Electronic Supplementary Material

AFM Characterization of Cellulose Nanocrystal Height and Width Using Internal Calibration Standards

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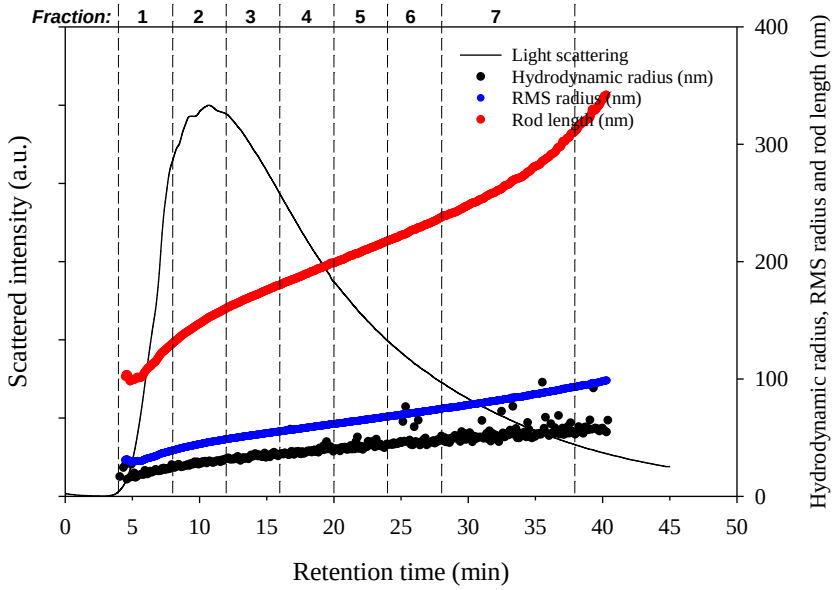
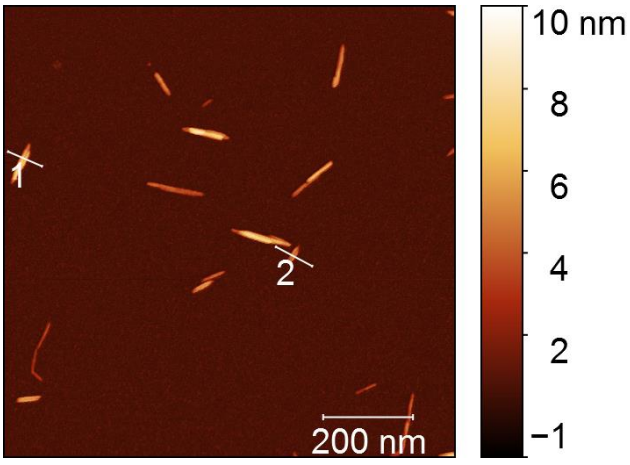
(A)



(B)



(C)

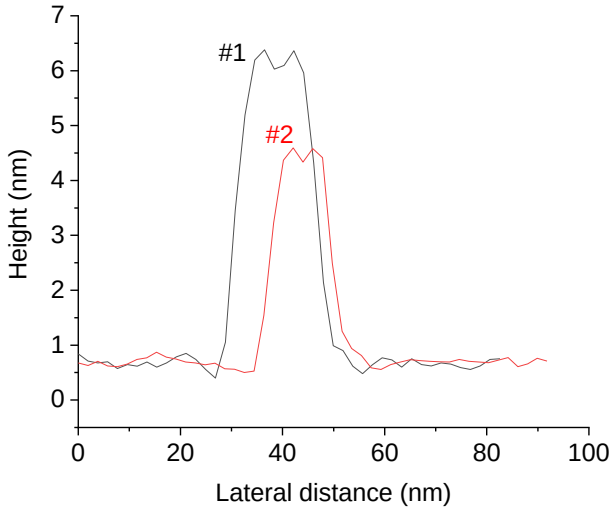
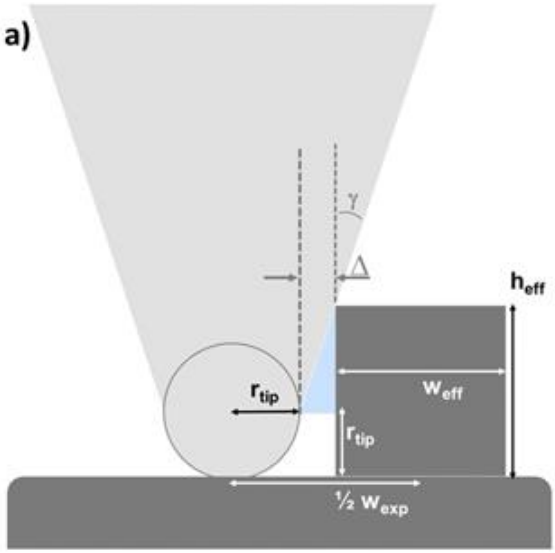


