HF_IDS_Cam: fast video capture with ImageJ for real-time analysis

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Installation

The installation procedure is available online at http://pccv.univ-tours.fr/ImageJ/HF_IDS_Cam2/.

Camera main functions

Frame rate (FPS): camera frame rate corresponds to the number of images captured by the camera every second and displayed by ImageJ. The frame rate is limited by camera capabilities, camera settings and data transfer rate to the computer.

Gain: Adjusts the brightness of the image.

Gain auto: when checked, the gain is automatically and dynamically adjusted, depending on the light captured by the camera. The reference mean grey level value is determined by the value of "brightness reference". Gain auto must be unchecked for quantitative fluorescence measurements.

Monochrome/Color Mode: monochrome/color mode sets the coloring and the pixel depth according to camera capabilities.

Binning: binning allows the reduction of the images spatial resolution by a defined factor (2, 4, 8…) to increase their brightness (pixel are pooled), and the diminution of the amount of data transferred to the computer. In some cases, binning can increase the maximum frame rate.

Subsampling: subsampling reduces the spatial resolution of images by a defined factor, which in turn diminishes the amount of data transferred to the computer. In some cases, subsampling can increase the maximum frame rate.

Pixel clock: determines at what rate pixels are acquired.

Exposure time: determines the exposure time for each pixel. A long exposure time increases the sensitivity, but decreases the acquisition rate.

Shutter auto: when checked, the exposure time is automatically adjusted depending on the light captured by the camera. As for "Gain auto", the reference mean grey level value is determined by the value of "brightness reference". Shutter auto must be unchecked for quantitative fluorescence measurements.
N.B. When both Shutter auto and Gain auto are checked, the exposure time and the gain are adjusted in this order.

Brightness reference: defines the target mean grey level value.

Area Of Interest (AOI): allows the limitation of the sensor area read by the camera. Selection of an AOI decreases the vision field of the camera and the amount of data transferred to the computer.

Tips to optimize video acquisition parameters

- To **increase the maximum frame rate**, several parameters can be optimized:
    - the AOI field must be limited to the object. To increase the size of the field, binning or/and subsampling can be used;
    - camera must be oriented in order to have the minimum number of horizontal pixel lines;
    - pixel clock can be increased;
    - USB3 has to be preferred to USB2 port;
    - exposure time can be reduced;
    - pixel depth may be set to 8-bits if measured light variation are quite important.

N.B. These setting may increase the noise in the video and decrease the light sensitivity.

- To **increase the sensitivity** (for low light recordings):
    - frame rate must be set to the minimum acceptable value;
    - exposure time must be set to maximum;
    - pixel depth can be set to maximum value (usually 16-bits);
    - gain should be set considering the maximum signal-to-noise ratio.

Keyboard shortcuts

Keyboard shortcuts are indicated in the camera control dialog box.

Shortcut	Function
P	**P**ause/Restart video capture
S	Video **S**napshot
A	Create an **A**OI on the selected Region Of Interest (ROI)
Z	**Z**oom on AOI
E	A**E**S (Auto Exposure) on ROI, automatically adjust the exposure for the current ROI defined by the selection
V	Start/Stop **V**ideo recording in RAM (recorded video is displayed at the end of the recording)
R	Predefine **R**ecording time or images number (the maximum duration/number of images is set by default regarding the free memory space)
"Space bar"	Write (mark) in the metadata of the currently recorded image

Synchronization of the camera with other devices (advanced use)

Introduction

Camera can be synchronized and interact with other devices able to send 3-5 V TTL input signals. To acquire this type of signal, a simple two-way "input box" has to be built and connected to the serial (COM) port of the computer.

Building the input box

Material:

- RS232 female socket

- 2x BNC female sockets

- LED

- ~1 kΩ resistor

- multi-strand shielded cable (4-wires)

