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# Reference to the code

***If the code provided here or modifications of it are used for scientific publications please refer to it as published in: U. Hohmann et al,* "A toolbox to analyze collective cell migration, proliferation and cellular organization simultaneously", *Cell Adhesion and Migration, 2022***

# 0. General remarks

The code provided here contains the migration analysis toolbox described in U. Hohmann et al, "A toolbox to analyze collective cell migration, proliferation and cellular organization simultaneously", Cell Adhesion and Migration, 2022.

It assumes that the folder and subfolder structure of the provided files is preserved, as some references between modules are present.

# 1. Contained files and folders

The following folders are provided. Please denote that the contained folders, sub-folders, and files within need to be stored as provided because scripts refer to each other.

1) Analyze Vector Fields

2) Cross Correlation (PIV)

3) Gen Flow Field Data

4) Plot Results

The folder “Sample Images + Analysis + Plots” contains example images and all outputs generated during any here provided step. This folder also serves as an example for the data structuring when applying the provided code.

# 2. Prerequisites for using the given code

## 2.1 Needed packages

The code provided was written in MatLab 2021a and relies on the following packages:

- System Identification Toolbox
- Image Processing Toolbox
- Statistics and Machine Learning Toolbox
- Curve Fitting Toolbox
- Parallel Computing Toolbox
- Signal Processing Toolbox
- Sensor Fusion and Tracking Toolbox

## 2.2 Prerequisites to images

To use the given code several pre-requisites for the images have to be fulfilled. The code in its current form is accepting “.tif”, “.png”, “.jpg”, “.pbm”, “.pgm”,”.ppm” or “.bmp” files that are grayscale. As the code reads any image of the given format in the given folder it should not contain additional unwanted images. Additional sub-folders do not pose obstacles. Image stacks are not supported. For full analysis of images data is expected to be ordered as follows: *One main folder, containing sub-folders. Each sub-folder contains time lapse images of one field of view.* See the sample-folder and Fig. 1 for illustration of ordering and image composition.

# 3. Analyze images using PIV, orientation analysis and/or cell division analysis

Navigate to the folder named “Cross Correlation” and open both the “ParameterFunctionMain.m” and “main.m” files. The respective functions are either used to run the image analysis or set input parameters,

including what kind of analysis to perform (e.g. only PIV, PIV + Cell Division, etc.). All parameters are described in the respective file.

When starting the analysis file it needs to be pointed a folder containing sub-folders with image series ("sub-folder 1", e.g. "Control" as in Fig. 1). Afterwards, the algorithm goes through each sub-folder and analysis the individual image series. Please denote that this step may take some time, depending on the number of images to be analyzed and the chosen settings.

In each folder with an image series up to three additional folders, termed "Results", "Results Cell Division Analysis" and "Results Orientation Analysis" are created, containing the raw-data for the respective analysis type, the settings used and in case of the cell division analysis an optional video of the image series together with detected divisions.

