Mitochondrial Reorganisation and Sub-Cellular Energy Demands
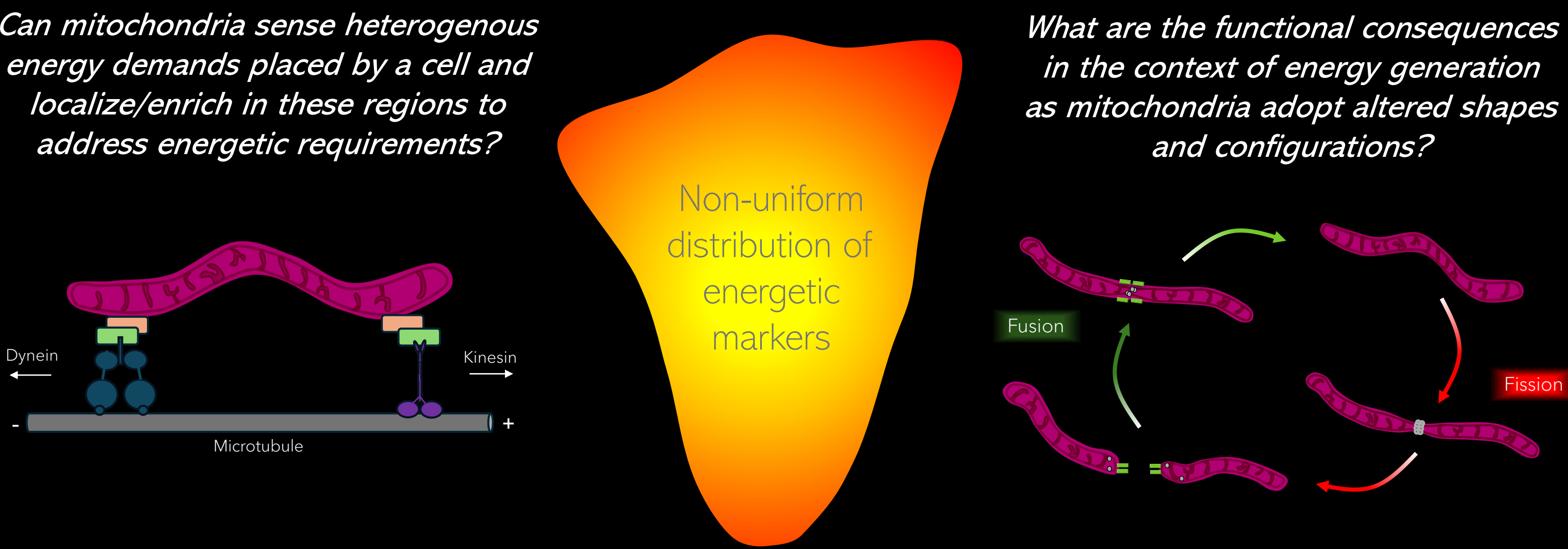
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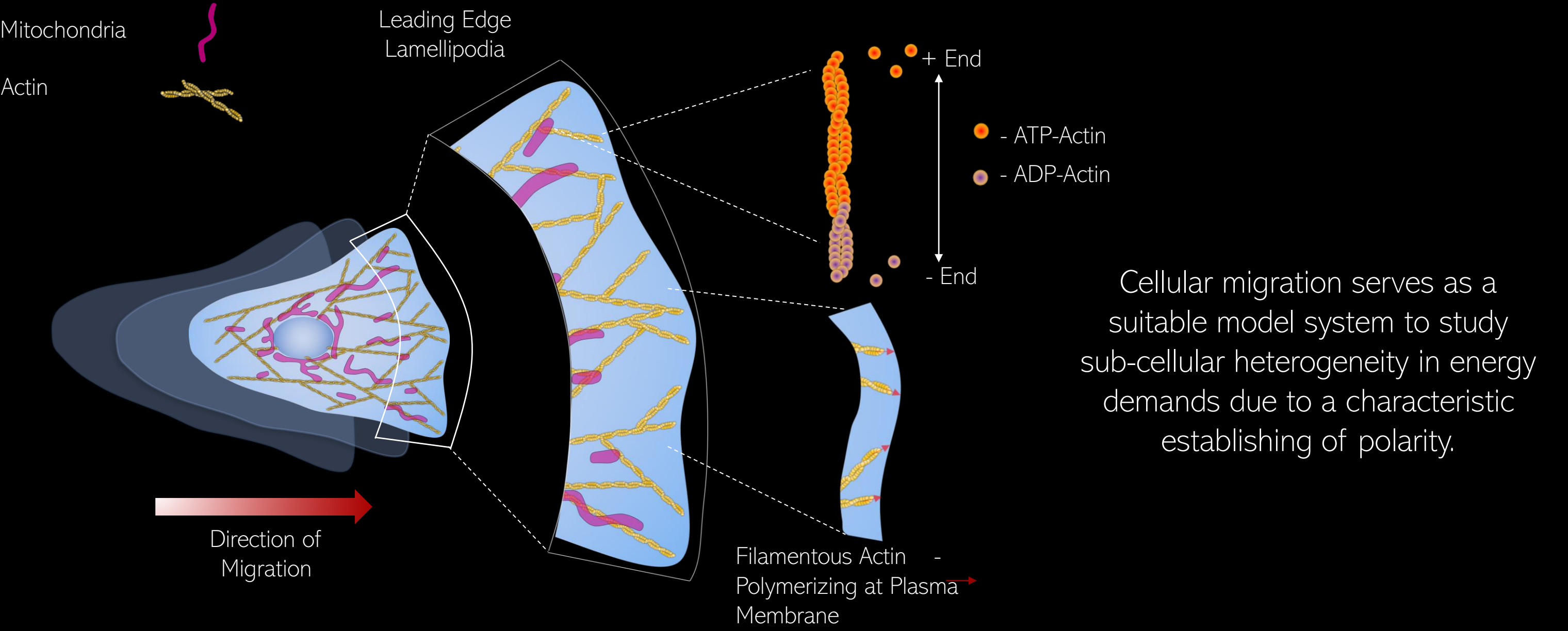Monash University[1], The University of Melbourne[2], ARMI[3]

