Standardizing Measurements for Cellulose Nanocrystal Particle Size Distributions

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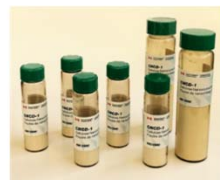
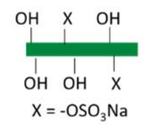
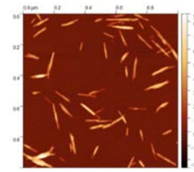
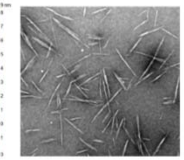
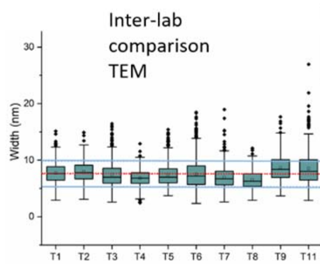
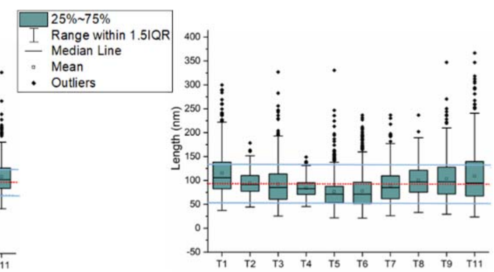












Standardizing Particle Size Measurements

Standards for characterization of cellulose nanomaterials are needed for:

- Quality control for production and applications
- Material specifications
- Environmental health and safety
- Occupational health and safety

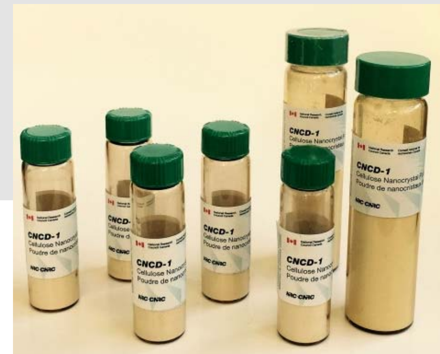
Particle size distribution is an important property for CNM (producer survey by ISO TC6 Task Group 1)

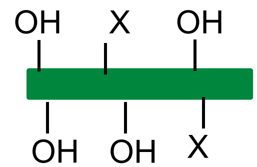
Interlaboratory comparisons are needed to assess reproducibility of measurement protocols and provide uncertainty estimates

Reference materials can be used to test methods and allow researchers to benchmark their measurements and/or materials

Microscopy Metrology Challenges

- Particle size is method-defined; number vs intensity-weighted particle size distributions; dry size vs hydrodynamic diameter
- Sample preparation – Do we select for a specific size (eg by washing or selective absorption)
- Agglomeration – How to minimize this on various surfaces used for microscopy? Which particles to count?
- Low EM contrast (vs metals), but staining may introduce artifacts and promote agglomeration
- Tip broadening for AFM-significant broadening of width; length should also be corrected for tip convolution
- Irregular-shaped particles (nanorods, not perfect cylinders or rectangles)
- Broad size distribution: analysis of large number of particles to define distribution and distinguish between different distributions (<u>define large</u>)





X = $-OSO_3Na$

from sulfuric acid hydrolysis of softwood pulp, spray-dried (supplied by CelluForce)

http://nrc.ca/crm

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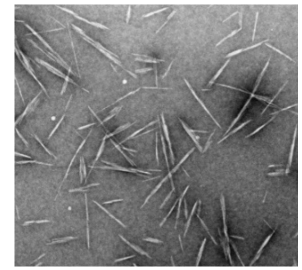
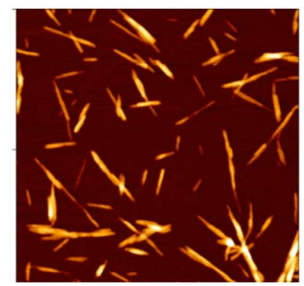
Determination of Particle Size Distribution for CNCs: VAMAS Interlaboratory Comparison



Objective. Validate the performance of image acquisition protocols and image analysis methods for determining CNC particle size distributions by atomic force microscopy (AFM) and transmission electron microscopy (TEM). Provide the pre-normative data for a technical specification on CNC particle size (TC 229 – Nanotechnologies: ISO TS 23151).



- Test image analysis methods on standard image set (manual analysis)
- CNCD-1 dispersed; samples prepared on mica & EM grids by pilot lab
- 10 participants for each method; detailed protocols that request analysis of 500 individual particles
- Complementary information: AFM gives length/height and TEM gives length/width
- Method to benchmark materials and methods; resource intensive for quality control

