Nuclear positioning and its translation dynamics is regulated by cell geometry

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INTRODUCTION

- The cytoplasm is a complex dynamic environment that is characterized by collective activities of several motor proteins and other active processes such as cytoskeletal re-organisation.
- These forces influence positioning and intracellular dynamics of various organelles in the cytoplasm and thereby, create unique biophysical signatures, which are altered in many diseases.

OBJECTIVE

To characterize the micro-rheological properties of altered intracellular environments using the nucleus, a cell organelle, as a probe particle.

APPROACH

I: Confine NIH3T3 fibroblasts cells expressing fluorescently labelled H2B which marks the nucleus, to a defined geometry using micro-patterned substrates to generate distinct cytoskeletal environments.

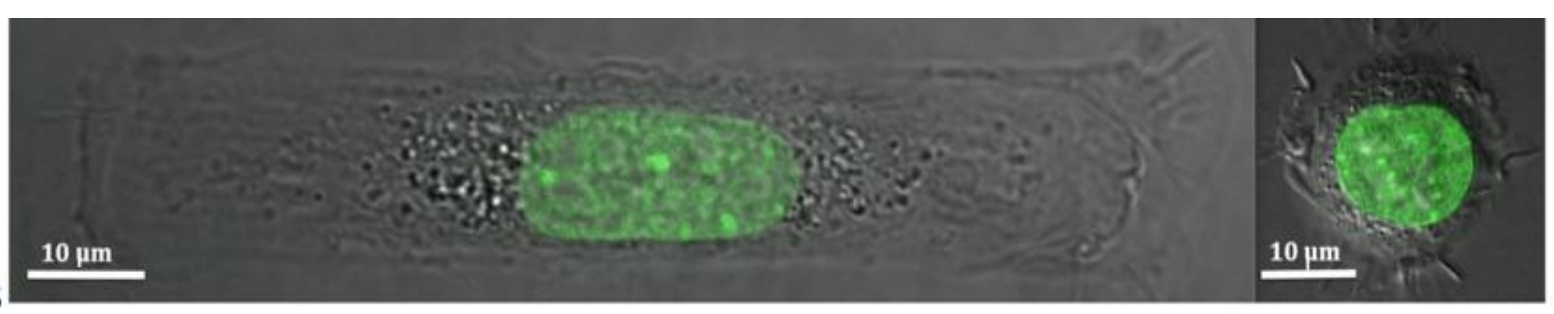
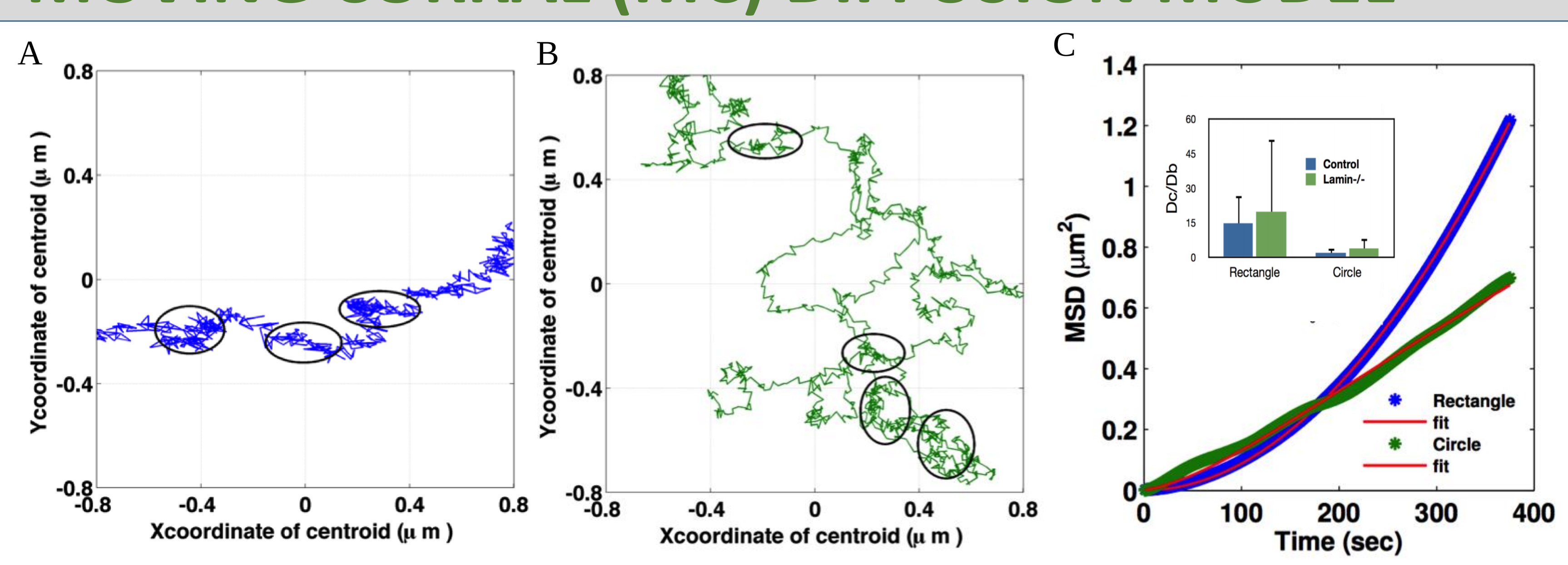