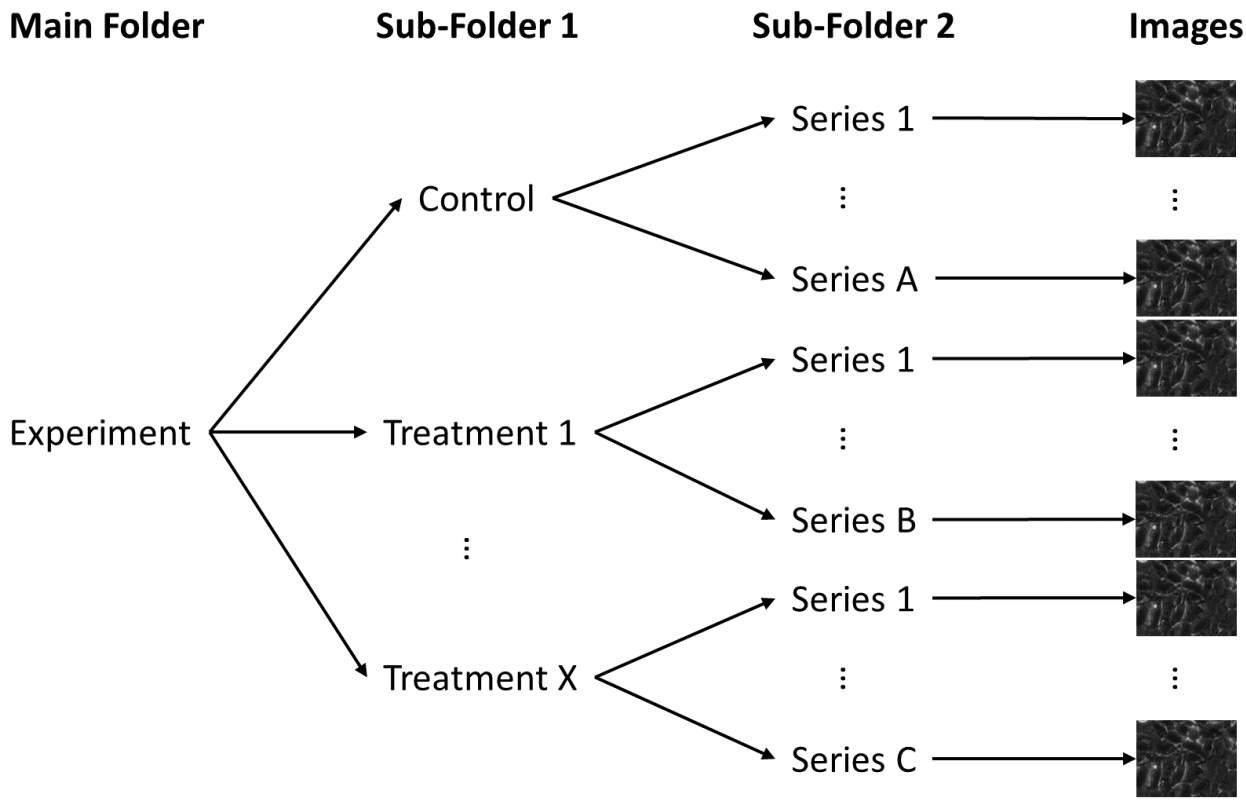


**Figure 1:** Example folder structure as used throughout the documentation.

## 4. Gather resulting data and create a joint output file for all fields of view of one experimental condition

Navigate to the folder named “AnalyzeVectorFields” and open both the “ParameterFunctionMain.m” and “AnalyzeFlowFields.m” files. The respective functions are either used to gather the results data from the previous step or set input parameters. All parameters are described in the respective file.

When starting the analysis file it needs to be pointed to a folder containing sub-folders with image series (“sub-folder 1”, e.g. “Control” as in Fig. 1). Afterwards, the algorithm goes through each sub-folder and gathers the data. It outputs up to six files in sub-folder 1 (see Fig. 1), containing the data regarding the velocity field, orientation field, cell division data and the respective settings used for analysis. The data stored here is just an intermediate gathering step.

## 5. Create a joint data structured array

Navigate to the folder named “Gen Flow Field Data” and open the “GenVelFieldData.m” file. The is used to gather the resulting data from the previous step.

When starting the analysis file it needs to be pointed the main folder containing all the sub-folders with different experimental conditions (“main folder”, e.g. “Experiment” as in Fig. 1). Afterwards, the algorithm goes through each sub-folder and gathers the data. It outputs up to 4 files in sub-folder 1 (see Fig. 1) with ordered data for all experimental conditions and image series:

CellDivData: Contains matrices for each image series of each experimental type with the data of the cumulative sum of cell divisions and the respective times of detection.

VecFieldData: Contains the angle of cellular orientation (AngleOrientation), of the velocity field orientation (AngleVelField) and relative orientation of the cellular orientation relative to the velocity field orientation (AngleVecField). All angles are given in degrees

VelFieldData: Contains the mean squared displacement scaling coefficient (pAll), root mean squared velocity (RMSVelAll), mean squared displacement over the whole time (MSDAll) and specific time windows (MSDTempAll), the order Parameter (QAll, QTempAll), 4-point-susceptibility (ChiAll, ChiTempAll) and the relative number of cells making new neighbors (NumNewNeighborsAll, NumNewNeighborsTempAll) for the whole time and specific time windows. All parameters are described in more detail in the manuscript referred to under “Reference to the code”.

TrackingMatrix All: x- and y positions of virtual particles for specific time windows as generated by the velocity field obtained from PIV.

## 6. Plot data

Navigate to the folder named “Plot Results” and open both the “ParameterFunction.m” and “Compare Groups.m” files. The respective functions are either used to gather the results data from the previous step or set input parameters. All parameters are described in the respective file.

For generating all possible plots, load the files created during the previous step into the workspace and run the script. It will generate and save multiple plots as .fig and .png files in the current working folder of MatLab.

## 7. License