

1. OVERVIEW

- a. Current release of Live imaging Tool Kit (LiT) package includes three executables (software files) named "**isems**", "**imgshift**", and "**qtenc**", sample images and manual (this file). **isems** estimates the shift of objects in time-lapse stacks and stores the shift values in .is2 files. **imgshift** converts original time-lapse stacks to shift-corrected stacks based on the information of .is2 file. **qtenc** encodes QuickTime movie files from time-lapse stacks.
- b. **isems** is an implementation of the iSEMS algorithm that was described in detail in [Kato and Hayashi 2008](#) (PMID:18422683 | doi:10.1111/j.1440-169X.2008.01029.x).

2. REQUIREMENT

- a. Basic knowledge of Unix commands is required to operate the tools in command-line.¹ All the tools are built as a universal binary and have been confirmed to work on PowerPC, and Intel based Macs running Mac OS X 10.4.11 or 10.5.2. Those tools do not work in other environments such as earlier versions of Mac OS X, Windows or Linux.

3. Install

- a. Move executables to your favorite directory
 - i. All the LiT tools are command-line applications, which runs on console window displayed by Terminal.app.²
 - ii. In the example shown in the next page, a directory named "imaging-bin"³ was created under home directory⁴ and executables were installed to this directory.
- b. Set path: **IMPORTANT**
 - i. In order for shell to find executables, shell should be configured properly. This can be done by placing **.bash_profile** just under your home directory⁵, using editor such as "vi" or "nano" that run on console. If those operations are not clear to you, consult UNIX

¹ Minimally you will need commands such as "ls" and "cd" to navigate through directories. Brief description can be found in the following sites: <http://www.computerhope.com/unix/overview.htm> (English), <http://cyberam.dip.jp/> (Japanese).

² "Terminal.app" is present in "/Applications/Utilities" directory of MacOS X.

³ Directory (folder) name can be arbitrarily chosen

⁴ # home directory is the directory named after your login name, placed under the /Users directory.

⁵ Files having "." at the beginning of file name is treated as invisible file by system, and you can find these 'dotted-file' by the command ls (list) with option -a. , You may need to create .bash_profile if it does not exist.

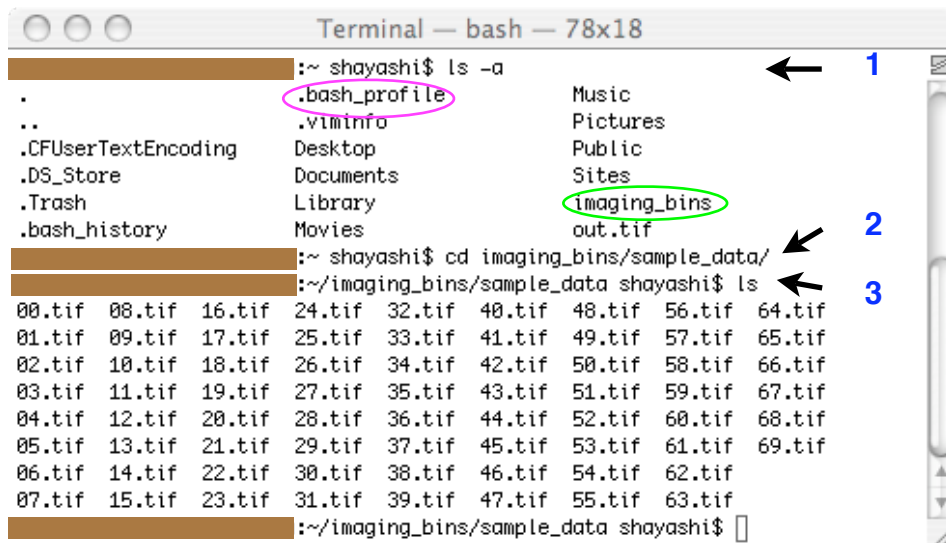
experts in your neighborhood. Edit **.bash_profile** as below. Terminal should be closed and re-opened to reload your setting.

```
PATH="$HOME/imaging_bin:$HOME/bin:$PATH"
export PATH
```

WARNING: Lines of **.bash_profile** should be terminated with LF (linefeed).

c. Example

Here the operator checked the content in the home directory including hidden files using `ls -a` command (1), changed directory to `imaging_bins/sample_data/` (2) and confirmed its content (3).