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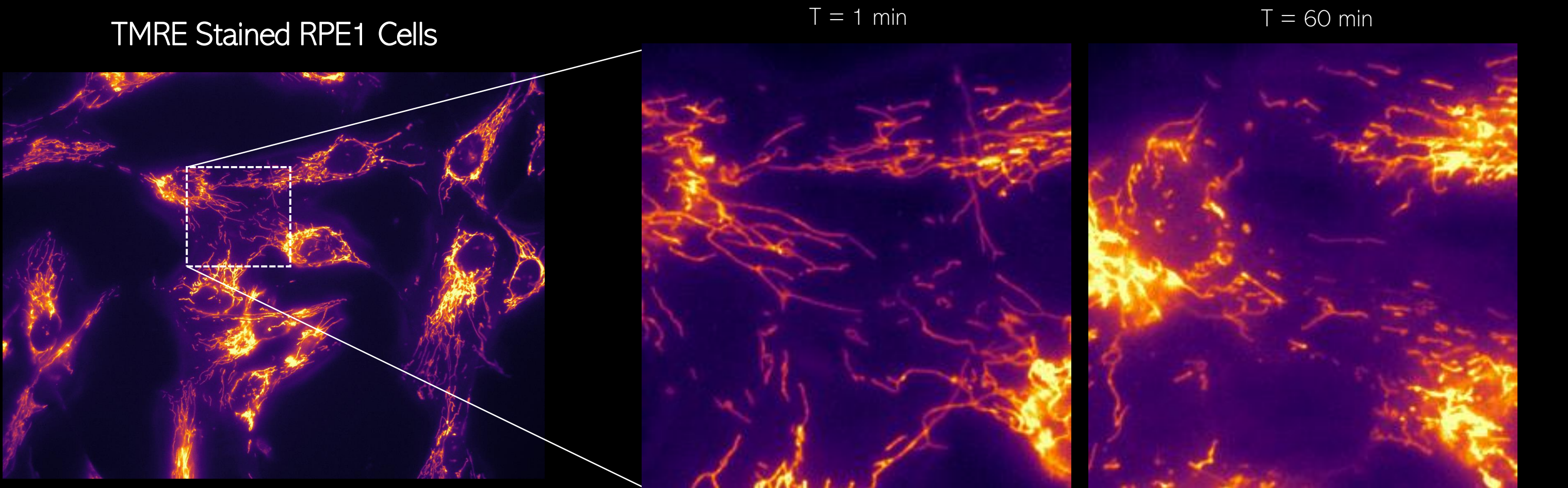
How Localised are Energy Demands in a Cell?

