

Smartphone Platform for Image-Based Cell Confluency Analysis

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Abstract—Monitoring cell confluency is a fundamental step in cell culture workflows, offering insights into cell growth dynamics, health, and density. In this study, we present a smartphone-based image analysis framework for automated confluency estimation using brightfield images. Unlike conventional systems that rely on deep learning or high-end microscopy, our approach applies image processing techniques based on variance and entropy filtering to identify and segment cell-covered regions. These filters provide robust texture-based differentiation between cell-populated and background areas without requiring prior training or hardware calibration. Binary masks are generated to compute confluency as the ratio of segmented area to total field of view. The method is lightweight, cost-effective, and adaptable to varying image conditions, making it ideal for portable and resource-limited settings.

Keywords—cell analysis, confluency, smartphone microscopy, image processing.

I. INTRODUCTION

Confluency analysis gauges the surface area covered by cells, a valuable metric in studies where uniform cell coverage is crucial [1], [2], [3]. The maintenance of uniform confluency is important for the standardization of cell culture protocols, the reproducibility of the experimental results, and the minimization of the variability in cell behavior [4]. For example, the evaluation of potential therapeutics can be performed by measuring cell proliferation. By comparing confluency levels between treated and control groups, researchers can determine the impact of a drug on cell growth [5]. Furthermore, bio-manufacturing processes, for instance, the generation of monoclonal antibodies, vaccines, and recombinant proteins, necessitate strict control of confluency for enhancing productivity as well as yield [6], [7], [8], [9]. Monitoring confluency within stem cell-based treatments facilitates the optimal differentiation, expansion, and viability for transplantation purposes [10], [11]. Tissue engineering tasks include seeding cells onto scaffolds for the development of artificial functional tissues or organs [12]. In such cases, confluency assessment maintains a uniform cell distribution, preventing inconsistencies in tissue formation that can affect structural integrity and function [13], [14]. Optimal confluency levels are crucial in genetic engineering and transfection study to maximize both the reproducibility and the efficiency of the transfection process [15], [16]. Cancer biology and toxicology research are largely dependent upon

confluency measurements to standardize cell growth-based proliferation assays and assess the effects of chemotherapeutic agents under uniform growth conditions [17], [18], [19], [20]. All these applications highlight the pivotal role of confluency monitoring as a quality parameter for experiment consistency, reproducibility, and maximizing the efficiency of cell-based research and production processes.

Manual confluency assessment, typically performed through visual inspection under a microscope, remains a widely used and label-free approach in cell culture studies. Although this method is simple and non-destructive, it is highly subjective, time-consuming, and prone to inter-operator variability. To overcome these limitations, various commercial systems have been developed that offer automated confluency analysis using either image processing algorithms or AI-based techniques. For example, platforms like CELENA® X and ZEISS Lab-scope utilize artificial intelligence for robust, morphology-independent analysis, while systems such as CKX-CCSW and Axion BioSystems apply classical image processing [21], [22], [23], [24]. However, these solutions often involve specialized infrastructure [25].

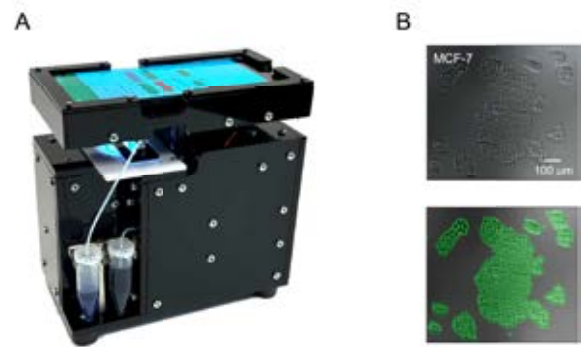


Fig. 1: (A) Image of the confluency analysis platform. (B) Segmentation result for breast cancer cells (MCF-7) cell culture image, showing green-highlighted regions indicating cell-covered areas.

