**NanoPro 1.0**

Part 2: Installation and Operation Manual



Updated 05.10.2021

## Section 1: Introduction

**NanoPro 1.0** is an affordable, high-quality, user-friendly, and do-it-yourself single-molecule microscope for super-resolution imaging.

**NanoPro 1.0** assembly and operation is described in two parts. Part 1 describes assembly and validation and is published here:

Danial, JSH, Lam, JYL, Wu, Y, Woolley, M, Cheetham, MR, Emin, D, and Klenerman, D. **Constructing a cost-efficient, high-throughput and high-quality single molecule localization microscope for super resolution imaging**.
*Nature Protocols*, 2021.

This is Part 2 of the **NanoPro 1.0** assembly and operation describing installation and operation of the control software.

Updates (i.e. variations) to parts 1 and 2 are published at:
https://github.com/jdanial/NanoPro 1.0

**Section 2: License**

MIT License

**Section 3: Use**

The **NanoPro 1.0** software can only be used to control the microscope described in:

Danial, JSH; Lam, JYL; Wu, Y; Woolley, M; Cheetham, MR; Emin, D; and Klenerman, D.
**Constructing a cost-efficient, high-throughput and high-quality single molecule localization microscope for super resolution imaging**.
*Nature Protocols*, 2021.

Or any variations thereof described in:

https://github.com/jdanial/NanoPro 1.0

Any data or publications arising from the use of **NanoPro 1.0** should cite the following paper:

Danial, JSH; Lam, JYL; Wu, Y; Woolley, M; Cheetham, MR; Emin, D; and Klenerman, D.
**Constructing a cost-efficient, high-throughput and high-quality single molecule localization microscope for super resolution imaging**.
*Nature Protocols*, 2021.

**Section 4: Hardware requirements**

All hardware (mechanical, optical and electronic) that can be used with the **NanoPro 1.0** software is described in:

Danial, JSH; Lam, JYL; Wu, Y; Woolley, M; Cheetham, MR; Emin, D; and Klenerman, D. **Constructing a cost-efficient, high-throughput and high-quality single molecule localization microscope for super resolution imaging**.
*Nature Protocols*, 2021.

Or any variations thereof described in:

https://github.com/jdanial/NanoPro 1.0

**Section 5: Installation**

**Note**:
Where Part 1 is referred to hereafter, see:

Danial, JSH; Lam, JYL; Wu, Y; Woolley, M; Cheetham, MR; Emin, D; and Klenerman, D. **Constructing a cost-efficient, high-throughput and high-quality single molecule localization microscope for super resolution imaging**.
*Nature Protocols*, 2021.

Or any variations thereof described in:

