Semi-automated image analysis framework on the morphology analysis of CNCs

Sezen Yucel
PhD Candidate, Georgia Institute of Technology
MINED (Materials Informatics for Engineering Design) Group

Team
Dr. Surya R. Kalidindi, GT, Schools of ME, MSE, and CSE
Dr. Robert J. Moon, Forest Products Laboratory
Dr. Linda J. Johnston, National Research Council Canada & University of Ottawa

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OUTLINE

- CNC particle size measurements
- Introduce SMART
  - Development
  - Proof testing
- Utilization of SMART
  - Comparisons to manual measurements
- Conclusion

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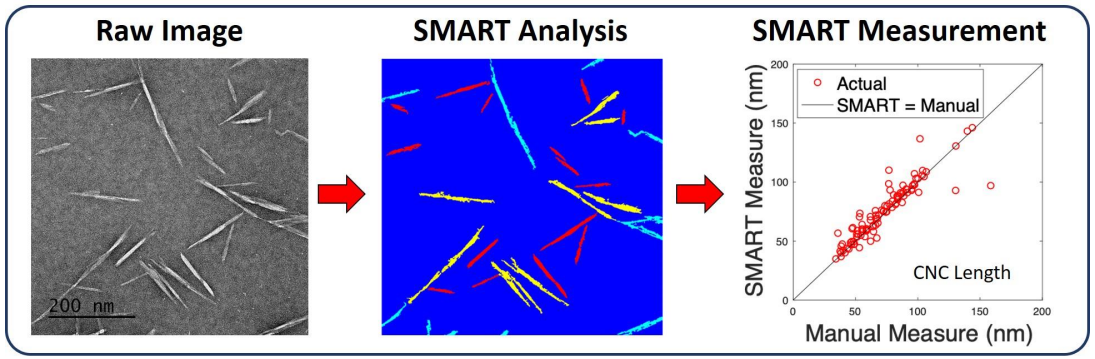
ORIGINAL RESEARCH

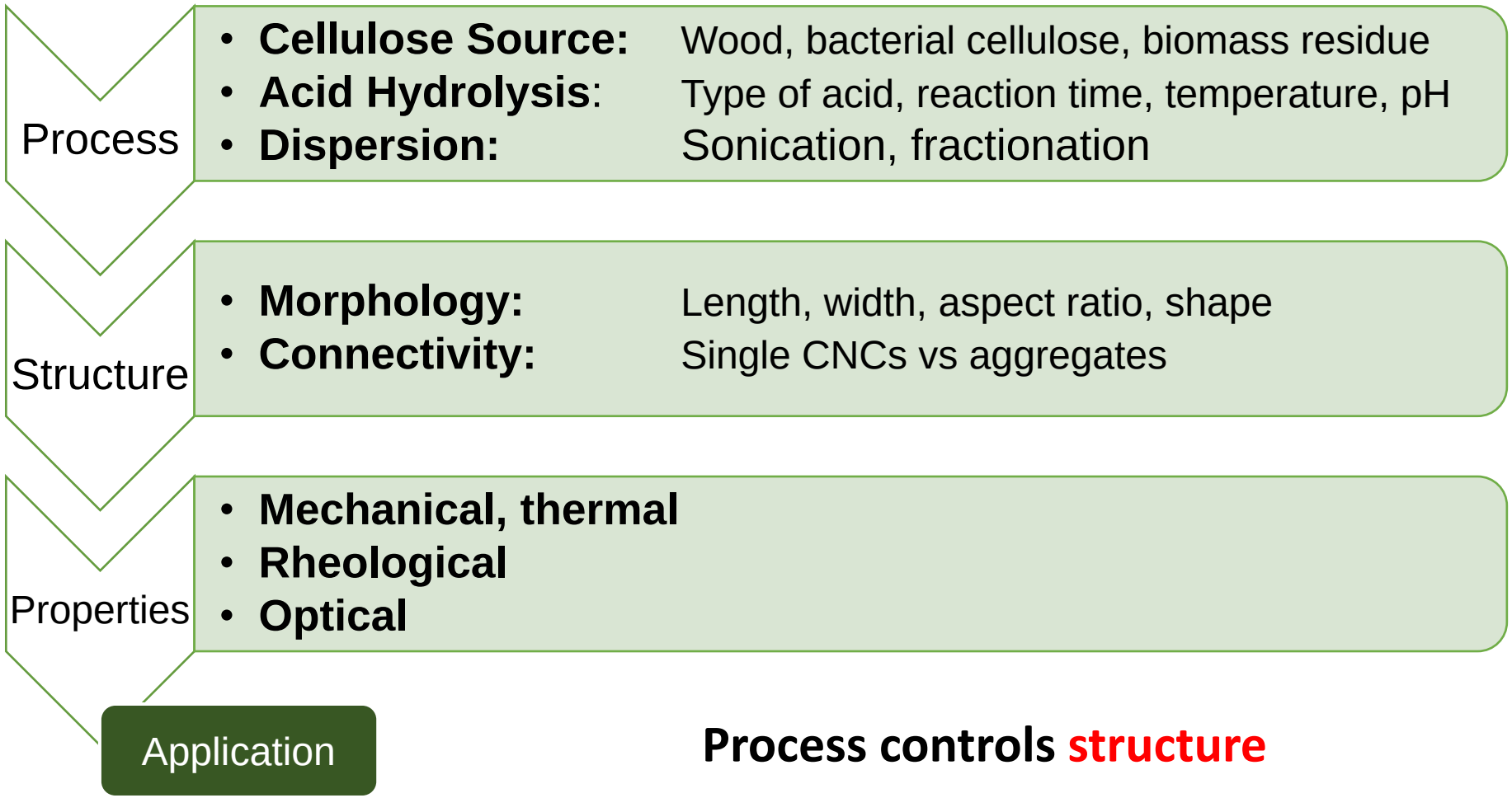
Semi-automatic image analysis of particle morphology of cellulose nanocrystals

Sezen Yucel · Robert J. Moon · Linda J. Johnston · Berkay Yucel · Surya R. Kalidindi



"As Good as Human"

Process – Structure – Property relation for CNCs

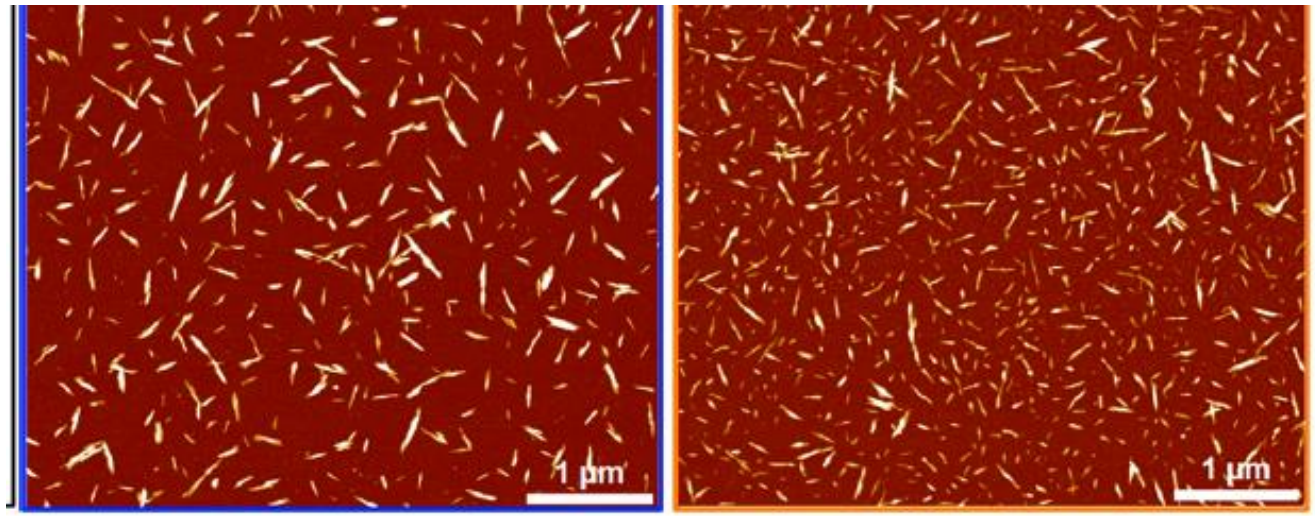


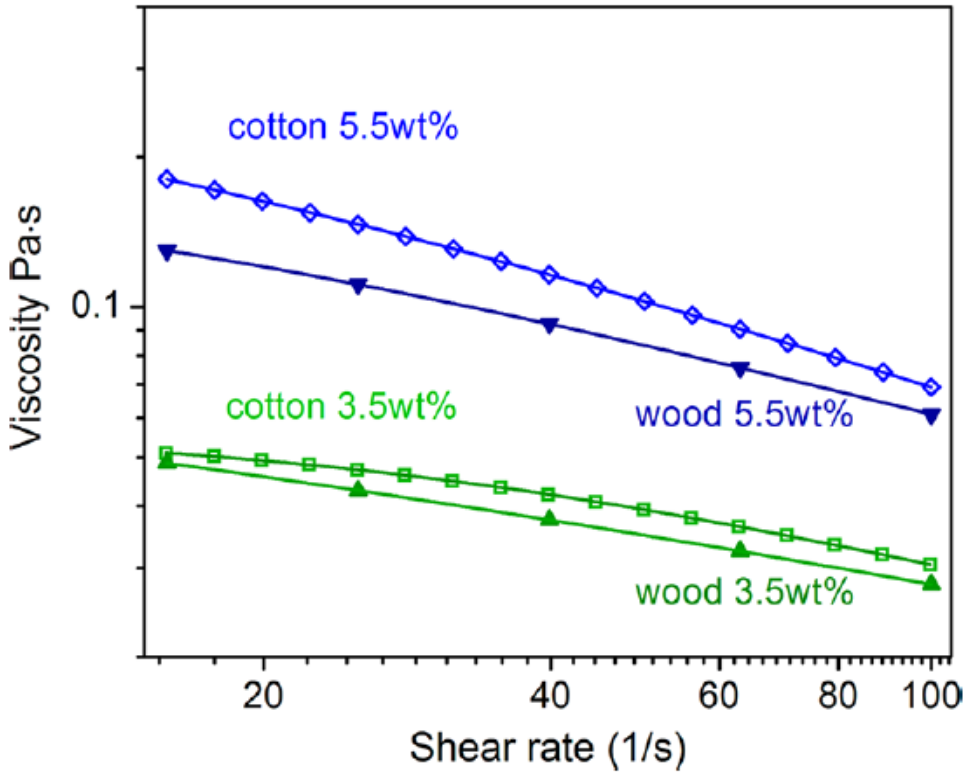
Process controls structure

Structure controls property

CNC morphology influences properties

Cotton Wood





Key Point:

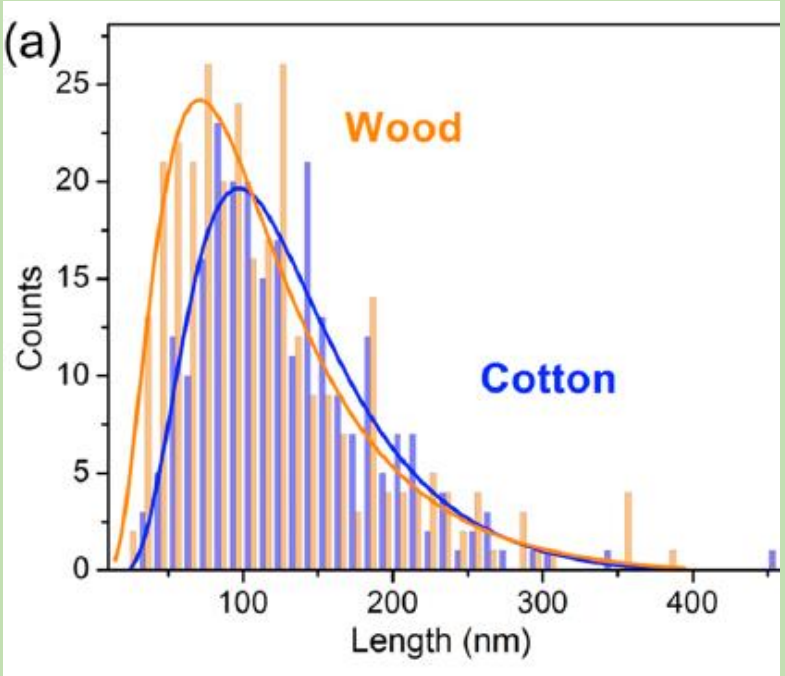
- Performance dictated by CNC size

Schutz et al. ACS Sustainable Chem. Eng. 6: 8317-8324 (2018)

Particle Size & Distribution Measurements

Particle Size

- Length, width, aspect ratio
- Distributions

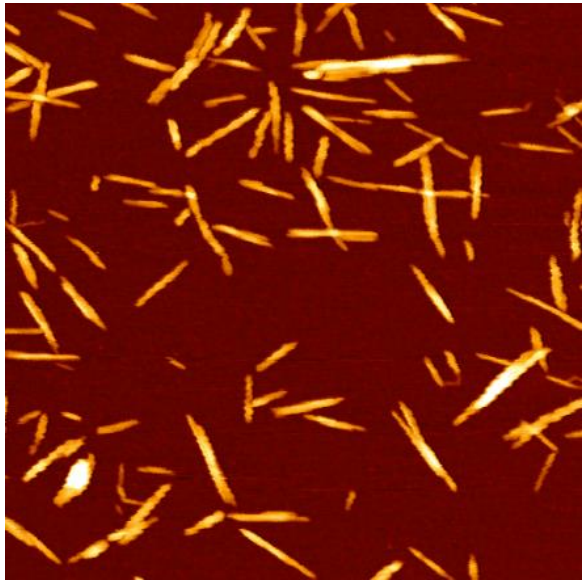


Techniques

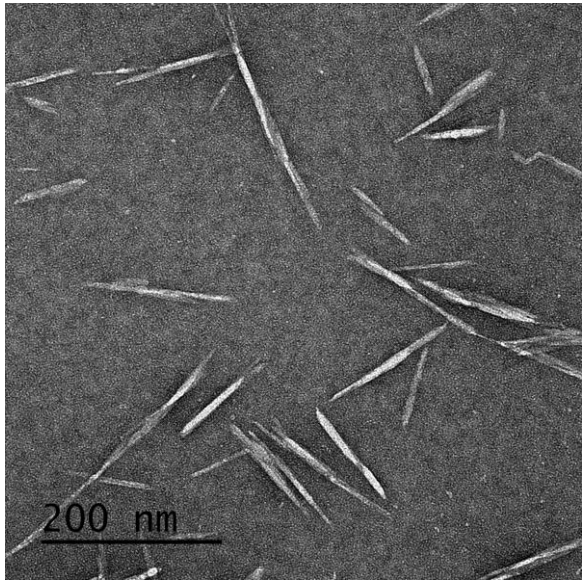
- Image Analysis
  - Optical
  - AFM, TEM, SEM
- Scattering..
  - DLS – Dynamic Light Scattering
  - Laser Diffraction Analyzer
  - Transmittance
  - Turbidity
- Fractionation
  - Centrifugation
  - Asymmetric field flow fractionation

Measuring CNC Morphology

AFM



TEM



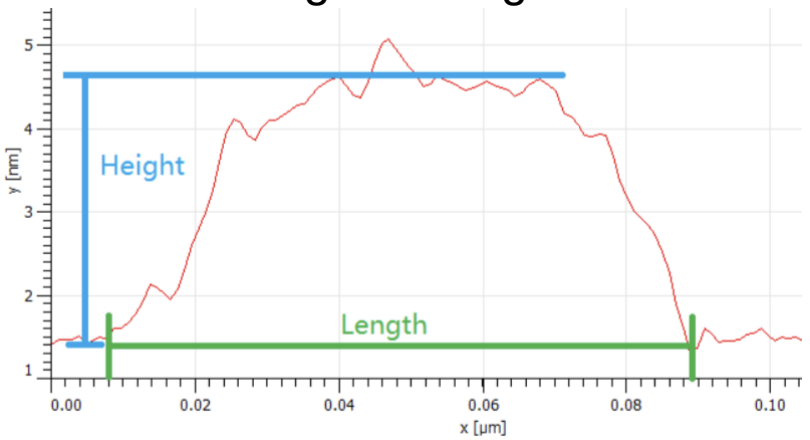
Problems with AFM & TEM analysis:

- Time consuming
- Subjective (user bias)
- Inconsistent

Challenges – Time Consuming

AFM

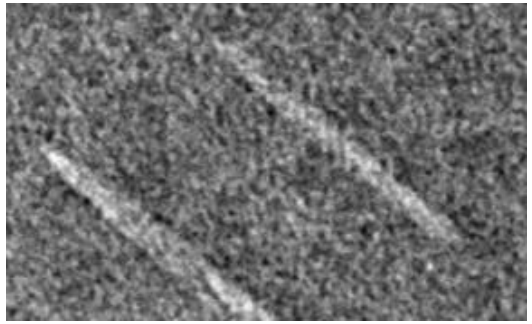
Length & Height



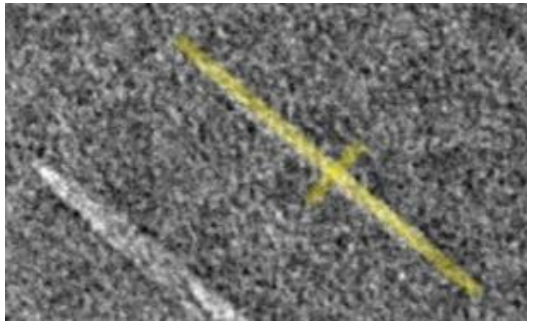
- One-by-one selection
- Line drawing
- ~30 sec/CNC
- ~500 CNCs*

  (recommended number)

TEM



Length & Width



Thesis: Leng, University of Ottawa (2016)

* Meija et al, *Analytical Chemi*stry (2020)

Challenges – Subjective (user bias)

- Interlaboratory comparison, CNC particle size analysis*
  - Reference CNC material, CNCD-1**
  - 6 analysts' measuring CNCs from the same 30 image TEM dataset

- Issues:
  - Different CNCs identified
  - Different mean lengths
  - Different mean widths

Lab	n	Length, nm			Width, nm		
		Mean	Std dev	median	Mean	Std dev	median
Lab 1, analyst 1	284	98.6	47.2	89.3	9.8	3.4	9.1
Lab 1, analyst 2	127	71.1	29.2	67.3	9.0	2.4	8.7
Lab 2	244	77.1	35.8	70.7	7.0	2.1	6.8
Lab 3	370	105	47.4	95.9	10.0	5.4	9.6
Lab 4	442	76.3	37.0	72.3	9.5	3.8	9.2
Lab 5	216	71	28	66.1	8.1	2.3	8.0