In this study, a smartphone-based automated platform is proposed for accurate and reproducible cell confluency analysis (Figure 1). Unlike traditional manual methods that are time-consuming and prone to subjectivity, the system integrates a lightweight image processing algorithm that eliminates the

need for deep learning or complex hardware. By minimizing user intervention and operator-dependent parameters, the platform ensures reliable segmentation of cell-covered regions, making it a practical and accessible tool for both research and clinical environments.

II. METHODOLOGY

A. High-Contrast Imaging

The confluency calculations offered by the platform are performed by creating high-contrast cell images through an integration of a custom-designed grid slab. In Figure 2A, the working principle of this grid slab is illustrated (see photograph), which contains a white paper with printed black grids placed between five microscope slides; for instance, four glass slides above the paper and one underneath, all of them securely bonded together to ensure stability and alignment. The grid slab with a thickness of 4 mm and a flow cell with a 3 mm acrylic layer are combined, creating a total distance of 7 mm between each sample and the grid. With this design, generation of a high-contrast background without capturing the grid in the imaging process is achieved. The visibility of transparent cells is augmented by black grids, which highlight their edges and features. The detection of other transparent cells is enhanced by creating variations of light intensity in such areas, where light can pass through or is blocked by the grid. The working principle of this technique is similar to phase contrast or differential interference contrast microscopy [26]. A comparison of the same samples of MCF-7 cells imaged by phase contrast microscopy is shown in Figure 2B, which clearly demonstrates the platform's capability of high-contrast imaging (Olympus CKX41) with a much simpler and more cost-effective methodology.

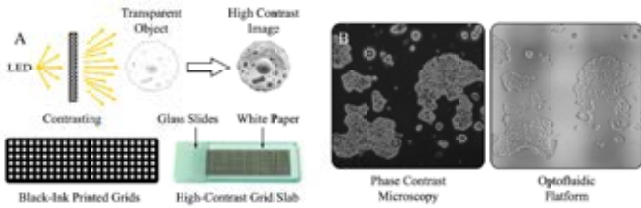


Fig. 2: (A) Schematic representation of the grid slab's working principle. (B) High-contrast confluency images produced by the optofluidic platform, offering visibility similar to phase-contrast microscopy.

B. Adherent Cell Culture

MCF-7 cells were cultured in RPMI 1640 medium (Sigma-Aldrich®), supplemented with 10% FBS (Sigma-Aldrich®), 100 units/mL penicillin (Sigma-Aldrich®), and 100 µg/mL streptomycin (Sigma-Aldrich®).

C. Segmentation Algorithm for Confluency Analysis

The flowchart in Figure 3 depicts the steps of the proposed image segmentation algorithm for confluency analysis. To attenuate noise and possible artifacts due to JPEG compression,

the input image with dimensions of 4000×3000×3 pixels (8 bits per pixel) is filtered with a 2D Gaussian smoothing filter (SD=2) in the preprocessing step. Thereafter, variance and entropy filters, two distinct filtering techniques, are applied to augment image features for segmentation. The local variance information is extracted from the image [27], which highlights the regions with prominent intensity variations, i.e., textures, corners, and edges. Due to the sharp intensity variations at cell boundaries, a variance filter is employed to improve the visibility of these regions. This filter calculates the local variance in a small area of each pixel within the Gaussian-smoothed image. For each target pixel, a 3×3 pixel window is extracted in the developed segmentation algorithm, and the output value of the central pixel of this pixel window is computed from the variance of these nine pixels. As a result, the variance of each local pixel window is represented by its output. To correct illumination and improve the contrast of the variance-filtered output, a gamma transformation with $\gamma = 0.6$ is applied. To ensure consistent intensity scaling, the final values are normalized to a range of [0, 255] [28]. Then, Sobel operators are applied to compute the gradient magnitude of the resulting image, highlighting intensity variations along cell boundaries [29]. Two 3×3 kernels are used by Sobel operators to estimate the image gradients in both x (horizontal) and y (vertical) dimensions, emphasizing rapid intensity changes at cell boundaries.

