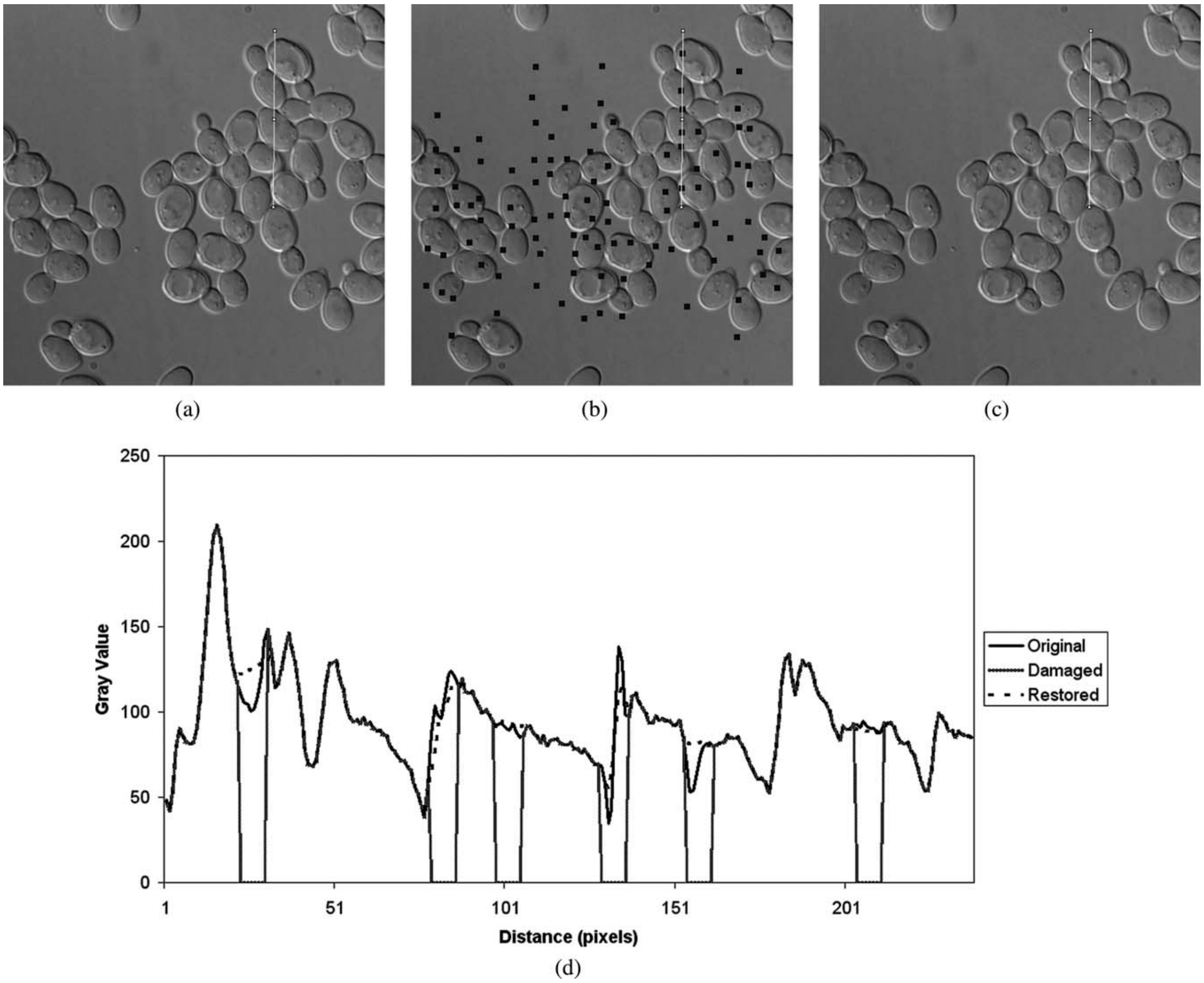


Fig. 6. Image of *Saccharomyces cerevisiae* collected by DIC (**a**) in original aspect, (**b**) with missing blocks due to transmission errors (2.51% data lost), (**c**) reconstructed by inpainting the missing regions, and (**d**) profiles along the same line in the three images.

signal reaching the detector drops, translating to missing regions in the collected image. In Figure 3b, we present the image reconstructed by inpainting the missing regions, in which the contribution of the artifacts is lowered, and the overall aspect is enhanced. This situation can be extrapolated to a considerable amount of microscopy techniques, where acquisition artifacts result in missing or defective regions of the collected image damaging its overall aspect.