Figure 1. NIH 3T3 cells expressing H2B-EGFP constrained on rectangular and circular geometries.

II: Analyse nuclear position and translational dynamics and study the sensitivity of its diffusive behaviour to cell geometry, nuclear rigidity, and TNFα cytokine stimulation

MOVING CORRAL (MC) DIFFUSION MODEL



In rectangular geometry the nucleus exhibits a strong corralled nature with subdiffusive motion in the corral.

Figure 5. A, B) Mean shifted nuclear centroid trajectories show defined corralled structures in rectangular geometry (A) compared to circular geometry (B). C) The MC model was fitted (red) to MSD curves for nuclei in rectangular (blue) and circular geometry (green) inset shows Dc/Db values.

CONCLUSION

Molecular links between perinuclear ASFs and nuclear envelope act as cables that direct the mobility of nucleus in rectangular cells and the lateral actin network confines the nucleus at shorter time scales. Whereas in circular cells, lower actin polymerization states and lower levels of laminA/C[1] lead to a highly dynamic nucleus.

Nuclear positioning dynamics is very sensitive to both the internal and external microenvironment of the cell.

Importantly, these results provide sensitive biophysical signatures for detecting cellular abnormalities using the nucleus as a probe particle.

REFERENCE

1.Ekta Makhija, D. S. Jokhun and G. V. Shivashankar, PNAS (2015)

ACKNOWLEDGEMENTS

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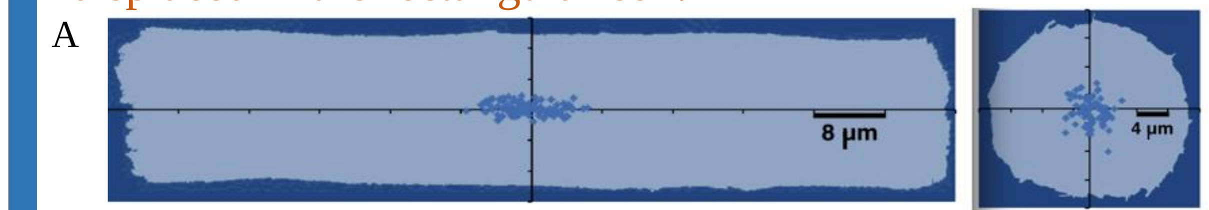
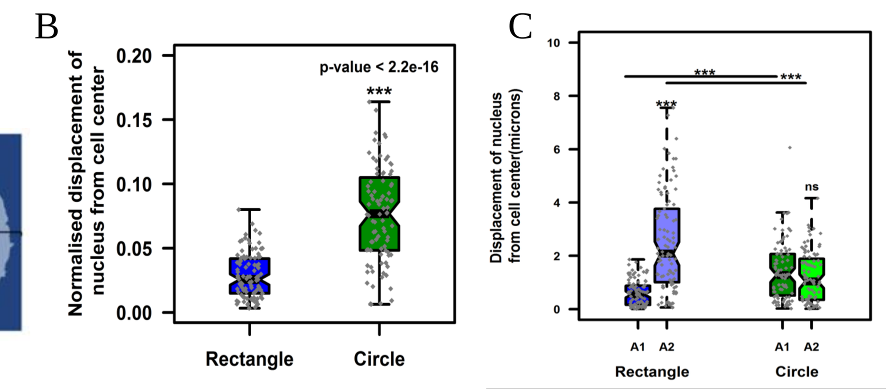
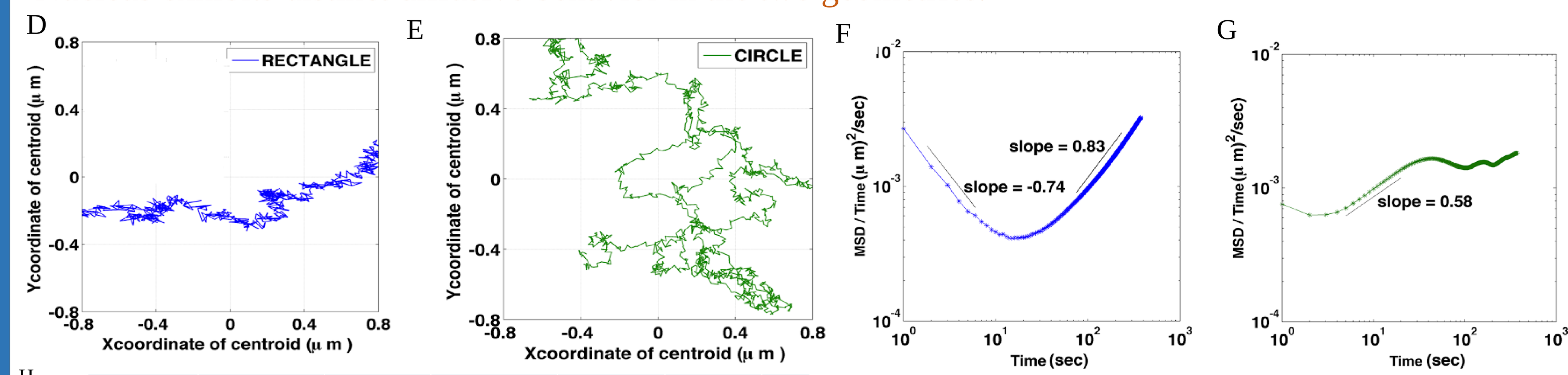
NUCLEAR POSITIONING AND DYNAMICS ARE SENSITIVE TO CELLULAR GEOMETRY

