Grishchuk Lab

Prepared by Vladimir Demidov, Oct 1st 2024

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## Description of the folder content

The zip-folder ‘UFFC software’ includes three folders: ‘Programs’, ‘Calibration files, and ‘Raw data example’. The ‘Programs’ folder has LabVIEW VIs for performing ultrafast force-clamp (UFFC) assay experiments. ‘Calibration files’ holds sample calibration files for converting QPDs voltage response into nanometer position coordinates. The folder ‘Raw data example’ features a sample of raw data collected from UFFC measurement on a pedestal coated with Ndc80c Bonsai.

|  |  |
| --- | --- |
| **File** | **Description** |
| UFFC software\Description\Description.docx | File containing description of the UFFC software package |
| UFFC software\Programs\AOD FC by FPGA 01.19.19\AOD FC by FPGA 01.19.19.vi | LabVIEW program for execution of the UFFC |
| UFFC software\Programs\AOD FC by FPGA 01.19.19\SubVI\V\_nm\_converter\_VD.vi | SubVI required to run AOD FC by FPGA 01.19.19.vi |
| UFFC software\Programs\FPGA\_FeedBack\_AOD\_control 09.04.2020\FPGA\_FeedBack\_AOD\_control 09.04.2020.vi | LabVIEW code used to generate FPGA firmware FPGA\_FeedBack\_AOD\_control 09.04.2020.lvbitx |
| UFFC software\Programs\FPGA\_FeedBack\_AOD\_control 09.04.2020\FPGA\_FeedBack\_AOD\_control 09.04.2020.lvbitx | FPGA firmware for Ultrafast force clamp feedback loop generated from FPGA\_FeedBack\_AOD\_control 09.04.2020.vi using LabVIEW FPGA Module |
| UFFC software\Raw data example\4.0pN\_.txt | Raw data file example generated using provided files |
| UFFC software\Calibration files example\QD1.txt | Example of the calibration file for detector monitoring bead1, x and y directions |
| UFFC software\Calibration files example\QD2.txt | Example of the calibration file for detector monitoring bead2, x and y directions |
| UFFC software\Calibration files example\QD3\_stage.txt | Example of the calibration file for detector monitoring pedestal bead, x and y directions |
| UFFC software\Calibration files example\QD3\_stage\_Z.txt | Example of the calibration file for detector monitoring pedestal bead, z direction |

## Calibration files

Prior to running the UFFC software it is required to carry out positional calibration of QPDs and stiffness calibrations of the traps. While stiffnesses of the traps 1 and 2 are typed in manually in the corresponding boxes as mentioned in section “**Description of the AOD FC by FPGA 01.19.19.vi**”, the output of positional calibration must be organized in the following files (the examples of the files are provided in “Calibration files example” folder of this package):

* 1. “QD1.txt” contains voltage-to-nanometer coefficients C0, C1, …, C7 in two rows. The parameters were obtained after trap1 AOD crisscross calibration (*1, 2*). The first and second rows respectively represent the coefficients for x-, and y-axes. The ultrafast force feedback is carried out along y-axis, so the x-axis direction coefficients are not used. However, the parameters are required to maintain file structure and successful run of the code.
  2. “QD2.txt” features two rows containing the voltage-to-nanometer coefficients C0, C1, …, C7 obtained after trap2 AOD crisscross calibration (*1, 2*). The structure of the file is identical to “QD1.txt”.
  3. “QD3\_stage.txt” consists of two rows having voltage-to-nanometer coefficients C0, C1, …, C7 obtained after pedestal bead stage crisscross calibration. This calibration is identical to the AOD crisscross calibration except the bead motion is driven by the piezo-stage (*3*). The structure of the file is identical to “QD1.txt” and “QD2.txt”.
  4. “QD3\_stage\_Z.txt” represents the voltage-to-nanometer coefficients C0, C1, …, C5 in a single row. The parameters were obtained during pedestal bead stage crisscross calibration mentioned above in (c) and z direction were saved separately.

## Description of the “AOD FC by FPGA 01.19.19.vi”

A screenshot of a computer

Description automatically generatedThe “AOD FC by FPGA 01.19.19.vi” located in folder “AOD FC by FPGA 01.19.19” is the primary program that initiates UFFC and stage stabilization feedback loops and allows adjustment of feedback parameters. Running the program requires calibration files and knowing stiffnesses of traps. It processes and stores QPD data read by FPGA. The front panel of this program is shown below.

