Analysis of membrane Piezo1 kinetics using GenEPi - A new tool for non-invasive imaging of Piezo1 activity

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Introduction

- **Piezo1** is an essential mechanosensitive ion channel and plays a critical role in many physiological processes [1]
- **GenEPi** is a genetically encoded fluorescent reporter of Piezo1-dependent activity which can be used accross scales [2]
- Total Internal Reflection Fluorescence Microscopy (**TIRFM**) is a powerfull tool to study Piezo1 membrane dynamics [3]

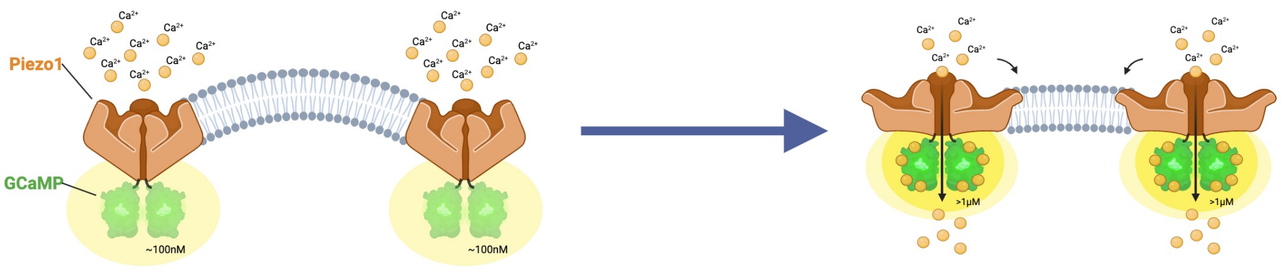


Fig. 1: GenEPi working principle.

Objective

Evaluate the localisation dynamics of Piezo1 using TIRFM imaging of GenEPi and subsequent MSD and JDD analysis

Conclusions

- Both MSD and JDD analyses suggest that the Piezo1 channel show anomalous diffusion
- Piezo1 channel displays an apparent diffusion coefficient of ~$4 \bullet 10^{-4}$ $\mu m^2/s$, in the range of previously reported values for wt-Piezo1
- Although MSD is more widely used to study diffusion dymanics, JDD can visualize whole and subpopulation motion distributions in different time lags

Relevance

- The analysis of diffusion at the molecular level for the interpretation of Piezo1 channel's localisation dynamics
- The algorithms used are Mean Squared Displacement (MSD) and Jump Distance Displacement (JDD) [4]

Analysis Workflow

Raw TIRFM images

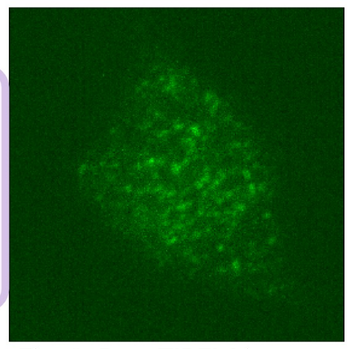


Fig. 2: Raw TIRFM images.

Denoising
Difference of Gaussians and Median filters (Fig. 3)

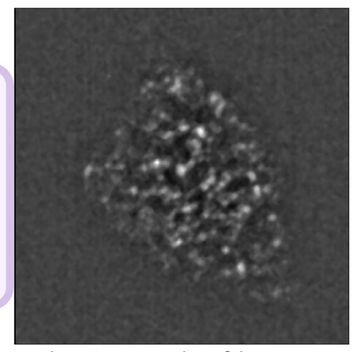


Fig. 3: Denoised images.

Segmentation
Otsu threshold and small object remover (Fig. 4)

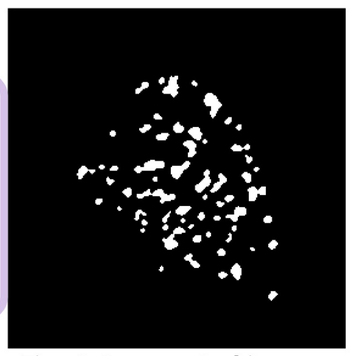


Fig. 4: Segmented images.

Tracking
Labelling and first moment of area (centroid localisation) (Fig. 5)

Fig. 5: Piezo1 centroids images.