https://github.com/jdanial/NanoPro 1.0

1. Download and install the National Instruments LabVIEW 2020 SP1 64-bit runtime engine from the following link (https://www.ni.com/en-gb/support/downloads/software-products/download.labview.html#369642).
2. Download and install the National Instruments VISA 20.0 from the following link (https://www.ni.com/en-gb/support/downloads/drivers/download.ni-visa.html#346210).
3. Download the **NanoPro 1.0** software file from (https://github.com/jdanial/NanoPro 1.0) and unzip its contents to a new folder on the desktop renamed to 'NanoPro 1.0'. Ensure the contents of the unzipped folder are as follows: two folders named [data] and [Dependencies], five files named [Configuration.csv], [Sequence.csv], [NanoPro 1.0_v1.3.aliases], [NanoPro 1.0_v1.3.exe] and [NanoPro 1.0_v1.3.ini].
4. Download and install the Kinesis 64-bit software for 64-bit windows from the following link (https://www.thorlabs.com/Software/Motion%20Control/KINESIS/Application/v1.14.28/KINESIS%20Install%20x64/kinesis_18247_setup_x64.exe). Copy the contents from the folder path [C:\Program Files\Thorlabs\Kinesis] and paste them into the desktop folder path […\Nano Pro\Dependencies\Thorlabs].
5. Install the [PVCamSDK_Setup.exe] and [PMQI-LabViewSamples_Setup_1.2.2.1.exe] software files provided with the sCMOS camera (item 14 in Part 1). Copy the files [pvcam_helpher_-coloured_v1.dll] and [PVCamNET.dll] from the folder path [C:\Program Files\Photometrics\PMQI-LabViewSamples\Examples\2018\Dependencies] and paste them into the desktop folder path […\Nano Pro\Dependencies\Photometrics].
6. Request the drivers' file from SmarAct (item 15 in Part 1). Unzip the contents of the drivers' file and install the file [CDM21226_Setup.exe]. Install the [MCS2_Installer_2.1.3.exe] software file provided with the MSC2 control system (item 17 in Part 1). During installation, ensure that the check boxes for 'MSC2 Tools and Programs' and 'Support for MSC2 with USB Interface' are checked.
7. Download and install the 64-bit version of the Phidget control software from the following link (https://www.phidgets.com/downloads/phidget22/libraries/windows/Phidget22-x64.exe). Copy the contents from the folder path [C:\Program Files\Phidgets\Phidget22] and paste them into the desktop folder path […\Nano Pro\Dependencies\Phidgets].
8. Download and install ImageJ (item 139 in Part 1) from the following link (https://wsr.imagej.net/distros/win/ij153-win-java8.zip). Download and install ThunderSTORM from the following link (https://zitmen.github.io/thunderstorm/). Follow the installation instructions provided.
9. Open the [Configuration.csv] file in the folder unzipped in step 4. Edit the file using the following information:
    a. Enter the serial number of the analog digital convertor device (item 44 in Part 1) in the field opposite to 'Phidget 1 Serial Number'. The six digit serial number is printed on a white sticker fixed to the analog digital convertor device.
    b. Enter the serial number of the control hub (item 45 in Part 1) in the field opposite to 'Phidget 2 Serial Number'. The six digit serial number is printed on a white sticker fixed to the control hub.

c. Enter the serial number of the position aligner (item 18 in Part 1) in the field opposite to ‘Photo Sensitive Detector Serial Number’. The eight digit serial number is printed on a black sticker fixed to the position aligner.
d. Enter the COM port number of the four-position slider (item 67 in Part 1) in the field opposite to the ‘Filter Slider COM port’. To find the COM port number of the slider, type ‘device manager’ in the search box of Windows 10 located at the bottom left corner of the screen. Left-click the {Device Manager} tab that appears under the Best Match list. A long list of components will be shown. Browse to the component named ‘Ports (COM & LPT)’ and left-click the grey arrow on the left side of the component. The COM port number will be dropped-down.

10. Run the **NanoPro 1.0** software by double-clicking the [NanoPro 1.0_v1.3.exe] software file in the ‘NanoPro 1.0’ folder. The **NanoPro 1.0** software window should appear (see **section 6: software window**) as well as another blank camera view window. The [message box] (see **section 6: software window**) on the **NanoPro 1.0** software window should display ‘Ready’. See Troubleshooting section in Part 1 if this message is not displayed.