A terminal window titled "Terminal — bash — 78x18" showing a series of commands and their outputs. The first command is `ls -a`, which lists the contents of the home directory, including hidden files like `.bash_profile` and `.viminfo`, and standard directories like `Music`, `Pictures`, and `Public`. The second command is `cd imaging_bins/sample_data/`, which changes the current directory. The third command is `ls`, which lists the contents of the `sample_data` directory, showing a grid of TIFF files named `00.tif` through `63.tif`. Annotations with arrows and numbers 1, 2, and 3 point to the first, second, and third commands respectively. In the first command's output, `.bash_profile` is circled in pink and `imaging_bins` is circled in green.

```
Terminal — bash — 78x18
~ shayashi$ ls -a
.          .bash_profile  Music
..         .viminfo       Pictures
.CFUserTextEncoding Desktop      Public
.DS_Store  Documents     Sites
.Trash     Library      imaging_bins
.bash_history Movies        out.tif

~ shayashi$ cd imaging_bins/sample_data/
~/imaging_bins/sample_data shayashi$ ls
00.tif 08.tif 16.tif 24.tif 32.tif 40.tif 48.tif 56.tif 64.tif
01.tif 09.tif 17.tif 25.tif 33.tif 41.tif 49.tif 57.tif 65.tif
02.tif 10.tif 18.tif 26.tif 34.tif 42.tif 50.tif 58.tif 66.tif
03.tif 11.tif 19.tif 27.tif 35.tif 43.tif 51.tif 59.tif 67.tif
04.tif 12.tif 20.tif 28.tif 36.tif 44.tif 52.tif 60.tif 68.tif
05.tif 13.tif 21.tif 29.tif 37.tif 45.tif 53.tif 61.tif 69.tif
06.tif 14.tif 22.tif 30.tif 38.tif 46.tif 54.tif 62.tif
07.tif 15.tif 23.tif 31.tif 39.tif 47.tif 55.tif 63.tif

~/imaging_bins/sample_data shayashi$
```

4. Execute

- isems**, **imgshift** and **qtenc** can process several image types supported by Cocoa framework, including TIFF, JPEG, PNG. multi-paged TIFF is not supported.
- To execute the tool, launch "Terminal.app" and type a name of executable followed by options and image file paths that you are going to process.
- For example, to simply estimate shifts of a sequence of tiff files with **iSEMS**, just type like below:⁶

```
$ isems gonnaprocess*.tif
```

⁶ Note that '\$' is the "prompt" put by shell and "*" means wildcard specifying multiple files. If you are not familiar with this kind of operation, please consult documentations on the UNIX environment.

- d. Each of tools has several options. Short description of available options are displayed by executing tool without any arguments.

5. **isems**

- a. **isems** calculates positional shift of overall objects in images by minimizing difference of each pixel values between two of adjacent images in a image array. Through the process, shift values are estimated for every pair of adjacent images, and stored in plain text named "shifts.is2" at the current directory. Path for the file can be modified optionally. **isems** itself does not shift the image, **imgshift** does it instead.
- b. Random noise in images that are acquired with high gain or PMT greatly affects shift estimation by **isems**. Try median and gaussian blur pre-filters available in **isems**. Try setting of 1 or 2 for each filters. Assignment of larger values greatly increase execution time.
- c. Options

-f PATH

Save shift-estimation result to file PATH. iSEMS stores estimation result in text file named "shifts.is2" as default.

