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INFLUX

MECHANOBIOLOGY & SOFT MATTER *group*

Neuroblastoma cell motility in tight spaces: Live imaging of chromatin distribution in deformed nuclei

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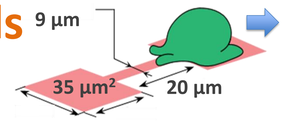
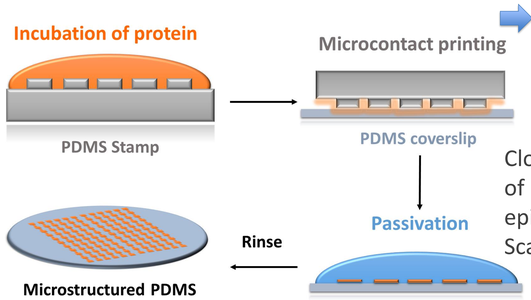
Neuroblastoma is the third-most common cancer in children after leukemia and brain cancer. Metastases arise from deficient cell populations able to migrate and squeeze their nucleus through complex microenvironments. Despite recent advances, the effect of spatial confinement on neuroblastoma cell migration (SH-SY5Y) and the nuclear mechanical response remains elusive. In this context, we developed original experimental microsystems for studying Neuroblastoma migration combining 2D adhesive micro-stripes and a fluorescence ratiometric imaging technique of chromatin (FRIC). Our results highlight the importance of the spatial distribution of adhesive sites in the modulation of the migration speed and validate the FRIC technique for studying the spatiotemporal organization of chromatin during migration.

2D adhesive micro-dumbbells

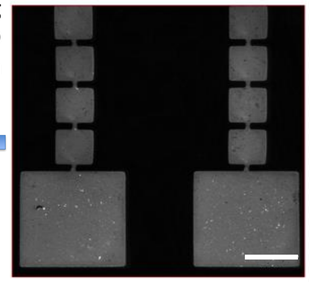
Microcontact printing



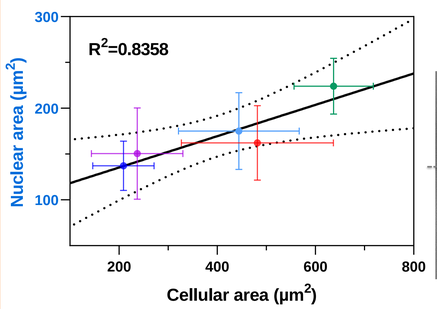
Schematic view of a cell on a micro dumbbell [1].



Microcontact printing allows to create 2D adhesive patterns with micrometric resolution[1].

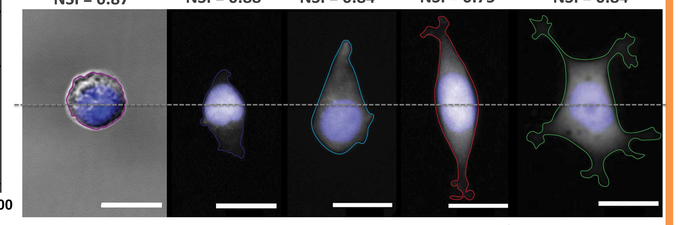


Close up image of a field of micropatterns in epifluorescence mode. Scale bar = 50 µm



Nuclear area increases linearly with the cell area.

Fibronectin	Fn +PLL	Laminin	Lam +PLL	Collagen I
CSI = 0.87	CSI = 0.55	CSI = 0.50	CSI = 0.25	CSI = 0.34
NSI = 0.87	NSI = 0.88	NSI = 0.84	NSI = 0.75	NSI = 0.84



Symmetry breaking events on collagen drive SH-SY5Y cell migration. Scale bar = 20 µm

Cell area

Asymmetry

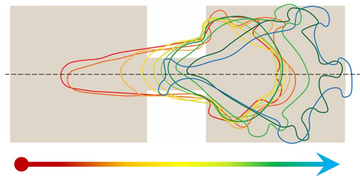
Conclusions and prospects

- Micro-dumbbells confine cells and orient their migration.
- Under 2D spatial confinement a transient increase in migration speed was observed.
- FRIC confirms that heterochromatin preferentially localizes in the nuclear periphery.
- Heterochromatin density is linked to nuclear deformation (NSI).