## Section 6: Software window

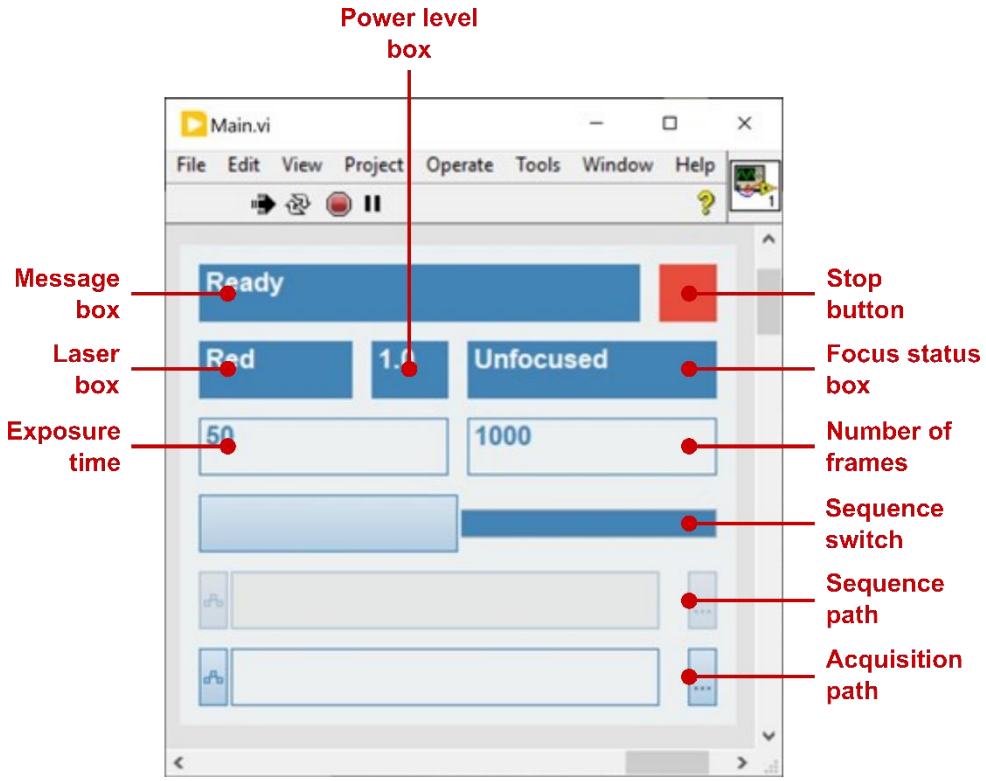


**Figure 1** the **NanoPro 1.0** software window. Components labelled in red are referred to between square brackets [] throughout the manual.

## Section 7: Joystick functions

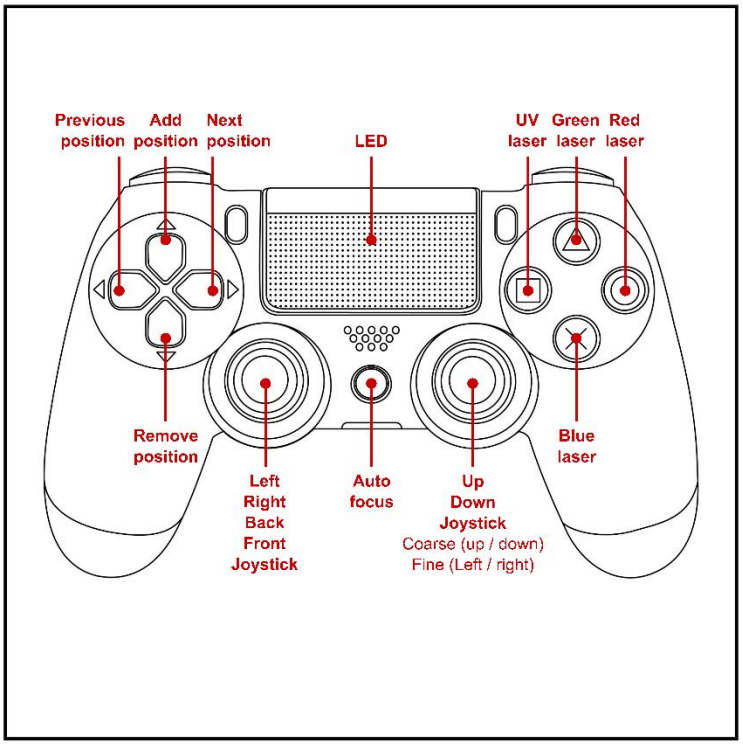


**Figure 2** front side of the control joystick (item 43 in Part 1). Components labelled in red are referred to between square brackets [] throughout the manual.

