Methods for Video Analysis in Bio-medical Images (an ImageJ based tutotial)

A few links : http://gibbs.engr.ccny.cuny.edu/technical/Tracking/RoachTrack.php

for MATLAB fans *or* http://fiji.sc/Fiji or http://icy.bioimageanalysis.org/ or http://www.ipol.im/

I chose Fiji. Install Fiji in your home directory if needed.
Resources: http://www.math-info.univ-paris5.fr/~lomn/Cours/CV/Material/ (if needed under CV/SeqVideo/Material/ also)

Part 1 : Optical Flow

- Start by applying a **small** rigid transform to an image like the *blobs.gif* one. ***Menu Transform/Interactive Rigid (exists in Fiji : rotate 5 degrees and translate 5 pixels if not available)***
- Save *blobs.gif* as *blobs1.gif* and the transformed one as *blobs2.gif*.
- Open the two images and make a stack out of both of them. ***Menu Images/Images to Stack (or so)***
- Apply the plugin ***Optical Flow/Gaussian approach*** *and check the Display color map box***.** Analyze the results. *(exists in Fiji in the menu Plugins/Optic Flow)*

→ What does it mean Gaussian here ?

- Do it again after applying an ***Integral Image Filter*** like the ***Mean***.

→ Are the results very different ? Do you know the difference between Mean and Median filtering ?

- From *books*, create an image sequence by importation of the folder *books (**Images to Track**)*. Then apply image processing on it for pre-processing and then use the ***Optical Flow*** menus.

- Then compute various optical flows with ***bij/FlowJ*** *(install the plugin from http://bij.isi.uu.nl/ and http://webscreen.ophth.uiowa.edu/bij/flowj.htm or my website, see also Plugins_Menu.pdf to see how to install a plugin in Fiji) (exists in Fiji in the menu Analyze/Optic Flow)*

Part 2 : ffmepg to manipulate video sequence

It is a tool very useful for video editing (linket to the codev library lib-av). Find below examples :

```
ffmpeg -i input.avi -c:a aac -b:a 128k -c:v libx264 -crf 23 output.mp4
ffmpeg -i slow.mp4 -s 320x240 -c:a copy smallslow.mp4
ffmpeg -i video.avi -an -vcodec rawvideo -y video2.avi
ffmpeg -i redcar.mp4  -i redcareverse.mp4 -filter_complex "blend=all_mode='overlay':all_opacity=1.0"  output3.mp4
```

You need to distinguish between the container mp4 and the coding scheme like h264 bitstream see an example from **https://stackoverflow.com/questions/7333232/concatenate-two-mp4-files-using-ffmpeg)**

```
ffmpeg -i input1.mp4 -c copy -bsf:v h264_mp4toannexb -f mpegts input1.ts
ffmpeg -i input2.mp4 -c copy -bsf:v h264_mp4toannexb -f mpegts input2.ts
ffmpeg -i "concat:input1.ts|input2.ts" -c copy output.mp4
```

- Please create the videos *redcaroverlay.mp4* video.
- If any issue in reading an *avi* file or displaying it, use this library to change parameters (like the frame rate etc.)

Part 3 : Tracking

- Open the image *Track_for_TrakMate* from the samples menu.
- It appears that the image is not always loadable. As a matter of fact, if you search for doc about this plugin you will find a video of biological spots moving in a sequence (like the one in bio2.avi). Then if not loadable, use the simul2.ijm to simulate ten random trajectories of spots and test the plugin.
- Project the stacks on one image with max intensity (Menu ***Image/Stack/ZProject***)
- Threshold it with Adjust threshold.
- Try to use the TrackMate plugin (https://imagej.net/Getting_started_with_TrackMate and https://imagej.net/TrackMate)
- Then launch the tracker ***MTrack2*** *(http://fiji.sc/MTrack2 and http://www.imagescience.org/meijering/software/mtrackj/)*
- Redo it with another threshold to remove or add noise.
- Which tracks to keep ?

- Explore the Python examples in the Repertory Python

  Use the lkOpticalFlow2.py program to track the 10 cells created by simul.ijm.
  And explain the parameters you set.