MSD - Calculated individual and average MSD, and fitted functions with Non-Linear Least Squares (NLS) method (Fig. 6)

JDD - Calculated Eucledian Distances and JDD for each type of diffusion. Found the best diffusion mode by Parameter Fitting (Fig. 6)

Results

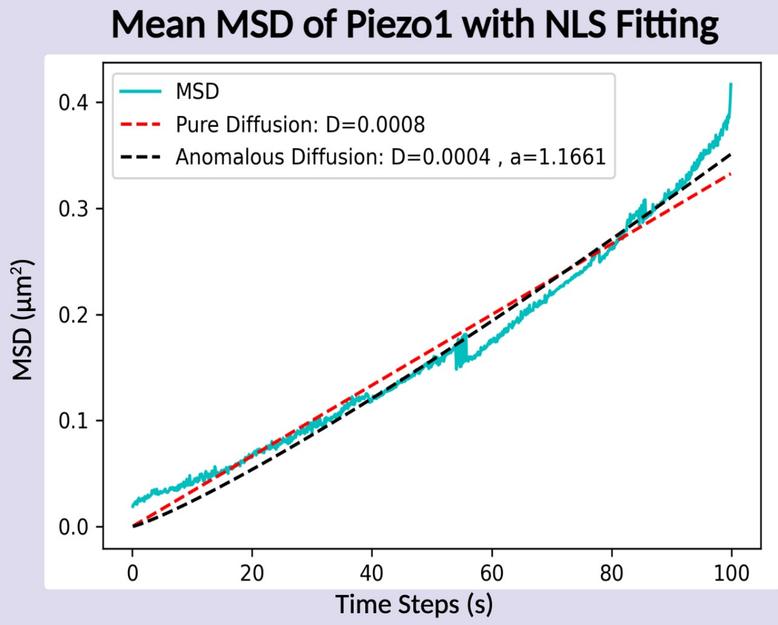
MSD calculation

- Find squared displacement with respect to a relative point over a time lag, τ
- Compute the mean of the squared displacement

JDD calculation

- Calculate the Euclidean distance of a point over a time lag, τ
- Perform parameter estimation for each of the diffusion models by Non-Linear Weighted Squares

Mean MSD of Piezo1 with NLS Fitting



JDD Diffusion model vs Jump Distance

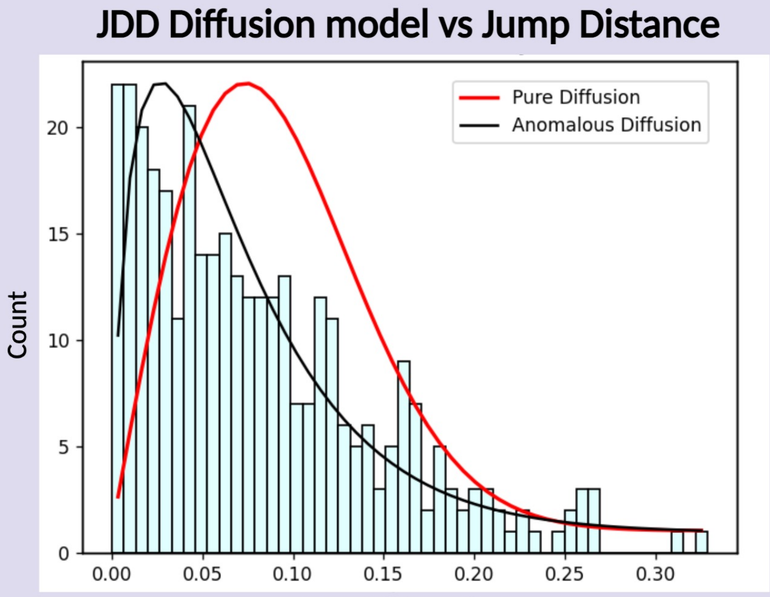


Fig. 6: MSD and JDD for localisation analysis of Piezo1 using GenEPi. (Left) Mean MSD (cyan), NLS fitting of pure diffusion (red), and NLS fitting of anomalous diffusion (black) from 5050 tracks. **(Right)** JDD histogram in cyan, and fitted pure diffusion (red) and anomalous diffusion (black).

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References
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