Guide: Standard C-Trap Dymo (in-solution assay) set up (Updated Jan 2024)

This step-by-step guide is intended as a reminder of what was covered during the full LUMICKS C-Trap training, and not as a stand-alone detailed operating protocol. This guide specifically addresses assays in solution (far from surface) such as DNA dumbbell experiments using the LUMICKS flowcell. Alternative steps due to typical differences between systems are highlighted in blue. Other differences may exist between systems and may not be captured here.

Step 0: Set up optics

- Clear the flowcell space (lower the objective, raise the condenser, slide the flowcell holder out)
  - If you have a nanostage, position it to the middle (x=100, y=100, z=100 µm)
- Add ~60 µL water on the objective
- Slide the flowcell holder back in
- Add 1-2 drops of condenser oil on the flowcell on the working area of interest
- Raise the objective until the water touches the bottom of the flowcell
- Visually inspect that the center of the objective is aligned with the area of interest (inside a channel)
- Close the C-Trap lid

Step 1: Adjusting the objective position

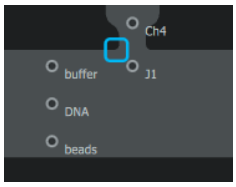
- Turn on the optical trap source box if not already on (as indicated by the lit up green button). Follow the instructions given to you, which are either 1) Key to 'on', push-twist-pull red button, press green button, or 2) Key to 'REM'
- Select the *Z-finder* camera
- Set *Trapping Laser* to 100% and *Overall Power* to 20% (optical trap source box should have orange light turned on)
- Verify that *Trap 1 split* is at 50% (this is needed for step 2)
- A signal should be visible on the *Z-finder*. Adjust the LUT (contrast settings) if needed
- Slowly raise the objective by manually rotating the knob clockwise until a first clear reflection is visible on the *Z-finder*
- Continue to raise the objective until a second clear reflection is visible. This second reflection should be very close to the first one (should require less than ½ turn of the knob). When in doubt, restart from step 0 and inspect visually again to make sure the objective is aligned (within a channel containing buffer, not just through glass)
- Once the second reflection is confirmed, continue to raise the objective until the reflection patterns are largest (which should correspond to about 50 µm, i.e. the middle of the flowcell).
  - Alternatively, move the nanostage down by 50 µm, preferably if the nanostage was set to **150 µm** in step 0, such that after this procedure, the nanostage remains at 100 µm

Step 2: Adjusting the condenser position

- Open the condenser control (drop down from *Tools* menu, or available after toggling the *Moon* camera)
- Lower the condenser to the *Approach* position
- Reduce the *Trapping Laser* to 30%
- Select the *Moon* camera. Adjust the LUT (contrast settings) if needed
- Step down with 0.100 mm (i.e. 100 µm) coarse steps while watching the moon image until the condenser touches the oil (visible event on the camera) or until the diameter of the lit area is similar to the "good moon" on the desktop
- Step down with 0.010 mm (i.e. 10 µm) fine steps until the number of bands and their overall patterns (curvature and thickness) matches the "good moon"
- Stepping down should decrease the number of bands while stepping up should increase them. If the reverse occurs, this likely indicates that you are too close to the flowcell and you should immediately *Raise* the condenser
- Adjust the *Trapping Laser* back to 100% (always 100% - except during step 2 - and use *Overall Power* to control the effective power delivered to the sample)

Step 3: Adjusting the Bright-field imaging and navigating the flowcell minimap

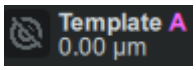
- Select the *Bright-field* camera
- Turn on the *Bright-field LED* to 15-30% (or to the desired power to have a good signal)
  - Adjust the LUT (contrast settings) or the bright-field camera settings if desired
- Move the microstage up (in Y- direction) until the top horizontal edge of the flowcell is visible on the bright-field camera
- Move the microstage right or left (in X+ or X- direction respectively) until the corner between the buffer channel and *Ch4* is visible on the bright-field camera
- Inspect the *Bluelake* microstage minimap on the right. Adjust the location of the blue small rectangle (right click, "*set as current…*") to the corresponding location displayed by the bright-field image
- Optional: move the microstage to the corner of *Ch5* to confirm the alignment