Nucleus is centrally positioned and anisotropically displaced in the rectangular cell.





Nucleus exhibits distinct diffusive behavior in the two geometries.



H

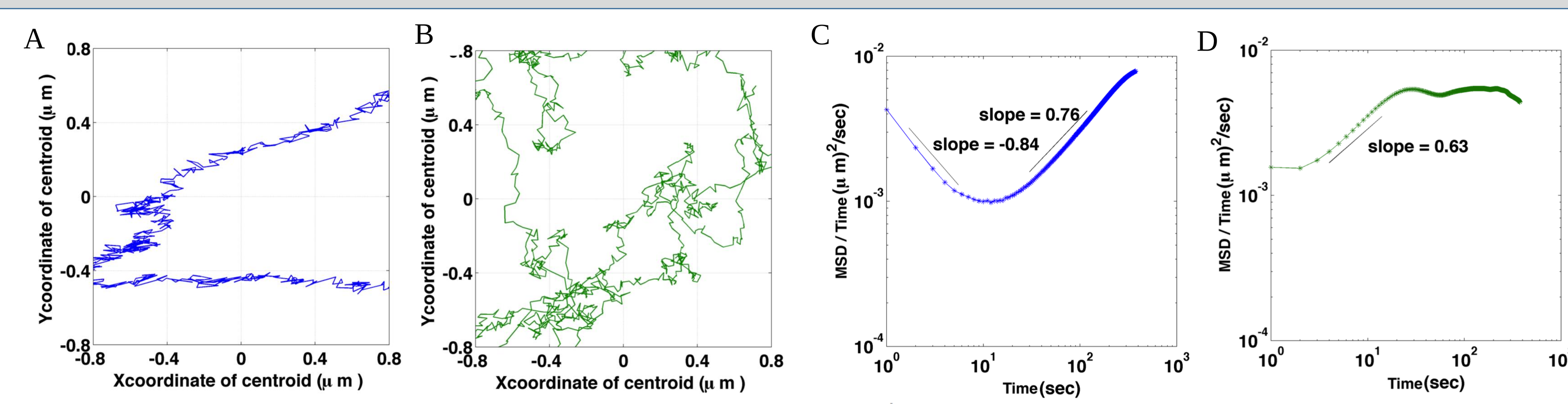
Geometry	Region 1:A1(τ) µm2/sec	α1	Region 2: A2(τ) µm2/sec	α2	τ crossover (sec)	n
Rectangle	1.5 × 10-3 − 2.6 × 10-4	0.26 ± 0.17	2.6 × 10-4 − 2.1 × 10-3	1.66 ± 0.17	18.6±14.6	19
Circle	-	-	6.4 × 10-4 − 2.2 × 10-3	1.54 ± 0.12	2.0±1.1	17

Rectangular cell nucleus exhibits two different regimes: a subdiffusive motion in shorter timescales and crosses over to superdiffusive motion

Circular cell nucleus exhibits superdiffusive behavior which crosses over to diffusive motion at larger timescales.

