PROFILING MORPHOLOGICAL AND BIOPHYSICAL SIGNATURES IN BLADDER CANCER CELLS FOR LABEL-FREE DIAGNOSTICS

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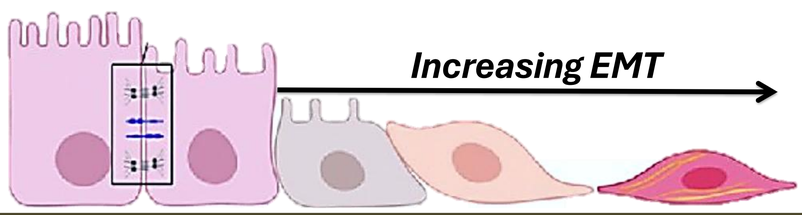
1 INTRODUCTION

Bladder cancer (BC) varies in cellular morphology and biophysical properties related to disease progression, particularly through epithelial to mesenchymal (EMT) transition. Urine cytology exhibits low sensitivity, close to 38%, particularly for low-grade tumours, leading to late diagnosis and reliance on cystoscopy, an invasive procedure [1]. Recent advancements in cell biophysics offer new ways for label-free and non-invasive BC detection [2]. In this study, we profiled morphological cues, light-metrics and clustering frequency of BC cell lines and clinical samples using imaging flow cytometry, aiming to identify morpho-biophysical biomarkers and their correlation with the EMT score and cancer progression.

2 PURPOSE

2.1) Profile morphological and clustering features of BC cell lines, control and clinical samples using imaging flow cytometry;

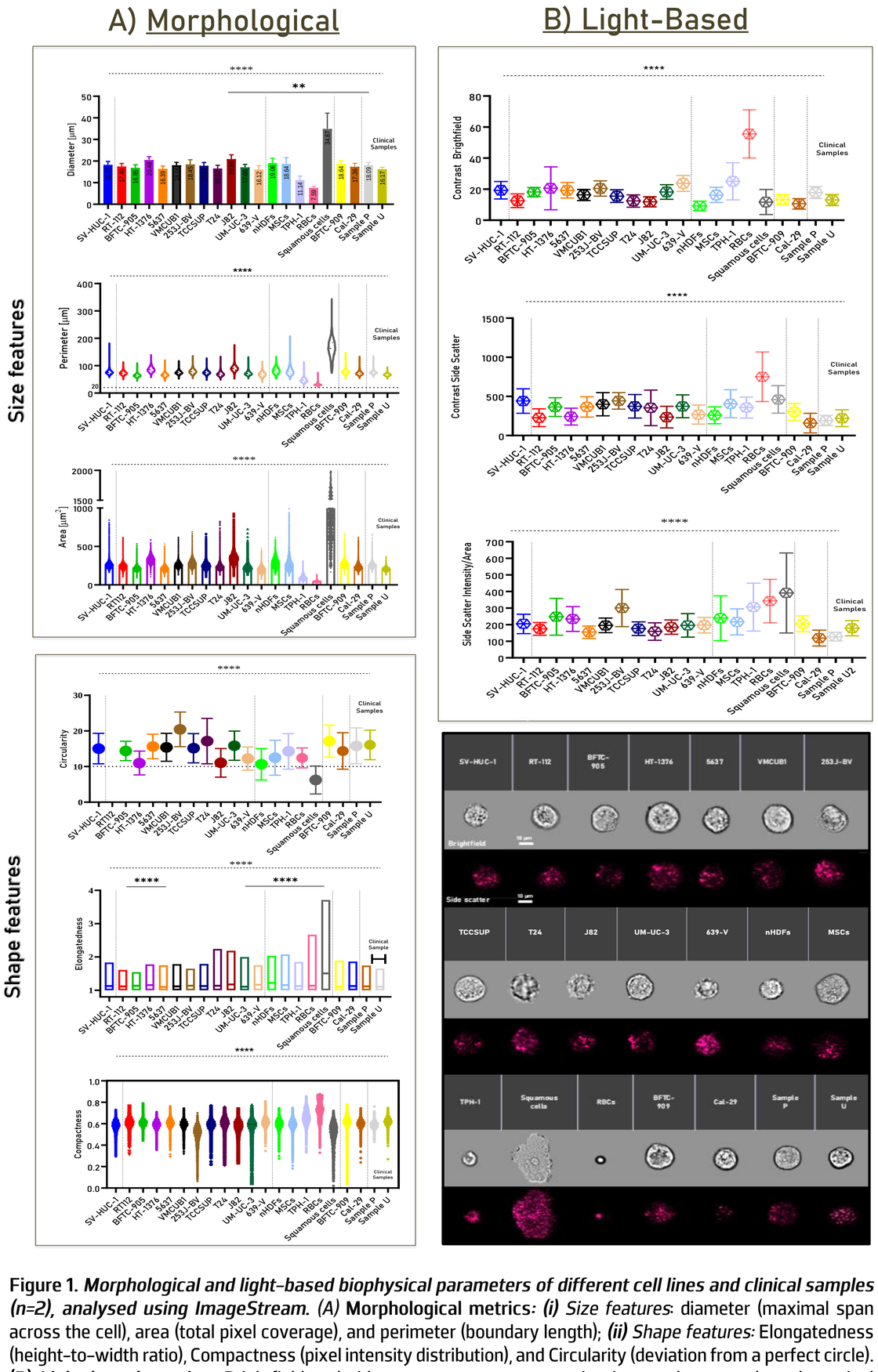
2.2) Assess the correlation between the evaluated morpho-biophysical cues and the EMT score of cancer cells



3 METHODS

Figure 1. Experimental design: Fourteen cell lines (one non-cancerous – SV-HUC-1 – and thirteen cancerous ones), primary dermal fibroblasts (nHDFs), mesenchymal stem cells (MSCs), a monocyte cell line (THP-1), red blood cells (RBCs), urine-derived squamous cells, and two clinical samples (Samples P and U) were cultured and then fixed for ImageStream analysis. Morphological criteria such as diameter, area, perimeter, elongatedness, compactness, circularity and light-based metrics, e.g., brightfield, light-side scatter contrast and intensity per cell area were quantified in single cell analysis. Self-clustering frequency was assessed by the ratio of clustered events (≥2 aggregated cells) to total events. Results represent mean quantification of ≥3 independent assays (N=3), each with over 7.0×10^4 events, per cell type. Data analyzed using two-way ANOVA with multiple comparisons, significance set at $p<0.001$.

