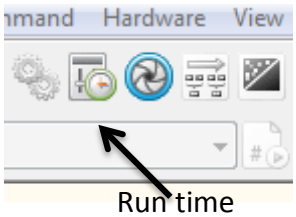
PALM/STORM set-up instructions

1. Switching on

Switch on:

a) microscope (front panel switch)
b) EMCCD camera (round button on the back of the camera)
c) ASI stage (back panel switch on joystick controller)
d) Lasers (mains switch on the wall)
e) C-focus (switch on front)
    I. *check offset voltage (using multimeter)*
    II. *adjust to between -1.0 and -0.5 V if necessary*
    III. *press 'set to midrange' red button*
    IV. *turn off multimeter*
f) Launch Andor Solis software
g) In Hardware/Temperature menu, switch on cooling to -80˚C (takes a few minutes to cool down the camera – double check this as cooling can sometimes switch itself off after a few seconds)
h) Click on 'Run-time' button, uncheck the gain box and set the exposure time to desired value (typically 10-50 ms). The EMCCD gain value can be changed, but should be switched off in 'Run time' whilst using the white light illumination
i) Launch Omicron Control Center (laser control). (In case any laser goes into 'interlock state', switch it off, try the 'reset' option and re-plugging its mini-USB cable)



Run time

2. Setting up PALM/STORM experiment

a) Mount the coverslip into the chamber, add >500 µl PBS **ensure the chamber <u>does not leak</u>!**
b) Place a drop of water on the objective and mount the chamber on the stage. Focus on the coverslip surface using brightfield illumination
c) Fix the chamber in the stage insert with metal clips
d) Replace PBS with 200 µl 0.01% poly-L-lysine (use ready made from Sigma, P4707) and incubate for 10 mins.
e) In the meantime, prepare 100 nm gold nanoparticles (microsphere-nanosphere.com or Sigma) by diluting stock particles 1:10 in PBS (approx. 200 µl required)
f) Replace polylysine with 200 µl diluted nanoparticles (fiducials)
g) Monitor particle sedimentation on the coverslip. After 1-2 mins place solution with imaging solution (>500 µl to ensure surface does not move as it dries)
    PBS for PALM
    STORM imaging buffer* for STORM

3. Calibration

a) Make sure the right filter cube is selected. In case 532 nm (good for FITC, AlexaFluor532,mGeos) is required set the filter wheel to position 1 –visible from left hand side. For 561 nm (MEos, PA-mCherry use cube 2). Either cube can be used for 405, 488 and 647 nm lasers. For PALM, the laser power is typically 40-70mW. For STORM, higher power may be required (100mW or higher)

b) In Andor Solis go to 'Setup Acquisition' –'Binning', select custom and specify 350x350 pixel image size. Position sub-image selection at the laser spot by moving selection box to the centre. Also, untick 'retain absolute coordinates'
c) Make a new folder (FOV1) on the hard drive and indicate its location in Andor Solis: 'Setup Acquisition'-'Autosave' –'location'
d) Find a field of view with a single or a few (well separated) fiducials on the coverslip
e) Switch on desired laser, focus, lock (using the c-focus lock button) and start calibration by launching 'calibration' (can be found from FILE/run program by filename - C:/andor_scripts/calibration.pgm)
f) After recording calibration, python script will ask 'Where was data spooled?' –select the correct folder. A selection of Z-slices will appear. Choose an image with a fiducial clearly visible and follow command prompt instructions to determine the PSF.

