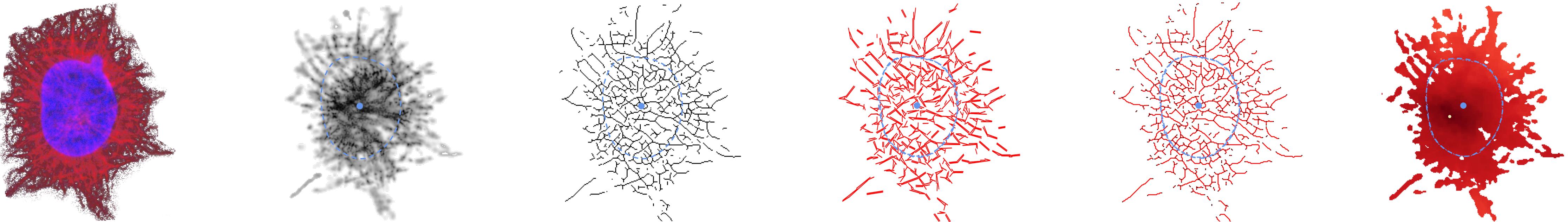


UNRAVELING THE CYTOSKELETAL ARCHITECTURE OF CANCER CELLS: DEVELOPMENT OF A NOVEL COMPUTATIONAL APPROACH



Authors

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BIOLOGICAL BACKGROUND - CYTOSKELETON