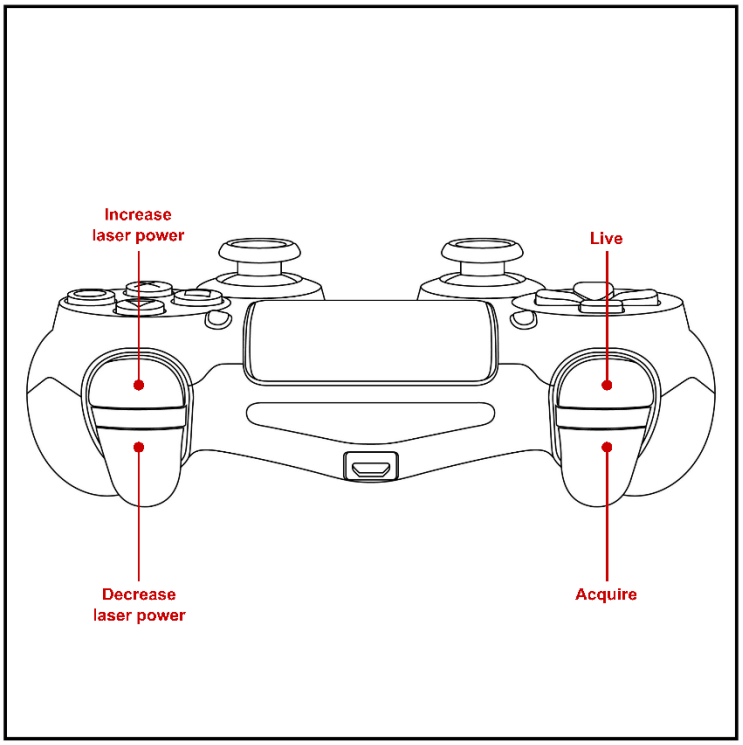


**Figure 3** bank side of the control joystick (item 43 in Part 1). Components labelled in red are referred to between square brackets [] throughout the manual.

## Section 8: Operation

**Note**:
Laser (excitation path) alignment, sample holder alignment, emission path alignment, focus stabilization system alignment, camera pixel size measurement, establishing microscope performance, and sample preparation & mounting are described in Part 1.

### *Subsection 8.1: Live imaging*

1. Switch on the desired laser, ultra violet (405 nm), blue (488 nm), green (561 nm), or red (638 nm) lasers (items 3, 5, 6, or 7 respectively in Part 1), by pressing the [UV laser], [Blue laser], [Green laser], or [Red laser] buttons, respectively, on the control joystick (**Figure 2**)**.** Alternatively, the LED can be switched on by pressing the [LED] button on the control joystick (**Figure 2**).
2. To activate live imaging, press the [Live] button on the control joystick (**Figure 3**). Laser / LED emission will start and 'Live' will be displayed in the [Message box] on the **NanoPro 1.0** software window (**Figure 1**).
3. To de-activate live imaging, press the [Live] button on the control joystick. Laser / LED emission will stop and 'Ready' will be displayed in the [Message box] on the **NanoPro 1.0** software window.

### *Subsection 8.2: Stage movement*

4. The stage (i.e. positioners) can be moved left, right, back, or front by moving the [Left Right Back Front] joystick on the control joystick (**Figure 2**) left, right, back, or front, respectively**.**
5. The stage can be moved up or down by moving the [Up Down] joystick on the control joystick (**Figure 2**) up, or down, respectively, for coarse movement, or left, or right, respectively, for fine movement.

### *Subsection 8.3: Position recording*

**Note**:
Only required for non-periodic multi-sample acquisitions.

6. Stage position can be recorded for multi-sample acquisitions by pressing the [Add position] button on the control joystick (**Figure 2**).
7. The last recording position can be removed by pressing the [Remove position] button on the control joystick (**Figure 2**).
8. The stage can be moved to recorded positions by pressing the [Next position] or [Previous position] buttons on the control joystick (**Figure 2**).