VAMAS

Nanoparticle Populations
Technical Work Area 34

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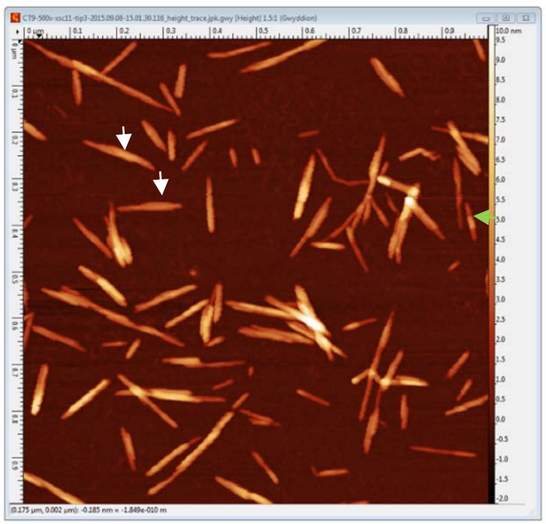
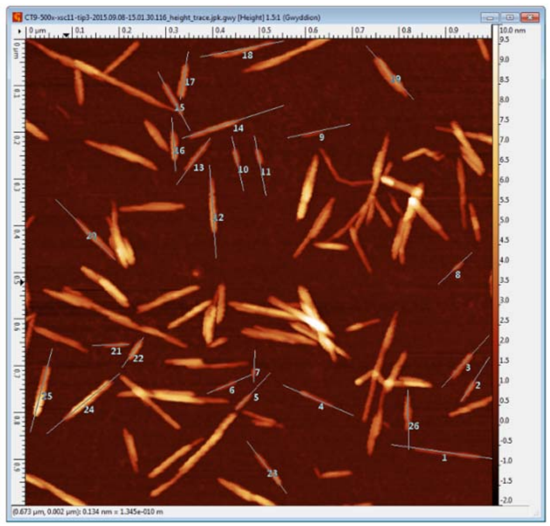
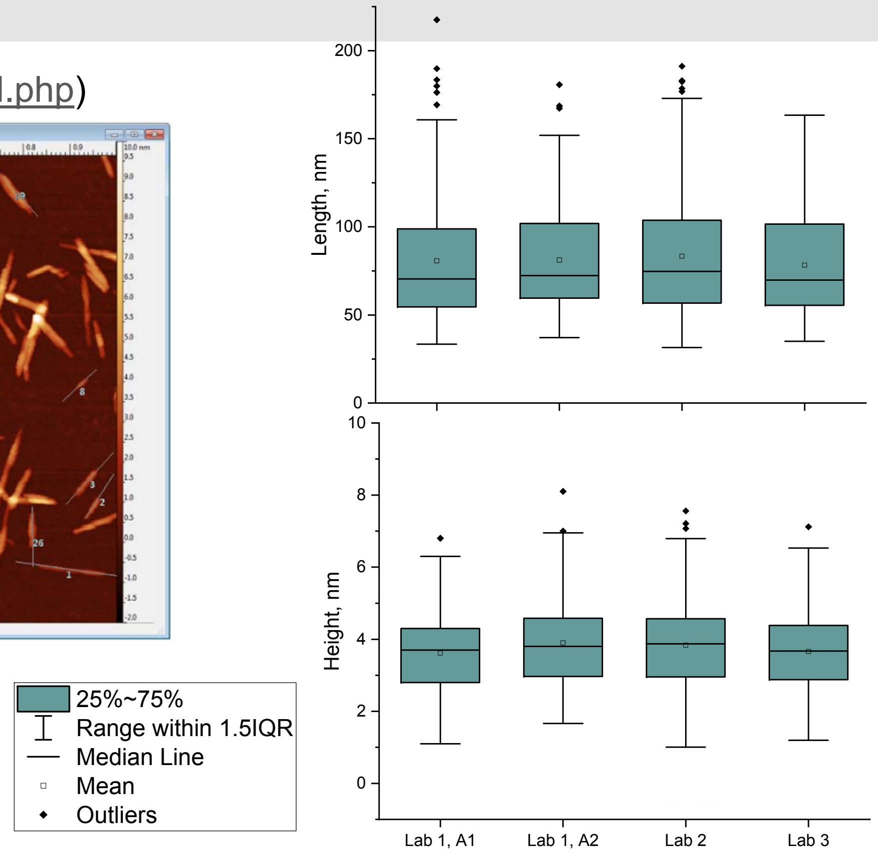
Testing AFM Analysis Method

Gwyddion (Version 2.52, http://gwyddion.net/download.php)