-m NUM

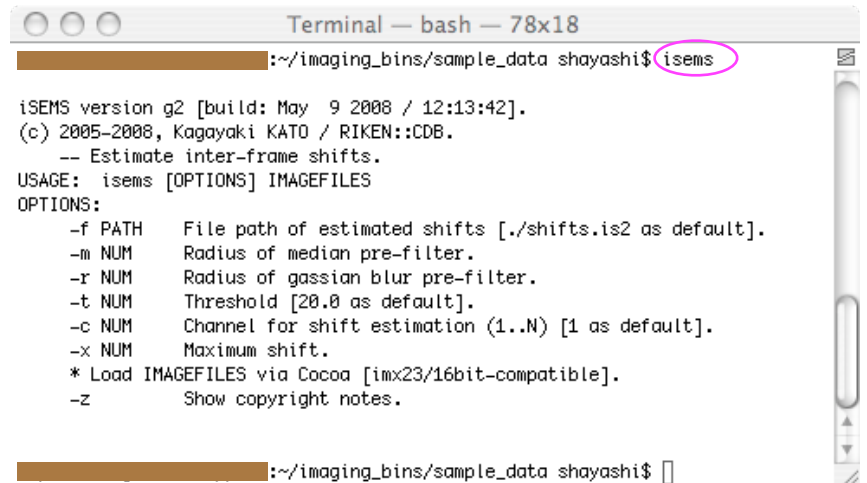
Apply median filter to every images before shift estimation. Radius of filter is specified by NUM. Note that given images are not altered by this operation.

-r NUM

Apply Gaussian blur filter to every images before shift estimation. Radius of filter is specified by NUM. Note that given images are not altered by this operation.

When both of pre-filter options (-m and -r) are turned on, iSEMS do median filter first, then apply gaussian blur.

-t NUM



```
Terminal — bash — 78x18
~/imaging_bins/sample_data shayashi$ isems
iSEMS version g2 [build: May  9 2008 / 12:13:42].
(c) 2005-2008, Kagayaki KATO / RIKEN::CDB.
-- Estimate inter-frame shifts.
USAGE: isems [OPTIONS] IMAGEFILES
OPTIONS:
  -f PATH    File path of estimated shifts [./shifts.is2 as default].
  -m NUM     Radius of median pre-filter.
  -r NUM     Radius of gaussian blur pre-filter.
  -t NUM     Threshold [20.0 as default].
  -c NUM     Channel for shift estimation (1..N) [1 as default].
  -x NUM     Maximum shift.
  * Load IMAGEFILES via Cocoa [imx23/16bit-compatible].
  -z        Show copyright notes.

~/imaging_bins/sample_data shayashi$
```

Set maximum limit of estimated variance to NUM. iSEMS puts shift to zero when the minimal variance⁷ of estimation is greater than this value. Default is 20.0.

-c NUM

Channel for estimation. NUM starts from one. This option is required for multi-channel images.

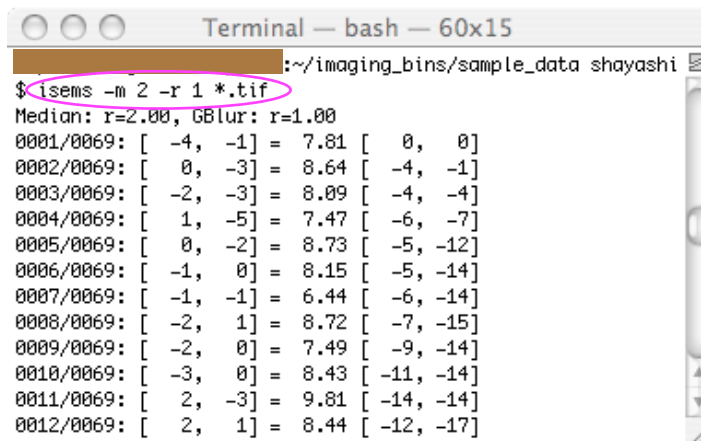
-x NUM

Set maximum pixels allowed to shift.

