AACR Poster #1073

AI-assisted high-dimensional cytological single cell analysis distinguishes normal and malignant urine samples using cell morphology images with high accuracy at the single cell level

Thierry P.P. van den Bosch[1*], Geert J.L.H. van Leenders[1*], Siamala Sinnadurai[1], Zhouyang Lian[2], Ryan Carelli[2], Matthew Nakaki[2], Vivian Lu[2], Janifer Cruz[2], Kelly R. Miller[2], Mahyar Salek[2], Maddison M. Masaeli[2], **Peter J. van der Spek**[1]

[1] Erasmus MC Dept of Pathology & Clinical Bioinformatics Rotterdam, NL, [2] Deepcell Inc. Menlo Park CA, USA

Introduction

Urothelial cancer (UCC) is the 4th most common malignancy in men. The current clinical problem in UCC management is the high interobserver variability of tissue grading. Moreover, low sensitivity of urine cytology for early detection of cancer, recurrences and disease progression remains a challenge.

Squamous-cell carcinoma (SCC) is a type of cancer that originates in squamous epithelial cells. Besides the epithelial lining of the skin, squamous cells also form the surface of the hollow organs that potentially are prone to viral infection. The organs most commonly affected by SCC are the skin, lungs, cervix and vulva, with rare occurrences (2-5%) also reported in the bladder.

High resolution images of single cells were captured using the Deepcell's REM-I platform.
We trained a deep learning model on a cohort of urine samples consisting of 54 healthy controls, 19 atypical cases, and 14 bladder cancer cases. Applications of this study include improved early detection and quantitative grading of bladder cancer based on distinct morphological characteristics of cells. This deep learning model can potentially be re-used for other cancers derived from e.g. lung, cervix and vulva.

Study Goals

- Label-free high-dimensional morphological profiling using Deepcell's REM-I to distinguish benign, atypical and malignant cells in urine cytology samples.
- Use Artificial Intelligence and Machine Learning for sorting and recognizing cells in urine, based on morphological differences.
- Count and collect the individual cells, belonging to distinct morphological clusters, to gain further insight in cell differentiation linked to bladder cancer.
- Sorted cells can be used for downstream analysis to study UCC/SCC relevant pathways, such as mutation detection (e.g. FGFR3), RNA expression profiling, immuno-histochemistry or proteomics.

REM-I Platform

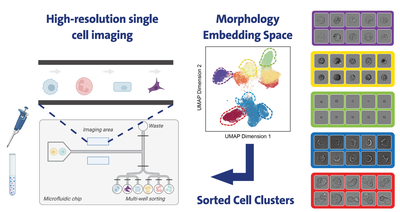
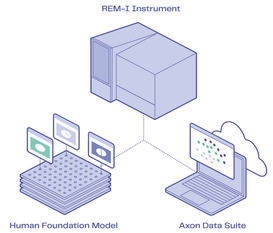


Figure 1. The REM-I workflow. A single cell suspension is loaded onto a microfluidic chip where images of single cells are captured and analyzed in real-time by the Human Foundation Model (HFM). High-dimensional morphological features are visualized in a UMAP. User-defined cell clusters can be sorted for downstream functional or molecular analysis. Morphology embeddings are used to profile the morphological heterogeneity of the cells, identify which morphological features differentiate each condition, and train a random forest classifier.

The REM-I Platform

A fully integrated solution

- **IMAGING:** High-resolution brightfield images captured at high speed
- **SORTING:** 6-way, label-free sorting
- **SINGLE CELL:** Real-time analysis of single cell morphology
- **HIGH-DIMENSIONAL:** Powered by Deepcell's Human Foundation Model
- **END-TO-END:** Comes with Axon, Deepcell's data suite



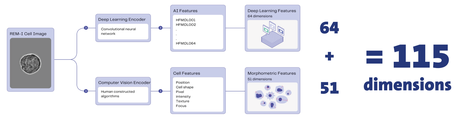


Figure 2. Human Foundation Model (HFM) is a feature extractor. Features of cell images are encoded into multi-dimensional numerical vectors. Morphometric features describe the metrics of cells such as surface area, diameter, shape, as well as biological characteristics such as vacuolization or pigmentation. Deep learning-derived features are information-rich metrics that describe cells. AI derived features can outperform traditional morphometrics in cell classification tasks through identification of non-human interpretable cell features.