Figure 2. A) Distribution of nuclear centroid from the cell centroid (the origin) in constrained geometries B) Normalized displacement of nucleus in rectangles C) Displacement of nuclear centroid along the short (A1) and long (A2) axis of the cell. The mean shifted trajectory of the nuclear centroid in rectangular geometry (D) and circular geometry (E). F,G)Plots of < r2(τ) >/ τ as a function of τ for the rectangular and circular geometry. (H) Table containing diffusion parameters

NUCLEAR DIFFUSION IS SENSITIVE TO LAMIN A/C LEVELS



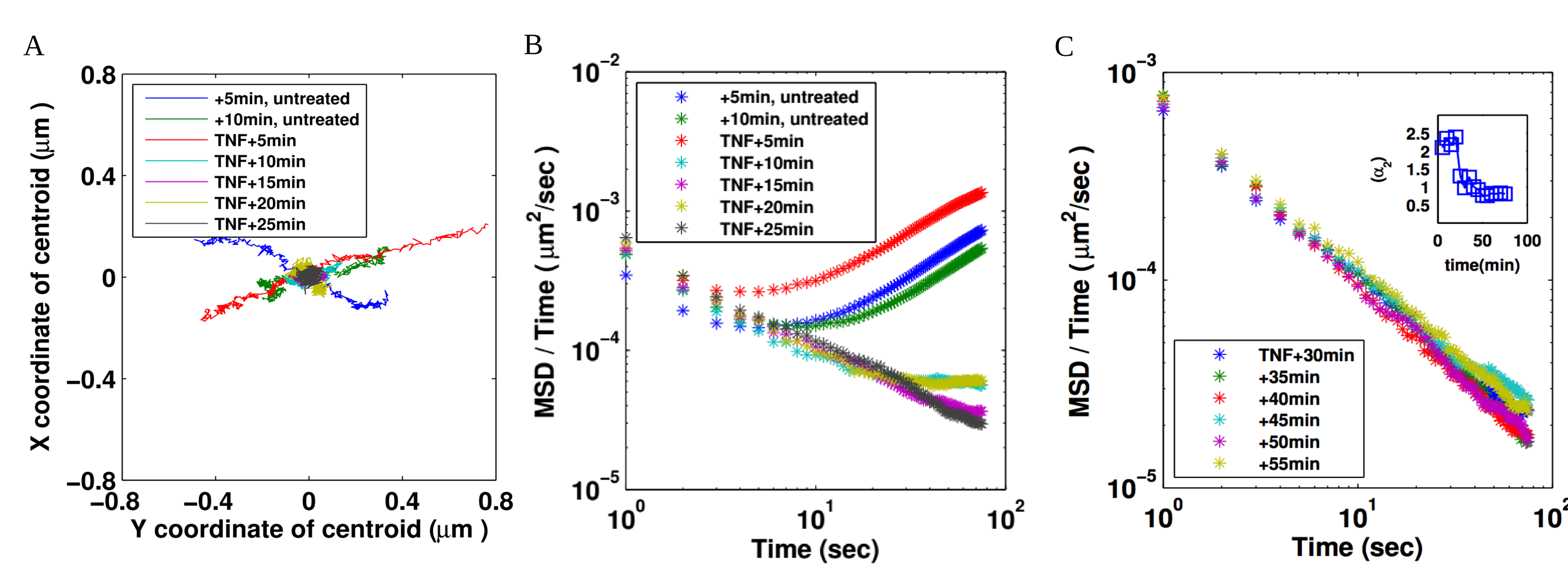
E

Geometry	Region 1:A1(τ) µm2/sec	α1	Region 2: A2(τ) µm2/sec	α2	τ crossover (sec)	n
Rectangle LaminA/C− /−	8.0 × 10-3 − 1.9 × 10-3	0.25 ± 0.06	2.0 × 10-3 − 7.5 × 10-3	1.5 ± 0.22	0.4 ± 3.8	5
Circle LaminA/C− /−	-	-	3.6 × 10-3 − 9.9 × 10-3	1.55 ± 0.12	3.5 ± 2.4	4

Nuclear diffusive behavior in lamin A/C deficient cells was similar to that observed in wild type cells on corresponding geometry. However, the lamin deficient nuclei explored larger regions.