- 50 Ω BNC cap

Connect the RS232 female socket as follow:
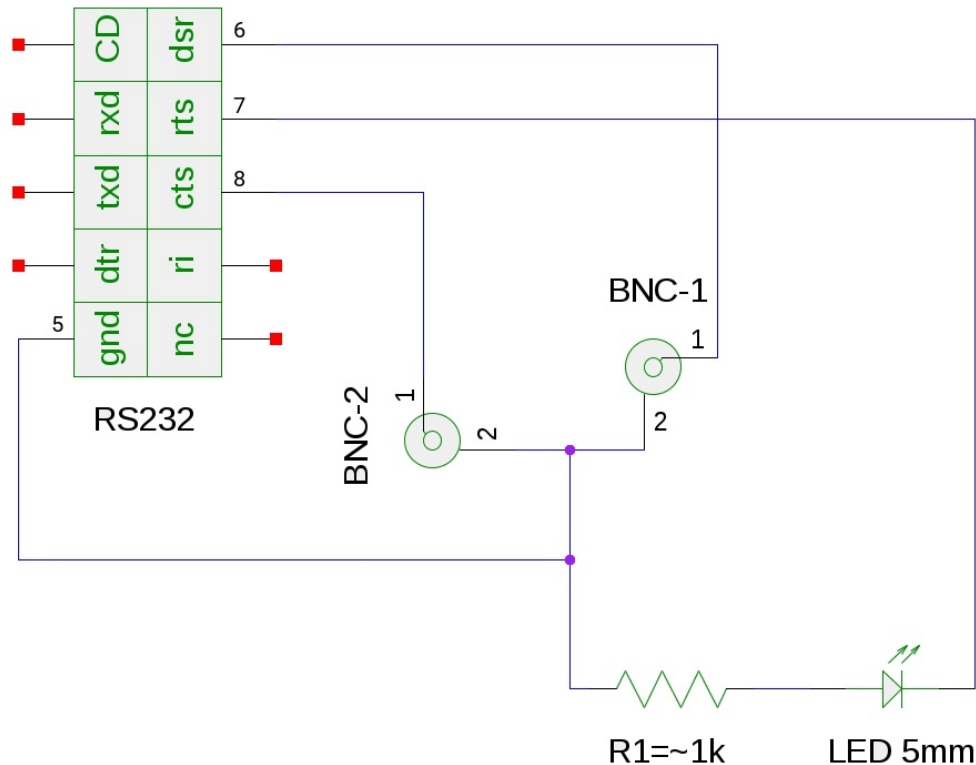
Connect the 50Ω BNC cap to the unused input.

N.B. Ready to use input box can be purchased on request from our laboratory.

Instructions to use the input box

First input (BNC-1: DSR)

The first input is not configurable. When activated, a 'sync' tag is automatically added to the metadata of the image recorded during reception of the signal. This input is dedicated to the acquisition of periodic and recurrent signals, for example in an excitable tissue to indicate when the preparation is electrically stimulated.

Experimental recordings of an electrically stimulated rat ventricular cardiomyocyte are available at http://pccv.univ-tours.fr/ImageJ/HF_IDS_Cam2/dl/Sync-Contraction.tif and http://pccv.univ-tours.fr/ImageJ/HF_IDS_Cam2/dl/Sync-FluoCa.tif.(ces liens ne fonctionnent pas) The cardiomyocyte has been loaded with a fluorescent calcium-sensitive dye (Fluo-4). It has then been superfused with a physiological Tyrode solution and electrically stimulated at 1 Hz with a pair of platinum electrodes, as previously described [1]. The 'synchronization output' of the stimulator has been connected to the first input (BNC-1) of the input box, in order to tag the image with 'sync' when a stimulation occurs. Transmitted light and fluorescence signals (505-525 nm) have been recorded with the IDS UI-3060-CP.

A macro showing a method to analyze such files is available on github repository https://github.com/PCCV/HF_IDS_Cam (Offline_analysis_macro_demo.ijm). When executed, the macro extracts each 'sync' and 'mark' (if applicable) in the meta-data of the video file. The mean grey level profile of each image is then plotted *vs.* time, and 'sync' and 'mark' positions are indicated by green and blue lines in the plot. The red cursor indicates the time position of the active slice.

Second input (BNC-2: CTS)

The user has to choose the action that will be executed by the software upon reception of the signal on the second input (BNC-2). For that, the user has to click the Set Synch Options button in the control panel, where he can select one among the following options:

1. **Tag the current image with 'mark' in the metadata**;

2. **Start/Stop the video acquisition** (can be done several times in a row);

3. **Take a picture** (with predefined parameters *e.g.*, exposure time, gain…);

4. **Beep**;

5. **Write value in IJPrefs file** (this is useful to start conditionally an action by script, *i.e.*, for online analyzes).The Online_analysis_macro_example.ijm plot the mean grey level profile of the selected region of interest of the following 100 images after the reception of 'sync' signal. A video showing an example of the macro running is available on github repository https://github.com/PCCV/HF_IDS_Cam (Online_video_analysis_demo.mp4).

Troubleshooting

- No beep: beep function is disabled by default under linux Gnome distribution, it must be reactivated.

- No 'synchronization option' in the camera control panel: java libraries are missing, see http://pccv.univ-tours.fr/ImageJ/getSync/.

- Abnormal input signals are captured: connect the 50 Ω BNC cap to the unused input.

References

1. C. Pasqualin et al., "SarcOptiM for ImageJ: high-frequency online sarcomere length computing on stimulated cardiomyocytes," Am. J. Physiol. - Cell Physiol. **311**(2), C277–C283 (2016) [doi:10.1152/ajpcell.00094.2016].