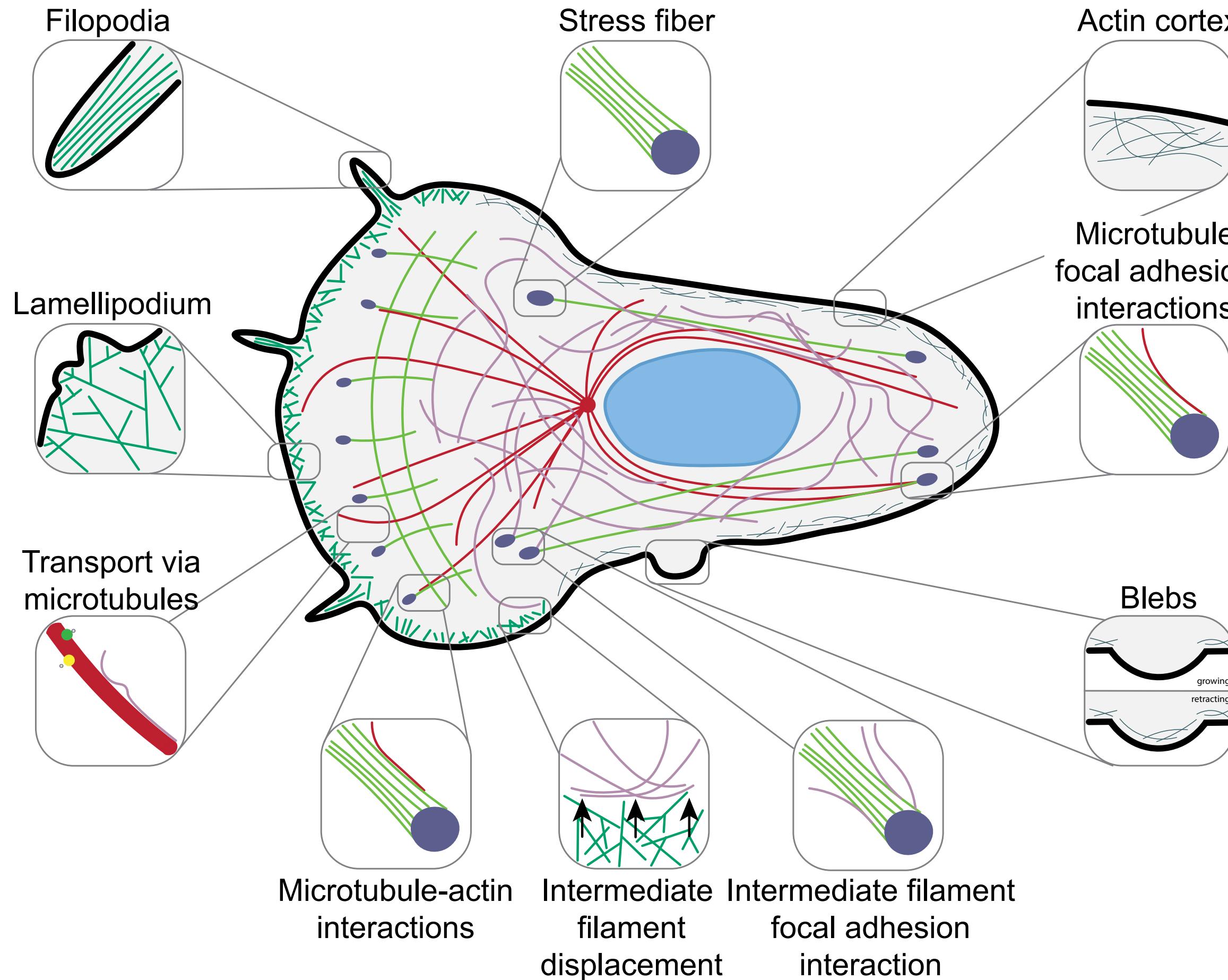


Figure 1: Proteins that form the cytoskeleton (actin, microtubules, and intermediate filaments) and their physical interactions form dynamic organizational structures inside the cell. Figure adapted from T. Hohmann and F. Dehghani, "The cytoskeleton—a complex interacting meshwork," Cells, vol. 8, no. 4, 2019.

During **cancer progression**, cells undergo **cytoskeleton re-organization** through the dynamic interplay of its components, including **microtubules**.

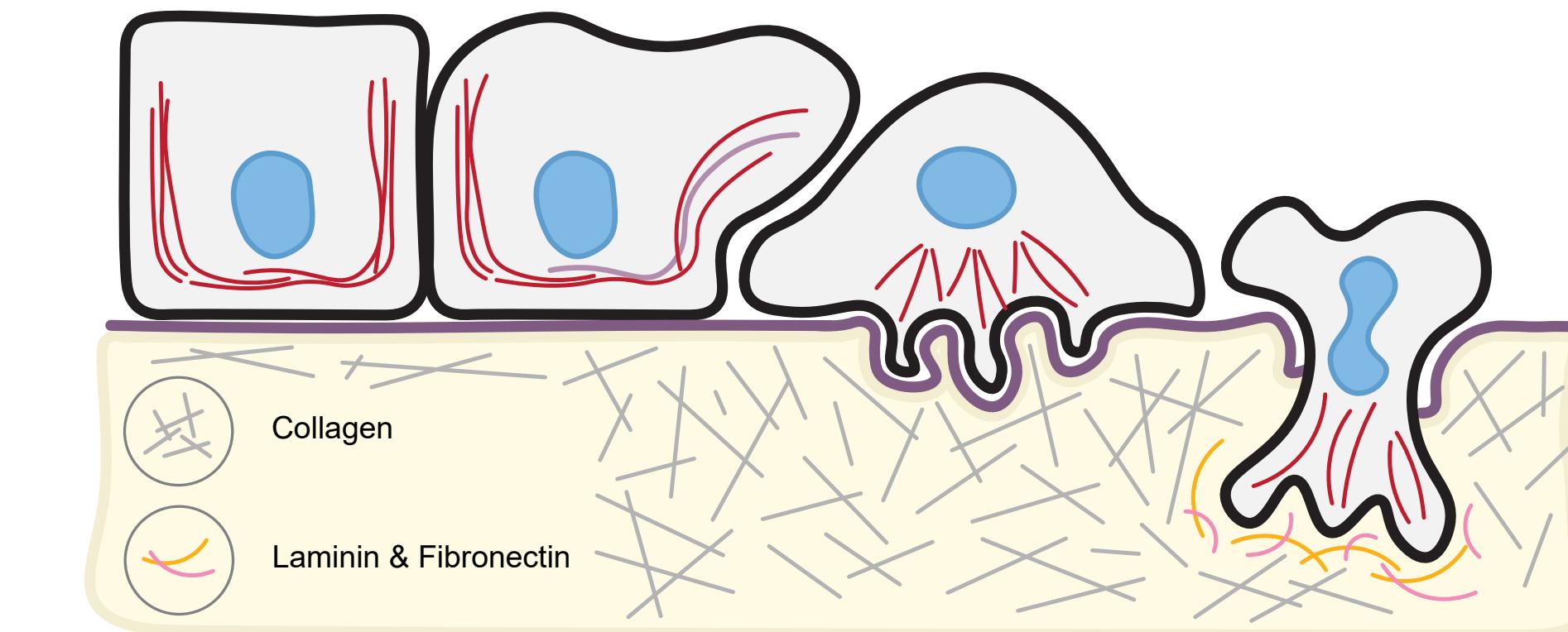


Figure 2: Representative image of normal and invading cells. While the cytoplasm, plasma membrane, and the majority of other small organelles may readily overcome constraints, the nucleus is the key limiting component due to its size and rigidity. Figure adapted from F. J. Calero-Cuenca, C. S. Janota, and E. R. Gomes, "Dealing with the nucleus during cell migration," Current Opinion in Cell Biology, vol. 50, pp. 35–41, 2018. and A. M. Moreira, J. Pereira, S. Melo, M. S. Fernandes, P. Carneiro, R. Seruca, and J. Figueiredo, "The extracellular matrix: An accomplice in gastric cancer development and progression," Cells, vol. 9, no. 2, 2021.