*Meija et al. Particle size distributions for cellulose nanocrystals measured by transmission electron microscopy: an interlaboratory comparison. *Analytical Chemis*try (2020)

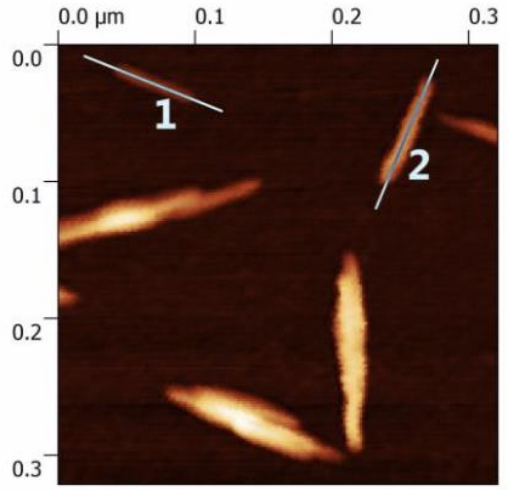
** National Research Center of Canada, http://www.nrc.ca/crm

Challenges – Subjective (user bias)

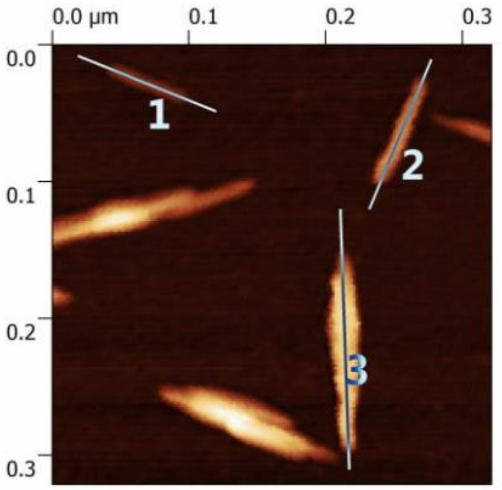
- 2 analysts' measuring 300 **total** CNCs from the same AFM images
- Measured different mean lengths & heights
- Identified **different** CNCs

	Analyst 1	Analyst 2	% Difference
Length (nm)	91.0 ± 32.4	109.4 ± 45.0	% 20.3
Height (nm)	4.2 ± 1.2	5.0 ± 1.7	% 19.1

Analyst 1



Analyst 2



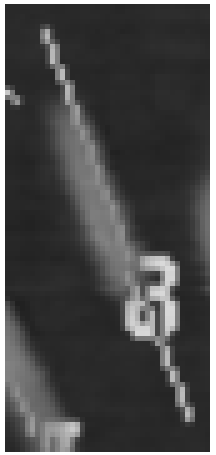
Thesis: Leng, University of Ottawa (2016)

Challenges – Inconsistent

- 2 analysts' measuring 100 **same** CNCs from AFM
- Measured different mean heights
- Dependent on placement of trace line

	Analyst 1	Analyst 2	% Difference
Length (nm)	98.4 ± 35.3	99.4 ± 34.0	% 1.3
Height (nm)	4.0 ± 1.2	4.5 ± 1.2	% 17.1

Trace line too closed to edge?



Thesis: Leng, University of Ottawa (2016)

OUTLINE

- Challenges in measuring CNCs

➔ Time-consuming, subjective, and inconsistent measurements;

➔ Lab-to-lab, person-to-person variability

- Introduce SMART
  - Development
  - Proof testing
- Utilization of SMART
  - Comparisons to manual measurements
- Conclusion

Semi-Automatic Image Analysis for CNCs

Develop an image analysis framework

- TEM & AFM images
- Combination of automated & semi-automated functions
- Automatically
  - detects/selects individual CNCs
  - Measures: length, width, height, area, etc.
  - plots/charts data
- Results are: accurate, consistent, and fast

Goal

- ***"As Good as Human"*** in the identification & measurement of CNCs

Welcome to SMART

SMART

- S – Standardized
- M – Morphology
- A – Analysis

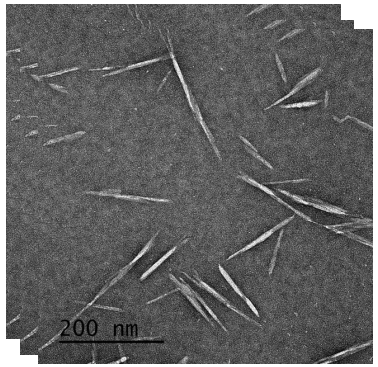
for

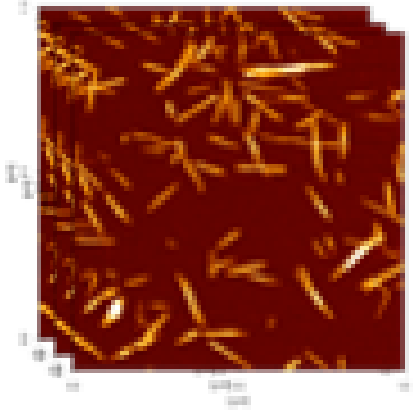
- R – Research

and

- T – Technology

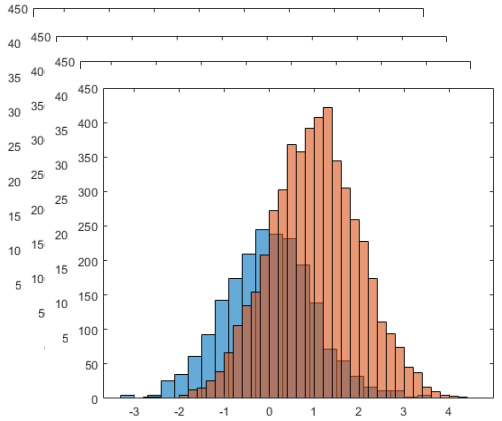
Micrographs

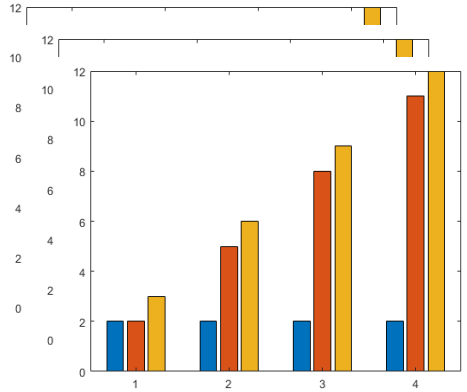




CNC SMART

Particle Morphology Info.



