Periodic Background Pattern Detection and Removal for Cell Tracking

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Abstract. The study of cell morphology and cell mobility variation when cells are grown on top of patterned substrates is becoming a very important factor in tissue regeneration.

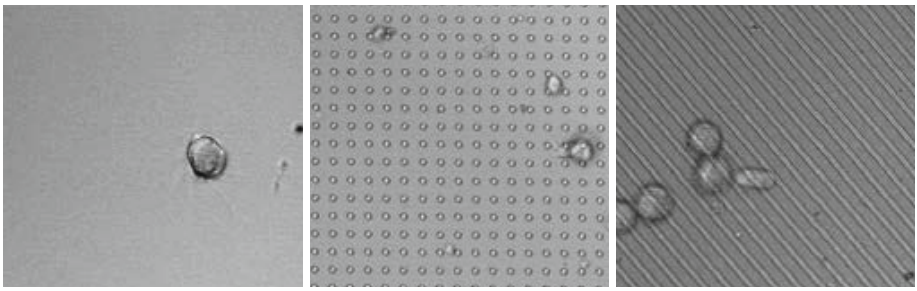
In this paper we present a novel approach to automatically detect and remove periodic background patterns in brightfield microscopy images. This background removal process is fundamental for the analysis of cell mobility as the periodic background pattern would otherwise lead to erroneous cell analysis. The detection of the background is performed by searching for the periodic background pattern organization through the analysis of keypoints automatically obtained from images. Using this information we are able to both detect and reconstruct the periodic background and finally remove it from the original images.

We tested the proposed approach on microscopy images with different periodic background patterns. The effectiveness of the method was validated both by visual inspection and by the cell tracking results obtained.

1 Introduction

The analysis of cell behaviour when interacting with different micropatterned surfaces has gained increasing interest in the last years [1,2]. Biologist researchers started producing micropatterned surfaces on biomaterials to study the possibility to modulate cell behaviour only through topography stimulus of biomaterials [1]. Micropatterned surfaces can be developed with controlled chemistry, roughness, thickness and textures to study its influence on cells (figure 1). Those cell/surface interactions are analyzed in order to access cell metabolic activity, adhesion morphology, proliferation and lineage differentiation [2]. Measurements of cell alignment, elongation and guided mobility on the surface

A. Campilho and M. Kamel (Eds.): ICIAR 2014, Part II, LNCS 8815, pp. 123–131, 2014.
DOI: 10.1007/978-3-319-11755-3_14



(a) (b) (c)

Fig. 1. Brightfield images with cells on top of different micropatterned surfaces: a) Flat surface; b) Pillar pattern surface; c) Line pattern surface

are essential to confirm these interactions [2]. Currently there is no automatic methodology to perform this measurements on patterned surfaces. Measurements are performed mainly by visual inspection alone [2].

The required cell mobility and morphology analysis is already performed on other studies where the background is flat [3–6]. Nevertheless it is known that there is an dependency between segmentation and interferences or changes in the image background like changes or distortions of image intensity or illuminance [7]. However, image background pre-processing steps are mainly concerned with intensity inhomogeneities and illumination [8–10].

Several other works tried to address similar problems with background pattern removal [11,12]. Andrew *et. al.* performed the removal of unwanted, non-periodic patterns from forensic images by registering the image under analysis with a control background pattern image [11]. This is not possible in our case since we do not have an available control image from the background pattern and the existing pattern varies between images. Considering the case of periodic background patterns it is possible to perform image background removal by filtering in the Fourier domain [12]. However, this only works for periodic stripe patterns, which does not applies to our pillar patterned images (figure 1 (b)). If we consider the existence of background pattern as texture, there are also several methods that can be used to address this problem [13]. Such methods perform texture classification and segmentation. However, they do not provide a simple way to synthesize or remove the background image.

In order to facilitate cell detection and tracking for mobility and morphology analysis we propose a new approach to automatically detect the periodic background pattern, synthethize the full background, and remove it from the original image. The proposed approach consists of four steps: first we evaluate if there is a periodic background pattern or not; then, if it exists, we detect the background pattern based on its periodicity; after that we synthesize the full background image; finally we subtract the background from the original image obtaining only information related to the existing cells.

