## **Supplementary material 1**

## Real-time phase-lag calculation system data flow



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- 1. Function generator: Generates a sinusoidal voltage at 20 Hz to drive the power source of the laser, also supplies the same signal to the PC audio card.
- 2. Camera: Silicon video 643M, EPIX Buffalo Grove IL, Supplies 8-bit grey scale image data of the FOV at 1000 fps to the image acquisition card on the PC.
- 3. Laser power source: Supplies oscillating current to drive the laser diode (2495-Y-5.0 W, 810 nm, JDSU, Milpitas, CA) based on the input signal from the function generator
- 4. PCI Audio card: SB0570L4, Creative Labs, Milipitas CA, converts the analog voltage signal from the function generator into digital values for use by the software bfcam.
- 5. PCIe Image aquistion card: PIXCI® SI1 EPIX Buffalo Grove IL, drives the camera and streams data from the camera into the PC.
- 6. Bfcam program: Custom made C# program for Unix/linux platfroms that relies on OpenCV 2.0, glade, portaudio.h and XCLIB camera library(EPIX Buffalo Grove IL) to drive the camera, perform image acquisition, calculate pixelsum in the ROI, convert the function generator signal through the audio card into an 8-bit digital value. This program extracts pixelsum of each frame and modulation signal intensity at that instant and pipes this data to realtimeanalyze programs.



bfcam\_realtime\_sum.zip

7. RealtimeAnalyze programs: Two Custom made C programs for UNIX/Linux platforms that rely on Gnuplot to collects data streams of pixelsum and modulation signal intensity from bfcam, fit sine functions to each of those signal, compute phaselag and amplitude of the cell response and saves that in a text file.



realtimefastanaly: pipe\_from\_bfcan

(\$ bfcam |./pipe\_from\_bfcam 250 20)

8. Calibration databank: Calibration data used by realtimeanalyze to compensate for systematic lag between camera data and audio signal data encoding. Threshold.txt supplies the minimum and maximum pixelsum limits, corner pixel thresholds to collect data only when exactly one cell in

the FOV. It also has initial values for fit parameters to help the fit function converge faster.

