Live Cell Imaging Video Making, Single-Cell Tracking, Data Analysis, and Cell Fate Simulation Software

The provided compressed files contain code related to papers published in *eLife* (Rancourt, A., Sato, S., and Satoh, M. S. (2022) Empirical single-cell tracking and cell-fate simulation reveal dual roles of p53 in tumor suppression. *eLife* 11, e72498 (2022)) and *BioRxiv* (Sato, S., Rancourt, A., Satoh, M.S. (2024) Cell Fate Simulation Reveals Cancer Cell Features in the Tumor Microenvironment. bioRxiv, doi: https://doi.org/10.1101/508705). The code, written primarily in C++ and Objective-C using Xcode (Mackintosh), is designed for Mac OS 12. There are 21 compressed files in total.

All code is authored by Masahiko Sato (Masahiko S. Satoh). The primary focus is to generate data, and as such, the code follows Masahiko Sato's personal coding conventions. There is redundancy in the code and frequent use of global variables. Comments are minimal, but the code is sufficiently readable for maintenance, bug fixes, and improvements by Masahiko Sato. However, if others wish to use it, some cleanup—such as reducing global variables and packaging functions—may be necessary.

A user manual is not available yet, and some training will be required to use the software. Although Masahiko Sato currently lacks sufficient resources to provide support, please contact Masahiko Sato for questions (masahiko.sato@fmed.ulaval.ca).

Software Overview

The software is divided into four groups:

1. Automated live cell imaging video-making
2. Associate software for video-making
3. Image segmentation and single-cell tracking
4. Data analysis and simulation

Automated Live Cell Imaging Video Making

This process is hardware (microscope) sensitive. We use a microscope controlled by Metamorph, which generates multilayer TIF files. This group includes the following software:

- **Imaging_Controller**
- **XY_Map**
- **FileUpLoad**
- **FileName_Assignment**
- **Focal_Image_Selection**
- **Contrast_Set**
- **Data_BackUp**

These tools can generate up to 16 independent live cell imaging videos in real-time for 1-4 weeks.

Imaging_Controller

Controls the live cell imaging video-making process and associated tasks. It operates in three modes:

1. Real-time automatic processing
2. Processing from saved TIF files created by Metamorph
3. Processing from focused images

Functionality:

- Creates a multidimensional image acquisition array (e.g., 5x5 arrays)
- Transfers images to a Macintosh computer via Ethernet
- Selects or generates focused images, adjusts contrast, and stitches images into videos
- Archives multilayer TIF and focused images

Processing Modes:

1. **Real-time automatic processing**: During live cell imaging, a multidimensional image acquisition array is created (e.g., 5x5 arrays), with each array viewed by Metamorph's Multi-dimensional analysis function. For example, if 5x5 arrays are set in 8 well chambers, Metamorph creates 200 multiplayer TIF files every cycle of image acquisition, repeating every 10 minutes for 14 days. These files are transferred to a Macintosh computer, where a focused plane is selected or a focused image is generated. The contrast is adjusted, and individual files are stitched into one image to make videos. Up to 16 live cell imaging videos can be made in real-time.
2. **Creating videos from saved TIF files**: This mode allows the creation of live cell imaging movies from archived TIF files.
3. **Creating videos from saved focused images**: This mode generates movies from saved focused images.

XY_Map

Sets up a multi-dimensional array for Metamorph's MDA (Multi-Dimensional Acquisition). It allows the selection of areas of interest and adjusts the position of each field of view (FOV). It supports 512x512, 1024x1024, and 2048x2048 images.

Functionality:

- Scans areas of interest using Metamorph to create a 5x5 image area (25 FOVs)
- Displays scanned data within the outline of a multi-well chamber
- Allows selection of areas of interest and adjusts FOV positions, especially useful when a multi-well chamber is removed and replaced on the microscope stage

FileUpLoad

Fetches multilayer TIF files created by Metamorph on a Windows computer.

FileName_Assignment

Assigns names to all files, including grayscale TIF image files and live fluorescence and indirect immune fluorescence images.

Focal_Image_Selection

Creates focused images by selecting a focused plane or generating an all-in-focus image.

Contrast_Set
Performs contrast adjustment and image stitching. It adjusts each FOV position considering CCD camera tilt and non-uniformity of image contrast and brightness.

Data_BackUp
Backs up multilayer TIF images to selected drives.

Associate Software for Live Cell Imaging

CellMovie, CellMovie2, CellMovie3, CellMovie4, CellMovie5, and CellMovie6: These tools display live cell imaging videos. CellMovie includes additional functions such as contrast adjustment, position adjustment if a shift occurs during imaging, and alignment of grayscale to fluorescent images. Versions 2-6 have identical functions, allowing convenient comparison of multiple videos.

FileConverter: Converts file arrangements from non-Metamorph platforms into formats compatible with Imaging_Controller and related software.

Single-Cell Tracking

Cell_Outline_Draw: Performs grayscale image segmentation using connectivity analysis.

Cell_Tracking: Controls the single-cell tracking process, creates a tracking database, assigns cell lineage and numbers, and corrects tracking errors.

Cell_Carving: Automatically tracks cells by analyzing segmentation data and using pattern information.

Data Analysis and Simulation Software

Lineage_Analysis: Analyzes data using the database generated by Cell_Tracking and performs cell fate simulations.

Cell3DDisplay: Simulates cell fate in 3D space.

CellMovieQuant: Analyzes images to quantify cell numbers, nuclei, and staining data.

Watson: Arranges HE staining images for processing by CellMovieQuant.

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