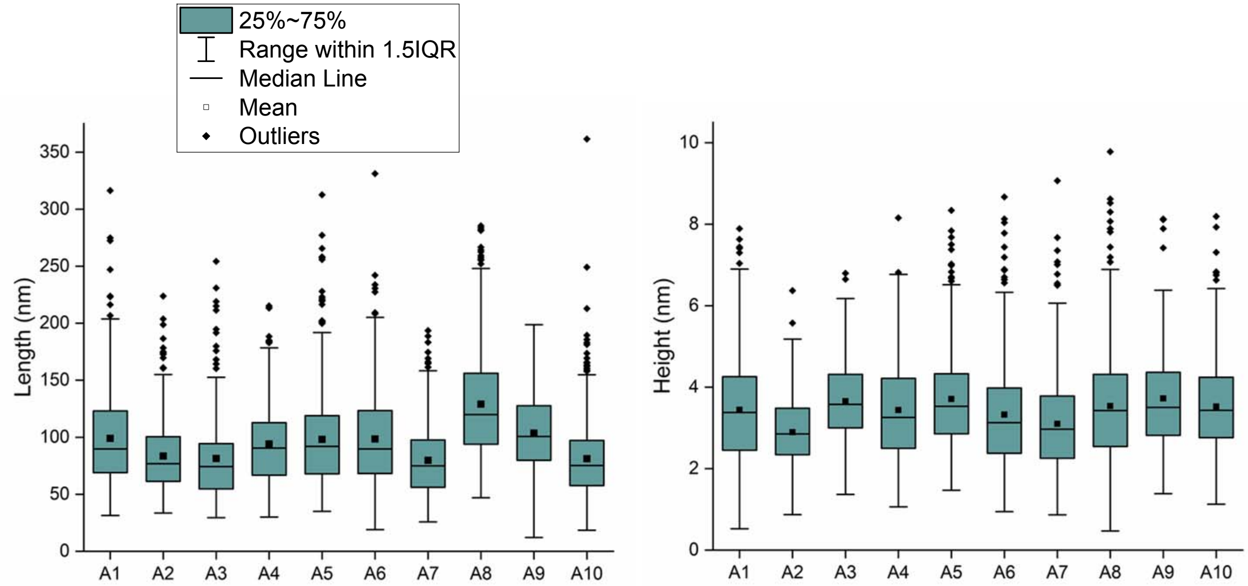
Analyze all single CNCs

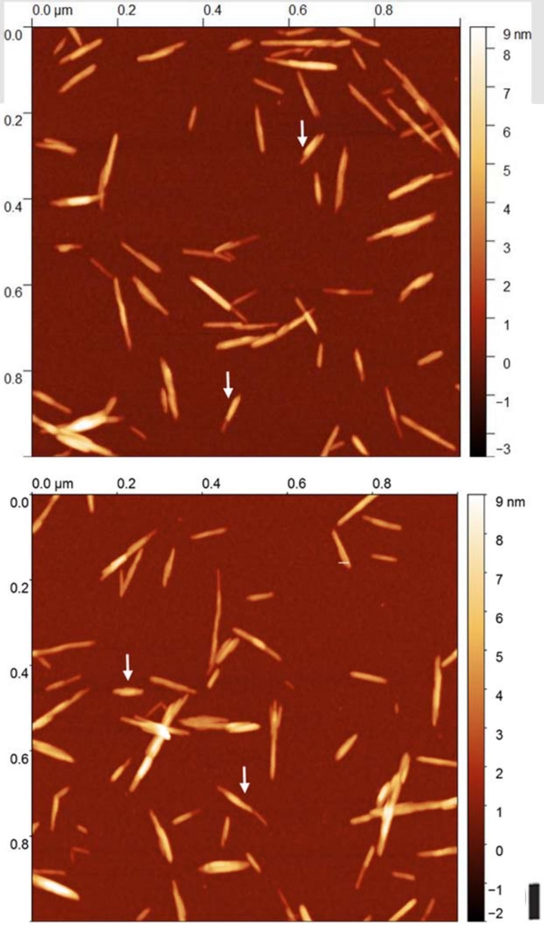
Exclude: clusters

CNCs on edge of image,

touching CNCs (unless edges are well-defined)