### *Subsection 8.4: Auto focusing*

9. The imaging plane can be locked in focus if 'Focused' is displayed in the [Focus status box] on the **NanoPro 1.0** software window (**Figure 1**). Press the [Auto focus] button on the control joystick (**Figure 2**) to lock the focus. 'Locked' should be displayed in the [Focus status box] on the **NanoPro 1.0** software window.
10. Focus can be unlocked by pressing the [Auto focus] button on the control joystick (**Figure 2**). 'Focused' should be displayed in the [Focus status box] on the **NanoPro 1.0** software window.

***Subsection 8.5: Light source modulation***

**Note**:
Step 10 applies to all lasers. Step 11 applies to LED.

11. Laser power can be increased or decreased by pressing the [Increase laser power] or [Decrease laser power] buttons, respectively. The voltage supplied to the intended laser should be displayed in the [Power level box].

**Warning**:
Do not increase the voltage supplied to the (638 nm) red laser (item 7 in Part 1) above 4V.

***Subsection 8.6: Single acquisition***

11. De-activate live imaging (step 3).
12. Set the [Exposure time] on the **NanoPro 1.0** software window (**Figure 1**) to the desired exposure time (in milliseconds). Set the [Number of frames] on the **NanoPro 1.0** software window to the desired number of frames to acquire.
13. Ensure a folder exists where the data is to be saved.
14. Select the acquisition path by pressing the button with the three dots on the [**Acquisition path**] on the **NanoPro 1.0** software window. A dialogue prompt will appear from which the folder where the date is to be saved can be selected.
15. Press the [Acquire] button on the control joystick (**figure 3**) to start acquisition. Ensure 'Acquire' is displayed in the [Message box] on the **NanoPro 1.0** software window. Gently rest the control joystick on the breadboard (item 64 in Part 1) to reduce perturbations to the microscope.
16. Wait until acquisition is complete or press the [Acquire] button on the control joystick to stop acquisition. Acquisition is complete or stopped when laser / LED emission is automatically de-activated and 'Ready' is displayed in the [Message box] on the **NanoPro 1.0** software window.

***Subsection 8.7: Multi sample acquisition***

**Note**:
Ensure a spreadsheet software, such as Microsoft Excel, is installed on the computer before proceeding with next steps.

17. Open the [Sequence.csv] in the 'NanoPro 1.0' folder created in step 3 of section 5.
18. Edit the file using the following information:
    a. Enter the number of times (i.e. repeats) the mounted sample is to be imaged in the field opposite to 'Number of repeats'. As an example, if you are imaging each well in an ibidi ® µ-Slide 8 Well chambers twice, enter 2 in this field.
    b. Enter the number of wells to be imaged in the direction parallel to the longer side of the optical table (item 63 in Part 1) in the field opposite to 'Number of X wells'. As an example, if you are imaging all wells in an ibidi ® µ-Slide 8 Well chambers, enter 4 in this field. Beware that if you would like to image at non-periodic locations, the entry in this field will be ignored. Use the control joystick to record positions to acquire at (steps 6 to 8).
    c. Enter the number of wells to be imaged in the direction parallel to the shorter side of the optical table in the field opposite to 'Number of Y wells'. As an example, if you are

imaging all wells in an ibidi ® µ-Slide 8 Well chambers, enter 2 in this field. Beware that if you would like to image at non-periodic locations, the entry in this field will be ignored.