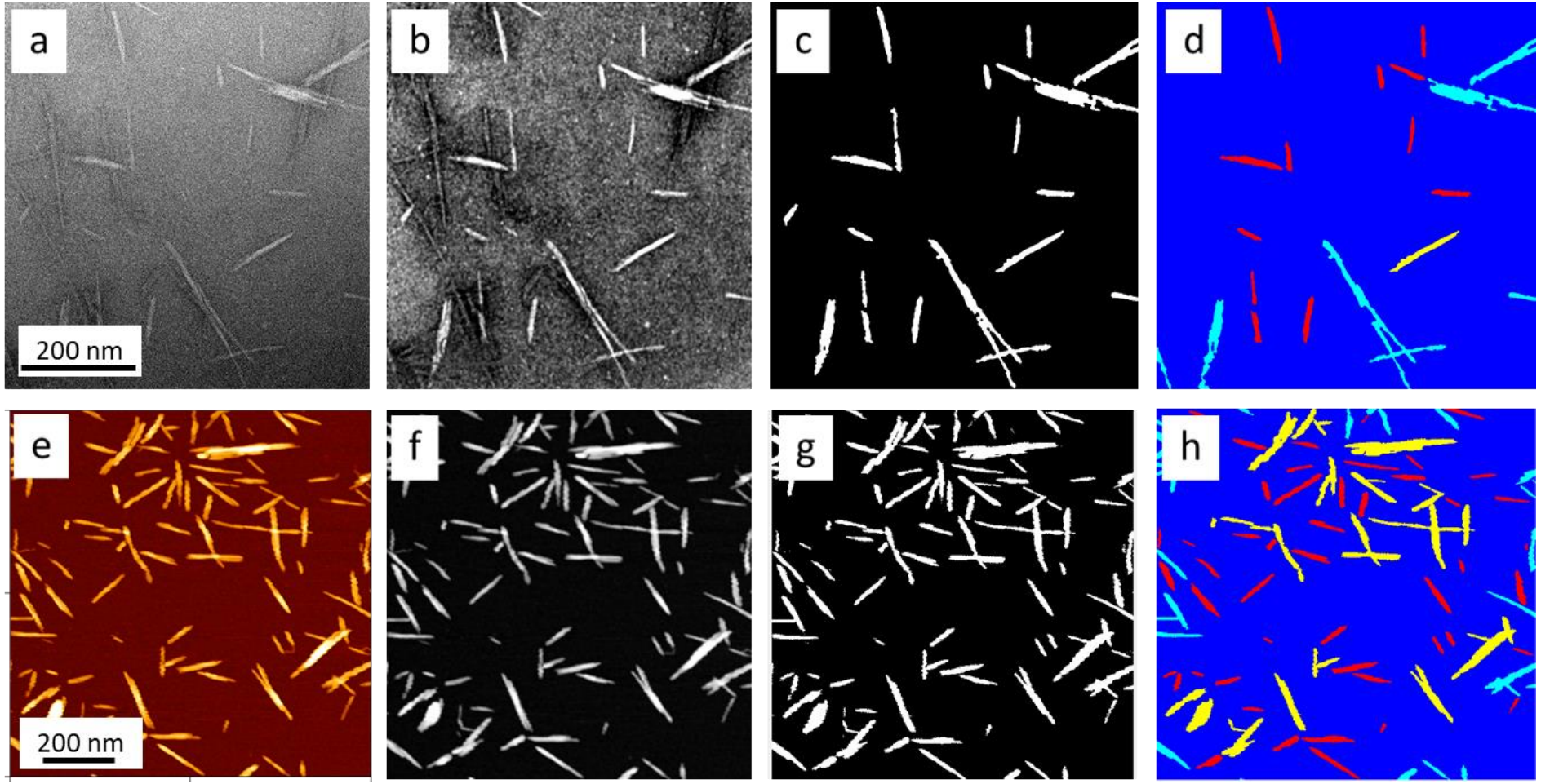


Approach

Raw image **Enhancement** **Segmentation** **Identification**

TEM

AFM



- Isolated CNCs
- Grouped CNCs
- CNCs at border

- Sharpening
- Smoothing

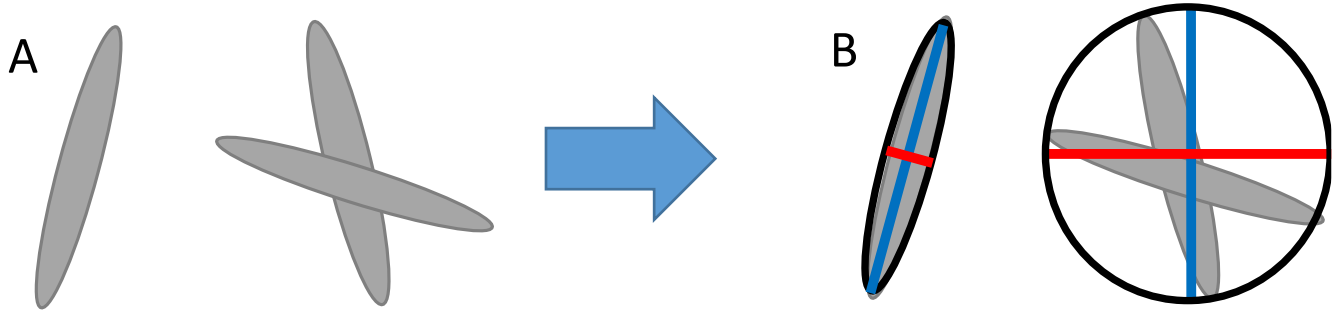
- BW image 0 or 1

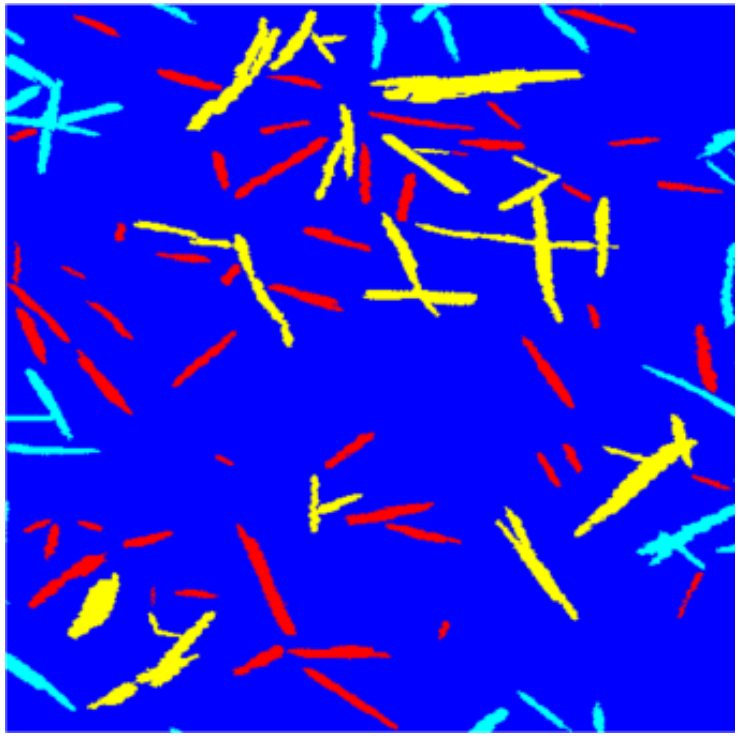
- Multi-step selection

Step1: Selection via Aspect Ratio

Isolated CNC selection

- SMART encircles continuous objects
- Measure major & minor axis lengths: L & W





TEM Criteria

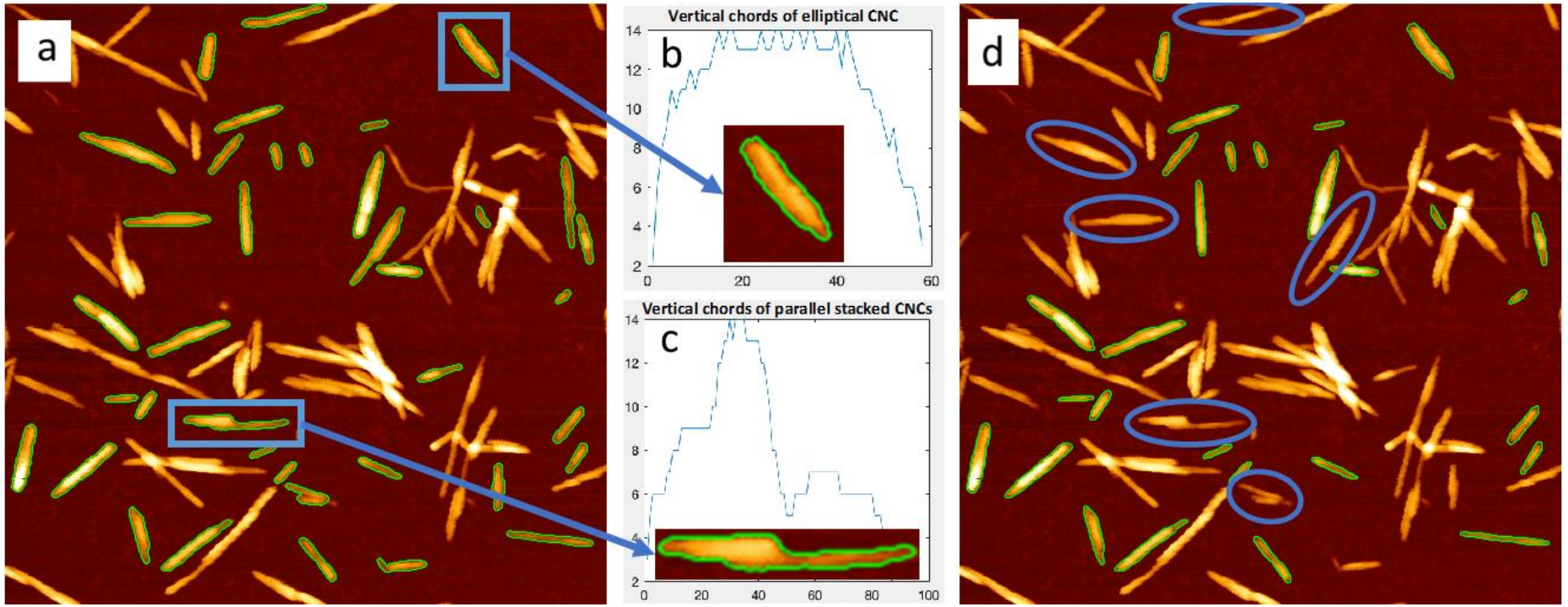
- Aspect ratio: L/W > 2.5
- Length: L = 15 to 250 nm
- Width: W < 15 nm