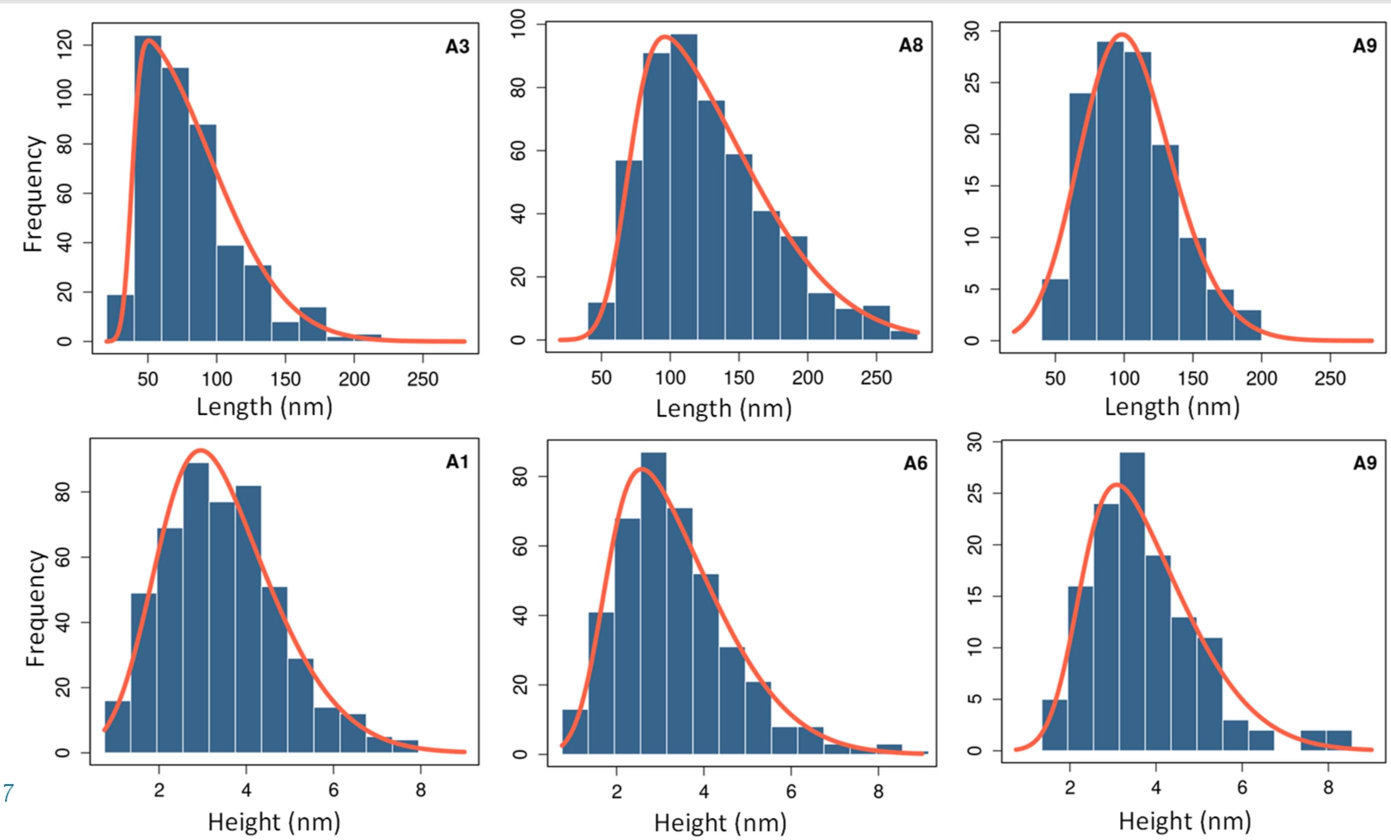
AFM Results: 10 Laboratories





- Mean length varies from 79 to 129
- Mean height varies from 2.9 to 3.7

Particle Size Distributions



Skew normal distribution model

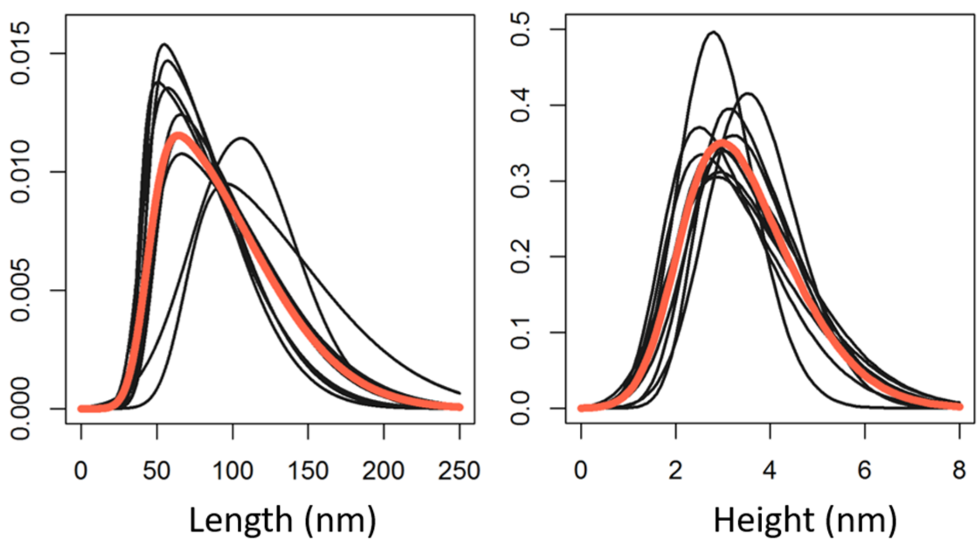
<u>3 parameters</u>:

Central location (mean)

Scale (distribution width, sd)

Shape factor, α (asymmetry)

Interlaboratory Comparison Results



AFM distributions: skew normal fits for 10 labs (black) and consensus (orange)

Length

	Mean, nm	Standard deviation, nm	Shape, α
Consensus value (std uncertainty)	**94.5 (0.4)**	**39.6 (0.3)**	**6.4 (0.3)**
Dispersion (std uncertainty)	**15.0 (0.6)**		

Height

	Mean, nm	Standard deviation, nm	Shape, α
Consensus value (std uncertainty)	**3.44 (0.01)**	**1.21 (0.01)**	**2.7 (0.1)**
Dispersion (std uncertainty)	**0.28 (0.02)**		

