

Determination of the Effect of Anticancer Laser Therapies on the Cancer Cell Migration and Invasion via Artificial Intelligence Methods

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Abstract—Photodynamic treatment (PDT) and photobiomodulation (PBM) demonstrate potential in targeting cancer cells and reducing their motility. This research examines the impact of photodynamic therapy (PDT) using Chlorin e6 (Ce6) as a photosensitizer, both with and without low-level laser therapy, on the migration of human prostate cancer cells (PC3), assessed through in vitro scratch wound healing assays. This integrative methodology facilitated objective and consistent measurement of cellular motility, offering insights into the effects of photodynamic therapy and PBM on prostate cancer cell migration, and illustrating their potential synergy as combinatory anti-metastatic therapies. The results demonstrate that the proposed method effectively facilitates the monitoring of cell migration dynamics and the evaluation of treatment efficacy.

Index Terms—Cancer Research, Photodynamic Therapy, Cell Migration, Image Processing, Prostates Cancer, Scratch Assay

I. INTRODUCTION

Cancer metastasis is the leading cause of cancer-related deaths worldwide, highlighting the urgent need for effective therapeutic options targeting the pathways that facilitate tumour cell migration and invasion [1]. Photodynamic therapy (PDT) is a promising minimally invasive treatment that uses photosensitizers activated by certain light wavelengths to make reactive oxygen species (ROS), which then kill cancer cells [4, 9]. PDT not only induces direct cellular apoptosis but also modulates various cellular processes, including migration, invasion, and adhesion [7, 8]. Recent research has examined the combination of PDT with photobiomodulation (PBM), a low-level laser treatment known for its impact on cellular metabolism and signalling, to improve therapeutic efficacy and diminish migratory consequences [7, 10]. It is important to look at how medicines affect the movement of cancer cells in order to improve treatment plans that will lower the risk of metastasis and improve patient outcomes. Accurate quantification of cell migration dynamics is essential for evaluating the efficacy of antimetastatic treatments. The in vitro scratch wound healing experiment is an established technique for examining collective cell migration by creating a uniform wound area in a confluent cell monolayer and monitoring its closure

over time [5, 11]. Manual examination of wound healing images is time-consuming, subjective and prone to variability between observers, limiting its reliability and efficiency [6]. To overcome these constraints, contemporary digital image processing methodologies integrated with machine learning algorithms have been formulated to automate and standardise the evaluation of cell migration tests [1, 3, 12]. Automated pipelines like these cut down on human error, make it easier to repeat experiments, and make it easier to screen a lot of therapeutic interventions at once. To quantify in vitro cell migration tests, the initial crucial step is the segmentation of input pictures to distinguish cellular regions from the wound area (non-cellular parts). After achieving this binary classification, the cell migration process can be quantitatively evaluated by determining the rate of scratch wound closure over time [2, 5].

This study investigated the impact of Chlorin e6-mediated PDT, both alone and in combination with PBM, on the migratory behaviour of PC3 cells. Cells were treated with diode lasers at 655 nm and 808 nm wavelengths at specified energy levels. A wound scratch assay was carried out, with imaging using light microscopy. A custom automated image analysis pipeline, which included grayscale conversion, noise reduction, segmentation, wound area masking, skeletonization, and feature extraction, allowed for precise quantification of cell migration dynamics, highlighting PDT and PBM's modulatory influence as potential anti-metastatic combinations.

II. MATERIALS AND METHODS

A. Cell Culture Experiments

PC3 cells were grown in RPMI-1640 medium with 10% FBS, 1% L-glutamine and 1% penicillin-streptomycin under normal conditions (37°C, 5% CO₂). Cells from passages 5–15 were sown at 110 cells per well in 96-well plates and adhered for 24 hours before treatment. Diode lasers at 655 and 808 nm (400 mW output) were used to achieve energy densities of 50 J/cm² and 5 J/cm², respectively. The laser parameters were chosen in accordance with previously reported ranges for PDT

and PBM in prostate cancer models, providing reliable photobiological responses while minimizing the risk of thermal artifacts [1,15]. Chlorin e6 (Ce6) was used as a photosensitizer in PDT methods. Five experimental groups were formed: control (no treatment), laser (655 nm irradiation), PBM (808 nm irradiation), PDT (Ce6 incubation followed by 655 nm irradiation), and PBM + PDT (808 nm pre-irradiation, Ce6 incubation, then 655 nm irradiation).

