How to Use FBI Software

An accompaniment to the manuscript:

Fluorescence Behavioral Imaging (FBI) tracks identity in heterogeneous groups of *Drosophila*.
Pavan Ramdya, Thomas Schaffter, Dario Floreano, and Richard Benton

Acquiring FBI data with *sQuid*.

sQuid is a general purpose C++/Qt software application permitting image acquisition from multiple cameras (in this case, an IR and a GFP camera) while controlling computer port output (in this case, parallel port control of LEDs). The software, installation guide, and user manual can be found at:

Download:
http://lis.epfl.ch/tschaffter/squid

For our experiments we run the cameras at 20 fps. To properly post-process data with the given shell scripts, be sure to name the pin controlling the excitation LED "blue" in the player settings file.

Processing FBI data.

STEP 1: sQuid raw data post-processing:
Following each experiment, it is necessary to build the required data types from raw *sQuid* data. This can be accomplished by installing and using the provided shell scripts.

Download:
http://lis.epfl.ch/FBI

1) batch_fluo_process
The user must be in the directory containing all experimental folders. This script runs the shell script *fluo_process* on each folder. It is adapted from a shell-script written by Kristin Branson of Ctrax.

2) fluo_process
NOTE: The user must change the name of folders pointing to the image files to the appropriate names for their cameras.

Makes a folder called "GFPimages" in the experiment directory.
Places all fluorescence images in this directory.
Makes a folder called "bkgd" in the experiment directory.
Places a subset of infrared (IR) images in this folder.

Makes a file "sequence.txt" containing all image names.
Converts IR .tif images into .jpeg for movie making
Makes an .mpeg video from IR images.
Deletes original image folders and images within.

STEP 2: Track movie.avi
Many different types of tracking software are available. For our experiments we used Ctrax [Branson et al. 2009]. We tracked flies in the video file 'movie.avi'. This was followed by error fixing using the Matlab script 'fixerrors.m' provided by Ctrax developers.

STEP 3: Automatic genetic identity determination using Matlab m-files
The goal of FBI m-files is to use a tracked movie and corresponding GFP images to automatically determine the genetic identity of each tracked fly. These scripts have been developed with Matlab R2011b on Mac OSX 10.6.8.

Download:
http://lis.epfl.ch/FBI

Each experimental folder should have the following folders and files within:

1) A folder containing all raw GFP images:
experiment_directory/GFPimages/

2) GFP images within this folder with the following names:
*modelCamGFP*_HR_MIN_SEC_MSEC_"blue".tif

3) A folder containing some IR images for background image creation:
experiment_directory/bkgd/

4) IR images within this folder with the following data names:
*modelCamIR*_HR_MIN_SEC_MSEC_pinID.tif

5) A matlab data file (.mat) containing tracking data (trx)
movie_FIX.mat
NOTE: file naming convention is important for later processing

6) A text file (.txt) containing the sequence of image names
experiment_directory/sequence.txt

If all of these files are present, the user can run **FBI_Process_BATCH.m** to process one or more experiments.

Details of FBI Matlab m-files.

Scripts for processing data

1) /FBI_PreProcess/FBI_PrepTrx.m

Fixes Ctrax first frame theta issue, & calculates median fly sizes.
NOTE: default input is movie_FIX.mat

Input: *Folder directory with one experiment*
Data In: *.mat file with trx data*
Data Out: *trx_PREP.mat (*variable structure name: *trx)*

2) /FBI_PreProcess/FBI_SequenceExtract.m

Extracts LED, timing, and frame name information from sequence.txt
NOTE: user must define camera name in line 83 for 'regexp'.

Input: *Folder directory with one experiment*
Data In: *sequence.txt file with image name data*
Data Out: *sequence.mat (*variables: *blueLEDoff; blueLEDon; ledSequence; nameSequence; timeSequence)*

3) /FBI_PreProcess/FBI_AlignImages.m

Determines the scale, shift, & rotation values to align IR & GFP images. Takes user input to align.
NOTE: Can be performed once and used for multiple experiments by placing resulting *alignParams.mat* file in the experiment folder or in the directory containing all experiments for batch processing.
NOTE: assumes flipped GFP images vs. IR in the y-dimension: line 153.
NOTE: initialized vector is end-user specific. lines 175, 176

Input: *Folder directory with one experiment*
Data In: *images from /GFPimages/ and /bkgd/*
Data Out: *alignParams.mat (*var: *xAdd; yAdd; TFORM; height; width)*

4) /FBI_PreProcess/FBI_MeasureFluo.m

Extracts image intensities at Front and Rear ROIs for each fly in GFP images.