Can mitochondria sense heterogenous energy demands placed by a cell and localize/enrich in these regions to address energetic requirements?
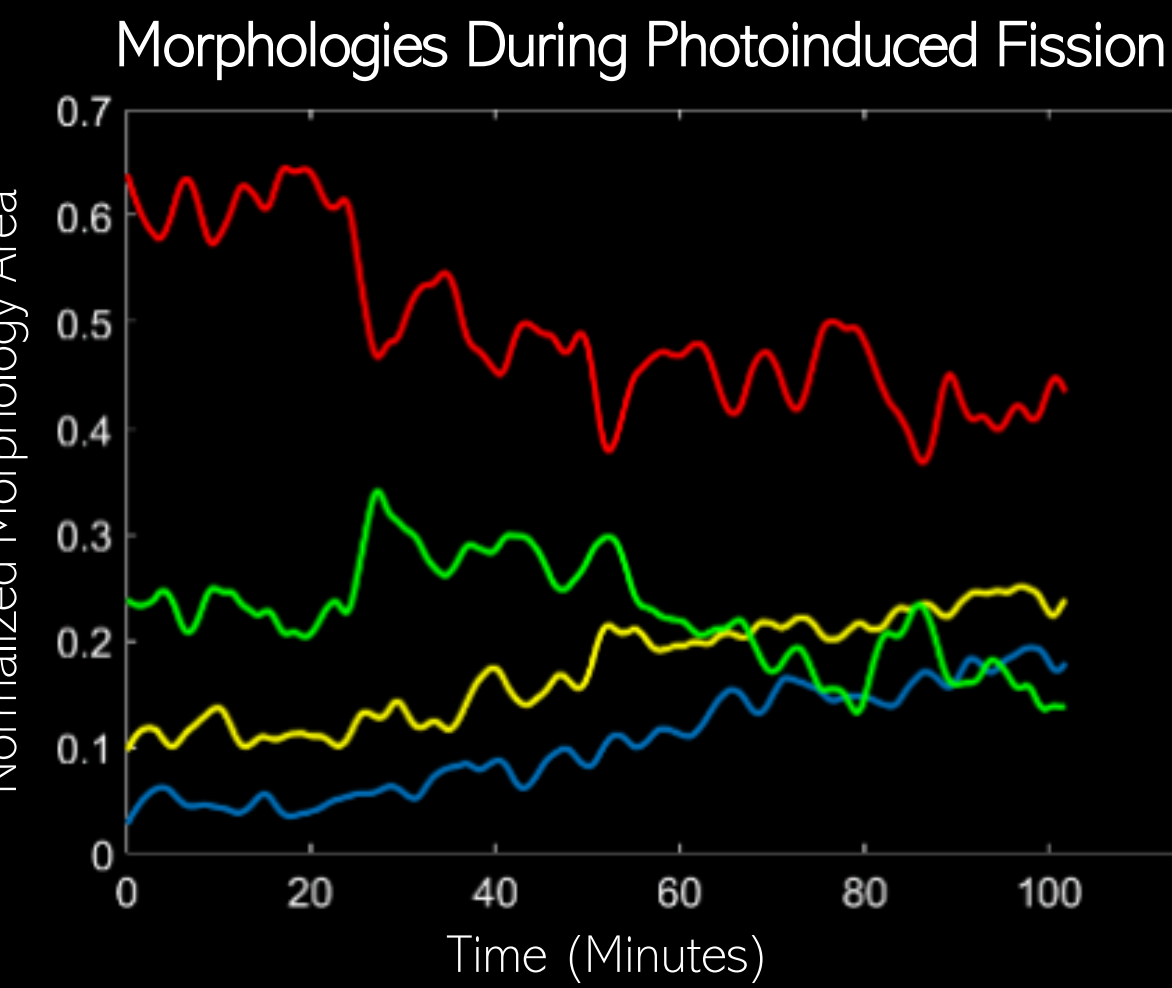
Non-uniform distribution of energetic markers

What are the functional consequences in the context of energy generation as mitochondria adopt altered shapes and configurations?

Dynein — Kinesin
Microtubule

Fusion — Fission

Model System: Cell Migration

Mitochondria
Actin
Leading Edge Lamellipodia
+ End
- ATP-Actin
- ADP-Actin
- End

Direction of Migration

Filamentous Actin - Polymerizing at Plasma Membrane

Cellular migration serves as a suitable model system to study sub-cellular heterogeneity in energy demands due to a characteristic establishing of polarity.

