**Installation and running guide for CBF analysis via Matlab script**

This guide describes briefly how to install and run scripts used in the manuscript : *Quantifying cilia beat frequency using high-speed video microscopy: Assessing frame rate requirements when imaging different ciliated tissues.* To run scripts please install the latest version of Matlab available. Download the scripts from GitHub page : github.com/ElvPan/CBF analysis

Please ensure to also download and unpack the Open Microscopy Environment (OME) Matlab toolbox on following page, as they will be used to open your microscopy files such as .czi, .oir, .nd2:

<https://www.openmicroscopy.org/bio-formats/downloads/>.

Next make sure that the analysis scripts and OME folder (bfmatlab) paths are added to the Matlab. As shown in below image, click on ‘Set Path’ within ‘HOME’ tab of Matlab and then click on ‘Add with subfolders’. Navigate to the folders containing downloaded scripts, including unzipped bfmatlab folder, and once you see that those paths were added, click on ‘Save’ then ‘Close’. Your Matlab will now know where to search for functions while running analysis.

Graphical user interface, application

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There will be two main functions used for analysis: ‘CiliaBeatingFrequencyFromMovies.m’ and ‘CiliaBeatingFrequencyFromKymographs.m’. First function is run on a single 2D+time movie file. The valid input formats include multi-tif file, czi and lsm (Zeiss), lif (Leica), nd2 (Nikon), as well as movie file formats such as mp4, mov and avi. To start a run please ensure to open the file as shown below, navigate to ‘Editor’ tab of Matlab and click on ‘Run’.

Text

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After data is loaded, the user will be prompted to select a sampling approach where CBF is to be calculated. The choices are multiple points selection, a line, a free hand line and a polygon. Is user selects ‘multiple points’ a new prompt of how many points to select will appear. Enter the number of point and when image appears, click with cursor which points to include in CBF calculation. Similarly proceed if selecting other spatial sampling approaches. Next user is prompted to enter the frame time in seconds, the cutoff signal-to-noise ratio for peak detection in CBF spectra as well as ‘peak prominence’. The peak detection in CBF spectra is done via Matlab functions ‘findpeaks’, so please refer to Matlab Help file for more information on the usage of this functions. The default values of these 2 parameters are set to 5 and 0.5, respectively. Please adjust if no or to many peaks are detected in CBF spectra. The script will proceed with calculation of CBF spectrum at each spatial sampling point and save the results in an excel file.

The second script ‘CiliaBeatingFrequencyFromKymographs.m’ is running on kymographs extracted from 2D+time data, and as such will not include any prompts to define the spatial sampling points. Its analysis is started in similar way as described above, except that user will prompted to enter the frame rate (frames per seconds), S/N of the peak in CBF spectrum, peak prominence and define along which dimension (1=row or 2=columns) is the axis of time in the input data.