**General Descriptions:**

**- Fitted decay rate (delta\_p), derived from the slope of the line fit to ln(I) vs. time.**

**Since the exponential decay model is:**

**I(t) = I₀ \* exp(-δₚ \* t),**

**it is linearized as:**

**ln(I(t)) = ln(I₀) - δₚ \* t**

**1. Check and input experiment-related information from the following three Excel files:**

- `global\_folder.xlsx`: *Specifies the directory paths for raw data, processed outputs, and result storage.*

- `well\_info.xlsx`: *Stores well-level metadata, including well IDs, doxycycline (Dox) induction times, and construct identities.*

*• Column 1: indicates the position of the well on the 96-well plate (e.g., A1, A2, etc.)*

*• Column 2: Construct and induction information — specifies the RNA construct used in the well and the corresponding doxycycline (Dox) induction time*

*• Column 3: Probe target(1 or 2) — indicates whether the intronic probe targets intron 1 or intron 2*

**2. Run `pre\_cellpose\_ad.m` in MATLAB to process the raw `.nd2` files generated from the experiment.**

This script converts the images into `.tif` format compatible with Cellpose, and performs intensity normalization to make the images suitable for segmentation.

**3. Use Cellpose to run pretrained model:**

- `20230707-decay-dapi1`: Segments all cells in the image.

**4. Run `decay\_main.m` in MATLAB to begin the main splicing analysis pipeline.**

- decay\_main.m depends on

**Matlab scripts**：

LoadCellBoundaries : *extract single-cell information from cellpose-generated masks*

AutoNucleusOtsu : *Nuclear segmentation by Otsu’s methods*

SelectTransfected : *Transfected Cell Selection*

SubtractBackground : *Background Correction*

QualityCheck : *This script is used to visualize the cells that were classified as transfected cells. Specifically, it displays the original images with overlays or masks to highlight selected transfected cells, enabling manual inspection of selection accuracy.*

SummaryAllwells: *summarizes the pre-mRNA intensities across all wells at each time point.*

SummaryAllcons\_mean: *performs fitting to determine the RNA decay rate from time-course data.*

**Matlab functions:**

autocontrast.m : *performs automatic contrast adjustment on images.*

**Example workflow for decay rate analysis pipeline:**

1) Initialize the environment:

$ source ~/.bashrc

2) Navigate to the directory containing MATLAB preprocessing scripts:

$ cd Desktop/Li\_96well/decay-codes

3) Run MATLAB script to preprocess raw `.nd2` images:

$ matlab -batch pre\_cellpose\_ad

4) Return to the parent directory:

$ cd ..

5) Activate the Cellpose conda environment:

$ conda activate cellpose

6) Run Cellpose segmentation using the pretrained model:

$ cellpose --dir 20231208-decay8/ad-images \

--pretrained\_model model/20230707-decay-dapi1 \

--chan 3 --chan2 0 --save\_png --no\_npy --verbose --diameter 36.355

7) Deactivate the conda environment:

$ conda deactivate

8) Return to the MATLAB code directory:

$ cd decay-codes

9) Run the main MATLAB analysis script:

$ matlab -batch decay\_main