Varying cell clusters are differentially represented across conditions

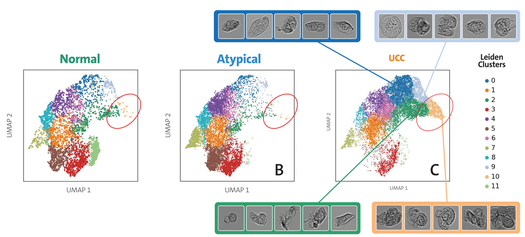


Figure 3. Morphology UMAP of urine samples showing 12 Leiden clusters in the three sample groups.
(A) Healthy controls (n=54); (B) Atypical (n=19); (C) Bladder cancer (n=4).

Morphology analysis reveals distinct morphotypes in normal vs pathological urine samples

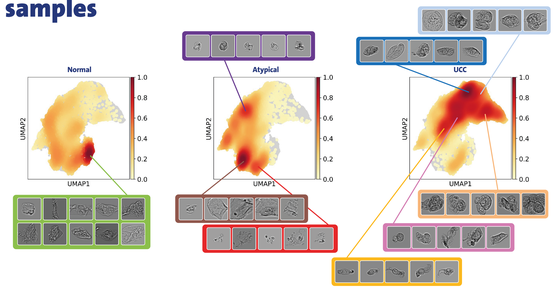


Figure 4. Morphology UMAPs showing high density region heatmap of corresponding conditions. Representative images of cells occupying the indicated UMAP space is shown.

High prediction accuracy for atypical versus UCC /SCC cases

Figure 5. Confusion matrix showing deep learning model performance in classifying atypical versus UCC. (n=8642), UCC (n=12637).

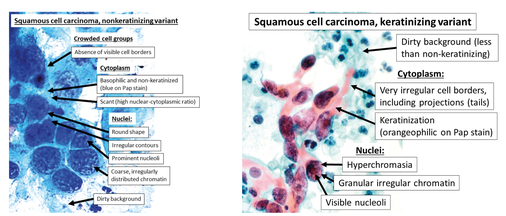


Figure 6. Cytonuclear differences between keratinizing and non-keratinizing SCC. UCC/SCC belongs to the non-keratinizing subgroup. (Images by Mikael Häggström, MD. Public Domain (CC0 1.0))

Molecular pathology

A gene frequently mutated in UCC is FGFR3. Figure 7a, generated through Omniviz software, shows the RNA expression profiles of the top 25 co-expressed transcripts with FGFR3 in 2158 solid tumors (GSE2109). These genes show high RNA expression (shown in red) in organs such as the bladder, cervix, lung and vulva. Using STRING database, these genes form a concise biological network linked to keratinization (Figure 7b). Moreover, the network includes OVOL1, which is involved in differentiation and maintenance of the urogenital system.

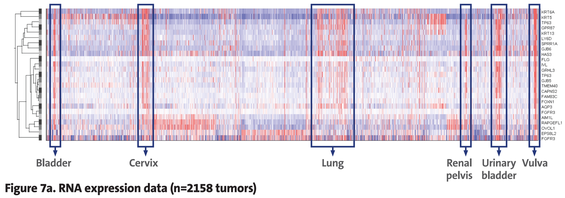


Figure 7a. RNA expression data (n=2158 tumors)

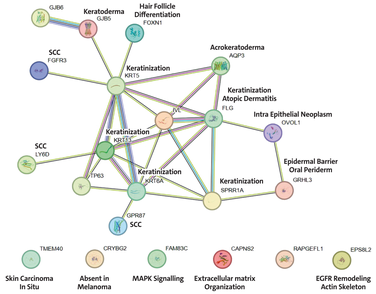


Figure 7b. Interaction network

Conclusions

Deepcell's REM-I platform identifies 12 distinct cell morphology clusters among normal and pathological urine samples.

AI-assisted high-dimensional morphology analysis distinguishes normal, atypical and bladder cancer cases with high accuracy at the single cell level for diagnostic purpose.

Changes in cell morphology are often linked to changes in transcription networks that drive differentiation related pathways, which is important for cancer detection and staging.

Erasmus MC
Universitair Medisch Centrum Rotterdam

Deepcell®