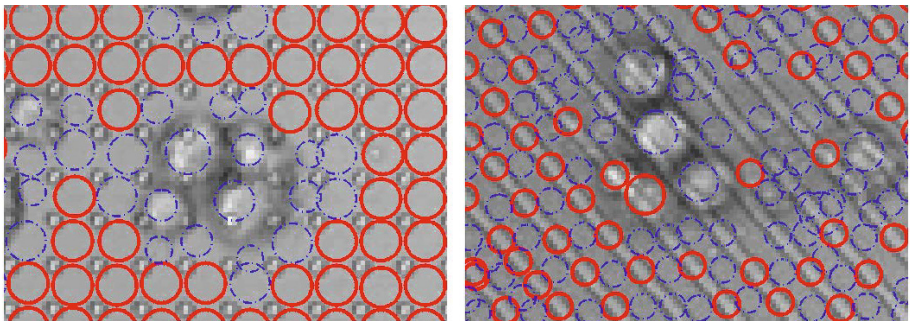


Fig. 2. Keypoint extraction from different background pattern brightfield images (colors represent different SIFT clusters).

2 Proposed Methodology

The first step of the proposed approach is to evaluate if there is a periodic background pattern in the image under analysis or if it has a flat background. We only perform the background pattern analysis if a periodic pattern exists.

2.1 Background Pattern versus Flat Background

In order to check if we have or not a periodic background pattern in the image under analysis we measure the image entropy:

$$E = -\sum_{i \in I} p_i . * \log_2(p_i), \tag{1}$$

where p is the histogram of image I and i is the pixel value. A high value indicates that we have high pixel values variation and we assume that it occurs in case of existing a periodic background pattern. Otherwise, a low value indicates that we are in presence of a flat background where pixel values do not differ to much from each other. We only consider the images for periodic background pattern detection that have entropy value over a defined threshold. Images in which the entropy value is low are assumed to have a flat background on which we only apply an illuminance and intensity inhomogeneities correction.

If a background pattern exists the first step for its detection is to extract keypoints from the image that will allow to infer the pattern periodicity.

2.2 Keypoints Extraction

In order to automatically obtain keypoints for periodic background pattern detection we use the Laplacian of Gaussian filter (LoG) [3,6]. This approach is based on the image scale-space representation and after applying this filter over several scales we search for local maxima of LoG response. We apply this approach to the images under analysis obtaining keypoints in positions that are related to both the background pattern and cell's position (figure 2). As we

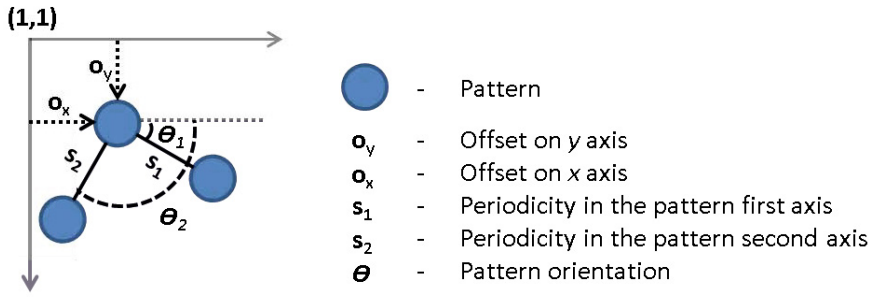


Fig. 3. Scheme used for detecting the pattern background periodicity. The background pattern is defined with a specific periodic interval s_1 and s_2, orientations θ_1 and θ_2, and image origin offset defined by (o_x, o_y).

observe in images from figure 2, as expected, the keypoints appear on locations with cells, however, they also appear in the regions of the background pattern. In the image from figure 2 (left) most keypoints are obtained with the same periodicity as the pillars from the background and in figure 2 (right) the same happens, in which most keypoints are obtained in the same orientation as the lines that compose the background.

After obtaining the keypoints we extract for each a SIFT descriptor [4]. We cluster the descriptors using k-means clustering ($k = 4$) and choose the largest cluster of descriptors that correspond to the keypoints obtained on the pattern repetition (figure 2 - red bold continuous circles). The selected keypoints will be used for the pattern periodicity analysis.

2.3 Background Pattern Periodicity Analysis

In order to detect the periodic background pattern we follow the scheme in figure 3. As we consider the periodic pattern to have two orthogonally independent periodicity spacings, we first search only over o_x, o_y, θ_1 and s_1 to find those that best fit our model. Given a set of values for o_x, o_y, θ_1 and s_1 we generate the predicted locations for our periodic pattern along the axis defined by θ_1. Given the selected LoG detection's coordinates in the image, we project each detection's location onto the axis defined by θ_1 along its normal. Each of those projections is then assigned to the nearest periodic pattern location, and the distance to the predicted periodic pattern is computed. As this distance calculation would favor smaller spacing in the periodic pattern it is normalized dividing by s_1. The set of parameters that lead to the lowest average distance are those assumed to represent the periodic pattern of the background in the image. Given o_x, o_y, θ_1 and s_1 we fix o_x, o_y and vary s_2 setting θ_2 to $\theta_2 = \theta_1 \pm 90$, but allowing for a 20 degree tolerance. If in this θ interval we find a second low average distance and the corresponding s_2 value is equal to s_1 value found previously, we assume that the background periodic pattern is pillar type (figure 2 (left)). We make this assumption because for both θ values (separated by ± 90 degrees) we

have the same keypoints periodicity. Otherwise, we assume a line background periodic pattern.

