1. Introduction:

The laser speckle contrast imaging is a widely used, non-invasive and promising tool to monitor blood flow in the human brain. This optical technique is based on the analysis of the back-scattered light signals from the human tissues to understand the blood flow dynamics in the real time. When the coherent light is reflected off of an object, a speckle pattern is formed as shown in the figure 1.

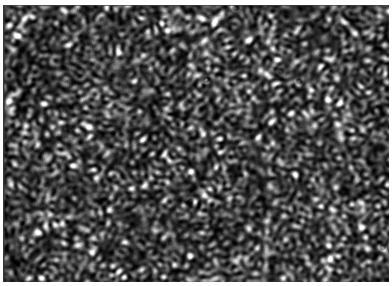


figure 1

2. Hardware details:

The essential hardware used to build the speckle contrast measurement setup are shown in the figure 2.

I.  Integrating sphere: Thorlabs- Part # 2P4

II.  Camera: XIMEA- Part # MC124MG-SY-UB

III.  700 micron diameter pinhole: Thorlabs- Part # P700K

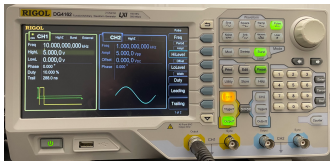
IV.  Rigol arbitrary waveform generator: DG4162