$$G_x = \begin{bmatrix} 1 & 0 & -1 \\ 2 & 0 & -2 \\ 1 & 0 & -1 \end{bmatrix}, \quad G_y = \begin{bmatrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{bmatrix} \quad (1)$$

Here, G_x and G_y are the gradient extraction kernels along the x - and y -directions, where their magnitude at each pixel location (i, j) is calculated via the Pythagorean sum as:

$$G_{\text{mag}}(i, j) = \sqrt{G_x(i, j)^2 + G_y(i, j)^2} \quad (2)$$

To ensure consistent intensity scaling, the gradient image is rescaled to a range of [0,255] and then processed by hysteresis-based double thresholding technique. This approach is more effective for segmentation of cell boundaries and reveals finer details, since it increases two intensity thresholds to differentiate strong edges while maintaining weaker, connected edges. This method attenuates the noise and isolates the most prominent edges adopting a higher threshold as “marker” (set to 0.8), and a lower threshold as “mask” (set to 0.5), capturing finer details and weaker edges. By using morphological reconstruction, the two output images are merged afterwards for more precise edge detection. By merging strong edge information with augmented details, this method enables more precise segmentation of cell boundaries, which balances noise/artifacts attenuation and preserves details. An approach based on entropy filtering is also introduced in this study, reinforcing the outcomes from the variance filtering technique.

The randomness or variability in local pixel intensity is evaluated by the entropy filter, which is a commonly used technique. This technique sets higher values for regions with