Input: *Folder directory with one experiment*
Data In: *trx_PREP.mat; alignParams.mat; /GFPimages/image files*
Data Out: *trx_EXTRACT.mat (*var: *trx)*

5) /FBI_Identify/FBI_SolveID.m

Calculates the likely identity of flies in experiment using metrics.

Input: *Folder directory with one experiment*
Data In: *trx_FBI.mat*
Data Out: *trx_ASSIGN.mat*

Script for batch processing:

To process multiple experiments serially:

1) /FBI_BatchProcess/FBI_Process_BATCH.m
Runs processing steps for each experimental folder in the parent directory.
NOTE: FBI_align_images.m can be performed for one experiment then *alignParams.mat* can be placed in Parent folder to use for all exp. directories.
NOTE: *assignParams.mat* should also be placed in the Parent folder in this manner.
Input: *Parent directory with all experiment directories*
Data In: *Parent directory name*
Data Out: *All outputs for each exp. directory.*

Scripts for visualizing data:
Allow the user to visually inspect results:

1) /FBI_Visualize/showtrx_GENO.m(fig)
Scroll through an experiment movie with a color-coded overlay of GFP and non-GFP flies. Red for non-GFP and blue for GFP.
NOTE: This is a modified version of Ctrax's showtrx.m. Therefore the Ctrax behavioural_microarray folder must be set in the Matlab path.
User Input: *location of .sbfmf & trx_ASSIGN.mat*
Data In: *.sbfmf; trx_ASSIGN.mat*

2) /FBI_Visualize/FBI_movie_overlay_ROI_GFP_forward.m
Makes a movie in which ROIs are overlaid on GFP images from first frame onwards.
NOTE: user can define the length of the movie.
User Input: *Folder directory with one experiment*
Data In: *alignParams.mat; /GFPimages/image files/ ;trx_FBI.mat*
Data Out: *FBImovie_GFP_ROI.avi*

3) /FBI_Visualize/FBI_movie_overlay_ROI_GFP_reverse.m
Makes a movie in which ROIs are overlaid on GFP images from last frame backwards.
NOTE: user can define the length of the movie.
User Input: *Folder directory with one experiment*
Data In: *alignParams.mat; /GFPimages/image files/ ;trx_FBI.mat*
Data Out: *FBImovie_GFP_ROI.avi*

Scripts for fixing data manually:
Allow the user to change an assignment for a given fly:

1) /FBI_Identity/FBI_set_identity.m
Allows the user to set the identities of all flies.
NOTE: Overwrites trx_ASSIGN.mat since assumes no solveID.
User Input: *Folder directory with one experiment*

Data In: *user defined trx.mat file*
Data Out: *trx_ASSIGN.mat*

2) /FBI_Identity/FBI_fix_identity.m
Allows the user to fix a mis-identified fly.
NOTE: Opens and uses existing trx_ASSIGN.mat
User Input: *Folder directory with one experiment*
Data In: *trx_ASSIGN.mat*
Data Out: *trx_ASSIGN.mat*

Example parameter files:
Files to modify to set initial parameters.
1) /FBI_example_params/alignParams.mat
From **FBI_AlignImages.m**. Parameters to overlay IR and GFP images.

TFORM – data structure for scaling operation
Height – image height
Width – image width
xAdd – additional shift in x axis
yAdd – additional shift in y axis

2) /FBI_example_params/assignParams.mat
For **FBI_Process_Batch.m**. Parameters for processing and identifying.

numDRK - number of non-GFP flies expected
numGFP - number of GFP flies expected
ratio - ratio of metrics *max5% ratio* / *skewness*
trxName – tracking (Ctrax trx structure) data naming convention used