Approximation of Missing Regions

Missing regions in an image collected by using a microscopy system can be present also due to the fact that image acquisition could not be performed for a particular region of the investigated area. An example in this case can be the acquisition of an image corresponding to an optical section by CSLM. If the laser beam performing the scan faces an "obstacle" in the volume of the specimen, before reaching the focal plane, Figure 4, this will result in a defective image region, affected by over saturation or over absorption. Related problematic scenarios regarding light attenuation in CSLM can be compensated by histogram modeling techniques (Capek et al., 2006; Stanciu et al., 2010), but only in the sense of enhancing the visualization of collected data and not by reconstructing or estimating missing data as it is possible in the case of inpainting. Other frequent scenarios in which the acquisition cannot be performed for a particular region that resides in the field of view are related to dust or water contamination of samples investigated by SPM techniques.

Filling in Missing Regions in Mosaic Images

Another scenario in which digital image inpainting could represent a solution is image mosaicing, which represents the technique of building a single image from the contents of many, and which has proven it's usefulness in microscopy imaging in several experiments (Ma et al., 2007; Thevenaz and Unser, 2007; Vercauteren, 2008). In this technique the individual images are regarded as tiles, and the resulting collection of assembled tiles forms the mosaic that covers a larger field of view than any individual tile. Sometimes in the stitched microscopy image, missing patches could be present due to the fact that no image acquisition was performed for those sample regions. This can happen because of various reasons, such as nonliniarities of the piezoceramic tube that performs the scanning in the case of scanning probe techniques, errors of motorized stage movement in the case of automated batch acquisition, or geometrical limitations in the case of rotating sample stages. In this case, inpainting the missing regions could give an estimate on them and contribute to the uniformity of the stitched image, Figure 5.

Compensating Transmission Errors

JPEG compression represents a solution in order to reduce the transmitted data volume in telemicroscopy sessions (Brauchli et al., 2002; Stanciu and Stanciu, 2008). JPEG divides the image into blocks of 8×8 pixels and calculates a 2D direct cosine transform, followed by quantization and Huffman Encoding (Wallace, 1991). In certain scenarios, the images are transmitted through a network block by block. During the transmission process faults can appear that translate to missing blocks in the received image. Usually, the packet loss rate occurs in a bursty fashion which means that the probability of having adjacent missing blocks is reduced. In Figure 6, we present an image collected by DIC representing cellular structures of *Saccharomyces cerevisiae* (Riffle and Davis, 2010), the received version of this image, with missing 8×8 pixels blocks and the reconstructed image by digital image inpainting.

We can observe in the profile presented in Figure 6d that the information introduced in the missing regions after inpainting is a good approximation of the original content. This translates to a restored image very similar to the original.

Inpainting can be regarded as a useful tool for computer vision applications. This family of applications is very important for microscopy imaging and in many of these methods image consistency, to which inpainting can contribute, plays a key role. Applications designed for tasks such as object detection and recognition, object tracking, image registration, image retrieval or image segmentation and indexing can be seriously affected by missing regions or acquisitions artifacts in the support image. An approximation of the missing content can allow such applications to achieve the results they were designed for.

Even though inpainting does not enhance the level of information in an image, the approximation of the missing regions results in a representation that allows nonetheless a more intuitive comprehension of the imaged scene. Choosing the inpainting technique to be used for any of the scenarios presented above is a subjective matter, as the results depend on the type of image content. However, observing the progress made in this domain in the last decade encourages us to think that inpainting techniques which could deal with any type of content specific to microscopy imaging already exist or will be soon available.

CONCLUSIONS

In this article, we introduce inpainting to the field of microscopy imagining. We have experimented digital image inpainting based on diffusion by "curvature-preserving" PDE's on images collected by several optical and scanning probe microscopy techniques, CSLM, DIC, FRET, AFM, STM and s-SNOM, containing different types of content specific to microscopy imaging. Along with the advantages and the good quality results provided by the tested method that were observed in the case of structured images with good contrast, we have noticed as well limitations of this technique when dealing with patterned images, images affected by mechanical noise or images of low contrast. Our experiments show that inpainting techniques can represent a solution in several scenarios common for microscopy imaging, such as the presence of artifacts, acquisition impossibility for particular sample regions or the occurrence of transmission errors in telemicroscopy sessions, and thus should not be neglected.

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