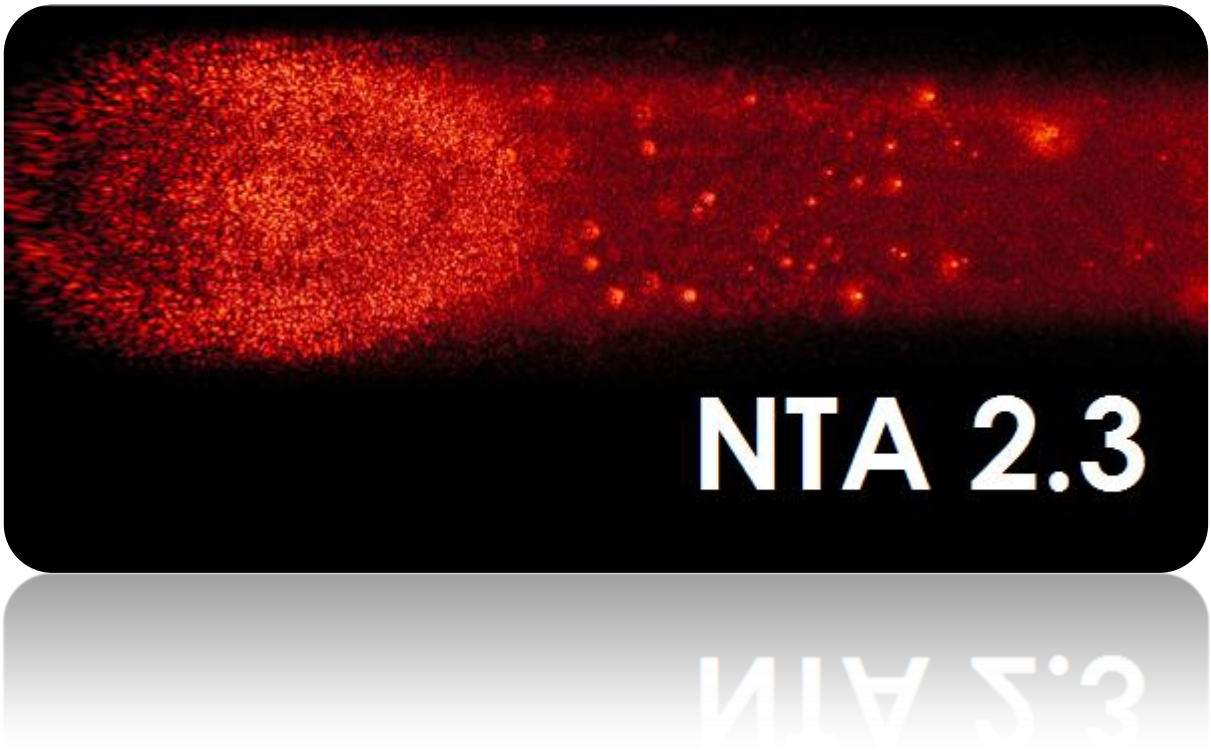
NTA 2.3 Analytical Software



Quick start guide

P560E

NanoSight Ltd., Minton Park, Amesbury, Wiltshire SP4 7RT, UK

Video capture and concentration

1. Navigate to the **capture** screen.

   Two modes of video capture are available: **basic** and **advanced**. This quick guide refers to using **basic mode.**

2. Select **basic mode**, increase **camera level** to maximum.

3. Find the 'thumbprint' flare spot where the laser beam emerges (see figs. 1 and 2). This can be used to check for vibration – the scatter pattern at the thumbprint should be static, any movement seen here indicates vibration.