AFM Criteria

- Aspect ratio: L/W > 2
- Length: L = 15 to 300 nm
- Width: W < 30 nm

Step2: Removal of “Stepped” Objects – Width

AFM

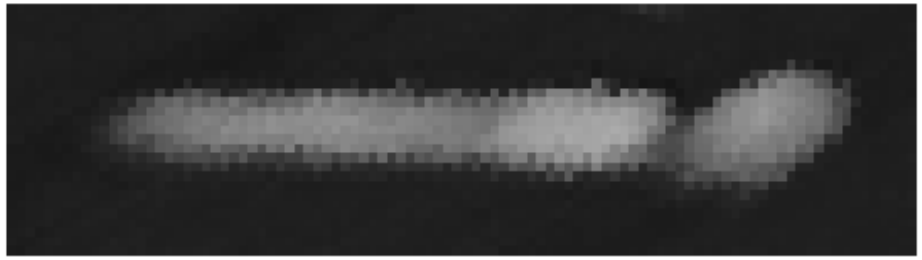


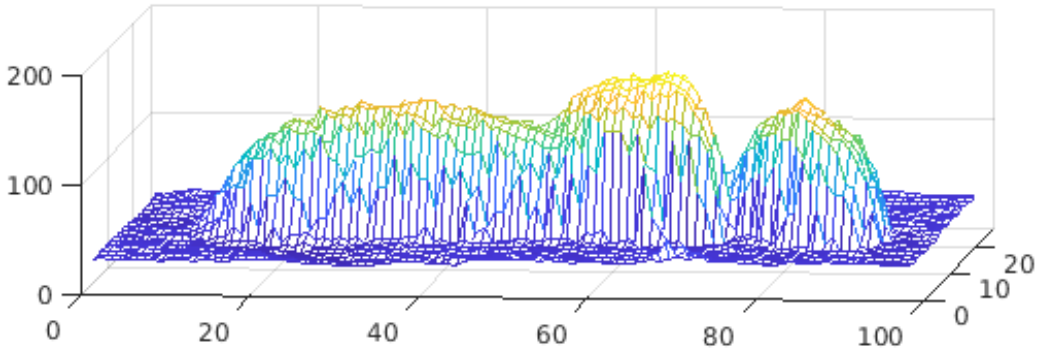
Width Stepped Approach

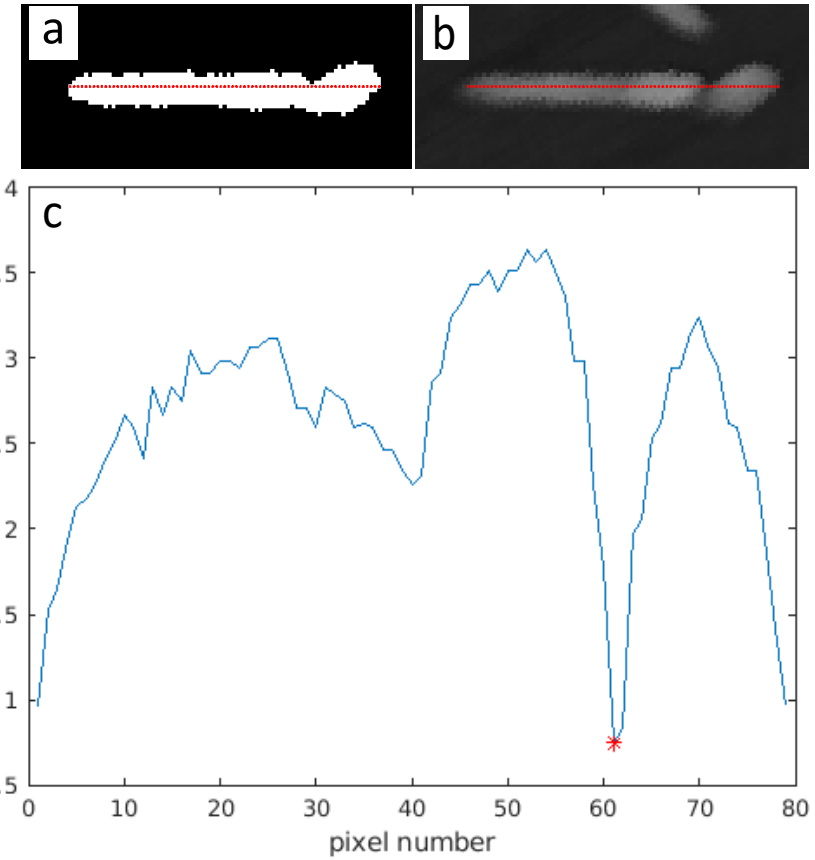
- AFM Images
- TEM Images

Step3: Removal of “Stepped” Objects – Height

AFM







Height Stepped Approach

- AFM Images only

Proof Testing: SMART vs Manual Measurement

Side by Side Comparison

- Commonly identified CNCs in SMART and Manual
- Measure: length, width, height
- Track each common CNC to see if any difference
- Differences only a result of the approach (SMART vs Manual)

Objective

- Test if we can identify and measure ***"As Good as Human"***

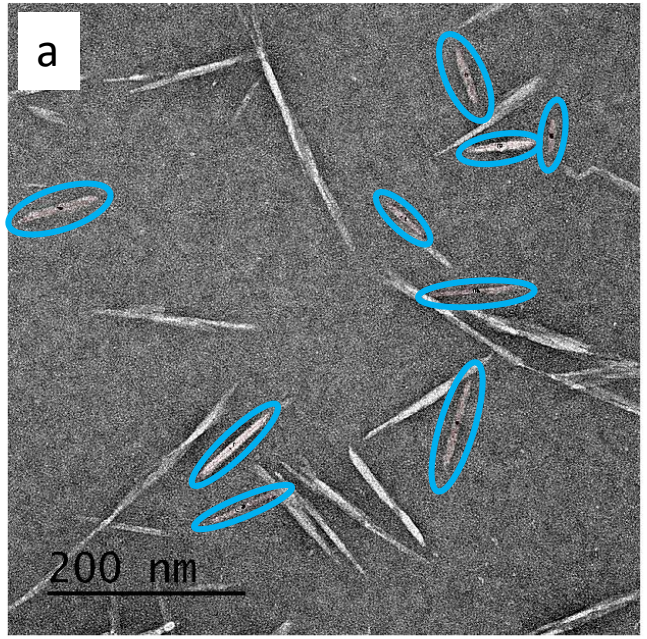
Dataset

- AFM and TEM images from *"Characterization challenges for a cellulose nanocrystal reference material: dispersion and particle size distributions"* by Jakubek et al (2018)

TEM

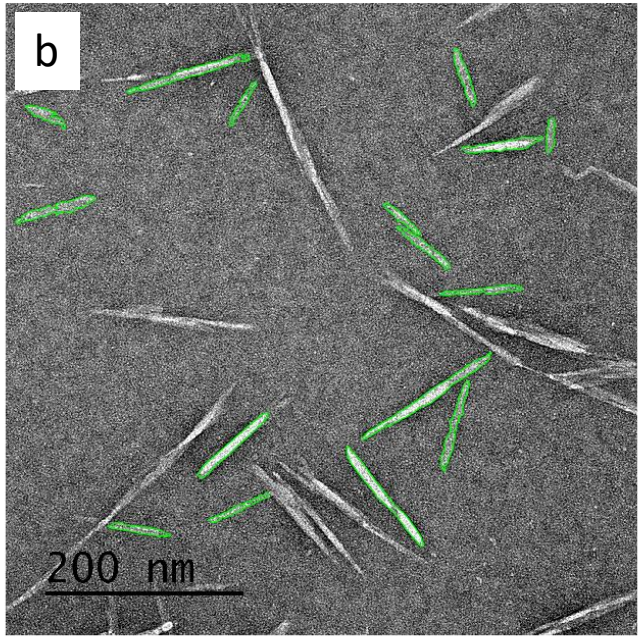
CNC Identification

Manual



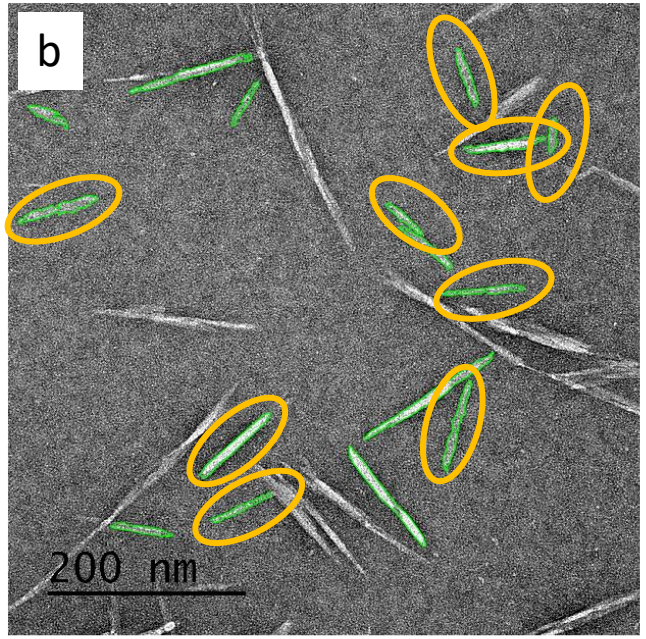
- 9 CNCs identified
- Blue ovals highlighting the selected CNCs were added by me, as it is difficult to see the manual measurements

SMART



- 14 CNCs identified
- Green outlines are SMART calculated perimeters of each identified CNC

Common CNCs



- 9 Common CNCs identified
- Orange ovals so the common identified CNCs between manual and SMART selection approaches

AFM

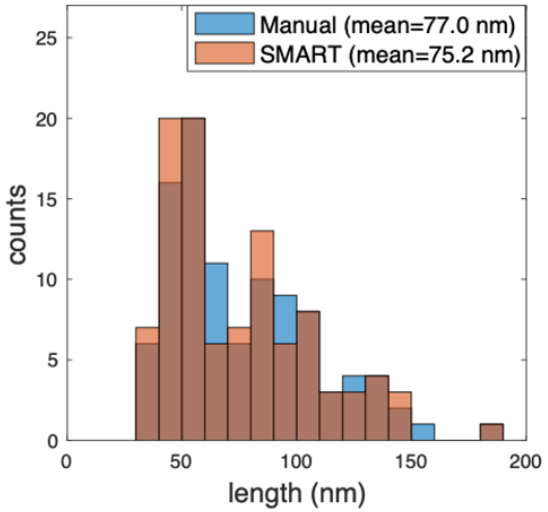
CNC Measurement

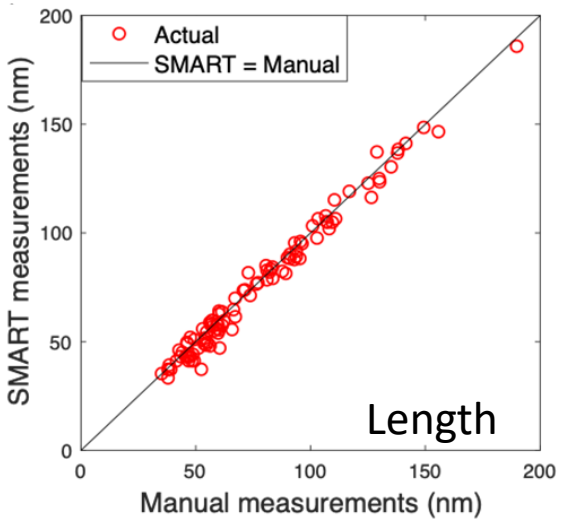
Dataset
- 5 AFM images
- 101 common CNCs