Fig. S1 Fractogram (A), AFM image (B) of CNC-F1A with the height profiles for the two cross sections (C). The two AFM images in (C) are digitally cropped from Figure S1B in order to visualize CNCs with the same z-scale

Alternate approach for calculation of the AuNP width

An alternate approach was used to calculate the width of one size of AuNPs using the tip radius calculated from the second size of AuNPs. This approach does not require the use of Eq. 2 which assumes a spherical NP. The method is based on Fig. 2 (parts a and b shown in the cartoons below) and associated text in the following paper: Canet-Ferrer, J., Coronado, E., Forment-Aliaga, A., Pinilla-Cienfuegos, E. Correction of the tip convolution effects in the imaging of nanostructures studied through scanning force microscopy. *Nanotechnology* **2014**, *25*, 395703.



$$½w_{\mathrm{exp}} = r_{tip} + \Delta + ½w_{eff}$$

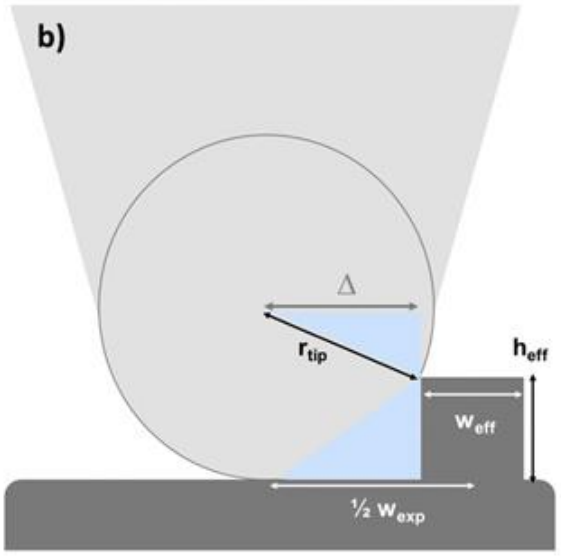
$$\Delta = (h_{eff}\text{-}r_{tip})\tan(\gamma)$$

This equation is applicable when h_{eff} is larger than $2r_{\mathrm{tip}}$.

To apply the above equation to AuNPs, the equation can be re-written as follows when H_{exp}>$2R_{\mathrm{tip}}$ (H_{exp} and R_{tip} are the AuNP height and the AFM tip radius, respectively):

$$W_{\mathrm{Cal\_AuNP}} = W_{\mathrm{eff}} = W_{\mathrm{exp}} - 2R_{\mathrm{tip}} - 2\tan(\gamma) \times (H_{\mathrm{exp}} - R_{\mathrm{tip}}) \qquad \textit{Eq. [S1]}$$

$W_{\mathrm{Cal\_AuNP}}$ values are the calculated widths of AuNPs. The tip angle (γ) value is 15 degrees based on the manufacturer's specifications.



When $H_{\text{exp}} < 2r_{\text{tip}}$,

$$W_{\text{eff}} = W_{\text{exp}} - 2 \times \sqrt{2r_{\text{tip}}H_{\text{eff}} - H_{\text{eff}}^2} \qquad \textit{Eq. [S2]}$$

Eq. S2 can be rewritten as *Eq S3* for AuNPs, assuming measured height H_{exp} for H_{eff}:

$$W_{\text{Cal\_AuNP}} = W_{\text{eff}} = W_{\text{exp}} - 2 \times \sqrt{2R_{\text{tip}}H_{\text{exp}} - H_{\text{exp}}^2} \qquad \textit{Eq. [S3]}$$

Depending on the AuNP's height and the AFM tip radius, either *Eq. S1* or *S3* is applied, with $\frac{H_{\text{exp}}}{R_{\text{tip}}} > 1.75$ or $\frac{H_{\text{exp}}}{R_{\text{tip}}} < 1.75$, respectively. Note that the $\frac{H_{\text{exp}}}{R_{\text{tip}}}$ value of 1.75 is arbitrarily chosen. The results are shown in Fig. S2 A and B, below.