Scan To View Timelapse Mitochondrial Data

Photogentle Widefield Microscopy

TMRE Stained RPE1 Cells
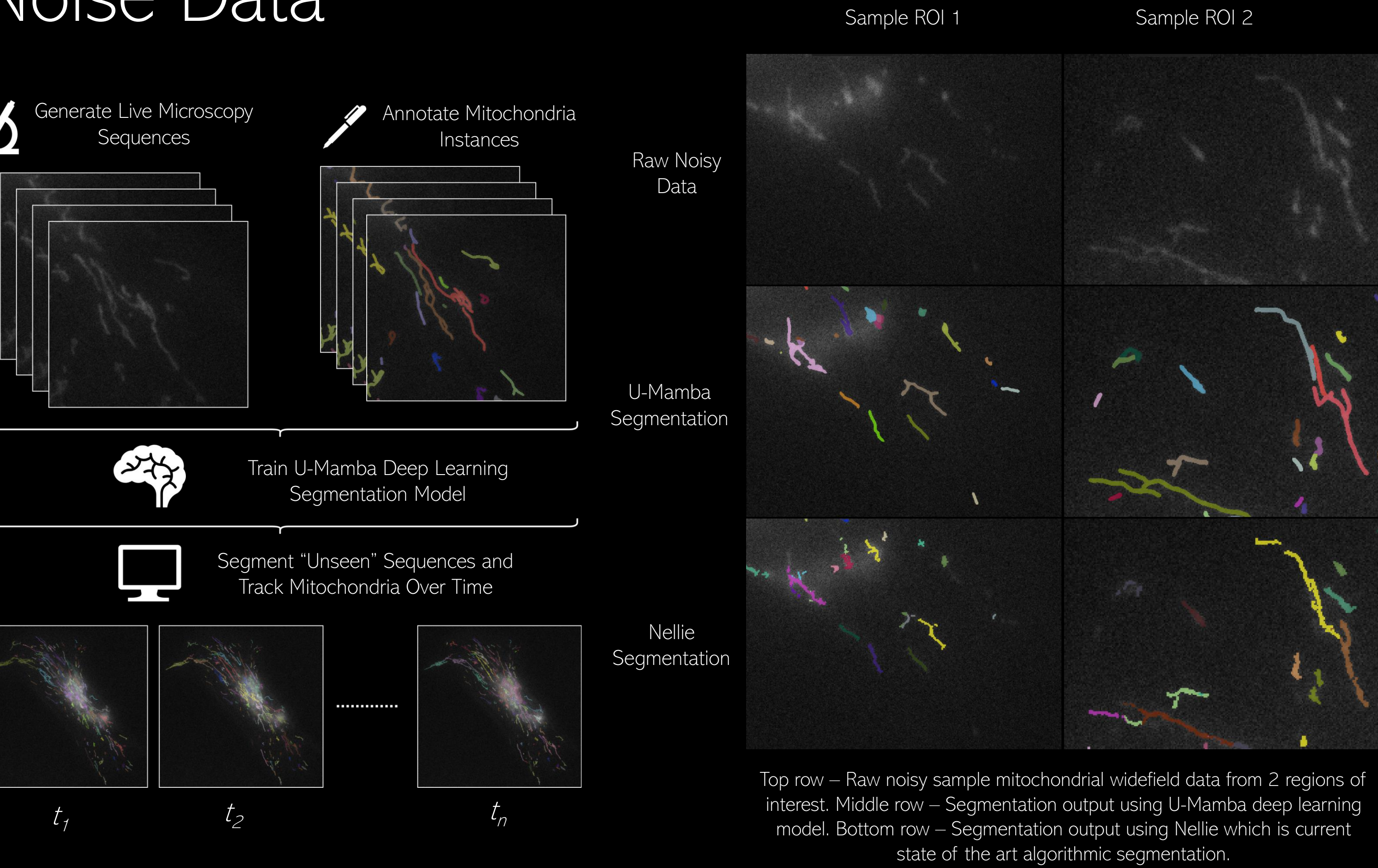
T = 1 min   T = 60 min

Left: Widefield images of RPE1 (Retinal Pigmented Epithelial) cells plated on fibronectin coated coverslips. Right: Zoomed comparison of region of interest in t=1 vs 60min.