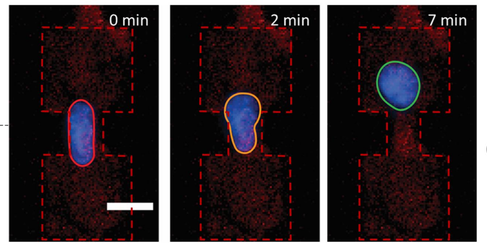
Taken together, these results point the way towards original strategies to study neuroblastoma cells and other cancer cell types in the context of cell migration

Cell migration under 2D spatial confinement

Time Lapse



Spatio-temporal evolution of an outlined SH-SY5Y cell migrating on a pattern.



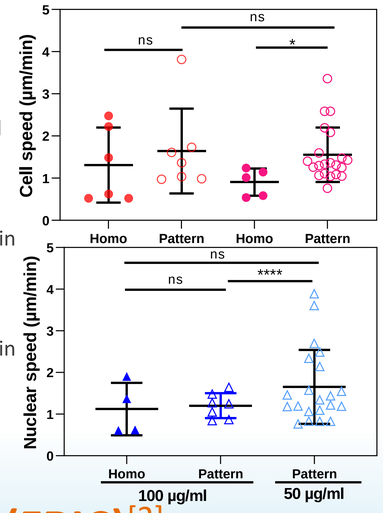
Adhesive Rear → **Rear Release**

Lateral compression of the nucleus exerted by stress fibers is released once the bridge is crossed by the cell. Scale bar = 20 µm

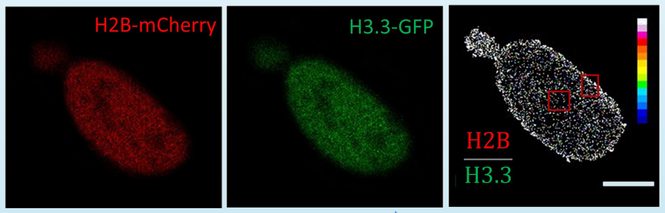
Migration speed increase

From (Homo) 0.90 ± 0.32 µm/min

To (Pattern) 1.55 ± 0.64 µm/min

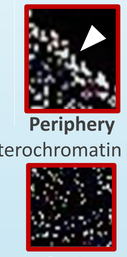


Fluorescence Ratiometric Imaging of Chromatin (FRIC)[2]

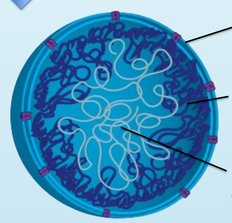


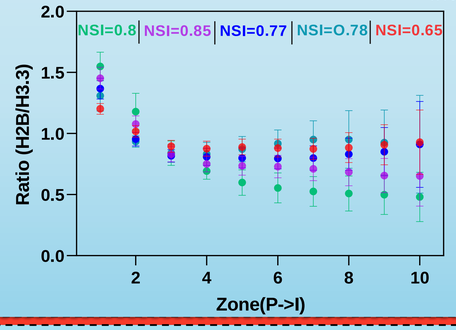
H2B and H3.3 histones stained with mCherry and GFP markers

Heterochromatin spatial distribution color coded for intensity. Scale bar = 5 µm



Schematic view of a nucleus

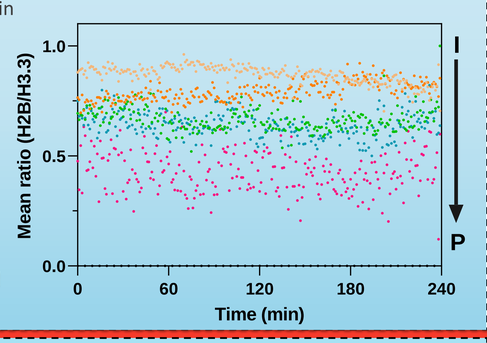




Nuclei are divided into 10 concentric zones. The ratio decrease from the periphery (P) to the interior (I) indicates an enrichment in heterochromatin.

Nuclear shape index values suggest that heterochromatin density is linked to nuclear deformation.

Heterochromatin amount is stable over time within the different zones



[1] Mohammed et al. Substrate area confinement is a key determinant of cell velocity in collective migration. Nature Physics (2019)

[2] Bergqvist et al. Monitoring of chromatin organization in live cells by FRIC. Nucleic acids research (2019)

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