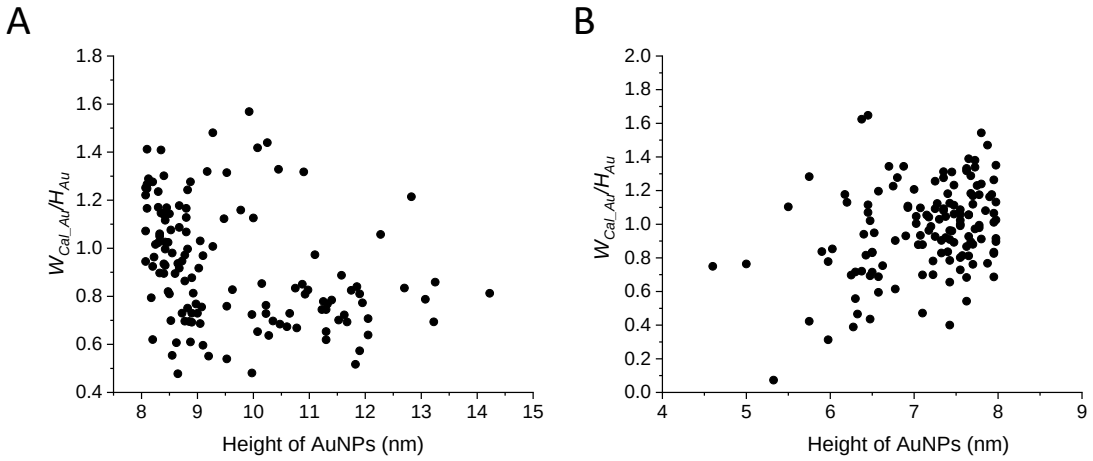


Fig. S2 Aspect ratio of the AuNPs plotted against AuNP height. Mean values of $W_{\mathrm{Cal\_Au}}/\mathrm{H_{Au}}$ are 0.92 ± 0.24 (Fig. S2A, AuNPs ≥ 8 nm) and 0.97 ± 0.27 (Fig. S2B, AuNPs < 8 nm).