figure 2

3. Speckle measurement setup:

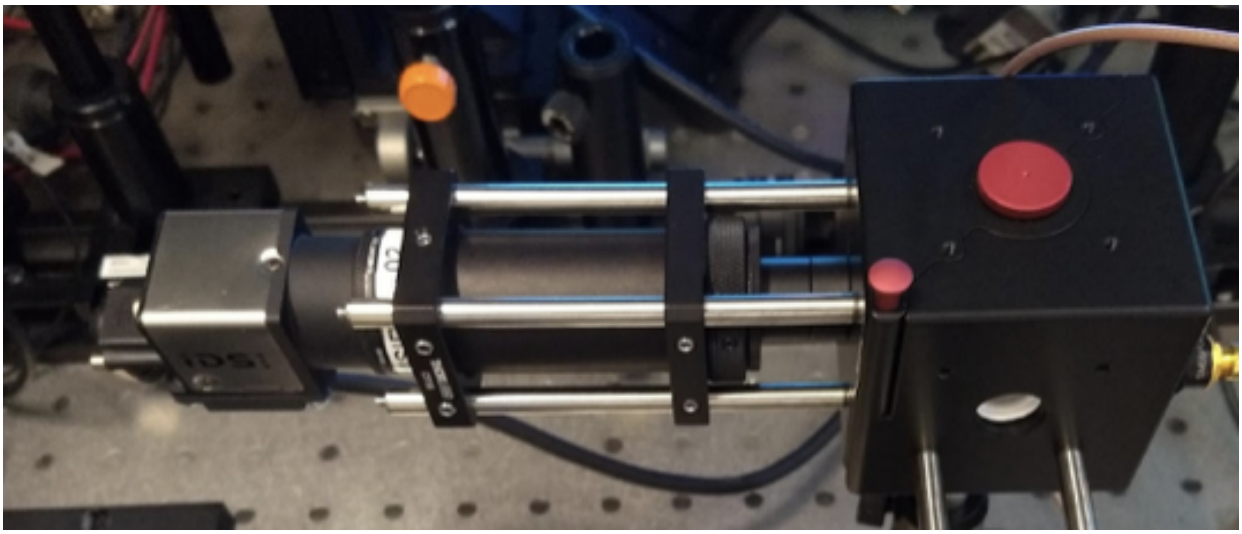


figure 3a

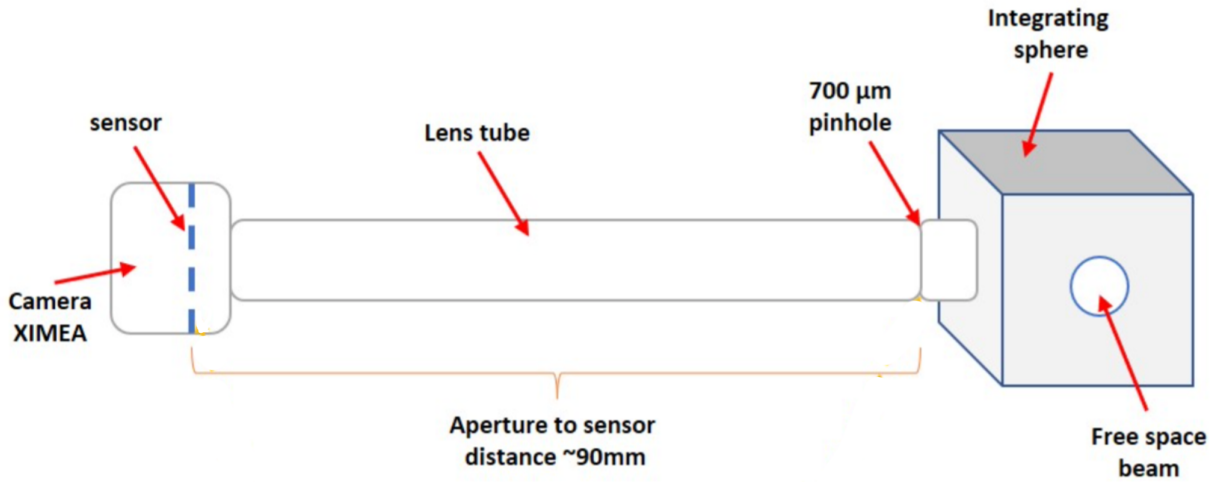


figure 3b

The figure 3a and 3b depict the setup for the speckle contrast measurements. The laser beam enters the integrating cubic integrating sphere. The multiple scattered beams make it through the 700 um pinhole ~90mm away from the XIMEA camera and form a speckle as shown in the figure 1.

4. XIMEA camera set-up and software settings:

4.1. Connect the camera's USB cable to the USB3 connection on the laptop or…

4.2. To synchronize the camera and the laser pulse, connect the IO-USB-Power port on the back of the camera to the trigger output of the laser driver (Thorlabs ITC4020) as shown in figure 4:

figure 4

4.2.1. The Ximea's trigger needs to be delayed to properly not clip the 100 us pulse. Therefore a delay generator (with the settings below) will need to be used. Connect the "TRIGGER OUT TTL 5V" output on the back of the Thorlabs ITC4020 to the "CH1: Mod/TSK/Trig" on the delay generator and the Output of Channel 1 should be connected to the camera's trigger cable (pink = ground/black, gray = positive/red).

figure 5a

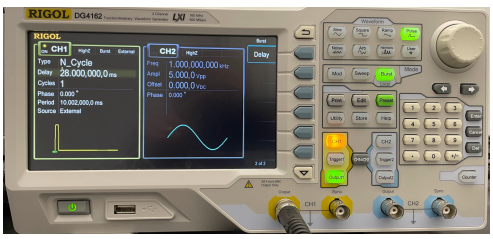


figure 5b

4.3. Open the “XIMEA CamTool” software.
4.4. Image below shows the software parameters (when connected) as explained below:

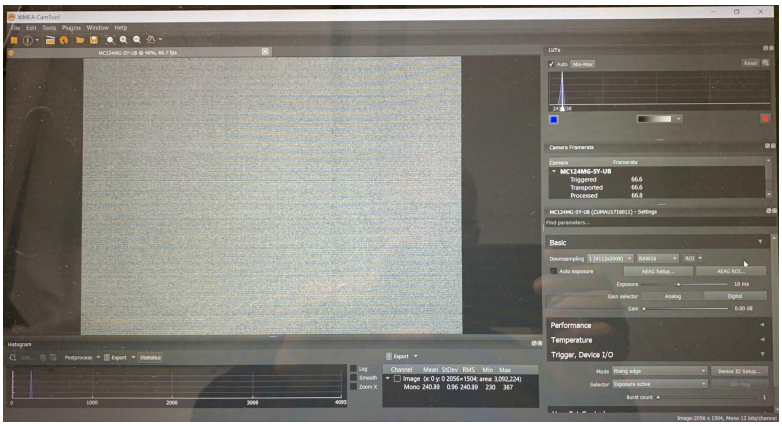


figure 6

4.5. Software settings: Before running the software, complete the following settings and then start image acquisition.

4.5.1. From the “Tools” menu at the top, make sure that the following features are selected: LUTs, Camera Freamerate, Histogram (statistics)

4.5.2. **Basic Setings:**
- 4.5.2.1. No downsampling, choose 1(4112x3008) option
- 4.5.2.2. From the next dropdown menu select “Raw16” option
- 4.5.2.3. Do NOT select “Auto exposure” option
- 4.5.2.4. Set the exposure time to 10ms
- 4.5.2.5. On Gain selector, select Analog
- 4.5.2.6. Set Gain at 0dB
- 4.5.2.7. ROI set on, defined as following (full-frame will not work properly at 30 Hz):
  - 4.5.2.7.1. Offset X: 1032
  - 4.5.2.7.2. Offset Y: 752
  - 4.5.2.7.3. Width: 2056
  - 4.5.2.7.4. Height: 1504

4.5.3. **Performance**:
4.5.3.1. On Control FPS select “free run” option

4.5.4. **Trigger, Device I/O**:
4.5.4.1. Mode: rising edge
4.5.4.2. Device I/O set up: On input for GPI1 select trigger
4.5.4.3. Selector: exposure active
4.5.4.4. Burst count: 0

4.5.5. **LUTs**:
4.5.5.1. Select the “Auto” box

4.5.6. **Other:**
4.5.6.1. Moving forward post Aug. 2022 we should be making sure that the gamma settings of the camera are set to 1.0 to remove any alteration of the raw values.

At this point start acquisition by pressing the run button at the top left of the software and make sure that camera frame rate is the same as laser repetition rate. If they are not the same, repeat “Trigger, Device I/O” settings one more time and also manually change the exposure time and set it back to 10ms again.