Legend

- | | | | | | |
|--|------------------------------|--|-----------------------------|--|------------------------------------|
| | Dendritic, bundled filaments | | Parallel, bundled filaments | | Antiparallel (contractile) bundles |
| | Cortex | | Focal adhesions | | Intermediate filaments |
| | | | | | Microtubules |

METHODOLOGY - IMAGE PROCESSING AND FEATURE EXTRACTION

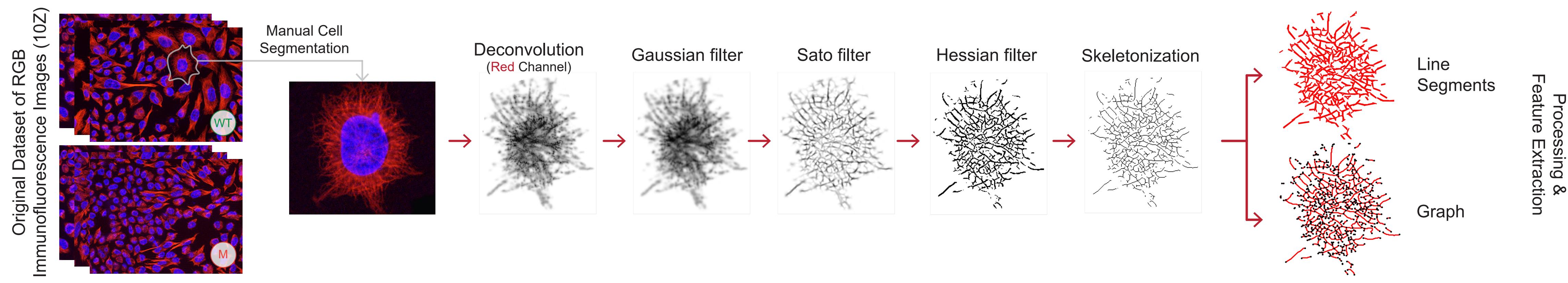


Figure 3: Overall pipeline to evaluate the cytoskeletal architecture of cancer cells. The processing pipeline involves the analysis of α -tubulin and DAPI immunofluorescent images. Preprocessing methods are subsequently applied to all the images for nuclei and cytoskeleton segmentation. After manual delimitation of cell boundaries, features are extracted and analyzed comparatively and globally.