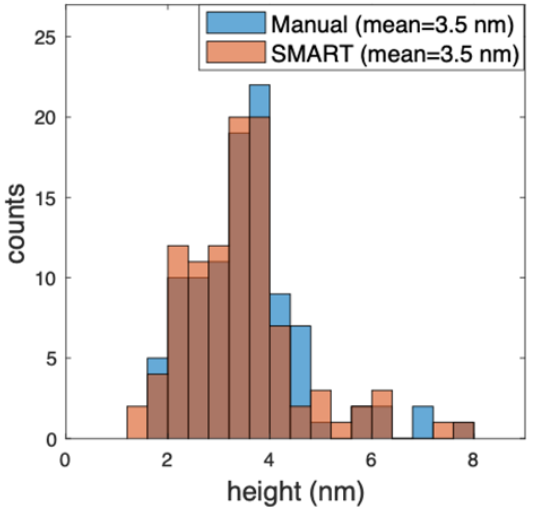
Mean
- Length: Δ = -1.8 nm
- Height: Δ = 0.0 nm

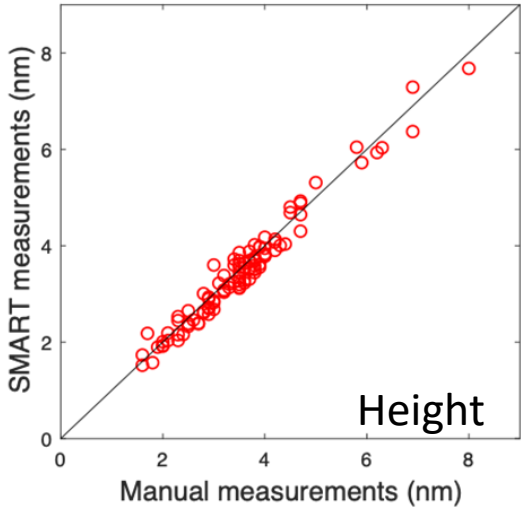
Distribution
- Length: same
- Height: same

Individual CNCs
- Length: same
- Height: same









Direct individual CNC to CNC comparison:
Points on the line indicate SMART = manual measurement

TEM

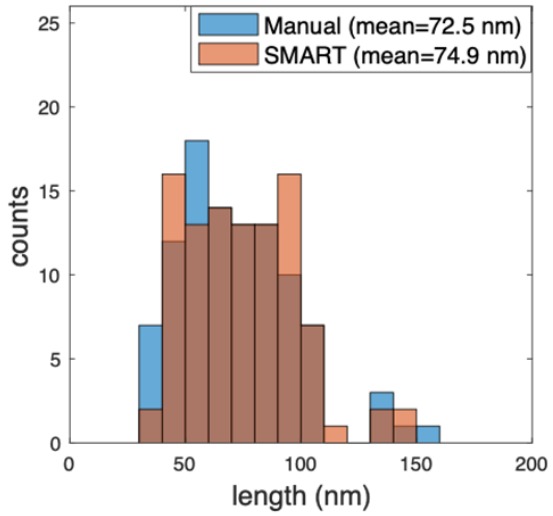
CNC Measurement

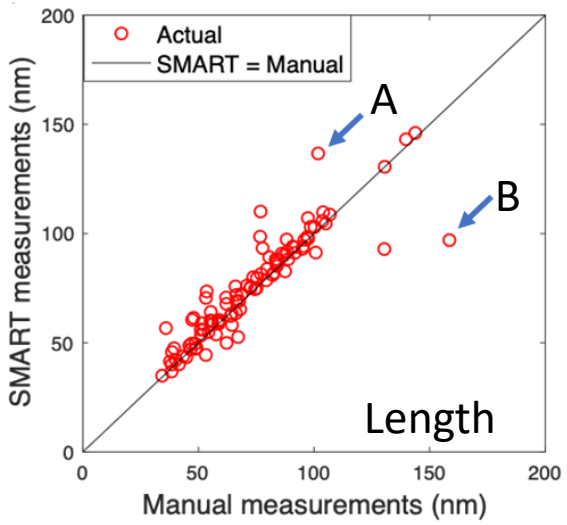
Dataset
- 15 TEM images
- 99 common CNCs

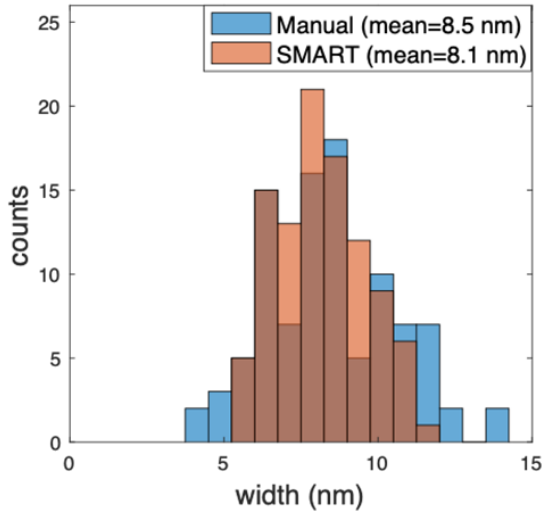
Mean
- Length: Δ = +2.4 nm
- Width: Δ = -0.4 nm

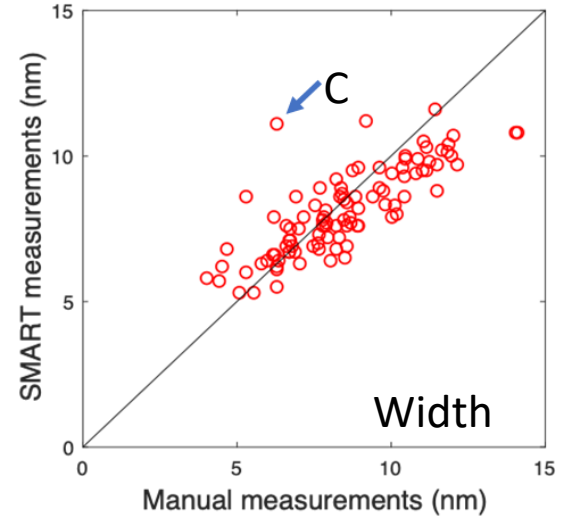
Distribution
- Length: Similar
- Width: Similar

Individual CNCs
- Length: very similar
- Width: low deviation



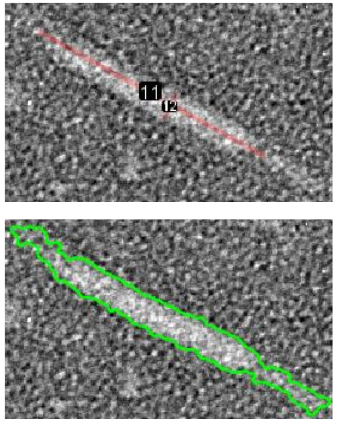






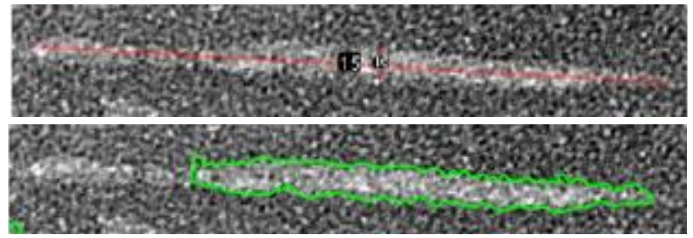
Direct individual CNC to CNC comparison:
Points on the line indicate SMART = manual measurement