4.6. Dark count and signal:
4.6.1. After the measurement is completed, you’ll find a couple of tabs on the software (shown below). Browse through the different tabs to find the dark count and signal. These counts can be found on the bottom of the software as labeled dark and signal. The tab titled “Image from script” contains the dark count and the one titled “MC124MG…” contains detected signals (dark included).

figure 7a

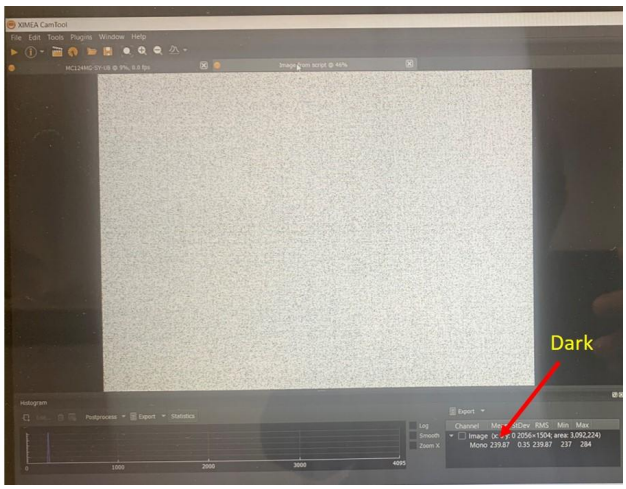


figure 7b

4.7. **Recording speckle contrast measurement**:

4.7.1. Using a script:

[XIMEA_Specke_Contrast]

software measures many images and records the speckle contrast for all images. To do so, from the “Plugins” menu on the top, select “Script Editor”. This opens the script (shown below). Number of images can be set to as many as necessary on the script. The default is 500 images. This script also measures the background noise.

4.7.1.1. https://www.ximea.com/support/wiki/apis/xiapi_manual

4.7.2. First make sure both the tapered amplifier and seed laser are turned off.

4.7.2.1. NOTE, a beam block is much preferred to turning both the seed and TA on and off between every measurement, which can introduce variability depending on how long the seed and TA are left on to stabilize between each measurement.

4.7.3. Next press the Run button on the script. This will measure the background noise

4.7.4. Now turn the seed laser and tapered amplifier and then press ok button to measure the speckle contrast

4.7.5. After the measurement is completed, click on the “Output” section on the lower part of the script and select all (shown below). Now all the data can be copied to an excel sheet.

4.7.6. Select the column with the data on the excel sheet and from the data tab of the excel sheet select “Text to columns” option and choose “comma”, “space” and “Tab delimited” and “finish”.

4.7.7. Now all the data is stored in columns for the 500 images measured. The last column is the speckle contrast, which is calculated by dividing the adjacent columns of standard deviation over average.

4.7.8. The mean and standard deviation of the 500 speckle contrast measurements can be calculated.

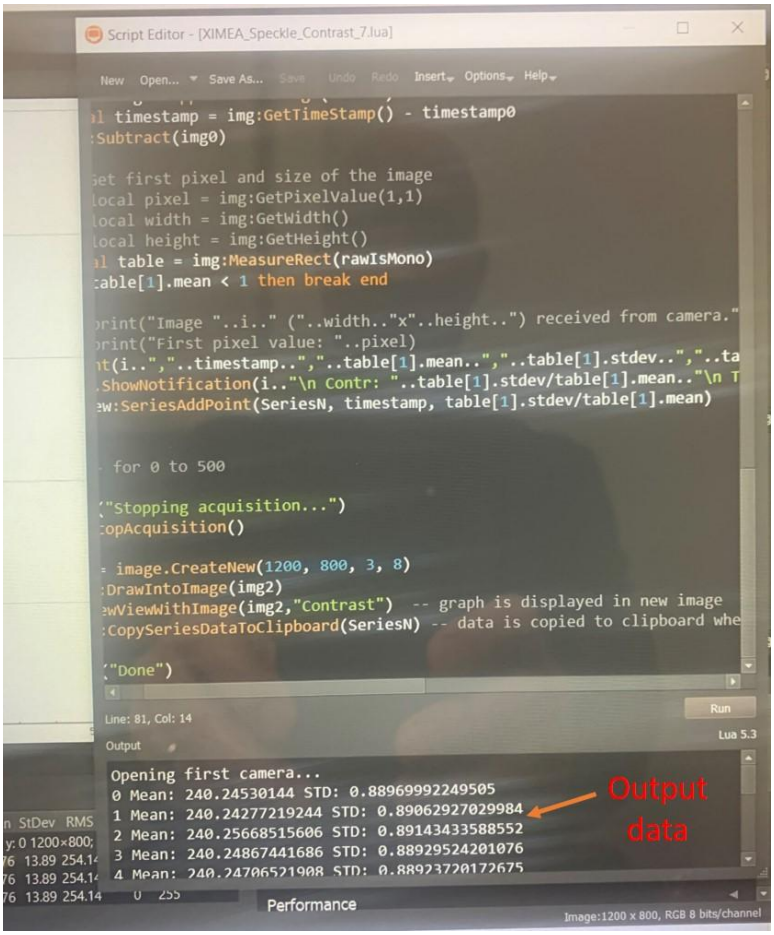


figure 8