

## Using 3D High-Content Analysis and Epigenetic Phenotyping of Cells in the Characterization of Human Prostate Tissue Heterogeneity

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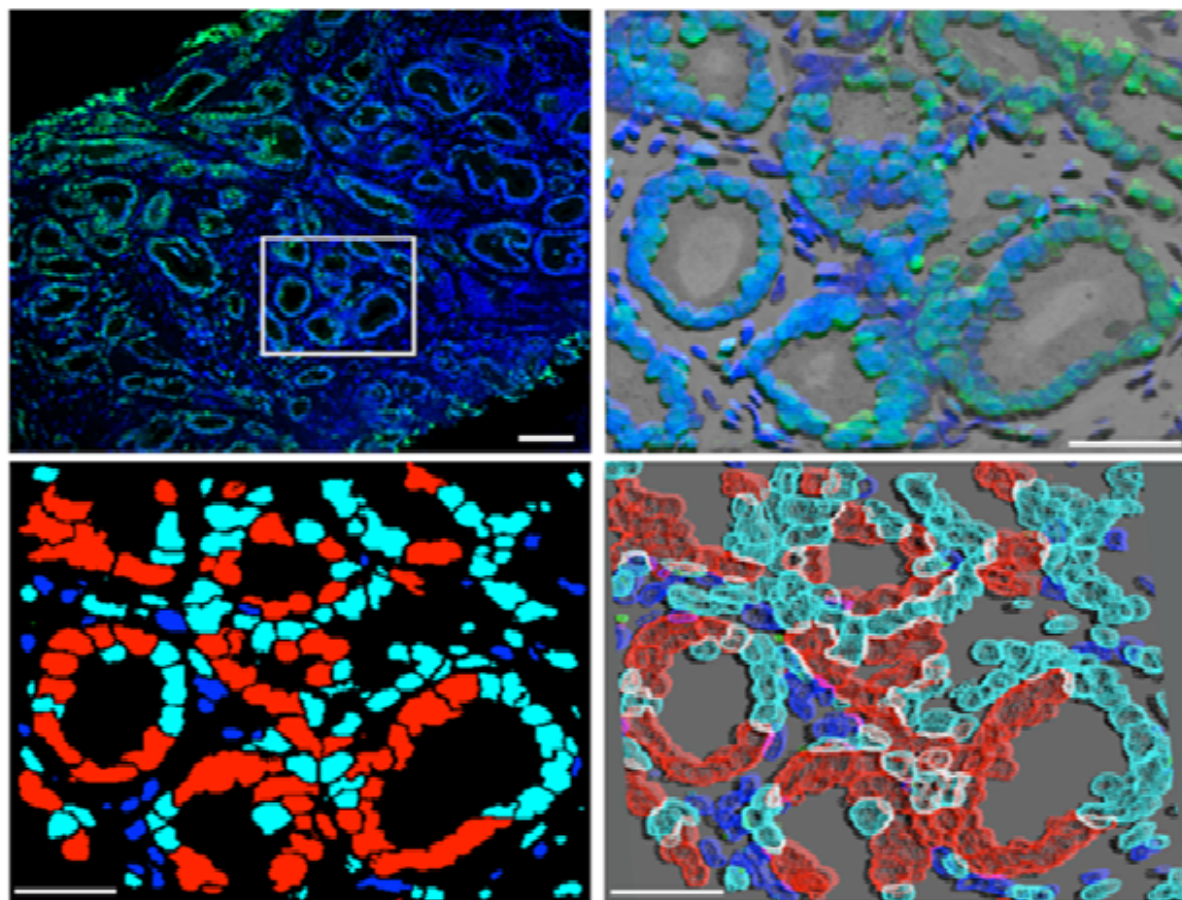
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### Clinical Image



Next-generation 3D high-content imaging and analysis enables the quantification and display of tissue heterogeneity regarding subcellular properties, and hence will improve the diagnostic characterization of biopsied tissues. **(Upper left)** Needle-biopsy section of human prostate adenocarcinoma, immuno-labeled for global DNA methylation (5methylcytosine, false-colored green) and counterstained for global nuclear DNA with 4',6-diamidino-2-phenylindole (DAPI, false-colored blue) and imaged with a 20X NA=0.7 lens. **(Upper right)** 3D reconstruction of a stack of confocal images taken from the boxed region (left sub-image with a 63X NA=1.3 lens, and processed using maximum intensity projection combined with shadow projection (CytoFx software, llucida LLC). **(Lower left)** Classification (phenotyping) of cells according to the levels of global nuclear DNA methylation using dedicated software; high (cyan) and mid levels (red) found predominantly in the epithelial-ductal cells, and low levels (blue) in stromal cells. **(Lower right)** 3D visualization of the classified heterogeneous cell population. Scale bars are 50  $\mu$ m.