Morphologies During Photoinduced Fission

- Punctate
- Rod
- Elongated
- Branched

Normalized Morphology Area vs Time (Minutes)

Mitochondrial morphology and dynamics are influenced by light dosage during imaging. Photo gentle imaging is necessary for studying mitochondrial dynamics at sub-second timescales over several hours in migrating cells.

Machine learning based morphological classification of mitochondria in region of interest over 1hr 20mins grouping mitochondria as either punctate, rod, elongated or branched.
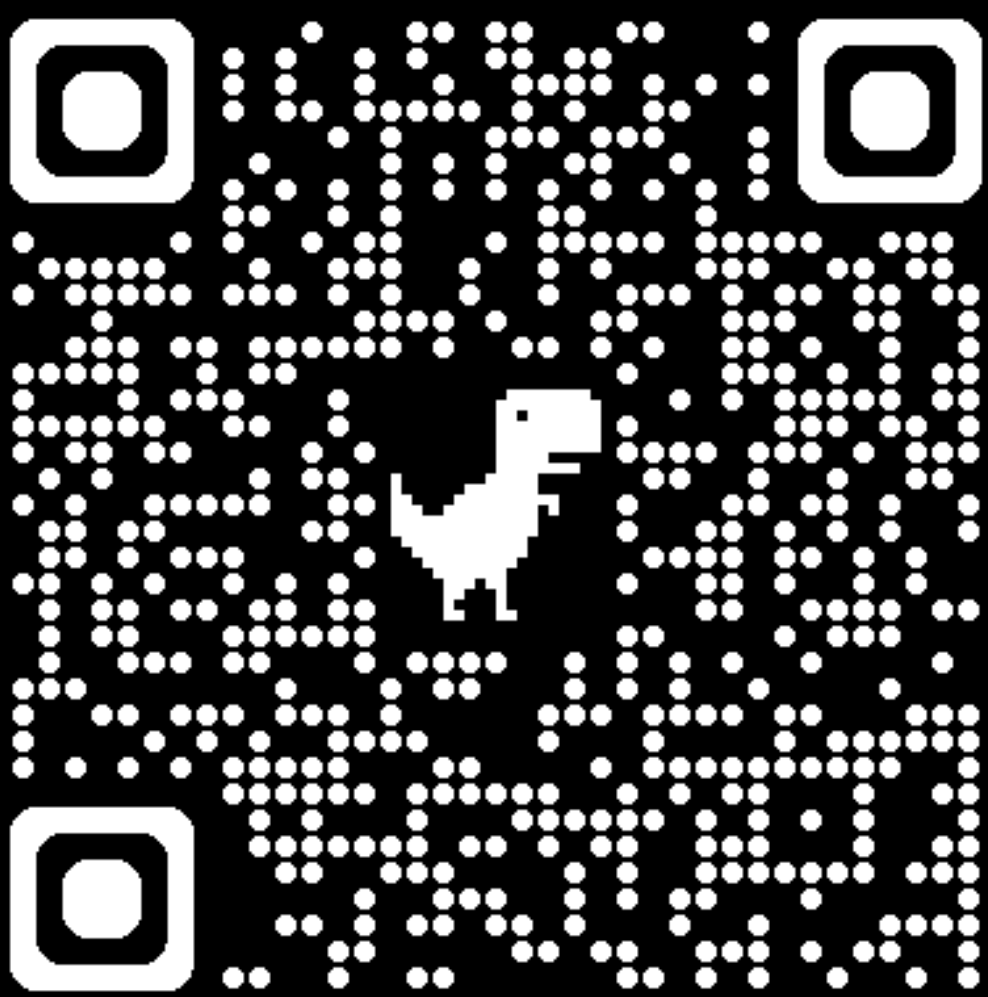
Cellular and Mitochondrial Dynamics

Cumulative Mitochondrial Mass and Protrusion Retraction Dynamics

Time: t_1 ... t_{end}

- Mitochondrial Mass
- Cumulative Mitochondrial Mass
- Cumulative Protrusions
- Cumulative Retractions
- Cell Trajectory

Polar plot representing the cell trajectory, and cumulative histograms over 2hrs for mitochondrial mass, cumulative cell boundary protrusions and retractions. Radial axis represents time.

Angular Trajectory of Cumulative Mitochondrial Vectors and Cell Vector vs Time

- Mitochondrial Trajectory
- Cell Trajectory

α Trajectory Angle (degrees) vs Time (minutes)

Trajectory angle plots for cell trajectory and cumulative mitochondrial vector trajectory.