Figure 3. The mean shifted trajectory of laminA/C deficient cells's nuclear centroid in rectangular geometry (A) and circular geometry (B). C,D)Plots of < r2(τ) >/ τ as a function of τ for the rectangular and circular geometry. (E) Table containing diffusion parameters

NUCLEAR DYNAMICS IS SENSITIVE TO CYTOKINE TNFα STIMULATION



The nucleus after TNFα stimulation shows a clear time dependent change in diffusive behavior and reaches a steady sub-diffusive motion after 30mins of treatment.

Figure 4. A) The mean shifted nuclear centroid trajectories in rectangular cells before and during TNFα treatment is presented at 5 minute intervals. B) Corresponding < r2(τ) >/ τ as a function of τ showing the transition from superdiffusive to subdiffusive motion over the first 25 minutes of treatment. C) Stabilized subdiffusive nuclear motion for the next 30 minutes. The inset shows α2 values over the time course of the experiment.

Mean square displacement (MSD) is given by $< r^2(\tau) >= \frac{1}{N-n} \sum_{k=1}^{N-n} [r((k-1)\Delta t + n\Delta t) - r((k-1)\Delta t)]^2$. Here r is the position vector of the particle at each time point(t) and N is the total number of measured points.
Time dependent diffusion coefficient A(τ) is given by $< r2(\tau) > / \tau = A\tau\alpha-1$
MCmodel: $< r^2(\tau) > = < r_c^2 >. \left(1 + \frac{4D_c\tau}{<r_c^2>}\right)\left[1 - \exp\left(\frac{-4D_b\tau}{<r_c^2>}\right)\right]$ where rc is the corral radius & Dc is the diffusion of the corral and Db is the diffusion in the corral

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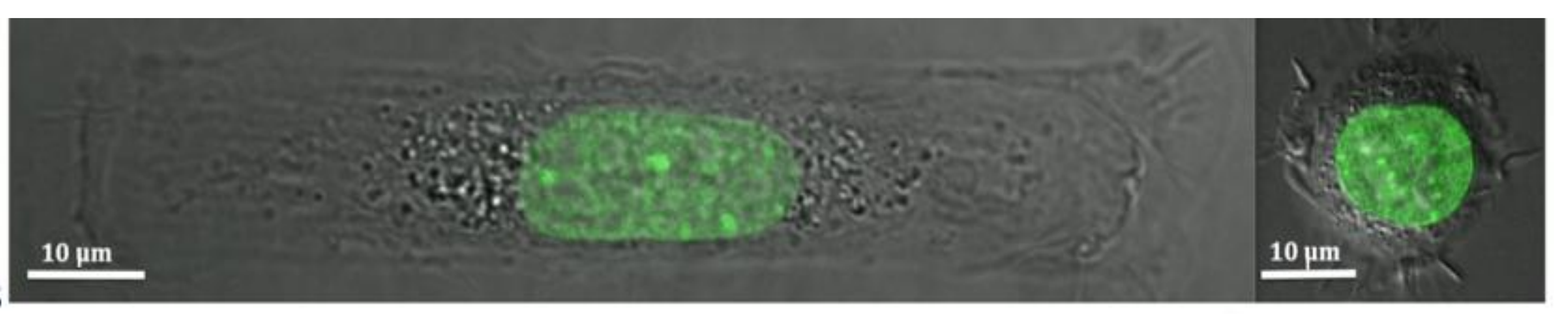

Figure 1. NIH 3T3 cells expressing H2B-EGFP constrained on rectangular and circular geometries.

II: Analysed nuclear position and translational dynamics and studied the sensitivity of its diffusive behaviour to cell geometry, nuclear rigidity, and TNFα cytokine stimulation

