Title:	Studying mRNA Inheritance using Time Lapse MIcroscopy Followed by end point SABER- FISH
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Abstract:	The ultimate aim of the work is to establish a protocol and an image analysis pipeline integrating time lapse microscopy to track cellular lineages and SABER-FISH to obtain mRNA counts. By integrating lineage data with mRNA counts, we can potentially study different aspects of inheritance of mRNA in single cells. In the established protocol, mouse embryo fibroblasts were transfected with a plasmid that imparts GFP fluorescence to the cytoplasm. Since the transfection efficiency is low for MEFs with the plasmid (1%), the cells were sorted using FACS (fluorescence-activated cell sorting). Further the cells were allowed to reattach to in an imaging slide and imaged for 48 hours to track them live. These cells were fixed immediately after the last frame was captured. Following permeabilisation of the cells, SABER-FISH was performed on these cells to detect the mRNA counts of a trial gene, NPAS2 on them. These were imaged by going to the same fields of view as the last frame of the time lapse video so as to capture the mRNA information for the cells whose lineage data was captured. After obtaining the fields of view, an image analysis pipeline was developed to integrate the two datasets. The 60x SABER-FISH images were first stitched to obtain a single image corresponding to the 20x timelapse frames. A CellPose model was optimised to segment the cells in both the timelapse and FISH images. TrackMate was used for manually tracking the cells in the time lapse video by importing the masks generated using the CellPose model. In order to match the cells in both the timelapse and SABER-FISH images, the images were first aligned using a control- point image registration algorithm on MATLAB. Further, the centroid of each cells in the time lapse image was calculated and mapped on the FISH images to map the cell identities between the two images. The RS-FISH plugin in FIJI was used for thresholding and marking the mRNA spots. A custom made Python code was used for counting the spots in the cells and providing an integrated dataset of sister cells and their mRNA counts. Sister cells showed the highest correlation in the levels of NPAS2 mRNA, followed by cells which were close together in space. Cells which were far apart did not show a significant correlation.
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