Front panel of the “AOD FC by FPGA 01.19.19.vi”

1. To run the “AOD FC by FPGA 01.19.19.vi” all parameters should be specified. Use the table below as a guide.

|  |  |  |
| --- | --- | --- |
| **Box name** | **Parameter description** | **Example** |
| Path to calibration file | System path to the folder with calibration files QD1.txt, QD2.txt, QD3.txt, QD3\_stage.txt, QD3\_stage\_Z.txt | C:\Experiment1\  chamber1\  dumbbell1 |
| Postfix | Text to be added to the end of the name of generated data file | Test |
| K\_QD1, pN/nm | Stiffness of the trap1, pN/nm | 0.08 |
| K\_QD2, pN, nm | Stiffness of the trap2, pN/nm | 0.08 |
| ped # | Number of the pedestal to be tested | 1 |
| Force, pN | Force in pN to be clamped on the dumbbell during measurement | 4 |
| time limit, s | Duration of the measurement in s | 30 |
| boundary, nm | Amplitude of force-driven oscillation in nm. Upon reaching this coordinate (both, in positive and negative direction), the force acting on the dumbbell will be reversed. | 175 |
| Feedback rate, μs | Time interval between acquired data points in μs | 15 |
| Control each nth data point | Parameter that regulates force feedback rate relative to feedback rate. The feedback calculation and trap adjustment are done every nth data point. If this parameter is set to 1, data will be collected, and traps will be adjusted on every data point. If this is set to n>1, the traps will be adjusted only on nth data point, but the data will still be collected for each data frame, i.e. frequency of trap force feedback will be equal to  (Feedback rate, μs)-1n-1. | 2 |
| b | UFFC feedback coefficient | -1 |
| b stab | Stage stabilization feedback coefficient | 0.3 |
| Rate QPD3 | Frequency of QPD3 data acquisition in Hz | 1000 |
| Samples to average, stage stab | Number of data points to average for stage stabilization feedback input | 10 |

1. After specifying the parameters, run the “AOD FC by FPGA 01.19.19.vi” program using Ctrl+R keys. To initiate measurement press “START EXP” button. Buttons “STOP” and “STOP. DON’T save” interrupt measurement with or with no saving the QPD data. The stage stabilization is controlled by the toggle “QPD3 Calibrated?” switch. If the experiment is carried out in the absence of pedestal bead, it should be turned off. Toggles “X(Y,Z) stab ON” switch on and off stage stabilization along indicated axes.
2. At the end of the measurement file 4.0pN\_.txt (force will correspond to the “Force, pN” parameter value) will be generated. This file contains data organized in rows in the following order:
3. Readout from QPD1 y-axis. Format: 10-bit signed integer. Divide by 3276.7 to obtain voltage in V.
4. AOD driver voltage input. Format: 10-bit signed integer. Divide by 3276.7 to obtain voltage in V.
5. Readout from QPD2 y-axis. Format: 10-bit signed integer. Divide by 3276.7 to obtain voltage in V.
6. Readout from QPD3 y-axis. Format: 10-bit signed integer. Divide by 3276.7 to obtain voltage in V.

Saved data is organized into vertical batches with size 4x1500 as explained on the scheme:

A black background with a black square

Description automatically generated with medium confidence

After the measurement is finished and the data are saved, the program will stop automatically.

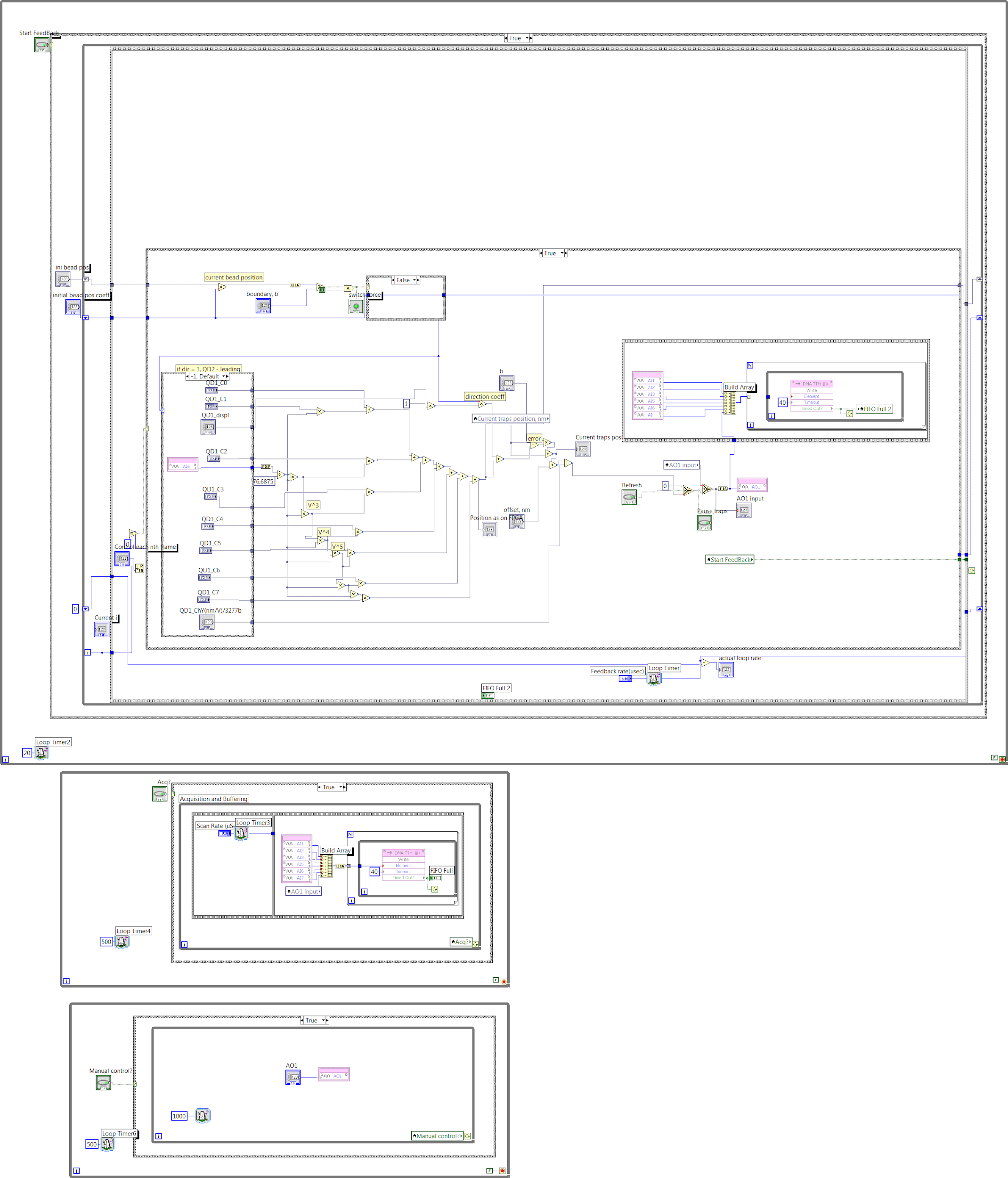
## Description of the “V\_nm\_converter\_VD.vi subVI”

