

## 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/squid>

For our experiments we run the cameras at 20 fps. To post-process data with the provided shell scripts, name the pin controlling the excitation LED “blue” in the *sQuid* 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* imaging data. This can be accomplished by installing and using the provided shell scripts. Mplayer/mencoder must be installed first.

#### **Download:**

<http://lis.epfl.ch/FBI>

#### **Script 1) batch\_fluo\_process**

The user must run this script in the directory containing all of the experimental folders. This script runs the post-processing shell script *fluo\_process* on each folder. This script is adapted from a script written for Ctrax batch processing (Kristen Branson).

#### **Script 2) fluo\_process**

NOTE: The user must update the name of folders pointing to image files to the appropriate names associated with their camera ID numbers.

*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 .avi 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. *Nat. Methods* 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. This yields a .mat file (e.g. movie\_FIX.mat) with the structure, *trx*, containing fly specific structures with x, y, orientation, and size data needed for FBI processing. To accommodate another data format with the same information, some simple modifications must be made to FBI post-processing Matlab scripts.

### **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: This file naming convention can be different but must be noted in *assignParams.mat* 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 in serial.

## **Post-processing FBI data**

### **General Matlab scripts:**

#### **Script 1) /FBI\_PreProcess/FBI\_PrepTrx.m**

Fixes first frame theta to second frame theta & 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*)

#### **Script 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*)

#### **Script 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*)

#### **Script 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*)

#### **Script 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*

### **Matlab scripts for batch processing:**

To process multiple experiments serially:

#### **Script 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.*

### **Matlab scripts for visualizing data:**

Allow the user to visually inspect results:

#### **Script 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 behavioral\_microarray folder must be set in the Matlab path.

*User Input:*             *location of .sbmf & trx\_ASSIGN.mat*  
*Data In:*                 *.sbmf; trx\_ASSIGN.mat*

#### **Script 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*

#### **Script 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*

### **Matlab scripts for fixing data manually:**

Allow the user to change an assignment for a given fly.

### **Script 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`

### **Script 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`

### **Matlab parameter files:**

Files to modify to set initial parameters:

#### **File 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

#### **File 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