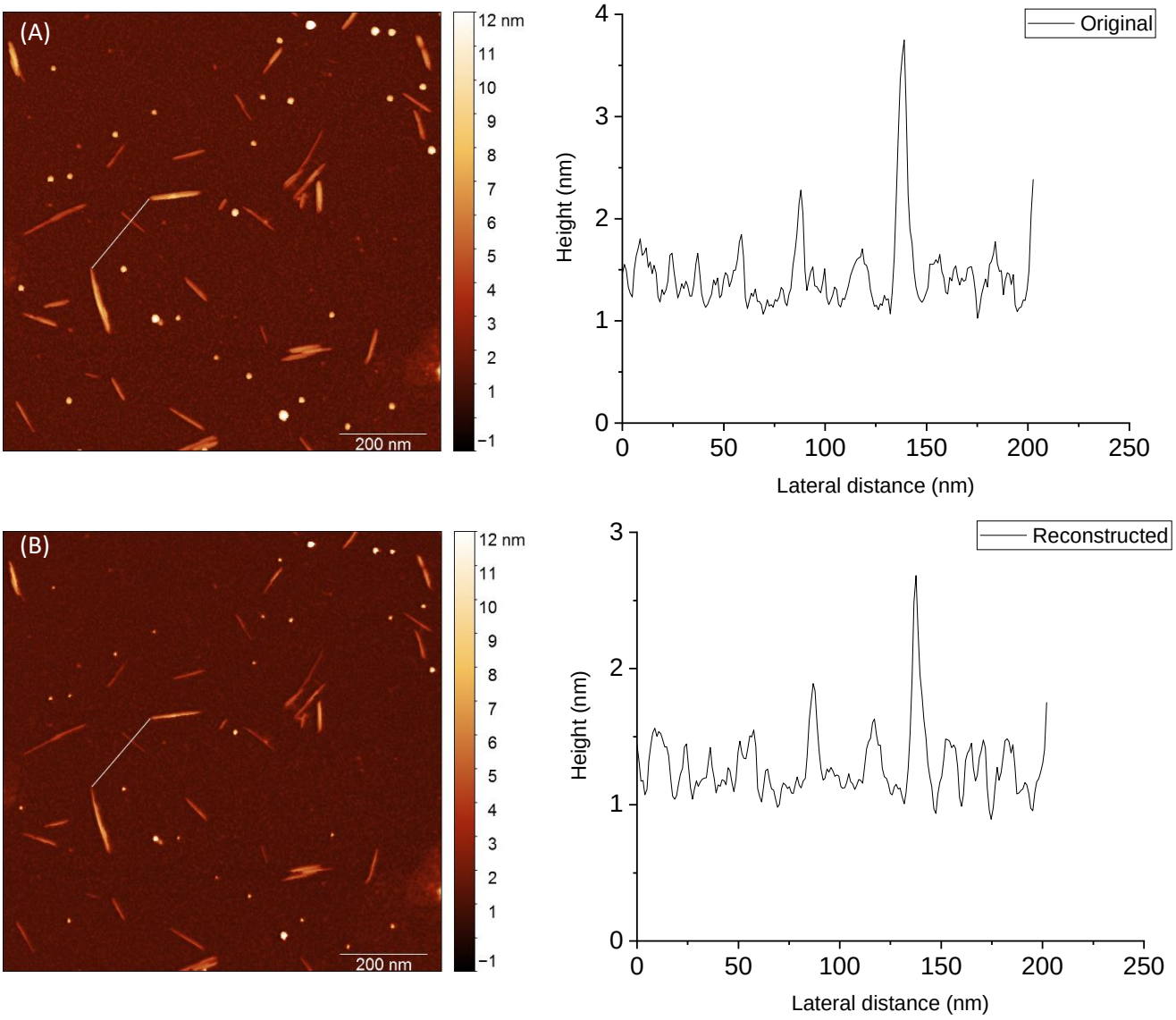


Fig. S3 Representative AFM image of CNC-F1A (A) and reconstructed image (B) by "Surface Reconstruction" tool with an AFM tip model of "a ball on stick" in Gwyddion. Corresponding profiles of individual CNC are shown on the right hand side. The profiles of the same CNC show that that the height of the CNC has changed due to the surface reconstruction, possibly due to the reconstruction algorithm used by Gwyddion (J. S. Villarubia, J. Res. Natl. Inst. Stand. Technol. 102 (1997) 425).