Scaling Towards Multicellular Systems

2D: Single cells on a coverslip Widefield

3D: Single cells on a coverslip Widefield

3D: Single cells in extracellular matrix Lattice Light Sheet

Side View
Top View

Raw TMRE stained mitochondria in a single cell in 2D.

Rendered 3D instance segmented volume of a single cell.

Raw 3D volume of single migrating cell acquired using LLSM.

3D: Multi-cellular models in native environment Airy Beam Adaptive Optics

Schematic of tumour spheroid invading engineered micro vessel

Schematic of Zebrafish 5 days postfertilization

Volumetric image of GFP labelled mitochondria in muscle sections in transgenic Zebrafish. 5 days postfertilization.

Cross-section through single zoomed in muscle section.

Specialized Analysis For Long-Term, High Noise Data

Generate Live Microscopy Sequences

Annotate Mitochondria Instances

Train U-Mamba Deep Learning Segmentation Model

Segment "Unseen" Sequences and Track Mitochondria Over Time

t_1 t_2 t_n

Sample ROI 1   Sample ROI 2

Raw Noisy Data
U-Mamba Segmentation
Nellie Segmentation

Top row – Raw noisy sample mitochondrial widefield data from 2 regions of interest. Middle row – Segmentation output using U-Mamba deep learning model. Bottom row – Segmentation output using Nellie which is current state of the art algorithmic segmentation.