Meija, et al, *Anal. Chem.*, **92,** 13434, 2020

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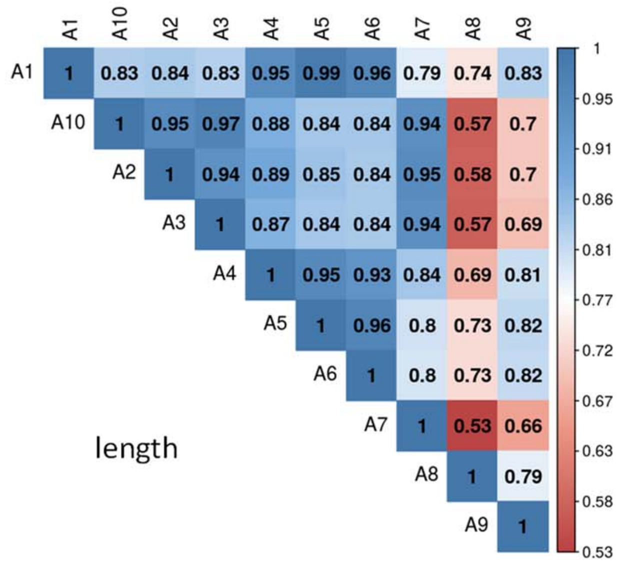
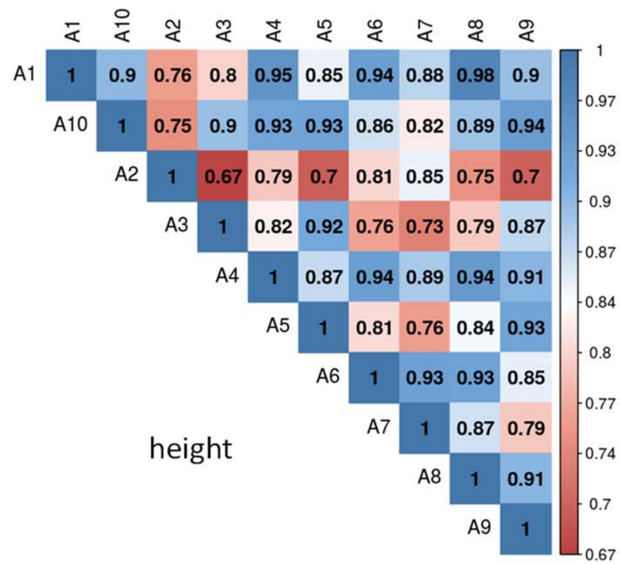
Interlaboratory Variability: Overlap Index

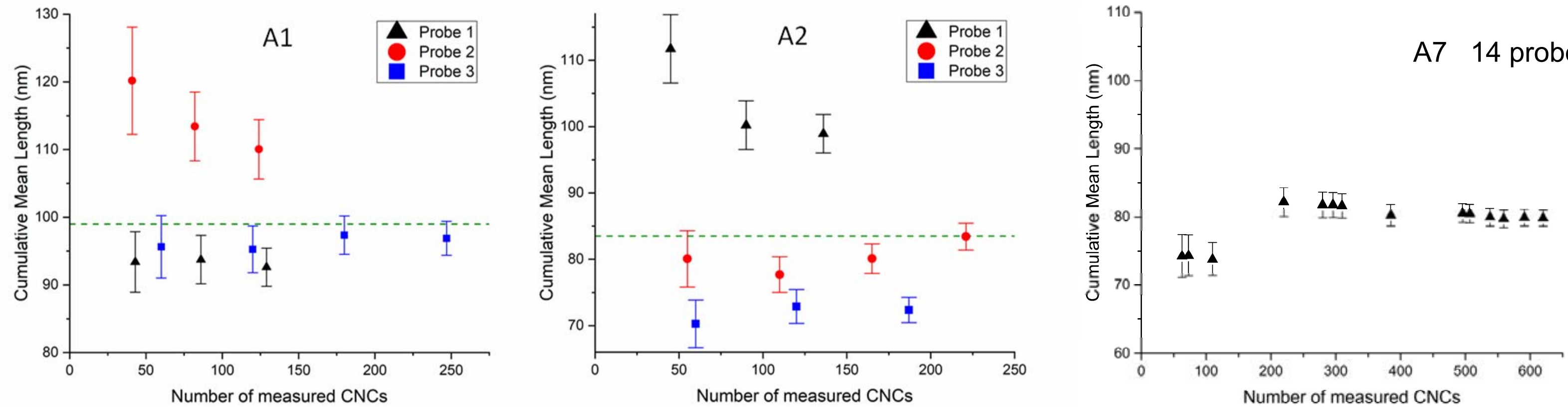




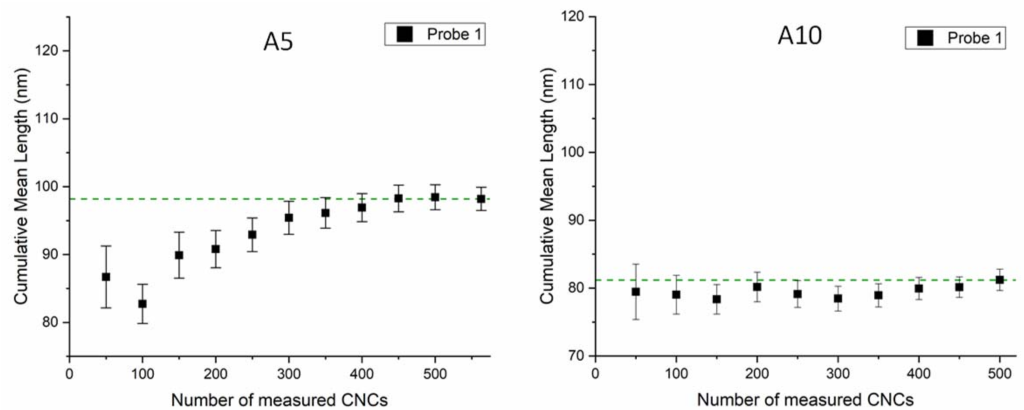
Overlap index, the common area between two distributions

Lab A8 – automated data acquisition, poor image quality – outlier?