RESULTS - CYTOSKELETON ORGANIZATION

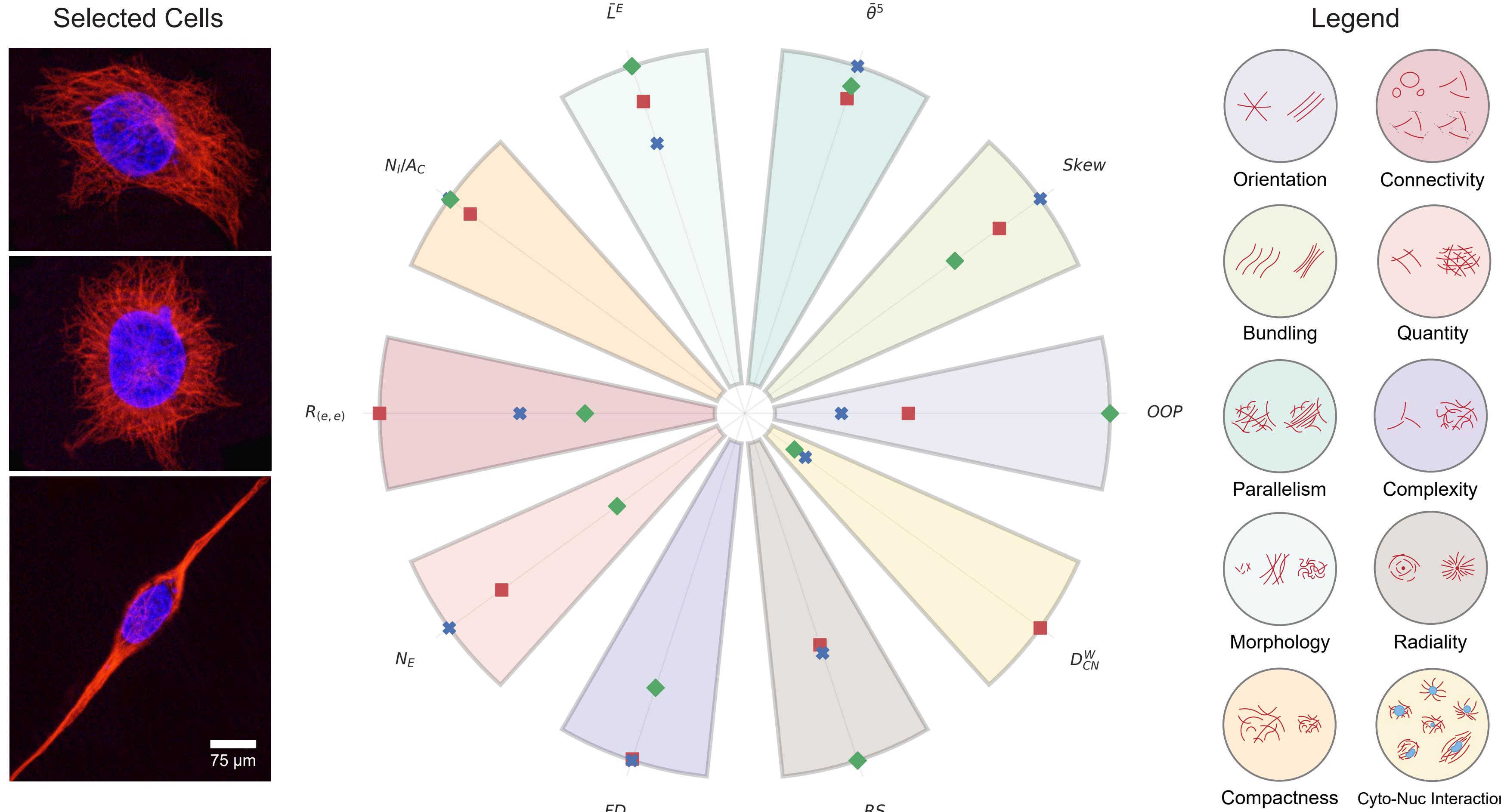


Figure 4: Partial cytoskeleton description and comparative analysis between three cells. Immunofluorescent images were obtained following α -tubulin (red) and DAPI (blue) staining.

RESULTS - GLOBAL ANALYSIS

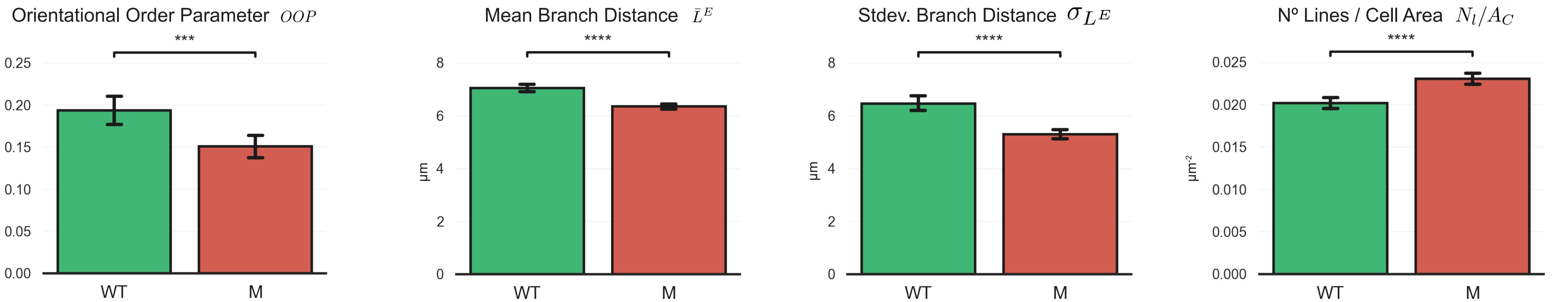


Figure 4: Quantification analysis of features related with cytoskeleton organization with statistically significant differences between groups. Bars represent the mean \pm 95% CI. ns, not significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$. WT, Wild Type; M, Mutant.

Microtubules of mutant cells (M) (invasive potential) were shown to have more **dispersed orientations**, to be **shorter**, to have **uniform length patterns** and to be more **compactly distributed**, compared to WT cells (without invasive potential).

FUTURE WORK

Methodology can
be applied

to other
cytoskeletal proteins

to morphologically
similar structures

in time-dependent studies or ML approaches, to assess the evolution of each
architecture aspect and make predictions that could help early diagnosis

actin
intermediate
filaments
microtubules

blood vessels
neuronal networks

from any cell type
(animal, vegetal, ...)

to **unravel** not only the cytoskeletal architecture of cancer cells,
but also many other vital cellular processes and mechanisms!