The pseudocode that follows summarizes the algorithm used for finding the periodic background pattern:

Algorithm 1. Pseudocode of the proposed approach

Require: *KeyPoints* extraction
  for o_x, o_y, θ_1, s_1 **do**
    Generate periodic pattern locations
    $distanceSum \leftarrow 0$
    for Each KeyPoint **do**
      Keypoint projection to obtain new coordinates in the axis defined by θ_1
      $minDist \leftarrow$ new coordinate distance to the closest pattern periodic location
      $distanceSum \leftarrow distanceSum + minDist/s_1$
    end for
    $distanceTotal(o_x, o_y, \theta_1, s_1) \leftarrow distanceSum$
  end for
  Find θ_1, s_1 and o_x, o_y (pattern information) that minimizes $distanceTotal$
  Repeat the s_1 and o_x, o_y estimation cycle to find s_2 and θ_2 with $\theta_2 = \theta_1 \pm 90 \pm 10$

Given the information found we classify each region as being periodic background pattern or foreground using template matching.

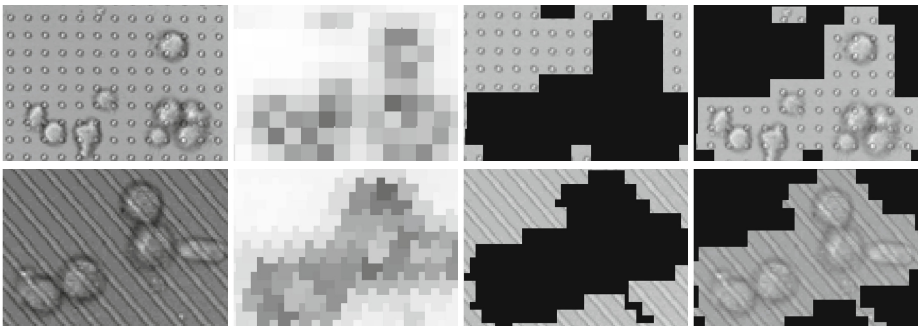
2.4 Foreground Removal

To detect the foreground regions we measure the similarity of each region with their 8 neighbour regions (according to the pattern periodicity found) using cross correlation:

$$\gamma(u,v) = \frac{\sum_{x,y}[f(x,y) - \overline{f}_{u,v}][t(x-u,y-v) - \overline{t}]}{\sqrt{\sum_{x,y}[f(x,y) - \overline{f}_{u,v}]^2[t(x-u,y-v) - \overline{t}]^2}}, \tag{2}$$

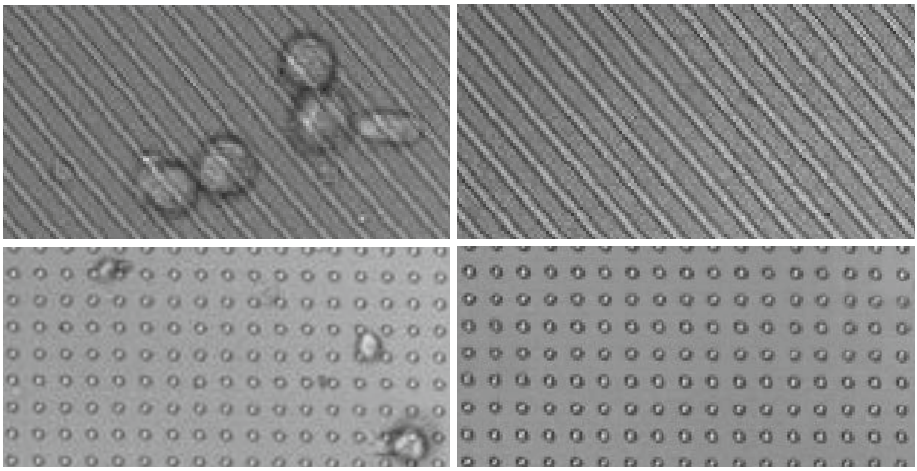
where $\overline{t}$ is the mean of the template and $\overline{f}_{u,v}$ is the mean of the image $f(x,y)$ in the region under the template [14]. A higher cross correlation coefficient (γ) indicates similar image regions.