4. Move to the optimal viewing region, where particles are seen next to the thumbprint (shown in fig. 1)

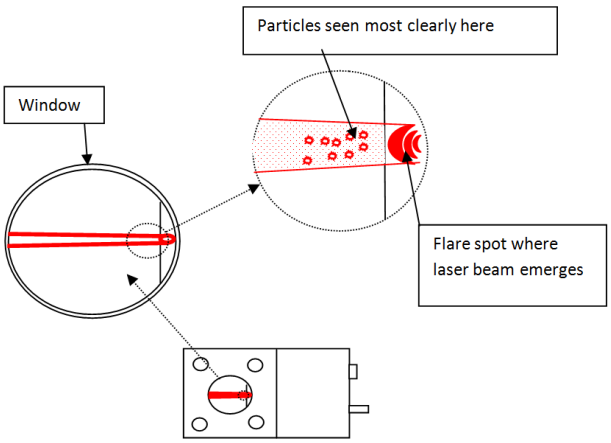


Figure 1 – Locating the thumbprint

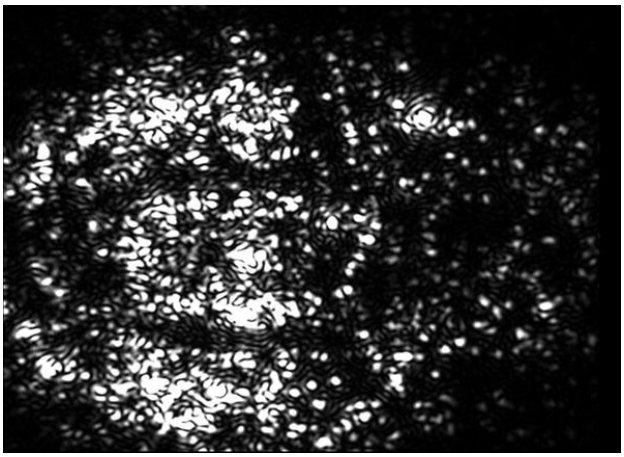


Figure 2 – 'Thumbprint' as seen in capture screen

5. Adjust focus so that particles appear as smooth spheres (fig. 3b). In practice it is difficult to get all visible particles to appear in focus – in this case it is better that the out of focus particles appear like fig. 3c rather than fig. 3a

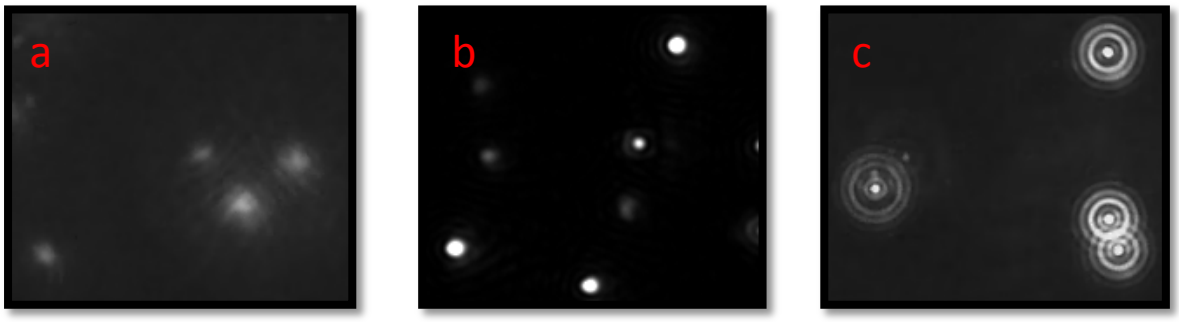


Figure 3 – Focus adjustment

6. Increase the **camera level** to maximum to ensure that all particles that exist in the sample are being visualised (fig. 4). Decrease the camera level back down, making sure to retain an image of the small particles, i.e. until the smallest particles disappear if the camera level is decreased further.

Example of overexposed image

Example of optimal images to allow accurate particle centering
Left – Monodisperse sample; Right – Polydisperse sample