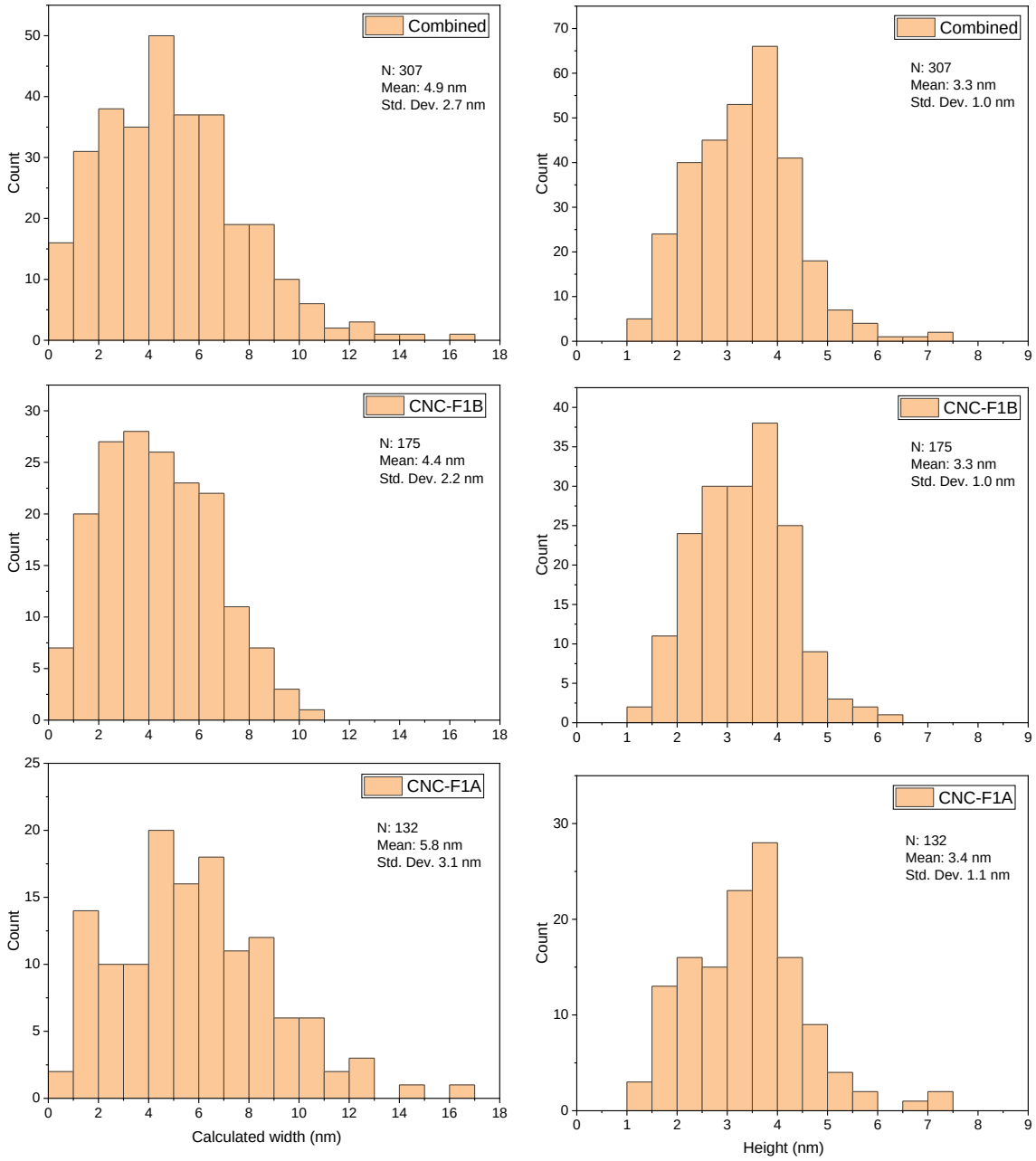


Fig. S4 Histograms for CNC calculated width and height for CNC-F1A, CNC-F1B and the combined data set. Mean and standard deviation for each distribution is provided on individual plots.

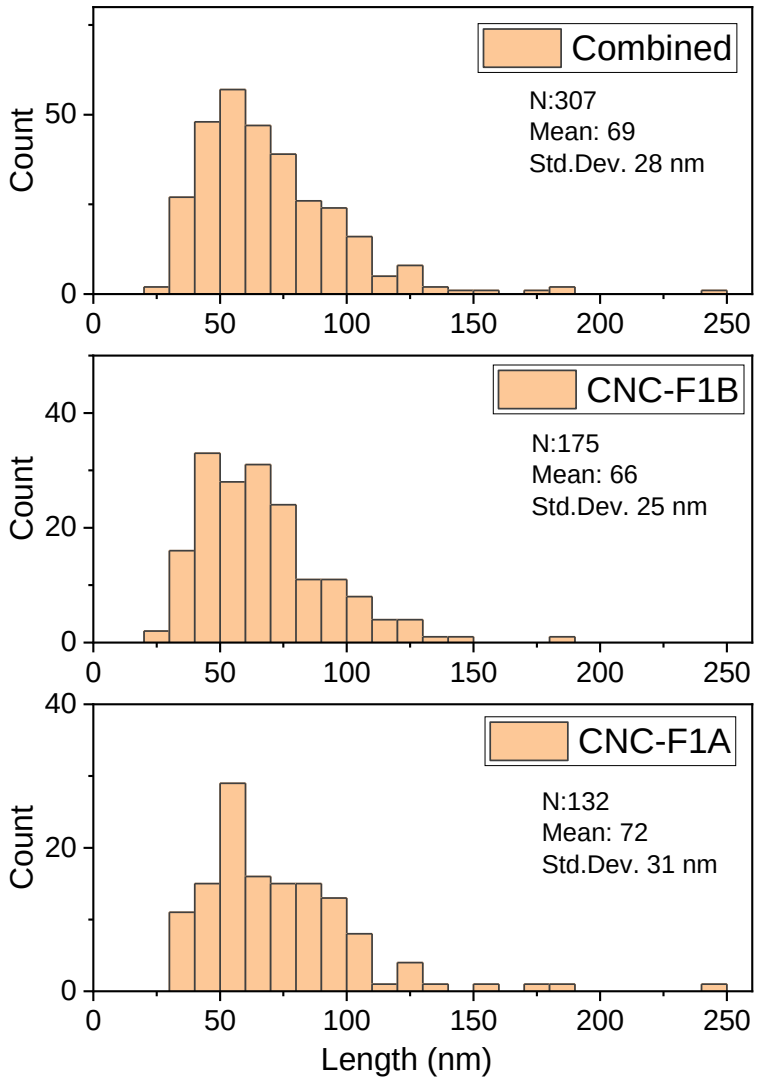


Fig. S5 Length of individual CNCs for CNC-F1A and CNC-F1B and the combined sample. The length values have not been corrected using the tip radius estimated from AuNPs.