d. Example

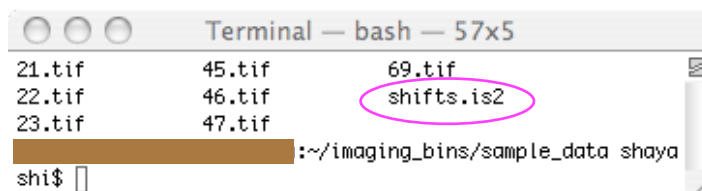
```
$ isems -m 2 -r 1 *.tif
```

- i. This operation applies median pre-filter (radius = 2) and Gaussian blur filter (radius = 1) to all TIFF files in the same directory and estimates image shift.



```
Terminal — bash — 60x15
~/imaging_bins/sample_data shayashi
$ isems -m 2 -r 1 *.tif
Median: r=2.00, GBlur: r=1.00
0001/0069: [ -4, -1] = 7.81 [ 0, 0]
0002/0069: [ 0, -3] = 8.64 [ -4, -1]
0003/0069: [ -2, -3] = 8.09 [ -4, -4]
0004/0069: [ 1, -5] = 7.47 [ -6, -7]
0005/0069: [ 0, -2] = 8.73 [ -5, -12]
0006/0069: [ -1, 0] = 8.15 [ -5, -14]
0007/0069: [ -1, -1] = 6.44 [ -6, -14]
0008/0069: [ -2, 1] = 8.72 [ -7, -15]
0009/0069: [ -2, 0] = 7.49 [ -9, -14]
0010/0069: [ -3, 0] = 8.43 [ -11, -14]
0011/0069: [ 2, -3] = 9.81 [ -14, -14]
0012/0069: [ 2, 1] = 8.44 [ -12, -17]
```

- ii. Shift estimation is shown in the Terminal window and stored as a shifts.is2 file in the same directory.



```
Terminal — bash — 57x5
21.tif      45.tif      69.tif
22.tif      46.tif      shifts.is2
23.tif      47.tif
~/imaging_bins/sample_data shaya
shi$
```

⁷ "variance" indicates the measure of difference between two adjacent frames used in iSEMS. It is the total variance of subtracted pixel intensities between the corresponding positions of two adjacent frames. See Kato and Hayashi, 2008 for more detail.

6. imgshift

- a. **imgshift** shifts given images by the pixel numbers specified in "shifts.is2", and write shifted images to current directory. Shifted images are stored as TIF format in the directory of original images.

b. Options

-f PATH [REQUIRED]

Path for shift values in ".is2" format, generated by iSEMS.

-m

Encode results as a QuickTime movie.

-r NUM

Set frame rate (frames per second) of movie. Work when '-m' option is turned on. 10 (fps) as default.

-S

Skip generating shifted TIF format images. In this case -m option should be turned on to see the result in a QuickTime movie.

-g

Draw grid lines on result images in every 10 pixels.

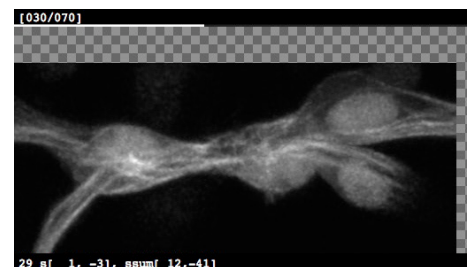
-b

NUM Set background gray level by floating point value from 0.0 through 1.0.

c. Example

i. `$ imgshift -f shifts.is2 -m -r 24 *.tif`

- ii. This operation shifts images based on the data in shifts.is2 and encode as QuickTime movie of 24 fps shown below (4). Shifted images are stored in the same directory (00.mov, magenta circle). Note that shifted images are saved in the same directory (*.shift.tif, green circle).



```
Terminal — bash — 62x8
~/imaging_bins/sample_data shayashi$
imgshift -f shifts.is2 -m -r 24 *.tif
70/ 70 [-094, 0060]
hayashishigekinokonpyuta:~/imaging_bins/sample_data shayashi$
ls
00.mov      23.tif      47.tif
00.shift.tif 24.shift.tif 48.shift.tif
00.tif      24.tif      48.tif
```

7. qtenc

a. **qtenc** is a terminal based QuickTime movie encoder. It loads given image files and encode them as a compressed movie by "Motion JPEG A" codec with "Best quality" option. Stacks of original TIF format images or processed images after **imgshift** treatment may be formatted for presentation/publication grade movies.

b. Options

-d STRING

Set annotation, drawn at the bottom-left of the movie frames.

-o PATH

Path for the encoded movie.

-i NUM

Set time stamps drawn at the top-right corner of the movie frames. NUM is frame per sec.

-r NUM

Set frame rate of the movie (in frames per second). 10 fps is default.

-s NUM

A scale bar of ten microns is drawn at the bottom-right corner of the movie. Set magnification by NUM (micron per pixel).

-l NUM

Set scale bar length in micron. Valid if '-s' turned on.