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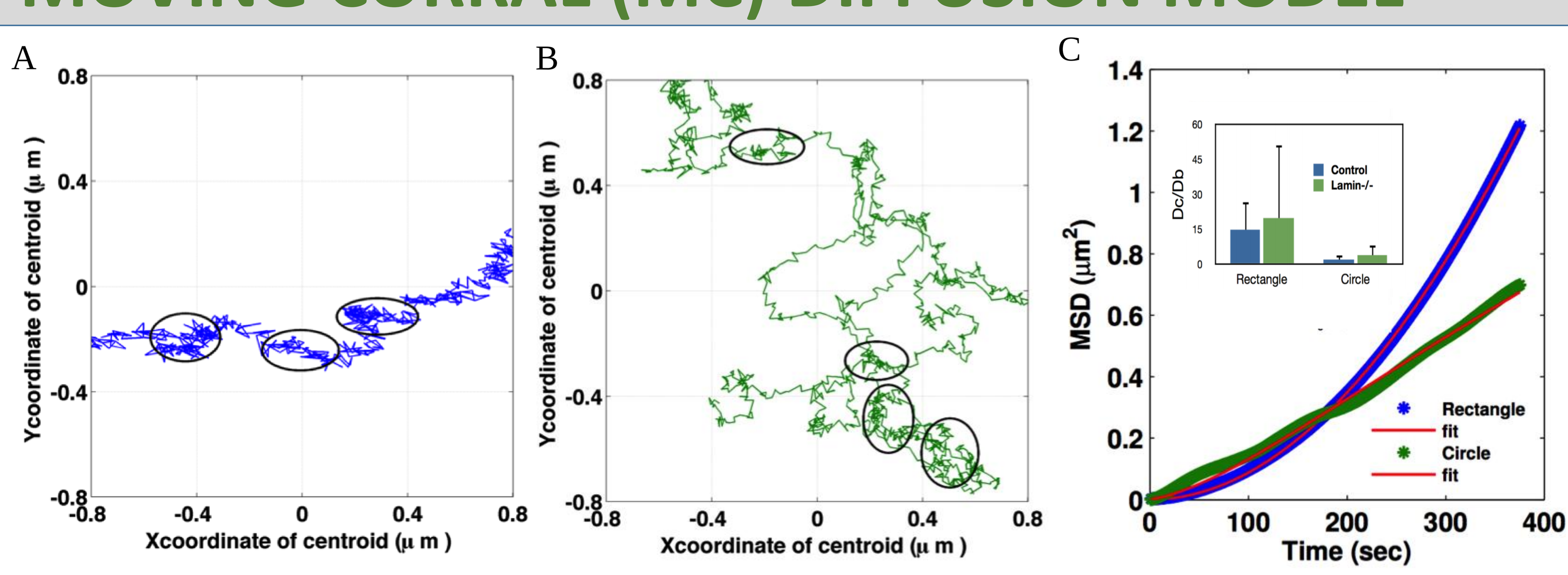

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CONCLUSIONS

Molecular links between perinuclear ASFs and nuclear envelope act as cables that direct the mobility of nucleus in rectangular cells and the lateral actin network confines the nucleus at shorter time scales.

Precise nuclear positioning is important for many cellular functions. In adherent immobile fibroblast cells, the central positioning of the nucleus evinces the presence of an intact mechanical homeostasis. Many diseases are characterized by alterations in elements of nuclear lamina and cytoskeleton leading to destabilization of this mechanical homeostasis. In this context, our results show that nuclear positioning dynamics is very sensitive to both the internal and external microenvironment of the cell. Importantly, these results provide sensitive biophysical signatures for detecting cellular abnormalities using the nucleus as a probe particle.