A



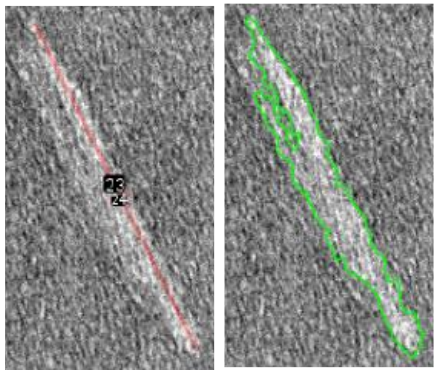
SMART is long

B



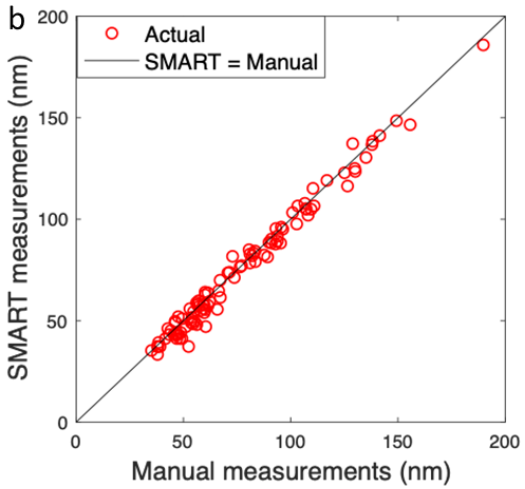
SMART is short

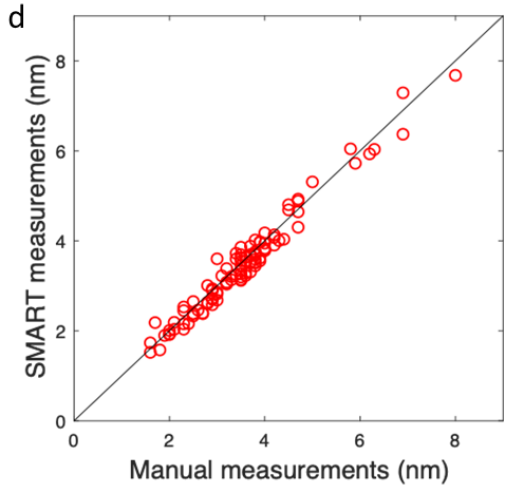
C



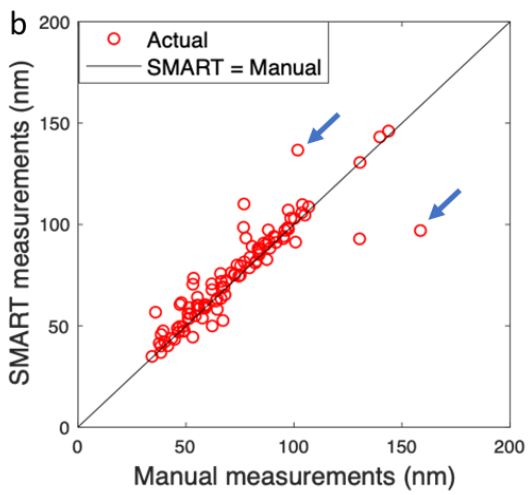
SMART is too wide

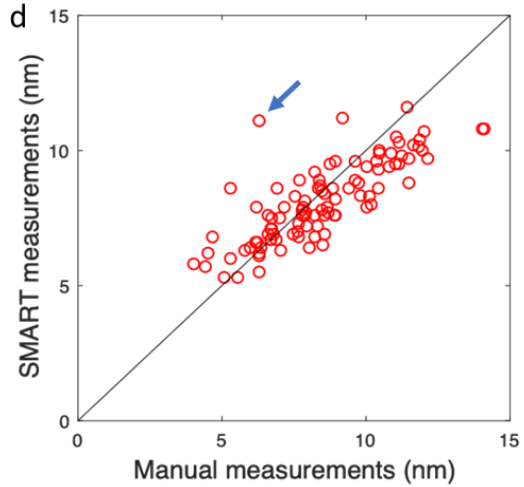
AFM





TEM





Key Result

- AFM image analysis: SMART nearly identical to manual
- TEM image analysis: SMART very similar to manual

OUTLINE

- Challenges in measuring CNCs
- Introduce SMART
  - Development
  - Proof testing

➔ Semi-automated image analysis framework
➔ Hierarchical multi-step process of isolated CNC identification
➔ SMART vs manual measurements
"As good as human"

- Utilization of SMART
  - Comparisons to manual measurements
- Conclusion

Utilization of SMART
Comparison to manual measurements

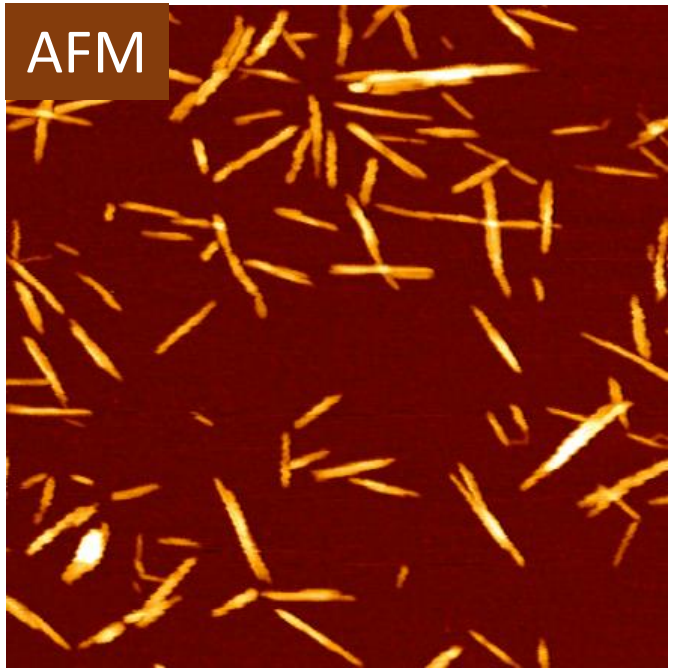
- A larger AFM and TEM dataset
  - Reference CNC material (fully characterized)
  - Trained/experienced in imaging and analysis
  - Idealized images (good contrast, well dispersed CNCs)
  - Measure: length, width, height
  - Comparing image analysis SMART vs manual

Dataset

"Characterization challenges for a cellulose nanocrystal reference material: dispersion and particle size distributions" by Jakubek et al (2018)

AFM Capability Example: Fast & Accurate

Raw Image



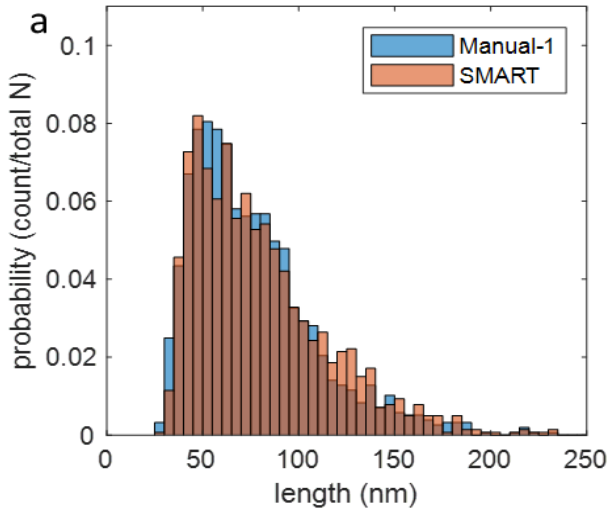
1 micron

Start → Finish

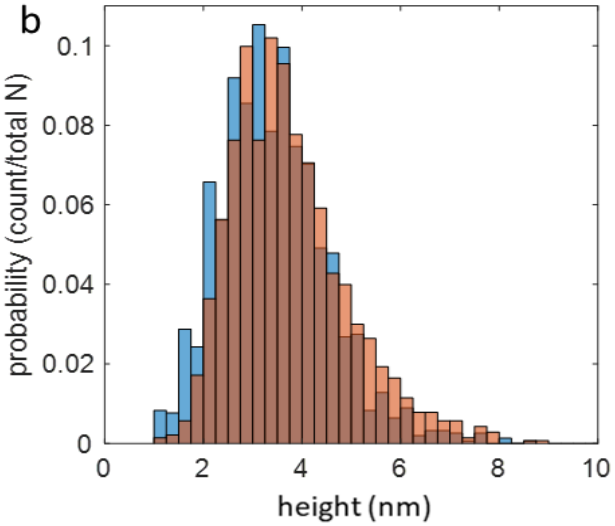
SMART:
- 66 AFM images
- 1403 CNC measured
- 7.5 mins
- 0.35 secs/CNC

