Integrative analysis of nuclear biomechanics by AI-enhanced quantitative phase microscopy in CAL27 oral carcinoma cells

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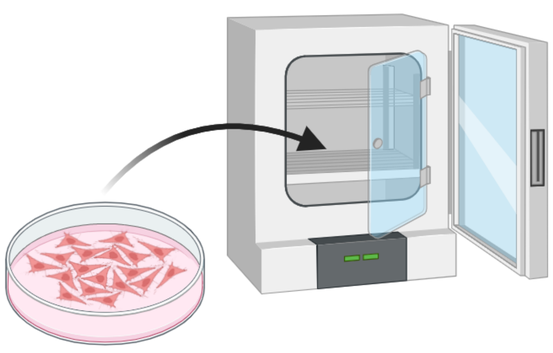
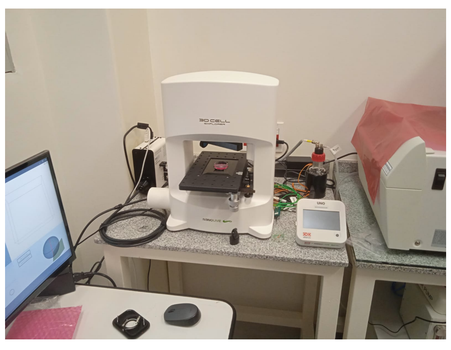
Introduction

The dynamics of the cell nucleus reflect important aspects of the internal organization and the pathophysiological state of cells. In this work, we built an artificial intelligence-enhanced approach for the quantitative analysis of nuclear rotation in CAL27 oral carcinoma cells cultured under different experimental conditions. Using label-free quantitative phase microscopy, we performed a time-lapse tracking of individual nuclei to compare rotational dynamics under stimuli such as hypoxia, chemotherapy (cisplatin treatment), and rotenone.

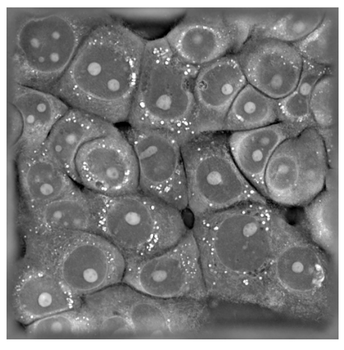
Materials and Methods



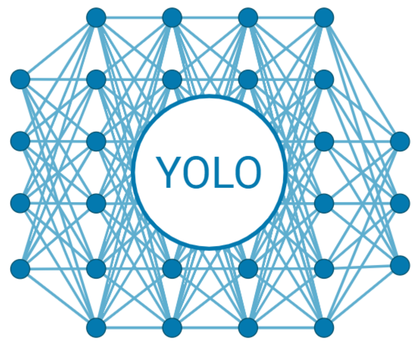
CAL27 cells were cultured in high-glucose DMEM medium at 37 °C, 5% CO_2, and seeded at high confluence.



Time-lapse images were acquired with a Nanolive 3D Cell Explorer microscope for 48 hours at one minute intervals.



Nuclei were modelled as two-dimensional objects, with the refractive index (RI) distribution treated as analogous to a mass distribution.



A YOLOv8n-seg deep learning model (Ultralytics v8.3.94) was trained in an environment with Python 3.11, PyTorch 2.6, and a Tesla T4 GPU with CUDA support. Training was performed for 100 epochs with a batch size of 8, using 512×512 pixel images. Datasets consisted of 38 training and 7 validation images defined in a custom YAML file with segmentation labels. The model was initialized with pretrained weights.