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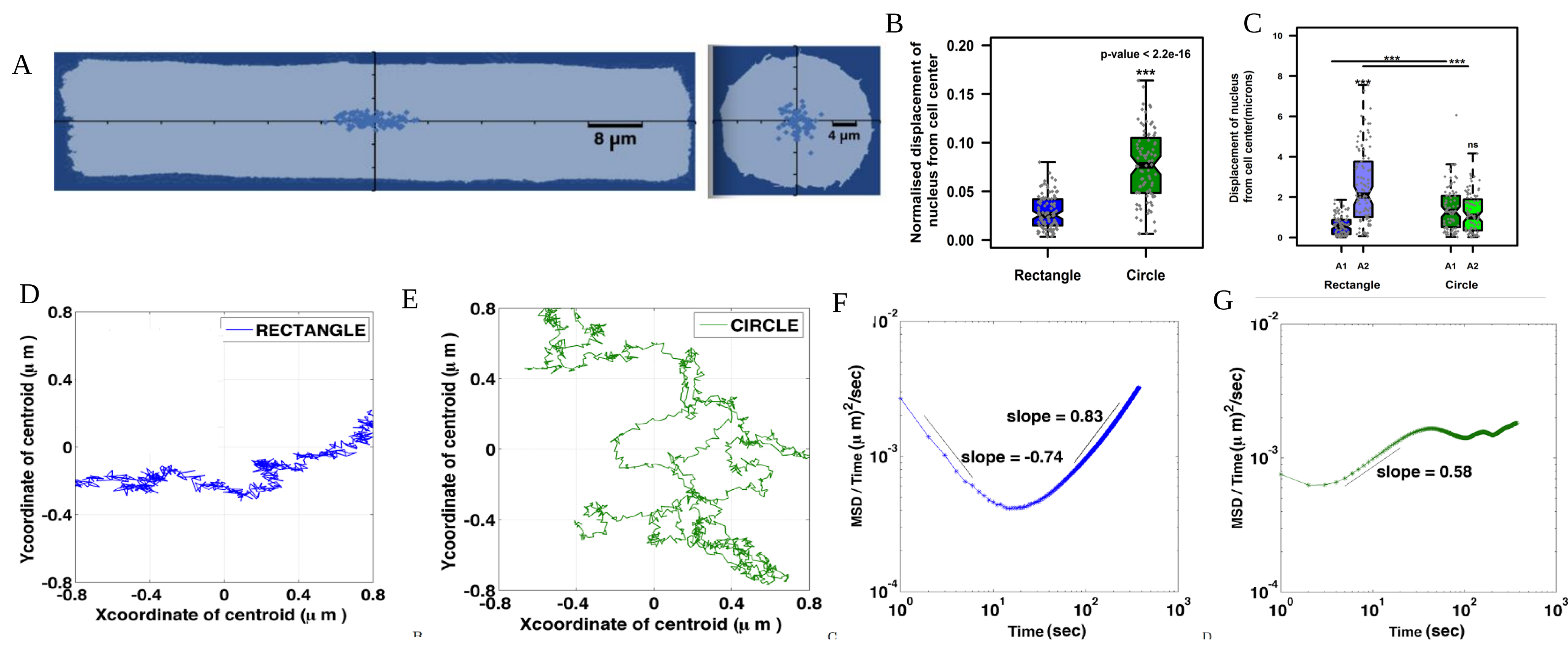
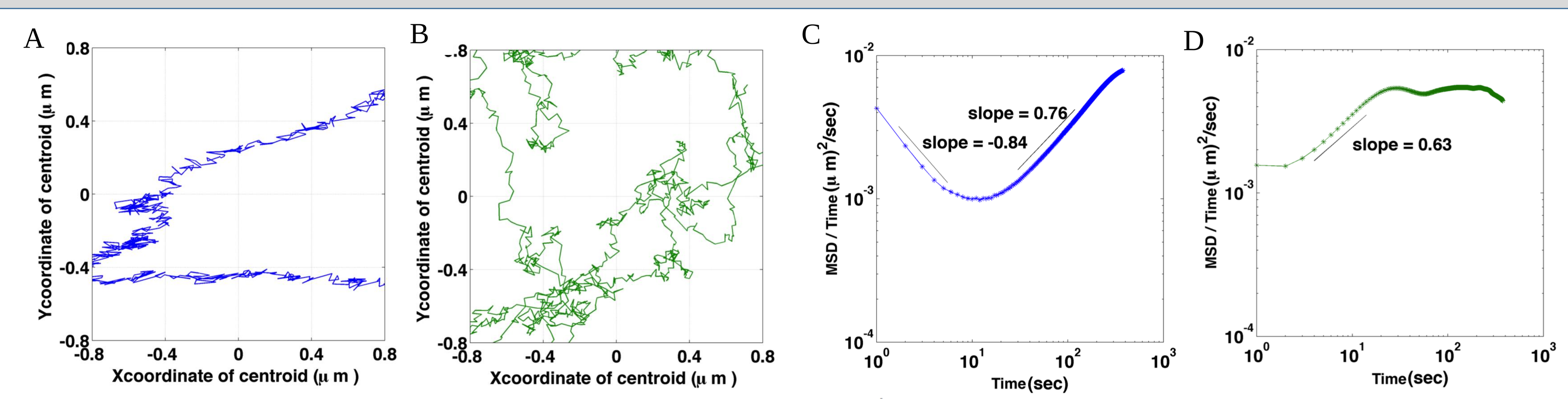
NUCLEAR POSITIONING AND DYNAMICS ARE SENSITIVE TO CELLULAR GEOMETRY



Nucleus is centrally confined the rectangular cell and exhibits two different regimes: a subdiffusive motion in shorter timescales and crosses over to superdiffusive motion. Circular cell nucleus exhibits superdiffusive behavior which crosses over to diffusive motion at larger timescales The absence of perinuclear actin cables in circular cells leads to an isotropic distribution of forces resulting in isotropic displacement of nucleus from the cell center in these cells.