The scratch wound experiment was carried out by making consistent scratches in confluent monolayers with a pipette tip. Following irradiation, cells were cultured under usual conditions, and scratch wound closure was examined at predetermined intervals using inverted light microscopy. A unique MATLAB image processing pipeline was used to provide quantitative analysis of cell migration, which was validated with manual measurements in ImageJ.

B. Image Preprocessing and Feature Extraction

To make the intensity distribution easier to understand and eliminate color information, all of the microscopic images were first changed to grayscale. This made the calculations less complicated. Figure 1. shows the whole pre-processing and feature extraction method. A Gaussian filter was used to get rid of high-frequency noise while keeping the structural boundaries. The Gaussian filtering technique is mathematically described as a convolution:

$$I_{\text{filtered}}(x, y) = (I * G_{\sigma})(x, y) \quad (1)$$

where $I(x, y)$ is the intensity of the image at point (x, y) and G_{σ} represents the Gaussian kernel with standard deviation σ .

To enhance the visibility of scratch wound boundaries and improve segmentation accuracy, histogram equalization was used. This process redistributes pixel intensities by transforming the image histogram into a near-uniform distribution, defined by:

$$s_k = T(r_k) = \sum_{j=0}^k \frac{n_j}{n} \quad (2)$$

where r_k is the input intensity level, n_j is the number of pixels with intensity r_j , and n is the total number of pixels. Adaptive thresholding, guided by Otsu's method, was employed for binarization. Otsu's method determines an optimal threshold t^* that maximizes inter-class variance:

$$t^* = \arg \max_t \left[\omega_0(t) \omega_1(t) (\mu_0(t) - \mu_1(t))^2 \right] \quad (3)$$

where ω_0 and ω_1 are class probabilities and μ_0 , μ_1 are class means. We then used a zero-crossing method to find the edges of the consolidated cellular areas and the scratch wound. This method finds boundaries by looking for places where the intensity of a filtered image varies. The length of the major axis was calculated for each edge that was found. The cellular region and the scratch wound were separated by the two longest edges.

Finally, a distance map was generated by computing the minimum Euclidean distances between opposing boundaries,

and the average value was used as a quantitative metric for scratch wound width.

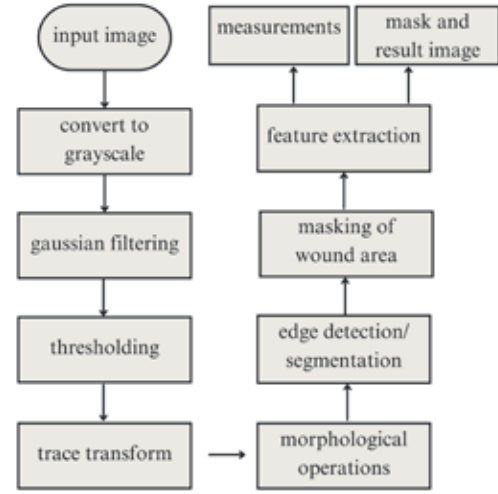


Fig. 1: Flowchart of the proposed image preprocessing and feature extraction algorithm

A local energy map was made to look at the shape of cells and the properties of the scratch wound area. This method looks at the local structural complexity in the grayscale image by looking at how the intensity and edge information changes in different areas. This gives us an idea of how the texture of the cells is spread out. The Trace Transform was also utilised to extract features. This is a more general version of the Radon transform that combines functions along straight lines at different angles and offsets. It lets you get invariant shape features that work even when the shape is rotated, scaled, or moved. Mathematically, it can be expressed as:

$$T(\phi, \rho) = \mathcal{T}[f(x, y)] = \int_{L_{\phi, \rho}} \tau(f(x, y)) ds \quad (4)$$

where $L_{\phi, \rho}$ is a line parameterized by angle ϕ and distance ρ from the origin, and τ is a trace functional such as mean, max, or entropy.

From the segmented scratch wound and cell regions, several quantitative features were extracted, including area (A), convex area (CA), eccentricity (Ecc), equal diameter (EqD), equal Ellipse area (EqElpA), minimum Feret diameter (MinFD), factor R (Rf), modified ratio (ModR), boundary box area (BBA) and shape factor 1 (SF1) [14]. The scratch wound area at each time point was used to calculate closure percentage as:

$$\text{Scratch wound Closure (\%)} = \left(\frac{A_0 - A_t}{A_0} \right) \times 100 \quad (5)$$

where A_0 is the initial calculated scratch gap feature and A_t is the calculated scratch gap feature at time t [22]. Cell migration distance and width changes are also tracked longitudinally to assess therapeutic impacts.