Effects of AFM Probe



Green line is data set mean



Similar effects for CNC height

Differences in probe properties and/or probe deterioration contribute to intra-lab and inter-lab variability

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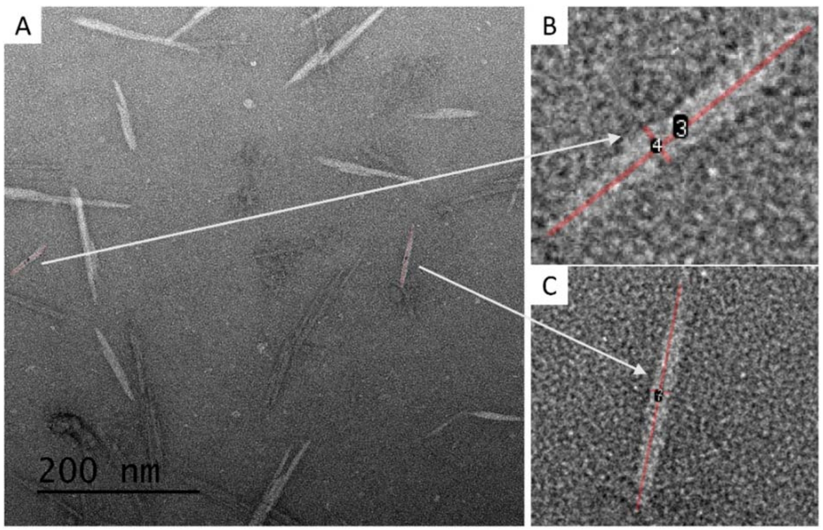
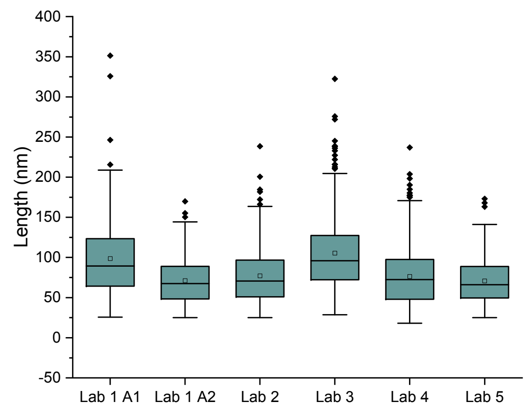
TEM Analysis Method

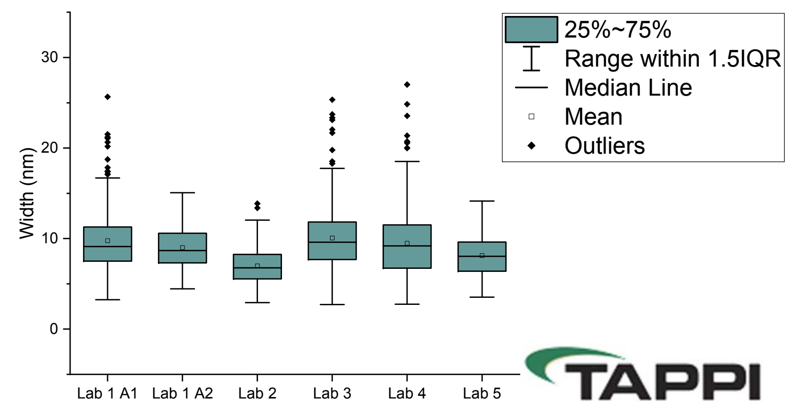
Custom ImageJ macro



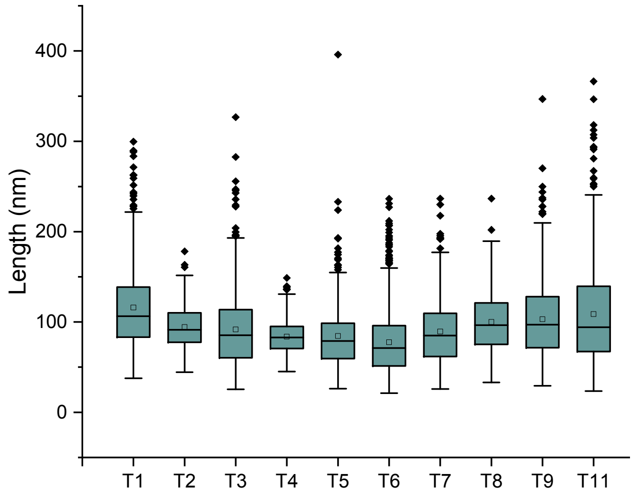
Examples of individual CNCs for analysis