As we measure the similarity between regions according to the pattern periodicity we will obtain high γ in presence of background pattern regions, and low values for foreground regions (figure 4 - b). From this analysis we then assume a specific *threshold* that separates the background pattern (figure 4 - c) from the foreground regions (figure 4 - d). From the detected background we then synthesize the removed foreground regions as background to obtain the full background image.



(a) (b) (c) (d)

Fig. 4. Foreground and background separation: a) Original image; b) γ similarity map (white - high value); c) Background detection d) Foreground regions

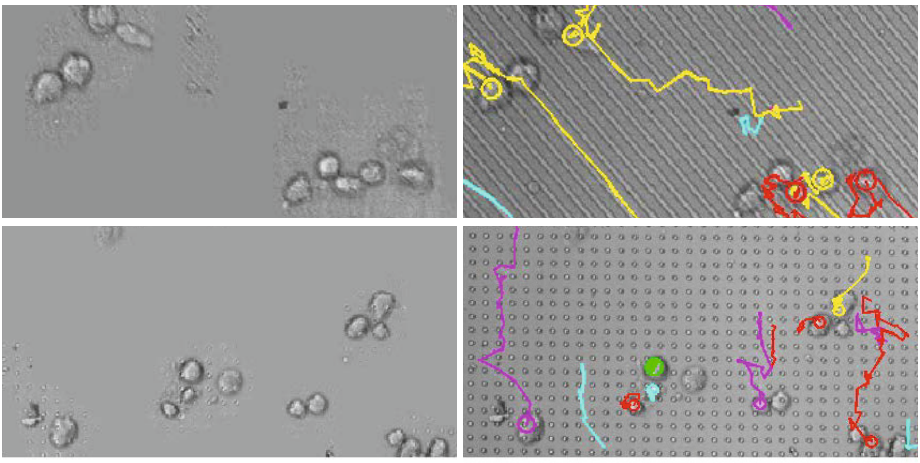


(a) (b)

Fig. 5. Background reconstruction: a) Original image; b) Periodic background pattern detection and reconstruction

2.5 Background Reconstruction

The final step to obtain the entire background pattern image is to reconstruct the foreground regions based on the pattern's periodicity. We define a patch (9×9) and search the image. When the center pixel of the patch is located at a foreground location we synthesize the periodic background pattern and replace it in the image. To synthesize the background we use the similar background patches located according to the parameters $(\theta_1,\ \theta_2,\ s_1,\ s_2,\ o_x, o_y)$ previously estimated. We average those similar patches to synthesise a valid background patch for that location and replace it in the background image. If pixels of that patch overlaps with already existing background, those pixels are combined



(a) (b)

Fig. 6. Background removal and cell tracking results: a) Result from subtracting the reconstructed background pattern from the original image; b) Cell tracks on top of the original image

weighting the new pixels with 0.1 and the old pixels with 0.9. Examples of the final results are given in figure 5.

3 Results and Discussion

We applied our approach on several brightfield microscopy images with different periodic background patterns and cells on top. For each original image we obtained the periodic background pattern and then we subtracted it from the original image and observed the results (figure 6 - a). From the results we were able to visualize that the differences are given mainly due to the cell presence which is an indicator that the background reconstruction is performing well.

Given the removal of the periodic background pattern we use the LoG filter to detect cells and we obtain detections only on locations with cells with no background pattern interference. From the cell detection result the cell tracking is then possible (figure 6 - b).

4 Conclusion

In this paper we proposed a novel approach to detect and extract the existing periodic background pattern in cell brightfield images. The approach is based on the analysis of keypoints periodicity obtained from each image. Once we found the existing periodic background pattern, it enables its removal from the original image by image subtraction.

Using the images from which the periodic background pattern was removed it is possible to perform cell detection and tracking. Future work will be done on the analysis of cell morphology and mobility which is now possible based on the development methodology for periodic background pattern removal. The influence of different background pattern (resultant of different micropatterned surfaces) on cell mobility and morphology will be quantified and compared.

Acknowledgments. This work has been financed by FEDER funds through the Programa Operacional Factores de Competitividade - COMPETE and by Portuguese funds through FCT - Fundação para a Ciência e a Tecnologia in the framework of the project PEst-C/SAU/LA0002/2013. T. Esteves is recipient of SFRH /BD / 80508/ 2011 by FCT. P. Quelhas is a *Ciência2008* awardee.

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