Manual:
- ~ 30 secs/CNC
- ~ 11 hrs to complete

Data & Statistics



Length

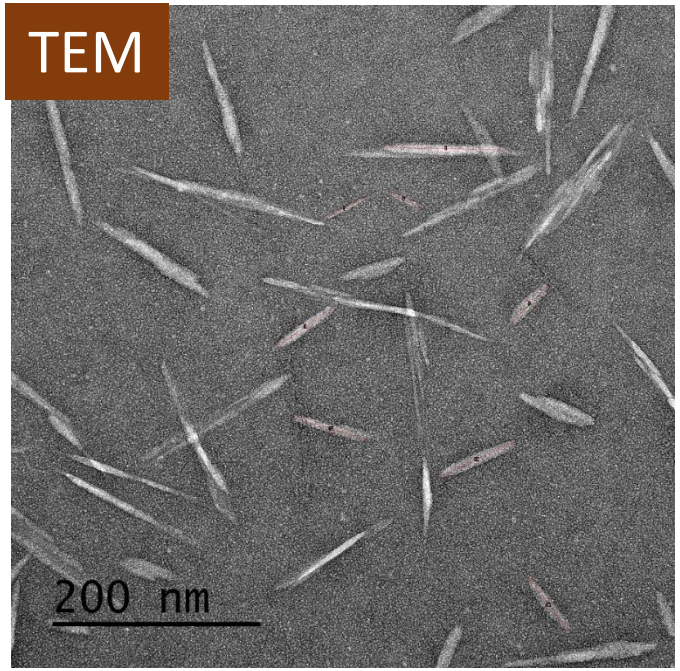


Height

Desktop Computer:
- i7 processor, 16 GB RAM, 3.7 GHz

TEM Capability Example: Fast & Accurate

Raw Image

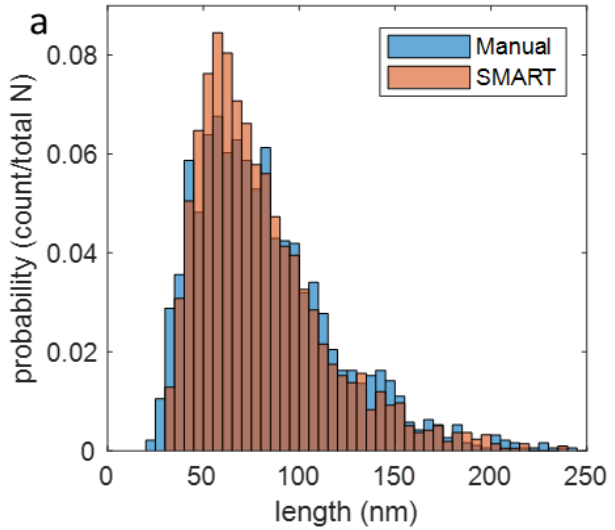


Start → **Finish**

SMART:
- 434 TEM images
- 2177 CNC measured
- 43 mins
- 1.2 secs/CNC

Manual:
- ~ 30 secs/CNC
- ~ 18 hrs to complete

Data & Statistics



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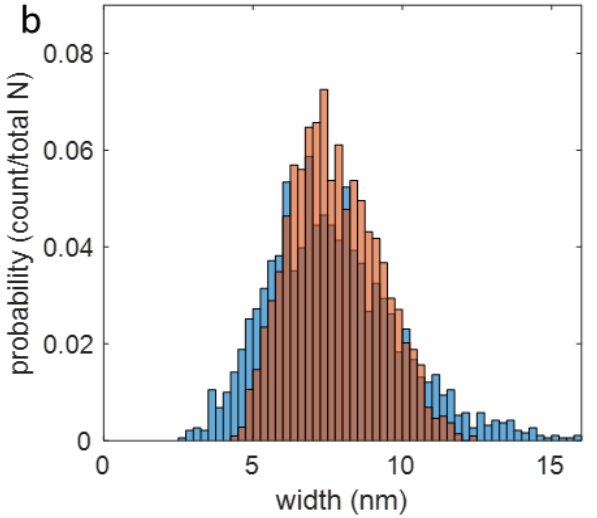
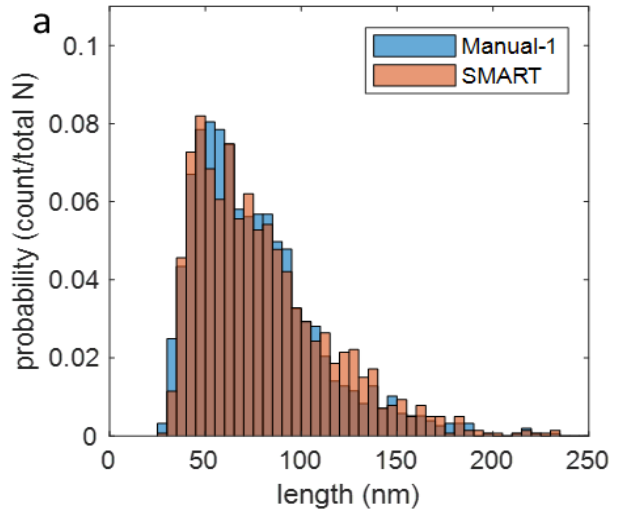
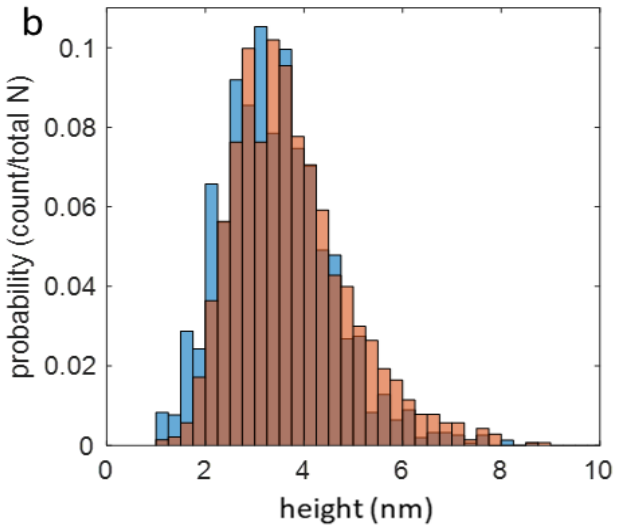


Width

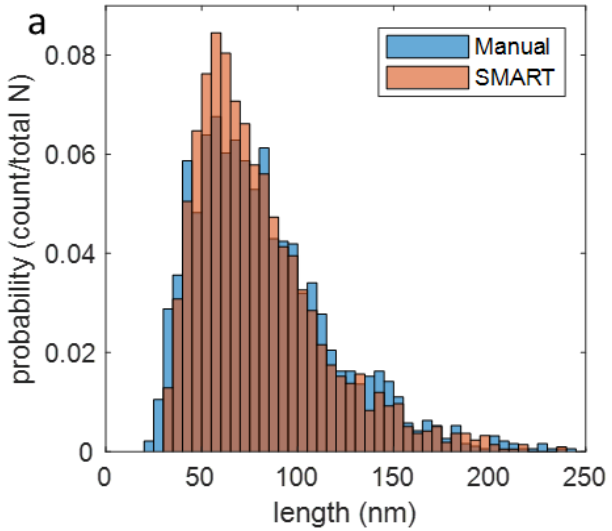
Desktop Computer:
- i7 processor, 16 GB RAM, 3.7 GHz

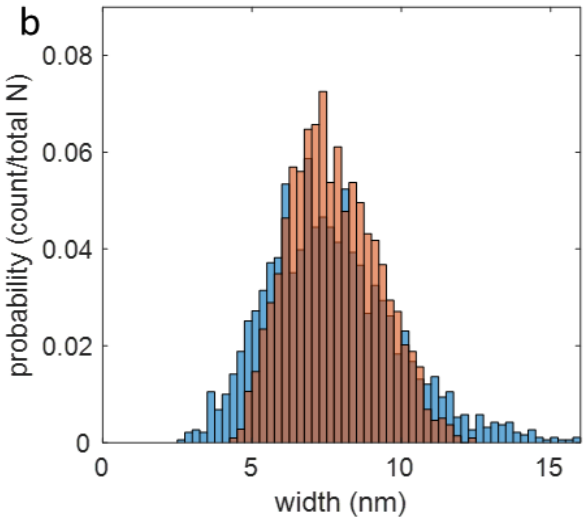
AFM





TEM





Key Result

- AFM and TEM image analysis: SMART is similar to manual
- SMART is much faster than manual approach

OUTLINE

- Challenges in measuring CNCs
- Introduce SMART
  - Development
  - Proof testing
- Utilization of SMART
  - Comparisons to manual measurements
- Conclusion

Testing SMART on large datasets

➔ Consistent results

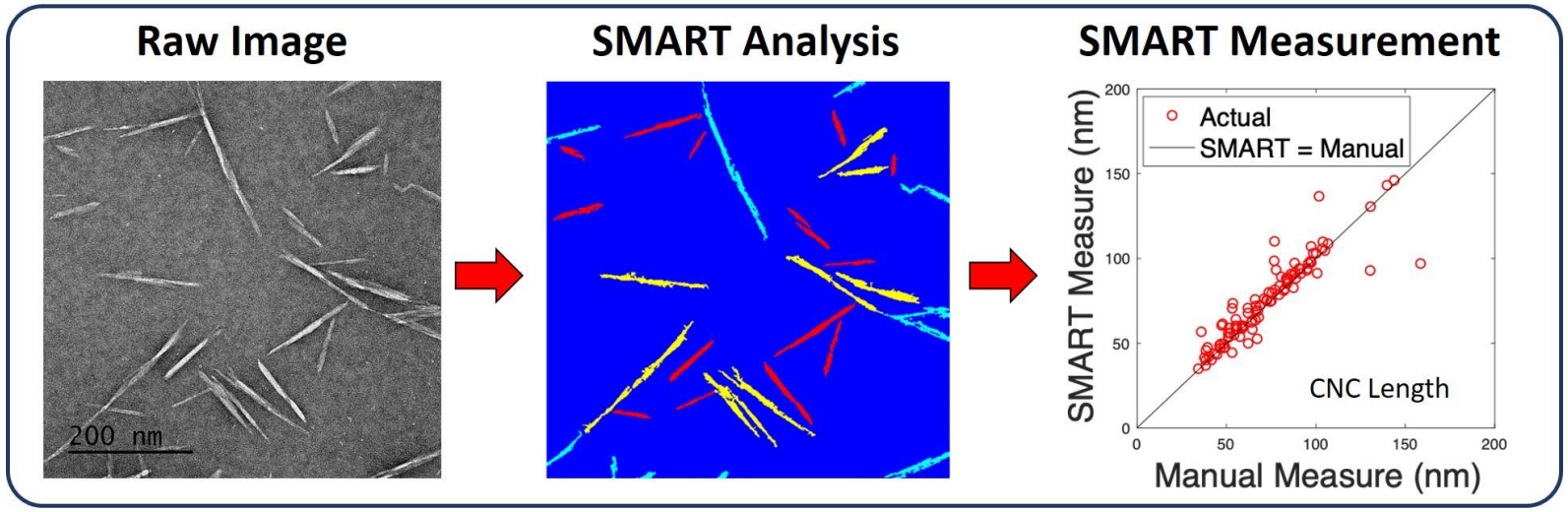
➔ Shorter analysis times

➔ SMART vs manual measurements
"As good as human"

Conclusion

SMART development & utilization:

- Demonstrated utility for AFM and TEM image analysis of CNCs
- Consistent and reliable results & >25 times shorter analysis times
- As good as human
- Additional grouping information



THANK YOU…

Questions & Comments