

**Supplementary Information for
„Dark field nanoparticle tracking analysis for size characterization of plasmonic and non
plasmonic particles“**

0. General

This document contains some information helpful to reproduce the measurements. The tables in section 2 specify all software settings used for the measurements. The references to the analyzed video files are listed in the table row “Source”. The video files are online available and under a creative commons licence. To reproduce the measurements you have to install Fiji/ImageJ and also the NanoTrackJ plugin. The version used in this paper is available under:

NanoTrackJ: Size characterization of freely diffusing nanoparticles by nanoparticle tracking. Thorsten Wagner, Martin Wiemann, Hans-Gerd Lipinski. [figshare](#).
<http://dx.doi.org/10.6084/m9.figshare.805052>

1. Naming convention

All files are named according to the following convention:

Type_T-X_C-X_FPS-X

with

- Type
 - DF = Dark Field (Cytoviva Condensor on an Olympus BX51 microscope)
 - NS = NanoSight LM 10 (green laser)
- P-X = Particle
 - P-Au: Gold
 - P-Ps: Polystyrol
- S-X = Nominal particle size in nm
- T-X = Temperatur in C°
- C-X = Camera Type
 - C-A: Andor-DL-658-OEM
 - C-E: PCO Pixelfly Edge
 - C-O: Canon EOS 5D Mark II
- FPS-X = Frames per Second

2. Software settings to reproduce the results

Figure 1A:

Software	Nanosight NTA 2.3
Video file	NS_P-Ps_S-100_T-22_C-A_FPS-30.avi (first 1800 frames)
Source	Wagner, Thorsten; Wiemann, Martin; Lipinski, Hans-Gerd (2013): Video recording of freely diffusing 100nm polystyrene nanoparticles using nanoparticle tracking analysis. figshare. http://dx.doi.org/10.6084/m9.figshare.879651

The settings used for this plot can be found in the supplementary files named as the video on the Journal website.

Figure 1B:

Software	NanoTrackJ
Video file	NS_P-Ps_S-100_T-22_C-A_FPS-30.avi (first 1800 frames)
Source	Wagner, Thorsten; Wiemann, Martin; Lipinski, Hans-Gerd (2013): Video recording of freely diffusing 100nm polystyrene nanoparticles using nanoparticle tracking analysis. figshare. http://dx.doi.org/10.6084/m9.figshare.879651
Center estimator	Maxima
Diffusion coefficient estimator	Covariance
Min. expected particle size	90 nm
Searching radius	13.34 px
Min. number of steps per track	20
Temperature	22,5 °C
Pixel size	164 nm
Frame rate	30 FPS
Linear drift corrected	True
Walker's method used:	True
Walker's method min size:	800nm
Mean size (Maxima Dialog)	3
Tolerance (Maxima Dialog)	15

Figure 1C:

Software	Nanosight NTA 2.3
Video file	NS_P-Ps_S-200_T-24_C-A_FPS-30.avi
Source	Wagner, Thorsten; Wiemann, Martin; Lipinski, Hans-Gerd (2013): Video recording of freely diffusing 200nm polystyrene nanoparticles using nanoparticle tracking analysis. figshare. http://dx.doi.org/10.6084/m9.figshare.879662

The settings used for this plot can be found in the supplementary files named as the video on the Journal website.

Figure 1D:

Software	NanoTrackJ
Video file	NS_P-Ps_S-200_T-24_C-A_FPS-30.avi
Source	Wagner, Thorsten; Wiemann, Martin; Lipinski, Hans-Gerd (2013): Video recording of freely diffusing 200nm polystyrene nanoparticles using nanoparticle tracking analysis. figshare. http://dx.doi.org/10.6084/m9.figshare.879662
Center estimator	Maxima
Diffusion coefficient estimator	Covariance

Min. expected particle size	190nm
Searching radius	9.37 px
Min. number of steps per track	20
Temperature	24.05 °C
Pixel size	164 nm
Frame rate	30 FPS
Linear drift corrected	True
Walker's method used:	True
Walker's method min size:	800 nm
Mean size (Maxima Dialog)	4
Tolerance (Maxima Dialog)	27

Figure 2 (left):

Software	NanoTrackJ
Video file	DF_P-Ps_S-100_T-24_C-E_FPS-30.avi
Source	Wagner, Thorsten; Wiemann, Martin; Lipinski, Hans-Gerd (2013): Video recording of freely diffusing 100nm polystyrene nanoparticles using dark field microscopy. figshare. http://dx.doi.org/10.6084/m9.figshare.878145
Center estimator	Maxima
Diffusion coefficient estimator	Covariance
Min. expected particle size	90nm
Searching radius	35.44 px
Min. number of steps per track	20
Temperature	24 °C
Pixel size	63 nm
Frame rate	30 FPS
Linear drift corrected	True
Walker's method used:	True
Walker's method min size:	800 nm
Mean size (Maxima Dialog)	1
Tolerance (Maxima Dialog)	5

Figure 2 (right):

Software	NanoTrackJ
Video file	DF_P-Ps_S-200_T-25_C-E_FPS-30.avi
Source	Video recording of freely diffusing 200nm polystyrene nanoparticles using dark field microscopy. Thorsten Wagner, Martin Wiemann, Hans-Gerd

	Lipinski. Figshare. http://dx.doi.org/10.6084/m9.figshare.878107
Center estimator	Maxima
Diffusion coefficient estimator	Covariance
Min. expected particle size	190 nm
Searching radius	24.72 px
Min. number of steps per track	20
Temperature	25 °C
Pixel size	63 nm
Frame rate	30 FPS
Linear drift corrected	True
Walker's method used:	True
Walker's method min size:	800 nm
Mean size (Maxima Dialog)	1
Tolerance (Maxima Dialog)	9

Figure 4:

Software	NanoTrackJ
Video file	DF_P-Au_S-60_T-24_C-O_FPS-25.tif
Source	Wagner, Thorsten; Wiemann, Martin; Lipinski, Hans-Gerd (2013): Video recording of freely diffusing 60 nm and 80 nm gold nanoparticles using dark field microscopy. figshare. http://dx.doi.org/10.6084/m9.figshare.879664
Center estimator	Maxima
Diffusion coefficient estimator	Covariance
Min. expected particle size	50 nm
Searching radius	18.03 px
Min. number of steps per track	20
Temperature	24 °C
Pixel size	182 nm
Frame rate	25 FPS
Linear drift corrected	True
Walker's method used:	True
Walker's method min size:	800 nm
Mean size (Maxima Dialog)	4
Tolerance (Maxima Dialog)	12

Figure 5:

Same configuration as used for figure 4. The orange colored distribution was measured using the color threshold with an upper hue of 60 and a lower hue of 0.

The green colored distribution was measured using the color threshold with an upper hue of 285 and a lower hue of 60.