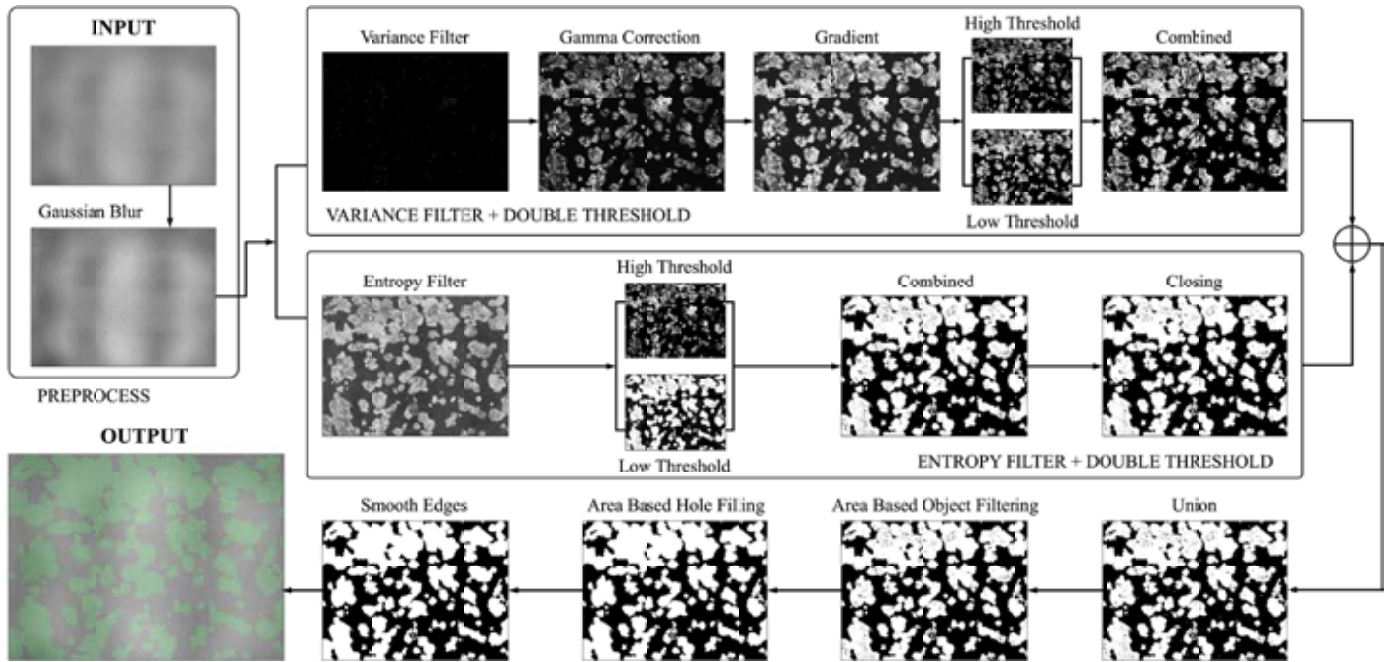


Fig. 3: Flowchart depicting the workflow of the image segmentation algorithm developed for confluency analysis.

intense variation. The entropy filter is a widely used technique for quantifying the randomness or variability in local pixel intensity [30]. With this technique, higher values are set to regions with marked intensity variation to accentuate areas rich in information, i.e., edges, corners and textures. From another perspective, complex textures and varying intensities of cells produce higher entropy values compared to the uniform background, which has lower entropy values. Owing to this difference, the selection of cell boundaries from the relatively uniform background is facilitated. In this study, this filter is applied through the computation of the local entropy within a 9x9 neighborhood around each target pixel. To partition regions of varying intensity, the filtering output is double-thresholded by using thresholds of 0.7 and 0.4. Then, the output images are merged using morphological reconstruction, followed by a morphological closing operation with a disk-shaped kernel of radius 3. Small internal holes across cell boundaries are filled by the closing operation, which augments the partition and preserves the integrity of the identified cell structures. A “union” operation is applied to merge binary outputs of variance and entropy filters for creation of a unified segmentation result. To enhance this output, small-connected objects are eliminated by implementation of an area-based object filter utilizing a connectivity value of 8. Thereafter, the internal sides of the identified cell boundaries are filled with area-based hole filling, providing a complete segmentation. For further augmentation of the output, a mean filter with a window size of 21 is applied to smooth the cell boundaries. Moreover, the resultant processed image is converted to the RGB color space, marking the detected cells in green. This green mask is then combined with the input image for a clearer visual representation of detected cells. Ultimately, confluency

(%) is computed by division of the number of white pixels by the total number of pixels in the final binary output image, i.e., $\text{White Pixels} / \text{Total Pixels}$. Ultimately, confluency (%) is defined as the ratio of white pixels to total pixels, where the values are measured from the image of final binary output.

III. RESULTS

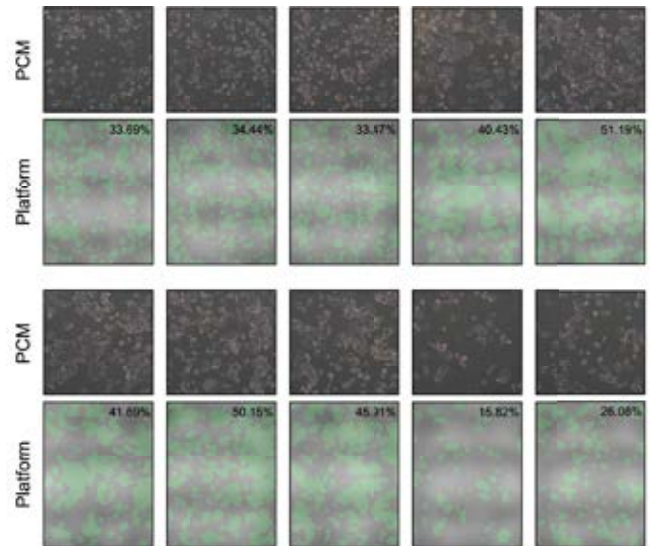


Fig. 4: Confluency analysis results of MCF-7 cells at different densities.

In Figure 4, the results of confluency analysis of MCF-7 cells at different confluency levels are illustrated. A phase contrast microscopy is utilized to capture the images in the first row (PCM), while the platform is used to acquire corresponding images shown in the second row, overlaid with