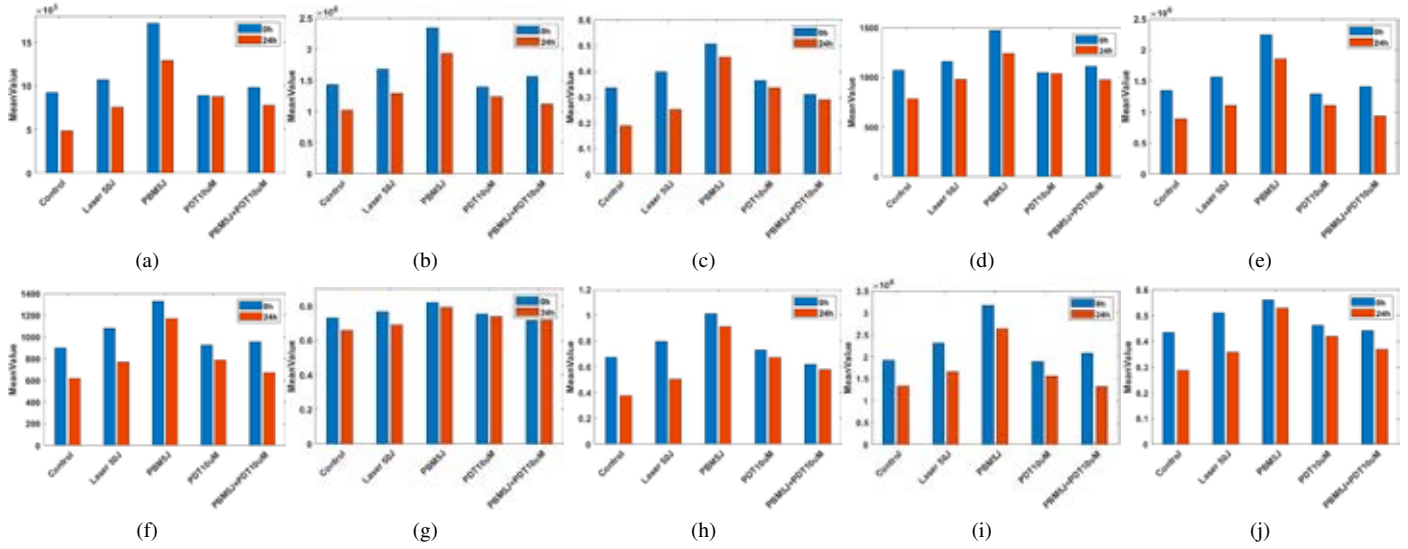


Fig. 2: Extracted quantitative features from segmented scratch gap and cell regions: (a) A, (b) CA, (c) Ecc, (d) EqD, (e) EqElpA, (f) MinFD, (g) Rf, (h) ModR, (i) BBA, and (j) SF1.

III. RESULTS

The image processing pipeline (Figure 1) encompassed a sequence of preprocessing operations, contrast enhancement, Gaussian smoothing, and histogram equalization followed by segmentation procedures incorporating adaptive thresholding and zero crossing edge detection, and culminating in quantitative feature extraction. This integrated methodology enabled precise delineation of scratch gap boundaries under variable imaging conditions and facilitated the derivation of morphological and geometric descriptors for subsequent quantitative analysis[3].

Quantitative features extracted from scratch assays at 0 h and 24 h were analysed across the five experimental groups (Control, Laser 50J, PBM5J, PDT10 μ M, and PBM5J+PDT10 μ M). As illustrated in Figure 2, the control group exhibited the most pronounced variations between 0 h and 24 h, whereas PDT-treated groups demonstrated minimal changes, indicating a significant inhibition of cell migration. Size-related features, including A, CA, EqD, EqElpA, MinFD and BBA [14], showed the largest reductions in the control group, reflecting rapid cell migration and scratch wound closure. In contrast, PDT10 μ M and PBM5J+PDT10 μ M groups maintained higher values at 24 h, consistent with delayed closure. Shape-related features (Ecc, Rf, ModR, SF1) decreased markedly in the control group, suggesting isotropic and uniform gap filling, while PDT-treated groups displayed only minor reductions, indicating irregular and incomplete migration.

To allow direct comparison of migration dynamics across treatments and features, percentage changes from 0 h to 24 h were calculated using Equation (6). In brief, for each treatment group and each quantitative feature, the mean value at 24 h was compared to the mean value at 0 h, and the relative change was expressed as a percentage. This approach, based

on the formula originally applied for wound area closure, was extended here to all extracted features, enabling normalization of differences in absolute scale between parameters [13]. The resulting values, summarised in Figure 3, show that the control group exhibited the largest relative changes in most parameters, while PDT-based treatments produced minimal changes, indicating substantial inhibition of migration. Laser 50 J/cm² and PBM 5 J/cm² groups displayed intermediate effects.