Step 4: Catch some beads to localize Trap 1 and Trap 2

- Adjust the *Overall Power* to the desired level. 20-30% typically works best for DNA-tether assays and ~4 µm beads
- Turn on the flow (valves on, pressure on) and wait to catch some beads
- Move the microstage to the buffer channel while keeping the beads in your traps, turn off the flow
- Adjust the location of Trap 1 and Trap 2 to the desired configuration using the caught beads as visual indicators
- Select the digital Trap 1 position. A red rectangle will appear. Hold Shift key, then left mouse click on where Trap 1 is. If available (depends on the specific system), perform the same procedure for Trap 2
- Tip: if both traps are on top of each other or very close by, you will find it difficult to catch beads. Try moving one trap at random to see if you are able to better catch two beads

Step 5: DNA-tether assay workflow



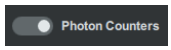
- Set up bright-field template tracking
  - Draw a new *Template* on the desired bead (supposedly a single bead, of the expected size), confirm and input the correct diameter
  - Select the *Tracking ROI* and extend it to generously cover the two beads with room for any bead movement, then, unselect to hide the ROI
  - The initially drawn template should be recognized on the corresponding bead with the highest correlation score (around 99%) while the other should be recognized with a high score (around 95%).
  - The vectorial distance between the center of the two templates minus 2x the bead radius is now being tracked under “Distance 1”. Different *Tracking modes* giving an additional “Distance 2” may be used for certain assays (e.g. Q-Trap)

- Set up force-distance visualization
  - On the data viewer screen (left), select the *F,d* tab
  - Add a new *eWLC* model of the corresponding contour length
  - Choose the *Force:* and *Distance:* of interest (typically, *Force 2x* and *Distance/Distance1*)

- Workflow
  - Catch new beads, move to buffer channel and *Re-calibrate* (F9 key) without DNA, without flow, and following the calibration guidelines instructed during training. Zero the forces after the calibration is applied
  - Turn on flow, then fish DNA (use Trap 1 to repeatedly decrease and increase the distance to ~0.6xLc to ~1.0 Lc to form a tether
  - Stop the flow (vent, and close channels), verify it is a single tether, move to imaging channel, etc.
  - See Step 6 for confocal imaging

Step 6: Confocal imaging

- While the C-Trap lid is shut, turn on the single-photon counter module (button in rack)
  - If the single-photon counter module does not have a button in the rack, it is enabled directly through *Bluelake*, near the confocal laser controls



- If the fluorescent laser module is visible in the rack, turn it on manually (key turn + button). Otherwise, it is directly turned on through *Bluelake*
- On *Bluelake*, on the confocal control area, enable the desired lasers and choose the desired percent powers (keep in mind the differences between true powers and displayed powers)
- Verify that the z-plane of the trapped bead is co-aligned with the z-plane of the confocal
  - Perform 2D scans covering both beads while changing Trap 1+2 z plane in incremental steps
  - Use the bead’s autofluorescence to approximate the right Trap 1+2 z plane, which is where the beads appear the biggest and their edges sharpest
  - And/or, if available, use the fluorescence signal from the DNA (from bound proteins, markers or intercalators). The best Trap 1+2 z plane is where the fluorescence spots on the DNA appear the sharpest (smallest, with the best signal-to-noise)
  - Keep in mind that this Trap 1+2 z plane will vary significantly based on a few factors, the most common ones being the bead size and the optical trap laser power used. However, for the same bead size and same optical trap, the correct Trap 1+2 z plane should remain very similar between experiments

- In general, use kymographs to image the DNA
  - The confocal 2D scans obtained (left screen) will show the true scanning area (as compared to the approximate blue-highlighted area on the bright-field). Select the kymograph mode and use the image on the 2D scan on the left screen to align the scanning line accurately before proceeding with kymographs
- Optimize the imaging settings by striking the balance between signal-to-noise, time resolution, and photobleaching/photodamage
  - Signal-to-noise can be improved with higher laser powers, longer *Pixel Time* and or removal of the background (by imaging out of the protein channel, or by optimizing the protein concentration and labelling)
  - Time resolution can be improved with shorter *Pixel Time* and smallest scanning dimensions
  - Photobleaching/photodamage can be reduced by using lower laser powers and/or introducing dwell times between scans by artificially setting a slower *Line Time* (or a slower *Image time* for 2D scans)
- Turn off the photon counters when the C-Trap lid is open, or when you are finished with confocal imaging