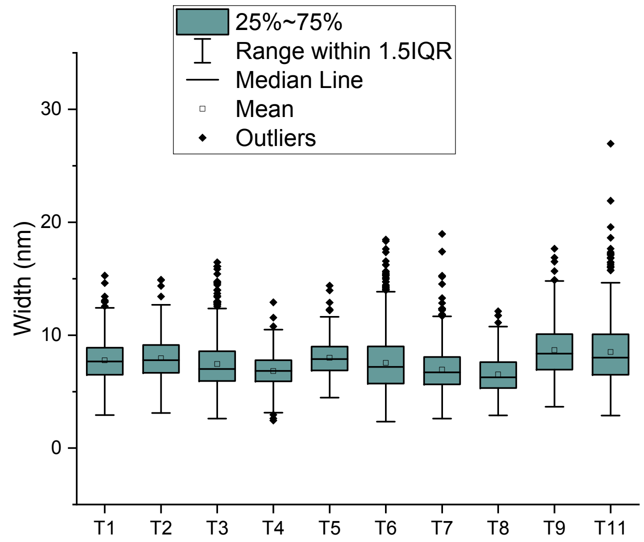


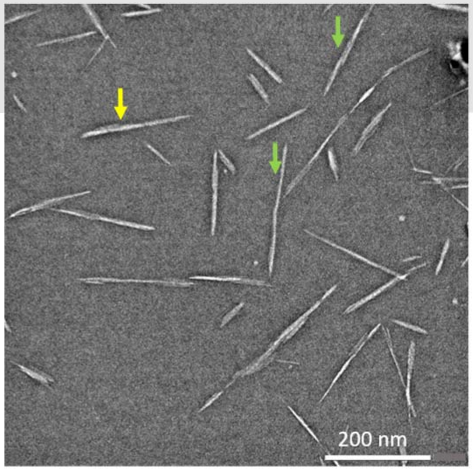
Means, 71 to 105 nm

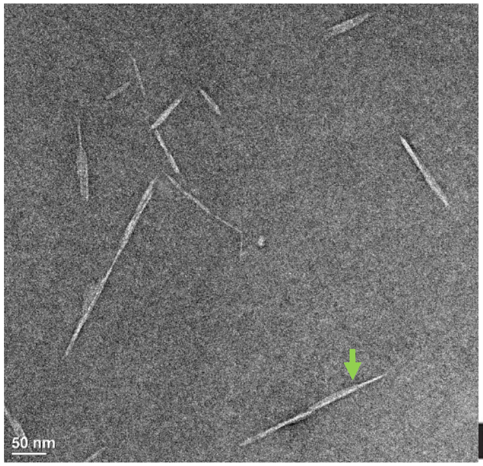


TEM Results: 10 Laboratories



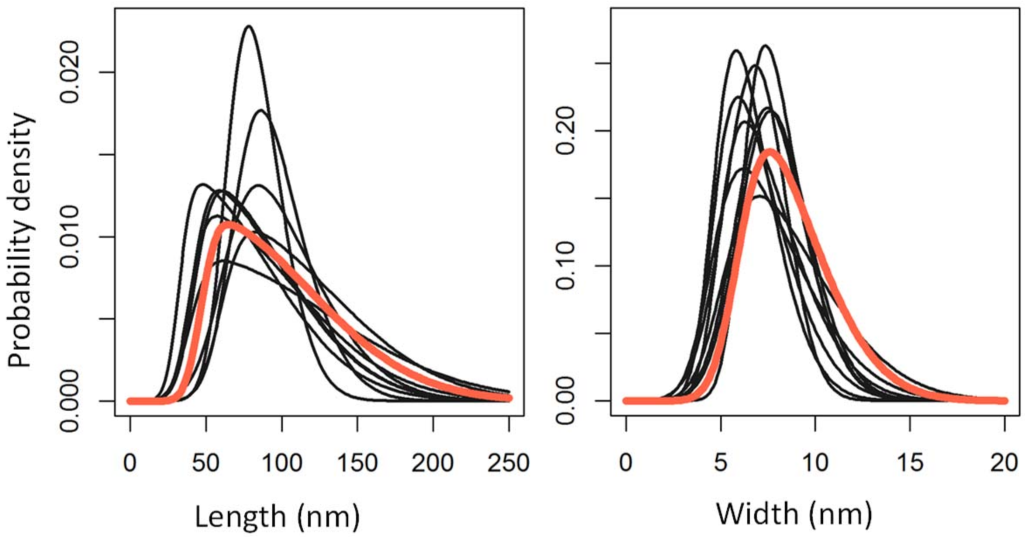






- Mean length varies from 78 to 116
- Mean width varies from 6.5 to 8.6

Interlaboratory Comparison Results



TEM distributions: skew normal fits for 10 labs (black) and consensus (orange)