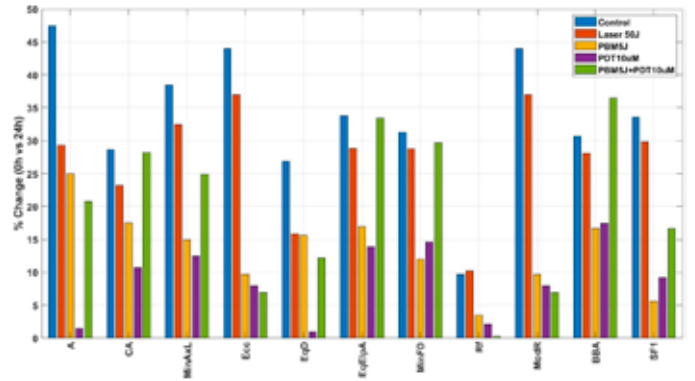


Fig. 3: Percentage change of measured features from 0h to 24h across the five experimental groups (Control, Laser 50 J/cm², PBM 5 J, PDT 10 μ M, and PBM + PDT). Changes in size- and shape-related metrics provide a quantitative assessment of cell migration dynamics and treatment effects.

Visual inspection of segmented scratch areas (Figure 4) supports the quantitative results. Control cells showed almost complete closure at 24 h, while Laser 50J and PBM5J groups had partial closure. In contrast, PDT10 μ M and PBM5J+PDT10 μ M retained wide gaps, confirming suppressed migration under PDT-based treatments.

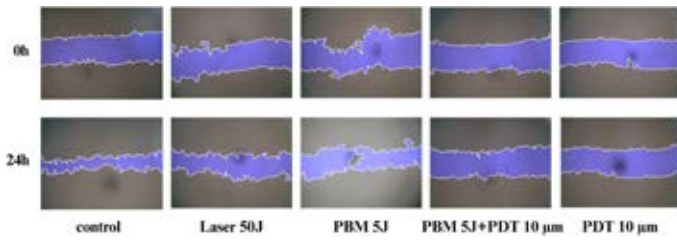


Fig. 4: Segmentation of cancer cell migration in five experimental groups at 0h (top) and 24h (bottom). Blue regions represent the segmented scratch wound areas.

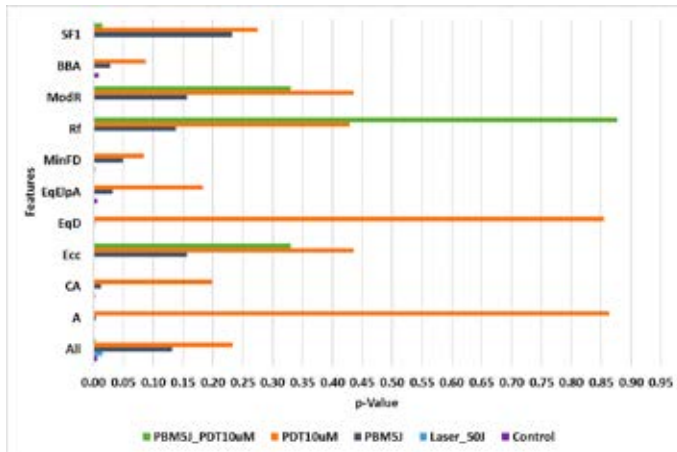


Fig. 5: ANOVA test results showing the p -values for different extracted features. “All” represents the p -values obtained by considering all concatenated size- and shape-related features, while the other labels correspond to individual feature sets. Significant differences ($p \leq 0.05$) indicate variation in migration dynamics among the experimental groups.

Quantitative features from scratch assays at 0h and 24h were analysed using one-way ANOVA ($\alpha = 0.05$). As shown in Figure 5, most features differed significantly between groups ($p \leq 0.05$), with the control group showing the largest changes, while PDT-treated groups maintained higher values, confirming significant inhibition of cell migration.

IV. CONCLUSION

This study applied a multi-step image processing pipeline to quantify migration in PC3 cancer cells under different treatments. PDT 10 μ M, alone or combined with PBM 5 J, strongly inhibited migration, while Laser 50 J/cm² and PBM 5 J alone had moderate effects. The pipeline provides a reproducible, quantitative assessment of migration and lays the groundwork for future AI-based studies, where feature-based classification and machine learning models can enhance automated analysis of cell migration.

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