d. Enter the number of Field Of Views (FOVs) to be imaged in the direction parallel to the longer side of the optical table in the field opposite to 'Number of X FOVs'. As an example, if you are imaging 4 regions of each well, enter 4 in this field. Beware that the product of this field and the field opposite to 'Number of Y FOVs' has to equal the number of regions to be imaged in each well.
e. Enter the number of Field Of Views (FOVs) to be imaged in the direction parallel to the shorter side of the optical table in the field opposite to 'Number of Y FOVs'. As an example, if you are imaging four regions of each well, enter 4 in this field. Beware that the product of this field and the field opposite to 'Number of X FOVs' has to equal the number of regions to be imaged in each well.
f. Enter the laser(s) to be used for imaging each region in the fields opposite to 'Lasers'. Lasers will be switched on according to the sequence in which they are entered. As an example, if you would like to image each region using the (638 nm) red, then (561 nm) green, lasers (items 3 and 7 in Part 1), enter 'Red' in the field opposite to 'Lasers' and 'Green' in the field opposite to 'Red'. Beware the first letter of each enter laser line has to be upper case.
g. Enter the distance between each well in the direction parallel to the longer side of the optical table in the field opposite to 'X wells distance (um)' in micrometres. As an example, if the distance between each well in the direction parallel to the longer side of the optical table is 4.5 mm, enter 4500 in this field. Beware that if you would like to image at non-periodic locations, the entry in this field will be ignored.
h. Enter the distance between each well in the direction parallel to the shorter side of the optical table in the field opposite to 'Y wells distance (um)' in micrometres. As an example, if the distance between each well in the direction parallel to the shorter side of the optical table is 4.5 mm, enter 4500 in this field. Beware that if you would like to image at non-periodic locations, the entry in this field will be ignored.
i. Enter the distance between each field of view in the direction parallel to the longer side of the optical table in the field opposite to 'X FOVs distance (um)' in micrometres. As an example, if the distance between each well in the direction parallel to the longer side of the optical table is 0.1 mm, enter 100 in this field.
j. Enter the distance between each field of view in the direction parallel to the shorter side of the optical table in the field opposite to 'Y FOVs distance (um)' in micrometres. As an example, if the distance between each well in the direction parallel to the shorter side of the optical table is 0.1 mm, enter 100 in this field.
k. Enter the number of frames to be imaged for each entered laser in the fields opposite to 'Frames'. Each laser will be switched on for the number of frames entered. As an example, if you would like to acquire 10 frames with the red laser, then 10000 frames with green laser, enter 10 in the field opposite to 'Frames' and 10000 in the field opposite to 10.
l. [Optional, only for STORM imaging]. Enter the increase in the power of the (405 nm) ultra violet laser (item 5 in Part 1) after a set duration (see step 18m) in the field opposite to 'UV activation step intensity (V)' in volts. As an example, if you would like to increase the power of the ultra violet laser in steps of 0.1 V, enter 0.1 in the field opposite to 'UV activation step intensity (V)'. Beware, if you are not performing STORM imaging, or would not like to use an activation laser, enter 0 in the field opposite to 'UV activation step intensity (V)'.

m. [Optional, only for STORM imaging]. Enter the time duration after which the power of the ultra violet laser is increased (see step 18m) in the field opposite to ‘UV activation step duration (min)’ in minutes. As an example, if you would like to increase the power of the ultra violet laser after every 10 minutes, enter 10 in the field opposite to ‘UV activation step duration (min)’. Beware, if you are not performing STORM imaging, or would not like to use an activation laser, enter 1000 in the field opposite to ‘UV activation step duration (min)’.

19. If periodic imaging is to be performed (i.e. no positions were recorded using the control joystick), move the positioners to the centre of the well on the back / right corner of the sample using the control joystick (step 4).
20. Switch on each laser entered in step 18f using the control joystick, one after the other (step 1). Increase, or decrease, the power of each laser (step 11).
21. Activate live imaging (step 2).
22. Move the positioners (item 15) downwards (coarse or fine) using the control joystick (step 5) to focus the sample as described in Part 1.
23. Activate the autofocus system (step 9).
24. Deactivate live imaging (step 3)
25. Toggle the [Sequence switch] on the **NanoPro 1.0** software window (**Figure 1**).
26. Select the [Sequence path] by pressing the button with the three dots on the [**Sequence path**] on the **NanoPro 1.0** software window. A dialogue prompt will appear from which the [Sequence.csv] file can be chosen.
27. Perform steps 12 to 16.

### *Subsection 8.8: Switching off software*

28. Press the [Stop] button on the **NanoPro 1.0 software window** (**Figure 1**). Close the **NanoPro 1.0 software window**.

**Note**:
Refer to Part 1 to switch off the entire microscope.