Figure 2. A) Distribution of nuclear centroid from the cell centroid (the origin) in constrained geometries B) Normalized displacement of nucleus in rectangles C) Displacement of nuclear centroid along the short (A1) and long (A2) axis of the cell. The mean shifted trajectory of the nuclear centroid in rectangular geometry (D) and circular geometry (E). F,G)Plots of < r2(τ) >/ τ as a function of τ for the rectangular and circular geometry. (H) Table containing diffusion parameters

NUCLEAR DIFFUSION IS SENSITIVE TO NUCLEAR RIGIDITY



When the molecular links between the CSF and nuclear envelope is disturbed by knocking out laminA/C in rectangular cells, there is loss of friction that previously restricted the nuclear dynamics leading to nuclei having higher diffusion constants. Whereas in circular cells, lower actin polymerization states and lower levels of laminA/C[1] lead to a highly dynamic nucleus.

Figure 3. The mean shifted trajectory of Lamin deficient cells's nuclear centroid in rectangular geometry (A) and circular geometry (B). C,D)Plots of < r2(τ) >/ τ as a function of τ for the rectangular and circular geometry. (E) Table containing diffusion parameters

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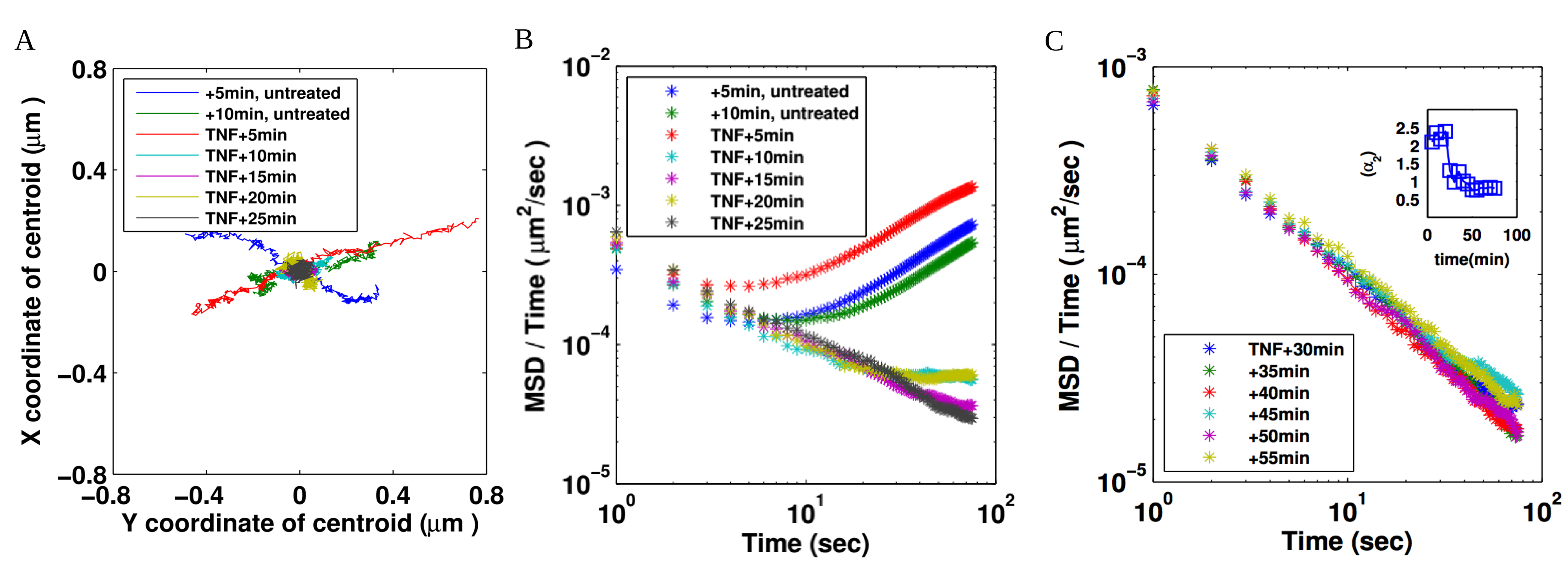

Following TNFα stimulation which can alter actin polymerization states, rectangular cells initially explores more space and then settles down to a subdiffusive motion

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