The “V\_nm\_converter\_VD.vi” subVI located in folder “AOD FC by FPGA 01.19.19\SubVI” is a supplementary program that is embedded into the “AOD FC by FPGA 01.19.19.vi”, runs automatically, and does not require any interaction with user. It converts QPD data read by FPGA from voltage into position in nm by plugging the QPD data into 7th order polynomial using formula , where is QPD y-axis reading in volts at time in the power of , – polynomial coefficient. It is ran automatically.

## Description of the “FPGA\_Feedback\_AOD\_control 09.04.2020.lvbitx”

The “FPGA\_Feedback\_AOD\_control 09.04.2020.lvbitx” is the field-programmable gate array (FPGA) bit file. It reconfigures the gate array logic and digital circuit of the FPGA to run the force-clamp feedback calculations and to adjust positions of the traps to maintain desired force acting on the leading bead using FPGA. It is loaded onto FPGA automatically when the main “AOD FC by FPGA 01.19.19.vi” is ran.

“FPGA\_Feedback\_AOD\_control 09.04.2020.lvbitx” bit-file was compiled using LabVIEW FPGA Module from the “FPGA\_Feedback\_AOD\_control 09.04.2020 .vi” force-clamp feedback program shown below.



1

2

3

4

Force-clamp feedback code. The principal components are highlighted in the red squares, each iteration they are executed in the order indicated by numbers. The general idea of each component is provided below.

1. In this part of code, the FPGA reads a voltage values from the y-axis output of QPD1 or QPD2. The QPD is chosen depending on which bead is currently leading. The calibration coefficients for the corresponding QPD are read as “QD1\_C0”, “QD1\_C1” … “QD2\_C2” variables.
2. In this part of code, the position of the leading trapped bead at -th data point is calculated using 7th order polynomial using formula , where is QPD y-axis reading in volts at time in the power of , – polynomial coefficient.
3. In this part of code, the new leading trap position is calculated using formula , where is current leading trap position, is negative feedback coefficient, is desired displacement of the leading bead from the leading trap’s center, calculated from desired force and leading trap’s stiffness, is position of the leading dumbbell bead.
4. In this part of code, the QPD1, QPD2, and QPD3 y-axis FPGA readouts are transferred to the FPGA memory.

## References

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2. S. K. Tripathy, V. M. Demidov, I. V. Gonchar, S. Wu, F. I. Ataullakhanov, E. L. Grishchuk, *Ultrafast Force-Clamp Spectroscopy of Microtubule-Binding Proteins* in *Optical Tweezers: Methods and Protocols , Methods in Molecular Biology, vol. 2478,* A. Gennerich, Ed. (Springer, 2022), pp. 609-650.

3. A. R. Carter, G. M. King, T. A. Ulrich, W. Halsey, D. Alchenberger, T. T. Perkins, Stabilization of an optical microscope to 0.1 nm in three dimensions. *Appl. Opt.* **46**, 421-427 (2007).