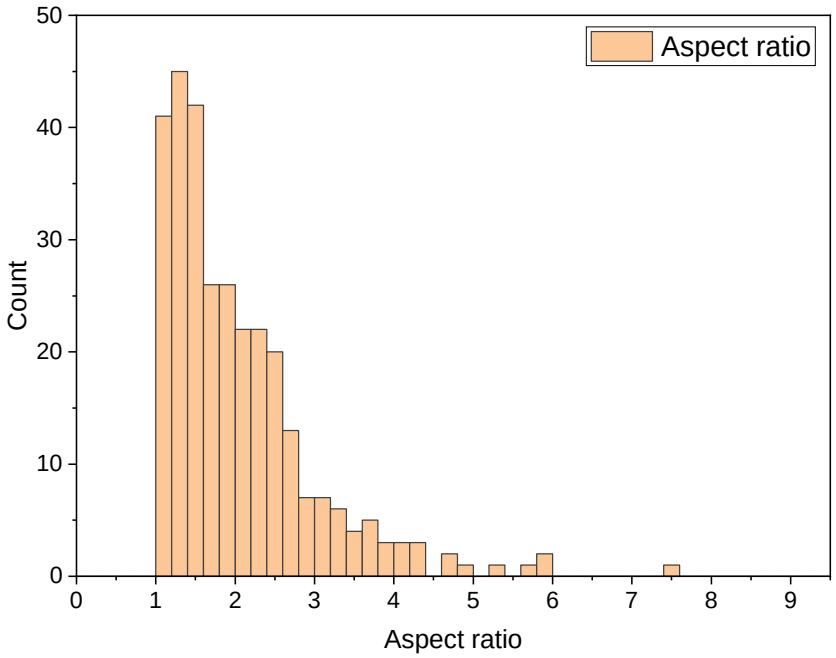


Fig. S6 Histogram of aspect ratio calculated as long axis/short axis (see Fig. 6 cartoon) from the calculated width and measured height for the combined data set.

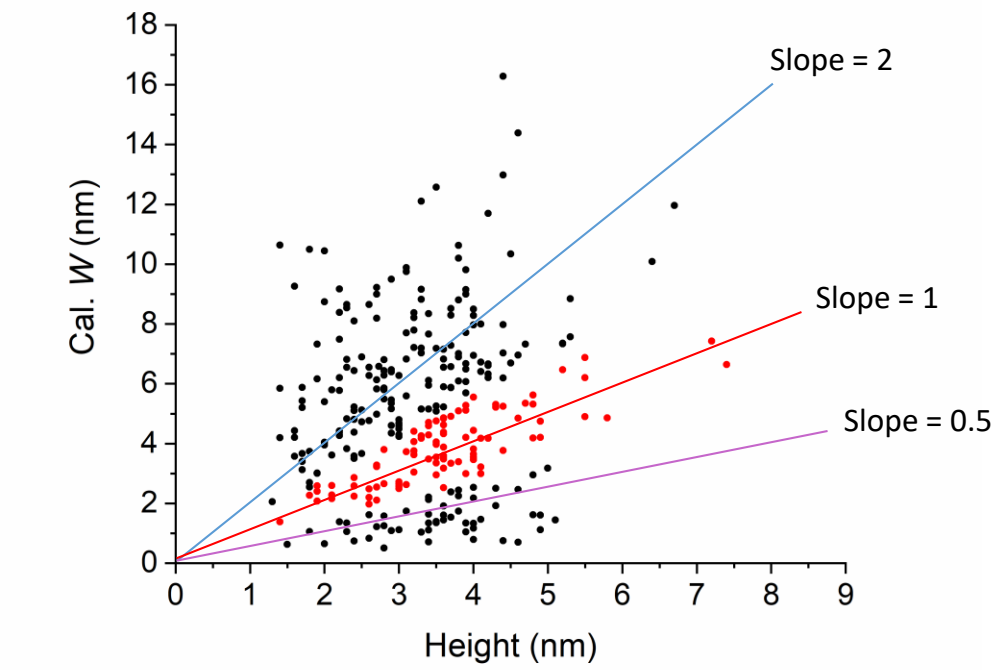


Fig. S7 Scatter plot showing the correlation between calculated width and measured height for fractionated CNCs. CNCs with calculated width/height between 0.7 and 1.4 are shown in red. The solid lines are a visual aid to illustrate the correlation between width and height for different CNC populations. AuNPs with heights < 8 nm were used to calculate CNC widths.