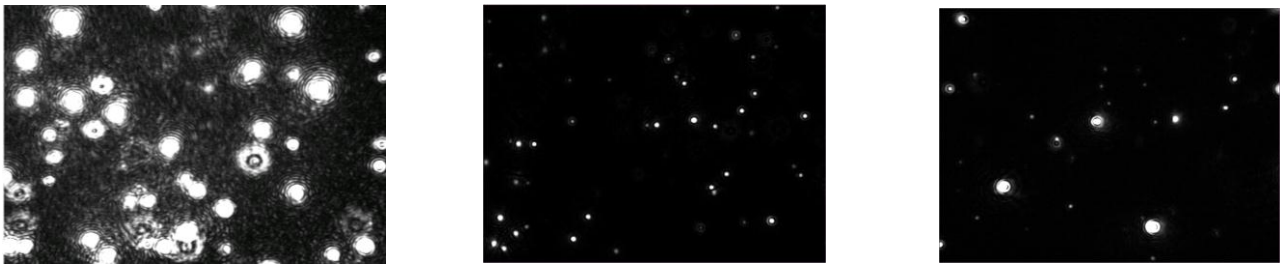
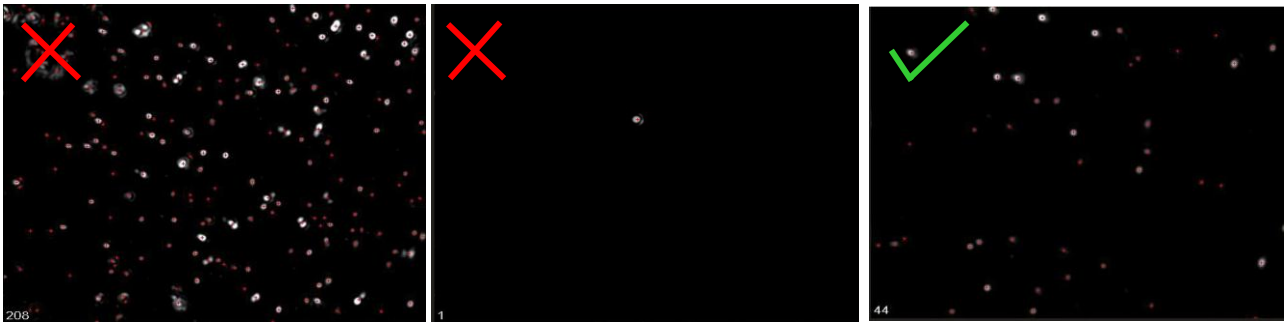


Figure 4 – Image brightness adjustment for monodisperse and polydisperse systems

7. Assess concentration visually:
   For **monodisperse samples** there should be around 20-100 particles seen in the field of view (fig. 5)



a) Concentration too high
208 particles identified

b) Concentration too low
1 particle identified

c) Ideal concentration
44 particles identified

Figure 5 – Sample concentration

When looking at larger/brighter particles sometimes interference is seen between particles close together – this can be removed by reducing the camera level, or by reducing the concentration of the sample.
In **polydisperse samples** this may cause some of the dimmest particles to be lost, in this case it is better to reduce the concentration of the sample.

- The NanoSight instrument can work with particle concentrations ranging from approximately 10^7 to 10^9 particles/ml
- Lower concentrations require longer analysis time due to limited statistics
- At high concentrations light scatter interference and cross-over between particles cause problems with tracking

In the event that the observed concentration is not ideal for analysis, alter the sample dilution accordingly, and re-do steps 2 to 7.

8. Set the capture duration based on approximate polydispersity, concentration and repeatability required using the table given in the capture screen.

9. Record the video, entering the sample temperature if prompted at the end of capture (read from the probe supplied with the LM10).

Analysis

1. Navigate to the analysis screen (click 'Main' button in capture) and load in a saved video using 'Open File'. (If you have just recorded a video this may be loaded into the analysis screen automatically).

   Like with capture, two modes of video analysis are available: **basic** and **advanced**. This quick guide refers to using **basic mode.**

2. **Screen Gain** can be adjusted to help see the dimmest particles in the system, which is a useful aid when setting the detection threshold.

   ! This function is only for visualisation only and has no effect on particle tracking

3. **Detection Threshold** should be set so that a red cross appears in each particle visible on screen.

   When setting the detection threshold one of two possible warnings, relating to the number of centres (red crosses) found in the first image may appear on screen:

The <5 objects warning may be seen when there are less than 5 centres found on screen – this indicates that the concentration of the sample is very low, or the **detection threshold** setting is too high.

The >200 objects warning may be seen in when there are more than 200 centres found of screen – this indicates that the concentration of the sample is very high, or the detection threshold setting is too low, detecting a significant amount of noise. Adjust the sample concentration if necessary or increase the **detection threshold** to remove noise.

   ! Information on other warning triangles is shown when hovering the mouse cursor over the warning triangle

4. To run the analysis with the chosen settings click the **Process Sequence** button – the particle size distribution will begin building up on the screen immediately.

For more detailed information on settings, advanced functions and data analysis please refer to the NanoSight NTA 2.3 Operation Manual which can be found in **Help** -> **About** in the NTA 2.3 software.

NanoSight Technical Support Contact Details
Telephone: +44 (0)1980 676060
Email: support@nanosight.com