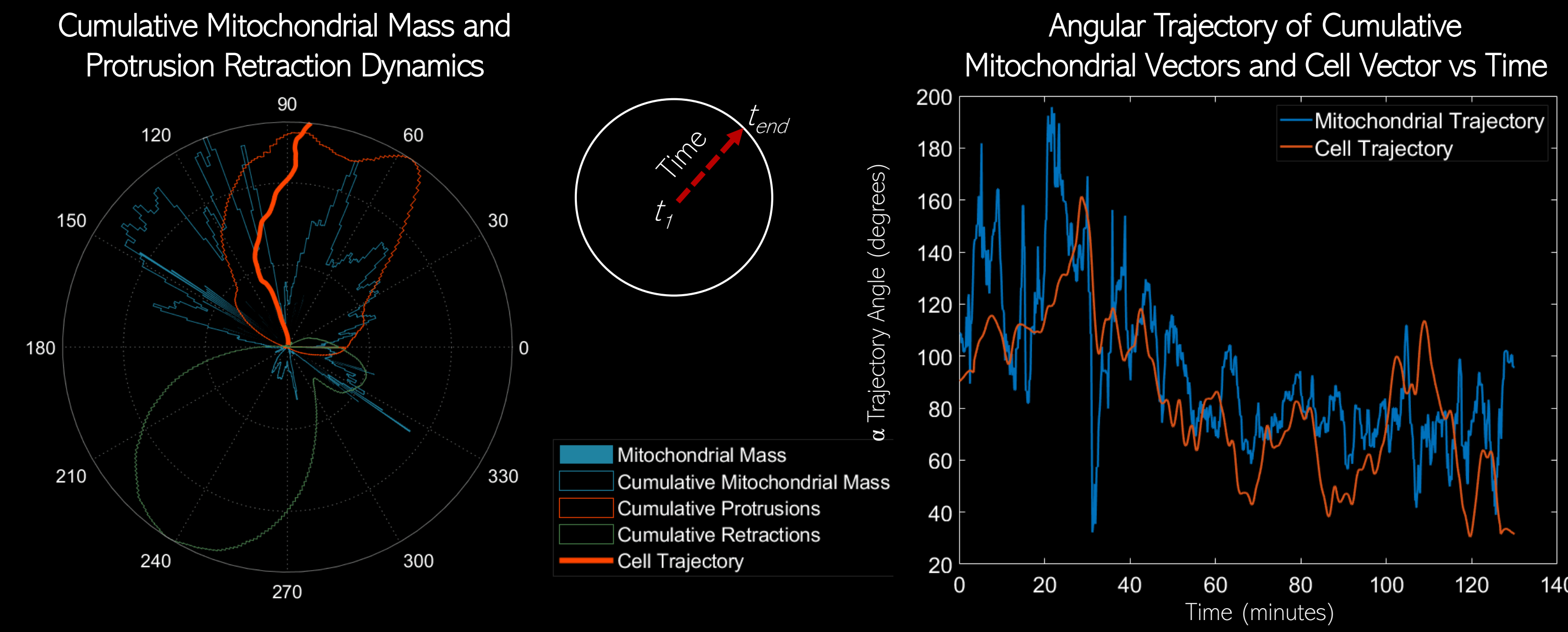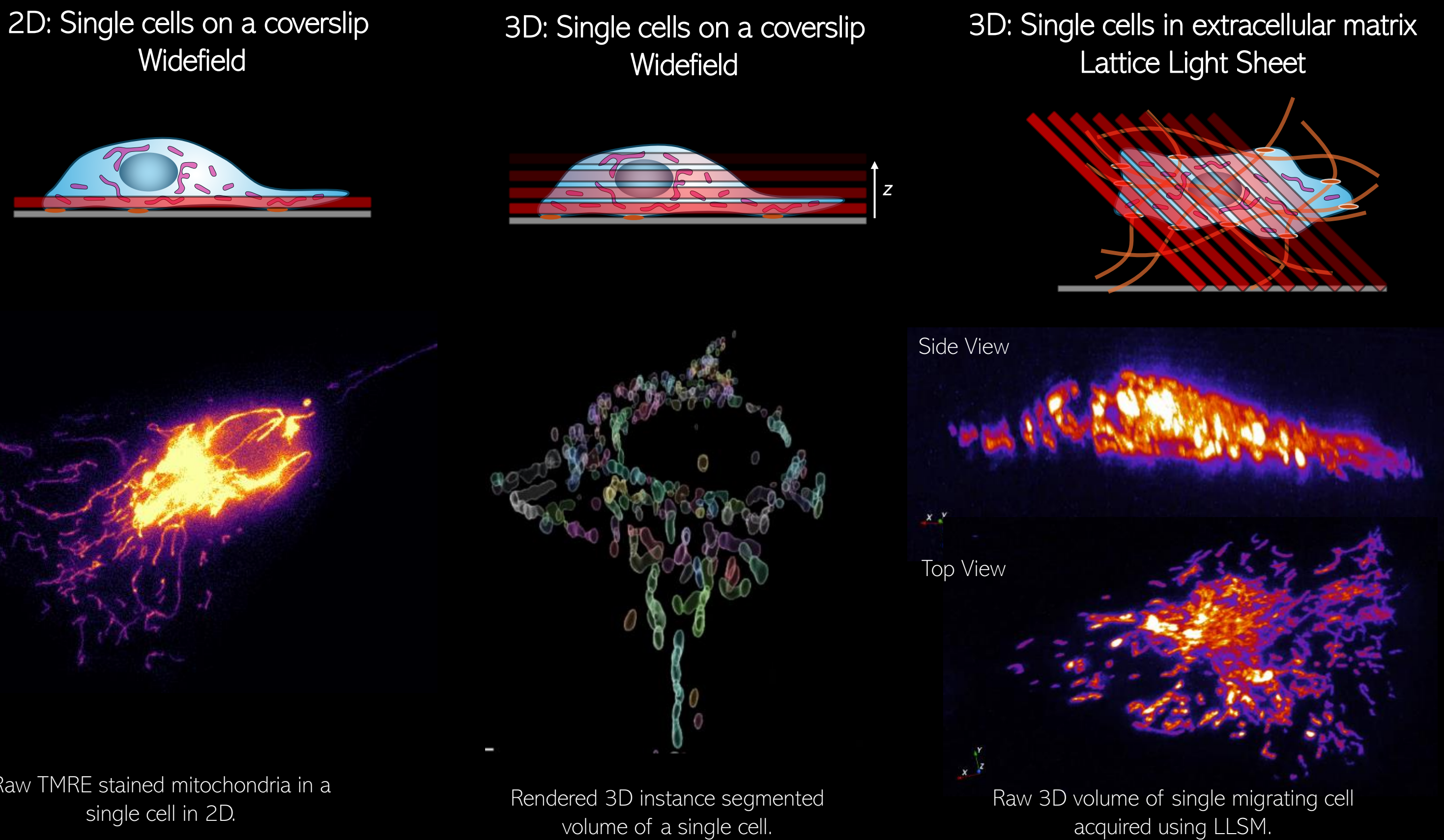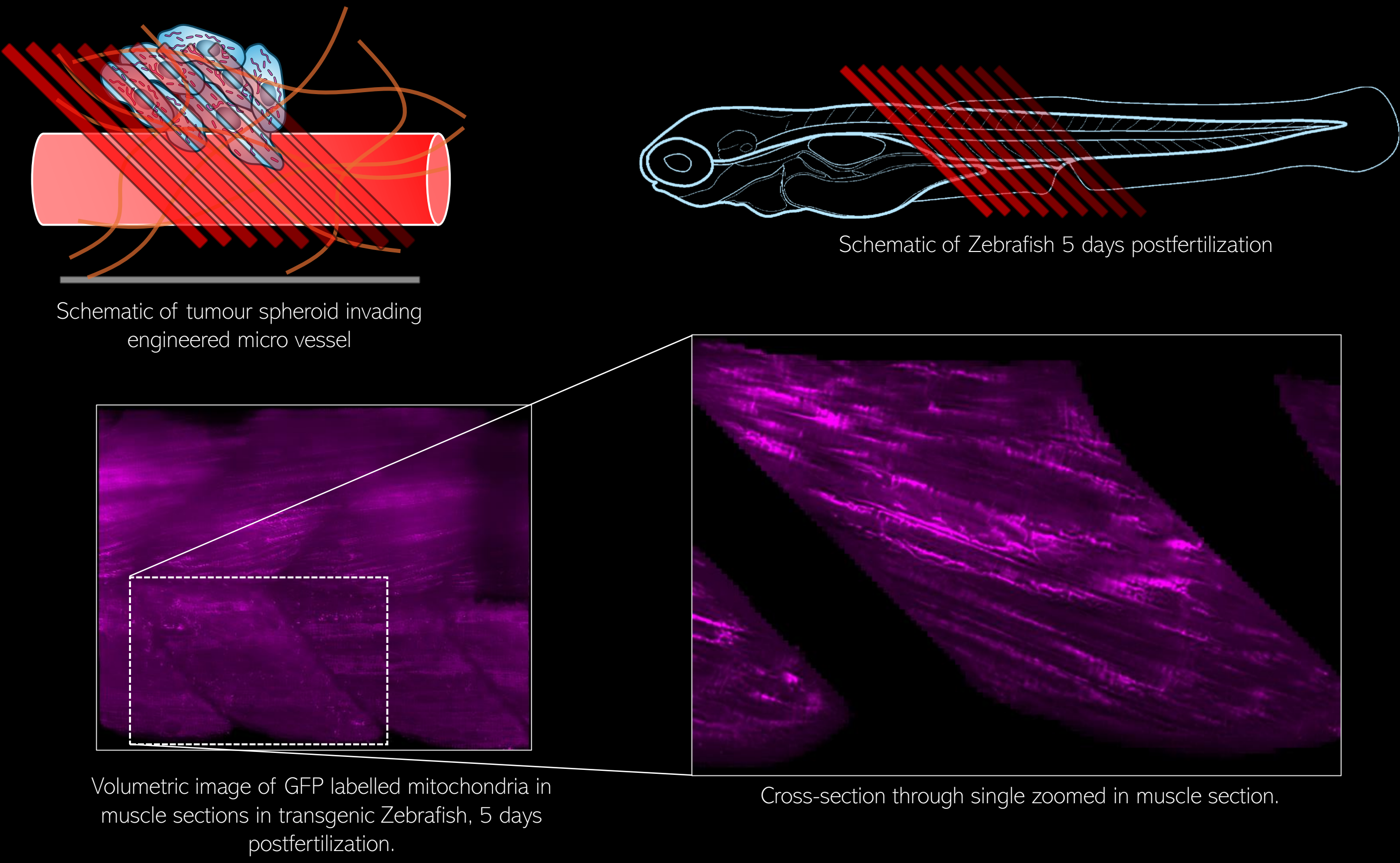
Selected Relevance References: **1.** Zhao et al. Int J Mol Med, 2019. **2.** Schuler et al. Mol Bio Cell, 2017. **3.** Penjweini et al. Redox Biol, 2020. **4.** Yong et al. eLife, 2019. **5.** Kruppa and Buss, J Cell Sci, 2021 **6.** Youle and Bliek, Science, 2016. **7.** Koncha et al. FEBS J, 2021. **8.** Pollard and Borisy, Cell J, 2003. **9.** Ma et al. arXiv, 2024. **10.** Lefebvre et al. arXiv, 2024. **11.** Chiu et al. Microsc. Microanal, 2022.