4 RESULTS



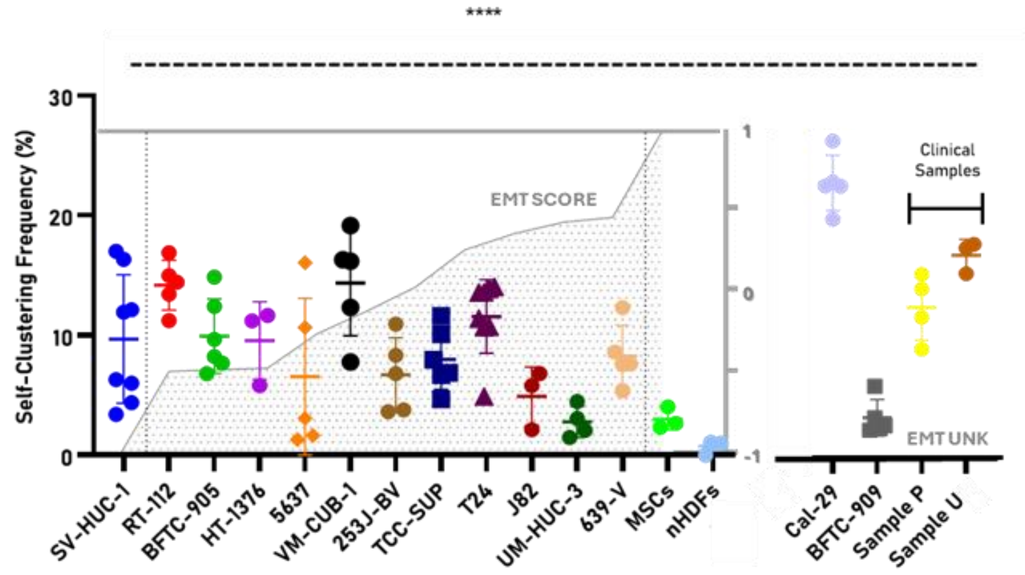


Figure 1. *Morphological and light-based biophysical parameters of different cell lines and clinical samples (n=2), analysed using ImageStream. (A)* **Morphological metrics**: *(i) Size features*: diameter (maximal span across the cell), area (total pixel coverage), and perimeter (boundary length); *(ii) Shape features:* Elongatedness (height-to-width ratio), Compactness (pixel intensity distribution), and Circularity (deviation from a perfect circle). **(B) Light-based metrics**: *Brightfield and side scatter contrast*, measuring image sharpness based on pixel intensity changes; *Mean intensity*: The ratio of fluorescence intensity to cell area, indicating average signal per pixel; **Cell image panel**: Light-based metrics organized by increasing EMT score, visually supporting data interpretation. Data are presented as mean ± SD, assessed using two-way ANOVA with multiple comparisons ($p < 0.001$). All groups showed statistically significant differences, enabling comprehensive phenotype assessment.

Figure 3. Self-*Clustering Frequency Analysis in different cell types, including BC cells, Control Groups (SV-HUC-1 for non-cancerous uroepithelial cells, Fibroblasts and MSCs for fibroblastic and mesenchymal phenotypes, blood and immune cells (RBCs and THP-1), and urine most common cells, the squamous cells. Two Clinical Samples, isolated from BC solid tumours, were also assessed.* The percentage of cluster events (% Cluster Frequency) was calculated as the ratio of clustered to total focused cells. Cluster frequency tends to decrease with an increasing EMT score varying from -1 to 1, meaning from epithelial to mesenchymal phenotypes, respectively. We considered the epithelial control (SV-HUC-1) as -1 and MSCs – mesenchymal control - as 1. Cal-29, BFTC-909, clinical sample P and U (without defined EMT scores) were included, and their approximated EMT score was quantified in this methodology. nHDFs had an absence of detectable clusters, which is characteristic of a fibroblastic phenotype. Results represent mean quantification from at least three independent assays (N=3) per cell type ($p<0.001$).

5 CONCLUSIONS

- **Cell Morpho-Biophysics as Diagnostic Tools:** The combination of morphological and light-based metrics can be used as a fingerprint of different BC phenotypes and may serve as key indicators for label-free determination of cancer progression risk.
- **Self-Clustering and Risk Stratification:** The inverse relationship between self-clustering frequency and EMT score suggests its potential applicability for risk stratification in BC diagnostic.

References.

[1] Zhu CZ, Ting HN, Ng KH, Ong TA. A review on the accuracy of bladder cancer detection methods. J Cancer 2019; 10(17):4038-4044. doi:10.7150/jca.28989. https://www.jcancer.org/v10p4038.htm

[2] Charpentier M, Gutierrez C, Guillaudeux T, Verhoest G, Pedeux R. Noninvasive Urine-Based Tests to Diagnose or Detect Recurrence of Bladder Cancer. Cancers. 2021; 13(7):1650. https://doi.org/10.3390/cancers13071650

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