Length

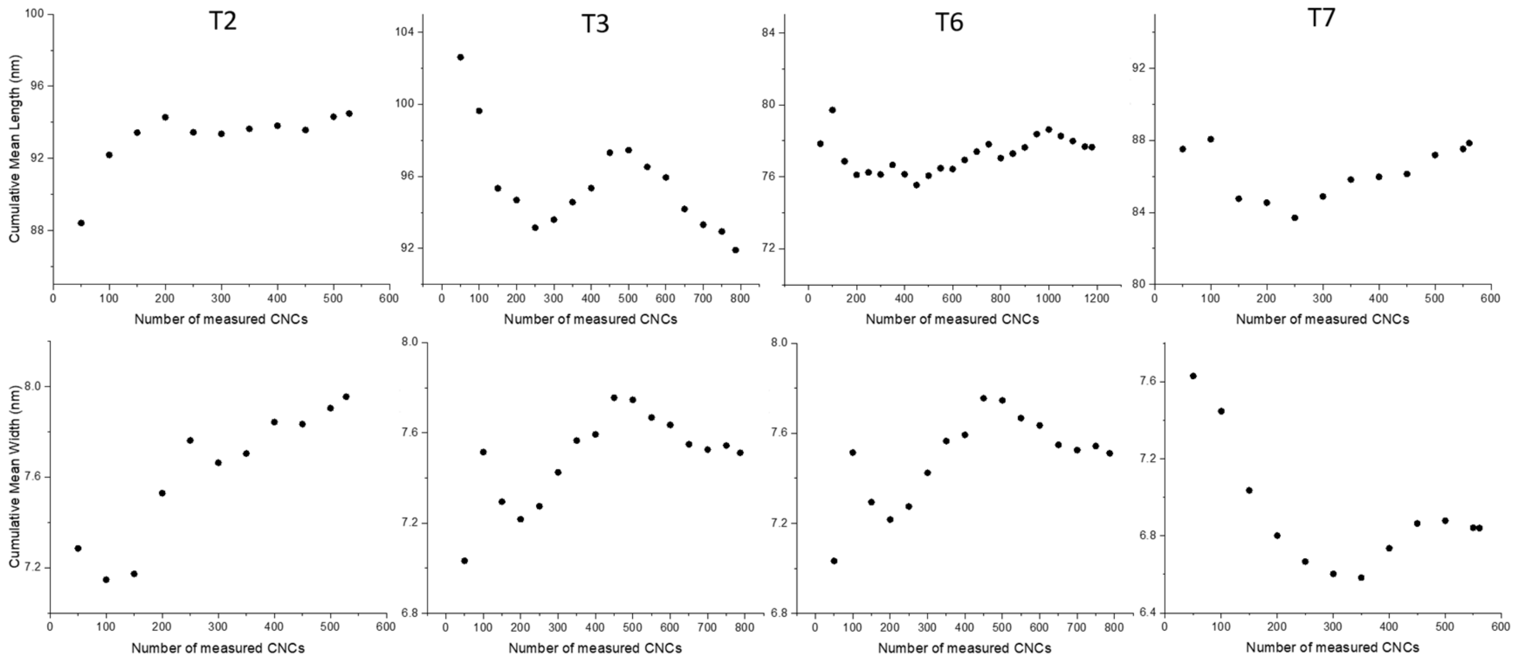
	Mean, nm	Standard deviation, nm	Shape, α
Consensus value (std uncertainty)	**95.8 (0.4)**	**39.0 (0.3)**	**4.8 (0.2)**
Dispersion (std uncertainty)	**12.3 (0.5)**		

Width

	Mean, nm	Standard deviation, nm	Shape, α
Consensus value (std uncertainty)	**7.65 (0.01)**	**2.20 (0.02)**	**2.8 (0.1)**
Dispersion (std uncertainty)	**0.78 (0.03)**		

Bushell, et al, *Cellulose*., **28,** 1387, 2021

How Many CNCs Should Be Analyzed?



- mean size stabilizes after ~300 analyzed CNCs for some cases, but not others
- sample heterogeneity (staining, agglomeration) and analyst bias

What Have We Learned?

AFM and TEM length agree; TEM width >2 times larger than AFM height (agglomeration of CNCs, multiple crystallites)

Lab-to-lab variability is larger than sample-sample variability for a single lab

Analyst bias contributes to variability and is more of an issue for TEM

TEM: - sample heterogeneity (agglomeration and staining) and subjectivity in selection of individual CNCs are main sources of ILC variability
- higher magnification gives smaller width (0.3 nm/pixel requested)

AFM: probe affects intra- and inter-laboratory variability (differences between probes, probe deterioration) but no obvious effect of imaging force

Decision on number of CNCs to analyze—requires careful monitoring of probe effects (AFM) and assessment of whether stable “size” is achieved (TEM)

Acknowledgements

NRC Metrology Research Center: Juris Meija, Mike Bushell, Maohui Chen, Martin Couillard, Zygmunt Jakubek + other group members

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Expert Team: ISO TS/23151 Particle size distributions for cellulose nanocrystals – in press

Natural Resources Canada, Forestry Innovation Program