NB: remember to unlock c-focus before searching for cell to image

4. Recording the first cell (FOV1)

a) Find a cell with at least one fiducial in the field of view (preferably two or three if imaging in multiple colours)
b) Set exposure time in the 'Run-time' menu (typically 10-50ms). Acquire and save widefield images. Make sure the EMCCD gain is switched off before turning on the brightfield illumination. Also, make sure the polariser insert on the left hand side of the microscope is moved out before recording fluorescence –otherwise single molecules will not be visible.

c) and d) - optional steps for jump tracking

c) Focus on a fiducial in the FOV and press 'zero' on the ASI stage controller. Using the ASI controller focus back on the cell/region of interest (ROI). Note the Z number on the ASI controller (difference in Z plane between 0 (the fiducial) and in-focus ROI)
d) Open the 'track-fid' program and adjust the slice position to the one noted above (NB: take care to include +/- where necessary and convert from mm to x100 nm *[i.e. 0.0001 mm → 1 x100 nm]* and save script, ctrl+s)
e) Press 'focus lock' on the C-focus. In case of severe drift, release focus lock by pressing it, refocusing and locking it again.
f) Start recording by running 'track-fid', launched by FILE/Run program by filename- C:/andor_scripts/track_fid.pgm. (Check script as this may have been altered by previous jump tracking –set slice position back to 0 if no jump tracking used)
g) After recording the first sequence (this will take approx. 20 mins), further sequences can be recorded by running the 'track_fid' program again. If 'dat' files from each sequence are automatically created these can be deleted to save space (this is until the 'sif' conversion issue is resolved)
h) Close python script window after the cell is recorded

5. Recording the next cell (FOV2)

a) Create a new folder (FOV2) and change file saving location in Andor Solis setup acquisition
b) Copy calibration files from FOV1 to FOV2 (or need to specify where python can find the calibration files as it automatically looks in FOV that collected the data)
c) Launch C:/andor_scripts/process_palm.py and indicate location of FOV2

d) Start recording by launching from FILE/Run program by filename- C:/andor_scripts/track_fid.pgm. and proceed as in FOV1

NB: A new calibration should be recorded for each laser used (if more than one)

6. Switching off

a) Turn off lasers, close Omicron Control Centre and switch off mains laser power
b) Release focus lock, if on, and switch off.
c) Switch off EMCCD cooling, the temperature indicator will go to 'OFF' state in a few seconds. Switch off the camera
d) Switch off the ASI stage controller
e) Switch off the microscope
f) Remove water from objective carefully using lens cleaning tissue
g) Check stage recess for dirt (this will tilt samples) and for leakage (this will damage the piezo head unit)
h) Bathe chamber in water and leave to dry

7. Data transfer

Move data from C-drive to S-drive as soon as possible to keep space free on the computer

8. Converting 'sif' to 'dat'

a) Start Andor Solis, go to File/Batch conversion and choose the folder containing sif files. Also, select the same folder as the destination for the converted files. Select conversion to 'Raw' format, highlight all files and click convert
b) A menu will pop up asking for the file bit length, select 16-bit, and click ok
c) Go to Palm3D software
d) Indicate the location of your data set and assign the metadata as follows

Z position:	0
Number of repeats:	n (50 if entire 'track_fid' program ran, but can be less if program cut short)
Number of records:	200
Number of tracking:	20
X size:	350
Y size:	350

e) Click process and follow the steps as described on the PALM3D website
In the initial step the molecules need to be localised (inspect selection parameters), by specifying NumSTD and NumStd_changed values (see PALM3D tutorial for help with this) – these values typically vary between 3.0-4.0.

*__STORM imaging buffer__ (keep on ice & make about 5 ml per STORMing session)
In order to STORM a reducing solution is needed which is made by mixing
5 ml Cell culture water
25 mg Glucose
50 µl 1M TRIS-HCl, pH 7.4
250 µl 1M NaCl
40 µl beta-mercaptoethanol
50 µl glucose oxidase & 5 µl catalase solutions (x1000 stocks, kept frozen at -20˚C)

Enyzme Stocks

Catalase 40 mg/ml (10 µl aliquots) –made up in 50 mM potassium phosphate + 50% glyerol solution
Glucose Oxidase (GLOX) 50 mg/ml (50 µl aliquots) – made up in 50 mM sodium acetate + 50% glycerol solution