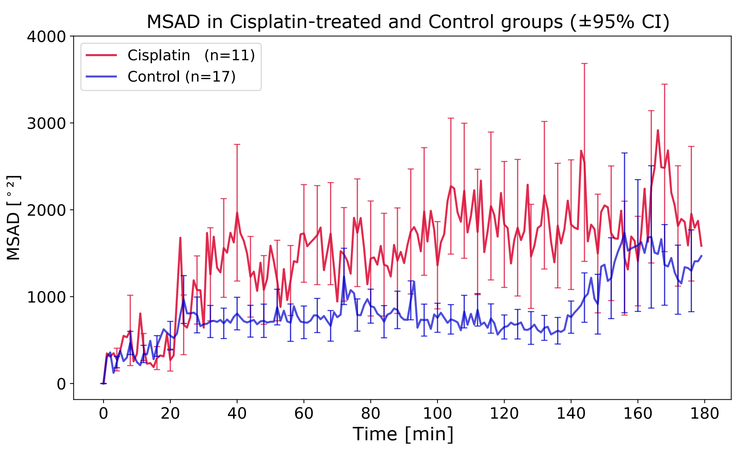
$$I = \begin{bmatrix} I_{xx} & I_{xy} \\ I_{xy} & I_{yy} \end{bmatrix}$$

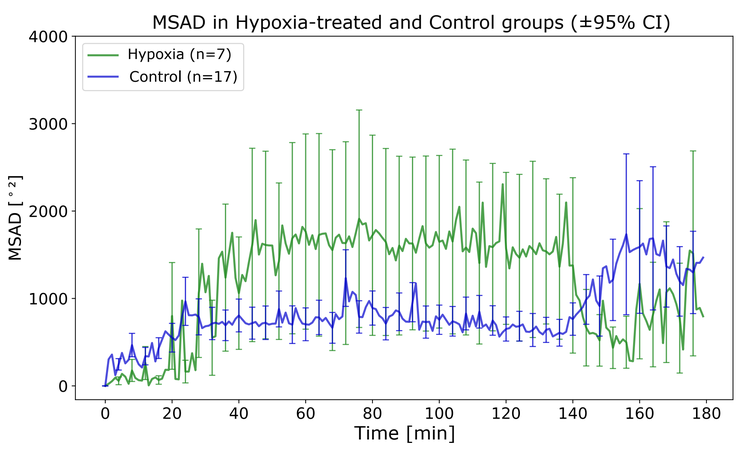
For each nucleus, the inertia tensor was calculated, and the resulting eigenvectors were used to estimate nuclear orientation over time.

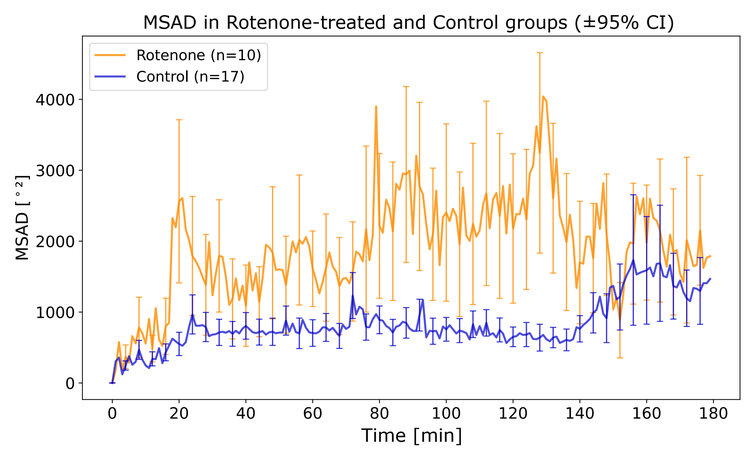
$$MSAD(k) = \frac{1}{N-k} \sum_{i=1}^{N-k} (\theta_{i+k} - \theta_i)^2$$

From this information, metrics such as the mean squared angular displacement (MSAD) were derived. MSAD is the rotational analogue of the mean squared displacement. It quantifies how much, on average, the orientation of a nucleus changes after a time lag *k*.

Results and Discussion







All treatments increased the MSAD over time compared with untreated cells, with the strongest effect observed under rotenone. This findings suggest a disruption of cytoskeletal structures responsible for maintaining nuclear orientation, possibly reflecting the loss of nuclear anchoring or a mechanical response to stress. Future analysis will incorporate 3D Nanolive data to capture volumetric nuclear features and evaluate dynamics with greater precision. This approach may uncover novel biophysical markers relevant to carcinogenesis.

References:

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