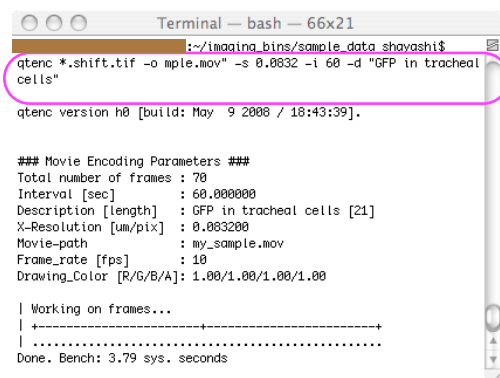
-c NUM NUM NUM NUM

Set color of drawing by color component (Red, Green, Blue, and Alpha) values from 0.0 to 1.0. Each of color components should be separated by white space. [1.0 1.0 1.0 1.0] as default.

c. Example

i. `$ qtenc *.shift.tif -o "my_sample.mov" -s 0.0832 -i 60 -d "GFP in tracheal cells"`

ii. Encode shifted images (*.shift.tif) as "my_sample.mov" with annotation string. Scale bar is set to 0.0832 microns/pixel (magnification of images) and time stamp is 60 seconds per frame.

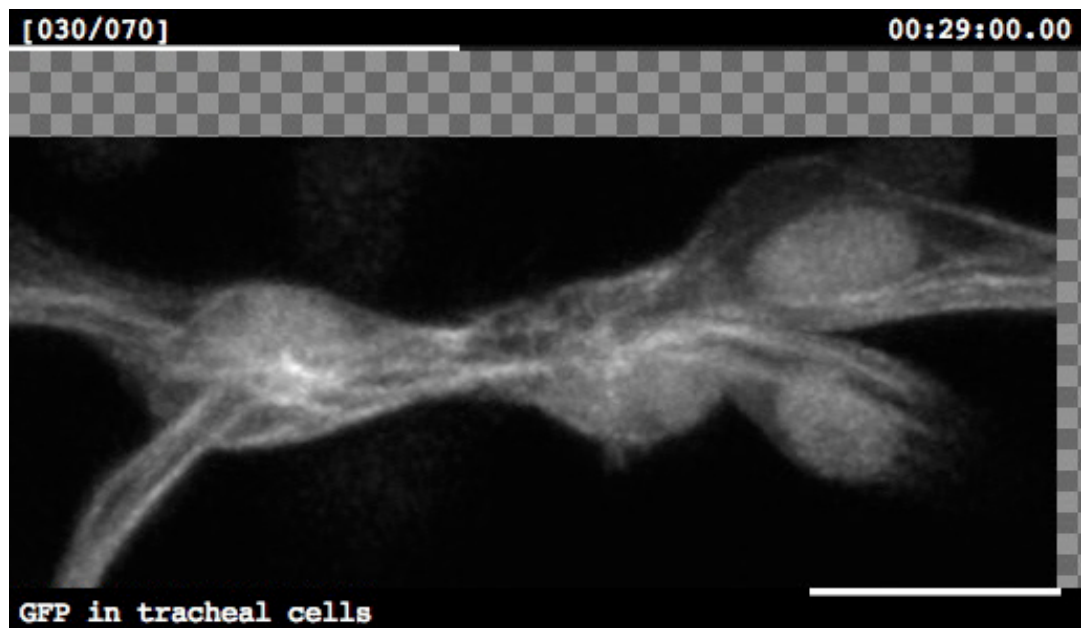


```
Terminal -- bash -- 66x21
~/imaging_bins/sample_data/shayashi$
qtenc *.shift.tif -o mple.mov" -s 0.0832 -i 60 -d "GFP in tracheal
cells"
qtenc version h0 [build: May 9 2008 / 18:43:39].

### Movie Encoding Parameters ###
Total number of frames : 70
Interval [sec] : 60.000000
Description [length] : GFP in tracheal cells [21]
X-Resolution [um/pix] : 0.083200
Movie-path : my_sample.mov
Frame_rate [fps] : 10
Drawing_Color [R/G/B/A]: 1.00/1.00/1.00/1.00

| Working on frames...
| +-----+
| .....
Done. Bench: 3.79 sys. seconds
```

iii. Example of isems-treated, annotated movie (snap shot).



iv. Original movie annotated by qtenc.

